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Journal Pre-proof

Dopamine-conjugated CuS/chitosan nanocomposite for targeted photothermal drug delivery: *In vitro* cytotoxicity study to establish bio-compatibility

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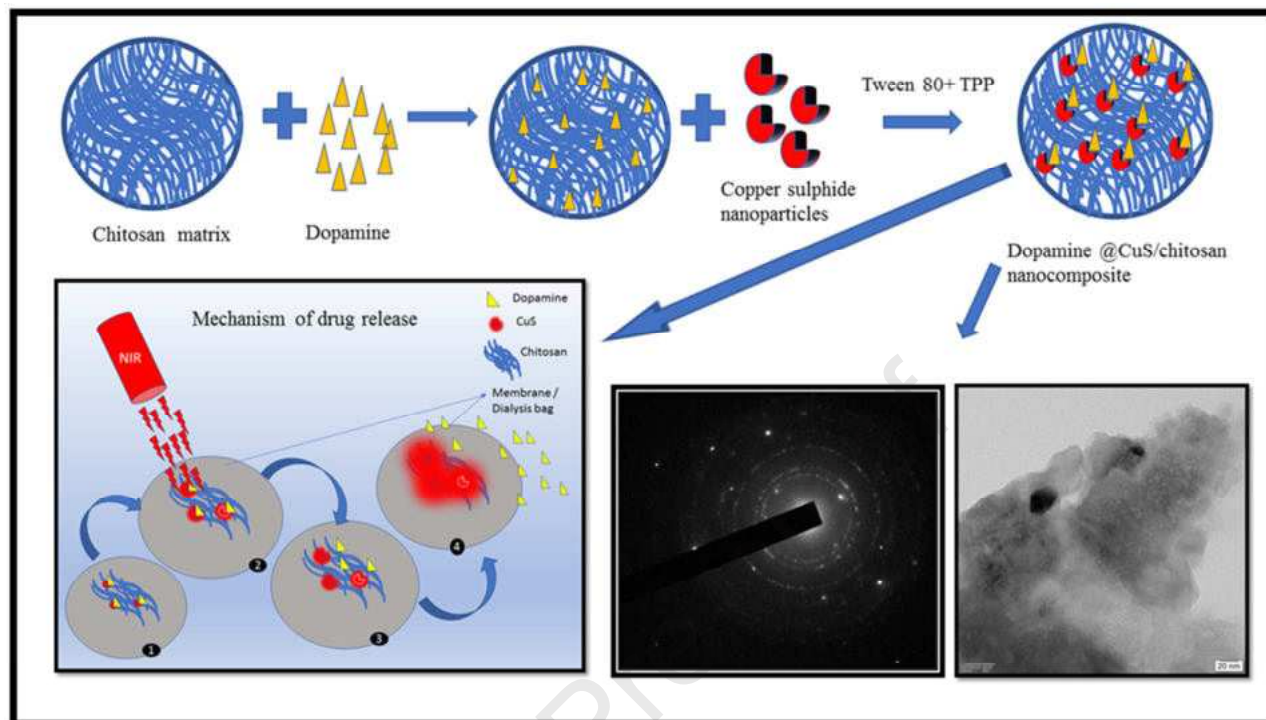
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Journal Pre-proof

Graphical Abstract



The synthesis of dopamine@ CuS/Chitosan is pictorially represented in the graphical abstract. Under mechanism of drug release, the drug encapsulated CuS/chitosan nanocomposite is introduced into the dialysis bag as indicated in inset 1. When irradiated with NIR light the CuS nanoparticles heat up as shown in inset 2. This dissipated heat causes the release of dopamine encapsulated in the nanocomposite (in inset 3). Finally, as indicated in inset 4 the drug is released across the membrane successfully.

Dopamine-conjugated CuS/chitosan nanocomposite for targeted photothermal drug delivery: *In vitro* cytotoxicity study to establish biocompatibility

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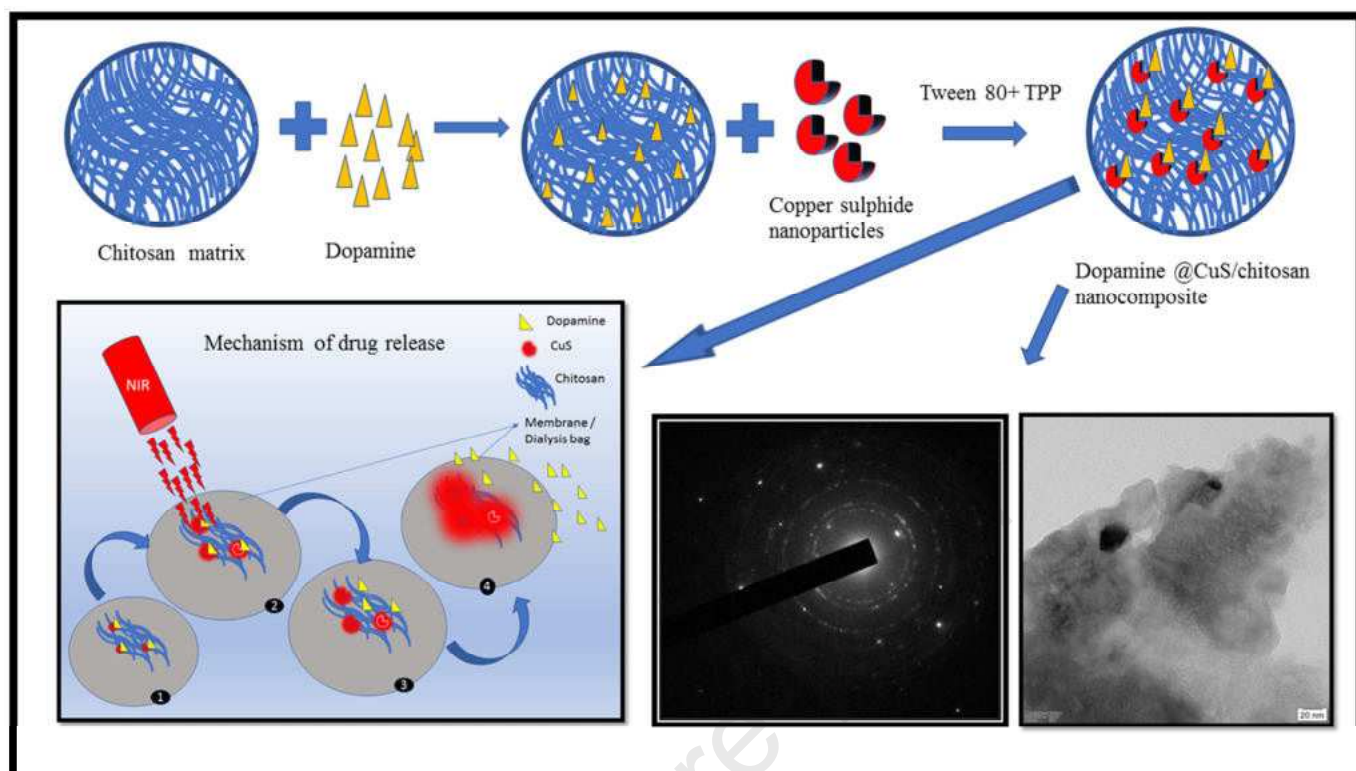
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Graphical Abstract

The synthesis of dopamine@CuS/Chitosan is pictorially represented in the graphical abstract. Under 'Mechanism of drug release', the drug encapsulated CuS/chitosan nanocomposite is introduced into the dialysis bag as indicated in inset 1. When irradiated with NIR light the CuS nanoparticles heat up as shown in inset 2. This dissipated heat causes the release of encapsulated dopamine from the nanocomposite into the medium, as seen in inset 3. Finally, as indicated in inset 4 the drug is released across the membrane successfully.

Abstract:

The Near-Infrared (NIR) photothermal therapy is used in biomedical applications like anti-cancer and anti-microbial treatments. In a biological system, NIR has deep penetrating ability without harmful side effects. The present work utilizes this property as a trigger in drug delivery applications. The ability of the drug to be released from the composite on application of NIR is examined. Nanospheres of covellite copper sulphide (CuS) with chitosan (CS) as base was synthesized by chemical method. Dopamine was conjugated to the CuS / CS system so that the CuS /CS would act as a nano drug carrier. NIR responsive behaviour of CuS is an ideal photothermal trigger to facilitate the release of the encapsulated drug. The encapsulation of dopamine in the composite was confirmed by FTIR and HRTEM analysis. The composite was further studied by X-ray diffraction technique and thermogravimetric analysis for the structural analysis and thermal stability. The NIR absorption showed the significant plasmonic peak of CuS. The dopamine@CuS/CS nanocomposite was introduced into a dialysis bag and the release of the drug through NIR triggering from the carrier was studied. Cytotoxicity/cell viability assay using MTT and cell cycle assay using flow cytometry were conducted separately for CuS nanoparticles and dopamine@CuS/CS nanocomposite in A549 (lung), L132 (cervical-derived) and SH-SY5Y (neuronal) cells. The viability of the cells and their cell cycle were not affected indicating the carrier as well as the nanocomposite are non-toxic. Thus, this photo-controlled technique is an ideal method to control and manage the targeted release of encapsulated drugs that are non-toxic especially in the context of neurodegenerative diseases.

Highlights

- Non-toxic CuS nanoparticles were synthesized and tagged with the scaffold chitosan and drug dopamine.
- The complex released the drug efficiently at pH4 combined with NIR radiation.
- Neither the nanocarrier nor the drug complex was toxic as revealed in cell viability and cell cycle assays.
- The preparation is highly recommended for targeted drug delivery under NIR photodynamic modulation.

Keywords: Photothermal therapy • CuS • drug delivery • chitosan • NIR triggering

1. Introduction

Novel drug delivery techniques are researched at large scale worldwide due to its numerous advantages. Many applications are seen primarily in cancer therapy where a specific site needs to be targeted for administering the drug[1,2]. Due to increased bioavailability, targeted delivery of drug and reduced site effects, these new therapeutic techniques are researched in treatment of various other ailments. The treatment of neurodegenerative diseases, in particular, demand efficient drug delivery techniques because of an impenetrable barrier called the blood brain barrier (BBB) that prevents or limits the permeability of drug molecules from the blood stream to the brain. Therapeutics for neuronal ailments has to be consumed in large amount of which only a smidgen is delivered or enters the specific site. The accumulation of drug may lead to side effects. Hence an efficient and selective mode of drug delivery application is necessary. A small molecule with dimension not greater than 200 nm and a negative zeta potential can act as a carrier of neurodegenerative therapeutics [3,4]. In case of Parkinson's disease, one of the major neurodegenerative disorders, the death of dopaminergic neurons in the substantia nigra leads to low dopamine levels resulting in impairment of motor control leaving the patients with uncoordinated and involuntary movements. This condition aggravates and the patients eventually lose control over all voluntary movements which deteriorate the quality of life, finally leading to death [5-7]. The current therapy like administering l-dopa and deep brain stimulation can prolong the lifetime but cannot control or cure or even better the quality of life of affected patients [8]. Therefore, the aim of current researchers worldwide is to target the drug in the desired area, control the release of the drug and reduce the side effects.

Nanotechnology has paved way for extensive research in the field of therapeutics and their delivery to specific sites in controlled amounts [9]. There are numerous aspects that control the release of these drugs. For example, temperature, pH, enzyme etc[10-15]. These triggers act as switches that control the delivery of drug to the site of action and sustained drug release thus reducing the side effects on normal cells [16-18]. The drawback of these techniques is that they are dependent of the *in vivo* environment and cannot be controlled or manipulated externally. As these factors are controlled by the environmental conditions within the organism, alternate approaches were studied and light-responsive triggered drug release was found to be one of the best approaches.

The biological window / therapeutic window also called the Near-Infrared (NIR) ranges between 650 to 1350 nm. This region is ideal because, radiation has its maximum depth of penetration into the tissue and is not harmful to living cells. Materials that are light responsive and non-toxic can therefore be classified as an ideal drug delivery carrier [19,20]. Copper

sulfide (CuS) is an inorganic semiconductor that displays NIR responsive behavior. There are reports where CuS has been employed for photothermal therapy for tumor and cancer cells[21]. CuS-based photothermal materials are excellent because of their simple preparation, high absorption coefficient, low cost, high stability, and good biocompatibility. This shows that NIR responsive property of CuS can be exploited for triggered drug release.

In the present work, CuS nanoparticles mimic a 'trigger' and / or 'switch' for photothermal release of dopamine into the biological environment. Conjugating CuS with a biocompatible polymer can further increase the efficiency of the nanocarrier.[22] Chitosan biopolymer acts as a matrix or a substrate on which the CuS and dopamine can be conjugated to form a nanocomposite. Chitosan a mucoadhesive, has high bioavailability and above all is pH responsive. Dopamine a neurotransmitter is encapsulated in the chitosan/CuS composite. The goal of this study is to explore and control the release of dopamine in the biological system. Hence an in-vitro environment was designed and an elaborate study for release of drug from the composite was recorded. The drug release characteristics from the composite in the presence and absence of NIR radiation in a phosphate buffer solution(PBS) maintained at pH 4 and pH 7 is explored. Thus, CuS conjugated with chitosan is a promising candidate to examine and study the applications of photo-controlled drug delivery systems.

2. Materials and methods:

The chemicals utilized in all the experiments are of analytical grade. Double distilled water was used throughout the experimentation process. Low molecular weight chitosan (~85% deacetylated), dopamine hydrochloride(2-(3,4-Dihydroxyphenyl)ethylamine hydrochloride, Copper chloride (CuCl₂) anhydrous powder with purity $\geq 99.995\%$, and high quality sodium sulfide cubic crystals (Na₂S) and trisodium citrate were purchased from Sigma Aldrich. 99% pure ethyl alcohol was bought from SRL chemicals.

2.1. Synthesis of dopamine@copper sulphide/chitosan nanocomposite (dopa@CuS/CS).

Optimal amount of CuCl₂ and Na₂S solution were prepared in the molar ratio 1:10. Na₂S solution, was added to the CuCl₂ solution drop wise. Trisodium citrate was used as ligand and the solution was maintained at 80 °C for 1 h in a hot water bath. The settled precipitate was washed and dried and CuS nanoparticles were collected.

The CuS / chitosan nanocomposite was synthesized by a simple chemical method. 0.35 g of chitosan was dissolved in 2% acetic acid solution. 5 wt % of the synthesized CuS was mixed with the solution and maintained at 80 °C for 1 h under constant stirring. The precipitate was washed several times and dried in hot air oven at 50 °C

The drug loaded dopamine@CuS/CS was synthesized using the procedure given elsewhere[23,24]. Approximately 5 wt% of dopamine hydrochloride and 5 wt% of synthesized CuS nanoparticles was added to the solution. The precipitate formed was washed, dried and its properties were analyzed. A flowchart of the synthesis procedure is given in figure S1.

2.2. Instrumentation

X-ray diffraction patterns were obtained between the range 5-60°, from GE X-RAY DIFFRACTION SYSTEM - XRD 3003 TT with CuK α_1 radiation ($\lambda=1.5406$ Å). The UV/VIS data was taken from PerkinElmer UV Win Lab 6.3.2.0749 / 2.02.06 Lambda 650 UV/VIS. Fourier transform infrared spectroscopy was recorded using FT/IR-6600 Fourier Transform Infrared Spectrometer from JASCO. A Tecnai instrument at 200 KV operating voltage recorded the HRTEM images. Perkin Elmer lambda 950 UV-VIS-NIR instrument with a deuterium lamp was used to record the NIR spectrum. The drug release from the nanocomposite was investigated using a 980 nm NIR laser diode with a power of 220 mW. The DLS spectra was recorded using Horiba Scientific Horiba SZ-100 to calculate the particle size

2.3. Cell culture

The cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, India, and the cells were grown in DMEM medium supplemented with 10% FBS, 1% antibiotic-antimycotic solution (10000 U penicillin, 10 mg streptomycin and 25 µg Amphotericin B per ml in 0.9% normal saline). The cells were maintained as a monolayer in 25cm² plastic tissue culture flasks at 37°C in a humidified atmosphere of 5% CO₂ in air.

2.4. Cell viability/cytotoxicity assay

This assay was performed to determine the cytotoxicity, if any, of the CuS and the nanocomposite towards the cells. The cells were plated at a density of 1x10⁶ cells/well in a 96-well plate at 37°C in 5% CO₂ in a CO₂ incubator. After 24 h of culture, the medium in the wells was replaced with the fresh medium containing the copper complex and the dopamine@CuS/CS separately in varying concentrations. After 24 h and 48 hr incubation, 100 µL of DMEM with MTT dye

solution (5 mg/mL in phosphate buffer, pH 7.4) was added to each well. After 4 h incubation at 37°C and 5% CO₂, the medium was removed and the formazan crystals were solubilized with 100 µL of DMSO and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader at 490 nm. The spectrophotometer was calibrated to zero absorbance, using culture medium without cells. The relative cell viability (%) related to control wells containing cell culture medium without copper complex or the drug complex were calculated using the following formula:

$$\% \text{ of cell viability} = 100 \times (\text{Sample absorbance} / \text{Control absorbance})$$

IC₅₀ value was calculated using graphpad prism 7.0.

2.5. Cell cycle assay

The cells were seeded in 6 well plates with high glucose DMEM medium. Once the cells reached the required growth, they were treated with the copper complex and the drug complex in the respective calculated IC₅₀ values of compounds in the medium for 24 hr. After 24 hr treatment, cells were trypsinized and resuspended with complete medium. Cells were collected and centrifuged at 1000 rpm for 5 min. Then cell pellet was washed with PBS twice and subsequently fixed with 1 mL of 70% of ice-cold ethanol overnight at 4 °C. Following removal of ethanol, the cell pellet was washed twice with cold PBS, and then incubated with 10 µL of RNase A for 30 min. After incubation it was washed with PBS and resuspended in 1 mL PBS with 50 µL Propidium iodide (1 mg/mL stock) for 30 min in dark. The cells were analysed to check the cell cycle phase using FACSVerse cytometer (Becton-Dickinson).

3. Results and Discussion

3.1. XRD studies:

Figure 1 shows the XRD pattern of CuS/chitosan and dopamine@CuS/CS. The prominent peaks at 29°, 31° and 48° with plane values (102), (103) and (110) respectively confirm that CuS has been conjugated with chitosan [25,26]. From these results it can be concluded that a covellite phase hexagonal system is formed according to JCPDS data 06-0464. Additional peaks present are also indexed in the figure 1A. The SAED pattern indicating the planes corresponding to the XRD data are given in figure S2 A. The fringe pattern and the corresponding d-spacing of 3.05 nm corresponding to (102) plane is depicted in figure S2 B.

In figure 1B, due to the dopamine@CuS/CS nanocomposite formation an extended broad peak is visible stretching from 10° to 22° and a sharp prominent peak at 20° represents the presence of dopamine in the composite. Due to the amorphous nature of chitosan the intensity of the CuS peaks though present are reduced considerably. The peak at 10° further confirms the presence of chitosan. The stability of CuS with temperature was analyzed by TGA and XRD and the results are shown in figure S3 and S4.

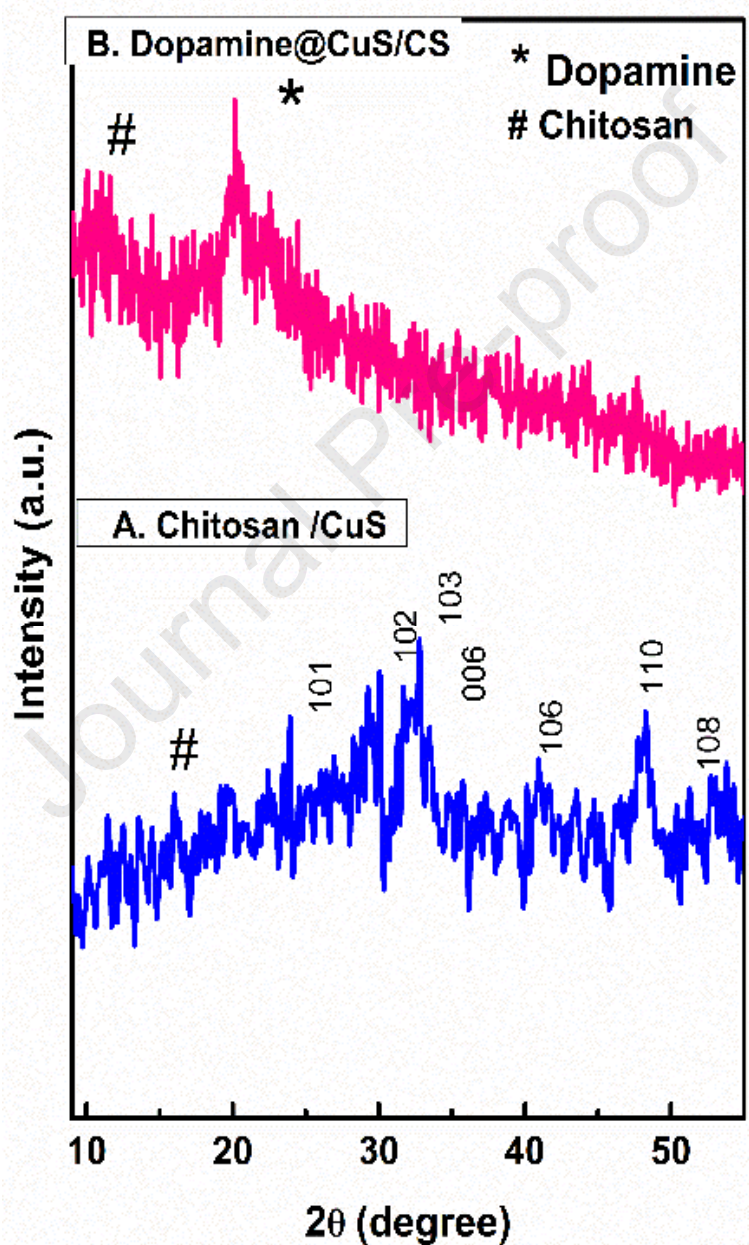


Figure 1. XRD pattern of A. chitosan / CuS nanocomposite B. dopamine@CuS/CS nanocomposite.

3.2. FTIR studies:

Figure 2 shows the FTIR spectrum of the synthesized samples. CuS is not IR active hence characteristic peaks specifically for CuS cannot be identified [27-29]. The broad bands stretching from 3000 to 3400 cm^{-1} correspond to the N-H and O-H stretching as well as the intermolecular hydrogen bonds. The peak around 2971 and 2865 cm^{-1} can be attributed to the C-H symmetric and asymmetric stretching respectively. Figure 2 A gives the FTIR spectra of chitosan / CuS and figure 2 B, gives that of dopamine@CuS/CS. The FTIR spectrum of dopamine encapsulated nanocomposite differs from that of free dopamine. In a typical spectrum of pure dopamine sharp peaks are seen between the range from 1500 cm^{-1} to 500 cm^{-1} . These peaks are not so prominent in the composite (fig 2B) because only a small weight percentage was analyzed and therefore the intensity of these peaks is considerably diminished. The bonding of dopamine to chitosan is through the oxygen atoms of the hydroxyl groups. Hence, the intensity of the hydroxy peak present at 3300 cm^{-1} is decreased. N-H bending of primary amine is seen at 1561 cm^{-1} and C-H₃ deformations are seen at 1386 cm^{-1} . The band at 1036 corresponds to C-O stretching and the band at 608 cm^{-1} might be due to Cu-S stretching vibrations. The shift and peak broadening seen at the regions indicated in the spectrum may be due to the composite formation.

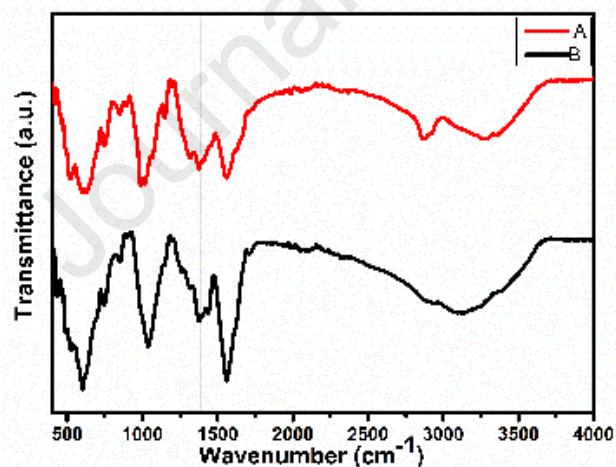


Figure 2. FTIR spectra of A. chitosan/CuS nanocomposite B Dopamine@CuS/CS nanocomposite.

3.3. NIR absorption spectra:

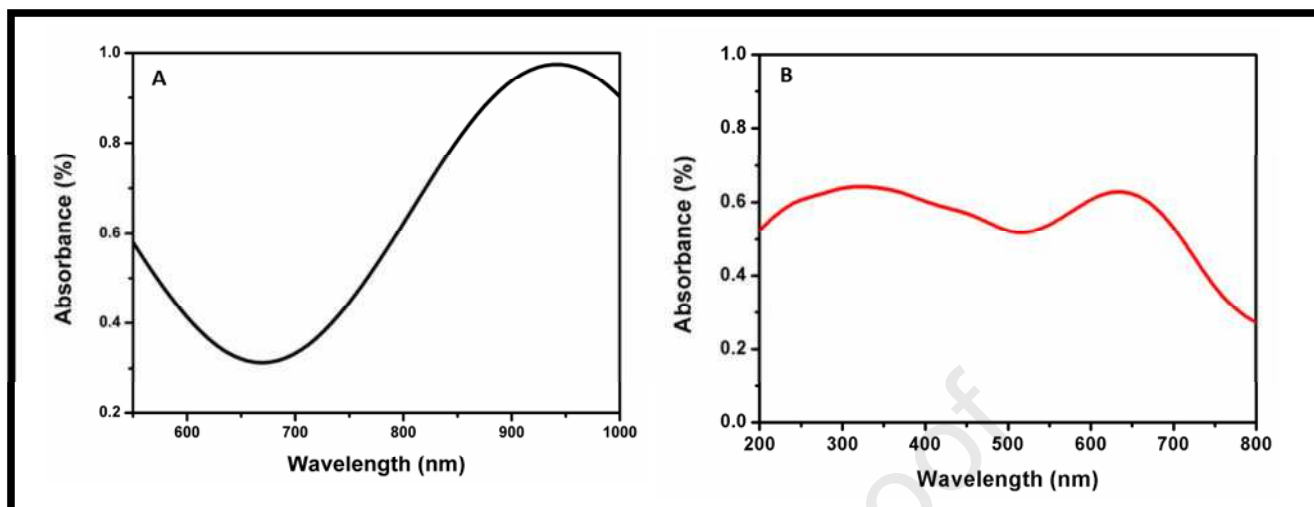


Figure 3. A. NIR absorption spectra of CuS nanoparticles B. UV-Visible absorption spectra of CuS nanoparticles

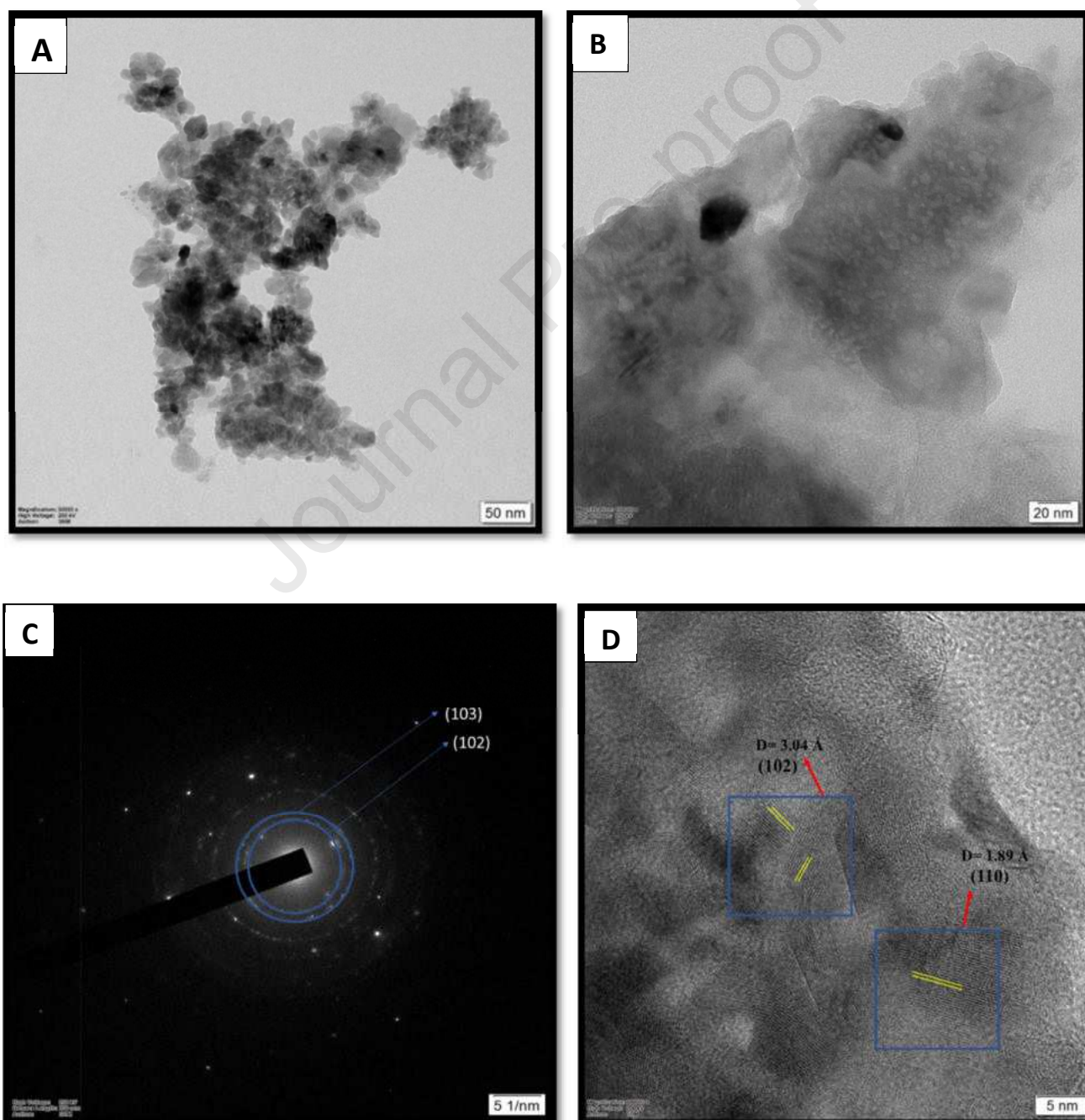
As shown in figure 3 A, CuS nanoparticles exhibit a strong absorption in the NIR region due to the localized surface plasma resonances (SPR) of valence-band free carriers (positive holes) of the CuS. This strong NIR absorption enables its application in drug release due to its photothermal ability. The strong peak is due to the d-d transitions of Cu^{2+} and its conjugation with other particles.[30-32]. The CuS nanoparticles display maximum absorption in the NIR region hence enabling their triggered release.

In figure 3 B, CuS nanostructures have two absorption peaks as reported in literature [33], around 200-400 nm and 600-700 nm. These are the characteristic peaks of covellite CuS. Morphological changes in CuS can also cause changes in the absorption peaks without altering the spectra. From the above results it can be stated that the synthesized CuS nanoparticles display absorption peak similar to that of CuS nanospheres. The sample stability due to the NIR radiation are discussed in the supplementary in figure S3 & S4.

3.4. HRTEM analysis:

The HRTEM images of the as-synthesized CuS particles were evaluated. The average particle size of the nanoparticles was evaluated from figure 4A. The SAED pattern and the fringes of CuS nanoparticles are shown in figures S2 A& B in the supplementary. Figure 4 B gives the HRTEM images of the dopamine@ CuS/CS nanocomposite. The contrast in the image clearly portrays the drug molecule and CuS embedded in the chitosan matrix. The SAED pattern shown in figure 4 C was

analyzed. The (102) and (103) planes show excellent correlation to covellite hexagonal phase of copper sulfide (JCPDS data 06-0464)[34]. These confirm the presence of CuS in the dopamine@CuS/CS nanocomposite. The outer rings present correspond to dopamine embedded in the nanocomposite. In figure 4 D, the d-spacing was calculated for the fringes in the image. The (102) plane with d-spacing 0.305 nm and (110) plane with d-spacing 0.189 nm are marked in the figure. These correspond to the CuS present in the nanocomposite and are in correlation with the XRD results. In figure 4 E and 4 F the particle size distribution histogram and DLS spectra of CuS nanoparticles were analyzed respectively and the average particle size was calculated to be ~15 nm.



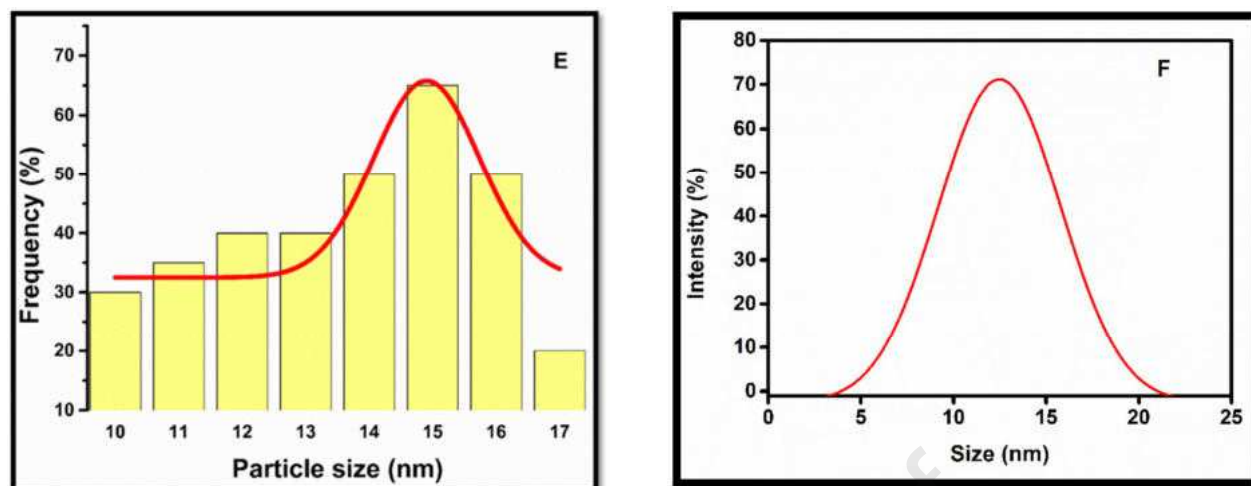


Figure 4 (A) HRTEM image of synthesized CuS nanoparticles (B) HRTEM image of dopamine@CuS/CS nanocomposite (C) SAED pattern (D) fringe pattern and d-spacing of dopamine@CuS/CS nanocomposite (E) Particle size distribution histogram and (F) DLS spectra of CuS nanoparticles.

3.5. Drug release studies:

The dopamine@CuS/CS is taken in a 6 cm dialysis bag (MWCO 1000 Da) and immersed in a beaker containing 60 mL of buffer, kept under constant stirring. 3 mL was removed from the 60 mL solution after different intervals of time and immediately replenished. 3 mL buffer solution removed are subjected to Ultra-Violet absorption studies and the intensity of absorption maxima at 280 nm which is the absorption maxima of dopamine was noted. A direct relation between the absorption maxima and concentration of drug is established. The release kinetics can be analyzed from the absorption maxima which in turn provides the concentration of drug released. The concentration of released drug is represented in percentage as the cumulative release. This technique was followed to examine the in-vitro drug release in PBS maintained at different pH (pH 4 and pH 7). The solution was constantly monitored for 24 h and readings were taken periodically every two hours to evaluate the pH dependent release. The above procedure to evaluate the drug release profile is explained elsewhere [24,35,36].

NIR triggered release of drug from the composite was observed in a similar manner. The dialysis bag containing the suspended nanocomposite was immersed in a 60 ml PBS solution and irradiated with an NIR laser for 1 minute with an interval of 20 minutes. Approximately 3 ml PBS was collected at each interval and stored for analysis. To compensate for the volume removed, fresh buffer solution of corresponding pH was added. This process was repeated continuously over a period of 5 hours. The mechanism of NIR controlled or photo-controlled release is depicted in the graphical abstract. Dopamine has a strong absorption peak at 280 nm. By measuring the intensity of the absorption peaks, the percentage of cumulative release can be calculated. Figure 5 A & 5 B shows the cumulative release observed at different pH values i.e. pH 7 and pH 4 respectively. In pH 4 a cumulative release of drug around 60 % was observed where as in pH 7 only 6 % of the encapsulated drug was released at the end of 24 h. This is because chitosan is pH responsive and the drug released from the composite varies at different pH level.

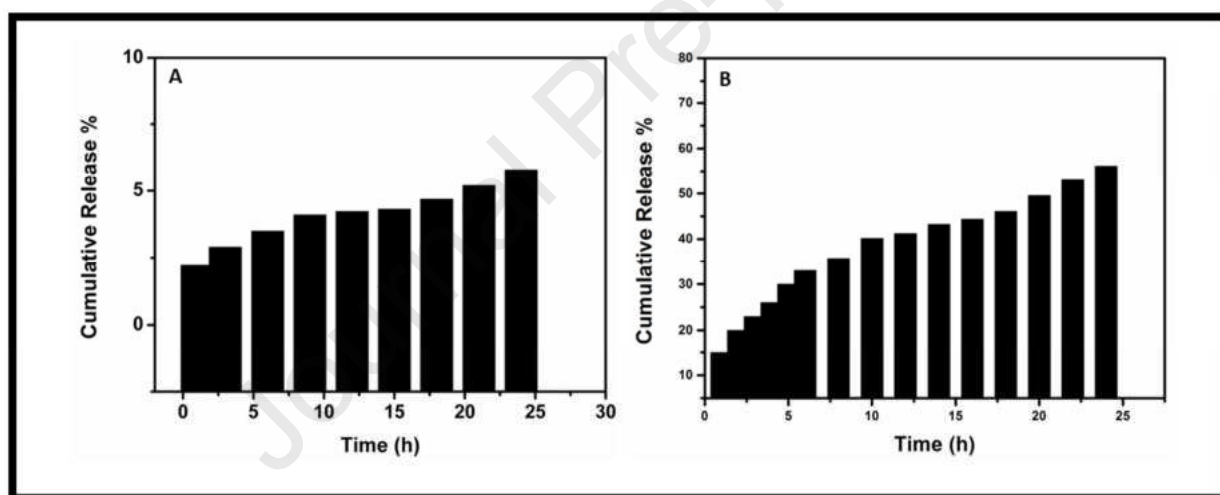


Figure 5 A. Cumulative release of drug in pH 7. B. Cumulative release of drug from nanocomposite in pH 4.

Photo-controlled or NIR controlled drug release was observed from the nanocomposite immersed in a PBS solution maintained at pH 7. In the absence of NIR radiation only 6 % of the drug was released as seen in figure 5 A. In contrast when irradiated with NIR light, approximately 50 % of drug is released within 300 min (5 hours) shown in figure 6. This release is observed due to the interaction of NIR radiation with the composite which facilitates immediate release of the drug. The CuS nanoparticles absorb the light and as a result begin to dissipate heat. This heat produced induces a disturbance in the composite facilitating the release of the drug.

Thus, this is an effective tool to control the release of the drug externally by varying the power of the irradiated NIR light and time of irradiation. [37,38]

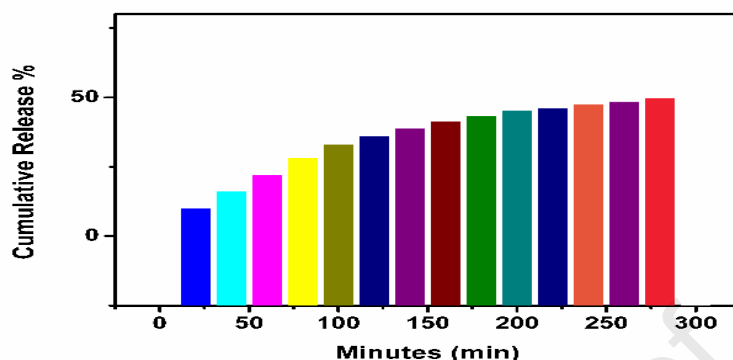


Figure 6. Cumulative release of dopamine from drug encapsulated nanocomposite in the presence of NIR radiation.

The supernatant before and after loading of dopamine on the CuS/CS composite was evaluated to determine the amount of free drug. The entrapment efficiency was calculated to be 89% and the drug loading efficiency was 35%. The absorbance percentage of the characteristic peak at 280 nm (dopamine absorption) was analyzed with known concentration and peak intensity and hence the amount of free drug was calculated.

The study employing dopamine@CuS/CS as a drug delivery carrier in photothermal drug delivery is novel and has not yet been studied. A discussion of comparison with similar hydrogel is elaborated. Pathania et. al. [39] reported fabrication of chitosan-g-poly(acrylamide)/CuS nanocomposite for controlled drug delivery at pH 2.2, 7.4 and 9.4 and maximum release of 76% was obtained at 2.2 pH at the end of 18 h. In the present study, maximum release of 60% was recorded at pH 4 with entrapment efficiency of 89%. Jie Yang et. al [40] studied macrocycle-capped CuS nanogates for synergistic chemo-photothermal therapy. In the presence of pulsed NIR radiation,

12% of the drug was released after 1 h in a pH 5 medium. In the present study, 50% of drug is released after 5 hours in the presence of pulsed NIR radiation at pH 7. From the above references we can decipher that dopamine@CuS/CS is an excellent nano-drug carrier and exhibits excellent drug release properties.

3.6. Cell viability / cytotoxicity assay

The toxicity of the synthesized materials was studied to evaluate the biocompatibility in a controlled *in vitro* environment by exposure to cultured cell lines. It is essential that both copper sulfide nanoparticles (CuS) and dopamine@CuS/CS (indicated as 'D5' in the following studies) nanocomposite does not induce any damage to the cells. Hence, both the synthesized CuS nanoparticles and the drug-loaded nanocomposite were separately tested to examine its toxicity, if any, in a biological environment. Cultured cells, A549 (lung cancer) and L132 (HeLa derivative), were used to test the interaction with the synthesized materials. The cell viability analysis was done for a period of 24 h and 48 h. Both materials were taken in five different concentrations (5, 25, 50, 75 and 100 μg) to test its toxicity. The cell cycle analysis was also carried out to examine the progression of cell division in the presence of the composite using the two cell lines. The statistical analyses of all the tests were tabulated respectively.

3.6.1. A549 cell line

The A549 cells is a pulmonary adenocarcinoma but is also useful as alveolar type II pneumatocyte in drug metabolism. The A549 cell line is considered an ideal cell line in respiratory cell research for decades. As shown in figure 7A and 7B, the cell viability measured from the MTT assay has been recorded. After a period of 24 and 48 h the cells are healthy and do not show toxicity. At 100 μg concentration of the both CuS and the nanocomposite, show 97% of cells are viable.

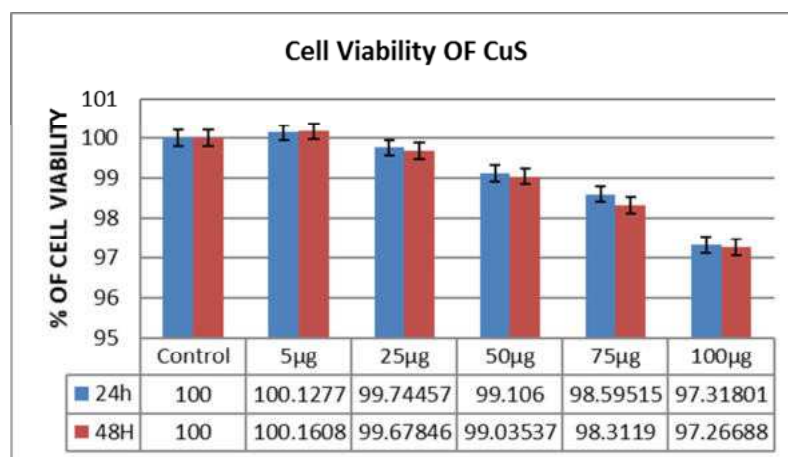


Figure 7 A. Cell viability after exposure to CuS nanoparticles at different concentrations for a period of 24 h and 48 h for A549 cells.

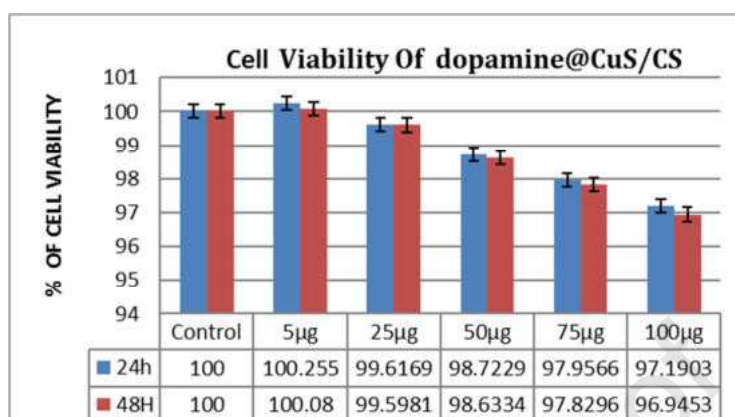


Figure 7 B. Cell viability after exposure to dopamine@CuS/CS nanocomposite at different concentrations for a period of 24 h and 48 h for A549 cells.

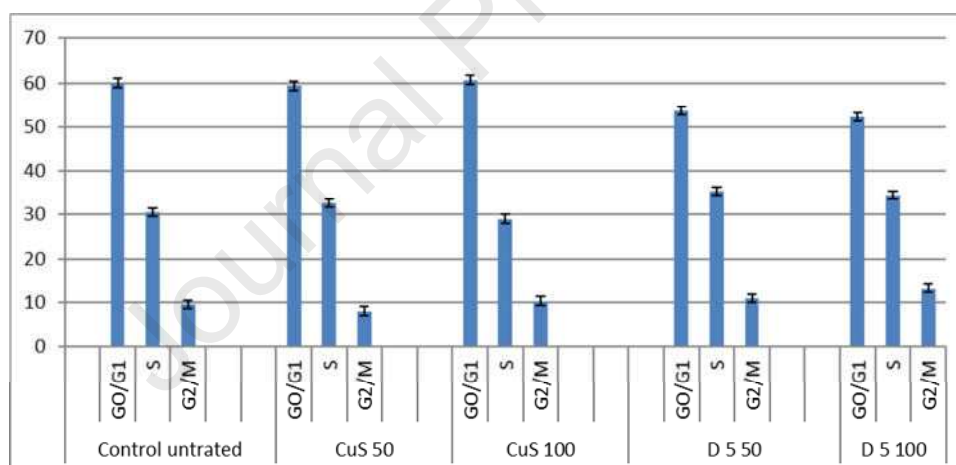


Figure 8. Effect of CuS and dopamine@CuS/CS(D5) nanocomposite on A549 cell cycle.

The cell cycle analysis for CuS and dopamine@CuS/CS nanocomposite in the concentration 50 µg/mL and 100 µg/mL was carried out. As shown in figure 8, the progression of the cell cycle with respect to *G0* (rest phase), *G1* (gap phase after previous cell division), *S* (synthesis of nucleotides and proteins for the next division; DNA replication occurs), *G2* (the second gap phase) and *M* (kinetic cell division phase) phase of cell cycle. Peaks at *S* phase in figures show duplicated DNA, meaning double the DNA content preparatory to chromatin replication and the kinetics of cell division. The treatment has not affected the cell cycle.

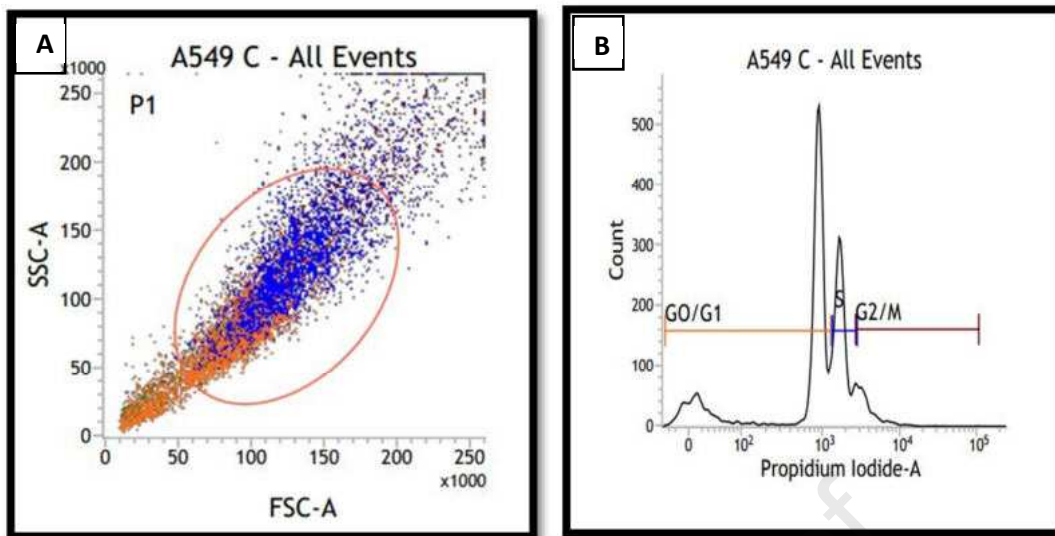


Figure 9 A. Cellular parameters of all events depicted by flow cytometry analysis. Pink, cells in 2n; blue, cells in 4n, ready to go into physical cell division of mitosis. **9 B** Histogram of cell cycle analysis of A549 cells

The relative size of cell or events using a known model is determined by Flow cytometry. In Figure 9 A the Forward scatter parameter (FSC) along X-axis gives the relative size for the cell. The side scatter parameter (SSC) along Y-axis depicts the particulates inside the cell. These parameters help in determining the size and internal complexity of one another based on control. Figure 9 B gives the histogram of cell cycle analysis.

Flow cytometry can also determine the percentage of a cell population in each cycle. Propidium Iodide (PI) helps to stain the DNA and RNA without disrupting the functioning of the nucleus when stained. By recording the fluorescence of the stained nucleus, the percentage of cell population in each phase of the cell cycle can be determined based on the DNA present.

3.6.2. L132 (HeLa derivative):

L132 is originally known as a human lung epithelial cell line derived from human embryonic lung, but later identified as a HeLa-derived cervical cell.

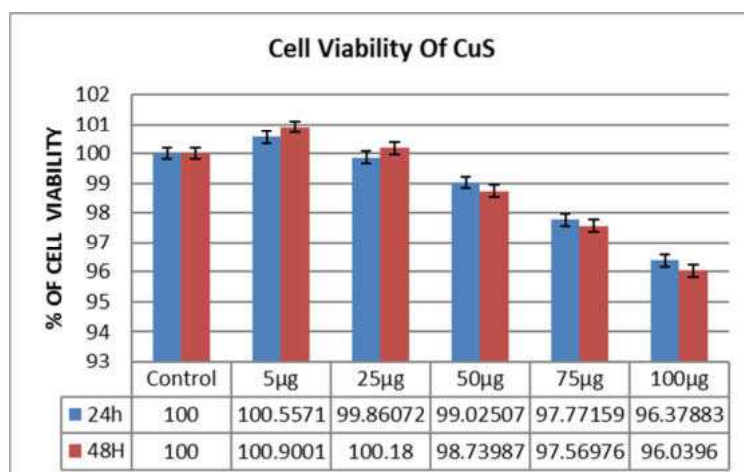


Figure 10 A. Cell viability for L132 cells on exposure to CuS nanoparticles at different concentrations for a period of 24 h and 48 h.

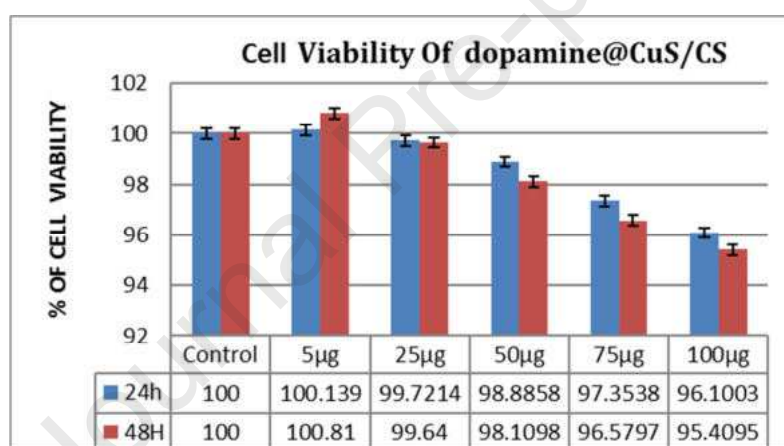


Figure 10 B. Cell viability of L132 cells on exposure to dopamine@CuS/CS nanocomposite at different concentrations for a period of 24 h and 48 h.

The cell viability results using the MTT assay was evaluated for the L132 cell line. As seen in figure 10 A and 10 B, the cells show 96% viability after a period of 24 h and 48 h.

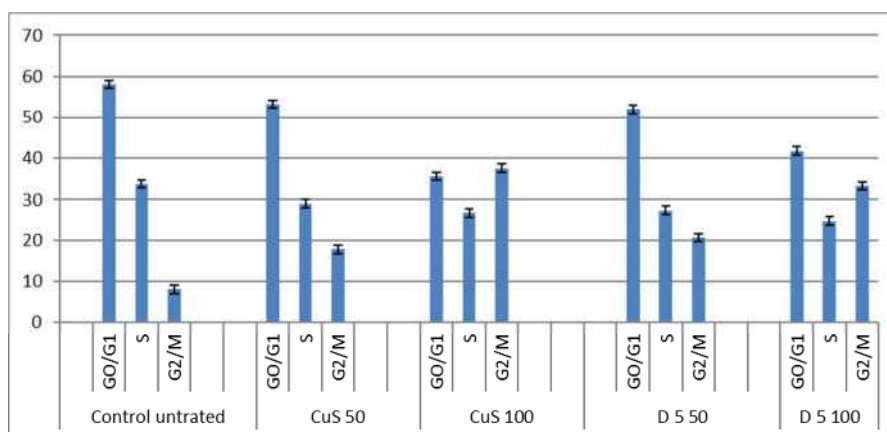


Figure 10 C. Effect of CuS and dopamine@CuS/CS(D5) nanocomposite on cell cycle for L132 cells.

The cell population of CuS at 50 μg concentration was similar to that of the control as shown in figure 10 C. For the CuS at 100 μg concentration there was a marginal decrease in the G0/G1 phase which indicates that there is insignificant change in the cell cycle parameters. But in the case of the dopamine@CuS/CS nanocomposite for 100 μg concentration the cell cycle was comparable to that of the control as seen in almost similar distribution of cells among different phases of the cell cycle. The cytotoxicity analysis on neuronal cells line (SH-SY5Y) was also carried out and the results are given in the supplementary figures S5 and S6.

4. Conclusions

Covellite hexagonal CuS nanoparticles were synthesized by simple chemical method. The CuS nanoparticles were conjugated with chitosan and the XRD spectrum of the as-prepared materials displayed corresponding characteristic peaks. The drug dopamine was encapsulated in the carrier and dopamine@CuS/CS was synthesized and characterized successfully. The UV and the NIR results proved that the CuS nanoparticles are NIR active and have a spherical morphology. The HRTEM images of CuS and dopamine@CuS/CS showed excellent correlation to the XRD results confirming the covellite hexagonal phase. The drug release profile revealed the pH responsive and NIR triggered drug release. In the pH controlled delivery, more amount of drug was released at lower pH i.e. 60% of the drug was released at pH 4 when compared to 6 % release in pH 7. NIR triggered release profile showed that almost 50 % of the drug was released within 5 hours and more NIR irradiation time can account for efficient release of drug from the nanocomposite. The *in-vitro* cytotoxicity test and cell cycle analysis for CuS and dopamine@CuS/CS nanocomposite was tested against three cells lines. The

A549, L132 (HeLa derivative) and SH-SY5Y(neuronal) cell line were cultured and the cytotoxicity results revealed that the materials were not toxic at the chosen concentrations (5, 25, 50, 75 and 100 µg) towards the cell lines. Hence the synthesized materials were found to be biocompatible. The results are conclusive evidence that these nanocomposites are ideal to be employed as neuro drug delivery carriers that function on photo-triggered response.

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Conflicts of interest

There are no conflicts to declare.

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Highlights

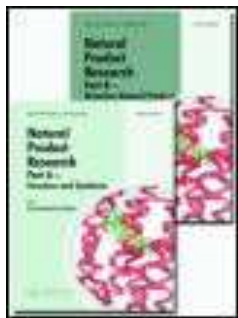
- Non-toxic CuS nanoparticles were synthesized and tagged with the scaffold chitosan and drug dopamine.
- The complex released the drug efficiently at pH4 combined with NIR radiation.
- Neither the nanocarrier nor the drug complex was toxic as revealed in cell viability and cell cycle assays.
- The preparation is highly recommended for targeted drug delivery under NIR photodynamic modulation.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Sulfation of chitosan from *Sepia kobiensis* as potential anticoagulant and antibacterial molecule

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SHORT COMMUNICATION



Sulfation of chitosan from *Sepia kobsiensis* as potential anticoagulant and antibacterial molecule

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ABSTRACT

The present work aimed to synthesis of chitin, chitosan and sulfation of chitosan from cuttlebone of cuttlefish *Sepia kobsiensis*. Principally chitin was extracted through sequential processes of demineralisation and deproteinization. Then chitosan was synthesized by a deacetylation and finally sulfated at semi-heterogeneous condition using chlorosulfonic acid in *N,N*-dimethylformamide. The synthesized macromolecules were characterized for its structural, physical and thermal (CHN, DDA, FT-IR, NMR, XRD, Viscometric analysis, SEM and DSC) properties. Apart from anticoagulant potential of the sulfated chitosan was tested using human plasma by means of activated partial thromboplastin time (APTT) and prothrombin time (PT). Further sulfated chitosan was tested for antibacterial potential by well diffusion method against eleven human pathogenic clinical isolates of both Gram positive and Gram-negative strains and minimum inhibitory concentrations (MIC) was calculated accordingly. The results of this study revealed the effectiveness of the sulfated chitosan at semi-heterogeneous conditions as a potent antibacterial and anticoagulant molecule.


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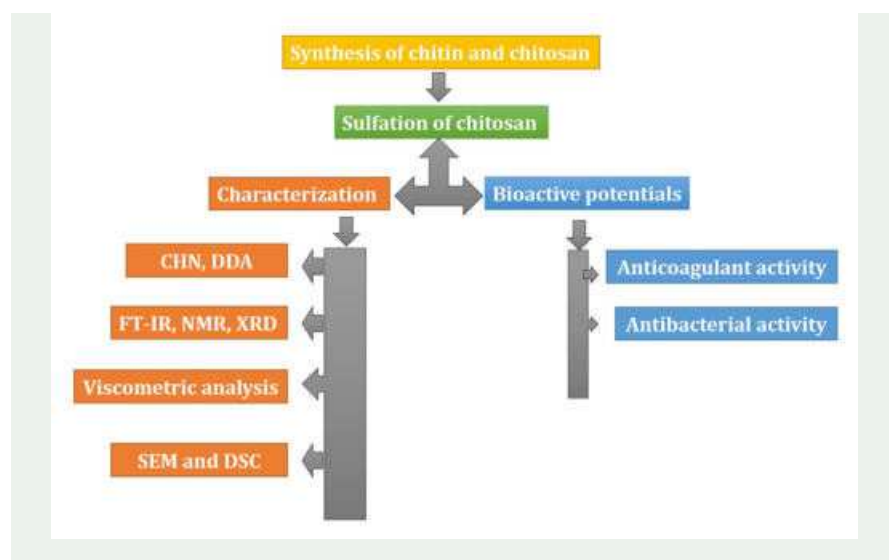
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Anticoagulant; cuttlebone; antibacterial; chitosan sulfate; cephalopods

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1. Introduction

Natural products are and have been a principal source in many cases, not only of nutrients, but also of remedies for millennia. An example is the growing interest towards the re-use of by-products from industrial processing of food and foodstuff to recover biologically-active molecules to obtain derived products originating from food matrices and that may be useful to support/supplement the diet. Nutraceuticals are an outstanding example of this emerging trend in the food chemistry area (Santini and Cicero 2020). Chitosan has been found one of the abundant polysaccharide in the ecosystem next to the cellulose. Since it has been known as a copolymer of (1-4)-2-acetamido-2-deoxy- β - D-glucan and (1-4)-2-amino-2-deoxy- β - D-glucan and acquired great attention from the researchers for its excellent biopotentials as biodegradable, immunobooster, antibiotic and strong antioxidant. The distinctive structural amino position on C2, C3 primary and C6 secondary hydroxyl groups projected its functional molecules in numerous beneficial end (Furusaki et al. 1996). The research continued to expose the structurally modified molecules of the parent chitosan and the resulted modified molecules proved its efficacy with additional functional properties particularly anti-viral, anti-inflammatory and anticoagulant (Jayakumar et al. 2007; Jordan et al. 2007).

The solubility of the chitosan was restricted in water and number of organic solvents and soluble well only in aqueous dilute acids. The solubility limitation of the chitosan causes as main preventive factor and reduced its efficacy to use as a pharmacological product. Hence, the development of improved chitosan needs further investigation on structural amendments to obtain highly water soluble derivative and should work on extensive pH range. The new sulfated chitosan was emerged through the structural amendments on amino and hydroxyl group and it resulted with extensive biological activities (Vikhoreva et al. 2005; Subhapradha et al. 2013).

Since there is an increasing need for the anticoagulant and antibacterial compound, this study was aimed to synthesis sulfated chitosan from the internal bone called as cuttlebone of cuttlefish with structural modification. Then the modified structure studied chemically and analyzed for its antibacterial and anticoagulant potentials. The

main intend for the selection of cuttlebone of cuttlefish is to minimize the contamination by the pathogenic agent of the mammalian sources. This research paper focused primarily on the synthesis of macromolecules chitin, chitosan from the cuttlebone of cuttlefish *Sepia kobeensis* with the structural modification by insertion of sulfo groups. Resulted macromolecules were characterized through Fourier transform-infra red (FT-IR), Nuclear magnetic resonance (NMR) spectral analysis, X-ray diffraction (XRD), Viscometric analysis, Scanning electron microscopy (SEM) and Differential scanning calorimetry (DSC), Carbon, Hydrogen, Nitrogen (CHN) content, degree of deacetylation (DDA) and the potentials as proved by both antibacterial and anticoagulant stuff.

2. Results and discussion

2.1. Spectrum of sulfated chitosan

Since the chitin skeletal structure has been proved to be very similar to heparin, the researchers paid their attentiveness to utilize the chitin for biological applications. The deacetylation of chitosan being a key process at various degrees could ensure with the generation of its derivatives similar to heparin. It was attained exactly through some structural modification on chitosan at a consistent states (Beck et al. 1992; Vongchan et al. 2003). The sulfation reaction was tried by the researchers on chitosan particularly in a kind synthetic chitin heparinoid (Nishimura and Tokura 1987). Even though, the attempted sulfation ends up with one or more inconveniences, which includes imperfect changeover of the chitosan with extreme degradability. The sulfation with N, N dimethylformamide (DMF) was evidenced more appropriate than reported others because of its stability, complexity and availability. The solubility of DMF was also proved for polymers, polysaccharides and polysaccharide derivatives (Vongchan et al. 2003).

Green FT-IR is based on the fact that the chemical bonds of the molecules have natural vibrational frequencies. In this context, the green infrared spectrophotometry stands out because it is a method that allows to quantify substances without organic solvents. It is suitable for drugs with solubility problems, since they can be analyzed in the solid form (Fanelli et al. 2018). In the present study eight major peaks have been detected between 464.56 and 3526.77 cm^{-1} with the FT-IR spectrum analysis of sulfated chitosan. The sulfo group peaks of 669.30 and 1161.64 cm^{-1} were identified in the spectrum of sulfated chitosan and allocated as C-O-S and S=O extended bonds, respectively (Figure S1). The large absorption bands in the range of 1161 in the FT-IR spectrum indicates the asymmetric stretch of N-S=O bond, which shows structural similarity to the FT-IR spectral analysis of sulfated chitosan (peak at 1160 cm^{-1}) (Han et al. 2016; Ramasamy et al. 2017).

The sulfated chitosan derived from the shell of bivalve *Donax scortum* were reported with the absorption peaks at 668.90 cm^{-1} and 1134.36 cm^{-1} and claimed because of sulfo group (Subhapradha et al. 2013). FT-IR spectrum of sulfated chitosan of pen *D. singhalensis* exhibited transmittance peak between 3428 and 470 cm^{-1} (Ramasamy et al. 2017). The spectrum peaks of *Ulva pertusa* found to be at 847 cm^{-1} , 1052 cm^{-1} , 1641 cm^{-1} and 3446 cm^{-1} because of sulfate in axial position C-O-S, stretching vibration of C-O, S-O of sulfate, C-O of uronic acids and O-H, respectively

(Qi et al. 2005). The specific signal at 1123 cm^{-1} of cuttlefish *Sepiella inermis* corresponds to the SO_3 (asymmetric stretch) and being a distinctive peak of sulfated chitosan (Vairamani 2010).

2.2. Anticoagulant potential of sulfated chitosan

The anticoagulant such as heparin and sulfated chitosan has the ability of blood coagulation both *in vivo* and *in vitro* conditions. The mechanism beyond anticoagulant has been demonstrated as well by Drozd et al. (2001) the derivatives of sulfated chitosan, non-fractionated heparin particularly induces the inactivation of thrombin and results in antithrombin III equimolar complex. The anticoagulant potential like APTT and PT of the sulfated chitosan synthesized from *Donax scortum* was reported as 6.45 IU/mg and 1.73 IU/mg, respectively (Subhapradha et al. 2013). Similarly, the APTT and PT activity of sulfated chitosan in *Sepia pharaonis* was slightly higher than the value reported earlier as 6.96 and 1.93 IU/mg, respectively (Jayalakshmi 2013). In the same way the anticoagulant efficacy of the sulfated chitosan obtained from the pen of squid *Doryteuthis singhalensis* was disclosed as 6.91 IU/mg (APTT) and 1.85 IU/mg (PT) and proved over again with our previous studies (Ramasamy et al. 2017). Though in the present investigation, the sulfated chitosan synthesized from the cuttlebone of *Sepia kobeensis* under semi-heterogeneous condition has possess stronger anticoagulant potential as 8.12 IU/mg of APTT and 2.45 IU/mg of PT. This research has been continuously focusing to find the influences of structural modified sulfated-chitins and sulfated-chitosan on anticoagulant activity. This results have also been supported the theory of sulfated chitosan and its sulfate group position would positively influence the APTT efficacy.

2.3. Antibacterial potential of sulfated chitosan

The role of deacetylated chitosan and its oligosaccharides derivatives in antibacterial efficacy towards pathogens was estimated about 89% than with natural habitants except in the case of lactic acid bacteria (Jeon et al. 2001). The maximum inhibition zone of 14 mm, minimum of 7 mm was reported against *V. cholerae* by the sulfated chitosan synthesized from *Sepioteuthis lessoniana* in 5 mg/mL concentration (Subhapradha et al. 2013). In the present investigation the synthesized sulfated chitosan has exposed a peak inhibition zone of about 15 mm (5 mg/mL or 100% concentration) against *Vibrio cholerae* and the minimum inhibition zone of 8 mm against *Pseudomonas aeruginosa* (Table S1 and Figure S2). Though the antibacterial efficacy was primarily depends upon the concentration of sulfated chitosan the higher concentration leads with higher efficiency. The same hypothesis was supported by the results of Liu et al. (2006).

There were no reports still on the systematic approach of chitosan and its derivatives on its antibacterial action. Though it was believed that the water soluble nature of chitosan derivatives have the enhanced skill to make a hole and interrupt the cell wall of the bacterial pathogen to burst out the cellular contents (Helander et al. 2001). In contrast, the insoluble form of chitosan molecules was precipitate and molded on

the outer cell wall membrane; it blocks the important channels of the bacterial pathogens and force to lethal condition. This severe condition may put off the transportation of vital fluids of cell and that influence the repair mechanism to recover back and leads oozing out cell constituents and cell death (Rhoades et al. 2006; Subhapradha et al. 2013).

2.4. Minimum inhibitory concentrations (MIC)

Minimum Inhibitory Concentration (MIC) method has been extensively applied to analyze the efficiency of a new drug. The MIC is being a more reliable and accurate technique for clinical management of a new drug when compare with usual disk or well tests (Ramasamy et al. 2013). The present research report reveals the MIC of sulfated chitosan required for complete mortality of the selected pathogens as follows, 60 µg/mL for *E. coli*, *S. pneumoniae*, 80 µg/mL for *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and 100 µg/mL for *Vibrio cholerae* (Table S2). When growth of the tested organism occurs on some concentration with sulfated chitosan, the MIC is recorded as superior as the maximum concentration experienced. The MIC is recorded as less than or equal to the lowest concentration when no growth occurs on any of the agar tubes but the growth control. Since the MIC phenomena are known to be drug adaptations, the occurrences might not vary among species and compound tested (Binder et al. 2019). MIC value helps as the basis for evaluating the category of susceptibility or resistance of the pathogen to a given antibiotic. The degree of susceptibility carries great epidemiological and clinical value, but it must be properly interpreted. The differences in the degree of a strain's susceptibility to antibiotics cannot be assessed by making a direct comparison of the MIC values obtained for such antibiotics, which, unfortunately, is sometimes done. Such an interpretation leads to the erroneous belief that the strain is most sensitive to the antibiotic for which the MIC is the lowest (Krochmal and Ruth 2021).

3. Conclusion

In the present investigation, the clear structural revelation of sulfated chitosan synthesized from *S. kobeensis* and the biological potential of sulfate group as a potent anticoagulant and antibacterial agents was evidently proved. This investigation has been led a route to sulfated chitosan use as an effective anticoagulant and antibacterial macromolecules. Sulfated chitosan from *S. kobeensis* cuttlebone may be considered as an alternative source for present anticoagulants due to its potent anticoagulant potentials. The sulfated chitosan may also be tried as a food supplement to develop its use in the nutraceutical or pharmaceutical industries in the future.

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Disclosure statement

There is no conflict of interest among the authors.

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Purification and partial characterization of carbohydrate-recognition protein C-type lectin from *Hemifusus pugilinus*

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ABSTRACT

A mannose binding lectin (C-type lectin) was detected in a molluscan snail *Hemifusus pugilinus*, this lectin molecule was isolated and purified from the plasma using mannose-fixed sepharose CL-4B column affinity chromatography. The purified protein corresponds to the molecular weight of 118 kDa on an SDS-PAGE gel. The divalent cation-dependent nature of the *H. pugilinus* lectin (Hp-Lec) evidenced through pH and thermal stability analysis using Circular Dichroism (CD) and Surface Plasmon Resonance (SPR) respectively. Functional investigations of the Hp-Lec reveal a broad spectrum of bacterial agglutination activity against wide range of Gram-positive and Gram-negative bacterial strains. Furthermore, Hp-Lec displayed the haemo agglutination activity against vertebrate red blood cells (RBCs) and its titers were recorded. Excitingly, microbial virulent pathogens such as fungal strains tested against the purified Hp-Lec (25 and 50 µg/ml), which exhibits the effective anti-fungal activity against tested fungal pathogens such as *Aspergillus niger* and *A. flavus*.

1. Introduction

Lectins are Ca²⁺-dependent carbohydrate-binding proteins possess the 'Carbohydrate Recognition Domain' (CRD) specific for a variety of monosaccharides [1,2] that are termed as C-type lectins (CTL). CRD domain helps to bind and fight with Pattern Recognition Molecular Patterns (PAMPs) located on the surface of microbial cell membranes. Lipopolysaccharides, Lipotechoic acids, Peptidoglycans and Glucans are the major PAMPs situated on the Gram positive, Gram negative and fungal cell walls acts like a triggering signal to the host immunity. Surface of microbial cells are characterized through the determinant moieties those are part of carbohydrates such as polysaccharides, glycoproteins and glycolipids consist of receptor like structures and situated on the cell membranes or bounded inside the membranes to recognize the invading pathogen and triggers the phagocytosis and other immune reactions.

Recently, research on innate immunity of invertebrates is growing and in order to find solutions for incurable diseases, researchers are trying to establish a relationship between both innate and adaptive

immune systems. This kind of approach would be helpful for the aquaculture industry for reducing losses and finding effective strategies against microbial pathogens [3,4]. C-type lectins (CTLs), which have been well studied among the lectin superfamily, were originally used to distinguish a group of Ca²⁺-dependent (C-type) carbohydrate-binding proteins from lectins. Lectins are classified into 13 groups: C-type, F-type, M-type, I-type, P-type, L-type, R-type, F-box lectins, chitinase like lectins, ficolins, galectins, calnexin, and intelectins according to their structural features and the overall domain organizations [5,6]. CTLs participate in many immune responses, such as microbial agglutination [7,8], induction of proPO [9], function in microbicidal, activation of respiratory burst [10,11] and antiviral response, and enhancement of opsonization as well as cellular encapsulation.

In recent years, an increasing number of CTLs have been identified from different species of crustaceans, such as *Macrobrachium rosenbergii* [12], *Procambarus clarkii* [13], *Eriocheir sinensis* [14–16], *Litopenaeus vannamei* [17], *Fenneropenaeus chinensis* [18,19] and two novel CTLs (MnCTLDcp2 and MnCTLDcp3) from *M. nipponense*, and they might be involved in prawn immune response against pathogen infection [20].

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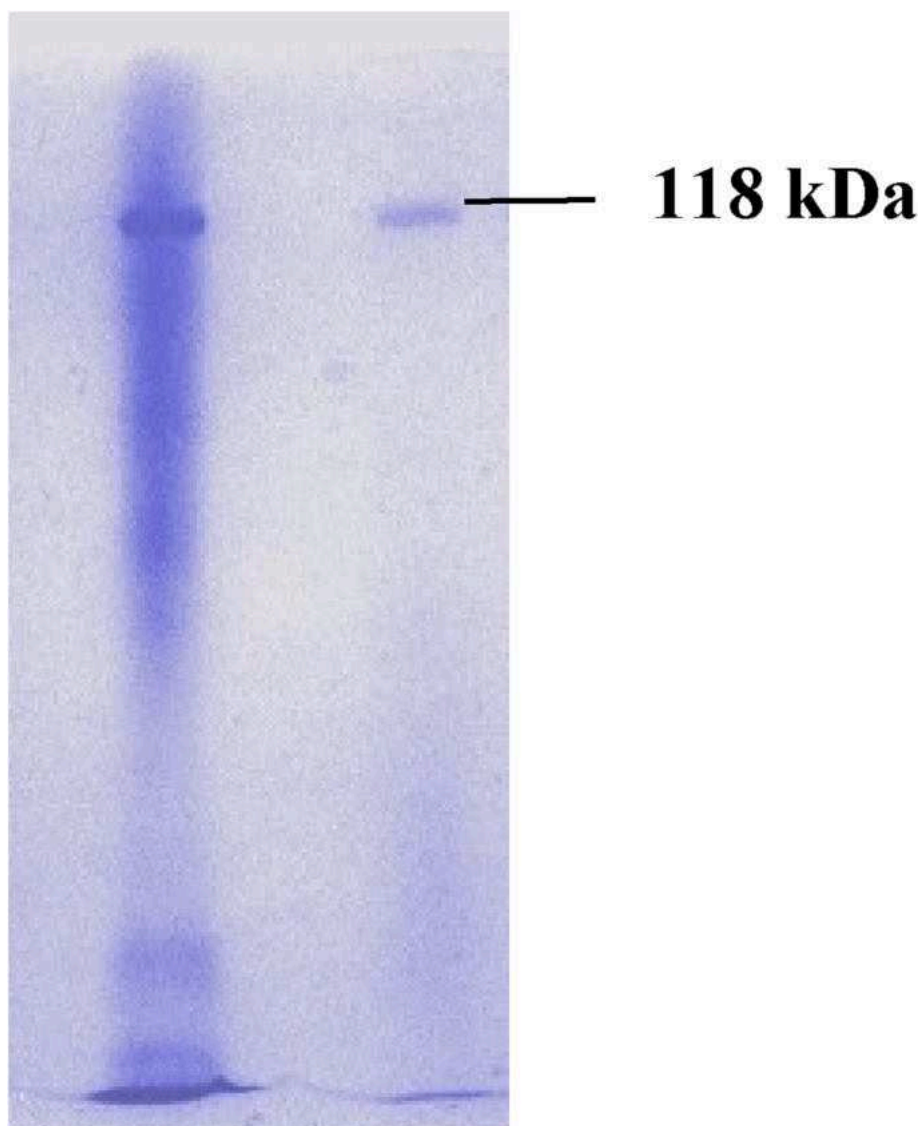


Fig. 1. Lectin purified from the haemolymph of the mollusk, *Hemifusus pugilinus* on SDS-PAGE. Lane I: protein molecular marker, lane II: purified lectin (molecular weight approximately 115 kDa).

C-type lectin (MrLec) with high expression in intestine is involved in innate immune response of *M. rosenbergii* [21]. L-type lectin in *L. vannamei* facilitates the clearance of *Vibrio harveyi* [22] and C-type lectin B regulates the expression of antimicrobial peptides and promotes phagocytosis in mud crab *Scylla paramamosain* [23]. This CTL also acts as a low-density lipoprotein for viral protein vitellogenin in *F. merguensis* was established by Pattamaporn Kwankaew et al., 2018 and it also involves in regulating shell and pearl formation in molluscs [24]. In crayfish *Procambarus clarkii* CTL exhibits the different types of CTL (CTL1, CTL2, CTL3, CTL4 and CTL5) amongst CTL2 involves in the proPO activating system [25,26] and other types of CTL involves in the destruction of invading microbial pathogens [27–33]. Newly CTL6 also reported in *P. clarkii*, which showed the serine rich region against towards the viral entry inside the host cell [34]. In addition, perlucin-like protein (LvPLP) was cloned from *L. vannamei* showed the response to *Vibrio parahaemolyticus* and *V. anguillarum*. The characteristics of LvPLP will gain new insights into functions of CTLs in crustacean innate immunity for sustainable aquaculture disease control management [35]. To our knowledge, there are no reports available regarding the role of lectins in snail *Hemifusus pugilinus*. Therefore, we purified lectin from *H. pugilinus* (Hp-Lec) through a mannose-coupled sepharose CL-4B

column and performed the biophysical characterization and immunological assays on this protein.

2. Materials and methods

2.1. Animals & protein purification

Fresh and alive *H. pugilinus* are collected (with the average sizes 4.96 cm) from the low tidal coastal area of Pampan Ramanathapuram, Tamil Nadu, India and transported to the Department of Animal Health lab and acclimatized for 5–7 days in aerated place with temperature of 28–30 °C. Haemolymph was collected by shell puncture [36] (Sauve et al., 2002). Haemolymph separation and handling was carried out in 4 °C to prevent cell aggregation [37]. Purification of Hp-Lec was performed using the protocol proposed by Nevens et al. (1992) and Alpuche et al. (2005) [38, 39], with slight changes. A mannose-coupled Sepharose 4B column (Bio-Rad Laboratories, Canada) was used for the purification of Hp-Lec. Polyacrylamide gel electrophoresis (12%) was performed on eluted fractions under reducing (β -mercaptoethanol) and non-reducing conditions [40]. The molecular weight of Hp-Lec was determined by comparison of its electrophoretic mobility with that of the molecular mass

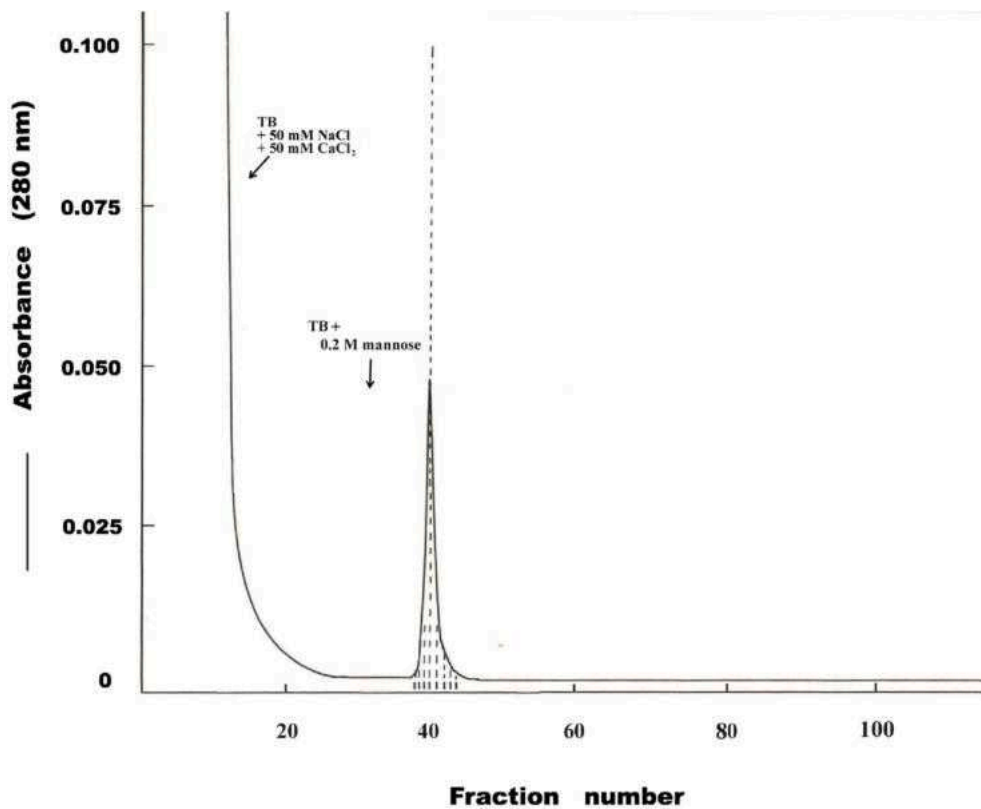


Fig. 2. HPLC analysis of purified lectin from the haemolymph of *H. pugilinus*.

Table 1
Haemoagglutination activity of *Hp-Lec* (50 µg/ml) against vertebrate RBC types.

RBC types and yeast cells tested	Agglutinating activity
Human O ⁺	0
Human A ⁺	32
Human B ⁺	64
Mice	16
Rat	16
Rabbit	128
Goat	64
Horse	0

Table 2
Agglutination of various bacterial species by the plasma *Hp-Lec* (50 µg/ml).

Bacterial species tested	Agglutination titre of plasma
<i>Vibrio parahaemolyticus</i>	16
<i>Pseudomonas</i> sp.	32
<i>Escherichia coli</i>	32
<i>Bacillus subtilis</i>	16
<i>Proteus vulgaris</i>	32
<i>Staphylococcus aureus</i>	16
<i>Lactobacillus</i> sp.	16
<i>Vibrio</i> sp.	16
<i>Enterococcus faecalis</i>	16

marker (Amersham Biosciences, USA). The homogeneity and molecular mass of *Hp-Lec* were also confirmed by gel filtration on a fast protein liquid chromatography (FPLC) column (ZorbaxBio-series GF-250, Du Pont, USA). Total protein concentration was determined based on the protocol by Bradford et al. (1976) [41] using bovine serum albumin (BSA) as a standard.

Table 3
Inhibition of agglutinating activity of plasma *Hp-Lec* (50 µg/ml) from the *H. pugilinus* by various carbohydrates.

CHO tested	Maximum concentration (mM)	Minimum concentration (mM)
Monosaccharides		
D-Glucose	200	–
D-Galactose	200	–
D-Mannose	200	–
D-Fructose	200	–
L-Fructose	200	–
Deoxy sugars		
2-Deoxy D-Glucose	200	–
2-Deoxy D-Galactose	200	–
Acidic Sugars		
D-Glucouronic acid	200	–
Glucouronic acid	200	–
N-Acetyl sugars		
GluNAC	200	0.15
GalNAC	200	0.15
ManNAC		0.05
Amino sugars		
Gal N	200	–
Glu N	200	–
Man N	200	–
Glycosides		
Methyl-β-D-Glucopyranoside	200	–
p-Nitrophenyl-α-D-Glucopyranoside	50	–
p-Nitrophenyl-β-D-Glucopyranoside	100	–
Disaccharides		
D-Maltose (glcα1, 4 glc)	200	–
Sucrose (glcα1, 2 fru)	200	–
Palatinase (gal α1, 6 fru)	200	–
D-Lactose (galα1, 6 glc)	200	–
D-Melibiose (galα1, 6 glc)	200	–

Table 4

Inhibition of agglutinating activity of plasma Hp-Lec (50 µg/ml) from the *H. pugilinus* by various polysaccharides against yeast cells.

Polysaccharides	Max.Inhibitory Concentration (mg/ml)	Minimum Inhibitory Concentration (mg/ml)
Laminarin (β 1-3 homopolymer of glucose)	1	–
Mannan (α 1-6 homopolymer of mannose)	1	0.0510
Dextran (α 1-6,3,2 homopolymer of glucose)	1	–
Inulin (α 2-6 homopolymer of fructose)	2	–
Colimanic Acid (α 2-8 homopolymer of Neu5Ac)	5	–

2.2. Effects of metal ions, temperature, and pH on hemagglutinating activity

We evaluated the hemagglutinating activity of Hp-Lec with and without divalent metal ions. To verify if temperature also influenced the hemagglutinating activity, Hp-Lec was incubated in a water bath at temperatures of 25, 40, 60 and 80 °C for 1 h. The effect of pH on the hemagglutinating activity of Hp-Lec was studied using Hp-Lec dialyzed for 24 h against phosphate buffer having different pH values, 2.0, 4.0, 6.0, 8.0, 9.0, and 12.0 [42].

2.3. Trypsin inhibition assay

In order to test the trypsin inhibition capacity of Hp-Lec, a 0.01 µg/ml protein solution was incubated with a 10-fold molar excess of trypsin for 1 h, and trypsin inhibition was determined based on the protocol proposed by Schwert and Takenaka (1955) [43]. In short, *N*-benzoyl-L-arginine ethyl ester (BAEE) was hydrolyzed by trypsin at the ester linkage, causing an increase in absorbance at 253 nm at 25 °C.

2.4. Effect of pH and temperature on structural stability

To test the effect of pH on the structural stability of Hp-Lec, purified protein samples were dissolved and incubated for 24 h in buffers with different pH values (2.0–12.0). Buffer formulations were adapted from Merrill (1990) [42]. After incubation, samples were analyzed by absorbance and fluorescence spectroscopy and by circular dichroism (CD). The effect of temperature on lectin stability was measured in the temperature range 25–85 °C by absorbance and fluorescence spectroscopy and CD. The surface plasmon resonance (SPR) binding constants were calculated using a bivalent ligand model, as previously described. Sensorgrams for the interactions between protein and the drug were analyzed using the BIA evaluation software.

2.5. Agglutination assays

Hp-Lec agglutinating activity was defined using different types of erythrocytes such as human RBCs (A, B, AB,O and vertebrates blood groups) and bacterial cells *Vibrio parahaemolyticus*, *Pseudomonas* species, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Lactobacillus* sp and *Vibrio* sp. were used as a pathogens. In a microtiter plate, 25 µl of diluted *H. pugilinus* purified lectin was mixed with 25 µl of tris-buffered saline buffer (TBS)/CaCl₂ containing RBCs (vertebrates) and incubated at 37 °C for 1 h. In the bacterial agglutination assay, we used both gram-positive and gram-negative above mentioned strains as test organisms. Each bacterial strain was suspended separately in TBS at a concentration of 2.5×10^9 cells/ml ($A_{600 \text{ nm}} = 1.6$) and titer values were equally recorded.

2.6. Sugar specificity assay

In agglutination inhibition assays, sugars, such as *N*-acetyl galactosamine (GalNAc), *N*-acetyl-glucosamine (GlcNAc), *N*-acetyl-mannosamine (ManNAc), mannosamine (Man-NH₂), galactosamine (Gal-NH₂), glucosamine (Glc-NH₂), glucose (Glc), galactose (Gal), mannose (Man), lactose (Lac), D-fucose, L-fucose, laminarin, mucin, and fetuin, serve as agglutination indicators. Competitive inhibitors were serially diluted and 20 µl of each inhibitor was mixed with 20 µl of the *H. pugilinus* agglutinin (1:16) in each well. The mixture was incubated for 30 min at 37 °C and then 50 µl of mammalian RBCs were added. The minimum inhibitor concentration required to block agglutination was calculated based on the calculation proposed by Sivakamavalli and Vaseeharan

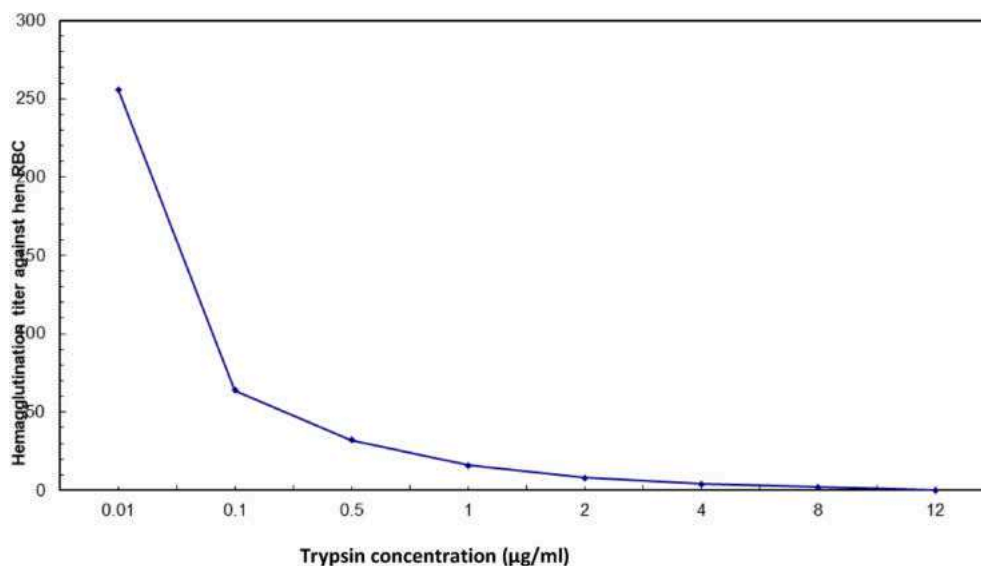


Fig. 3. Dosimetry hemagglutination of trypsin with various concentrations. Serum samples incubated with Hp-Lectin at the specified final concentration in the reaction mixture for 30 min at 37 °C and tested for agglutinating activity against hen RBCs.

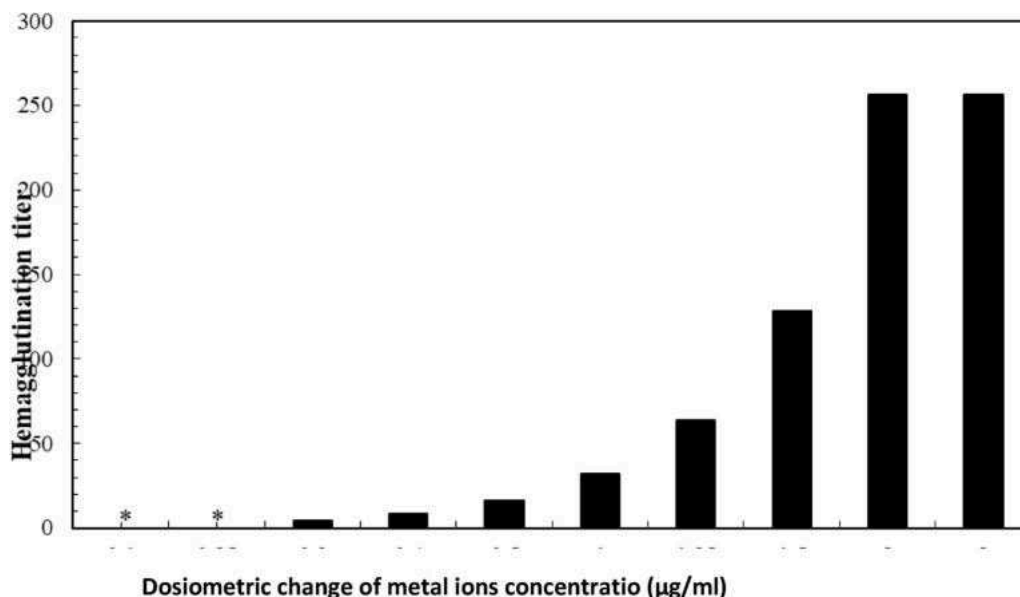


Fig. 4. Dosimetry hemagglutination of metal ions (CaCl₂) with different concentrations. *Hp*-Lectin incubated with metals at the specified final concentration in the reaction mixture for 30 min at 37 °C and tested for agglutinating activity against hen RBC.

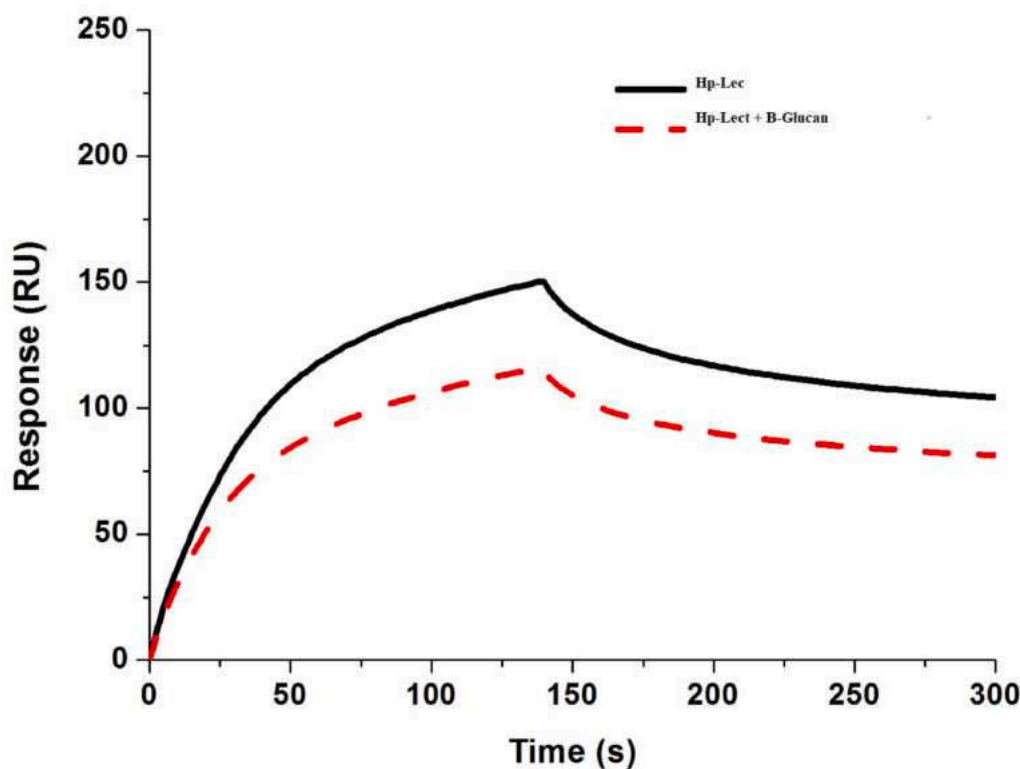


Fig. 5. Sensorgrams of the interaction between the *Hp*-lectin and *Hp*-lectin with metal ions.

(2013) [4].

2.7. Antifungal activity of *Hp*-Lec

The antifungal activity of *Hp*-Lec was evaluated using the methodology proposed by Refs. [44,45]. Differently sized *Hp*-Lec with various concentrations (25–50 µg *Hp*-Lec/ml PDA) was used in a colloidal solution and added to the sterilized potato dextrose agar (PDA) medium. Control dishes contained only PDA. The mycelia of *A. niger* and *A. flavus*

were placed in the center of each Petri dish and incubated at 27 ± 1 °C. Measurements of the colony diameter (cm) were performed at 24 h intervals. The experiment was concluded when the mycelia reached the edges of the control dish. The antifungal index (AI) was calculated at the end of the experiment using the following formula:

$$AI (\%) = (1 - D_1/D_2) \times 100$$

Where *D*₁ is the colony diameter in the test dishes and *D*₂ is the colony diameter in the control dish.

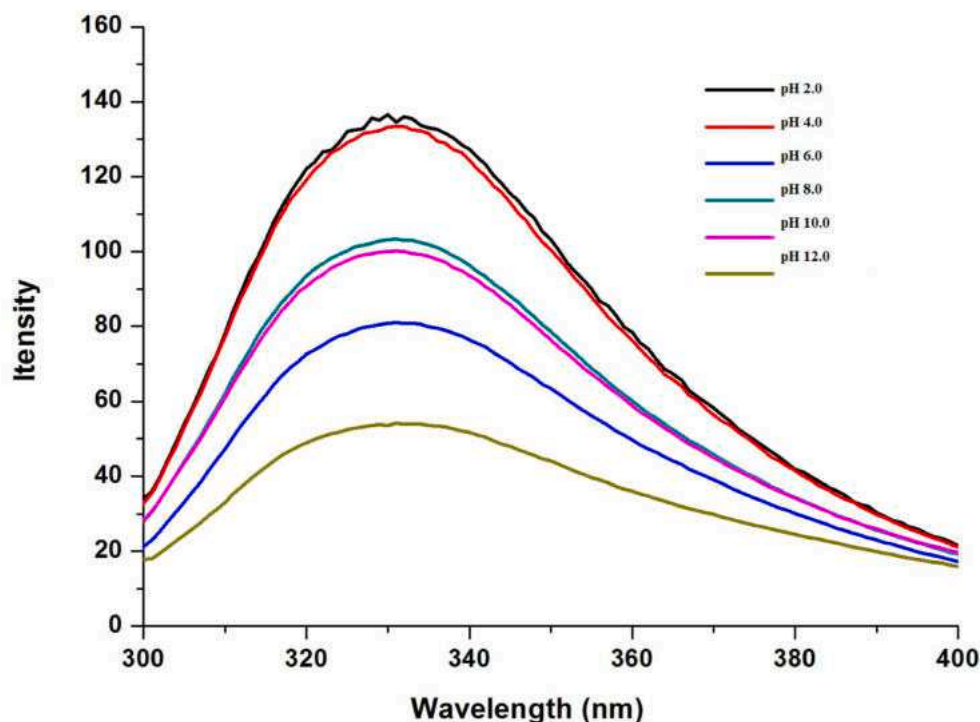


Fig. 6. Thermal stability of Hp-lectin with different pH ranges, analyzed through CD spectra.

3. Results

H. pugilinus plasma consists of divalent cation and metal-dependent proteins and a sharp band was observed at 115 kDa in the SDS-PAGE (Figs. 1 and 2). The molecule was named “mannose-binding protein lectin” (Hp-Lec), indicating that mannose was a better ligand when compared to GlcNAc. Hp-Lec bound better to Sepharose CL-4B when compared to other agarose beads. Hp-Lec produced five peptides through amino acid sequencing and important matches in NCBI or Marine Genomics databases. Hp-Lec showed effective hemagglutination (HA) of vertebrate RBCs, which was inhibited by three amino-derived sugars (Table 1). *H. pugilinus* mannan-binding protein (Hp-Lec) protein was excised and submitted to amino acid sequencing. Amino acid sequence data resulted in 9 fragments (data not shown). Unfortunately, these segments did not match with the reported ones. The highest HA was observed with rabbit RBCs and weaker HA in titer plates were observed with other RBCs (Table 1). Similarly, human blood groups reacted with the protein based on their type. Bacterial agglutination has been tabulated in Table 2. Results showed that mannan is a potential inhibitor of Hp-Lec, followed by Glc-NH₂ and GalNAc. However, no inhibitory activity was observed with Man, Glc, Gal, fructose (Fru), GlcNAc and sucrose, as shown in Tables 3 and 4.

For the agglutination inhibition assay the trypsin was used to inhibit the reaction at various concentrations assay, Hp-Lec was mixed with trypsin at ratios of (Hp-Lec: trypsin 1:1 and 10:1) and remarkable inhibition was observed (Fig. 3). Hp-Lec HA activity was improved upon using 10 mM CaCl₂ or MgCl₂ through Surface Plasmon Resonance the stability and the interactions of glucan with lectin molecule were detected (Figs. 4 and 5). The effect of pH on Hp-Lec HA was analyzed through preincubation in buffers with pH 2.0–12.0 (Fig. 6). Glucan recognizing ability of Hp-Lec further cross verified through the fungal activity using the fungal mycelium inhibition level. Further, Hp-Lec showed antifungal activity against the tested fungal pathogens *A. niger* and *A. flavus* (Fig. 7A and B), and this was attributed to its ability of recognizing chitin and glucan molecules on fungal cell wall. This carbohydrate-recognizing ability gives Hp-Lec the power to identify multiple pathogens.

4. Discussion

In this present study, lectins were purified from the plasma of the marine snail *H. pugilinus* through a mannose-coupled affinity column chromatography; the purified lectins showed functional activity in the presence of divalent cations, especially calcium. These kinds of calcium dependent lectins are called C-type lectins, and contain a calcium-binding domain in their structure [45,46]. At the same time, another concept denotes proteins with an affinity for mannan EPN pocket identified as MBP-like proteins, which are reported in many invertebrates [47–49]. MBPs are members of the lectin group that require calcium for their functioning [50]. Vertebrate MBPs are multimeric and trigger the complement system, thereby performing a crucial role in immunity [50]. These proteins act in a manner similar to that of pentraxin-like lectins that are purified from horseshoe crabs, *Limulus polyphemus* and *Tachypleus tridentatus*.

Lectins have been purified from giant tiger shrimp *Penaeus monodon* [51], kuruma shrimp *P. japonicas* [52], banana shrimp *P. merguensis* [53], Pacific white shrimp *Litopenaeus vannamei* [46] Chinese shrimp *Fenneropenaeus chinensis* [54,55], and Chinese mitten crab *Eriocheir sinensis* [44,56]. In molluscs, the relatively conserved specificity of lectins for Gal has been found in the clam *Tridacna maxima* [57], the pearl oyster *Pinctada fucata martensii* [58], *Ostrea chilensis* [59], the penguin wing oyster *Pteria penguin* [60], and *Crassostrea virginica* [61]. Other molluscs contain a heparin-binding lectin, such as the clam *Anadara granosa* [62], and a lactose-specific lectin from *Octopus vulgaris* [60]. In addition to the novel self-protective functions, a classical function reported in snail egg proteins is the one showed by the lectin of *Pomacea canaliculata*, which confers protection from bacterial invasions [63]. The lectins of mollusc gastropods, in particular *Helix pomatia* have been thoroughly purified and characterized, both functionally and structurally [64]. Two C-type lectins have been isolated from pulmonate *Biomphalaria glabrata* [65] and *Pomacea flagellata* snail [66,67]. The activity of agglutinin from the eggs of *Pomacea urceus* and *Pomacea paludosa* was activity inhibited by galactose and its derivatives. Similar results were observed in *Pila ovata* [67]. Hp-Lec showed a high affinity towards mannose and its derivatives, and it displayed affinity towards the

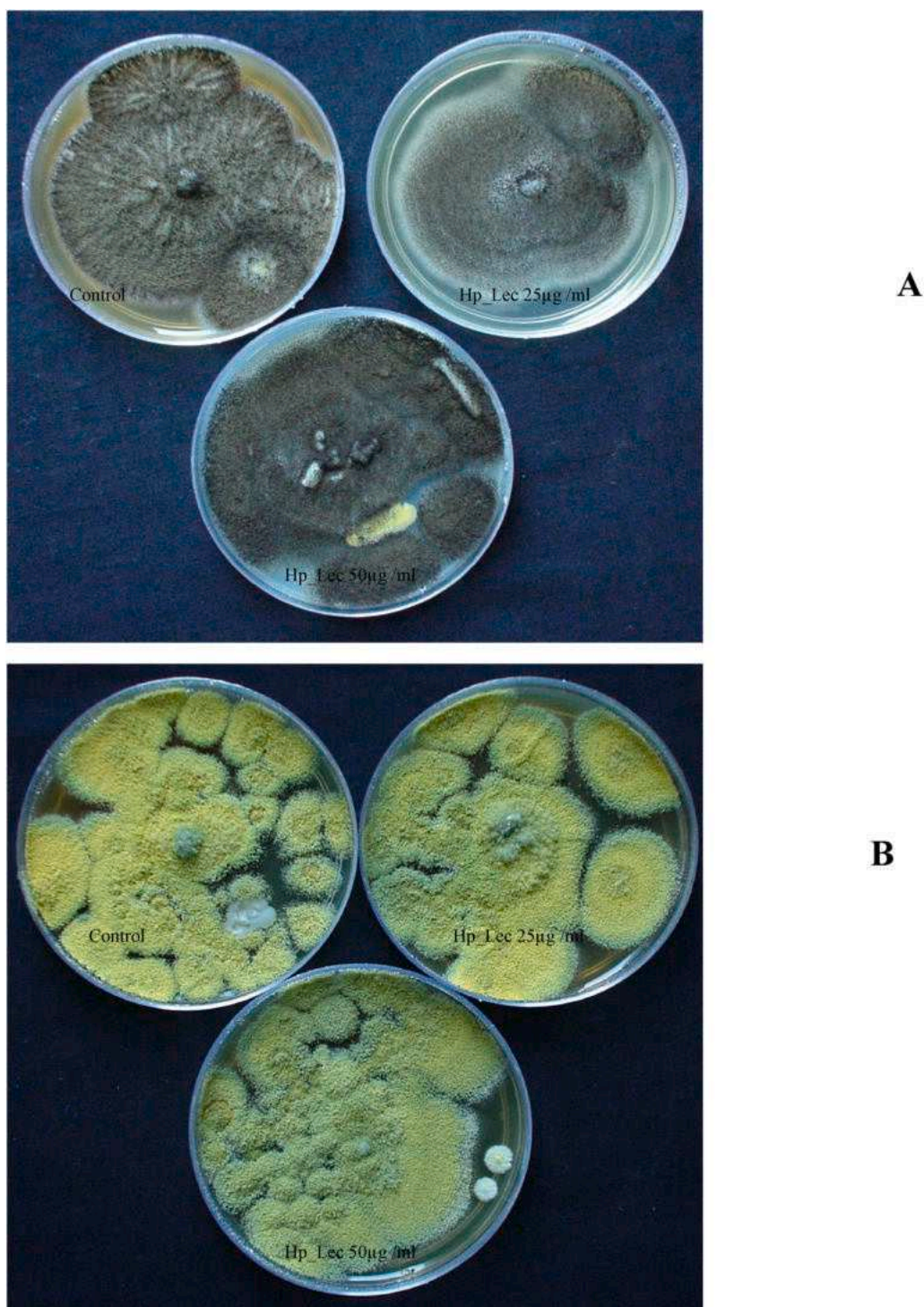


Fig. 7. Activity of purified Hp-lectin against the fungal strains, *Aspergillus niger* (A) and *Aspergillus flavus* (B).

Sephacrose 4B column. This was in agreement with the results for an already reported sialic acid-binding protein HaNBP [68].

Hp-Lec showed a strong HA activity in rabbit blood cells and a moderate HA activity in human A and B RBCs; these results correlate with the results obtained for agglutinins of ampullariid snails *P. canaliculata*, *P. ovata* and *P. urceus* [69]. Our inhibition experiments indicated that Man-NH₂, Gal-NH₂, and GlcNAc are powerful inhibitors of Hp-Lec. A selective affinity for Gal and Gal-derived sugars was reported in *Pomacea* snails, *P. urceus* and *P. flagellata* [65,66,71], and *Achatina* [70]. Overall, the results regarding the inhibition of HA activity in

Hp-Lec revealed that the protein specificity towards galactose is conserved in this mollusc [71]. HA activity of Hp-Lec with human A and B groups was also in agreement with this conserved specificity for galactosides. The agglutinating activity of Hp-Lec was pH-stable (between pH 4.0 and pH 8.0) and independent of the presence of divalent metals. *P. flagellata* agglutinins showed similar results at pH values of up to 10 [65,66].

In this study, Hp-Lec exhibited a good HA inhibition activity towards sugar molecules, especially mannan and *N*-acetyl based sugars, such as GalNAC, GlcNAC, and ManNAC. These results were in accordance with

those already reported for HaNBP [70–74]. Cornick and Stewart reported that a hemocyte agglutinin distinguished equine RBCs, but did not report any inhibition with any sugars. Fortunately, the ligands recognized by this protein, mannan and GlcNAc, are components of microbial cell walls. Yeast, fungal, and bacterial cell walls contain mannan, chitin, and peptidoglycan, respectively, and all possess a GlcNAc moiety [75,76]. Hp-Lec exposed to the fungal pathogens *A. niger* and *A. flavus* possibly inhibited fungal growth and disrupted the fungal mycelium layer; the same result was observed in case of a lectin from the tunicate *Clavelina picta* [76], though it could not detect chitin or peptidoglycan, nor did it agglutinate any of the tested bacteria. In conclusion, this report describes a novel Hp-Lec purified from the marine snail *H. pugilinus* and characterized it based on biochemical characterization and thermal stability analysis assessed at different pH values, and CD analysis of temperature changes. The functional activity of the molecule was elucidated through agglutination reactions and fungal activity studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carres.2020.108224>.

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Role of aposomes and epididymosomes in sperm quality control: A light and transmission electron microscopic study in an experimental rat model

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Abstract

The epididymis responds to adverse conditions of misshapen spermatozoa resulting from pathological changes or toxic insults by secretion of a dense matrix that segregates the latter for complete disintegration and dissolution. The objective of this study was to find the source of this matrix and the role-player of disintegration and dissolution of misshapen spermatozoa. We chose Wistar strain male rat model to tackle this issue, and the rats were administered with aflatoxin B1 for 55 days so as to increase the incidence of misshapen spermatozoa. At the end of the treatment, different segments of epididymis were processed for microscopic observations. We found that parallel with abundant misshapen spermatozoa in the epididymis the principal cells of the initial segment secrete enormous membrane-bound apical blebs called aposomes, which contain epididymosomes. The aposomes were found to coalesce so as for the content to merge and form a dense matrix that entangles the misshapen spermatozoa and segregates them from viable spermatozoa. The epididymosomes associate with the misshapen spermatozoa, and the latter is processed to disintegration and total dissolution. Therefore, we assign the role of segregation of misshapen spermatozoa from viable ones to the dense matrix of aposomes and their disintegration and dissolution to the epididymosomes.

KEYWORDS

apocrine secretion, aposome, dense matrix, epididymosome

1 | INTRODUCTION

Though apparently simple, the mammalian epididymis, formed of a single long convoluted and contorted ductus epididymidis, is in reality an amazingly complex organ (Cornwall, 2009). This complexity is contributed by the heterogeneity of epithelial cell types, consisting of principal, clear, narrow, apical, basal and halo cells (de Souza et al., 2018), and also its antero-posterior differentiation into anatomical [head (caput), body (corpus) and tail (cauda)], and histological and functional segments viz. initial segment, intermediate zone [not reported in human], caput, corpus and cauda (Robaire & Hermo, 1988). Adding to the complexity, the individual cell types

perform separate and integrated functions (Cornwall, 2009). The major role attributed to the epididymal epithelium is secretion of several proteins, in a generally segment-specific manner, which contribute greatly to post-testicular physiological maturation of spermatozoa into motility and fertilising ability (Dacheux & Dacheux, 2002). There are other roles such as receptor-mediated and fluid-phase endocytosis of proteins and solutes, maintenance of the continually changing microenvironment (Hinton & Palladino, 1995), preventing reactive oxygen damage to the spermatozoa (Cornwall, 2009), detoxification of harmful substances, immune surveillance of sperm antigens and sperm transport from caput to cauda and then to the vas deferens during ejaculation

(Harper, 1994). The cauda epididymidis further provides for the storage of spermatozoa until ejaculation (Akbarsha et al., 2015; Jainudeen & Hafez, 2000).

Under normal physiological conditions, there are many spermatozoa that are deformed/defective/dead due to disrupted spermatogenesis and post-testicular sperm processing mechanisms (Menkveld, 2010). The incidence of misshapen spermatozoa increases several folds in the context of pathological manifestations and/or toxic insults (Nixon et al., 2019), including in the human at times of infertility/subfertility (unpublished observation). In this context, the epididymis, in its versatility, is endowed with a sperm quality control mechanism by the way of secretion of a dense material that segregates the sperm cargo for complete disintegration and liquefaction, so as to safeguard the normal/viable spermatozoa (Faisal & Akbarsha, 2008), perhaps from the hostile environment of reactive oxygen species (Sullivan et al., 2005). Our aim was to find the source of the dense material and the critical role-player in the disintegration and liquefaction of the misshapen spermatozoa, for which we used an experimental animal model, the rat, induced into plenty of misshapen spermatozoa by treatment with aflatoxin B1 (Faisal & Akbarsha, 2008; Faisal et al., 2008), although untreated animals would also have a few-to-many misshapen spermatozoa (Cooper & Hamilton, 1977).

The epididymal epithelium, especially in the caput region, besides the classical exocrine secretory process, contributes to apocrine secretion as well (Hermo & Jacks, 2002). This secretory pathway involves formation of apical cytoplasmic blebs that detach and arrive at the luminal compartment. The apical blebs, the aposomes, disintegrate and liberate their content which includes small membrane-bound vesicles, the epididymosomes (Hermo & Jacks, 2002; Sullivan, 2015; Sullivan & Saez, 2013). Epididymosomes have a roughly spherical shape and a bi-layer membrane, and they are heterogeneous in size and content. Their size varies from 50 to 500 nm in diameter and are rich in cholesterol:lipid ratio (Frenette et al., 2003), and show complex electrophoretic patterns of associated proteins (Nixon et al., 2019; Sullivan et al., 2005). Epididymosomes are enriched in sphingomyelin and polyunsaturated membranous fatty acids, especially in arachidonic acids, in mice (Rejraji et al., 2006). Epididymosomes have been described in an increasing number of mammals, including human (Frenette et al., 2005), mouse (Rejraji et al., 2002), horse (Arienti et al., 1998), sheep (Gatti et al., 2005), chimpanzee (Frohlich & Young, 1996), hamster (Yanagimachi et al., 1985), bull (Frenette et al., 2002), cat (Rowlison et al., 2018) and rat (Belleannée, 2015). The functional maturation of the spermatozoa is accomplished via extensive crosstalk between the spermatozoa and the epididymal luminal content, and distinctly occurs in the complete absence of de novo gene transcription or protein translation (de Souza et al., 2018; Zhou et al., 2018). Therefore, it is ideal to implicate epididymosomes as the complex trafficking machinery of enzymes, structural proteins, immunological proteins, chaperones, cytokines and small noncoding RNAs (Reilly et al., 2016; Sharma et al., 2016; Sullivan & Mieusset, 2016; Zhou et al., 2017). Proteins of epididymosomes are selectively transferred to spermatozoa

(Frenette et al., 2002; Frenette & Sullivan, 2001). Some of these proteins are GPI-anchored (Frenette & Sullivan, 2001) and have been shown to be essential for the acquisition of sperm-fertilising ability (Sullivan et al., 2005; Yanagimachi et al., 1985), whereas others have been suggested to modulate the motility of maturing spermatozoa (Frenette et al., 2005, 2006) or protection from oxidative stress (Frenette et al., 2005, 2006).

We serendipitously found lead for the dense material in which misshapen spermatozoa are embedded to contain epididymosomes. This study traces adopting light and transmission electron microscopy (TEM), epididymal epithelial aposomes to contribute to the dense material and the epididymosomes present in the aposomes to contribute to the disintegration/dissolution of the misshapen spermatozoa.

2 | MATERIALS AND METHODS

The methodology in respect of the animal study was essentially the same as described previously (Agnes & Akbarsha, 2003; Faisal & Akbarsha, 2008; Faisal et al., 2008). Briefly, ninety-day-old Wistar strain male rats, raised from a stock obtained from Indian Institute of Science, Bangalore, Karnataka, India, were used and fed on aflatoxin-free pellet feed (Sai Durga Feeds & Foods) and water ad libitum. Aflatoxin B1 (AFB1) was obtained from Sigma Chemical Company. The toxin was dissolved in a minimum quantity of ethanol and diluted in olive oil. The preparation was administered to 10 rats in the experimental group, through *intramuscular* route, at a daily dose of 20 µg/kg body weight (Agnes & Akbarsha, 2001, 2003; Faisal et al., 2008) for 55 days, the duration of one spermatogenic cycle (Clermont, 1962). This treatment is known to affect spermatogenesis and viability of epididymal spermatozoa and thus increase the load of misshapen spermatozoa (Faisal & Akbarsha, 2008; Faisal et al., 2008). Control rats of equal number received the vehicle. At the end of the experiment, control and treated rats were transcardially perfused with Karnovsky fluid under pentothal sodium anaesthesia and dissected to remove the epididymides.

Thin slices of different segments of the epididymis (initial segment, intermediate zone, caput, corpus and cauda) were fixed in 2.5% glutaraldehyde in cacodylate buffer and post-fixed in 1% osmium tetroxide. After thorough wash in the buffer, the tissue slices were dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in thin viscosity resin (Araldite CY212, SPI-Chem™). Semithin sections (1 µm thick) obtained in a Leica ultramicrotome were stained with Toluidine Blue-O (TBO; pH 4.4) for observation in a Carl Zeiss research microscope. Photomicrographs were obtained in a CCD camera (Sony) connected to a computer loaded with AxioVision (Carl Zeiss) software. To measure the size of the aposomes and epididymosomes, 100 randomly selected aposomes/epididymosomes, circular in profile, from multiple sections, were measured using AxioVision software and represented as mean ± SD. The areas of interest were chosen from the semithin sections and

subjected to ultrathin sectioning in the same microtome. The sections were stained with 0.1% lead citrate and 6% uranyl acetate. The sections were analysed in a transmission electron microscope (Philips 201C), and images of interest were photographed. The animal experiment was approved by the Institutional Animal Ethics Committee of Bharathidasan University.

3 | RESULTS

While exploring meticulously through the tissue sections, we found principal cells of initial segment and intermediate zone of epididymis to associate with roughly elongated to spherical electron-lucent bodies of size $3.9 \pm 2 \mu\text{m}$ (Mean \pm SD; $n = 100$), less profuse in the control rats (Figure 1a,b) compared to treated rats (Figure 1c–f), and plenty of such bodies were also seen in the lumen such as to almost fill the latter. These are known in the literature as aposomes (Rejraji et al., 2006). The aposomes present in the lumen of the epididymis and were found loosely distributed; spermatozoa were found to lie scattered amongst the aposomes (Figure 1a,f). The aposomes provided evidence of merger or coalition (Figure 1d,e) to form into conglomerates (Figure 1f). Thus, the aposomes tended to lose their

identity and form the dense matrix. The spermatozoa and cell debris were seen entangled in the dense matrix (Figure 1f). The darkly stained narrow cells were protruding deep into the lumen, and some of these protrusions were appearing as pinching off such as to lie free among the luminal aposomes (Figure 1c–e).

Transmission electron microscopic observation confirmed the spherical membrane-bound aposomes in the epididymal lumen, and in most cases, spermatozoa were found scattered among the aposomes (Figure 2a). Looking into the aposomes, at high magnifications, it was seen that they contained homogeneous spherical bodies of approximately 130 nm size (Figure 2a–d). These are the epididymosomes known in the literature (Rejraji et al., 2006). The densities of epididymosomes in the aposomes were reflecting different grades, compact (Figure 2b), moderate (Figure 2c) and scattered (Figure 2d). Incidentally, an evidence that the aposomes are produced as blebs of epididymal epithelial cells could be seen in the aposomes containing cell inclusions such as mitochondria (Figure 2b–d).

The spermatozoa embedded in the dense matrix had undergone disintegration and/or dissolution (Figures 3a,b, 4a–d and 5) to different grades. There were also normal and intact spermatozoa outside the boundary of the dense matrix (Figures 3a,b and 4a–c). The sperm chromatin and outer dense fibrils are apparently tough to

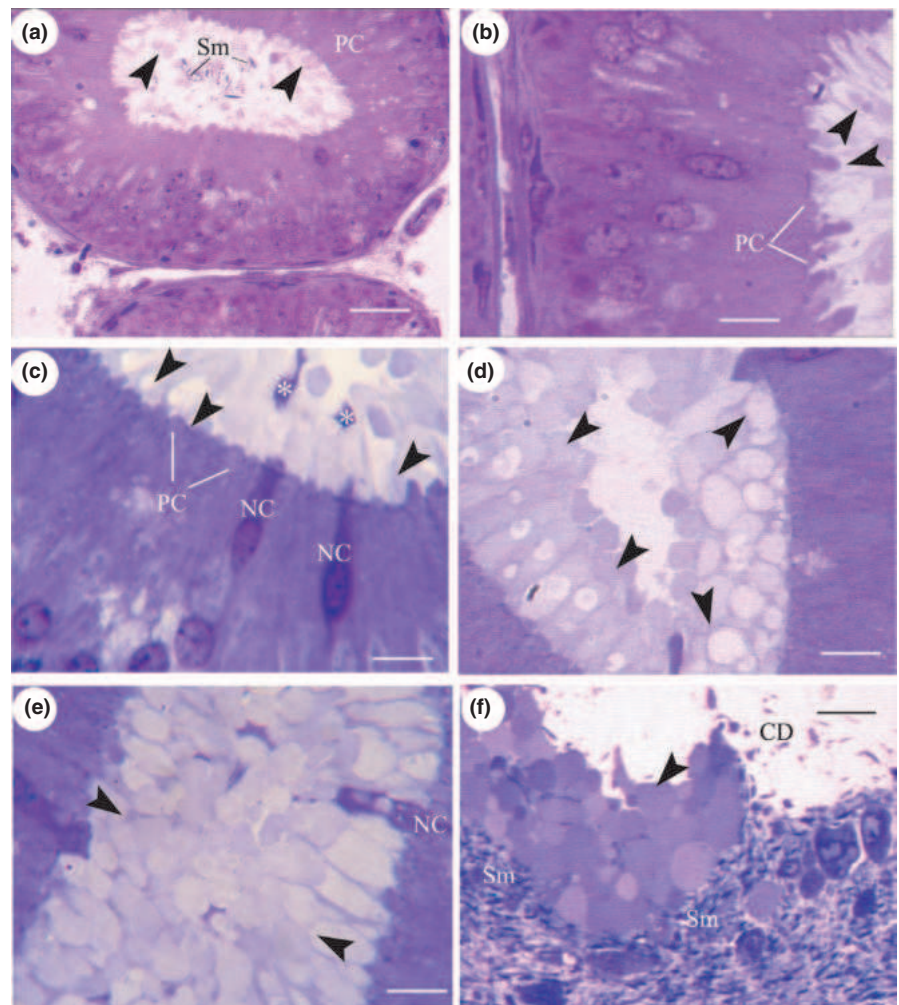


FIGURE 1 TBO-stained semithin sections of initial segment of rat epididymis. (a, b) Control rat. Principal cells (PC) secrete fewer aposomes (arrowhead); the lumen, clearly visible, contains the discharged aposomes (arrowhead) and spermatozoa (Sm). (c–f) AFB1-treated rat. (c–e) Principal cells (PC) show evidence of secretion of aposomes profusely (lightly stained; arrowheads) into the lumen, such as to almost obliterate the latter; narrow cells (NC), with apical protrusions, and their portions lying in the lumen (asterisks), are seen in (c). (f) The aposomes appear to associate among themselves by merger and form into conglomerates (arrowhead). The spermatozoa (Sm) and cell debris (CD) can be seen entangled in the dense matrix. Scale bar (a): 2.2 μm ; (b–f): 1.1 μm (TBO: Toluidine Blue-O; AFB1: aflatoxin B1)

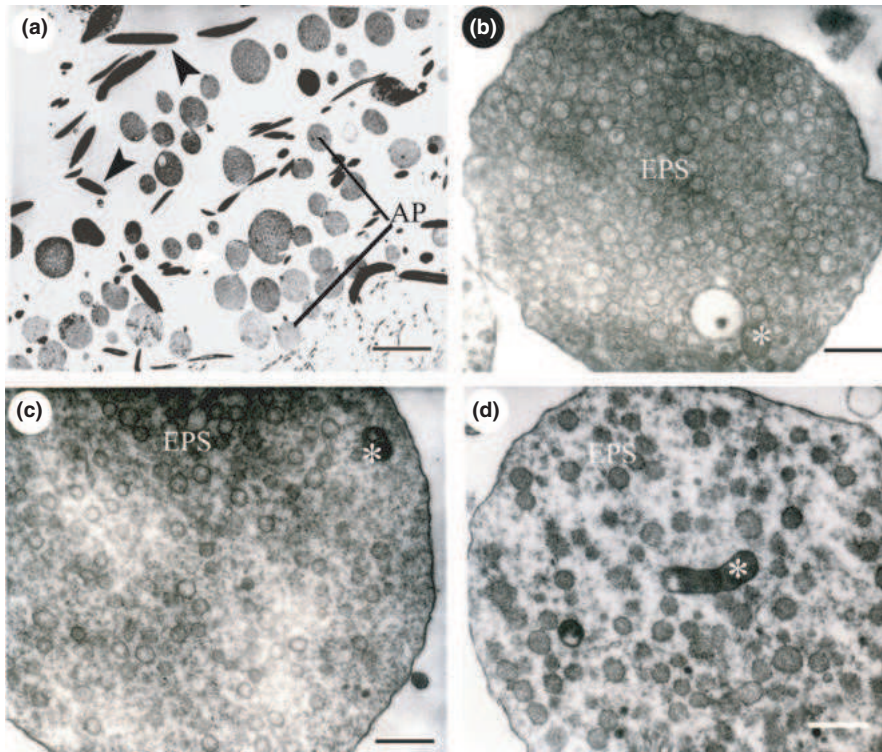


FIGURE 2 AFB1-treated rat. TEM of initial segment of epididymis. (a) Aposomes (AP) are abundantly present in the epididymal lumen, and spermatozoa (arrowheads) are scattered among them. (b–d) The aposomes contain homogenous spherical bodies, epididymosomes (EPS), which contain cell inclusions such as mitochondria (asterisks); the densities of epididymosomes in the aposomes reflect different grades, compact (b), moderate (c) and scattered (d). Scale bar (a): 6.2 μm ; (b, c): 260 nm; (d): 210 nm (TEM: transmission electron micrographs)

disintegrate/dissolve (Figures 3a,b and 4a,b). But, the spermatozoa gave impression that they ultimately undergo total dissolution such as to be present as a heterogeneous material in clear vacuole-like spaces (Figure 4c,d) or as electron-dense/electron-lucent densities comparable in appearance to lipofuscin inclusions (Figure 5).

Epididymosomes were found in the dense matrix that had entangled the spermatozoa that indicate dissolution/disintegration and were seen even to be in direct contact with the spermatozoa/sperm cargo (Figure 5).

4 | DISCUSSION

Epididymosomes are known in the literature and have been reported in a variety of mammalian species, including man, as mentioned elsewhere. However, this is the first time a newer role to epididymosomes in epididymal sperm quality control is attributed, apart from their known role in protein and other content transfer to sperm surface towards its post-testicular physiological maturation (Nixon et al., 2019). We have produced evidence for the profuse discharge of epididymosomes in large membrane-bound vesicles when the lumen abounds with misshapen spermatozoa as in the context of aflatoxin toxicity, more profuse than in the control rats, and this coincides with the abundance of matrix-entangled spermatozoa in the epididymal lumen.

Yanagimachi et al. (1985) were the first to describe adopting electron microscopy, membranous vesicles and the epididymosomes, in the hamster epididymal lumen, that interact with the sperm surface, and these authors suggested that the said vesicles are involved in sperm plasma membrane cholesterol transport. The epididymal

principal cells occasionally produce material in a granular form and release them by lysis of their apical membrane at the instances of toxic infliction (Agnes & Akbarsha, 2001; Akbarsha et al., 2015). These are adaptations involving structural changes mostly associated with specific functional attributes.

Rejraji et al. (2006) analysed apocrine secretion activity in the five segments of mouse epididymal epithelium and observed the formation of vesicular structures, affirming an earlier observation (Hermo & Jacks, 2002). The latter authors observed the principal cells to emit apocrine protrusions along the entire epididymal tract, but very prominently in caput epididymidis, as in the present study. In agreement with these observations, the proximal epididymis (i.e. segments I–III of the caput) was very active in apocrine secretions. However, contrary to what was reported initially by these authors, it was later found that the corpus and cauda epididymides were only very poorly active (Rejraji et al., 2006). Free vesicles resulting from blebs emitted by principal cells were also observed in the epididymal lumen. The content of the smaller vesicles was observed to contain uniformly distributed electron-dense granular material, whereas the larger vesicles looked rather empty. Even though a cytoplasmic continuity between these vesicles and epithelial cell cytosol was noticed during the apocrine secretion, the content of both these entities greatly differed from each other. A few of the vesicles had granular material aggregated in the vicinity of their membrane. Thus, their ultrastructure was almost the same as that observed for human prostasomes or prostasome-like vesicles in rat epididymis, stallion semen or bovine seminal vesicles (Arienti et al., 1998; Minelli et al., 1998). We presume that these variations in the ultrastructure, including those observed in our study, are in accordance with the heterogeneity of epididymosomes in terms of their encapsulation and interaction, discussed elsewhere.

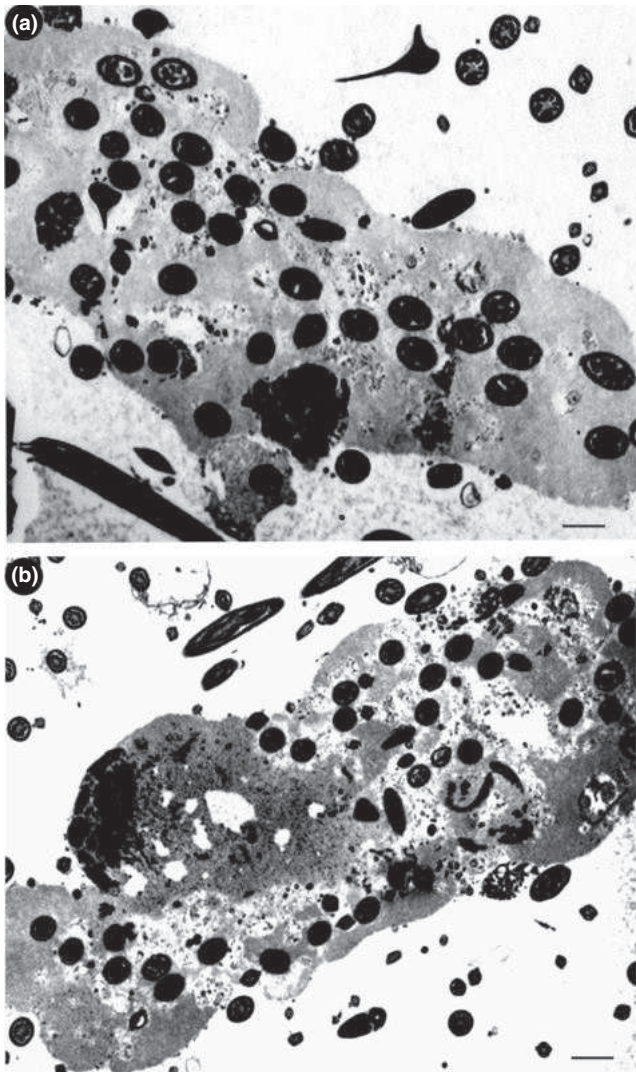


FIGURE 3 AFB1-treated rat. (a–b) TEM of dense matrix present in the caput epididymal lumen and the spermatozoa embedded in it can be seen to undergo disintegration/dissolution to different extents. Normal spermatozoa can be seen outside the boundary of the dense matrix. Scale bar (a): 1.5 μm ; (b): 2.1 μm (TEM: transmission electron micrographs)

In the bovine, two heterogeneous populations of epididymosomes have been characterised, each possessing the capacity to differentiate their investment between live and dead spermatozoa (Baskaran et al., 2020; Frenette et al., 2010). One such population is defined by their smaller diameter (~10–100 nm) and an abundance of CD9 protein (Grigor'eva et al., 2017). This population of epididymosomes exhibits preferential interaction with live spermatozoa, thus implicating them in sperm maturation/storage (Caballero et al., 2013). The other epididymosome population lacks CD9, but is loaded with epididymal sperm-binding protein 1 (ELSPBP1) and has a proclivity to interact with dead or dying spermatozoa through ELSPBP1/BLVRA (biliverdin reductase) complex (Akbarsha et al., 2015; Baskaran et al., 2020; de Souza et al., 2018; Sullivan, 2015). It is assumed that this difference in the markers may also correspond to the difference in granularity observed in the

present study. The acquisition of ELSPBP1/BLVRA complex by the maturing spermatozoa from epididymosomes enables tagging of spermatozoa that must be eventually expelled, while protecting the live spermatozoa from the detrimental effects of dead/dying spermatozoa (Sullivan, 2015). Therefore, the role of epididymosomes in sperm quality control is almost well established and perhaps it is wielded via ELSPBP1-rich population of epididymosomes and through the dense matrix-associated dissolution and disintegration. Nevertheless, ELSPBP1 is not the first protein known to be associated with the misshapen spermatozoa.

Proteomic analysis of human epididymosomes identified a spectrum of 146 large molecular weight proteins of varied functions including wads of proteins involved in the elimination of defective spermatozoa (Thimon et al., 2008). Amongst the various identified epididymosomal proteins, those involved in the elimination of defective spermatozoa include programmed cell death 6 (PDCD6) (Thimon et al., 2008), encoded by apoptosis-linked gene 2 (ALG/2), which is concerned with apoptosis (Vito et al., 1996). Yet another protein, Annexin V, present in the epididymosomes (Thimon et al., 2008) is also concerned with apoptosis (Gorczyca et al., 1998) and for segregation of functional spermatozoa from defective ones (Hoogendijk et al., 2009).

Ubiquitin is also associated with the epididymosomes in humans and bovine, and binds preferentially to the surface of defective spermatozoa. This has been reported in men, bulls, rhesus monkeys and mice (Sutovsky et al., 2001). The percentage of defective spermatozoa decreases as the spermatozoa descend through the epididymal passage, because of the disposal of such spermatozoa along with the ubiquitinated cytoplasmic droplets by the epididymal epithelium (Faisal & Akbarsha, 2016; Roussel et al., 1967). Thus, among its many functions, ubiquitin plays a role in extra-lysosomal protein degradation also (Robaire & Hermo, 1988). Glutathione peroxidase type 5 (GPX5) is an enzyme secreted in association with epididymosomes, and it protects the spermatozoa from oxidative stress and preserves DNA integrity during epididymal transit (Rejraji et al., 2002). This incarnates a pivotal role for epididymosomes in recognition and elimination of defective spermatozoa. Thus, in the light of earlier studies and based on the protein content of epididymosomes, there exists an alternative pathway for the elimination of misshapen spermatozoa modulated via epididymosomal interaction, that is in the dense matrix of the epididymal lumen.

Cooper and Hamilton (1977) were the first to describe and discuss in detail a 'flocculent material' or 'dense matrix' that entangles disintegrating/dissolving spermatozoa in untreated rat, mouse, hamster and guinea pig, supported by exquisite electron micrographs, and drawing strength from several earlier publications. As in the present study, these authors also noted that when the degenerating/disintegrating spermatozoa were entangled in the dense material they were in different grades of dissolution, and the intact spermatozoa were secluded from the dense material by a clear halo. It is comprehensible from this paper that sperm disintegration/dissolution and a dense matrix in which such spermatozoa are embedded would occur in normal nonseasonal males, apart from seasonal breeders

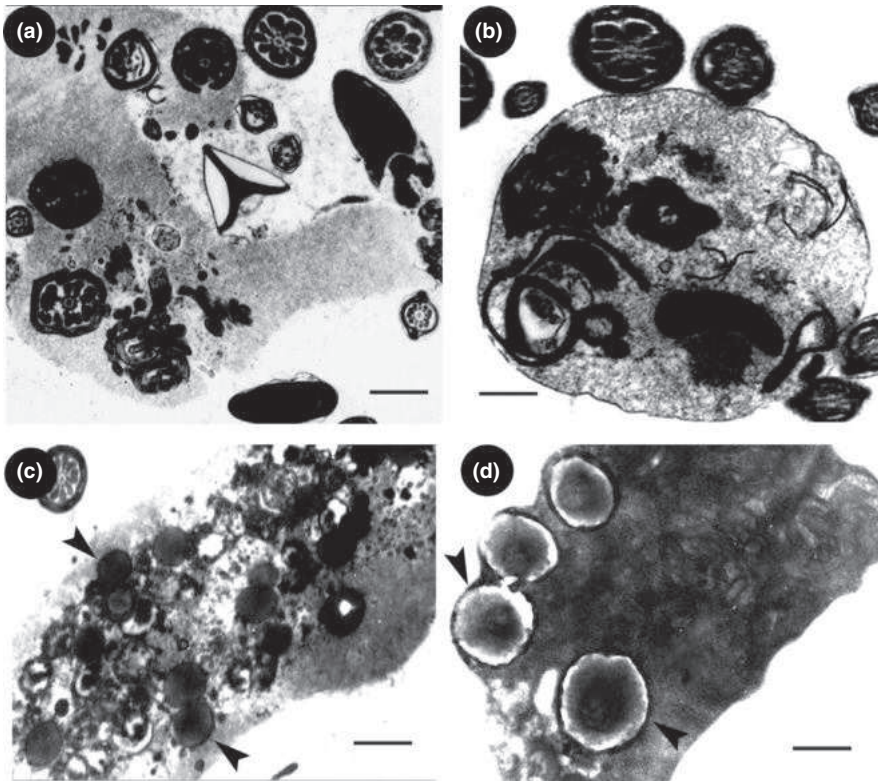


FIGURE 4 AFB1-treated rat epididymis. TEM of spermatozoa embedded in the dense matrix. The spermatozoa are in different extents of disintegration/dissolution. The liquefied material has a heterogeneous appearance (arrowheads). (a, b): caput; (c): corpus; (d): cauda. Scale bar: (a, c): 1.2 μm ; (b): 2.0 μm ; (d): 2.4 μm (TEM: transmission electron micrographs)

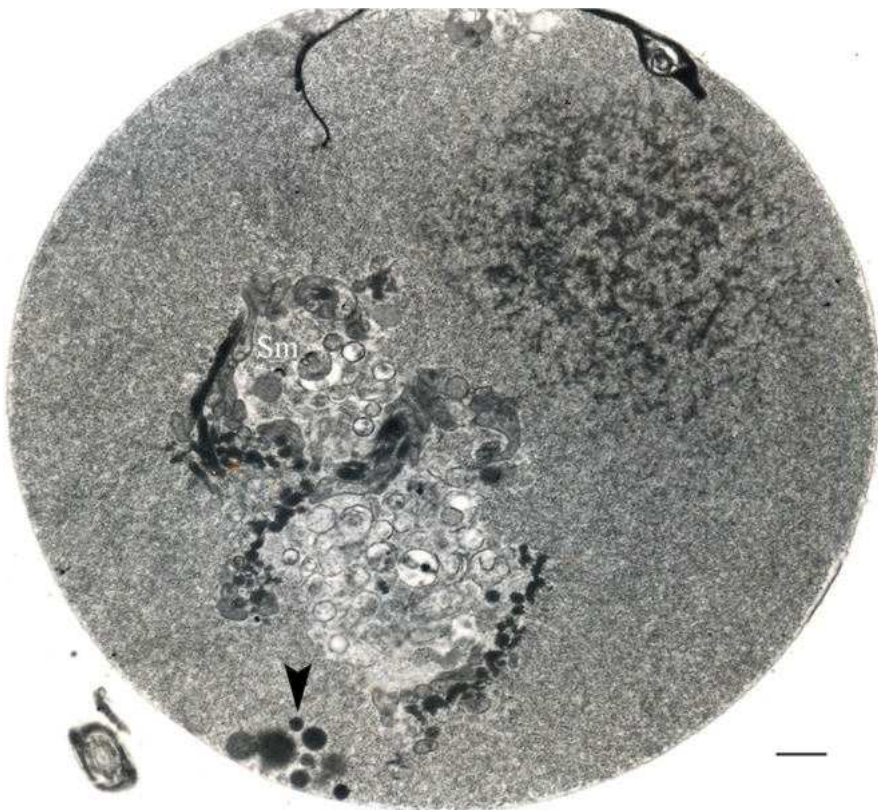


FIGURE 5 AFB1-treated rat epididymis. TEM of matrix-embedded spermatozoa, in the lumen, which have undergone almost complete disintegration and dissolution resulting in a fibrous/homogeneous dense material and a lipofuscin-like material (arrowhead). Epididymosomes are found in the dense matrix which entangles the disintegrating spermatozoa (Sm). Scale bar 80 nm (TEM: transmission electron micrograph)

and animals subjected to experiments. But this article having been published much before anything was known about aposomes and epididymosomes, there is no mention about the source of the dense material. We have bridged this gap by reporting the presence of

varying numbers of abundant dense masses in the lumen, from caput epididymidis onwards. Spermatozoa were found entangled in the dense masses, and these spermatozoa were in different degrees of disintegration (Faisal & Akbarsha, 2008). Quite a few reports

support the role for a dense material in the disintegration of spermatozoa in epididymal milieu (Alexxander, 1972; Martan, 1969; Olson et al., 2004; Simeone & Young, 1931). Therefore, the dense material which in turn formed from the aposomes could be a device to mass-ubiquitinate the misshapen spermatozoa and to seclude the viable spermatozoa from the ubiquitination system, to facilitate the removal of misshapen spermatozoa.

Thus, this study links aposomes of epididymal epithelial principal cell origin to the dense matrix, which entangles the misshapen spermatozoa towards a sperm quality control mechanism, and suggests a newer pathological role for epididymosomes present in the aposomes, apart from their known physiological roles, to contribute to disintegration/dissolution of dead/defective spermatozoa. The precise biochemical and molecular mechanisms underlying disintegration/dissolution of misshapen spermatozoa by epididymosomes and how the viable spermatozoa get excluded from the dense matrix are worthy of further investigation.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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Research Article

In Vivo and *In Vitro* Toxicity Profiles of Hexane Extract of *Alpinia malaccensis* Rhizome in Rat and Cell Line Models

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The objective of the study was to evaluate the potential toxicity of crude *n*-hexane extract of *Alpinia malaccensis* rhizome. The *in vivo* acute oral toxicity was evaluated by administering a single oral dose of the extract at 0, 300, or 2000 mg/kg body weight to female Wistar rats according to modified OECD Test Guideline 423. For the *in vitro* cytotoxicity study, A549, HepG2, 3T3, and COS-7 cell lines were exposed to different doses of *A. malaccensis* extract and cell viability was assessed adopting MTT assay followed by AO/EB staining, Hoechst staining, and comet assay with a view to compare the cellular and molecular mechanisms underlying the toxicity, if any. It was found that administration of 2000 mg/kg bw dose in *in vivo* oral acute toxicity study did not produce significant toxicity or mortality. No significant ($p < 0.05$) differences were observed for body weight and hematological and biochemical parameters compared to control after 14 days of treatment. No changes in behavior, body weight, hematological and biochemical parameters, and aspects of histopathology were observed when compared to the control. Thus, the possible oral lethal dose for *A. malaccensis* extract is above 2000 mg/kg body weight. The *in vitro* cytotoxicity analysis showed nontoxicity concentrations of the extract to be 2, 1.4, 30, and 1.4 $\mu\text{g/mL}$ for A549, HepG2, 3T3, and COS-7 cells, respectively, where no apoptotic/necrotic cell death and DNA damage were observed. In conclusion, the extract of rhizome of *A. malaccensis* did not produce apparent cytotoxicity or acute oral toxicity, confirming the scope to use *A. malaccensis* as a safe food preservative and a natural therapeutic product after further subacute and chronic toxicity studies.

1. Introduction

Medicinal plants have been widely used in conventional or traditional medicinal practices as a reliable cure to diseases since prehistoric days. The World Health Organization also has recognized plants as a reliable source of therapeutics [1]. The therapeutic property of the medicinal plants lies in the bioactive compounds they contain. It is believed that the plant-derived

compounds, when used as therapeutics, are less harmful compared to the synthetic drugs [2]. Thus, the medicinal plants offer great scopes to discover newer drugs. Nevertheless, surveys have indicated that many plants used in traditional medicines can potentially produce adverse effects [3, 4], and some of them have been shown to contain toxic compounds [5]. Therefore, not all medicinal plants are safe. Hence, there is a pertinent need to assess the toxicity of medicinal plants to ensure safety.

Alpinia malaccensis, popularly known as Malacca ginger and “Rankihiriya,” belongs to the family Zingiberaceae. It is a perennial South Asian medicinal plant cultivated widely in the tropical regions of Asia including Indo-China, Bangladesh, and Sri Lanka [6]. *A. malaccensis* has traditionally been used in the preparation of medicines to cure nausea, vomiting, and wounds and also as a seasoning ingredient in the processing of meat [7]. Somarathna et al. [8] evaluated the bioactive chemical constituents of the hexane crude extract of *A. malaccensis* rhizome and found 82.87% 1'-acetoxychavicol acetate (1'ACA) as the major active chemical compound. Crude *A. malaccensis* extract as well as the pure compound 1'ACA showed strong antibacterial and anti-biofilm activities against *Listeria monocytogenes* and *Staphylococcus aureus* [8, 9].

However, Primahana et al. [10] reported that trans-methyl cinnamate found in *A. malaccensis* produces moderate toxicity to brine shrimp at 120.47 µg/mL LC₅₀. Further, Thu et al. [11] reported that fruit of *A. malaccensis* induces cytotoxicity in A549 and HepG2 cell lines at 4.8 and 5.5 µg/mL doses, respectively. It was suggested that the inconsistency of the chemical composition may be due to the differences in the plant parts used, age, variety, edaphic factors, and geographical regions where cultivated [12, 13]. Also, Chan et al. [14] reported that the rhizome accumulates more secondary chemicals than the other plant parts. Therefore, evaluation of toxicological aspects of *A. malaccensis* rhizome will be a rewarding area of research.

The present study was aimed at investigating the potential toxicity of *n*-hexane extract, which would contain the most nonpolar compounds, of rhizome of *A. malaccensis* by finding the acute *in vivo* toxicity using the rat model and *in vitro* cytotoxicity using cell lines.

2. Materials and Methods

2.1. Plant Collection and Authentication. *A. malaccensis* plants were collected from the medicinal garden of the Nature Secret (Pvt) Ltd, Millewa, Horana, Sri Lanka, and a voucher specimen (2012/APE/02) was deposited in the herbarium of Department of Agricultural Plantation Engineering, the Open University of Sri Lanka. The plant was identified using the key to species by Dassanayake and Forsberg [15].

2.2. Extraction of *A. malaccensis*. Fresh *A. malaccensis* rhizomes were washed in running water, and the outer skin was peeled off. The rhizome was sliced and oven-dried at 40°C for 24 h. The dried slices were pulverized using a grinder (National Super Blender, Taiwan, Model MX-TIIOPN) for 1 minute at 30 seconds interval in each cycle for five times. The powder was stored at -20°C until use. *n*-Hexane was used as the solvent for the extraction. The extract was prepared by adding 10 g of *A. malaccensis* powder to 100 mL of *n*-hexane and agitated (160 rpm) for 24 h at 30°C in a rotary shaker (Stuart® orbital shaker SSL1, UK). The mixture was centrifuged at 4500 ×g for 10 min (Centurion Scientific Ltd, UK), and the supernatant was filtered using #1 Whatman filter paper. The filtrate was evaporated under vacuum at

40°C using a rotary evaporator (KIA RV 5, Switzerland) and filter-sterilized through a 0.45 µm filter unit (Millex® HA, Germany). The filtrate was N₂-fluxed under heat (40°C) for 3 h until a hexane-free extract was obtained. Finally, the concentrated extract was stored at 4°C until use.

2.3. Cell Culture. Human lung carcinoma cell (A549), hepatocarcinoma cell (HepG2), normal mouse fibroblast cell (3T3), and monkey kidney tissue cell (COS-7) were obtained from the National Center for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and with 20 mL each of penicillin and streptomycin as antibiotics, in a humidified atmosphere of 5% CO₂ and 95% air, in a CO₂ incubator (Thermo Scientific, USA) [16]. The cytotoxicity studies were conducted at Mahatma Gandhi-Doerenkamp Center for Alternatives at Bharathidasan University, Tiruchirappalli, India.

2.4. MTT Assay for Assessment of Cell Viability. Cell viability, which would reveal the cytotoxic property of the extract, was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay with slight modifications [17]. Cells were seeded in 96-well plates at 5 × 10³ cells/well and incubated for 24 h at 37°C. The cells were treated with the extract at increasing concentrations within 0–1000 µg/mL for 24 h, at 37°C. The extract was quantitatively dissolved in minimum quantity of DMSO and diluted in the culture medium to prepare the stock solution, which was then made up in the culture medium so as to have the final extract at DMSO concentration of 0.1%. This concentration of DMSO is known not to affect the cell viability [18]. DMSO at this concentration was also used as the solvent control. Experiments with each extract concentration were conducted in triplicates on the same batch of cells. After 24 h incubation, 20 mL of MTT (Sigma-Aldrich, St. Louis, MO, USA) solution (5 mg/mL in PBS) was added to each well and incubated for 3 h at 37°C. The medium was then removed, and 100 mL of DMSO was added to each well to dissolve the purple formazan product. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, Hercules, CA, USA). The percentage inhibition was calculated from these data using the following formula, and IC₅₀, defined as concentration of the test substance at which cell viability is decreased to 50%, was calculated.

Percentage of cell inhibition

$$= \frac{\text{mean OD (control)} - \text{mean OD (treatment)}}{\text{mean OD (control)}} \times 100. \quad (1)$$

2.5. Morphological Assessment of Cell Death Using AO/EB Fluorescent Assay. The morphological characteristics of cells in respect of apoptosis and necrosis were assessed by AO/EB staining [19]. The A549, HepG2, 3T3, and COS-7 cells were cultured in 6 well plates and incubated for 24 h with IC₅₀ and

no toxicity concentrations of the extract, as found in the MTT assay. The treated and untreated cells were centrifuged (3000 rpm for 4 min) and incubated with acridine orange (AO) and ethidium bromide (EB) solutions (1 part of 100 mg/mL each of AO and EB in PBS) and observed in a fluorescent microscope (Carl Zeiss, Jena, Germany) using a UV filter (450–490 nm). Three hundred cells per sample were counted, in duplicate, and scored as viable or dead, and if dead whether by apoptosis or necrosis as judged from nuclear morphology and cytoplasmic organization. Then, the percentages of apoptotic and necrotic cells were calculated. The morphological features of interest were photographed.

2.6. Assessment of Nuclear Morphological Features Using Hoechst 33528 Staining. The nuclear morphological features of the cells were assessed using Hoechst 33528 staining [20]. The cells were cultured in 6-well plates and treated with *A. malaccensis* at its IC₅₀ concentration and no toxicity concentration, for 24 h. After incubation, the treated and control cells were harvested and stained with Hoechst 33258 (1 mg/mL in PBS) for 5 min at room temperature. A drop of cell suspension was placed on a glass slide and covered with a cover slip. Three hundred cells, each in triplicate, were observed at x400 in the fluorescent microscope fitted with a 377–355 nm filter. The percentage of cells reflecting pathological changes was calculated.

2.7. Genotoxicity Assessment Using Comet Assay. Genotoxicity assessment was performed using comet assay where single-cell gel electrophoresis was performed to quantify the DNA damage [21]. The cells were treated with the IC₅₀ concentration and no toxicity concentration of the extract for 24 h. The harvested cells were suspended in low melting point agarose in PBS and pipetted out to microscope slides on precoated layer of normal melting point agarose. Another layer of medium melting agarose was laid on top of the low melting agarose. Slides were immersed in prechilled lysis buffer (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris, 0.2 mM NaOH (pH 10), and Triton X-100) and incubated overnight at 4°C in order to lyse the cells and allow DNA unwinding. Then, the slides were exposed to alkaline buffer (300 mM NaOH, and 1 mM Na₂-EDTA (pH > 13)) for 20 min to allow DNA unwinding and then subjected to electrophoresis at 7 V. The slides were washed with neutralization buffer (0.4 M Tris, pH 7.5) for 2 min and observed in the fluorescent microscope. The triplicate data, each from one hundred and fifty cells, from each treatment group, were collected manually. The images were used to evaluate the degree of DNA damage representing the fraction of total DNA in the tail, according to Gayathri et al. [16].

2.8. Acute Oral Toxicity Study Using Wistar Rats

2.8.1. Animals. The acute oral toxicity test was carried out using female Wistar rats. The number of animals and sex used for each test were determined considering both

Organization of Economic Cooperation and Development (OECD) guidelines and the opinion of the Ethics Review Committee of Medical Research Institute (2016/22), Sri Lanka, based on the 3R concept. All animals were maintained under standard laboratory conditions including 20°C to 24°C temperature, 50% to 70% relative humidity, and a light regimen of 12 h light and dark throughout the experimental period. Standard size, polycarbonate, and transparent cages were used for animal housing. Three rats from the same sex were kept in one cage. Sterilized wood shavings were used as the bedding material. All animals were fed with MRI rabbit and rat formula prepared according to WHO guideline given by Saboudry [22]. The formulations were prepared at the Medical Research Institute using locally available ingredients. All rats were acclimatized to laboratory conditions for a period of 7 days prior to dosing.

2.8.2. Acute Oral Toxicity Test. Acute oral toxicity test of the crude *n*-hexane extract of rhizome of *A. malaccensis* was carried out according to the method described in OECD Test Guideline 423. Three-month-old female Wistar rats, weighing 150–200 g, were randomly divided into three groups, control ($n=3$) and two treatment groups ($n=3$ each). The animals were maintained in an air-conditioned and light-controlled room with access to water and feed *ad libitum* throughout the experiment. Animals were kept fasting overnight with free access to water the day prior to starting the experiment. Animals were individually weighed. Rats in the control group were administered olive oil (1 mL). Doses of 300 and 2000 mg/kg BW of crude extract were used for the limit test. The administered volume was adjusted to 1 mL/kg BW per rat.

After administration of *A. malaccensis* extract, rats were observed for first 30 min, and first 24 h with special attention during first 4 h, and thereafter daily for 14 days. The rats were weighed and observed for signs of toxicity including mortality, changes in behavioral pattern (salivation, lethargy, and sleep), physical appearance, injury, pain, and signs of illness during the observation period. In addition, the body weight, feed intake, and water consumption were recorded throughout the experimental period. Animals were mildly sedated using gaseous anesthesia, and 1 mL of blood was collected by the lateral tail vein puncture technique for biochemical and hematological analysis. The serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) were measured with commercially available estimation kits (Pointe Scientific, Colombo) using a semiautomated biomedical analyzer (Stat Fax 3300, Ramsey, MN, USA). At the end of the experiment, the rats were humanely euthanized by overdosing gaseous anesthesia, and the liver, kidney, lung, heart, and spleen were collected. The relative organ weights of rats were recorded and also examined macroscopically. The organs were preserved in a fixation medium of 10% solution of formalin for histopathological study.

2.9. Histopathological Study. Portions of formalin-fixed liver, kidney, lung, heart, and spleen were dehydrated with alcohol, embedded in paraffin wax, cut into 4-5 μm thick sections, and stained with hematoxylin and eosin. Slides were examined in a light microscope at x40 magnification. The microscopic features of the organs of control and treated rats were compared. The study was carried out in the Veterinary Institute, Gannoruwa, Sri Lanka.

2.10. Statistical Analysis. All values are expressed as mean \pm SEM. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using SPSS statistical software. A p value of <0.05 is considered significant.

3. Results

3.1. Cytotoxic Property of Crude *n*-Hexane Extract of *A. malaccensis*. The cytotoxic property of *A. malaccensis* *n*-hexane extract was analyzed against A549, HepG2, 3T3, and COS-7 cells at different concentrations to determine the respective IC_{50} by MTT assay. The IC_{50} values of different concentrations of *A. malaccensis* extract are graphically represented in Figure 1. The IC_{50} values in respect of A549, HepG2, 3T3, and COS-7 cells were 7.25, 22.5, 62.75, and 8.25 $\mu\text{g}/\text{mL}$, respectively. The extract showed 0% inhibition at concentrations 2, 1.4, 30, and 1.4 $\mu\text{g}/\text{mL}$, for A549, HepG2, 3T3, and COS-7 cells, respectively, and these concentrations are considered as nontoxic.

3.2. Microscopic Features of Apoptosis and/or Necrosis. Microscopic features of apoptosis were observed adopting AO/EB staining and are shown in Figure 2(a). In general, dead cells are permeable to EB and fluoresce red, whereas live cells are permeable to AO only and therefore fluoresce green. The viability and membrane integrity of the cells were determined based on the fluorescence pattern. The morphological changes observed in the treated cells were classified based on the fluorescence emission as follows: (i) viable cells having highly organized nuclei fluoresced green; (ii) early apoptotic cells which showed nuclear condensation fluoresced orange green; (iii) late apoptotic cells with the highly condensed chromatin or fragmented chromatin fluoresced orange to red; and (iv) necrotic cells fluoresced orange to red without chromatin fragmentation. Data on cells indicating apoptotic and necrotic morphologies, induced on treatment with the IC_{50} concentration and no toxicity concentrations of *A. malaccensis* for 24 h, and collected from manual counting are presented in Figure 2(b), which revealed that *A. malaccensis* at IC_{50} is efficient in bringing about early apoptosis (less than 50%), but little necrosis was produced. However, *A. malaccensis* at no toxicity concentration did not induce any remarkable apoptosis or necrosis (less than 10%) for the cell lines tested (Figure 2).

3.3. Microscopic Features of Nucleus. Hoechst 33528 staining was adopted to find the morphological changes in the nucleus as caused by treatment of IC_{50} and no toxicity concentrations of *A. malaccensis*, and the features are shown in Figure 3(a). Data collected from manual counting of cells with normal and abnormal nuclear features are shown in Figure 3(b). In the control cells, the nuclear chromatin was full while after treatment with the IC_{50} of the extract, changes such as chromatin marginalization, condensation, and fragmentation were noticed. These observations revealed that exposure of *A. malaccensis* to IC_{50} concentration alone led to chromatin fragmentation which is a characteristic feature of apoptosis. However, treatment with no toxicity concentration (IC_0) of the extract indicated no difference with the control.

3.4. Identification of DNA Damage as Revealed in Comet Assay. Comet assay was adopted to detect the cellular DNA lesions or genotoxicity. Comet assay is very sensitive of strand breaks in DNA. DNA damage was analyzed based on DNA tail size, shape, and migration pattern, and the data are shown in Figure 4(a). *A. malaccensis* at IC_{50} caused moderate damage to DNA. However, at the no toxicity concentration, there was only negligible damage to DNA. Since the tail length and density reflected the extent of strand breaks in DNA, the percentage of DNA in the tail provided a quantitative measure of DNA damage as shown in Figure 4(b).

3.5. Acute Oral Toxicity as Revealed in Animal Experiments. A single dose of 300 or 2000 mg/kg of *A. malaccensis* extract of the rhizome administered through oral route did not produce any mortality in rats during the 14-day observation period. Further, with regard to the behavioral patterns, rats treated 2000 mg of *A. malaccensis* extract were cleaning their face very frequently for the first 4 hours. However, after 4 h, the behavioral pattern was the same as in the control group.

3.6. Organ Weights of Tested Rats. Data on the organ weights between control and treated rats are shown in Table 1. The mean body weight of rats that received the two doses of *A. malaccensis* extract was not significantly different from the control group. The rats treated with the *A. malaccensis* extract at a dosage of 2000 mg/kg bw had liver weights significantly ($p < 0.05$) higher than control liver weight.

3.7. Biochemical Responses. Data on serum biochemical parameters of the rats are shown in Table 2. No significant difference ($p > 0.05$) in serum biochemical parameters was observed between rats treated *A. malaccensis* and control. Packed cell volume percentage (PCV%) was not significantly different between treated and control groups. Serum AST (u/L) and ALT (u/L) levels were not different between groups.

3.8. Hematological Responses. Data with regard to hematological parameters of rats are presented in Table 3. No significant difference ($p > 0.05$) was observed between treated and control groups.

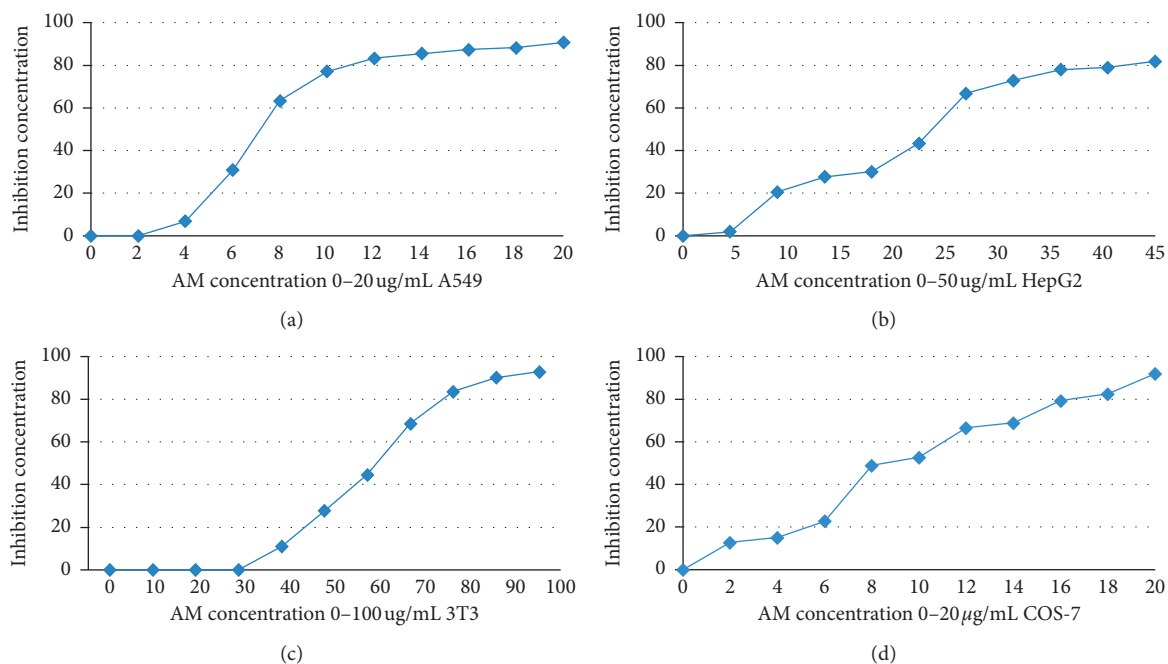


FIGURE 1: Dose response curve of *A. malaccensis* extract against different cell lines.

3.9. Histopathological Observations of Organs. The results of histopathological examination of liver sections of rats treated with olive oil (control) and different doses of the extract are shown in Figure 5. The liver tissue displayed normal hepatic cords and portal vein (Figure 5(a)) in the control groups. Liver tissue of rats administered the extract at 300 mg/kg did not show any alteration (Figure 5(b)), while the tissues of rats treated with 2000 mg/kg body weight showed mild congestion (Figure 5(c)).

The results of histopathological examination of kidney sections are shown in Figure 6. The kidney tissues presented intact tubules in control rats (Figure 6(a)). Rats administered the extract at 300 mg/kg body weight showed normal glomeruli and tubules (Figure 6(b)), while those in the 2000 mg/kg group showed glomeruli with mild lymphocytic infiltration (Figure 6(c)).

The histopathological examination of heart muscle sections of control rats revealed no pathological changes (Figure 7(a)). The rats in extract treatment at 300 mg/kg group also did not show any abnormality (Figure 7(b)), while those in the 2000 mg/kg group showed mild congestion (Figure 7(c)).

Lung sections of control rats are shown in Figure 8(a). The rats administered 300 and 2000 mg/kg extract did not show any perceivable microscopic changes (Figure 8(b)).

4. Discussion

Evaluation of toxicity, if any, of *A. malaccensis* *n*-hexane extract is the principal objective of this research since there is little scientific and clinical data on the effectiveness and the safety of *A. malaccensis* rhizome extract. Single oral dose administration of 2000 mg/kg body weight of crude *A. malaccensis* *n*-hexane extract did not induce mortality or

clinical signs of toxicity in rats throughout the observation period of 14 days. This indicated that the LD₅₀ value of *A. malaccensis* extract is greater than 2000 mg/kg body weight. Although all treated rats showed normal behavior during 24 h, some animals showed sign of mild distress during the first 4 h and drank too much water during the first 3 h after oral administration of the extract. According to Teo et al. [23], changes in the body weight can be considered as markers of adverse effects upon oral administration of drugs and chemicals. More than 10% body weight loss from the initial body weight is considered as significant ($p > 0.05\%$) [24]. In our study, none of the animals showed such reduction in body weight, but their body weights increased during the observation period indicating that the plant extract did not produce any adverse effect on the body weight of rats in terms of acute oral toxicity. Similar observations were reported by Karunarathne et al. [13] when they administered 2000 mg/kg body weight of crude *Alpinia galanga* *n*-hexane extract on rats, which is a plant extract of the same genus having 1'ACA as the major chemical compound. Furthermore, no change of organs was observed in the treated rat with the exception being the liver of rats treated with 2000 mg/kg bw day of *A. malaccensis* crude extract. Assessment of liver and kidney function is a very vital index in evaluating the toxicity of the plant extracts. The serum urea, creatinine, and liver enzyme (ALT, AST, and ALP) concentrations of the blood were the indices used to evaluate the function of kidney [25]. The results in respect of BUN, creatinine, ALT, and AST after 14 days of oral administration were not significantly different from the control group and revealed that there was no abnormality of the kidney for all the treated rats. Hematopoietic system is one of the most susceptible targets of toxic compounds, especially the bone marrow where the production of red blood cell occurs [26]. Acute administration of the plant

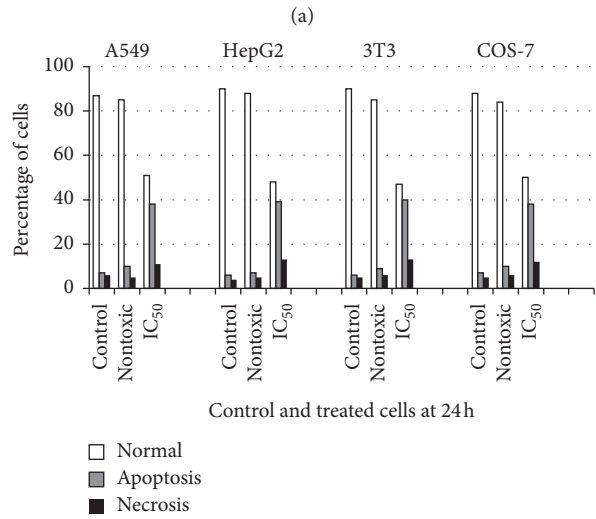
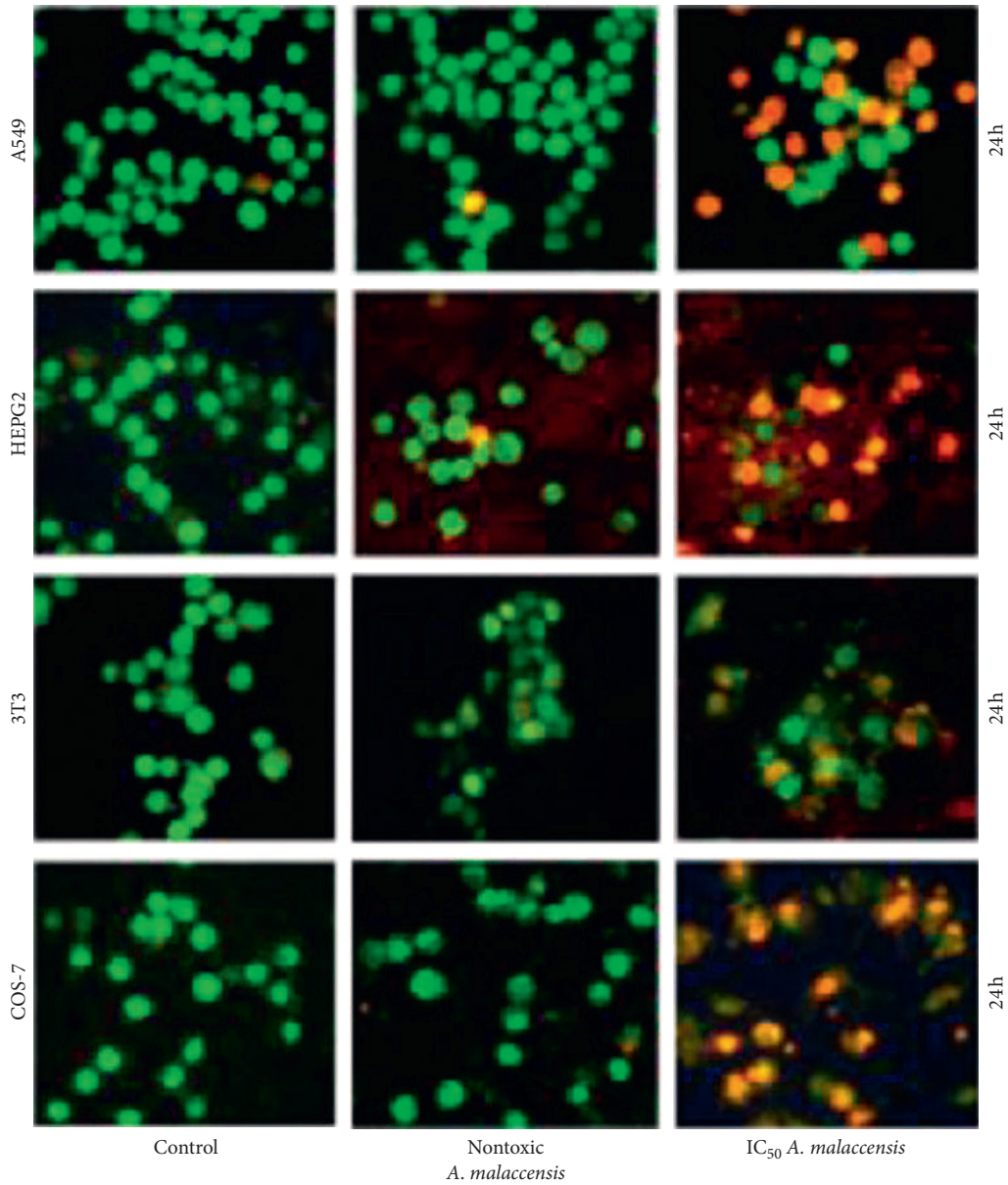
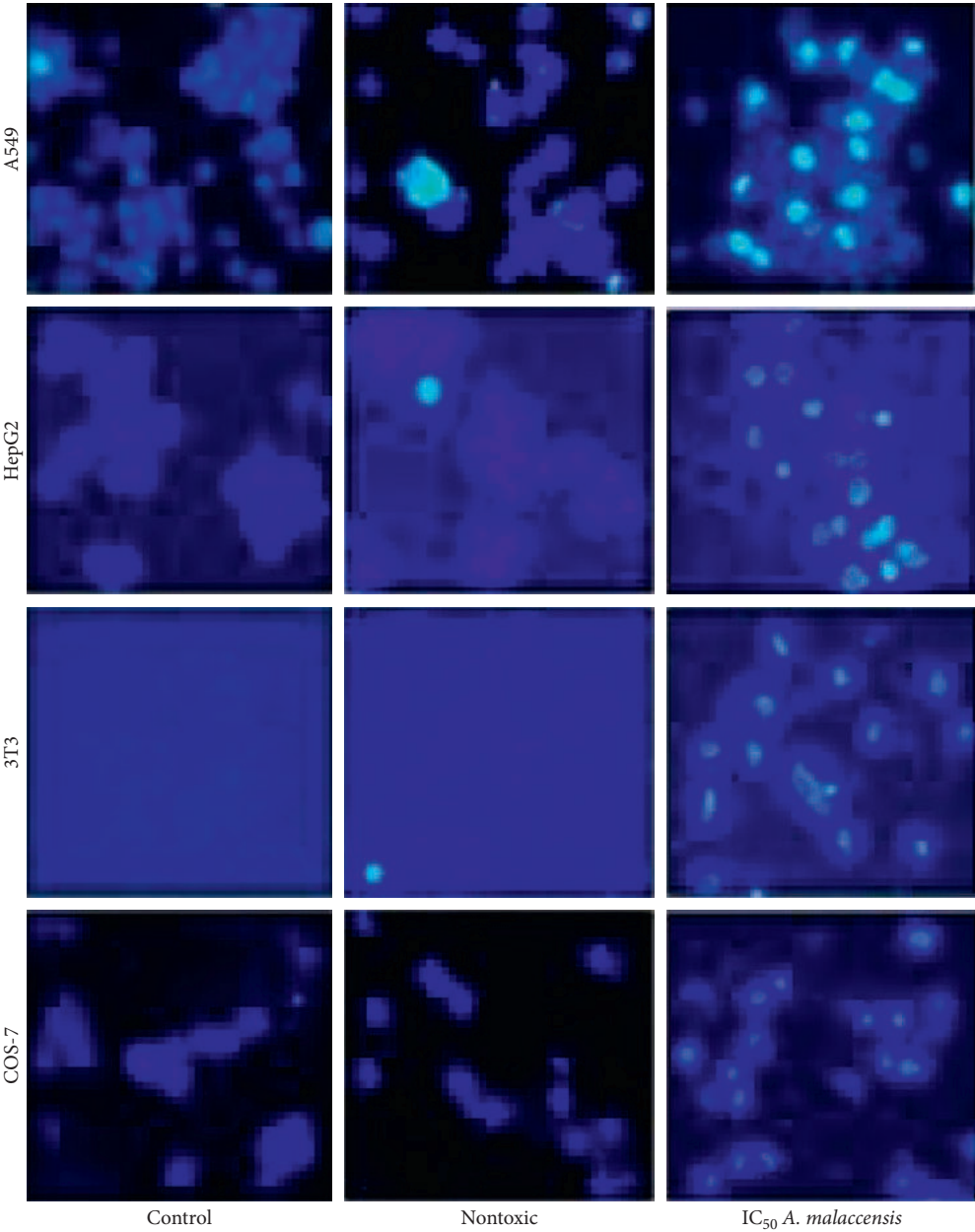


FIGURE 2: Assessment of apoptosis and necrosis. (a) Cells exposed to *A. malaccensis* extract and observed adopting AO/EB staining. Control cells are viable and fluoresce uniformly in green; cells in apoptosis fluoresce from yellow to orange; necrotic cells are swollen and fluoresce in bright red. (b) Percentage of normal, apoptotic, and necrotic cells. Data are expressed as the average of three independent observations.



(a)
FIGURE 3: Continued.

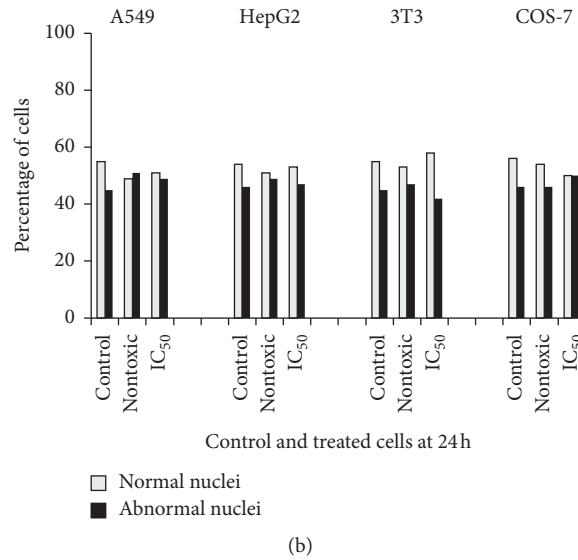


FIGURE 3: (a) Nuclear features of cells as revealed in Hoechst staining. (b) Percentage of cells with normal and abnormal nuclei. Data are expressed as average of three independent observations.

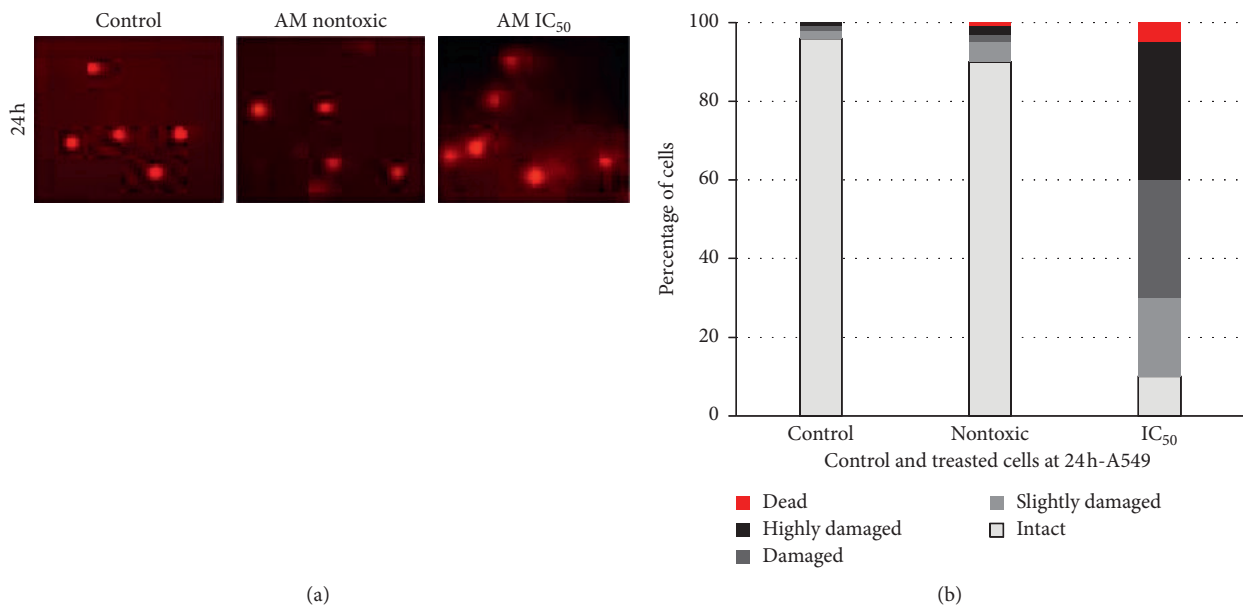


FIGURE 4: Assessment of DNA damage in A549 cells adopting comet assay. Data for the other cells are not shown since all showed similar trend. (a) DNA damage induced by *A. malaccensis* extract. Treated cells in which DNA has undergone strand breaks look like comets. (b) DNA damage as defined according to the DNA in the tail. The multiple parts of each column (from the bottom to the top): intact (0–20%), slightly damaged (20–40%), damaged (40–60%), highly damaged (60–80%), and dead (80–100%). Data are expressed as average of three independent experiments.

TABLE 1: Organ weights (g) of female rats.

Organ	Weight in grams	<i>A. malaccensis</i> extract (mg/kg)	
	Control	300	2000
Liver	6.05 ± 0.40 ^b	6.09 ± 0.42 ^b	7.12 ± 0.18 ^a
Left kidney	0.56 ± 0.06	0.53 ± 0.01	0.67 ± 0.06
Right kidney	0.57 ± 0.09	0.54 ± 0.38	0.66 ± 0.03
Lungs	0.98 ± 0.08	1.00 ± 0.05	1.16 ± 0.05
Heart	0.68 ± 0.04	0.68 ± 0.04	0.75 ± 0.04
Spleen	0.44 ± 0.04	0.42 ± 0.02	0.50 ± 0.02

Data with different lower case letters are significantly different ($p < 0.05$).

TABLE 2: Serum biochemical parameters of female rats.

Biochemical parameter	<i>A. malaccensis</i> extract (mg/kg)		
	Control	300	2000
PCV (%)	43.00 ± 1.41*	43.66 ± 0.47	43.66 ± 0.47
ALT(u/L)	34.33 ± 2.50	31.06 ± 1.55	28.40 ± 5.39
AST (u/L)	117.00 ± 2.08	117.00 ± 0.00	119.65 ± 1.05
BUN (urea/mg)	15.53 ± 2.69	15.23 ± 2.20	16.03 ± 1.10
Creatinine (mg/dL)	0.63 ± 0.09	0.93 ± 0.09	0.87 ± 0.12

*Results are expressed as mean ± SEM of 3 samples.

TABLE 3: Serum hematological values of female rats.

Hematological parameter	Control	<i>A. malaccensis</i> extract	
		300 mg/kg	2000 mg/kg
Hb (g/dL)	12.60 ± 0.14*	14.00 ± 0.84	13.13 ± 0.71
WBC (×10 ³ mm)	9.00 ± 0.71	11.20 ± 1.57	8.66 ± 0.30
RBC (10 ⁶ /L)	6.78 ± 0.89	7.20 ± 0.21	6.53 ± 0.41
Neutrophils (10 ⁹ /L)	16.33 ± 0.76	15.66 ± 5.43	13.00 ± 0.81
Lymphocytes (10 ⁹ /L)	81.33 ± 8.99	82.66 ± 5.55	83.66 ± 0.41
Erythrocytes (10 ⁹ /L)	0.33 ± 0.47	0.33 ± 0.47	1.00 ± 0.81
Monocytes (10 ⁹ /L)	2.00 ± 0.82	1.33 ± 0.47	2.33 ± 0.47

*Results are expressed as mean ± SEM of 3 samples.

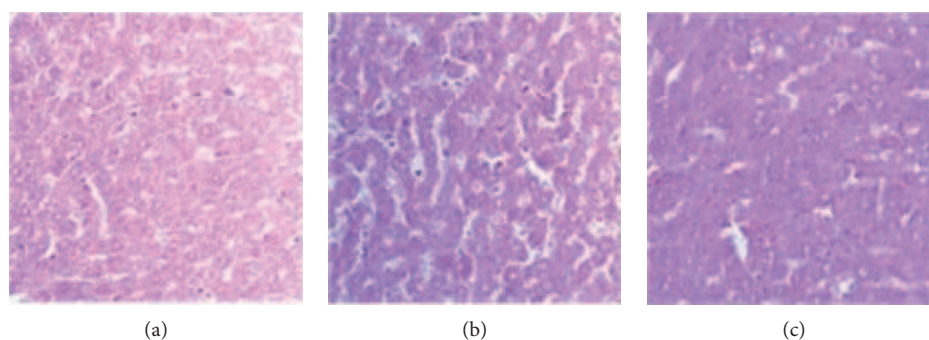


FIGURE 5: Photomicrographs of liver sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.

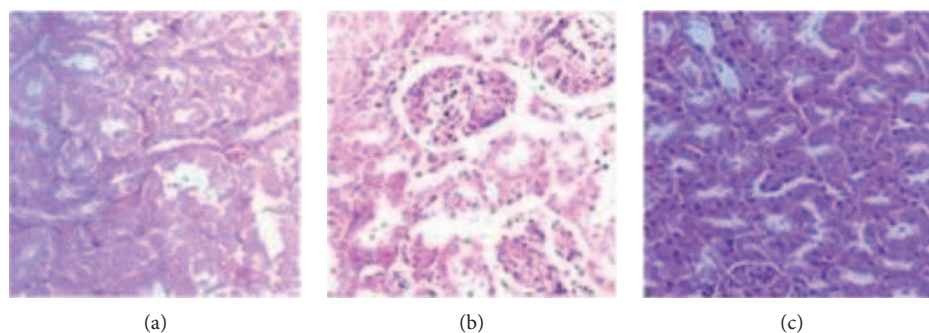


FIGURE 6: Photomicrographs of the kidney sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.

extract did not cause any significant changes in the hematological profile of rats which received the *A. malaccensis* extract at different doses, suggesting that the plant extract does not affect the hematopoietic system. The treatment did not bring about any serious histopathological change in the

liver, kidney, heart, and spleen. Therefore, it is to be inferred that the plant extract, at least up to 2000 mg/kg, is not toxic in respect to acute oral administration. However, before a final decision, subacute and chronic toxicity testing of *A. malaccensis* will be highly relevant.

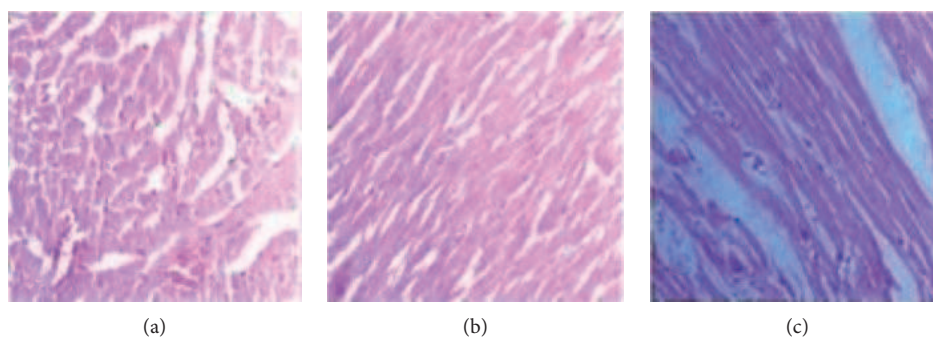


FIGURE 7: Photomicrographs of the heart sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm .

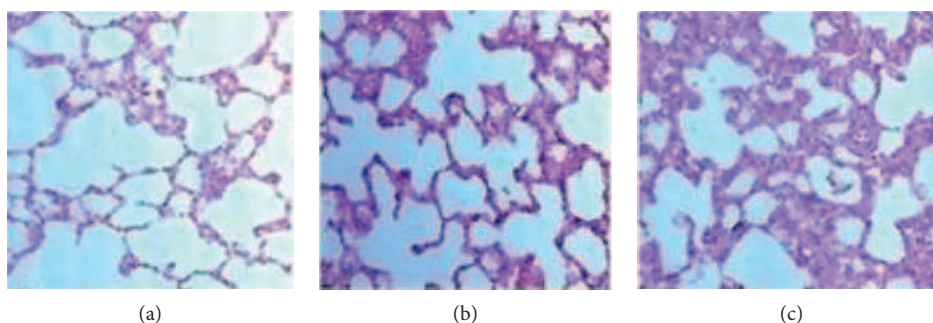


FIGURE 8: Photomicrographs of the lung sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm .

In vitro studies are equally important in deciphering if a material is toxic or not. Different cell lines might exhibit different sensitivities towards a compound if it is cytotoxic. Therefore, use of more than one cell line is considered necessary in the detection of cytotoxic effects [27]. So, we found the IC_{50} values of *A. malaccensis* *n*-hexane extract for A549, HepG2, 3T3, and COS-7 cell lines which were 7.25, 22.5, 62.75, and 8.25 $\mu\text{g}/\text{mL}$, respectively, and the zero-toxic concentration (IC_0) was 2, 1.4, 30, and 1.4 $\mu\text{g}/\text{mL}$, respectively. This is important since so far no toxicity data have been reported in literature for *A. malaccensis* [28].

Further, the mode of cell death, whether apoptosis or necrosis, induced by the plant extract was assessed. Double staining of AO/EB for nontoxic concentration of *A. malaccensis* showed nuclear and morphological changes of apoptosis only in less than 10% of the cells. However, more than 10% apoptosis was observed for cells treated the IC_{50} concentration of *A. malaccensis* extract. Similar observations were made for Hoechst staining. However, comet assay revealed that 7.25 $\mu\text{g}/\text{mL}$ of *A. malaccensis* IC_{50} induced severe damage to DNA of A549 cell. In contrast, plant extracts at non-cytotoxic concentration(s) did not induce DNA damage in A549 cell. Thus, our results clearly demonstrated that *A. malaccensis* at nontoxic doses did not exert genotoxicity in A549 cells. In fact, the extract at these nontoxic concentrations could be used as preservative of food stored for human consumption without any adverse effect on human health. Thus, considering the nontoxic concentrations, acceptable daily intake (ADI) of the extract could be

approximately calculated using NOAEC (no-observed adverse effect concentration) divided by uncertainty factor (usually 10 to extrapolate from animal to human and 10 for interindividual differences in sensitivity). NOAEC is defined as the concentration at which there is no risk (nontoxic level) [29]. The approximate ADI value for *A. malaccensis* *n*-hexane extract is 55.41 mg/day (data not shown). However, bio-availability of the extract(s), animal data, or PBPK (physiologically based biokinetic model) is necessary to derive an appropriate ADI for humans. Therefore, we recommend further investigations on subchronic and chronic toxicity testing to find the reliability of the estimated ADI of the plant extract when used as food preservative and/or therapeutic. Put together, the most nonpolar (*n*-hexane) extract of *A. malaccensis* offers great potential as a food preservative as well as therapeutic which could be further established through clearly directed studies.

5. Conclusion

The oral toxicity studies in the rat model revealed that *A. malaccensis* *n*-hexane extract at a single dose of 2000 mg/kg body weight did not produce any serious adverse effect with respect to general behavior, body weight, feed intake, biochemical parameters, and organ histology. In addition, cytotoxicity analysis showed that nontoxic concentration of *A. malaccensis* (2, 1.4, 30, and 1.4 $\mu\text{g}/\text{mL}$, respectively) does not affect cell viability or the DNA. Therefore, these concentrations may be practiced for human consumption

without any adverse health effect. However, we recommend subchronic and chronic toxicity tests to further exonerate the adverse effects by repeated administration of *A. malaccensis*. Further, it is possible that higher doses of *A. malaccensis* extract would potentially damage DNA, and therefore, we recommend further research towards standardization of *A. malaccensis* as preservative of food meant for human consumption and/or as a therapeutic.

Data Availability

The data of organ weights, serum biochemical parameters, serum hematological values of female rats, *in vitro* inhibition concentration of *A. malaccensis* on different cell lines, morphological assessment on apoptosis and necrosis, nuclear features of cells, assessment of DNA damage, and photomicrographs of internal organ sections used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

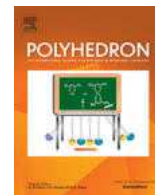
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Identification of cytotoxic copper(II) complexes with phenanthroline and quinoline, quinoxaline or quinazoline-derived mixed ligands

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ABSTRACT

A series of mixed ligand copper(II) complexes, formulated as $[\text{Cu}(\text{L1-L5})(\text{phen})(\text{H}_2\text{O})](\text{ClO}_4)_2$ (**1-5**), where phen = 1,10-phenanthroline, L1 = 2-pyridin-2-yl-quinoline, L2 = 2-pyridin-2-yl-quinoxaline, L3 = 6,7-dimethyl-2-pyridin-2-yl-quinoxaline, L4 = 4-phenyl-2-pyridin-2-yl-quinoline, and L5 = 4-phenyl-2-pyridin-2-yl-quinazoline, were synthesized and characterized. The molecular structure of **3**, which alone formed into appreciable crystals, was determined by single-crystal X-ray studies, and the coordination geometry around Cu(II) was nearly square pyramidal (τ , 0.092). DNA and protein binding, DNA cleavage and *in vitro* cytotoxicity of the mixed ligand complexes **1-5** were investigated and compared with their analogue bis-complexes $[\text{Cu}(\text{L1-L5})_2\text{H}_2\text{O}](\text{ClO}_4)_2$ **6-10**. All five mixed ligand complexes exhibited efficient DNA and protein binding, wherein **5** was the most potent. DNA cleavage studies revealed that all mixed ligand complexes engage in self-activated DNA cleavage, with **2** producing full conversion of supercoiled DNA to nicked circular form. Complex **5**, with the highest DNA- and protein-binding efficiencies, demonstrated the highest cytotoxicity to A549 non-small human lung carcinoma cell ($\text{IC}_{50} = 3.85 \mu\text{M}$), three times more potent than cisplatin. Metal-assisted reactive oxygen species (ROS) were found to be responsible for cytotoxicity of the complexes. Fluorescent staining assays showed that all complexes induce apoptotic cell death along with some degree of necrosis. Western blot analysis of caspase-3 expression of cells exposed to Cu(II) complexes **1** and **5** revealed that both promote apoptosis, with **5** demonstrating more potency. Thus, the mixed ligand copper complexes demonstrated efficient biological activity compared to bis-complexes, with **5** holding promise for future investigation towards development as a cancer therapeutic.

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1. Introduction

Cancer is a deadly disease that affects the population at global scale [1]. While organic drugs have been most widely used, the discovery of metal-based anticancer drug cisplatin [2] has shown that inorganic compounds can also be reliable anti-cancer drugs.

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Although cisplatin is used to treat certain types of cancers [3–5], it produces side effects and has limited selectivity for cancers [6,7]. To overcome these drawbacks, scientists have put intensive effort to identify appropriate alternative non-platinum metallic anticancer drugs [8]. Among the several putative non-platinum metals copper, an essential metal, has received considerable attention because it plays a key role in endogenous biological systems [9]. Some experimental results also indicate that metabolism of copper is severely affected by the neoplastic disease [10]. Numerous anticancer agents approved for clinical use are molecules that damage deoxyribonucleic acid (DNA) or block DNA synthesis [11]. In addition to DNA, proteins are also important molecular targets for anticancer agents. Recent literature reports have shown that cytotoxicity of most of the copper(II) complexes originates by

way of formation of reactive oxygen species (ROS) through Cu(II)/Cu(I) redox process [12–17]. Thus, there is motivation to design and synthesize copper(II) complexes that can bind to DNA and proteins, cleave DNA, and raise the level of cellular ROS as cytotoxic agents [8,18–24].

Numerous ternary copper(II) mixed chelates have been identified as cytotoxic agents [25–28]. Recently, our group reported several mixed ligand copper(II) complexes containing 1-proline and diimines that bind and cleave DNA/protein and induce cell death via ROS [29]. We found that one of the complexes [Cu(L-pro)(5,6-dimethyl-1,10-phenanthroline)]⁺ in the series was involved in production of the highest level of cellular ROS, oxidative DNA cleavage, and self-activated protein cleavage. This complex induced higher cytotoxicity than cisplatin.

Although various ligands are used to tune the copper centre to become biologically active, the heterocyclic derivatives such as quinoline, quinoxaline, and quinazoline warrant special consideration. The pharmacological importance of these heterocyclic derivatives has motivated many scientists to develop novel ligands and corresponding metal complexes for anti-cancer applications [30]. Numerous quinoline derivatives and their metal complexes have been attributed with anticancer property [31–35]. Also, biological applications and anti-cancer activities have been demonstrated for quinoxaline compounds and their copper complexes [36–39]. Mitsopoulou and colleagues recently tested the DNA binding prop-

erty and cytotoxicity of two mono- and bis-Cu(II)-2-(2'-pyridyl) quinoxaline complexes and concluded that the bis-complex was more cytotoxic than the mono-complex and cisplatin [40]. Likewise, several quinazoline compounds have also been identified as effective anti-proliferative agents [41–43]. However, mixed ligand complexes with these heterocyclic compounds have not yet been explored for anticancer activity. Recently, Velusamy and his co-workers have synthesized and characterized some of the quinoline- (**L1** and **L4**), quinoxaline- (**L2** and **L3**), and quinazoline-based (**L5**) ligands (where **L1** = 2-pyridin-2-yl-quinoline, **L2** = 2-pyridin-2-yl-quinoxaline, **L3** = 6,7-dimethyl-2-pyridin-2-yl-quinoxaline, **L4** = 4-phenyl-2-pyridin-2-yl-quinoline, and **L5** = 4-phenyl-2-pyridin-2-yl-quinazoline), and isolated bis-copper(II) complexes of the type [Cu(L1-L5)₂(H₂O)](ClO₄)₂ (**6–10**) (Scheme 1). Such complexes have been used for simultaneous fixation and sequestration of atmospheric carbon dioxide under mild conditions [44].

In the present research, we focussed on preparation of mixed ligand copper(II) complexes containing ligands **L1–L5**, as described above, and 1,10-phenanthroline (phen), together formulated as [Cu(L1-L5)(phen)(H₂O)](ClO₄)₂ (**1–5**). DNA and protein binding, DNA cleavage efficiency and *in vitro* cytotoxicity were investigated and compared to the corresponding bis-analogues [Cu(L1-L5)₂(H₂O)](ClO₄)₂ **6–10**. The DNA binding and cleavage properties of the complexes were observed by allowing the complexes to interact with commercially available calf thymus (CT) DNA and supercoiled plasmid DNA pUC19, respectively. The protein binding efficiency of the complexes was ascertained using BSA. All complexes **1–10** were tested for cytotoxicity on non-small human lung carcinoma cell A549. Interestingly, all the mixed ligand complexes **1–5** displayed higher cytotoxicity, through ROS production, than the corresponding bis-complexes (**6–10**). Among the five mixed ligand complexes, the complex **5** that bound most efficiently with DNA and protein displayed the highest cytotoxicity compared to the other complexes, and the order of efficacy was **5** > **1** > **2** > **3** > **4**. Also, **1** and **5** which alone were subjected to western blot analysis caused increased expression of caspase-3 wherein the performance of **5** was much better.

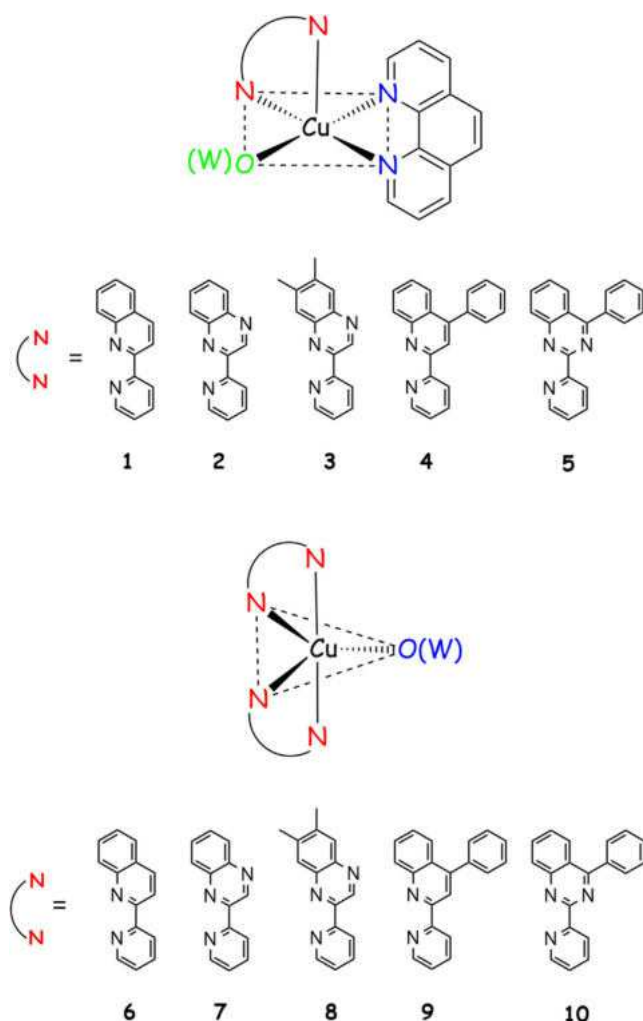
2. Experimental

2.1. Materials

All the analytical grade reagents and chemicals were used as purchased, without any further purification. Copper(II) perchlorate hexahydrate was purchased from Alfa Aesar (MA, USA). 1,10-phenanthroline (phen), ethidium bromide (EthBr) and agarose (molecular biology grade) were purchased from HiMedia (Mumbai, India). Calf thymus (CT) DNA (highly polymerized; stored at 4 °C) was purchased from Sigma-Aldrich (MO, USA). Bovine Serum Albumin (BSA) and pUC19 supercoiled DNA (caesium chloride purified) were obtained from Genei (Bangalore, India). All the commercial grade solvents were distilled by standard procedures [45] and used for preparation of the ligands and complexes. Ultrapure water (18.2 μΩ) was used for the preparation of all buffers and in biological experiments.

2.2. Experimental methods

UV-visible spectra of all complexes were obtained in UV-2450 Shimadzu (Koyoto, Japan) spectrophotometer using a quartz cuvette at 1 cm length. Fluorescence emission titration was performed using G9800A Cary Eclipse fluorescence spectrophotometer of Agilent Technologies (CA, USA). Thermo Fisher (MA, USA) Q-Exactive Plus-Quadrupole-Orbitrap Mass Spectrometer was used for the HR-MS analysis. Elemental analysis was performed on a 2400



Scheme 1. Schematic representation of the ligands and their copper(II) complexes **1–5** and **6–10** [44].

CHN Perkin-Elmer (MA, USA) analyser. The viscosity of the DNA solution was measured in an Ubbelohde viscometer (Cannon, PA, USA) maintained at a steady temperature of 20 °C in a thermostatic water bath. The DNA cleavage experiment was performed using agarose gel electrophoresis system of Genei (Bangalore, India). The cleaved DNA fragments were visualized by Vilber Lourmat gel documentation system (Marne-la-Vallee Cedex, France). For anticancer studies, a CO₂ incubator (Heraeus, Hanau, Germany) and an ELISA plate reader (Biotek Instruments, Inc., VT, USA) were used. ROS measurement was made using TECAN (Männedorf, Switzerland) fluorescent microplate reader.

2.3. Synthesis of mixed ligand copper(II) complexes

During handling of the perchlorate salts of metal complexes with organic ligands, extreme care was taken to avoid the possible explosion.

2.3.1. Synthesis of [Cu(L1)(phen)(H₂O)](ClO₄)₂ (**1**)

A solution of ligand L1 (0.21 g, 1.0 mmol) and phen (0.18 g, 1.0 mmol) in methanol was added drop by drop to a stirred solution of Cu(ClO₄)₂·6H₂O (0.19 g, 0.5 mmol) in the same solvent. The resultant mixture was stirred for 3 h, when a green precipitate formed. This was filtered, washed with cold methanol and then dried under vacuum (Yield: 80%). Anal Calcd for C₂₆H₂₀Cl₂CuN₄O₉: C: 46.82%, H: 3.02%, N: 8.40%. Found: C: 46.65%, H: 3.14%, N: 8.64%. HR-MS (CH₃OH), positive mode, (*m/z*): C₂₆H₁₈CuN₄ [M]²⁺, calcd: 224.541; found: 224.540.

2.3.2. Synthesis of [Cu(L2)(phen)(H₂O)](ClO₄)₂ (**2**)

Complex **2** was prepared by adopting the same procedure as above but used L2 (0.21 g, 1.0 mmol) instead of L1, and isolated as a green solid with the yield of 89%. Anal Calcd for C₂₅H₁₉Cl₂CuN₅O₉: C: 44.96%, H: 2.87%, N: 10.49%. Found: C: 45.09%, H: 2.76%, N: 10.35%. HR-MS (CH₃OH), positive mode, (*m/z*): C₂₅H₁₇CuN₅ [M]²⁺, calcd: 225.039; found: 225.038.

2.3.3. Synthesis of [Cu(L3)(phen)(H₂O)](ClO₄)₂ (**3**)

Complex **3** was prepared by adopting the same procedure as for **1** but used L3 (0.24 g, 1.0 mmol) instead of L1. After the resulting clear solution was slowly evaporated, brown needle-shaped **3** crystals, suitable for X-ray diffraction, were obtained with a yield of 85%. Anal Calcd for C₂₇H₂₃Cl₂CuN₅O₉: C: 46.60%, H: 3.33%, N: 10.06%. Found: C: 46.49%, H: 3.21%, N: 9.89%. HR-MS (CH₃OH), positive mode, (*m/z*): C₂₇H₂₁CuN₅ [M]²⁺, calcd: 239.054; found: 239.054.

2.3.4. Synthesis of [Cu(L4)(phen)(H₂O)](ClO₄)₂ (**4**)

Complex **4** was prepared by adopting the same procedure as for **1** but used L4 (0.28 g, 1.0 mmol) instead of L1 and isolated as a yellow solid with the yield of 78%. Anal Calcd for C₃₂H₂₄Cl₂CuN₄O₉: C: 51.73%, H: 3.26%, N: 7.54%. Found: C: 51.96%, H: 3.34%, N: 7.45%. HR-MS (CH₃OH), (*m/z*): C₃₂H₂₂CuN₄ [M]²⁺, calcd: 262.557; found: 262.556.

2.3.5. Synthesis of [Cu(L5)(phen)(H₂O)](ClO₄)₂ (**5**)

Complex **5** was prepared by adopting the same procedure as for **1** but used L5 (0.28 g, 1.0 mmol) instead of L1 and isolated as a dark green solid with the yield of 84%. Anal Calcd for C₃₁H₂₃Cl₂CuN₅O₉: C: 50.04%, H: 3.12%, N: 9.41%. Found: C: 49.89%, H: 3.05%, N: 9.30%. HR-MS (CH₃OH), positive mode, (*m/z*): C₃₁H₂₁CuN₅ [M]²⁺, calcd: 263.054; found: 263.054.

2.4. Crystallographic analysis

This was limited to complex **3**. A fine needle-shaped crystal of the complex was mounted on glass fibres for data collection. Data were collected on an Oxford Diffraction Xcalibur Eos Gemini Diffractometer (Malvern Panalytical, Malvern, UK) at ambient temperature using graphite monochromated Mo-K α radiation ($\lambda = 0.7107 \text{ \AA}$), solved using direct methods with SHELXS software and refined using SHELXL-2013. The graphic interface package PLATON was used, and the figures were generated using ORTEP 3.07 generation package. The positions on all the atoms in a complex were obtained by SHELXS software [46]. Metal atoms in the complex were located from the E-maps, and non-hydrogen atoms were refined anisotropically. The hydrogen atoms bound to the carbon were refined by adopting an already reported method [47]. The crystallographic data and details of data collection for the complex are given in Table S2.

2.5. In vitro DNA binding and cleavage experiments

All the metal complexes are partially soluble in water but completely soluble in 10% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 (10% DMF-Tris-HCl buffer). Hence, to perform DNA binding and cleavage studies, concentrated stock solutions of metal complexes were prepared by dissolving them in 10% DMF-Tris-HCl buffer and diluting them with the corresponding buffer for all experiments. Preparation of CT-DNA stock solution and determination of its concentration were carried out in accordance with the procedure already reported [48].

2.5.1. Ethidium bromide displacement assay

In a typical binding experiment, to 2 mL solution of EthBr 125 μM CT-DNA solution was added to get the maximum fluorescence intensity of EthBr (excitation at 450 nm; emission range 500–600 nm) in 10% DMF-Tris-HCl buffer. Subsequently, 10 μM aliquots of solutions of the complexes 1–10, which are not autofluorescent (Fig. S1), in 10% DMF-Tris-HCl buffer, were added to the EthBr-bound CT-DNA solution. Thereupon, the emission intensity decreased based on the ability of metal complexes to break the EthBr-DNA bond. The values of the apparent DNA-binding constant (K_{app}) were determined from the plot of these intensities against concentration of the complexes [49], using the equation

$$K_{\text{EthBr}}[\text{EthBr}] = K_{\text{app}}[\text{Complex}]$$

where K_{EthBr} is $4.94 \times 10^5 \text{ M}^{-1}$ [50], the concentration of EthBr is 12.5 μM , and the concentration of the complex is that used to achieve a 50% reduction in the fluorescence strength of DNA-bound EthBr.

2.5.2. Viscosity experiments

To find the nature of molecular interaction of the complexes with the CT-DNA, the change in viscosity of DNA was measured by Ubbelohde viscometer by maintaining a constant temperature at 20 °C. The complex was added gradually in increasing concentration from the 1/R 0 to 0.5 to 100 μM of DNA stock solution present in 10% DMF-Tris-HCl buffer and the viscosity was measured for each addition. The mixture was equilibrated for 5 min before each addition. The data thus obtained were plotted as $(\eta/\eta_0)^{1/3}$ versus 1/R, where η and η_0 are the viscosity of DNA in the presence of the complex and the viscosity of DNA alone in buffer solution, respectively.

2.5.3. Agarose gel electrophoresis

The interaction of complexes **1–10** with supercoiled pUC19 DNA was monitored using agarose gel electrophoresis. The plasmid

DNA (SC form, 40 μM) in 10% DMF-Tris-HCl buffer was treated with copper complexes prepared in the same buffer. In each experiment the plasmid DNA was treated with the complexes and the cleavage of plasmid DNA in the absence and presence of an activator and a reductant, separately, was observed and monitored using agarose gel electrophoresis. The samples were then analysed for the cleaved products using gel electrophoresis. $1 \times$ Tris-Acetate-EDTA (TAE with 40 mM Tris base, 20 mM acetic acid, 1 mM EDTA) buffer and 1% agarose gel containing $1.0 \mu\text{g mL}^{-1}$ ethidium bromide were used. The gels were viewed in a Gel Documentation System (Vilber Lourmat, France) and photographed using a CCD camera (Alpha InfoTech Corp). The observed less intense plasmid DNA was corrected by a factor of 1.47 in view of the lower capacity to bind ethidium bromide [51]. The plasmid DNA cleavage efficiency was measured by densitometric calculation using ImageJ software which determines the ability of the complexes to convert supercoiled DNA form (I) to nicked circular form (II) and linear form (III).

2.6. BSA binding experiments

The protein binding study was performed by tryptophan fluorescence quenching experiments using bovine serum albumin (BSA, 5 μM) as the substrate in phosphate buffer (pH 7.1). Quenching of the emission intensity of tryptophan residues of BSA at 344 nm (excitation wavelength at 295 nm) was monitored using complexes **1–10** as quenchers with increasing complex concentration. The plot of I_0/I vs. [complex] was constructed using the corrected fluorescence data taking into account the effect of dilution. The excitation and emission slit widths were 5 and 5, respectively. Fluorescence measurements were performed using a 1 cm quartz cell on a Fluorescence Spectrophotometer (Agilent Technologies). Stern-Volmer quenching constant K_{sv} was obtained from the following equation [52]:

$$I_0/I = 1 + K_{sv}[Q]$$

where I_0 and I are the BSA fluorescence intensities in the absence and presence of the complexes, and K_{sv} is the Stern-Volmer quenching constant.

2.7. Cell culture

The A549 lung cancer cell was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in RPMI 1640 medium (Biochrom AG, Berlin, Germany) loaded with 10% fetal bovine serum (Sigma-Aldrich, MO, USA), mitomycin C (Sigma-Aldrich, MO, USA), cisplatin (Getwell Pharmaceuticals, Gurugram, India) and antibiotics 100 U mL^{-1} penicillin and 100 $\mu\text{g mL}^{-1}$ streptomycin (HiMedia, Mumbai, India) in 96 well culture plates. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in a CO_2 incubator (Heraeus, Hanau, Germany). All experiments were performed using cells from passage 15 or less. The copper(II) complexes were dissolved in DMSO and diluted with appropriate quantities of the culture medium.

2.7.1. MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay [53] was employed to measure the cytotoxicity of the complexes. The copper complexes were dissolved in minimum quantity of 100% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA). The concentration range of complexes at 0–100 μM was prepared to final DMSO dilution of 0.02%, and added to the well, 24 h after seeding 5000 cells (A549) per well of 96-well plate. DMSO (0.02%) was used as the solvent control and cisplatin was used as the positive control. After 24 h incubation, 20 μL of MTT solution [5 mg mL^{-1} in phosphate-buffered saline (PBS)] was added to each well, and the plates were wrapped with aluminium

foil and incubated for 3 h at 37 °C. The purple formazan product was dissolved by addition of 100 μL of 100% DMSO to each well. The absorbance was examined at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, Hercules, California, USA). Data were collected for three replicates, each in triplicate, wherein the three average values were used to calculate the means and the standard deviations. The percentage of inhibition was calculated from this data using the following formula:

Percentage inhibition

$$= \frac{\text{Mean OD of untreated cells (control)} - \text{Mean OD of treated cells}}{\text{Mean OD of untreated cells (control)}} \times 100$$

From the values thus obtained, the IC_{50} values for 24 h treatment, for A549 cells, were deduced from the curves obtained by plotting percentage inhibition against concentration.

2.7.2. ROS assay

Untreated A549 cancer cells at 70–80% confluence were seeded at 0.1×10^6 in the wells of a 96-well culture plate and treated with 5 μM concentration of complexes **1–10** and incubated for 12 and 24 h. At the close of the experiment cells were rinsed with PBS. Then, the adherent cells were detached using accutase and centrifuged at 1000 rpm for 5 min. Each pellet was suspended in PBS containing 10 μM dichlorofluorescein diacetate (DCFH-DA) (Sigma) and then incubated for 30 min at 37 °C in the dark. After the removal of excess DCFH-DA, the pellets were washed three times with PBS. Finally, the cells were suspended in PBS. The DCF fluorescence was measured immediately at 485 nm excitation and 530 nm emission (fluorescein isothiocyanate filter) using TECAN fluorescent microplate reader. Data were analysed using Sigma Plot (SigmaPlot by Systat software, UK). The amount of ROS was determined as the mean fluorescence intensity. The measurements were repeated thrice. Mean values are shown in the figures, and standard errors are shown as error bars. Comparisons between treatments were analysed by One-way ANOVA using Sigma Plot. P values are labelled in the figures and data from experimental and control groups were compared where $P < 0.05$ was considered statistically significant.

2.7.3. Acridine orange (AO)/ethidium bromide (EthBr) fluorescent assay

AO/EthBr staining was conducted as described by Spector *et al.* [54]. The cultured A549 lung cancer cells were taken to a 6-well plate and treated with IC_{50} concentrations of the complexes for 24 h, when DMSO (0.02%) was used as solvent control. The treated and untreated cells (25 μL of suspension containing approx. 5000 cells) were incubated with acridine orange (AO) and ethidium bromide (EthBr) solutions (1 part of 100 $\mu\text{g/mL}$ each of AO/EthBr in PBS) and examined in a fluorescent microscope (Carl Zeiss, Jena, Germany) using a UV filter (450–490 nm). Three hundred cells per sample were counted, each in triplicate and scored as viable or dead and, if dead, whether by apoptosis or necrosis as judged from the nuclear morphology and cytoplasmic organization. The percentages of apoptotic and necrotic cells were then calculated. Morphological features of interest were photographed.

2.7.4. Annexin V-Cy3 staining

Cancer cells were cultured on cover slips and treated with IC_{50} concentrations of complexes **1–5** and incubated for 24 h. The cell pellet was harvested and washed with PBS and then with $1 \times$ binding buffer. The washed cell pellets were incubated in a solution containing 50 μL of double labelled Annexin-Cy3 and 6-CFDA [55] and kept in dark for 10 min. After 24 h incubation, the excess label was removed by washing the cells with $1 \times$ binding buffer. Approximately 300 cells were mounted to a fluorescent microscope and the labelled cells were counted. This assay reflected

the percentage of dead cells and it facilitated detection of live cells (green), necrotic cells (red), and apoptotic cells (red nuclei and green cytoplasm). Data were collected from duplicate experiments.

2.7.5. Western blot analysis

The MTT assay showed that among the Cu(II) complexes **1–10**, the IC₅₀ of **1** & **5** were the lowest, in the order **5** < **1**. To throw light on specific biochemical targets, cells exposed to **1** & **5** were subjected to caspase-3 assay adopting western blot. A549 cells were treated with the complexes **1** & **5** at the respective IC₅₀ concentrations. After 24 h incubation, cells were washed with ice-cold PBS and lysed in RIPA lysate buffer. The proteins were extracted by a modified Lowry's method [56], separated out in SDS-PAGE, and transferred to a nitrocellulose membrane. The membranes were blocked with 5% casein in Tris buffer for 90 min, washed with TBS/0.05% Tween-20 buffer and then incubated overnight at 4 °C with the primary antibody for caspase-3 (Genei, Bangalore, India) at 1:500 dilution. Thereafter, the membrane was washed four times with TBS/0.05% Tween-20 and incubated with the secondary antibody conjugated with peroxidase or alkaline phosphatase for 1 h. After extensive washing, the reaction product was developed with NBT-BCIP-alkaline phosphatase buffer.

3. Results and discussion

3.1. Properties of the copper(II) complexes in solution

The synthesis and characterization of L1–L5 and their bis-copper (II) complexes **6–10** have already been reported [44]. The ligands L1–L5 and bis-complexes **6–10** of the same batch were used for this work. The mixed ligand copper(II) complexes **1–5** were prepared by adding copper(II) perchlorate hexahydrate methanolic solution to a mixture of L1–L5 ligands and phen in methanol. With good yields, each complex was isolated. Formulae of the complexes **1–5** were determined by elemental analysis, further supported by a single crystal X-ray structure of **3**. The HR-MS data were recorded in methanol as shown in Fig. S2 which reveals that complexes **1–5** retain their structure even in solution, **1**: *m/z*, 224.540 for C₂₆H₁₈CuN₄ [M]²⁺; **2**: *m/z*, 225.038 for C₂₅H₁₇CuN₅ [M]²⁺; **3**: *m/z*, 239.054 for C₂₇H₂₁CuN₅ [M]²⁺; **4**: *m/z*, 262.556 for C₃₂H₂₂CuN₄ [M]²⁺ and **5**: *m/z*, 263.054 for C₃₁H₂₁CuN₅ [M]²⁺. The single crystals of **3** were isolated from methanol solution by gradual evaporation. The other complexes did not crystallize to good finish, and yielded only the polycrystalline solids. The **1–10** complexes are partly water-soluble but clearly soluble in 10% DMF-Tris-HCl buffer.

Typical five-coordinated square-pyramidal Cu(II) complexes are most likely to display a broad d-d transition in the visible region which may or may not be associated with a low-energy shoulder at $\lambda > 800$ nm [57]. The electronic absorption spectra of complexes **1–10** in 10% DMF-Tris-HCl buffer were recorded. The mixed ligand complexes **1–5** exhibited one low intensity absorption band in the visible region (661–708 nm) with a low-energy shoulder, as indicated above, which is typical of electronic metal-centred d-d transition of square-pyramidal Cu(II) complexes (Fig. S3, Table S1) [58]. Compared to mixed-ligand complexes, the bis-complexes **6–10** showed an identical pattern of a slightly low-energy d-d transition band (752–795 nm). This is due to incorporation of the planar aromatic phen ligand which exerts more ligand field strength around copper as a result of d-d band shifting to high-energy region. These complexes also showed a weak ligand-copper(II) charge transfer transition (LMCT) in the range 485–533 nm, and showed strong intra-ligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in the range 252–342 nm. In order to determine the stability of the complexes **1–10** in aqueous medium, UV–visible spectra were recorded at various time intervals of 0, 1, 6 and 24 h in 10% DMF-Tris-HCl buffer.

No major changes were observed in the UV–visible spectrum between 0 and 24 h for all the complexes. One representative spectrum is shown in Fig. S4 for complex **1**. This shows that all complexes **1–10** are stable in the buffer medium [59,60].

3.2. Crystal structure

3.2.1. Crystal structure of [Cu(L3)(phen)(H₂O)](ClO₄)₂ (**3**)

The molecular structure of complex **3**, with atom numbering, is depicted in Fig. 1. X-ray crystallographic data and selected bond distances and angles are given in Table S2 and Table 1. The complex [Cu(L3)(phen)(H₂O)](ClO₄)₂ (**3**) crystallized as monoclinic with *P*2₁/*n* space group. The coordination environment around the copper(II) centre can be described as a square pyramidal or trigonal bipyramidal geometry as defined by the angular structural index geometric parameter τ , using the equation $\tau = [(\beta - \alpha)/60^\circ] = 0.092$, where $\alpha = 168.41^\circ$, and $\beta = 173.98^\circ$. The τ value has been used to describe the degree of structural distortion from square pyramidal geometry with $\tau = 0$ to trigonal bipyramidal geometry, $\tau = 1$. The calculated τ value for complex **3** was found to be 0.092, which indicates the existence of a nearly square pyramidal geometry. The copper(II) centre is five-coordinated with the four corners of the basal plane occupied by two phenanthroline *N*-atoms (N4, N5), one pyridyl *N*-atom from L3 ligand (N1) and one oxygen atom (O1) of the water molecule. The apical position is

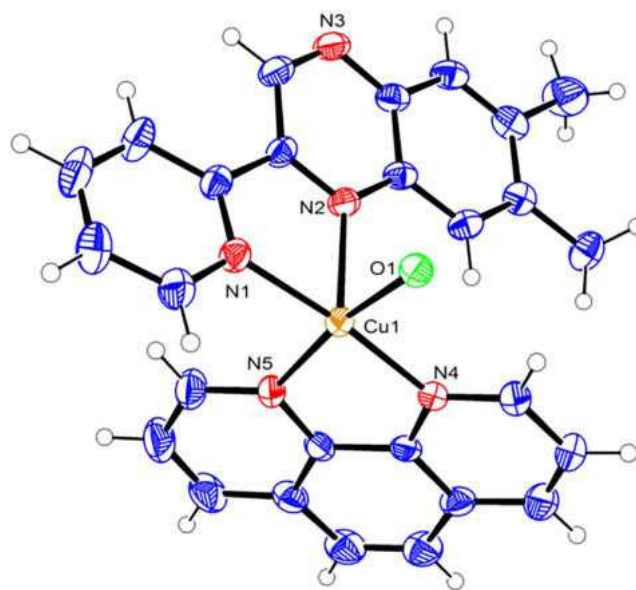


Fig. 1. ORTEP diagram of complex **3**. Thermal ellipsoids are drawn at 40% probability level, and ClO₄ anion is omitted for clarity.

Table 1
Selected inter-atomic distances (Å) and angles (°) for complex **3**.

Bond distances (Å)		Bond Angles (°)	
Cu(1)–N(1)	1.990(4)	N(1)–Cu(1)–N(5)	174.18(16)
Cu(1)–N(5)	1.994(4)	N(1)–Cu(1)–O(1)	92.17(16)
Cu(1)–O(1)	2.028(4)	N(5)–Cu(1)–O(1)	91.94(15)
Cu(1)–N(4)	2.027(4)	N(1)–Cu(1)–N(4)	92.43(17)
Cu(1)–N(2)	2.305(4)	N(5)–Cu(1)–N(4)	82.78(17)
		O(1)–Cu(1)–N(4)	168.40(16)
		N(1)–Cu(1)–N(2)	77.12(16)
		N(5)–Cu(1)–N(2)	106.67(16)
		O(1)–Cu(1)–N(2)	94.42(14)
		N(4)–Cu(1)–N(2)	96.98(16)

occupied by *N*-atom (N2) of the quinoxaline ring from ligand L3. The *N*-atom (N2) coordinates axially at a rather long-distance Cu-N2_{quinoxaline} (2.305(4) Å) presenting an elongated square pyramidal geometry as it was observed in previous reports. The bond angles N1-Cu1-N4 173.98(15)°, N5-Cu1-O1 168.41(15)° and N1-Cu1-N2 77.01(15)°, N5-Cu1-N2 97.04(15)°, and N4-Cu1-N2 106.75(14)° deviate from the ideal square pyramidal bond angles of 180° and 90° and, hence, slight distortion is observed in the Cu(II) coordination geometry.

3.3. DNA-binding studies

3.3.1. Ethidium bromide displacement assay

This assay is a simple approach for determining the binding efficiency of metal complexes with DNA. Complexes that are capable of replacing the EthBr intercalator from the DNA-bound EthBr adduct are considered strong DNA binding complexes [61]. Unbound EthBr has lesser fluorescence than when bound with DNA [61]. The binding of the metal complexes was ascertained based on the degree to which the metal complexes quenched EthBr emission (Fig. 2). Incremental addition of existing metal complexes **1–5** and **6–10** to DNA-bound EthBr ($R = [\text{DNA}]/[\text{EthBr}] = 10$) in 10% DMF-Tris-HCl buffer resulted in a decrease in the fluorescence of DNA-bound EthBr for both complex series (Fig. 2b and 2c). For phen-containing mixed ligand complexes, the measured K_{app} values were in the order $5 > 1 > 2 > 3 > 4$ (Fig. 2b and Table 2). Complex **5**, which contains quinazoline, displayed a higher binding affinity to DNA than the other complexes in the sequence. The higher reduction of emission intensity by **5** suggests that the moiety of quinazoline assisted the partial intercalation of phen ligand with DNA. Likewise, bis-complexes **6–10** showed decrease in emission intensity in the order $9 > 10 > 8 > 6 > 7$ (Fig. 2c), but these com-

Table 2

Fluorescence spectral properties of copper(II) complexes bound to CT-DNA and Stern-Volmer quenching constants for the interaction with BSA.

Complex	K_{app} ($\times 10^5 \text{ M}^{-1}$) ^a	K_{sv} ($\times 10^4 \text{ M}^{-1}$) ^b
[Cu(L1)(phen)(H ₂ O)] ²⁺ 1	3.76	0.123 ± 0.018
[Cu(L2)(phen)(H ₂ O)] ²⁺ 2	3.70	0.110 ± 0.002
[Cu(L3)(phen)(H ₂ O)] ²⁺ 3	3.63	0.109 ± 0.005
[Cu(L4)(phen)(H ₂ O)] ²⁺ 4	3.60	0.181 ± 0.08
[Cu(L5)(phen)(H ₂ O)] ²⁺ 5	4.18	0.245 ± 0.005
[Cu(L1) ₂ (H ₂ O)] ²⁺ 6	3.01	0.083 ± 0.041
[Cu(L2) ₂ (H ₂ O)] ²⁺ 7	2.89	0.072 ± 0.005
[Cu(L3) ₂ (H ₂ O)] ²⁺ 8	3.12	0.093 ± 0.009
[Cu(L4) ₂ (H ₂ O)] ²⁺ 9	3.27	0.096 ± 0.011
[Cu(L5) ₂ (H ₂ O)] ²⁺ 10	3.16	0.084 ± 0.011

^aApparent DNA binding constant from EthBr displacement assay using increasing concentrations (0–60 μM) of **1–10**.

^bStern-Volmer constant for tryptophan quenching of the complexes in the presence of BSA; concentration of protein = 5 μM; increasing concentrations (0–60 μM) of **1–10**.

plexes showed lesser quenching efficiency than their mixed ligand counterparts, analogues **1–5**. The poor DNA binding affinity of complexes **6–10** could be due to the possibility that those complexes bind the groove/surface of DNA.

3.3.2. Viscosity measurements

Viscosity experiment was performed to examine the binding mode of **1–10** with DNA by measuring the viscosity of CT-DNA before and after addition of the metal complexes. There would normally be an increase in viscosity of DNA when associating with intercalatively binding metal complexes [62]. On the other hand, binding of complexes at the surface or groove leads to a decrease in viscosity of DNA due to the kinks or bends. The relative viscosity of the CT-DNA was determined when the complexes of interest

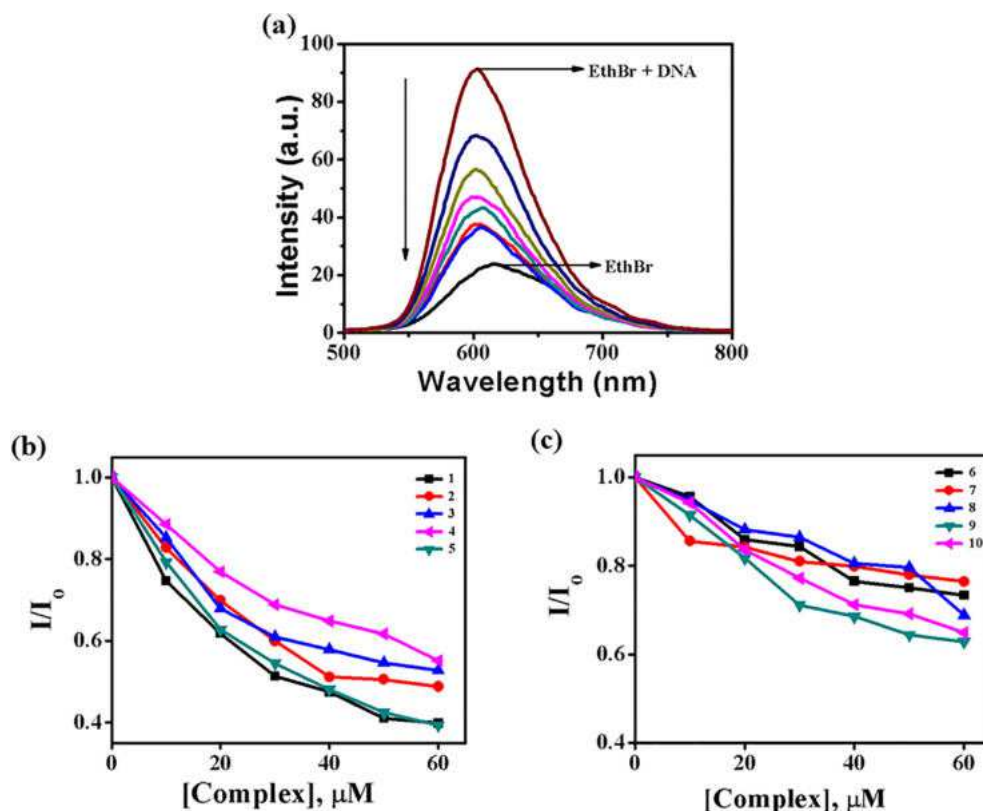


Fig. 2. (a) Influence of complex **1** on emission intensity of CT DNA-bound EthBr (12.5 μM) at different concentrations of the complex in 10% DMF-Tris-HCl buffer. (b) and (c) Effect of the addition of complexes **1–10** on emission intensity of CT DNA-bound EthBr.

were titrated with CT-DNA (100 μM) with concentration of the complex increasing from $1/R$ 0 to 0.5 ($1/R = [\text{complex}]/[\text{DNA}]$) in 10% DMF-Tris-HCl buffer. The relative viscosity values $(\eta/\eta_0)^{1/3}$ were plotted against $1/R = [\text{complex}]/[\text{DNA}]$ (Fig. 3). The order of increase of viscosity, $\text{EthBr} > 5 > 3 > 1 > 2 > 4 > 9 > 8 > 10 > 7 > 6$, indicates a higher viscosity increase in phen-containing complexes **1–5** than bis-complexes **6–10**. This may be due to the involvement of partial intercalative binding mode of the aromatic planar phen ring present in complexes **1–5**. However, with respect to the possible intercalator EthBr, all the complexes displayed only lesser increase in viscosity. Interestingly, **5** displayed higher viscosity increase compared to the remaining complexes in the series which is attributable to the primary ligand quinazoline that assists the intercalation of phen moiety with DNA, compared to the other primary ligands. The EthBr displacement assay (cf. *vide supra*) further substantiates this inference.

3.4. DNA cleavage studies

The ability of self-activating DNA cleavage of mixed **1–5** and bis **6–10** complexes was assessed by incubation of supercoiled (SC) pUC19 DNA (40 μM in base pairs) with 100 μM complex **1–10** concentrations (Figs. 4, 5; Tables S3 and S4) in 10% DMF-Tris-HCl buffer for 4 h in the absence of activating agents such as ascorbic acid, H_2O_2 , etc. Upon gel electrophoresis of the reaction mixture, the

complexes showed different cleavage efficiencies and the order of DNA cleavage was assessed on the basis of conversion of supercoiled DNA (SC, form I) to the nicked circular (NC, form II) and the linear circular (LC, form III) forms. Interestingly, all five Cu(II) metal complexes were able to engage in DNA cleavage effectively, while quinoxalin-containing mixed-ligand complex **2** exhibited a higher percentage of DNA cleavage, converting supercoiled (SC, form I) to 100% nicked circular (NC, form II). It also demonstrated similar DNA cleavage efficiency of the established self-activating DNA cleavage agent $[\text{Cu}(\text{dpq})_2(\text{H}_2\text{O})]^{2+}$. On the other hand, complexes **6–10** showed only moderate DNA cleavage by converting SC DNA to nicked circular (NC, Form II) and linear circular (LC, Form III). It is interesting to note that the complexes exhibiting NC and LC forms before converting all SC DNA forms indicate that bis-complexes **6–10** are involved in double-strand DNA cleavage [63,64] rather than single-strand cleavage [65]. Although complex **2** exhibited efficient DNA cleavage, it did not produce linear LC form and, therefore, the complex **2** proceeded with a single-strand rather than a double-strand DNA cleavage. The cleavage studies led to the inference that mixed and bis-metal complexes engage in different DNA cleavage modes. However, the ligation experiment has to be performed to confirm the double-strand DNA cleavage [63,64]. In addition, complex **2**, at increasing concentrations (0–80 μM), was incubated with a fixed concentration of DNA, 40 μM , to determine the effect of concentration of the metal complex on DNA

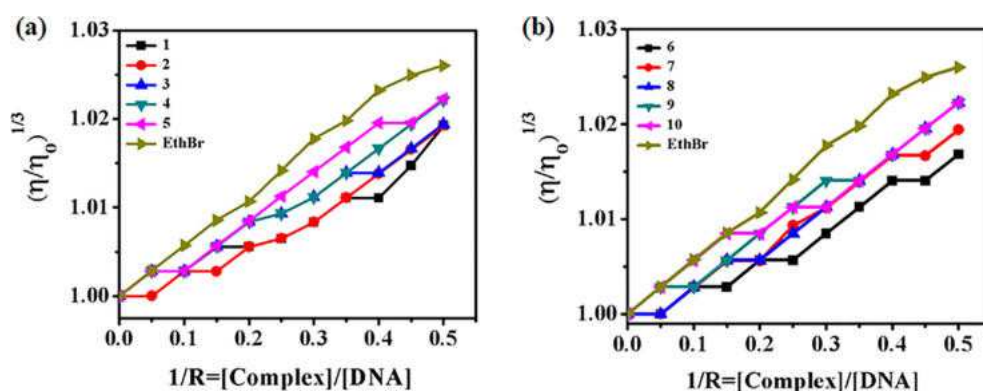


Fig. 3. (a) and (b) The effect of addition of complexes **1–10** on the viscosity of CT DNA in 10% DMF-Tris-HCl buffer; Relative specific viscosity vs. $1/R$; $[\text{DNA}] = 100 \mu\text{M}$.

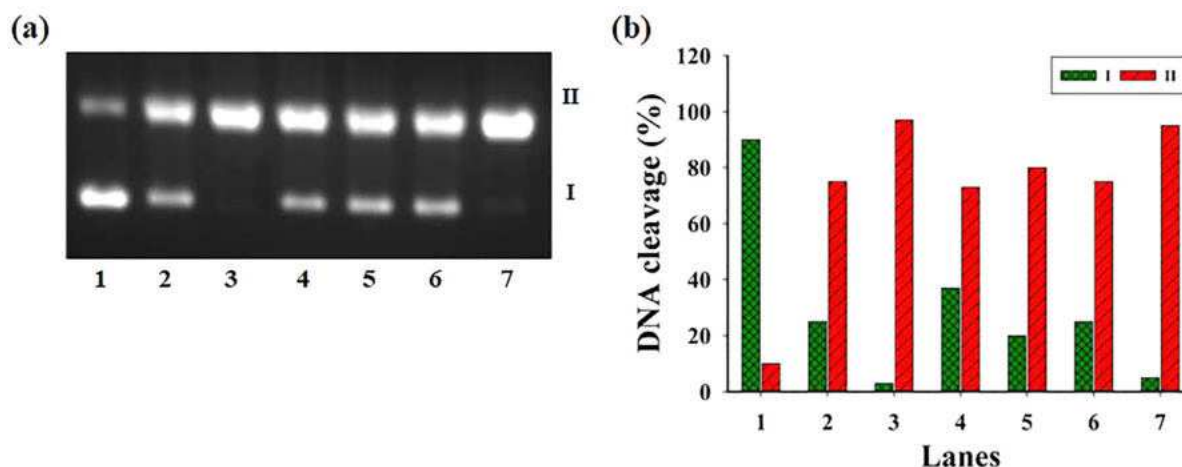


Fig. 4. (a) Gel electropherogram showing cleavage of supercoiled pUC19 DNA (40 μM base pair) by complexes **1–5** (100 μM) in 10% DMF-Tris-HCl buffer at 37 $^{\circ}\text{C}$ for incubation time of 4 h in the absence of any reducing agent. Lane 1, DNA; lane 2, DNA + **1**; lane 3, DNA + **2**; lane 4, DNA + **3**; lane 5, DNA + **4**; lane 6, DNA + **5**; lane 7, DNA + $\text{Cu}(\text{dpq})_2(\text{H}_2\text{O})^{2+}$. (b) Percentage efficiencies of DNA cleavage showing increase in Form II with an incubation time of 4 h. Forms I and II are supercoiled and nicked circular DNA, respectively.

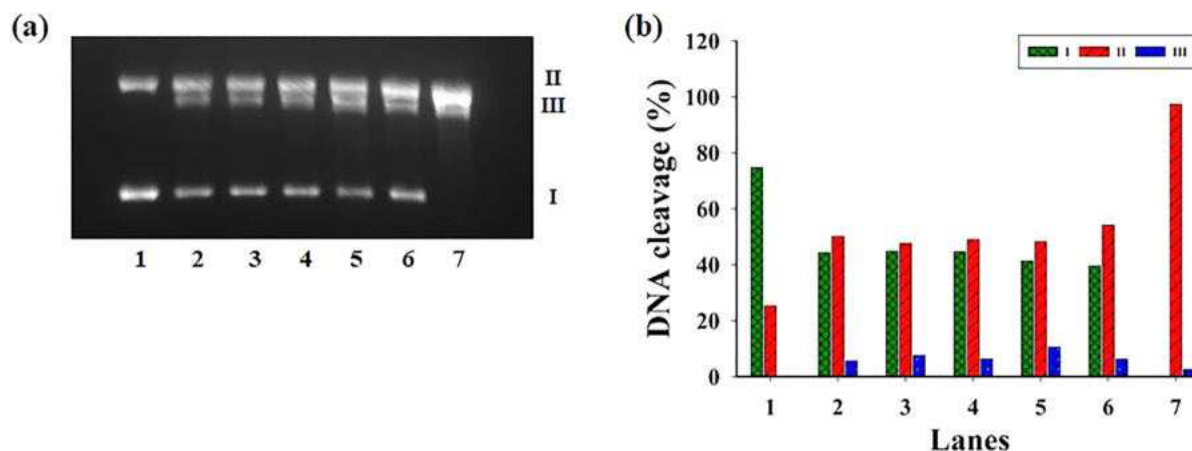


Fig. 5. (a) Gel electropherogram showing cleavage of supercoiled pUC19 DNA (40 μ M base pair) by complexes **6–10** (100 μ M) in 10% DMF-Tris-HCl buffer at 37 $^{\circ}$ C for incubation time of 4 h in the absence of any reducing agent. Lane 1, DNA; lane 2, DNA + **6**; lane 3, DNA + **7**; lane 4, DNA + **8**; lane 5, DNA + **9**; lane 6, DNA + **10**; lane 7, DNA + Cu(dpq)₂(H₂O)₂²⁺. (b) Percentage efficiencies of DNA cleavage showing increase in Forms II and III with an incubation time of 4 h. Forms I, II and III are supercoiled, nicked circular and linear circular DNA, respectively.

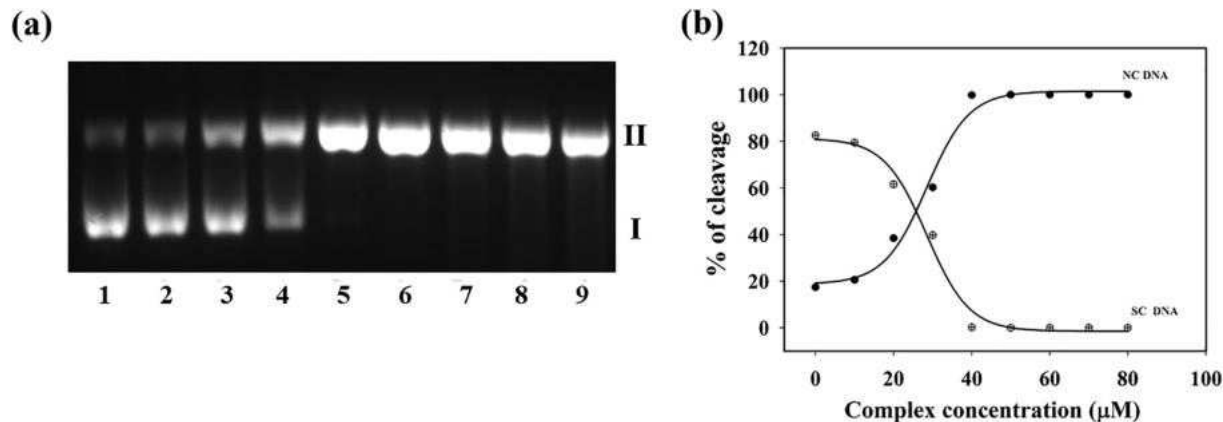


Fig. 6. (a) Cleavage of supercoiled pUC19 DNA by copper(II) complexes in a buffer containing 10% DMF, 5 mM Tris-HCl and 50 mM NaCl at 37 $^{\circ}$ C for 4 h. Lane 1, DNA; lane 2, DNA + **2** (10 μ M); lane 3, DNA + **2** (20 μ M); lane 4, DNA + **2** (30 μ M); lane 5, DNA + **2** (40 μ M); lane 6, DNA + **2** (50 μ M); lane 7, DNA + **2** (60 μ M); lane 8, DNA + **2** (70 μ M); lane 9, DNA + **2** (80 μ M). Forms I and II are supercoiled and nicked circular forms of DNA, respectively. (b) Cleavage of supercoiled pUC19 DNA showing decrease in Form I (SC DNA) and formation of Form II (NC DNA) with incubation time.

cleavage. Interestingly, at a metal complex concentration of 40 μ M, there was a 100% conversion of SC form to NC form (Fig. 6a, 6b; Table S5). This indicates that complex **2** and DNA are involved in a cleavage reaction of 1:1 stoichiometry.

The capability of oxidative DNA cleavage of the complexes **1–10** was investigated in the presence of the reducing agent ascorbic acid. In this study, complexes **1–5** did not show appreciable DNA cleavage (Fig. 7; Table S6), while bis-complexes **8** and **9** showed moderate DNA cleavage with NC and LC forms (Table S7; Fig. S5). However, both showed diminished DNA cleavage compared to the standard compound [Cu(dpq)₂(H₂O)₂]²⁺. Incorporation of the extended aromatic ligand phen, particularly with quinoxalin-containing complex **2**, has the ability to interact strongly with DNA and produced more DNA fragments than the other complexes.

3.5. Protein binding of the complexes

To assess protein binding of the complexes, the interaction of **1–10** metal complexes with bovine serum albumin (BSA) was monitored using a tryptophan quenching assay [66]. A decrease in the endogenous BSA tryptophan emission intensity was observed when complexes **1–10** were added to BSA in phosphate buffer (pH 7.1). This is due to changes in the secondary protein structure

as well as the tryptophan environment of BSA on the binding of the complexes [29]. The magnitude of the fluorescence intensity of BSA, as expressed by the Stern-Volmer constant K_{sv} , is considered to be a measure of the binding affinity of the protein complexes in the tryptophan residue region [29]. The value of K_{sv} obtained (Table 2) as the slope of the linear plot of I_0/I vs. [complex] from Fig. 8 and the ability of the complexes to quench the emission intensity of BSA are in the order $5 > 4 > 1 > 2 > 3 > 9 > 8 > 10 > 6 > 7$. As a result, quinoxaline- and phen- containing mixed-ligand complex **5** exhibited the highest protein-binding affinity relative to the other complexes. This could be due to the fact that the quinoxaline moiety present in the complex can enhance the hydrophobic interaction of the complex with the protein chain. This finding is also consistent with the trend in DNA binding studies.

3.6. Cytotoxicity

3.6.1. MTT assay

The cytotoxicity of the mixed-ligand complexes **1–5** and bis-complexes **6–10** was tested in the human non-small cell lung carcinoma cell line A549, with cisplatin used as a reference drug under identical conditions, using the MTT assay. The IC₅₀ values

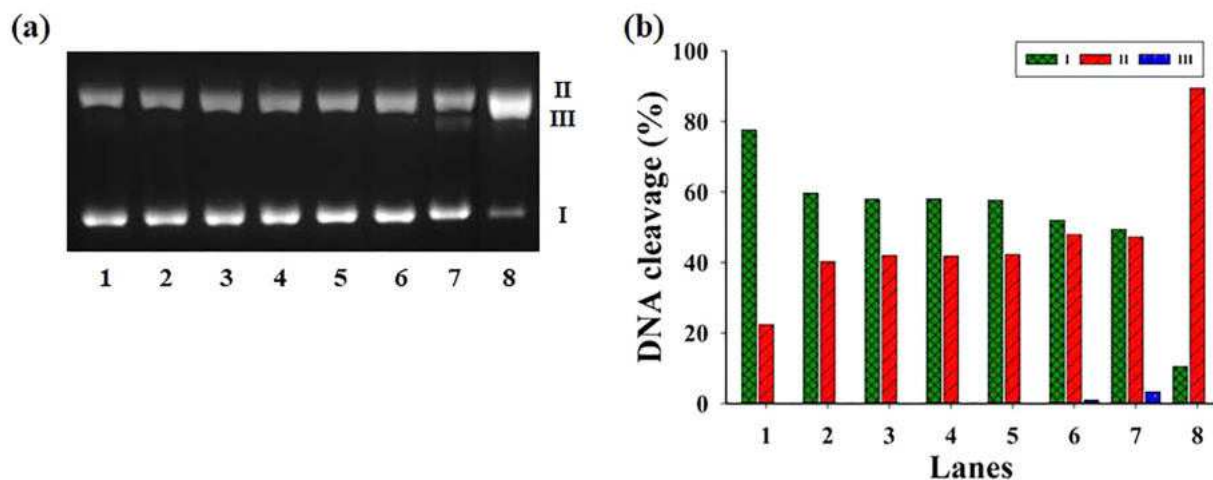


Fig. 7. (a) Gel electropherogram showing oxidative cleavage of supercoiled pUC19 DNA (40 μM base pair) in the presence of ascorbic acid (10 μM) by complexes **1–5** (30 μM) in 10% DMF-Tris-HCl buffer at 37 $^{\circ}\text{C}$ for an incubation time of 1 h. Lane 1, DNA; lane 2, DNA + H₂A + **1**; lane 3, DNA + H₂A + **2**; lane 5, DNA + H₂A + **3**; lane 6, DNA + H₂A + **4**; lane 7, DNA + H₂A + **5**; lane 8, DNA + H₂A + Cu(dpq)₂(H₂O)₂²⁺. (b) Percentage of oxidative DNA cleavage efficiency of complexes **1–5** showing increase in Form II with an incubation time of 1 h. Forms I, II and III are supercoiled, nicked circular and linear circular DNA, respectively.

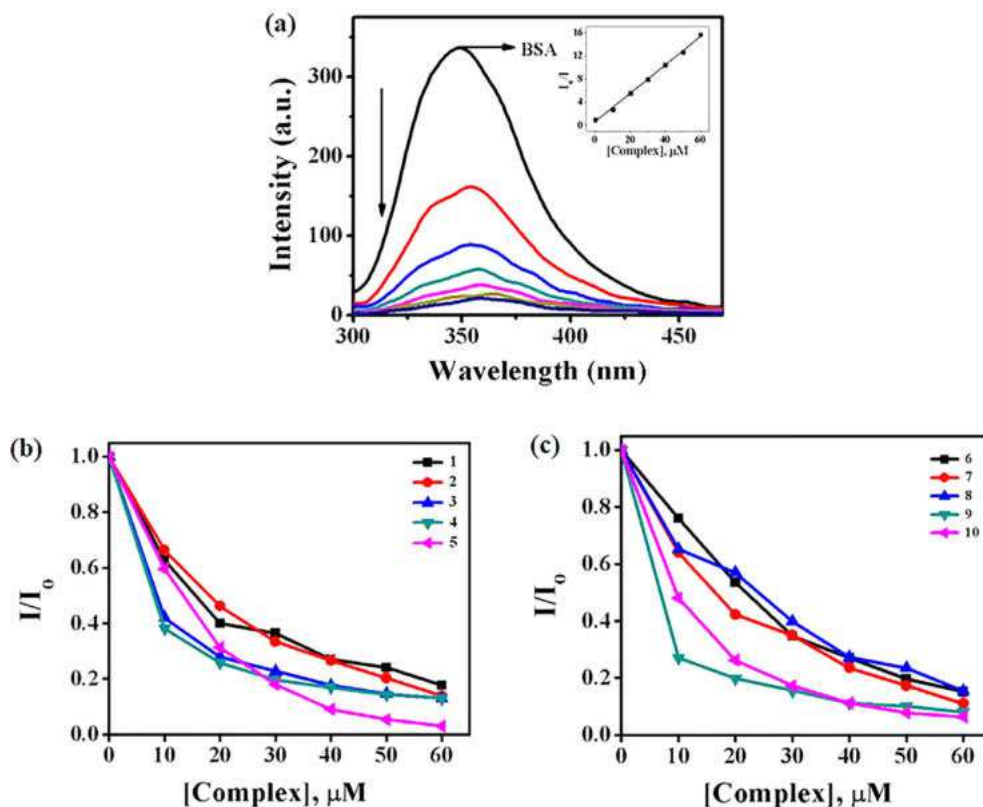


Fig. 8. (a) Effect of complex **5** on fluorescence quenching of BSA at various concentrations (0–60 μM) in phosphate buffer at pH 7.1. Inset: Plot of [complex] vs. I_0/I . (b) and (c) Fluorescence quenching was observed at 295 nm as BSA titrated with complexes **1–10** in phosphate buffer at pH 7.1.

(Table 3) obtained by plotting the cell viability against different concentrations of the complexes revealed that the cytotoxicity of the mixed-ligand complexes **1–5** was more intense than that of the reference drug cisplatin and bis-complexes **6–10** for 24 h incubation. Yet, all bis-complexes **6–10** showed higher IC_{50} values than cisplatin. Among the mixed-ligand complexes, complex **5** showed the highest cytotoxicity [order **5** (3.85 μM) > **1** (4.50 μM) > **2** (5.0 μM) > **3** (7.75 μM) > **4** (8.85 μM)] and was 3 times more potent than cisplatin. The cytotoxic efficiency of the present complexes

was also compared with the Casiopeínas [Cu(4,7-dimethylphenanthroline)(glycinate)]NO₃ (Casiopeína IIgly or Cas IIgly) complex that entered phase I clinical trial against HeLa cells [67–69]. Interestingly, the mixed-ligand complexes **1** (4.50 \pm 0.05 μM , **2** (5.00 \pm 0.05), and **5** (3.85 \pm 0.05) showed greater cytotoxicity than Cas IIgly (IC_{50} , 6 μM against A549 cell line) [70]. The highest cytotoxicity of complex **5** could be attributed to its efficient DNA- and protein-binding properties. Yet, all complexes **1–5** were more cytotoxic than cisplatin, so all five might be worthy of further

Table 3MTT assay for finding viability of A-549 cells treated for 24 h with copper(II) complexes **1–10**.

	IC ₅₀ values (μM)
[Cu(L1)(phen)(H ₂ O)] ²⁺ 1	4.50 ± 0.05
[Cu(L2)(phen)(H ₂ O)] ²⁺ 2	5.00 ± 0.05
[Cu(L3)(phen)(H ₂ O)] ²⁺ 3	7.75 ± 0.05
[Cu(L4)(phen)(H ₂ O)] ²⁺ 4	8.85 ± 0.05
[Cu(L5)(phen)(H ₂ O)] ²⁺ 5	3.85 ± 0.05
[Cu(L1) ₂ (H ₂ O)] ²⁺ 6	21.75 ± 0.05
[Cu(L2) ₂ (H ₂ O)] ²⁺ 7	47.50 ± 0.05
[Cu(L3) ₂ (H ₂ O)] ²⁺ 8	35.75 ± 0.05
[Cu(L4) ₂ (H ₂ O)] ²⁺ 9	45.00 ± 0.05
[Cu(L5) ₂ (H ₂ O)] ²⁺ 10	46.75 ± 0.05
Cisplatin	13.0 ± 0.02

investigation towards drug development. The greater cytotoxicity of the mixed ligand complexes compared to the bis-complexes likely relates to the phen ligand.

3.6.2. Mechanism of cell death

3.6.2.1. Intracellular ROS generation. Copper complexes are known for redox potential [71] and induction of cell death by intracellular ROS generation [72]. This is a mechanism that can potentially trigger apoptosis in cancer cells [73]. For these purposes, ROS generation capacity of the complexes **1–10** was investigated by incubating 5 μM of the complexes with A549 cells for 12 and 24 h (Fig. 9). The intracellular ROS levels were detected using the DCFH-DA fluorescent probe [74]. In the presence of ROS, non-fluorescent dichlorofluorescein (DCFH) was oxidised to fluorescent dichlorofluorescein (DCF). The extent of increase in fluorescent intensity of the treated solution compared to untreated is related to efficiency of producing higher level of ROS. Complex **1**, which had the next highest level of cytotoxicity, produced the highest level of ROS compared to other complexes. All the complexes caused production of higher amount of ROS compared to control in the order **1** > **4** > **2** > **3** > **9** > **7** > **6** > **10** > **5** > **8** at 12 and 24 h incubation. Thus, the complex **1** appeared to engage in higher

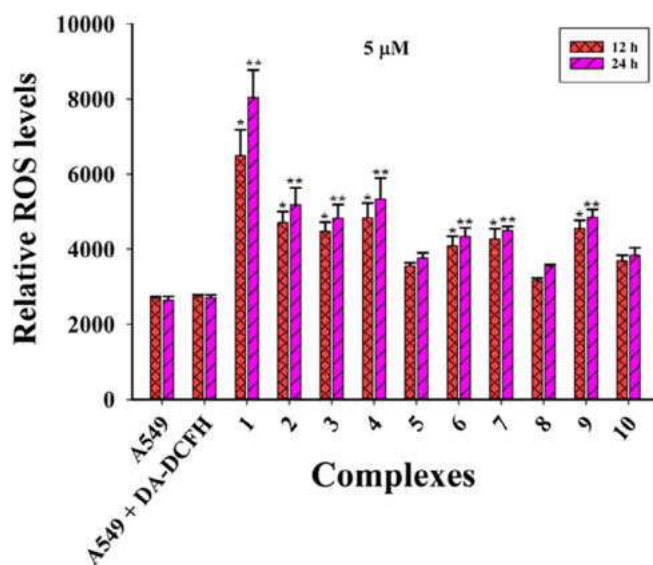


Fig. 9. ROS production induced by copper(II) complexes **1–10** in A549 cell with treatment for 12 & 24 h at 5 μM concentration. Each data is average of data obtained from three independent experiments. Thus, the results are means of three independent experiments and show the standard error of the mean. Statistical analysis was done using one way ANOVA, * = ($p < 0.05$), ** = ($p < 0.05$) indicate significantly different from untreated control.

ROS production than all other complexes at both 12 and 24 h incubation at 5 μM concentration (Fig. 9). On the contrary, prominently DNA- and protein-binding and cytotoxic complex **5** showed lesser ROS production. Further investigation is needed to understand the difference of the manifestations of these complexes. Nevertheless, it could be generalized that cytotoxicity of the present complexes is mediated by or due to ROS.

3.6.2.2. AO-EthBr staining. In the context of cancer biology, a compound that induces predominantly apoptotic mode of cell death may be more appropriate than one which induces necrosis [75]. In order to examine the mode of cell death following the treatment of the metal complexes, acridine orange (AO) and ethidium bromide (EthBr) fluorescent assays have been used to detect the mechanism of cell death, either apoptosis or necrosis (Figs. 10, 11). The fluorescent AO dye permeates living as well as early apoptotic cells that have an intact cell membrane and binds DNA to fluoresce green. On the other hand, EthBr enters the cells only when the integrity of the membrane is lost, and binds to DNA via intercalation and fluoresces brightly. Cancer cells were treated with all complexes **1–10** at their respective IC₅₀ for 24 h. The different morphologies of the cells were identified and categorized using acridine orange and ethidium bromide (AO/EthBr) staining assay and fluorescent microscopy on the basis of the available literature [54,76] (Fig. 10a, 11a). These morphological changes in and data from manual counting (Fig. 10b, 11b) of cells treated with **1–10** suggest that cells are committed to effective apoptotic cell death and marginally to necrosis. The order of efficiency of the complexes to induce apoptosis is set out as follows: **2** > **7** > **4** ≈ **9** ≈ **6** > **10** > **1** > **5** > **8** > **3**. Complex **2**, which exhibited self-activated DNA cleavage, showed a higher percentage of apoptotic cell death than the other complexes. Complex **5**, with the highest cytotoxicity, induced only a lower percentage of apoptosis.

3.6.2.3. Annexin V-Cy3 staining. In order to further confirm that the complexes induce apoptotic cell death, the Annexin V-Cyanin 3 assay was performed. Since the mixed-ligand complexes were shown to inflict higher cytotoxicity than the bis-complexes **6–10**, only complexes **1–5** were used in this analysis. The rapid translocation of the membrane phospholipid, phosphatidylserine, from the cytoplasmic leaflet to the extracellular surface and accumulation there is an early indicator of apoptosis [77]. The combination of Annex V-Cy3 (red emitting) and 6-CFDA (green emitting) makes it possible to differentiate viable cells (Annexin V-negative, 6-CFDA-positive), early apoptotic cells (Annexin V-positive, 6-CFDA-positive) and necrotic cells (Annexin V-positive, 6-CFDA-negative) [78]. When cancer cells were treated with the transition metal complexes **1–5** there was a significant increase of Annexin V-Cy3 and 6-CFDA double stained (red and green fluorescence) cells indicating an early stage of apoptosis as shown in Fig. 12. The findings of Annexin V-Cy3 assay further support the apoptotic mode of cell death induced by the transition metal complexes.

3.6.2.4. Western blot analysis. The western blot analysis was performed to investigate if the present copper(II) complexes trigger the caspase-dependent apoptosis or programmed cell death pathway [79]. When the intracellular ROS level is elevated by the copper complexes (cf. *vide supra*), mitochondrial membrane potential (MMP) is disrupted [80–82]. As a consequence, mitochondria release cytochrome-c into the cytosol, leading to the formation of a large complex consisting of Apaf-1 (apoptotic protease activating factor-1) and caspase-9 known as, apoptosome, which subsequently cleaves procaspase-3 and initiates apoptosis by activating effector caspase-3 [80–82]. In this context, based on the order of cytotoxicity, the top two copper complexes **1** and **5** were selected and incubated with the A549 cells. The whole cell protein lysate of

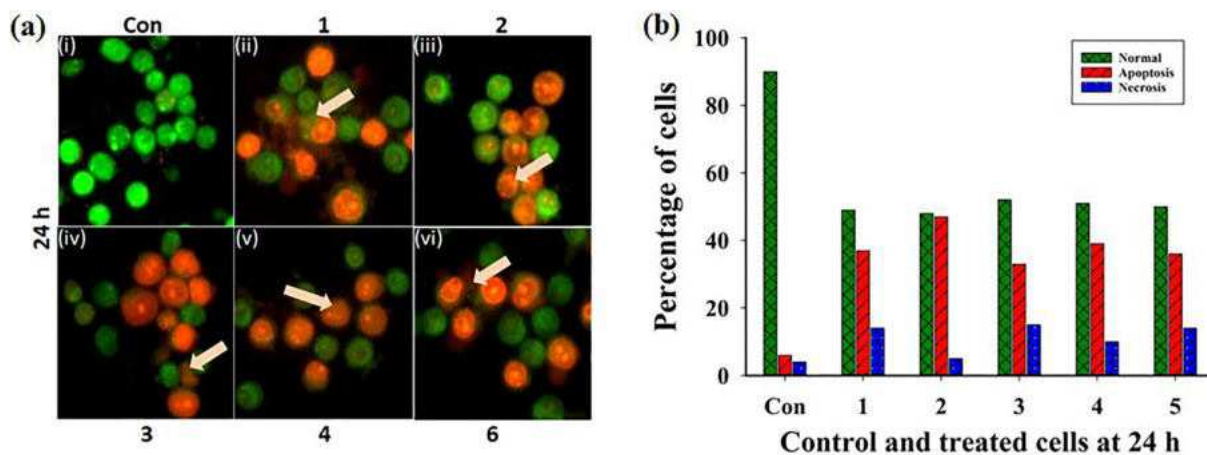


Fig. 10. (a) Photomicrographs showing AO/EthBr stained A549 cells. (i) Untreated cells, (ii-vi) treated with 1-5 for 24 h. Arrows show apoptotic body formation. (b) Graphical representation of percentage of viable, apoptotic and necrotic cells.

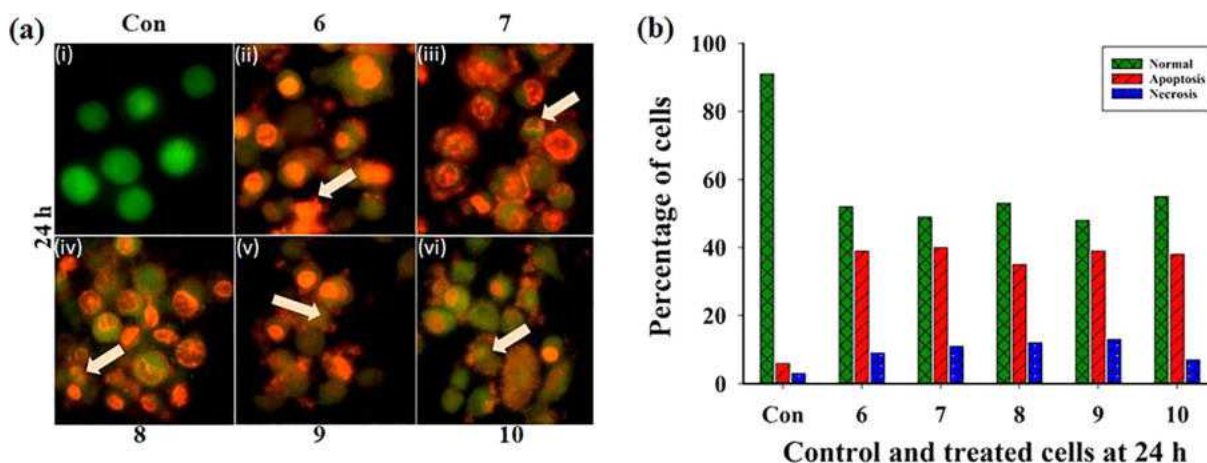


Fig. 11. (a) Photomicrographs showing AO/EthBr stained A549 cells. (i) Untreated cells, (ii-vi) treated with 6-10 for 24 h. Arrows show apoptotic body formation. (b) Graphical representation of percentage of viable, apoptotic and necrotic cells.

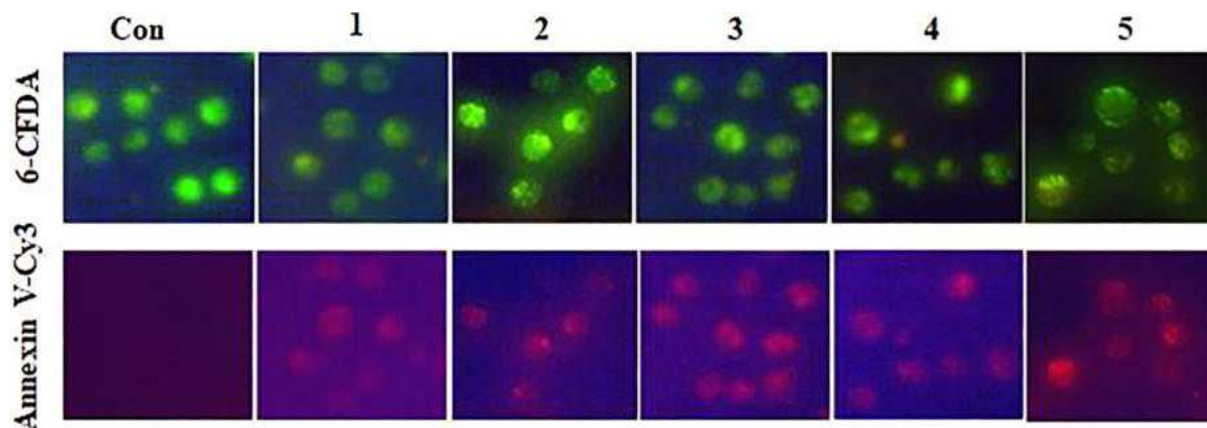


Fig. 12. Photomicrographs showing Annexin V-Cy3/6CFDA stained A549 cells treated with complexes 1-5 for 24 h, reflecting indications of early changes of apoptosis. Green, 6-CFDA stained live cells; red, annexin V-Cy3 stained necrotic cells; red and green, annexin V-Cy3 stained apoptotic cells.

complex 1 and 5-treated A549 cells were subjected to SDS-PAGE and the western blot analysis was subsequently performed in a qualitative manner to find the expression of caspase-3. Compared to control lane the western blot showed that the complex 5 trig-

gered more amount of caspase-3 than complex 1. The result indicates that the copper(II) complexes 1 and 5 cause increasing caspase-3 expression levels in A549 cell, where complex 5 is the most efficient (Fig. S6).

4. Conclusions

Mixed ligand copper(II) complexes **1–5** containing quinoline/quinoxalin/quinazoline derivatives and 1,10-phenanthroline were characterized and their biological activity compared to their bis-complexes **6–10** analogues. One of the mixed ligand complexes, **3**, was structurally characterized and the coordination geometry around Cu(II) was predicted to be almost square pyramidal. DNA and protein binding studies showed that quinazoline-containing mixed ligand complex **5** has a higher DNA- and protein-binding efficacy than the other complexes. DNA cleavage studies showed that quinoxaline-containing mixed ligand complex **2** resulted in significantly higher self-activated DNA cleavage than the remaining four mixed ligand complexes and all five bis-complexes. The efficiency of cytotoxicity of all complexes **1–10** was tested in human non-small lung carcinoma cell A549 and the most efficient DNA and protein binding complex **5** showed relatively higher cytotoxicity, 3-times more than cisplatin, compared to the other complexes, but the IC₅₀ of all five complexes indicated all these compounds as more potent than cisplatin. The DCFDA assay confirmed that the metal-assisted-generated reactive oxygen species (ROS) is responsible for cytotoxicity of the complexes. AO/EthBr staining assay, supported by Annexin V-Cy3 staining assay, revealed that all metal complexes have the ability to induce apoptotic cell death with some degree of necrosis, with the best efficiency proved by metal complex **2** but the others are not any much inferior. The western blot result demonstrated that copper (II) complex **5** would promote apoptosis in lung cancer cells better than complex **1** by regulating the expression of caspase-3. Based on these observations, we conclude that mixed ligand complexes with quinoline/quinoxaline/quinazoline derivatives and 1,10-phenanthroline ligands are biologically active compared to their bis-complex analogues. These results provide justification for further development of mixed ligand Cu(II) complexes as anticancer agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.poly.2020.114886>.

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Observations on Dag-like defect of spermatozoa induced by treatment of the phytotherapeutic *Quassia amara*/quassin in the mouse model

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Abstract

Gross alterations in the morphology of spermatozoa, teratozoospermia, invariably render them incapable of fertilisation. One of the contributory factors to teratozoospermia is failure of spermatozoon to shed the cytoplasmic droplet even after their arrival at epididymis. *Quassia amara* and quassin are of medicinal value with special reference to malaria. Nevertheless, there are also reports implicating *Quassia*/quassin in male reproductive toxicity. We were interested in finding if its therapeutic application would jeopardise male fertility. So, we tested it for male reproductive toxicity by analysing, among other aspects, abnormal sperm morphologies, and made a systematic analysis of the spermatozoa of treated mice before they are spermiated and until they arrive at the cauda epididymis. The spermatozoa not only failed to shed the cytoplasmic droplet during epididymal transit but swell to a very large size and were angulated, resulting in Dag-like defect or lasso shape. A link between cytoplasmic droplet that was retained and lasso shape of tail was indicated. This article traces the structural changes in spermatozoa that lead to angulation, flexion and coiling of the tail, caused due to retention of cytoplasmic droplet, and explains one of the mechanisms of toxicant-induced teratozoospermia.

KEYWORDS

cytoplasmic droplet, Dag-like defect, Hermes body, lasso spermatozoon, *Quassia amara*, quassin, teratozoospermia

1 | INTRODUCTION

Spermatozoa are cells unique in several respects, one of which is their morphology. Their size and shape are unique for each species. Gross alterations in the morphology invariably render them incapable of fertilising the ovum. Such spermatozoa are one of the aspects of teratozoospermia and one of the contributing factors of male infertility (Jensen et al., 2018). The causative factors of teratozoospermia include therapeutics (Ypsilantis et al., 2003), microbial infection (Mehta et al., 2002), toxic insults (Akbarsha et al., 1996; Faisal et al., 2015) and genetic factors (Coutton et al., 2015). Spermatozoa lose their cytoplasm greatly during spermatogenesis, before spermiation, as a residual body and it is later phagocytosed by Sertoli cell

(De Kretser & Kerr, 1994), yet while leaving the testis they still retain a small portion of cytoplasm called the cytoplasmic droplet (CD) or recently rechristened as Hermes body (Au et al., 2015). However, the failure of spermatozoa to get rid of the CD even after their sojourn through epididymis such that the ejaculated spermatozoa retain the CD is one of the contributory factors of teratozoospermia. The organisation of CD and its displacement from sperm head to the terminal part of mid-piece during its epididymal transit is a hallmark of epididymal maturation (Moore et al., 2010). Albeit the epididymal segment for the migration of CD varies depending on the species, mostly it happens when sperm leaves corpus. The mechanics of CD migration along the flagellum during epididymal transit still remains elusive. It is suggested to be effected by the shear forces rendered

by the peristaltic activity of ductus epididymidis on highly concentrated spermatozoa in the viscous luminal fluid (Cooper et al., 2004) or result from the deprived fucosidase activity (Veeramachaneni et al., 1998). The CD thus shed is endocytosed by clear cells of the cauda epididymidis (Hermo et al., 2019).

Since spermiation and before it is shed in the epididymis, the CD has been attributed with physiological roles. One such role is the aromatisation of androgen into estrogen, which enhances the fluid reabsorption by the epithelium of ductuli efferentes, ensuring increased sperm density at the epididymis (Hess et al., 1995). Yet another role of CD is the volume regulation; during epididymal sojourn spermatozoa acquire osmolytes accumulated by the epididymal epithelium by means of regulatory volume increase (RVI) brought up by the increasing tonicity of luminal fluid from the initial segment to cauda. After ejaculation into the comparatively hypotonic female tract, these osmolytes are expended for regulatory volume decrease (RVD) (Xu et al., 2013; Yeung et al., 2006). In most cases, the CD is shed before ejaculation, once it has fulfilled its role(s). Failure to shed the CD thereafter renders the spermatozoon to carry an extra load of cytoplasm which might hinder its motility (Kuster et al., 2004; Rengan et al., 2012).

Quassia amara, belonging to the family Simaroubaceae, is a small tropical tree, traditionally used against a variety of health problems as an herbal remedy. *Q. amara* and the biologically active secondary metabolite in it, quassin, are of medicinal value. Quassin is under investigation for antimalarial property (Ajaiyeoba et al., 1999; Bertani et al., 2007; Houël et al., 2009). There are several reports, including from our own lab, implicating *Q. amara* and quassin in male reproductive toxicity, and it has been hypothesised that quassin could be developed into a male contraceptive agent (Faisal et al., 2006, 2015; Njar et al., 1995; Obembe et al., 2014; Parveen et al., 2003; Raji & Bolarinwa, 1997). We were interested in finding if the therapeutic application of *Q. amara* and/or quassin would jeopardise male fertility and, so, we tested *Q. amara* methanol extract (QAE) and quassin for male reproductive toxic property at a higher level of analysis. One of the approaches was to look at the abnormal sperm morphologies in QAE-/quassin-treated mice, when we found that the spermatozoa not only failed to shed the CD during epididymal transit but they swell to a very large size. Added to this, a majority of spermatozoa were angulated and/or coiled, resulting in a lasso/Dag-like shape, and looked further more abnormal in morphology (Faisal et al., 2006). A link between retention of the CD and the lasso shape of tail was indicated. This article traces the structural changes in the spermatozoa that lead to angulation, flexion and coiling of the tail as caused due to retention of the CD/Hermes body, which is swollen to a large size, and explains one of the manifestations of toxicant-induced teratozoospermia.

2 | MATERIALS AND METHODS

Institutional Animal Ethics Committee (IAEC) of Bharathidasan University had approved the experiment. The bark of *Q. amara* was

a gift from Late Dr. BMJ Pereira, IIT Roorkee, Uttaranchal, India. The methodology in terms of extraction and animal experiments are the same as reported previously (Faisal et al., 2015). In short, 200 g of the powdered bark of the plant was extracted (20 cycles) in 1 L methanol using a Soxhlet apparatus and the extract was condensed utilising a rotary evaporator (Büchi Labortechnik AG, Switzerland) to about 50 mL and further condensed by air drying at room temperature to yield approximately 2 g of crude extract, which was stored at 0°C till use. Quassin was a gift from TRIFOLIO-M GmbH, Germany. The condensed methanol extract and quassin were dissolved separately in a minimum quantity of ethanol and diluted with phosphate-buffered saline (PBS). Ninety-day-old male Swiss albino mice, each weighing 30–35 g, were assigned to three groups of 10 each. Mice in the common control group were administered with PBS, and those in the remaining groups were injected, as a daily dose, with methanol extract (100 mg/kg body weight) or quassin (1 mg/kg body weight), based on our previous studies (Faisal & Akbarsha, 2016; Faisal et al., 2006, 2015), through intraperitoneal (*ip*) route for 35 days, the length of one complete spermatogenic cycle in mouse (Oakberg, 1956). At the completion of the treatment, five mice from each group were dissected under mild sodium pentobarbital anaesthesia, the testicles and epididymides were removed and washed in normal saline. One micro-litre of cauda epididymal fluid was collected in a micropipette and diluted with 99 μ L PBS, pH 7.4 (Akbarsha et al., 2000). In order to evaluate the morphology of the spermatozoa, fresh spermatozoa from the above preparation were subjected to acridine orange (AO) and ethidium bromide (EB) staining (Faisal et al., 2008; Spector et al., 1998). The preparations were observed in a Carl Zeiss research microscope (Germany), with fluorescence adapter, at $\times 400$ or $\times 1000$ magnification, and the unstained preparations were observed in phase contrast illumination. Images of spermatozoon with lasso tail or trend towards this abnormality were chosen to reveal a sequence, and assembled. To calculate the *per cent* spermatozoa with lasso tail abnormality, 100 randomly chosen spermatozoa from each slide were examined and categorised into normal and abnormal with lasso tail. Mean values and the standard deviations were calculated and paired sample *t*-test was carried out. Selected images of interest were recorded in a computer with Carl-Zeiss Axiovision (Germany) image-analysis software connected to a Sony DXC-151AP CCD camera (Tokyo, Japan).

Further, we made a systematic analysis of the spermatozoa of QAE-/quassin-treated mice from the point of spermiation until they arrive at the cauda epididymidis and stored, adopting light and transmission electron microscopy (TEM). For this, testicles and epididymides were excised from the remaining five animals from each group and perfused with Karnovsky's fluid in cacodylate buffer. Thin sections of testicles and different segments of epididymis were fixed in glutaraldehyde in cacodylate buffer and post-fixed in osmium tetroxide. After dehydration in ethanol and clearance in propylene oxide, the tissues were embedded in Araldite CY212 (SPI-ChemTM, Switzerland). Semithin sections of 1 μ m thickness were obtained in a Leica ultramicrotome (Jena, Germany) and stained in toluidine blue-O (TBO) (pH 4.4) for microscopic examination. Ultrathin

sections were obtained in the same microtome and stained with lead citrate (0.1%) and uranyl acetate (6%). These preparations were meticulously examined in a transmission electron microscope (Phillips 201C; The Netherlands) and photomicrographs were obtained.

3 | RESULTS

When in the control mice hardly any cauda epididymidal spermatozoa retained the CD, with none in lasso shape, a majority of the spermatozoa of quassin-treated mice retained the CD, and all such spermatozoa had lasso shape (Figure 1a, b). In *Q. amara* extract (QAE) treated mice also the spermatozoa retained the CD and all such spermatozoa possessed lasso shape, but the incidence was lesser (Table 1). In all the cases of lasso shape, the flagellum was re-curved at mid-piece-principal piece junction and formed a loop where the CD was retained. The tip of the flagellum emerged out from underneath the head to become free. Thus, the entire spermatozoon had an 8-shaped configuration. These spermatozoa exhibited only a sluggish motility, and it followed a circular pattern.

The Figure 2 illustrates the tentative sequential changes in the formation of the lasso configuration. The most characteristic change was swelling of the CD to a size larger than the head. This swelling occurred even before the migration of CD to distal parts of the flagellum, that is when it was closer to the head (Figure 2a). With the CD remaining swollen, and swelling to a further larger size, the CD could be located at the midpiece-principal piece junction (Figure 2b, c). At this point, the flagellum was seen to develop a kink, which intensified so as for the spermatozoon to assume the lasso shape (Figure 2d-f). The CD contained a dense body in a translucent cytoplasm (Figure 2d-j). It looked as if the outer phase of the membrane of the CD was contracted so as to render the flagellum to re-curve in a definite pattern such that the spermatozoa attained the lasso shape (Figure 2d-j). At the fold of the flagellum, the CD connected the two limbs of the fold (Figure 2g-l). The Figure 3 illustrates the different morphological manifestations of the lasso spermatozoa, in comparison with a spermatozoon with normal shape.

Lasso-shaped spermatozoa, with the retained CD, were not limited to the cauda epididymidis (Figure 4a), but also found in the proximal segments of the epididymis (Figure 4a, b) and rarely in the lumen of the seminiferous tubules (Figure 4c-e). The latter seminiferous

tubules manifested histopathological changes such as loss of germ cells and thinning of the seminiferous epithelium (Figure 4d).

The transmission electron microscopic observations supported the findings made in the light microscopic preparations described above. The corpus epididymidis of control mice invariably contained spermatozoa of normal shape, as revealed in the transverse sections wherein a section passed through the CD with a single axoneme, and rarely two axonemes, that is the axonemes of the recurved flagellum (Figure 5a). On the other hand, in the QAE/quassin-treated mice the sections of corpus epididymidal spermatozoa revealed two axonemes in a common cytoplasm, the axoneme of the recurved flagellum present in the CD that connected the two limbs of the flagellum (Figure 5b-d). At a higher magnification, it was seen that these sections passed through the CD, where the characteristic membrane elements were present (Figure 5c) or beyond the level of the membrane elements (Figure 5d). In transmission electron micrographs, which depicted sperm cut longitudinally, the re-curved of the flagellum clearly revealed to be due to contraction of the CD, bringing the two limbs of the recurved flagellum closer (Figure 5e-g).

4 | DISCUSSION

The peculiarities of sperm morphology in this study included failure of the CD to being shed and swelling of the latter to a size larger than the head, and angulation of the flagellum at the place of CD. This cascade of events happened in a coherent way, and led to a culmination point namely lasso-shaped spermatozoa, resulting in the limited and circular pattern of motility. Angulated and coiled sperm tail deformities has already been reported in infertile men who are

TABLE 1 Retention of cytoplasmic droplet by QAE-/quassin-treated mice spermatozoa (values: mean \pm SD)

Treatment	Spermatozoa with cytoplasmic droplet (%)
Control	1.3 \pm 0.4
QAE 100 mg	34.0 \pm 7.5***
Quassin 1 mg	64.7 \pm 7.8****

Note: *** and **** indicate $p \leq 0.005$ and $p \leq 0.0001$, respectively, control versus treated.

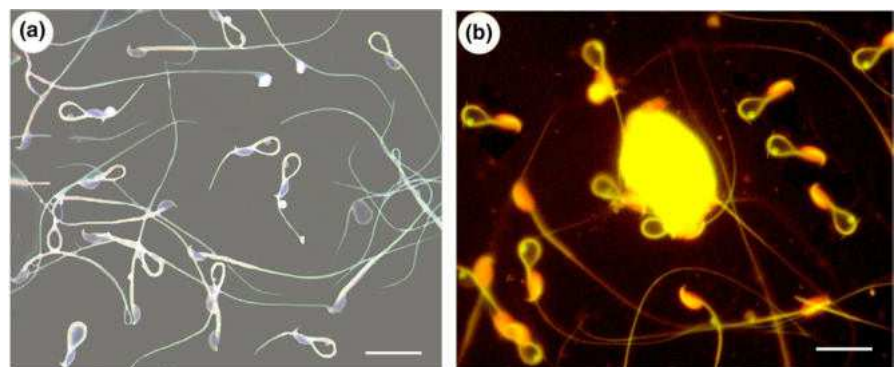


FIGURE 1 Unstained (phase contrast) (a) and AO-EB stained (b) cauda epididymidal spermatozoa of quassin-treated mice showing lasso shape. Scale bar: 49 μ m

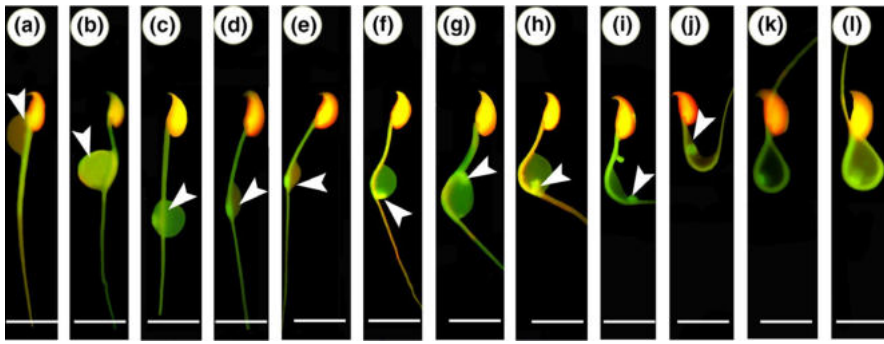


FIGURE 2 AO-EB stained cauda epididymal spermatozoa of QAE-/quassin-treated mice showing sequential changes in the formation of lasso configuration. Scale bar: 33 μ m

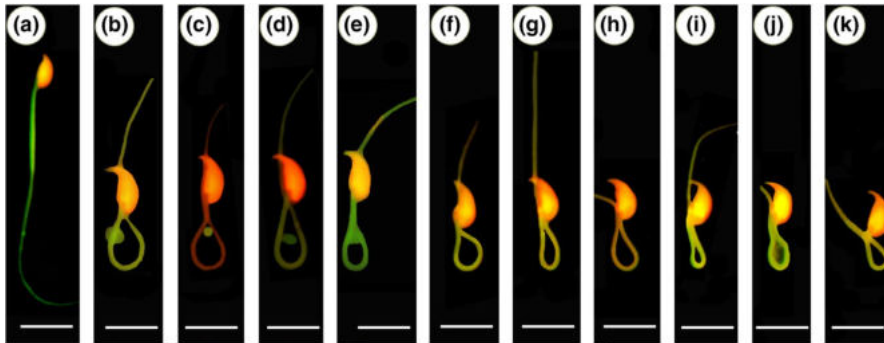


FIGURE 3 AO-EB-stained cauda epididymal spermatozoa of control (a) and QAE-/quassin-treated (b-k) mice displaying different morphological manifestations of the lasso spermatozoa (b-k). Scale bar: 33 μ m

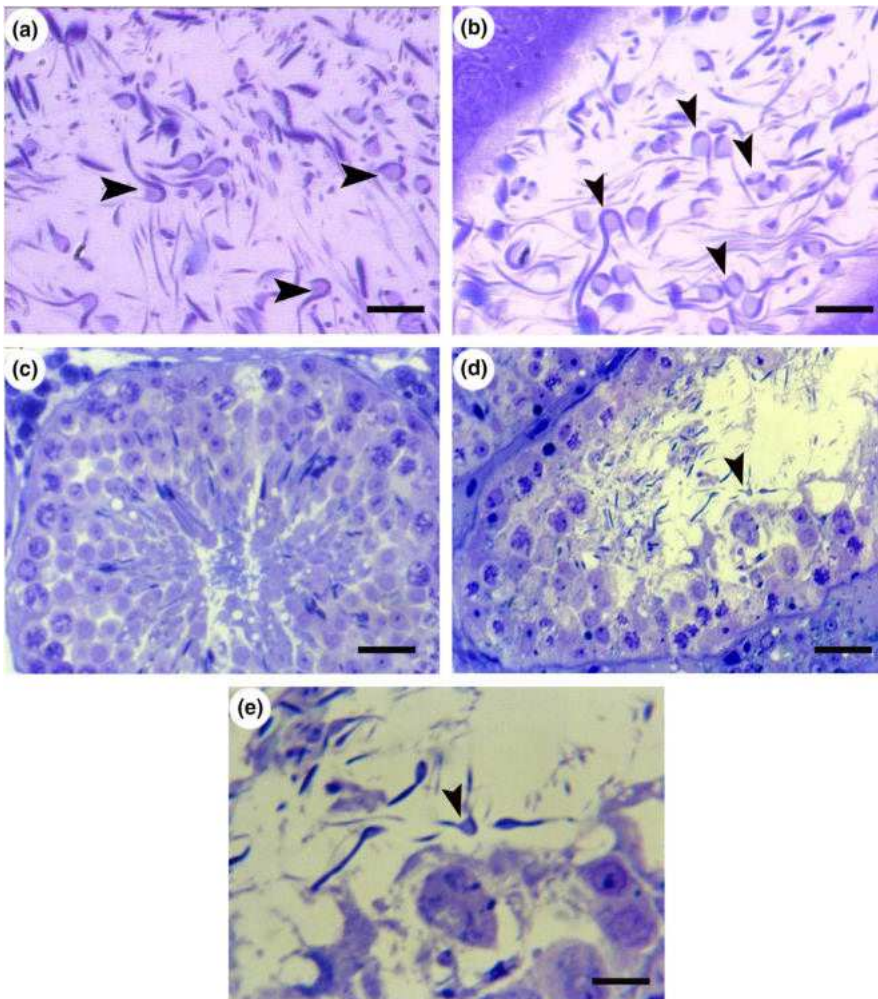
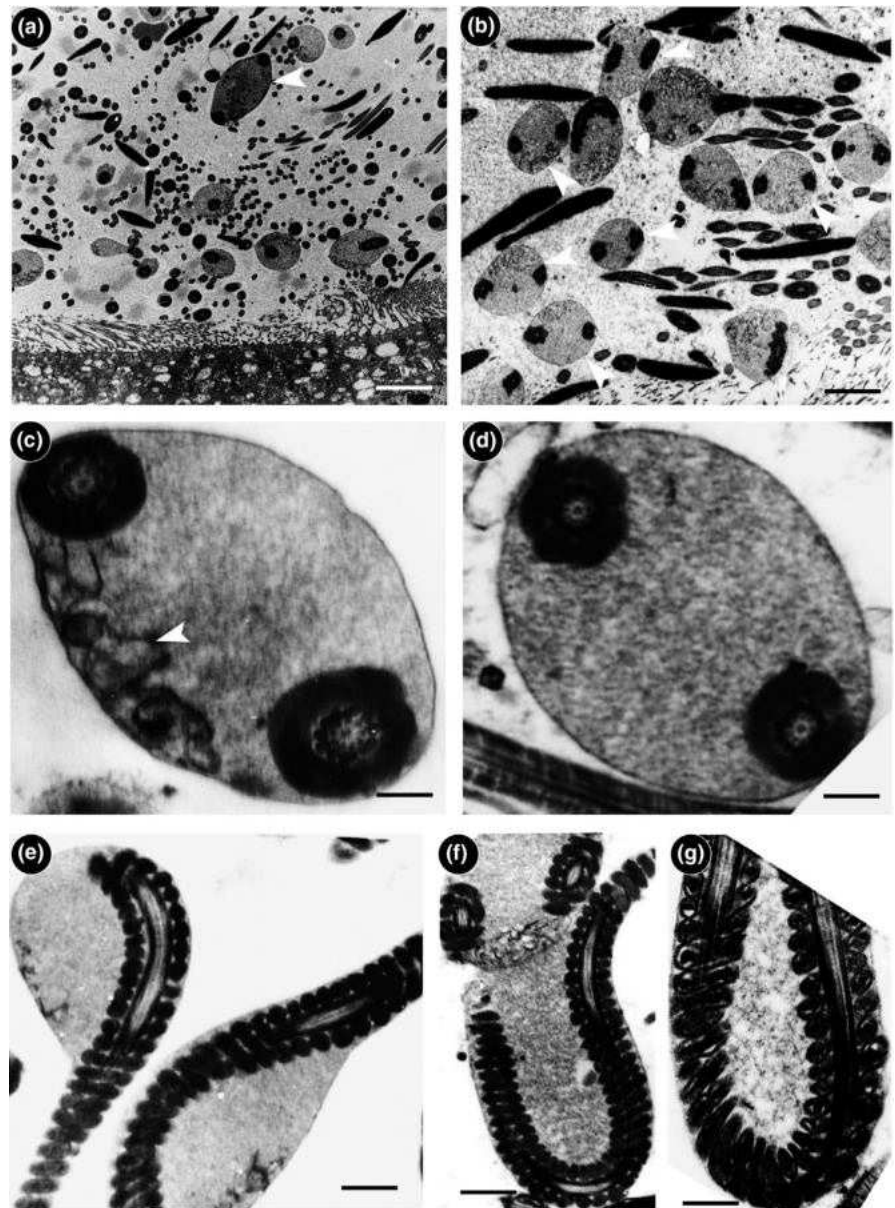


FIGURE 4 TBO-stained semi-thin sections of epididymis/testis of control (c) and QAE-/quassin-treated mice (a, b, d, e). (a): T.S. of cauda epididymidis showing lasso spermatozoa (arrowheads) in the lumen; (b): T.S. of intermediate zone showing lasso spermatozoa (arrowheads) in the lumen; (c): T.S. of seminiferous tubule of control mouse; (d, e): T.S. of seminiferous tubule of treated mouse showing a lasso-shaped spermatozoon (arrowhead). Scale bar (a, b, e): 21 μ m; (c, d): 40 μ m

FIGURE 5 TEM of T.S. at various levels of epididymis of control (a) and QAE-/quassin-treated mice (b-g). (a): Corpus epididymidis of control mouse invariably contains normal spermatozoa, and rarely abnormal ones (arrowhead); (b): T.S. of corpus epididymidis of treated mouse showing spermatozoa with two axonemes (arrowheads); (c): Lasso-shaped spermatozoa contain characteristic membrane elements (arrowhead) in the CD (c) or beyond the level of the membrane elements (d); (e-g): L.S. of the lasso-shaped spermatozoa showing tentative sequence of re-curvature of the flagellum. Scale bar (a): 10 μm ; (b): 6 μm ; (c, d): 1 μm ; (e, f): 2 μm ; (g): 1.2 μm



astheno-zoospermic and/or terato-zoospermic, laboratory animals subjected to experimentation and in some domesticated animals. The spermatozoa with angulated or coiled tail matched the 'Dag-defect' or 'Dag-like defect' reported in cattle, first time reported in bull spermatozoa by Blom (1966). In this case, almost all the spermatozoa had strongly coiled or broken tails (Brito et al., 2010; du Plessis et al., 2014; da Silva et al., 2013; Yeung et al., 1999). The Dag-like defect is a primary abnormality that is common during testicular devolution and is usually inherited, especially in Jersey bulls, in which it is relatively common (Blom, 1966). It has also been found sporadically in most other domestic animals, either as a permanent defect, in which case it is probably inherited transiently as a response of testis to toxic insult. As per the original report, the Dag-like defect in the bull is due to the abnormality of outer dense fibres in the spermatozoon and high zinc content (Blom & Wolstrup, 1976), and has a close relation with CD, but the authors were of opinion

that it arises in the distal caput epididymidis (Koefoed-Johnsen & Pedersen, 1971).

This abnormal sperm morphology matches that observed in the mice fed excess selenium-containing diet (Shalini & Bansal, 2008). In these mice, the authors frequently observed the equidistant cross-sectioned mid-pieces of the tail enclosed in a common cytoplasm, and interpreted this as fusion of sperm tail or double-tailed spermatozoon or as due to the folding of mid-piece region. The light microscopic observations in the present study were corroborated by the TEM evidences showing the spermatozoa with equidistant, cross-sectioned mid-pieces/ midpiece-principal piece of the tail enclosed in a conjoint cytoplasm. Though we also observed double-tailed spermatozoon and spermatozoa fused in pairs (Faisal et al., 2015), the incidence was rare compared to the number of spermatozoa with the above-mentioned abnormal morphology and, therefore, it implies that the section could be of those spermatozoa which are

folded at the region of the CD. Whereas in the case of Se-deficient mice, aside from the above mentioned defects, they had other structural deformities like loose contact of the mitochondrial helix with the outer membrane, mitochondrial aberrations and retention of CD; however, the prevailing deformity was the presence of both mid-piece and principal-piece enclosed in a conjoint plasma membrane (Shalini & Bansal, 2008).

Roughly half of the *azh/azh* mutant spermatozoa were having detached heads and tails (Akutsu et al., 2001) and the same abnormality has also been observed due to the toxicity of quassin (Faisal et al., 2015). Although tail deformities of the epididymal spermatozoa of these mice were categorised into different types, all of them had some sort of angulation and one among them was lasso-like. The sperm tail folding in *azh/azh* deficient mice resulted in the coalition of opposite plasma membranes and ensuing enclosure of tail segments within a common cytoplasm (Mochida et al., 1999). This version has been supported by the TEM images showing equi-distant cross-sectioned mid-piece of tail enclosed in a conjoint cytoplasm of spermatozoa in the epididymal lumen, as reported in the first cases of Dag-like defect (Koefoed-Johnsen & Pedersen, 1971), which was again observed in this study as discussed *vide-supra*. This sperm tail deformity was more often detected in epididymal spermatozoa rather than testicular spermatids and the authors associated these tail deformities to the impaired epididymal sperm maturation. *Alox15* mice lacking the *15Lox* gene also showed similar morphology due to altered epididymal sperm maturation and CD migration (Moore et al., 2010). Taking leads from the previous studies (Rodriguez & Stewart, 2007; Shalini & Bansal, 2008; Varesi et al., 2013), we explicitly make it clear that the problem could originate right in the testis as evidenced in the light micrographs showing lasso spermatozoa in the lumen of seminiferous tubule.

Suzuki-Toyota et al., (2004) reported the appearance of coiling of the tail at the posterior end of GOPC-deficient mice, which in turn would induce other tail deformities during epididymal passage. Though these authors link the problem with the epididymal transit they also agree that there was an issue with the posterior ring after the spermiogenesis, which implies that the problem begins right from the testis. As seen from our data, it could be conceived that the outcome of *Quassia* treatment in this study is comparable to this, since we did notice spermiated spermatozoon in the seminiferous tubules do indicate the manifestation, but the genetic infliction is beyond comprehension.

The water channels, otherwise termed as 'aquaporins' (AQPs) have been conceived as potential role players of sperm volume regulation in view of their highly specialised function in water permeability (Curry et al., 1995). The AQP 3, 7 and 8 are the major AQPs implicated in sperm volume regulation and are expressed in spermatozoon and epididymis/testis of many species (Bonilla-Correal et al., 2017; Ishibashi et al., 1997; Ma et al., 2000). However, knocking out of AQP7 and 8 did not end up in apparent defects in the sperm function (Sohara et al., 2007; Yang et al., 2005). *Aqp3* null spermatozoon is capable of normal motility activation against

hypotonicity but are incapable of proper volume regulation (Chen et al., 2011) and attained a morphology which resembles Dag-like defect and the ones in the present study. The authors attributed this sperm tail bending due to AQP3 deficiency, after entering uterine environment, to the intrinsic defect in response to physiological hypotonic stress. Cooper (2011) also pointed out that retention of CD at ejaculation is a cause of reduced fertility or infertility due to failure of spermatozoa to negotiate the utero-tubal junction environment. Further, the authors suggested that CD, apart from sensing the variations in extracellular tonicity to impart necessary signals required to motility activation, plays a role in filtering out the unqualified spermatozoa by introducing tail deformities that cannot tolerate natural hypotonic stress and thereby assuring fertilisation with a healthy spermatozoon. However, we presume that retention of the CD itself (as discussed *vide-infra*) and the swelling that ensued can be an inherent mechanism in spermatozoa towards a quality control to circumvent any chance of fertilisation with any defective spermatozoon. This view has been supported by the presence of an entire set of protein degradation mechanism in the CD including ubiquitin, all proteasome subunits, P97 and highly abundant cullin 3, apart from the hydrolytic enzymes and their possible role in the decomposition of defective spermatozoa (Au et al., 2015; Varesi et al., 2013). Altogether, it can be reasoned that the occurrence of lasso spermatozoa in the QAE/quassin-treated mice might be most likely due to an impairment of AQP3 or to a certain extent problem in AQP 7/8 induced by the toxicity.

It is envisaged that due to defective spermatogenesis the spermatozoa may fail to respond adequately to the changing luminal microenvironment of the epididymis (Cooper, 2011) and this can lead to inadequate sperm osmolyte loading and impaired RVD. Indeed, if volume regulation fails osmotic entry of water into the CD will not be adequately balanced by RVD, whereupon spermatozoa will develop flagellar coiling or angulation at the site of the CD due to swelling. This has been reported in mouse and bull to cause impaired motility and fertility (Cooper, 2011). In the mouse spermatozoa, visible signs of swelling depend on the presence and absence of CD (Cooper & Yeung, 2003). Therefore, the angulation or coiling of the sperm tail is a well-known consequence of sperm swelling (Yeung et al., 1999). Thus, what we have found in this study can also be ascribed to the deranged osmolyte loading during the ductus epididymal sojourn of spermatozoa or inadequate RVD due to the probable defective spermiogenesis caused by the toxic insults. This view is supported by Barth and Oko (1989) who suggested that the underlying cause for angulated tail might be an abnormal development in spermiogenesis, although abnormal epididymal secretions can also cause the same.

According to Cooper et al., (2004) and Yeung et al., (2006), the angulation and coiling of the spermatozoon from infertile *c-ros* knockout mice arose due to impaired volume regulation ensuing from an altered epididymal milieu. Apart from the altered epididymal milieu, some transgenic mouse models also had coiling and angulation of the flagellum, such as due to CD retention in

Herc4 ^{β -geo/ β -geo} mutant animals (Rodriguez & Stewart, 2007), due to lower osmolality in GPX5-Tag2 (Cooper et al., 2004), due to structural mal-development in the *Gopc*^{-/-} mice (Suzuki-Toyota et al., 2004), due to cytoplasmic retention during spermiogenesis in the *Spem1*^{-/-} mice (Zheng et al., 2007), etc. However, the outcome of the present study is retention of CD by spermatozoa of QAE-/ quassin-treated mice and their angulation when in testis itself. As proposed by Cooper and Yeung (2003) and Cooper et al., (2004), the CD is greatly deviated in its ultrastructure in *c-ros* knock-out mouse as reflected by the appearance of large electron-dense vesicles, and a comparable abnormality was also noticed in the spermatozoa of aflatoxin B1-treated mouse (Agnes & Akbarsha, 2003). Thus, all these perspectives of CD show that there is a possibility of having an additive deleterious effect of QAE/quassin during epididymal transit of spermatozoa cannot be excluded. It was conjectured that once the CD has fulfilled the role of adding P450 aromatase, it has to be removed, as the presence of estrogen in the later segments of the epididymis would be harmful to the maturing epididymal spermatozoa. Cooper (2011) has suggested that epididymal dysfunction, consociated with poor osmolyte loading of spermatozoa prevent timely loss of droplet. Further, the unshed CD is an added burden for spermatozoa and its retention at ejaculation would greatly hamper sperm motility and fertility (Cooper, 2011; Kuster et al., 2004). Therefore, we hypothesise that QAE/quassin treatment interferes with the osmoregulation of the spermatozoa whereupon they swell and the CD increases in its volume and it might have caused the failure to perform its function of aromatisation of P450. As the CD has not yet completed its role, the spermatozoa might not receive the signals to shed the CD and therefore the CD is retained.

Thus, this paper reiterates that the use of *Q. amara* or quassin as a therapeutic in any context would potentially produce serious health effects, particularly male reproductive toxicity, as seen in the development of lasso spermatozoa, affected in morphology and motility. The basic problem in the development of lasso shape of sperm lies in the swelling of the CD, when right in the testis, and the failure in the CD to being shed during epididymal transit. The lasso shape of sperm results due to angulation of the flagellum at the midpiece-principal piece junction caused due to contraction of the swollen CD followed by membrane fusion.


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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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One step preparation of green reduced copper oxide nanorods using *Citrus sinensis* L. peel extracts and evaluation of their photocatalytic degradation of Rose Bengal dye and antibacterial activity

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ABSTRACT

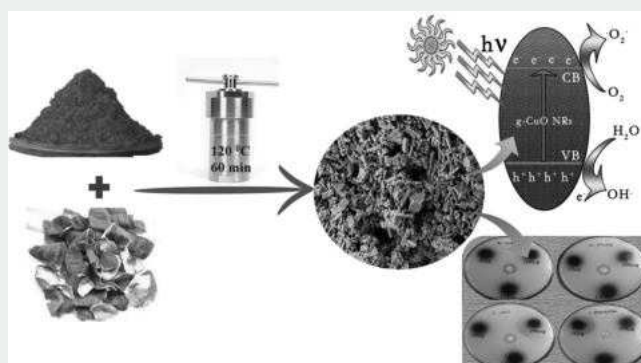
Modern development in nanoscience and nanotechnology has contributed to the varied uses of metal and metal oxide nanoparticles in numerous arena of sciences, research organisations and industries. Among all metal oxides, copper oxide nanoparticles (CuO NPs) has reached more responsiveness as a result of its unique properties and applications. Herein, we report a one-step hydrothermal preparation of a photocatalytically stable copper oxide nanorods (g-CuO NRs) in the presence of *Citrus sinensis* L. peel extracts. The as prepared g-CuO NRs was used for photocatalytic activity in degradation of Rose Bengal (RB) under visible light irradiation. Upon 30 min of visible light irradiation, the g-CuO NRs photocatalyst exhibits an excellent photocatalytic activity (94.5%) for degradation RB. In addition, the g-CuO NRs were further tested for their antibacterial activity against gram-negative *Escherichia coli*, *Klebsiella pneumoniae* and gram-positive *Staphylococcus aureus*, *Streptococcus pneumoniae* bacteria and the test results showed improved antibacterial activity with the increase concentration of g-CuO NRs. The outcome of this work highlights the g-CuO NRs to be an interesting material for treatment of organic dyes and biomedical applications.

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Introduction

The foremost important problem intimidating the existing world is contamination in various ecosystems [1,2]. Owing to the development of textile industries and dye houses, huge amounts of waste discharges are mixed with aquatics. Textile discharges containing a variety of synthetic organic contaminate dyes, posing serious health risks to humans and other living things [3,4]. The dye-polluted wastewater is very hazardous, causing genetically irreversible illnesses such as breast and colon cancer, heart disease, and haemophilia, etc [5–7]. There are many approaches have been applied to address the problem of wastewater contamination [8]. Among others, photocatalysis is an advanced

method for dye degradation due to its simple experi-

mental setup, low cost and eco-friendly nature [9].

Currently, semiconducting properties of the materials have appealed growing consideration for their extensive ecological, attractive photocatalytic, photo-electrochemical, optical and chemical properties such as low toxicity and chemical stability [10–14]. It has been a reliable aim chased by chemists to convert chemicals powerfully with solar energy. Also the biological nanoparticles are prepared with the assistance of medicinal plants as reducing agents and is an attractive investigation field by preparing various types of metal/metal oxide nanoparticles like, calcium, copper,

gold, iron, silver and zinc [15–20]. Among them, CuO NPs have received great attentions in many applications due to its optoelectronic, catalytic, sensing, and superconducting properties [21]. Aside the previously mentioned usage, CuO NPs showed exclusive anticancer, antimicrobial and antioxidant efficacy which renders them a promising tool for biomedical applications. These properties have initiated exhaustive research into photocatalysts and antibacterial for their possible applications in the fields of environmental remediation [22–25].

Different methods available to prepare CuO NPs, namely, sol–gel technique, sonochemical, alkoxide-based route, electrochemical methods, precipitation-pyrolysis, microwave irradiations, solid-state reaction method, thermal decomposition of precursor, etc. However, these existing techniques sometime contain harmful aspects such as contamination with impurities, use of strong acid or strong alkali solutions, or vacuum environments. Therefore, an environmentally benign fabrication process is desired to obtain high-quality products via a low-cost method. Hydrothermal synthesis of the green reduced CuO NPs as a new green technology method has the potential to solve these problems. The synthesis of green assisted copper oxide (CuO) nanomaterial using plants source are eco-friendly, cost-effective and more stable. However, the research going on with this technique is very seldom. The reason green assisted plant extracts work so well in the preparation of nanomaterial is because they act as reducing agents as well as capping agents. Different plant/parts of plant have been exploited for the fabrication of copper oxide NPs are shown in Table 1.

In recent years, many researches have shown promising results on the activity of nanoparticles as a potent antimicrobial substance that can act either individually or combined with other materials [32,33]. Recently, the work on $\text{Ag}_3\text{PO}_4/\text{TiO}_2$ heterostructure exhibited high antibacterial activity towards *E.coli* bacterial strain [34]. Likewise, incorporation of nanocrystalline titania as an integral part of silicone network structure was applied for reduction in *S. aureus* biofilm formation and the mechanism of inhibition of bacterial colonisation is envisaged to involve disruption and puncturing of the bacterial membrane [35]. The antibacterial activities of AgNPs anchored to the thiol-group of functionalised polymer were reported and the results demonstrate the nanohybrid structure play a synergistic role against *E.coli* strain [36]. Similarly, the solar disinfection by photocatalysis to destroy bacteria by W, Nd and Zn doped titania materials were studied. The antimicrobial characteristic of the free radicals was explored to investigate the inhibition of the growth rate of *E. coli* bacteria the results showed that the hydroxide-free radicals have high toxicity for microorganism [37]. The anti-microbial

nanoparticles have been reported for titania-coated nickel ferrite, neodymium-doped titania coated-nickel ferrite and neodymium-doped titania coated-nickel ferrite composite nanoparticles. The doped composite materials acts better activity against the *E. coli* pathogen in comparison with undoped TiO_2 nanoparticles [38–40].

In this work, we report a green route for preparation of g-CuO nanorods photocatalyst using *Citrus sinensis L.* peel extract for the efficient degradation of Rose Bengal (RB) dye under visible light irradiation. Four bacterial strains including two gram-negative *E. coli*, *K. pneumoniae* and two gram-positive *S. aureus*, *S. pneumoniae* microorganisms bacteria were used to investigate the in vitro antibacterial property of g-CuO nanorods. To the best of our knowledge, there is no report of *Citrus sinensis L.* peel extract mediated green synthesis of g-CuO nanorods and application in photocatalytic and antibacterial activity to date.

Experimental procedures

Materials and reagents

All reagents were analytical grade purchased from Sigma-Aldrich and used without further purification. All solutions were prepared with 18.2 M Ω cm deionised Milli-Q water.

Preparation of plant extract

Fresh peel of *Citrus sinensis L.* were collected from local fruit juice shop near Periyar University campus, Salem District, Tamilnadu, India. The peel was properly cleaned with tap water followed by deionised water to avoid dust and other contaminated particles and kept in an oven at a temperature of 60°C for 10 days and then the dried peel were powdered well using a mixer grinder and the powder was stored in dark at ambient temperature. To prepare the aqueous extract, around 30 g of the dried peel powder were taken in a 250 ml of beaker having 150 mL of double distilled water and refluxed at 70°C for 30 min under magnetic stirring. The mixture was then centrifuged (Sigma 2–16PK centrifuge) for 10 min at 7000 rpm and then filtered using Whatman No.1 filter paper. The clear solutions of plant extracts thus obtained were stored in a refrigerator until further use.

Preparation of g-CuO nanorods

The CuO NRs reduced and stabilised with the extract of *Citrus sinensis L.* peel were synthesised following a methodology previously described [41,42]. 1 M of copper nitrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) were dissolved in 20 ml of deionised water. Afterwards, 30 ml of *Citrus sinensis L.* peel extract were added to the solution for

Table 1. Summary of plant-mediated CuO nanoparticles.

S. No.	Plant name	Family	Part used	Phytochemical constituents	Size (nm)	Ref.
1	<i>Punica granatum</i>	Lythraceae	Peel extract	Alcohol, phenols, amide, and othe molecules containing carbonyl group	40	[26]
2	<i>Tribulus terrestris</i>	Zygophyllaceae	Fruit extract	Alkaloids, flavonoids, tannins, ascorbic acid and phenolic compounds	5–22	[27]
3	<i>Cordia sebestena</i>	Boraginaceae	Flower extract	Phenol, flavonoid, tannin, phytosterol, coumarin, fumaric acid, a-tocopherol	20–35	[28]
4	<i>Hibiscus rosa sinensis</i>	Malvaceae	Flower extract	Tannins and phenolic proteins, triterpenoids, 2, 3-hexanediol, nHexadecanoic acid, 1,2-Benzenedicarboxylic acid and squalene.	12	[29]
5	<i>Eucalyptus globulus</i>	Myrtaceae	Leaf extract	Phenols, terpenoids, flavonoids and tannins	5–24	[30]
6	<i>Pterolobium hexapetalum</i>	Fabaceae	Leaf extract	Phenols, flavonoid, terpenoids, tannins, alkaloids, carbohydrates, and glycosides	10–50	[31]

the reduction of copper ions. The pH value 7.0 adjusted for the mixture by the addition of NaOH (1 N) solution. Then, binary mixture was heated at 120°C in a sealed flask for 1 h in the hotplate for 60 min and kept at room temperature. The reaction showed colour change from green to dark brown solution. The prepared CuO NRs were separated after centrifugation at 5000 rpm for 1 h and the product was re-dispersed by ultrasonication for 15 min and then dried in an oven for 3 h. Finally, they were calcinated at 300°C for 5 h and the final product was stored at room temperature for further characterisation and applications studies.

Characterisation techniques

The crystal phase of the as-prepared CuO NRs photocatalyst was carried out using X-ray diffraction (RIGAKU Powder X-Ray Diffractometer) and the data were collected in the 2θ range of 10 to 80°. Scanning electron microscope (SEM, JEOL, JSM-5600) and a transmission electron microscope (TEM, FEI, Tecnai G20) were performed in order to investigate the size, size distribution and morphology of the synthesised nanoparticles. The EDX spectroscopy was conducted using Oxford 6587, Oxford Instruments. FTIR measurements were carried out, in a range of 4000 to 450 cm^{-1} by Frontier FTIR, PerkinElmer, USA. UV-Vis Diffuse reflectance spectroscopy (UV-Vis DRS) was recorded in the range 200–800 nm with a Perkin Elmer Lambda 25 spectrometer. The solid-state photoluminescence measurements were carried out using a spectrofluorimeter (Varian, Cary Eclipse Fluorescence Spectrophotometer).

Photocatalytic degradation of methylene blue (MB) dye

The photocatalytic activity of the synthesised green assisted g-CuO NRs catalyst was evaluated through photodegradation of Rose Bengal (RB) dye solution under solar light illumination. In order to assess the degradation process, 30 mg of the catalyst was dispersed in 50 mL of RB solution under ultrasonication for 1 min. Before illumination, the suspension mixture was gently agitated for 30 min in dark in order to achieve adsorption-desorption equilibrium of the dye with the catalyst. Afterwards, the resulting solution was kept under direct sunlight. At given intervals, 3 mL of the suspension was withdrawn and centrifuged to remove the dispersed catalyst powder (Note that the change in dye concentrations was used to determine the adsorption level of dye in dark.). The concentration of the clean transparent solution was determined by using UV-Vis spectrophotometer (Shimadzu, UV-2450). The concentration of

remaining dye after photocatalytic experiment was determined by measuring the absorbance of dye. The photocatalytic efficiency rate was calculated using the relation degradation (%) = $[(C_t - C_0)/C_0] \times 100$. Where C_t and C_0 are the concentrations of RB dye after time 't' and at the start, respectively.

Antibacterial activity

The antibacterial activities of g-CuO NRs were assessed by agar well diffusion method [9,43]. Four different human pathogenic bacterial strains such as gram positive *Staphylococcus aureus*, *Streptococcus pneumoniae* and gram negative *Escherichia coli*, *Klebsiella pneumonia* bacteria strain were used as the test microorganisms. The tested bacterial species were obtained from microbial type culture collection centre, Chandigarh, India. A set of three concentrations (50, 100 and 150 $\mu\text{g ml}^{-1}$ in sterile distilled water) of the prepared nanorods were added to the, respectively, labelled wells. The zone of inhibition (ZOI) around the well were measured after 24 h.

Results and discussion

XRD analysis

The crystal structure of the g-CuO NRs was determined by X-ray diffraction (XRD). As evident from Figure 1, all diffraction peaks were intense which indicates the synthesised g-CuO NRs was highly crystalline. The typical diffraction peaks were well matched with the standard data of CuO (JCPDS Card No. 10-0399). All diffraction peaks were assigned to monoclinic phase. It is notable that different diffraction peaks corresponding to the planes (120), (220), (040), (230), (201), (240), (150), (041), (250) and (200), respectively. The presence of carbon is due to the acids, alcohols, proteins and many other phytochemicals that already exists in the plant extracts.

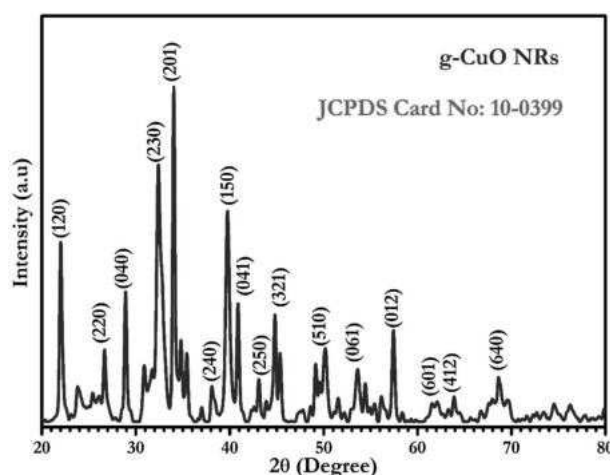


Figure 1. XRD patterns of green synthesised g-CuO NRs.

Furthermore, the crystallite size of g-CuO NRs is determined using Debye–Scherrer's formula [44]. The lattice parameters obtained for synthesised g-CuO NPs were $a = 9.502$, $b = 11.97$, and $c = 3.240$. The calculated crystallite size of g-CuO NRs was been found to be 34 nm.

Morphological studies

The morphology, structure, and size of the g-CuO NRs were investigated using FE-SEM and HR-TEM. As shown in Figure 2(a-c), as-obtained g-CuO has a rod-shape and irregular structures with particle size range from 60 to 80 nm. Also, observed, the images indicated that the NPs were both agglomerated and mono-dispersed with almost spherical morphologies. Such variation in particle shape and size distribution is associated with the chemical composition of the *Citrus sinensis* L. peel extract. Furthermore, elemental distributions of the green assisted nanorods was analysed by EDX spectroscopy. From the EDX spectrum (Figure 2 (d)), the elemental compositions were recorded, which they are confirming the presence of Cu, O and C elements in the g-CuO NRs. No other signals were observed, indicating the good purity of g-CuO NRs. The TEM images of the g-CuO NRs at different magnifications are shown in Figure 3. TEM micrographs (Figure 3(a-e)) reveals that the g-CuO are rod-like structure with average size of 65 nm. Figure 3(f) shows the representative selected area electron (SAED) pattern for g-CuO NRs achieved with the peel extract. SAED patterns show various diffraction rings of monoclinic g-CuO. The ring pattern shows that these g-CuO NRs are randomly oriented, and the strong intensities reveal the high crystallinity of the sample. All of the obtained rings in SAED pattern may be attributed to the diffraction peaks of monoclinic structure of g-CuO NRs indicating that the obtained nanorods are in the pure phase of CuO, supporting with the XRD results.

Fourier transform infrared (FTIR) analysis

FTIR spectroscopy was performed to identify the presence of functional groups on the surface of g-CuO NRs. The FTIR spectrum (Figure 4) showed main characteristic bands at 3401, 2971, 1608, 1400, 1272, 1056, 568 and 499 cm^{-1} respectively. The FTIR spectrum of plant extract assisted g-CuO NRs shows that the broad absorption band at 3401 cm^{-1} attributes to the hydroxyl (OH) functional group in alcohols and phenolic compounds. These bands are attributed to the protein residue present on the surface of NPs after synthesis process. In the present work, we assumed that the proteins present in the leaf extract was responsible for the reduction acetate group of the metal salt to NPs and acted as stabilising and capping agents for g-CuO NPs. The small band at approximately

2971 cm^{-1} is due to $\text{O} = \text{C} = \text{O}$ stretching vibration. Band at 1608 cm^{-1} can be assigned to aromatic bending of alkene group ($\text{C} = \text{C}$). The absorption peak around at 1272 cm^{-1} may be correspond to $-\text{OH}$ bending vibrations of $\text{Cu}-\text{OH}$. The main characteristic band of g-CuO at 1400 and 1056 cm^{-1} are assigned to $\text{C}-\text{O}$ stretching vibrations. The peaks at 568 and 499 cm^{-1} are indicated that the formation of characteristic stretching vibrations in monoclinic $\text{Cu}-\text{O}$.

Optical spectroscopy

In order to determine the light absorption properties and bandgap energy of the prepared g-CuO NRs, UV-Visible diffuse reflectance spectroscopy (UV-Visible DRS) was performed in the range of 200 to 800 nm as showed in Figure 5(a). The presence of molecules such as proteins in the leaf extract was responsible for the reduction of Cu metal salt to form g-CuO NPs. The UV-Vis spectrum of the g-CuO NRs dispersed in water exhibiting the maximum absorption peaks at about 380 nm. In the spectrum, the peak at 380 nm are due to surface plasmon absorption of metal oxide. The surface plasmon absorption in the metal oxide nanoparticles is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. This type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter. Surface plasmon absorption band with a maximum at 380 nm indicates the formation of g-CuO NRs.

Energy band gap is usually attained from optical edge of absorption spectrum, which is nothing but the minimum energy necessary to excite an electron from the maximum occupied molecular orbital to the minimum unoccupied molecular orbital. From the absorption spectrum the band gap energy was found using Tauc's plot formula [45], $\alpha h\nu = A(h\nu - E_g)^n$. Where h , ν , α and C are Planck's constant, photon frequency, absorption coefficient and a constant, respectively. By extrapolating the linear region of $(\alpha h\nu)^2$ versus the photon energy axis the optical band gaps can be estimated. Figure 5(b) shows expected band gap energy and it was found to be 1.87 eV. This banned band arrangement induced fresh energy level in the band gap of g-CuO NRs.

Figure 5(c) shows the PL excitation and emission spectrum of g-CuO NRs. The PL property is a potent instrument to examine the optical properties of the metal oxides semiconductor, particularly the PL emission properties, owing to most of applications of optoelectronic devices. The PL property was influenced by the formation, particle size and morphology of the g-CuO NRs. Generally, the reported emission spectrum of g-CuO NRs consists of many bands in the range 200–700 nm. The spectrum showed UV as well as visible emissions, exhibit UV emission peak at 300 nm and visible emission peak in the violet region

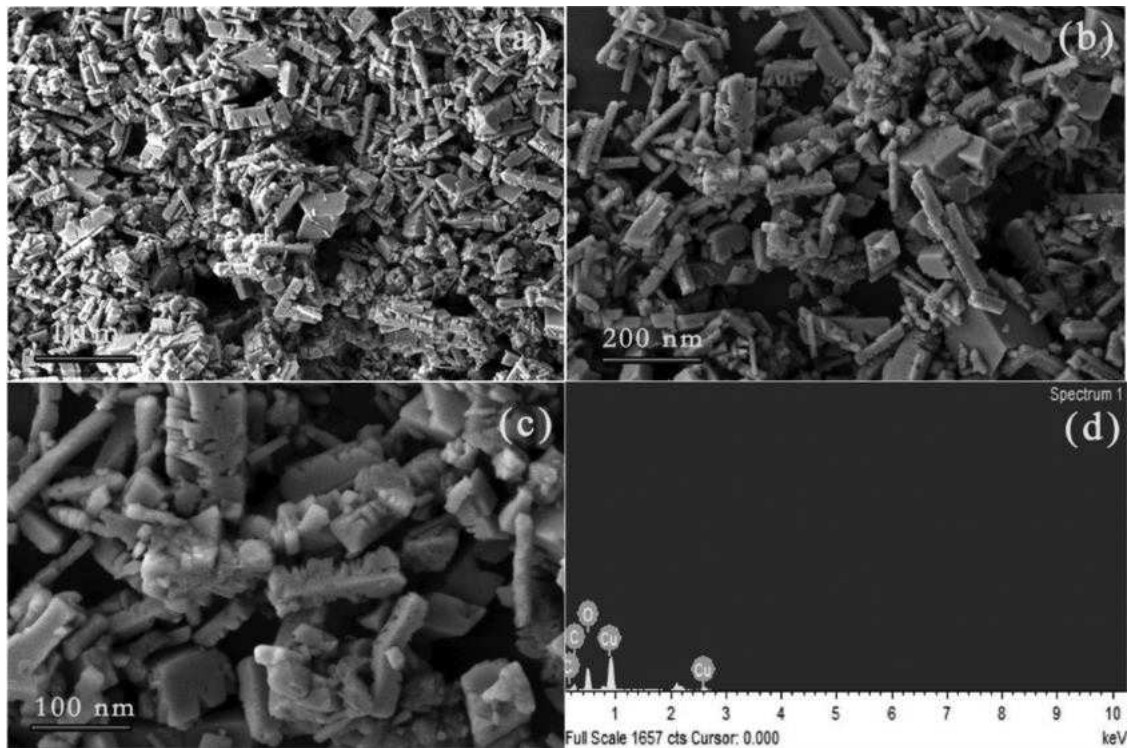


Figure 2. (a-c) FE-SEM images and (d) EDS spectrum of g-CuO NRs.

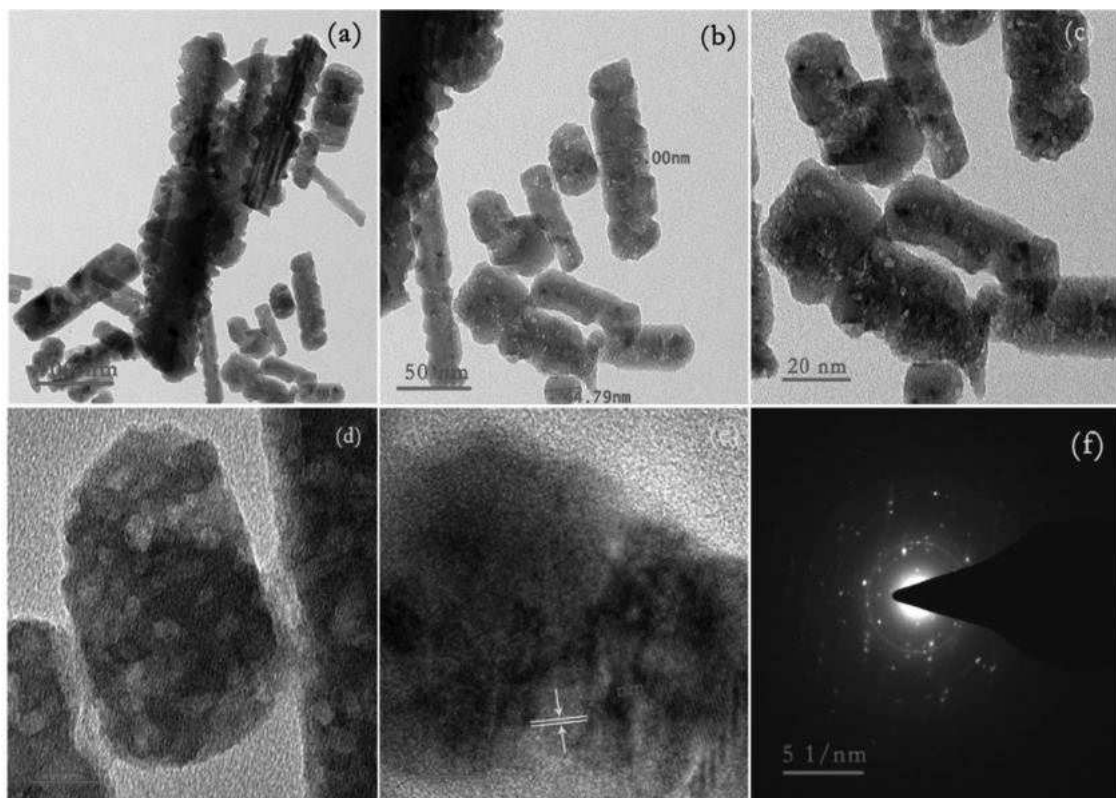


Figure 3. (a-e) HR-TEM images and (f) SAED pattern of g-CuO NRs.

(400 nm), respectively. The origin of UV emission in g-CuO NRs is owing to the recombination of electron-hole pair in free-excitons [46]. The photoluminescence blue bands at 413 nm were investigated by transition vacancy of oxygen and interstitial oxygen.

This photoluminescence spectrum is useful for understanding the electron transition energies within g-CuO NRs through determining the emission peaks and using them to estimate the corresponding electronic energy levels.

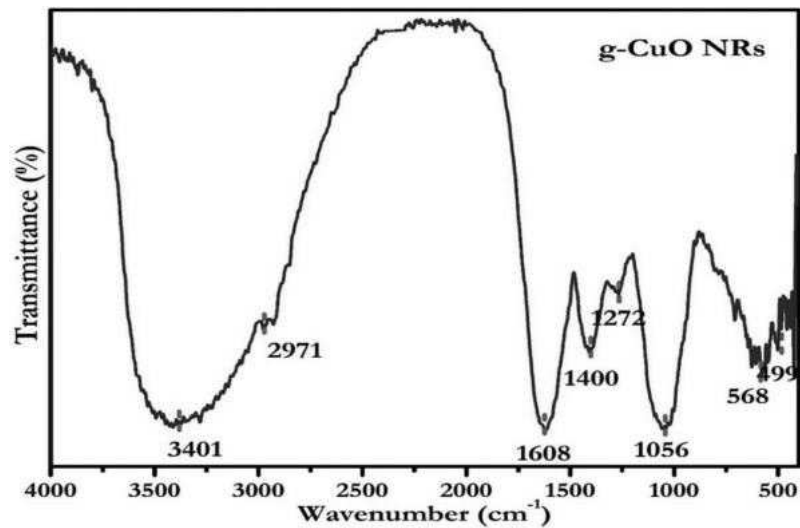


Figure 4. Fourier transform infrared spectroscopy (FTIR) spectrum of g-CuO NRs.

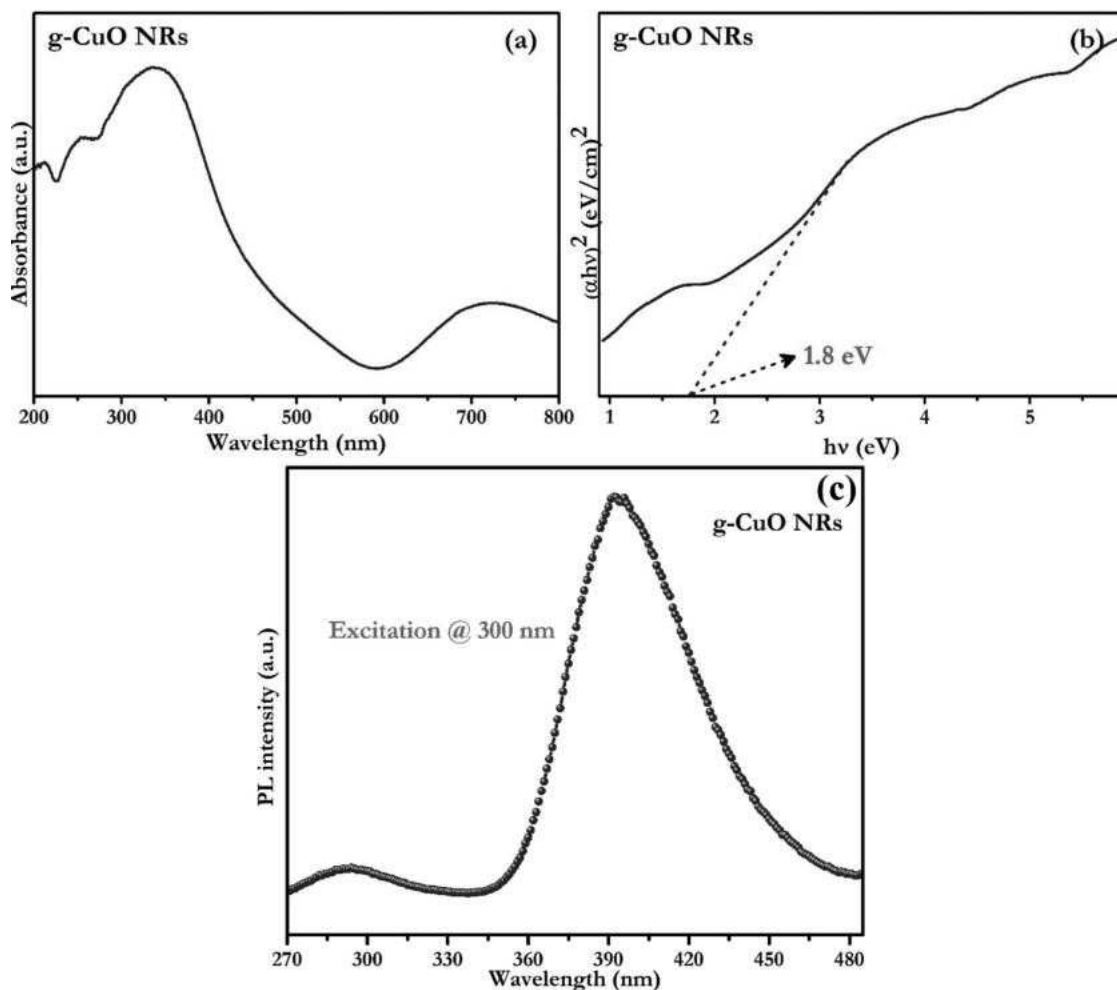


Figure 5. (a) UV-vis diffuse reflectance spectrum, (b) band gaps and (c) Photoluminescence Spectrum of g-CuO NRs.

Photocatalytic studies

To highlight the potential catalytic efficiency of green-assisted g-CuO NRs towards environmental remediation and elude the wastewater contaminant,

photocatalytic tests of Rose Bengal (RB) aqueous solution was investigated under sunlight irradiation (Figure 6(a-c)). Under optimal research circumstances, normally 50 mL sample of wastewater containing 0.1 g of g-CuO nanophotocatalyst and

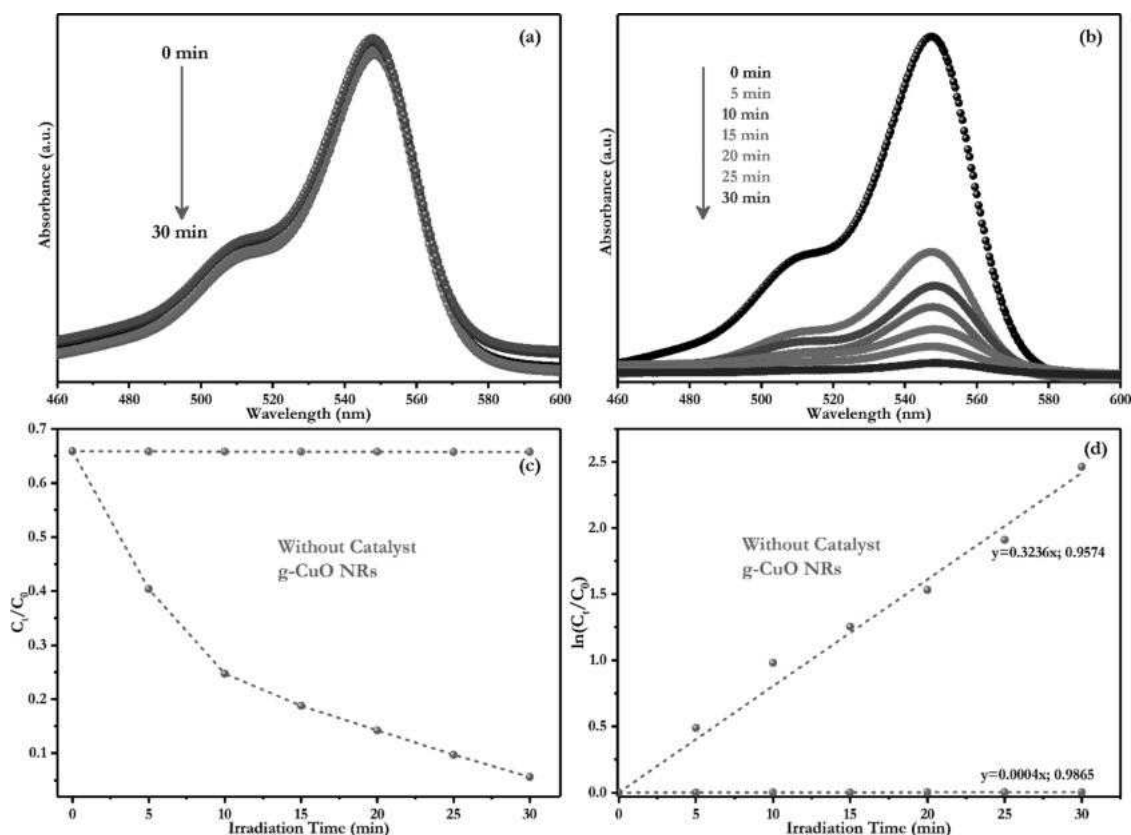


Figure 6. UV-vis spectra of photodegradation of RB dye using (a) Blank and (b) g-CuO NRs at different sunlight exposure time, (c) Photodegradation efficiency curves of RB degradation using without and with g-CuO NRs, (d) Plots of $\ln(C_t/C_0)$ versus irradiation time for the photodegradation processes of RB using without and g-CuO NRs.

10 ppm of RB was placed into the beaker. Prior to illumination, the suspensions were magnetically stirred in the darkness for half an hour to reach the adsorption/desorption equilibrium. After sunlight irradiation for interim times, the suspension was tested and the degradation process of RB was examined through a UV-Vis spectroscopy. The control experiment displays that the absorbance of RB is extremely slow without photocatalyst (Figure 6(a)). The synthesised g-CuO NRs from pyroreduction exhibited photocatalytic activity against RB dye and this dye degradation demonstrated that the nanorods of bio-synthesised g-CuO could efficiently degrade 94.5% within 30 min under sunlight irradiation (Figure 6 (b)) indicating that the RB is stable enough to be the probe for the investigation of the photocatalytic properties of the as-prepared photocatalysts. Usually, photocatalytic degradation processes are carried out only in wastewater because the radicals can only react with the RB dye dissociated. Therefore, the degradation of dye is likely related to their dissociation degree of RB dye in wastewater.

Furthermore, the kinetics of dye degradation was estimated and the results are shown in Figure 6(d). The kinetics models of the pseudo-first-order model were tested to determine the kinetics rate in the degradation process of RB dye onto the g-CuO NRs and are commonly expressed as the following equation, $\ln(C_t$

$/C_0) = kt$. Where k is the photodegradation rate constant (min^{-1}) and C_0 and C_t are the concentrations (mg/L) of dye after self-photolysis and at different irradiation times, respectively. The results showed linear curve fit between $\ln(C_t/C_0)$ and reaction time t of different dyes follows a pseudo-first-order kinetics behaviour [47,48]. The correlation coefficients (R^2) were higher than 0.9576, which indicates that the photodegradation of dyes fits well with the kinetic model. The rate constants of without and g-CuO NRs are 0.0004 and 0.3236 min^{-1} , respectively.

A recycling test was conducted for RB degradation in order to check the stability of g-CuO NRs for practical application. In this experiment, the removed photocatalyst after photodegradation process was washed with water and acetone for five times followed by drying at 100°C and then used for the further cycle. The results of recycling experiments are presented in Figure 7. The as-prepared g-CuO photocatalyst showed relatively higher photocatalytic activity in photodegradation of RB after four repeated cycles of reactions. That is to say, the photocatalytic activity changes little from the first time to the fourth time. The slight decrease of photocatalytic activity after four cycles was probably due to a small loss of g-CuO NRs from the samples during the photodegradation and recycling process.

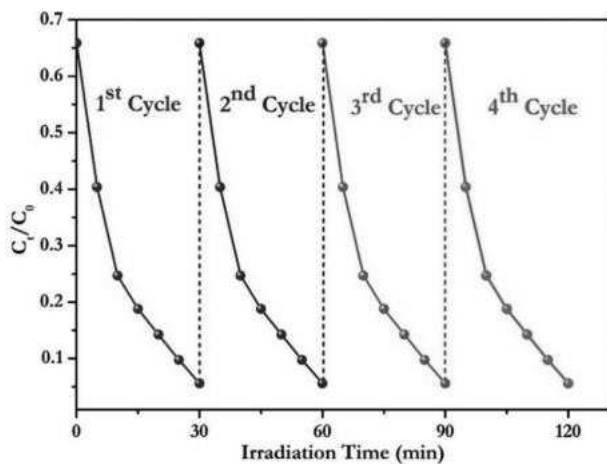
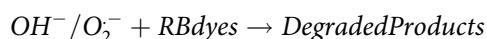
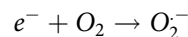
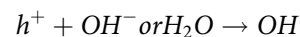
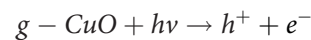


Figure 7. Recycled photocatalytic activity of g-CuO NRs for degradation of RB dye under sunlight irradiation.

In general, photocatalytic activity is a quite difficult behaviour that contains numerous steps: (i) generation of the electron-hole pairs (related to photon absorption), (ii) separation and diffusion of the charge carriers to the active surface sites, (iii) reaction of holes with defects or with the RB on the surface-solution interphase, and (iv) electron scavenging. Hence, the perceived different quantum efficiencies could be owing to the dissimilar periods of the electron-hole pairs generated by the photons interrupting on the g-CuO catalysts. Based on g-CuO NRs results, a probable reaction mechanism of g-CuO NRs function in the photocatalytic elimination of RB is depicted in Figure 8. Under sunlight irradiation, electrons transfer from valence bond (VB) to the conduction band (CB) in the illuminated g-CuO semiconductor is led to electron-hole pair formation which in turn generates superoxide radical anion (O_2^-) and hydroxyl radicals ($\cdot OH$) in the presence of H_2O and O_2 molecules [49]. The relevant reaction formulas are shown as follows:



Antibacterial activity

The antibacterial activities of g-CuO NRs was studied with two pathogens of gram-negative *E.coli*, *K. pneumoniae* and two gram-positive *S. aureus*, *S. pneumoniae* bacteria. Figure 9 shows the zone of growth inhibition on bacteria due to the effect of g-CuO NRs for three different concentrations and the diameters of inhibition zone (in mm) are tabulated in Table 2. Increase in the size of zone of inhibition was noticed for all bacteria tested with increasing concentrations of g-CuO NRs indicating that the g-CuO NRs is a potent antimicrobial agent carrying more capability to kill the tested organisms. The maximum growth inhibition zones for *S. aureus* and *S. pneumoniae* are 10.30 and 9.24 mm at the concentration of $150 \mu g ml^{-1}$, whereas inhibition zones for gram-negative *E. coli* and *K. pneumoniae* are 9.22 and 10.36 mm, respectively. It can be observed that, the g-CuO NRs exhibited better antimicrobial activity for *S. aureus* and *K. pneumoniae* pathogens as compared to that of *S. pneumoniae* and *E. coli* bacterial pathogens and it can be concluded that these nanorods are more active against both gram-positive and gram-negative bacterial strains. The effective antibacterial activity for g-CuO NRs was due to the adherence of nanosize of g-CuO on the bacteria membrane, degrades lipopolysaccharide molecules, causes large increases in membrane permeability, and then enters inside the bacterial cell, resulting in DNA damage and leads to bacterial cell death.

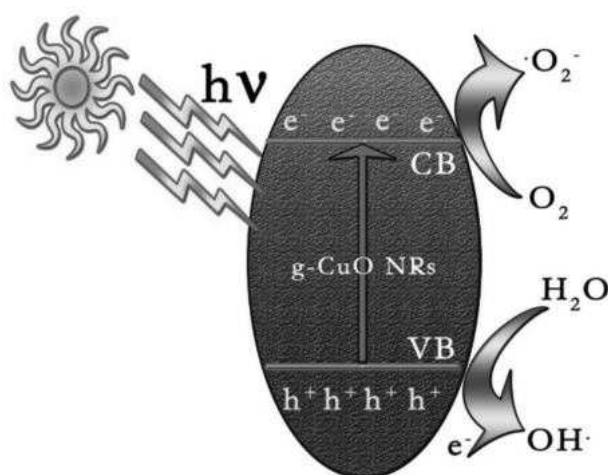


Figure 8. Possible mechanism for photocatalytic degradation of RB dye using g-CuO NRs.

Conclusion

The g-CuO nanorods were prepared in a facile manner and characterised using various techniques and the results confirmed that the formation of highly pure and well-dispersed g-CuO NRs with average particle size of 60–80 nm. The as-obtained nanorods exhibited better photocatalytic activity in degradation of RB under sunlight irradiation. Moreover, it was demonstrated that the g-CuO NRs was stable enough to be recycled several times. Furthermore, g-CuO NRs were used to study the antibacterial activity for gram-positive and gram-negative bacteria that have shown better results at three different concentrations. Therefore, the green prepared g-CuO nanorods may find

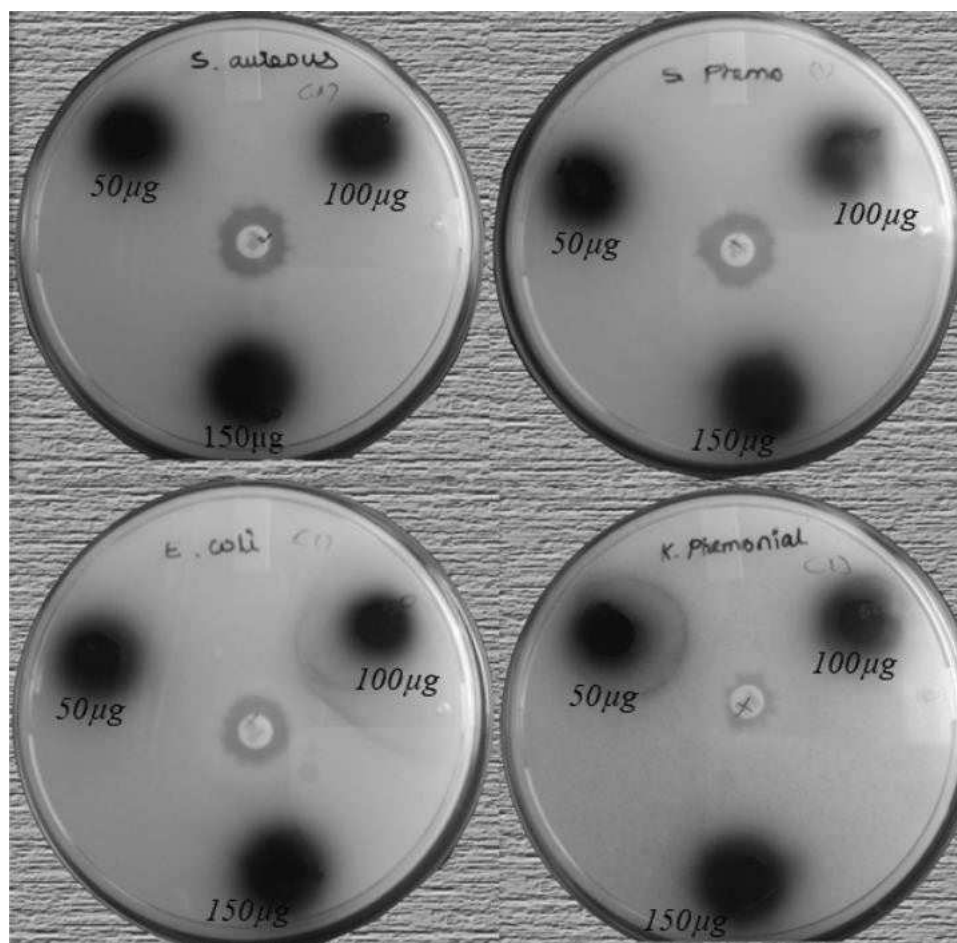


Figure 9. Growth inhibition of gram-positive (*S. aureus* and *S. pneumoniae*) and gram-negative (*E. coli* and *K. pneumoniae*) bacteria strains caused by g-CuO NRs.

Table 2. Diameters of the inhibition zone of as-prepared g-CuO NRs.

Bacterial strains	Zone of inhibition (in mm) g-CuO NRs (Average of three measurements).		
	Concentration of the g-CuO NRs		
	50($\mu\text{g ml}^{-1}$)	100($\mu\text{g ml}^{-1}$)	150($\mu\text{g ml}^{-1}$)
<i>S. aureus</i>	7.21 \pm 0.19	8.45 \pm 0.24	10.30 \pm 0.14
<i>S. pneumoniae</i>	6.36 \pm 0.43	7.50 \pm 0.52	9.24 \pm 0.51
<i>E. coli</i>	7.45 \pm 0.75	7.11 \pm 0.42	9.22 \pm 0.26
<i>K. pneumoniae</i>	8.81 \pm 0.11	9.81 \pm 0.13	10.36 \pm 0.53

promising applications in wastewater treatment and various biomedical applications.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the author(s).

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EXTRACTION, ISOLATION, CHARACTERIZATION, *IN-SILICO* MOLECULAR DOCKING AND ANTICANCER EVALUATIONS OF SOLANOPUBAMINE FROM *SOLANUM PUBESCENS* WILLD

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ABSTRACT

The present work deals with the extraction, isolation, characterization, *in-silico* molecular docking and anticancer activity of Solanopubamine from leaf explants of *Solanum pubescens*. The extraction was carried out by Soxhlet apparatus using DMSO as solvent. The DMSO leaf extracts were purified by column chromatography. The functional groups and structure of the isolated Solanopubamine was confirmed by Fourier transforms infrared spectroscopy (FT-IR), ¹H-Nuclear magnetic resonance spectroscopy (¹H-NMR) and high performance liquid chromatography (HPLC). The isolated Solanopubamine was separately docked into two different active sites of EGFR and ER α . The docking results by the interaction with EGFR, showed only two hydrogen bond interactions; (i) the side chain hydrogen atom of the negative charged residue of ASP 776 were well interacted with oxygen atom (O-H) of Solanopubamine with bond length of 1.996Å and (ii) hydrogen atom (N-H) of Solanopubamine molecule were well interacted with hydrophobic residue of LEU 694. The results showed that the isolated Solanopubamine compound is potential for anticancer activity. *In-vitro* anticancer activities of the isolated Solanopubamine were screened against human breast cancer cell line (MCF-7) using MTT assay. The IC₅₀ values were found to be 86.33µg/ml. The present study reveals that the isolated Solanopubamine shows a significant *in-vitro* anticancer activity against MCF-7 cells and also it is less toxic for human cells.

Keywords: Solanopubamine, Alkaloid, ¹H-NMR, HPLC, MCF-7.

1. INTRODUCTION

Solanum pubescens belongs to the family Solanaceae and very closely related to the Turkey berry (*Solanum torvum*). *Solanum pubescens* is commonly known as Kaattusundaikaai in Tamil (Fig.1). It is a medicinally useful plant and is particularly known for its therapeutically bio-active compound; alkaloids. A variety of compounds are reported in *S. pubescens* and some of them are myricetin methyl ethers [1] Solanopubamine [2] and solanopubamides A & B [3]. The tribal people used *S. pubescens* plants for the treatment of liver disorders, diarrhoea and cancer disorders [4], however, there is no report on the anticancer activity research in this plant.

Cancer is a major cause of death and breast cancer is one of the common malignancies leading to death in women around the world. The disease occurs almost entirely in women, but men are also prone to it. Radiation is a well documented risk factor for breast cancer and its exposure induces the formation of free radicals.

As a result, novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions [5]. The three dimensional structure of the protein ligand composite could be served as a considerable source of understanding the way of interaction of proteins with one another and the performance of their biological functions.

Computational homology based on the modeling methods, is the best and fast technique to predict the protein structure from a known 3D template when there is no solved structure [6]. Historically virtual screening of the drug development process is divided into the structure and ligand-based algorithms [7].

The functional groups and structure of the isolated Solanopubamine was confirmed by FT-IR, ¹H-NMR and HPLC. The isolated Solanopubamine was separately docked into two different active sites of EGFR and ER α . The docking results showed that the isolated Solanopubamine compound is potential for anticancer activity.

Finally, the *in-vitro* anti-cancer activity of isolated Solanopubamine from *Solanum pubescens* against breast

cancer cell line (MCF-7) was confirmed.



Fig. 1: *Solanum pubescens* plant a) Plant habit; b) Fruits and Flower.

2. MATERIAL AND METHODS

2.1. Collection and authentication of plant materials:

The aerial parts of *Solanum pubescens* were collected from Sirumalai hills, a part of the Western Ghats region, Dindigul district, Tamilnadu. The species was authentically identified and compared with herbarium specimen available in BSI, Coimbatore, and Tamilnadu. Specimen No: BSI/SRC/5/23/2017/Tech/2985.

2.2. Preparation of Plant Extracts

The leaves of *S. pubescens* were dried in hot air oven at 40-50°C for a week. The dried plant material was powdered using mixer grinder and subjected to soxhlet extraction with 99% methanol for 24 hours.

2.3. Column chromatography

Solanopubamine was isolated from the condensed extracts using Column chromatography and TLC and structure was confirmed by FT-IR, ¹H NMR and HPLC. Separation of Solanopubamine was carried out using petroleum ether as a solvent, as this particular extract showed four different spots. The separation of Solanopubamine from the extracts was carried out by column chromatography [8].

The column was packed by using wet packing technique with silica gel (300 g) as the adsorbent. Slurry was prepared using hexane and the slurry was poured in to the column. Fractions were concentrated and TLC was performed [9].

The R_f value of the spots were calculated using the formula (1)

$$R_f = \text{Distance traveled by solute} / \text{Distance traveled by solvent (1)}$$

2.4. Characterization:

2.4.1. FT-IR

Fourier transforms infrared (FT-IR) spectroscopy (Nicolet 6700 Thermo Fisher) was used to identify the functional groups present in the isolated sample (Solanopubamine) and the scanning rate was recorded in the range of 400-4000 cm^{-1} .

2.4.2. ¹H NMR

¹H-NMR analysis of the isolated Solanopubamine was performed on Varian Mercury 300 (300 MHz for ¹H) and Varian Inova 750 (750 MHz for ¹H) instruments (Agilent Technologies, Palo Alto, CA), equipped with a 5 mm probe.

2.4.3. HPLC

Isolated *Solanopubamine* were subjected onto the HPLC analysis using Shimadzu Model LC2010 AHT Auto Sampler (UV-Vis Detector).

2.5. Cell Lines and Cytotoxicity

MCF-7 cells was obtained from the National Centre for Cell Science (NCCS), Pune, India, and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-anti-mycotic solution (10000U per ml penicillin, 10,000 $\mu\text{g}/\text{mL}$ streptomycin and 25 $\mu\text{g}/\text{mL}$ amphotericin B in the culture medium). The cells were maintained as monolayer in a 96 well microplate at a density of 4 x 10⁴ cells in 100 mL of culture medium,

and incubated overnight at 37°C under 5% CO₂ atmosphere. The cell lines were maintained at 95% humidity in a CO₂ incubator and the cultures were allowed to grow till 80-90% confluency before drug treatment. Solanopubamine were loaded in each well at a final concentration of 10, 20, 40, 80, 160 and 320 µg/mL. After incubation for 24 and 48 h, unreacted and treated cells were washed with 1% Phosphate-Buffered Saline (PBS). After that, 10 µL of 12 mM MTT stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide was added to each well and incubated for 3h. Then the medium was carefully removed without disturbing the formazan crystals, and the intracellular purple formazan crystals were dissolved in 100µL of acidified isopropanol. The plates were read for measurement of absorbance at 590 nm.

2.6. Molecular Docking

2.6.1. Preparation of Ligand Structure:

Chemdraw assistance has been taken to sketch the plant isolated molecules (<http://www.cambridgesoft.com>). The sketched was then prepared by Ligprep 2.7 [10] where 2D structure gets organized into 3D structure and different tautomers were also the result of Ligprep 2.7. Ligands were ionized at a pH range about 7.0 ± 2.0 to preserve the qualities for molecular docking [11].

2.6.2. Qik Prop

QikProp [12] is a quick, accurate, easy-to-use absorption, distribution, metabolism, excretion and Toxicity (ADMET) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

2.6.3. Selection of target protein EGFR and ER α

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals and some funding agencies such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (*i.e* secondary) databases that categorize the data differently. For example both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize struc-

tures based on genes.

Prior to perform any molecular interaction study, preparing target molecule is significant. The crystal structure of cancer protein EGFR (PDB ID: 1M17) and ER α (PDB ID: 3ERT) were retrieved from protein data bank [13, 14]. Maestro is Schrodinger's powerful united multi-platform Graphical User Interface. Import a ligand/protein co-crystallized structure, typically from the Protein Data Bank, into Maestro. Locate any waters you want to keep then delete all others. Determine whether the protein-ligand complex is a dimer or other multimer containing duplicate binding sites and duplicate chains that are redundant. Adjust the protein, metal ions, and cofactors. If there are bonds to metal ions, delete the bonds, then adjust the formal charges of the atoms that were attached to the metal as well as the metal itself. Set charges and correct atom types for any metal atoms, as needed. Set bond orders and formal charges for any cofactors, as needed.

Finally the protein structure energy was minimized reached until the average root mean square deviation non bonded hydrogen atom 0.30Å [15], the prepared protein was input file for molecular docking. Grid files represent physical properties of a volume of the receptor (specifically the active site) that are searched when attempting to dock a ligand. The complex for this exercise is actually in two files, one containing the receptor and one containing the ligand. In the Van der Waals radii scaling section, we choose Scaling factor default value of 1.00 (no scaling). The grid box X=3.524, Y= 3.241, Z= -1.342 was set for protein.

2.6.4. Examining Glide Data

Glide is employed for the rapid docking of plant isolated molecules into the active site of the target receptor. Grid was prepared for protein with the exact same center and the size of the bounding box set on 30Å the Glide algorithm is operates with a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using funnel type approach and follows a unique scoring method. Glide score and glide energy was analyzed using XP visualizer [10, 12]. Glide results are examined with an emphasis on visual rather than numerical appraisal. The set of exercises uses the Glide XP Visualizer panel to display information on the terms in the Glide XP scoring function that contribute to the ligand binding. From the Glide Docking window panel we can view docked complex can analyses with its parameter for PI3K and generated conformers with ligands.

3. RESULTS AND DISCUSSION

3.1. Isolation of Solanopubamine

Column chromatography of plant extract sample endowed with number of fraction. The alkaloids isolated from leaf extracts of *Solanum pubescens* were subjected to a detailed evaluation. The chromatographic evaluation indicated that the isolated compound from *Solanum pubescens* was pure as a single spot was obtained in TLC, whereas the compound from *Solanum pubescens* was found to be alkaloids. The range of sample R_f value on 5.8 cm, 4.3 cm and 0.74, have been perfectly correlated with Compound exhibited positive response for Dragendorff's reagent which as indicates that the isolated compound was an alkaloid. The melting point of these compounds was found to be 157-158°C.

3.2. Functional group confirmation by FT-IR spectroscopy

FT-IR spectroscopy is a useful tool for the identification of presence/absence of functional groups in the isolated Solanopubamine and its obtained spectrum is shown in Fig.2. From the results, the sample contained a broad band which appeared at 3370 cm^{-1} indicates the hydroxyl stretching (-OH); the band at 2949 cm^{-1} is attributed to N-H (stretching vibration) group frequency. A band which appeared at 1113.99 cm^{-1} was attributed to C-N stretching vibration. This FT-IR study confirms the presences of functional groups such as hydroxyl, amino and methylene group in the prepared compound. The observed result matched very well with the literature values [16]. The functional groups corresponding to their wavelength were listed in table 1.

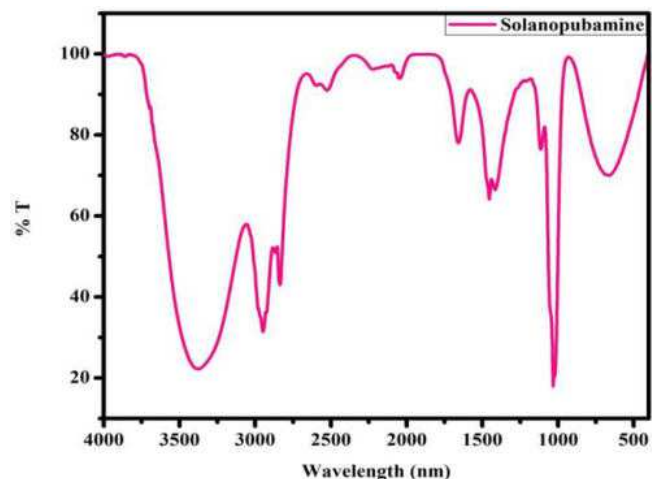


Fig. 2: FT-IR characterization of isolated compound (Solanopubamine)

Table 1: FT-IR characterization of isolated compound

Wavelength in (cm^{-1})	Name of the functional group	Functional group
3370	Alcohol	O-H
2949	Alkane	C-H
2835	Alkane	C-H
2866	Alkane	C-H
2525	Acid	O-H
2221	Alkyne	-C≡C-
2025	Alkenes	-C=C
1665	Alkene	C=C
1453	Aromatic	C=C
1414	Aromatic	C=C
1113	Amine	C-N
1031	Ether	C-O
657	Alkyl halide	C-C

3.3. Structural conformation of Solanopubamine by $^1\text{H-NMR}$

$^1\text{H-NMR}$ spectra were recorded using DMSO- d_6 solvent and the $^1\text{H-NMR}$ spectrum of isolated Solanopubamine presented in Fig.3. According to the literature survey, the proton NMR signal was assigned (ref). Ring A: 1.44 (1H, m, 2-H), 1.54 (1H, m, 2-H), 1.70 (1H, m, 4-H), 1.14 (1H, m, 4-H), 1.70 (1H, m, 1-H), 1.09 (1H, m, 1-H); 3.34 (1H, m, 3-H); Ring B: 1.30 (2H, m, 6-H), 1.91 (1H, m, 7-H), 1.23 (1H, m, 7-H); Ring A/B: 1.26 (1H, m, 5-H); Ring C: 1.54 (1H, m, 11-H), 1.44 (1H, m, 11-H); 1.98 (1H, m, 12-H), 1.63 (1H, m, 12-H); Ring B/C: 0.69 (1H, m, 9-H), 1.57 (1H, m, 8-H); Ring D: 1.95 (2H, m, 15-H); Ring C/D: 1.44 (1H, m, 14-H); Ring E: 2.27 (1H, m, 20-H); Ring D/E: 1.91 (1H, m, 17-H), 2.73 (1H, m, 16-H); Ring F: 2.80 (1H, m, 26-H), 2.22 (1H, dd, $J = 3.5$ Hz, 7.1 Hz, 26-H), 1.54 (1H, m, 24-H), 1.95 (1H, m, 24-H), 4.1 (1H, m, 23-H); Ring E/F: 2.18 (1H, m, 22-H); Methyl: 0.83 (3H, s, 19-H) 0.87 (3H, s, 18-H), 0.98 (3H, d, $J = 5.58$ Hz, 21-H), 1.23 (3H, d, $J = 7.3$ Hz, 27-H); the observed $^1\text{H-NMR}$ spatral valus were matched with the repots suggested by Adnan *et al*, 2013[17]. The outcome of this reports which us similaer to our report.

Positive HRESI-MS: found for $\text{C}_{27}\text{H}_{47}\text{N}_2\text{O}$: 1.39 (1H, m, 11-H); 1.82 (1H, m, 1.61 (1H, m, 8-H); Ring D: 1.84 (2H, m, 15-H); Ring C/D: 1.24 (1H, m, 14-H); Ring E: 2.24 (1H, m, 20-H); Ring D/E: 1.57 (1H, m, 17-H), 2.81 (1H, m, 16-H); Ring F: 2.78 (1H, m, 26-H), 2.06 (1H, dd, $J = 3.5$ Hz, 7.4 Hz, 26-H), 1.61 (1H, m, 24-H), 1.08 (1H, m, 24-H), 3.85 (1H, m, 23-H); Ring

E/F: 1.85 (1H, m, 22-H); Methyl: 0.90 (3H, s, 19-H) 0.97 (3H, s, 18-H), 0.99 (3H, d, J = 6.5 Hz, 21-H), 1.26 (3H, d, J=7.5 Hz, 27-H); and ¹³C NMR (CDCl₃; dC): Ring A: 34.50 (C-2), 37.90 (C-1), 41.10 (C-4), 51.80 (C-3); Ring B: 29.60 (C-6), 32.20 (C-7); Ring A/B: 36.70 (C-10), 46.30 (C-5); Ring C: 22.10 (C-11), 37.80 (C-12); Ring B/C: 36.60 (C-8), 55.60 (C-9); Ring D: 28.0 (C-15); Ring C/D: 42.40 (C-13), 58.60 (C-14); Ring E: 31.90 (C-20); Ring D/E: 63.50 (C-17), 71.0 (C-16); Ring F: 28.60 (C-25), 33.30 (C-24), 59.70 (C-26), 67.40 (C-23); Ring E/F: 79.90 (C-22); Methyl: 12.60 (C-19), 17.40 (C-18), 18.70 (C-21), 22.10(C-27); 12-H), 1.61 (1H, m, 12-H); Ring B/C: 0.76 (1H, m, 9-H), [17].

Proton NMR spectroscopy was used for the confirmation of structure of isolated compound and it shows the presence of 50 hydrogens in the compound [18]. The aromatic protons are observed, and the five methoxy groups were also prominent ¹H NMR (300 MHz, DMSO-d₆) δ [ppm]: 8.02(2H,d,H2',H6'); 7.16 (2H,d,H3',H5'); 6.77 (1H,s,H3); 4.02 (3H,s,OCH₃); 3.97-3.78 (12H,m, 4xOCH₃) [19].

3.4. High Performance Liquid Chromatography (HPLC)

The quality control parameter studies clearly indicate the purified Solanopubamine. The total alkaloid fraction was also subjected to HPLC studies and results were presented in Fig.4. The qualitative HPLC profiles were detected at a wavelength of 254 nm due to sharpness of

the peaks and proper baseline and recorded its percent area and heights. The Fig.4 shows one major peak at 3.725 retention time. The major peak at 3.725 retention time confirmed the presence of Solanopubamine. However, there is a need to carryout advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of active bio compound of this plant in the future [20].

3.5. Biological Activities

3.5.1. In silico Anticancer analysis

The crystal structure of EGFR (PDB ID: 1M17) and ER α (PDB ID: 3ERT) was recovered from protein data bank. The plant isolated chemical compounds Solanopubamine were separately docked into active site of the EGFR and ER α . The crystal structure of the 3D docking simulation of Solanopubamine with EGFR, the following two residues were mainly involved in hydrogen bond interaction: (i) EGFR ASP 776 with bond length (1.996Å) and (ii) hydrophobic residue of LEU 694. Fig. 5(a) shows that the side-chain protonated oxygen atom (O-H) well interacted with negatively charged residue of ASP 776 and nitrogen atom (N-H) interacted well with hydrophobic residue of LEU 694. Fig. 5(b) shows the 3D docking simulation of Solanopubamine into ER α formed only one hydrogen bond interaction. The side chain hydrogen atom (O-H) interacted well with negative charged residue of ASP 351 with a bond length of 1.994 Å.

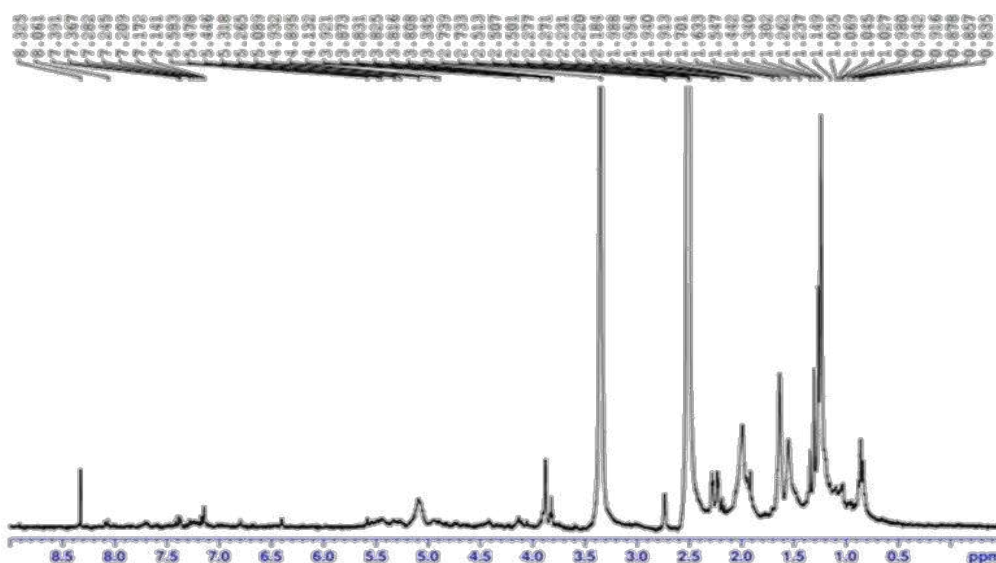


Fig. 3: ¹H-NMR Spectra of isolated compound Solanopubamine

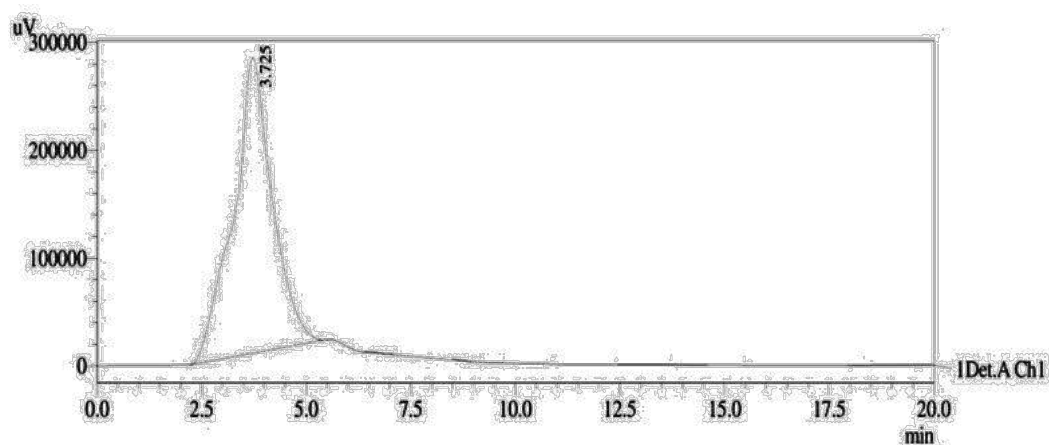


Fig. 4: HPLC- Spectra Conformation of Solanopubamine compound

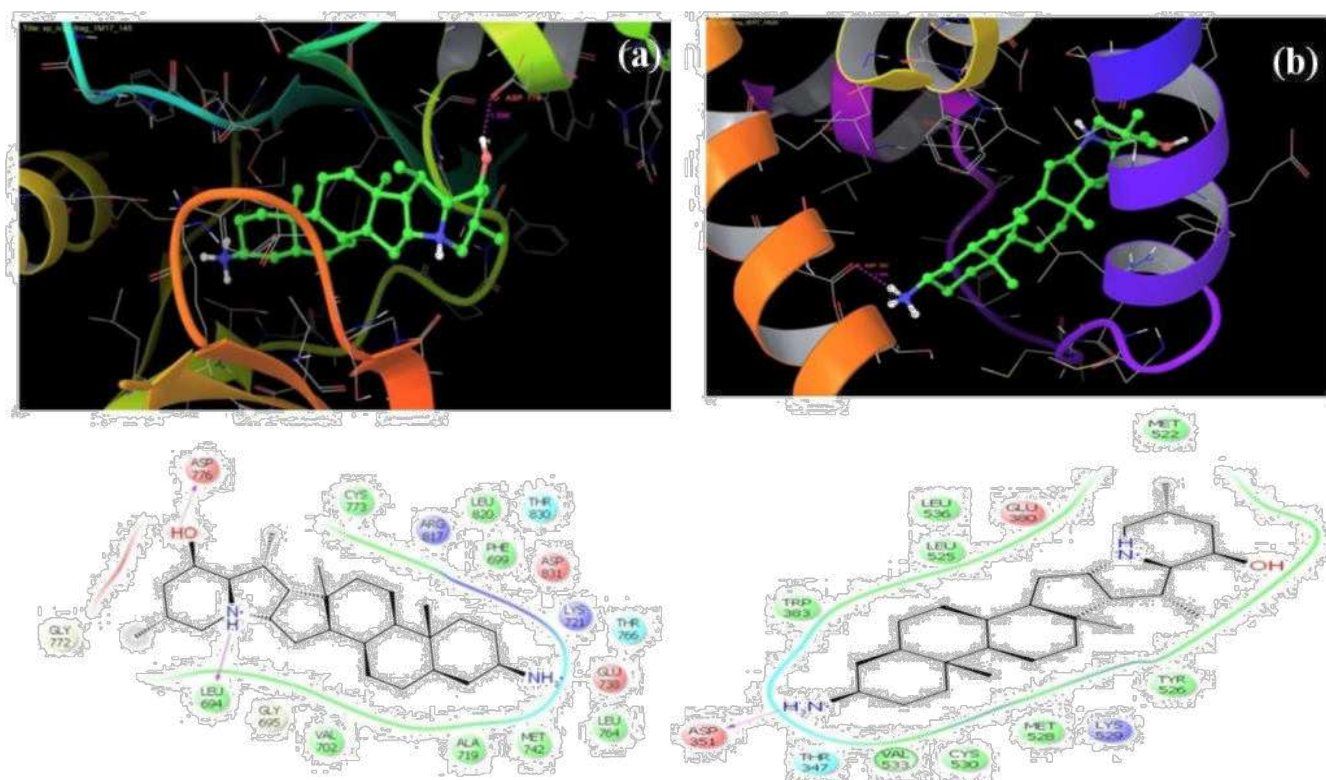


Fig. 5: 3D and 2D Docked structure of Solanopubamine target with (a) EGFR (PDB ID: IM17) protein and (b) ER α (PDB ID: 3ERT) Protein.

Interestingly, the residues TRP 383, LEU 536, MET 522, MET 528, CYS 530, CYS 773, LEU 820, MET 742 were mainly involved in hydrophobic interactions with the active site of the ER α and EGFR. The glide score and glide energy of the compounds were, as reported in Table 2. The glide score of EGFR and ER α are -4.421Kcal/mol, and -5.152Kcal/mol respectively, and the glide energy of EGFR and ER α are -30.13Kcal/mol

and -32.61Kcal/mol respectively. The observed docking simulation was matched with the reports suggested by Bari *et al*, 2012 [21].

3.5.2. Anticancer activity

In vitro exposures of MCF-7 cells with different concentrations (10, 20, 40, 80, 160 and 320 μ g/ml) of Solanopubamine DMSO extracts significantly sup-

pressed MCF-7 cancer cell growth and decrease in cells count was observed with increase in concentration of the extracts tested. A dose dependent increase in cytotoxic activity for all the concentrations was observed. The maximum inhibition of 90.3 % of MCF-7 cells was observed at a concentration of 320 $\mu\text{g}/\text{ml}$ of the plant extracted tested. *In-vitro* anticancer activity of isolated Solanopubamine is shown in Fig. 5, 6 and Table 3. The isolated Solanopubamine were shown IC_{50} value of 86.33 $\mu\text{g}/\text{ml}$. Anticancer drugs with minimal side effects on normal cells are highly desirable for therapeutic purposes [22] hence; the current study also emphasized that the efficiency of TC DMSO extract-

mediated suppression of cell viability on cancer cells. Indeed the concentrations of DMSO extract of TC that was cytotoxic to human breast cancer cells failed to induce apoptosis cell death.

The main aim of analyzing crude plant extracts is either to isolate bioactive agents for direct use as drugs or to identify bioactive compounds that can be used as lead substances in the preparation of semi synthetic drugs [23]. A large number of novel anticancer drugs have been discovered from natural products in the past and new ones are continually being developed thereby improving their efficacy or reducing their toxicity.

Table 2: Docking score of Solanopubamine were retrieved by Glide.

Protein name	PDB ID	Compound Name	Glide Score	Glide Energy	No. of Hydrogen bond interaction	Interacting residue	Distance (\AA)
ER α	3ERT	Solanopubamine	-5.152	-32.61	1	ASP 351 (Aspartate)	1.994
EGFR	1M17	Solanopubamine	-4.421	-30.13	2	ASP 776 (Aspartate) LEU 694 (Leucine)	1.996 -

Table 3: Determination of cytotoxicity of Solanopubamine on MCF-7 cell line by MTT Assay

Cell line: MCF 7				
Sample	Conc. ($\mu\text{g}/\text{ml}$)	OD @ 590nm	%Inhibition	IC_{50}
Control	control	0.69	0.0	
Solanopubamine	10	0.65	6.3	86.33 $\mu\text{g}/\text{ml}$
	20	0.60	12.5	
	40	0.50	27.1	
	80	0.30	46.0	
	160	0.18	74.3	
	320	0.07	90.3	

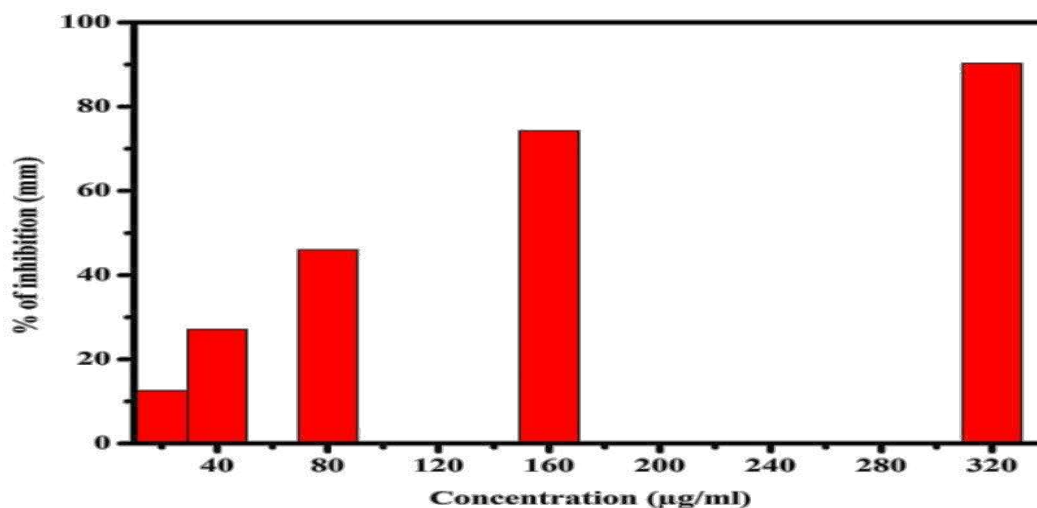


Fig. 6: Percentage (%) of Inhibition activity of isolated Solanopubamine compound with different concentrations (10-320 $\mu\text{g}/\text{ml}$)

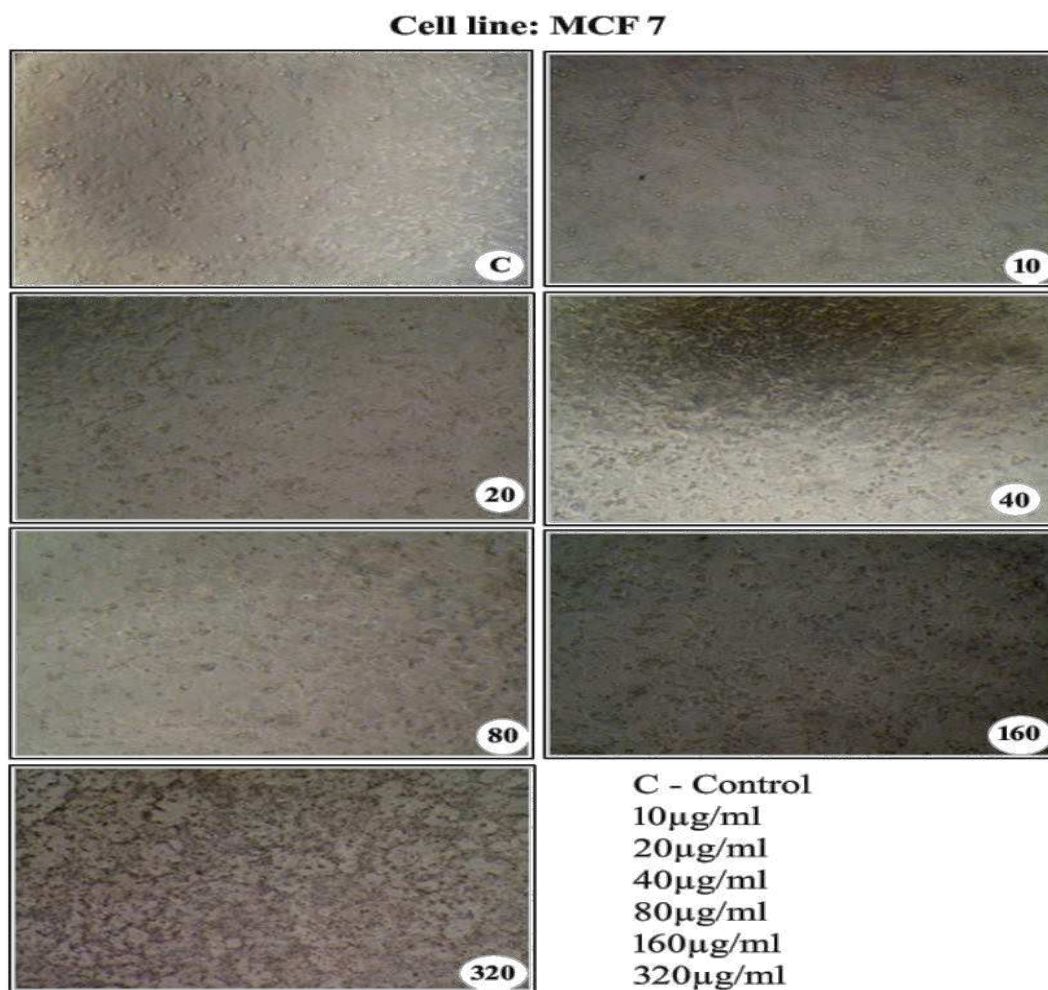


Fig. 7: In-vitro anticancer activity of isolated Solanopubamine compound with different concentrations (10-320 µg/ml)

4. CONCLUSION

On the basis of preliminary characterization studies, the isolated Solanopubamine compound has good physical and chemical properties (colour, state, solubility, melting range and R_f value) which are identical to the standard Solanopubamine. Spectral data shows that the isolated compound is mostly similar to standard Solanopubamine. IR peaks of various functional groups of Solanopubamine are found in this isolated compound. 50 protons were found in the $^1\text{H-NMR}$ spectra of isolated compound. So, it can be concluded that the isolated compound has the molecular formula $\text{C}_{27}\text{H}_{47}\text{N}_2\text{O}$ which corresponds to the molecular formula of Solanopubamine. The above results confirmed that the isolated compound is Solanopubamine. The isolated Solanopubamine was separately docked into active site of the EGFR and ER α . Further, the isolated Solano-

pubamine showed a significant *in-vitro* anti cancer activity against MCF-7 cells.

Conflict of interest

No potential conflict of interest was reported by the authors.

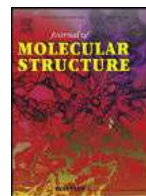
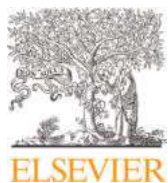
5. ACKNOWLEDGEMENT

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New monomeric mixed-ligand complex of iron(III)-3-chloropyridine: Synthesis, structure, luminescence, electrochemical and magnetic properties

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ABSTRACT

The monomeric aquobis(3-chloropyridine)tri(chloro)iron(III) 3-chloropyridine complex, $[\text{Fe}^{\text{III}}(\text{3-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3]\cdot\text{3-Clpy}$ is synthesized and characterized using spectroscopic techniques, single crystal X-ray diffraction, magnetic susceptibility and cyclic voltammetry techniques. The structural and spectroscopic features help to identify the formation of the mixed-ligand high spin complex. The theoretical calculation to determine the electronic, magnetic and optical properties were performed by Density Functional Theory using VASP calculation. The variable temperature magnetic susceptibility indicates the presence of a paramagnetic iron(III) center in the complex. Electrochemical studies of the complex with cyclic voltammetry showed cathodic peaks corresponding to a Fe^{III} to Fe^{II} reduction. To the best of our knowledge, this is the first report on the synthesis and characterization of monomeric iron(III)-3-chloropyridine complex.

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1. Introduction

The rich diversity of chemistry of metal complexes provides exciting prospects for the design of a number of coordination compounds with valuable characteristics. In many cases, transition metal ions and their complexes play a central role in controlling the reactivity and mechanism of the chemical reactions of interest. Research concerning the coordination chemistry of Fe(III) has intensified in recent years due to its important role in numerous chemical and biological systems [1–3]. The structural models for active sites of various iron-containing enzymes have added a wealth of knowledge to our understanding of various aspects of iron chemistry with respect to structural, electrochemical and magnetic properties [4,5]. The unique features of mononuclear iron(III) complexes need clarification to understand the structural and functionalities in chemistry, biology and medicine [6]. Design of the pyridine framework of iron(III) complexes containing water and chloride ion results in substantial weakened metal–ligand in-

teraction, which causes bond length and bond angle distortions. Moreover, mixed ligand complexes were used in moderating surface implantation of metal ion [7], by creating an appropriate potential gradient for electron transfer to occur and as electrochemical response unit in heterogeneous photocatalysis [8]. However, the mechanism of interaction between the iron(III) center with the solid surface remains obscure.

Recently, we have reported [8–11] on the emission, electrochemical and ferromagnetic behaviors of monomeric cobalt(III), iron(III), nickel(II) and zinc(II) complexes with a series of alkyl or aryl amine ligands. Photoluminescence, magnetic and electrochemical studies in the organometallic framework is an interesting area of research. Moreover, a great deal of development has been reported in the molecular magnetism of mononuclear or polynuclear transition metal complexes [12]. However, studies on the magnetic behavior of monomeric iron(III) complexes are limited. Hence, it is interesting to figure out the temperature dependent magnetic behavior of prepared iron(III) complex.

To the best of our knowledge hetero ligand complexes of iron(III) with heterocyclic aromatic amine and two different coordinated ligands are rare [13]. A mixed ligand can be a synthetic challenge to tune the physicochemical properties of the transition metal complexes [14,15]. There are relatively few reports

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available on iron with mixed ligands complexes [16]. The $[\text{Fe}^{\text{III}}(3\text{-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3]\cdot 3\text{-Clpy}$ (1) crystal has an interesting and unique crystalline and molecular structure. Herein, the structure, electronic, luminescence and magnetic properties of complex 1 have been investigated by various instrumental techniques and Density Functional Theory (DFT).

2. Experimental

2.1. Materials

Iron(III)chloride hexahydrate (97%), 3-chloropyridine (3-Clpy) (99%) and KBr (spectral grade) were purchased from Sigma Aldrich. All other chemicals were obtained from S. D. Fine chemicals (India). All the chemicals were at least analytical grade and used with no further treatment. Solvents were triply distilled and purified before use.

2.2. Preparation of complex 1

A mixture of extra-pure samples of 5.4 mg (2 mmol) $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$, 2.82 mL (5.7 mmol) 3-chloropyridine (3-Clpy) and 20 mL ethanol/chloroform (10 %v/v) were taken in a 50 mL round bottom flask with cork. The contents were stirred for 30 min to produce a deep reddish orange solution, which was then kept in the dark. Orange colored microcrystalline solid separated for a period of ten days, which was isolated *via* suction filtration and washed with a small amount of methanol. The product was repeatedly recrystallized in 15 mL of 70% 3-Clpy/ethanol binary solvent and the resulting solution was filtered *via* gravity. The crystals were washed with cold ethanol, dried and preserved in desiccators containing phosphorus pentoxide.

Complex 1; Recrystallized from 70% 3-Clpy/ethanol to give orange colored crystals, yield 3.85 mg, 74%. Repeated recrystallization ensures purity upto 99.9%. *Anal. Calc.* for $\text{C}_{15}\text{H}_{14}\text{OFeN}_3\text{Cl}_6$: C, 34.59; H, 2.71; and N, 8.07%. Found: C, 34.56; H, 2.69; and N, 8.05%. FTIR (KBr disc, cm^{-1}): 3634(b), 3538(b), 3167(sh), 2999(w), 2944(w), 2316(sh), 2292(m), 2249(m), 1708(sh), 1632(m), 1573(sh), 1466(m), 1443(s), 1427(s), 1410(s), 1367(w), 1035(m), 965(w), 933(w), 924(w), 901(w), 895(w), 871(w), 852(s), 828(m), 816(m), 798(m), 774(s), 738(s), 706(s), 698(s), 670(m), 653(m), and 615(s). Raman shift (cm^{-1}): 80, 216, 301, 369, 424, 480, 604, 634, 736, 808, and 1024. UV vis λ_{max} (ϵ_{max} $\text{M}^{-1}\text{cm}^{-1}$) in acetonitrile (32.78 μM): 360 (7657), 311 (7321), 265 (15863), 240 (14948), and 210 (19737).

2.3. Physical measurements

Elemental analyses were performed on Thermo Scientific FLASH 2000 Organic Elemental Analyzer. The FTIR spectral investigations in the range 4000–400 cm^{-1} were made using KBr pellet on a Thermo Nicolet-6700 FTIR instrument. Raman spectral data were collected on the Jobin Yvon Horibra LABRAMR1100 micro-Raman spectrophotometer. Electronic absorption spectral studies were undertaken on a Shimadzu 2450 double beam spectrophotometer with quartz cells using prepared complex (~150 μM) in acetonitrile. Steady-state fluorescence emission was recorded on the Spex FluoroLog-3 spectrofluorometer (Jobin-Yvon Inc.) using 450 W xenon lamp and equipped with a Hamamatsu R928 photomultiplier tube. The instrument works on the principle of time-correlated single-photon counting technique. Time-resolved fluorescence decay measurements were carried out using 295 nm nano-LED sources for excitation. The photons were collected from the front face of the sample with TBX-4-X single-photon-counting detector. The fitting analysis was performed using the commercially available DAS6 v6.2-Horiba Jobin Yvon.

X-ray crystal data were collected on an Oxford Diffraction Xcalibur Diffractometer using graphite monochromated Mo- $K\alpha$ radiation ($\lambda = 0.71073$ Å). A suitable crystal with size 0.20 mm \times 0.15 mm \times 0.15 mm was selected and mounted on a diffractometer. A total of 7764 reflections were collected at 293(2) K. The structure was solved by direct methods and refinement by full-matrix least squares on F^2 using 32-bit Olex2-1.1 version program [17]. Hydrogen atoms were located from difference Fourier syntheses and included in the structure factor calculations with a riding model.

Cyclic voltammetry was carried out using the Autolab interface electrochemical analyzer consisting of three-electrode configuration in an electrochemical cell, with a Pt (0.3 mm diameter) working electrode, a Pt counter electrode, and Ag/AgCl-KCl (sat.) reference electrode. The cell used was a glass vial with a fitted-top equipped with four holes to accommodate three electrodes and a gas purging tube. The Pt working electrode surface was polished with alumina (Sigma-265497-25G, assay 99.5 % trace metals basis, avg. particle size ≤ 10 μm) powder ensuring a clean, uniform electrode and surface rinsed thoroughly with distilled water and acetone. 0.1 M tetra-(n-butyl)ammonium perchlorate (TBAP) was employed as the electrolyte in di-methyl formamide (DMF). The system was deoxygenated by purging with nitrogen gas for 10–15 min before measurement at sweep rates from 50 mV s^{-1} to 350 mV s^{-1} . The EPR spectra were recorded at room temperature on a JEOLJES-TE100EPR spectrometer operating with X-band frequency (9.407 GHz, power: 1 mW and field: 250 ± 250 mT) using crystalline complex at room temperature. The instrument was operated at 100 kHz field modulation to obtain the first derivative EPR spectrum. α -diphenyl- β -picryl hydrazyl (DPPH) was used as the standard for magnetic field correction for g-factor calculations. Magnetic measurements were carried out using Lakeshore-7404 VSM in powder form using vibration frequency 82.5 Hz and dynamic range 1×10^{-7} to 10^3 emu. Magnetic susceptibilities on selected crystalline samples of complex 1 in *dc* magnetic field were obtained under the selected field of 500 Oe at the 20–300 K range.

2.4. Theoretical calculations

The theoretical calculation to determine the electronic, magnetic and optical properties were performed by DFT using Vienna *ab initio* simulation package (VASP) [18–20]. The electronic density of states in the complex 1 was determined by the Density Functional Theory (DFT) using Generalized Gradient Approximation (GGA) with Perdew, Burke and Ernzerhof (PBE) as exchange correlation functions. The geometric crystal lattice volume and atomic coordinates were relaxed totally before performing the density of states calculation. The reciprocal space was sampled with k-point of $6 \times 6 \times 6$ grid of Monkhorst-pack and 400 eV was used for plane wave basis set. Forcite module in Material Studio 7.0 was used for the total energy calculation of bond and non-bonding structures along with the electronic density determination [21].

3. Results and discussion

3.1. X-ray crystal structure analysis

The crystal structure of the compound consists of discrete neutral complex 1 molecule with uncoordinated free 3-chloropyridine (3-Clpy) moiety (Fig. 1). Solvated 3-Clpy moiety and neutral $\text{Fe}^{\text{III}}(3\text{-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3$ complex are held together by hydrogen bond interactions between a hydrogen atom from a coordinated H_2O molecule and N from 3-Clpy moiety (Fig. S1). The coordinated 3-Clpy ligands are on the opposite vertices of the coordinating octahedron with their pyridine planes nearly parallel to each other; the dihedral angle between them is $2.7(0.4)^\circ$. The packing of the crystal is stabilized through intermolecular hydrogen bonds. Further,

Table 1
Crystal data and structure refinement for complex 1 measured at 293 K.

parameters	data
Empirical formula	C ₁₅ H ₁₄ Cl ₆ Fe N ₃ O
Formula weight	520.84
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	a = 8.7508(11) Å alpha = 73.972(12) deg. b = 9.0006(14) Å beta = 84.509(10) deg. c = 13.9318(17) Å gamma = 79.790(12) deg.
Volume	1036.7(2) Å ³
Z, Calculated density	2, 1.669 Mg/m ³
Absorption coefficient	1.511 mm ⁻¹
F(000)	522
Crystal size	0.2 mm × 0.15 mm × 0.15 mm
Theta range for data collection	2.75 to 25.00 deg.
Limiting indices	-10 ≤ h ≤ 10, -10 ≤ k ≤ 10, -16 ≤ l ≤ 16
Reflections collected / unique	7764 / 3651 [R(int) = 0.0791]
Completeness to theta = 25.00	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.91 and 0.666
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	3651 / 4 / 241
Goodness-of-fit on F ²	0.990
Final R indices [I > 2σ(I)]	R ₁ = 0.0590, wR ₂ = 0.1201
R indices (all data)	R ₁ = 0.0899, wR ₂ = 0.1422
Largest diff. peak and hole	0.684 and -1.006 e Å ⁻³

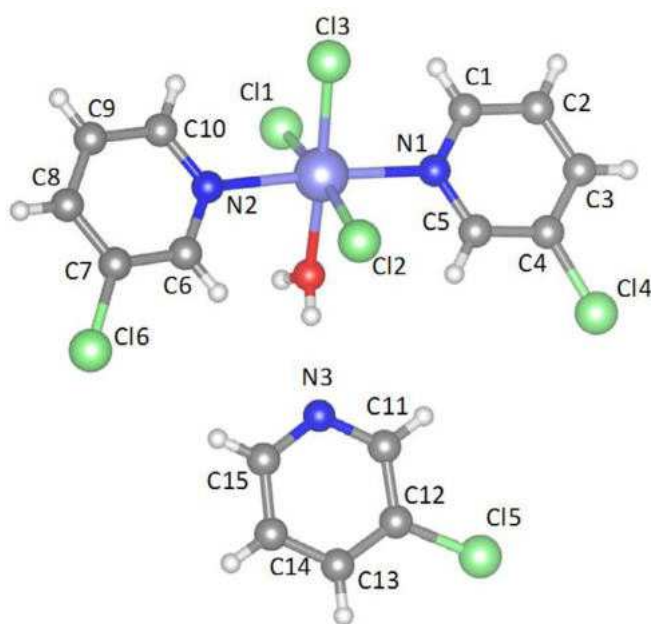


Fig. 1. Complex 1 showing the atom-numbering scheme.

there is slipped π - π interaction between the centro-symmetric pairs of free 3-Clypy with an inter-planar distance of 3.407 Å and the centroid-centroid slip of 1.367 Å (Fig. 2). However, the pairs do not further link for extended stacking.

Details of the data collection and X-ray crystal structure refinement are illustrated in Table 1. Selected bond lengths and bond angles are presented in Table 2. The Fe-N (ave.) distance for the complex is 2.193 Å, which is slightly smaller than that found for Fe1-N1 (2.199 Å), which is compensated by the Fe1-N2 (2.187 Å) bond distances on the opposite site. Similarly, the Fe-Cl (ave.) distance for the complex is 2.313 Å, which is slightly smaller than that found for Fe1-Cl2 (2.369 Å), and is compensated by the Fe1-Cl1 (2.2961 Å) and Fe1-Cl3 (2.2743 Å) bond distances. The dissimilar Fe-N bond length of the two 3-Clypy moieties and Fe-Cl bond

Table 2
Selected bond lengths and angles for complex 1.

bond	length/Å	bond	angle/°
C(1)-N(1)	1.343(6)	C(5)-C(4)-Cl(4)	118.1(5)
C(4)-Cl(4)	1.715(6)	C(3)-C(4)-Cl(4)	122.3(4)
C(5)-N(1)	1.352(6)	C(6)-C(7)-Cl(6)	118.3(5)
C(6)-N(2)	1.329(6)	C(8)-C(7)-Cl(6)	121.3(4)
C(7)-Cl(6)	1.729(6)	C(13)- C(14)- Cl(5)	122.6(6)
C(10)-N(2)	1.330(6)	C(15)- C(14)-Cl(5)	119.6(6)
C(11)-N(3)	1.328(8)	C(1)- N(1)-C(5)	119.0(5)
C(14)-Cl(5)	1.703(7)	C(1)-N(1)-Fe(1)	122.2(4)
C(15)-N(3)	1.336(8)	C(5)-N(1)-Fe(1)	118.8(3)
N(1)-Fe(1)	2.199(4)	C(6)- N(2)-C(10)	118.1(5)
N(2)-Fe(1)	2.187(4)	C(6)- N(2)-Fe(1)	118.8(4)
O(1)-Fe(1)	2.085(3)	C(10)- N(2)-Fe(1)	123.1(4)
Cl(1)-Fe(1)	2.296(16)	C(11)- N(3)-C(15)	118.0(6)
Cl(2)-Fe(1)	2.369(16)	Fe(1)- O(1)-H(1A)	122(3)
Cl(3)-Fe(1)	2.274(15)	Fe(1)- O(1)-H(1B)	121(3)
-	-	N(2)- Fe(1)-N(1)	176.00(5)
-	-	N(2)- Fe(1)-Cl(3)	92.84(11)
-	-	N(1)- Fe(1)-Cl(3)	90.95(12)
-	-	N(2)- Fe(1)-Cl(1)	89.48(12)
-	-	N(1)- Fe(1)-Cl(1)	88.80(12)
-	-	N(2)- Fe(1)-Cl(2)	89.42(12)
-	-	N(1)- Fe(1)-Cl(2)	91.59(12)

length lead to distortions [15,22]. Such metal-ligand bond lengths were used to obtain the spin state of the metal center [23]. The Fe-N (ave.) bond distances in iron(III) complexes are around 2.2 Å in high spin complexes, and less than 2.0 Å, which was reported for low spin complexes [24]. The Fe-N (ave.) bond distances for complex 1 is 2.193 Å, it shows that the present complex is unambiguously high spin d^5 system. One of the co-crystallized 3-Clypy molecules is located in hydrogen-bonding distance from the group. The Fe(1)-O(1) bond distance for present complex is 2.09 Å, which is almost similar with reported complexes [Fe(bipy)Cl₃(DMSO)] and [Fe(phen)Cl₃(DMSO)] are 2.09 Å and 2.07 Å, respectively. The Fe-O bond distance in [Fe(phen)Cl₃(H₂O)] was reported to be 2.19 Å, which is considerably longer than the Fe-O bond distances in present complex, [Fe(bipy)Cl₃(DMSO)] and [Fe(phen)Cl₃(DMSO)] complexes [23,24]. This elongation in the former complex is due to hydrogen bond formation. However, there are no hydrogen bonds

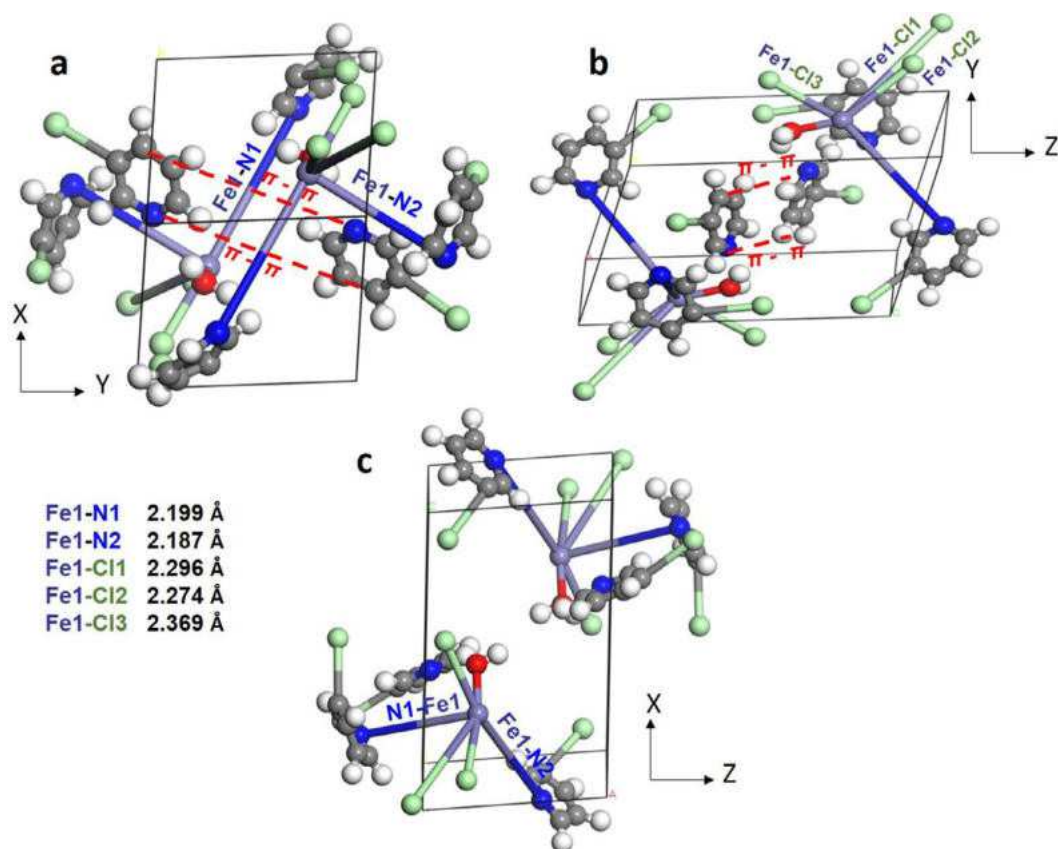


Fig. 2. Packing diagram of complex 1 showing the π - π interaction. (a) XY (b) YZ, and (c) XZ orientations, respectively.

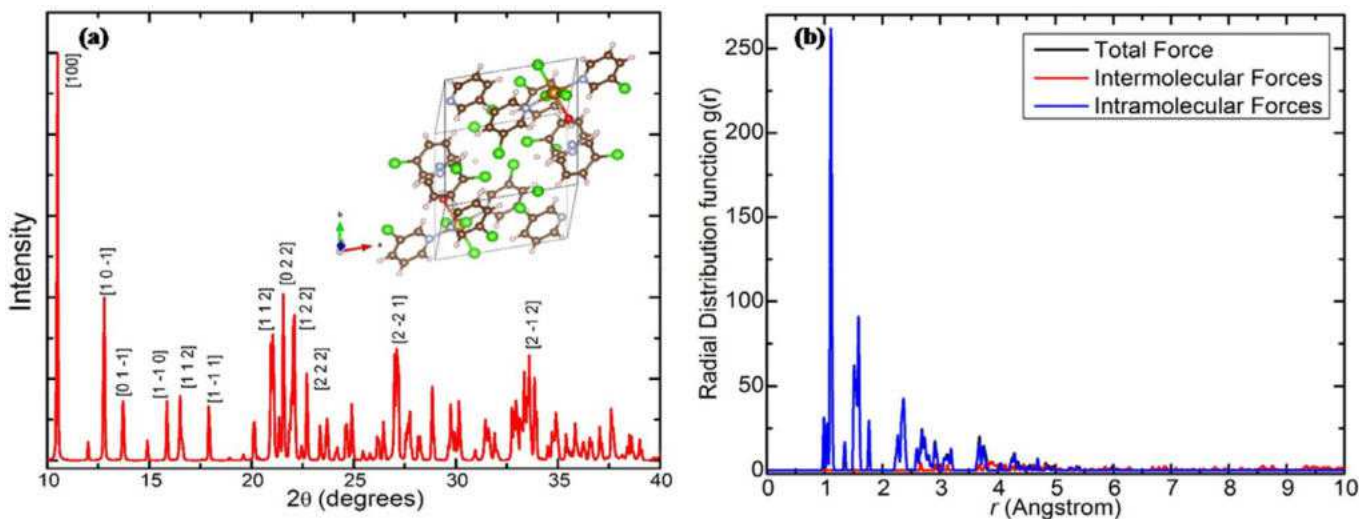


Fig. 3. (a) Powder XRD diffraction pattern with peak indexing and (b) radial distribution function of complex 1.

present in later reported complexes [24]. However, there are hydrogen bonds present in our prepared complex although it doesn't affect bond lengths. In a layer of the crystal, every molecule of complex 1 is surrounded by two ($Z = 2$) other mutually and symmetrically related molecules, which are in good agreement with those found in similar reports on iron(III) complexes [25,26].

The powder XRD pattern simulated from the crystal structure of the monomeric complex 1 is shown in Fig. 3a with the pattern indexing data. From the Forcite structural analysis calculation, we confirm the formation of triclinic structure with lattice parameters of b/a and c/a ratio of 0.3995 and 1.215. The total formation en-

ergy of the structure is determined as 714.125 kcal/mol and with the total contributions to total energy distribution as shown in Table S1. The radial distribution function (RDF) is defined as the ratio of local density to average density of atomic groups in a system. Local density of molecules in region near the molecule will be different from the average density of the system. But, when the region is located far from the molecule, the density will be same as average density which means that the value of R is large, the RDF is 1 $g(r)$ is normally defined as geometric distribution of surrounding particles in space given the coordinates of one particle. Mostly, intermolecular forces include hydrogen bonds and van der

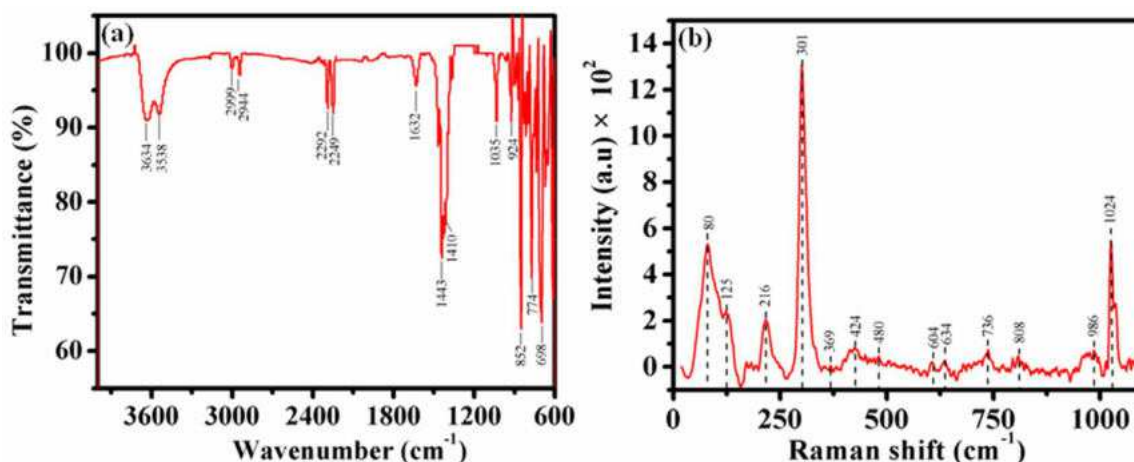


Fig. 4. (a) FTIR and (b) Raman spectra of complex 1.

Waals forces. The hydrogen bond length is 3–4 Å. The intermolecular and intramolecular forces are shown through the radial distribution function shown in Fig. 3b.

3.2. FTIR, Raman and electronic spectral analyses

FTIR and Raman spectra of complex show several characteristic features supporting the given composition and structure. The infra-red spectrum of free 3-Clpy ligand bands are observed at 3436, 3131, 2971, 1919, 1344, 1016, 915 and 896 cm^{-1} , which were shifted to higher energy at 3634, 3538, 2999, 2249, 1427, 1367, 1035, 924 and 901 cm^{-1} (Fig. 4a), respectively. This indicates that the 3-Clpy ligand predominantly coordinated with iron(III) central metal ion. This result is consistent with previous reports [27–29]. Other important vibrational modes observed in the range 1466–1443 cm^{-1} and 1632 cm^{-1} are due to water molecules. The experimental Raman spectrum of pure 3-Clpy in the region 250–1250 cm^{-1} has already been reported [30]. The 729 cm^{-1} band is a mixed-mode of (C1, C4) and (C2, C5) carbon atoms for free ligand, and it is shifted to 736 cm^{-1} in the present complex (Fig. 4b). The C–Cl(stretching), C–N(bending), trigonal bending and ring-breathing are also largely appeared at 426, 616, 729 and 1035 cm^{-1} for free ligand and corresponding values are shifted to 424, 634, 736 and 1024 cm^{-1} in the present complex. The coordinated Raman modes of Fe–N, Fe–Cl and Fe–O are assigned at 255, 301 and 369 cm^{-1} , respectively. The FTIR and Raman spectral studies confirm that the 3-Clpy, H_2O and Cl ligands were coordinated with iron center in complex 1.

The electronic absorption spectrum of complex 1 recorded in acetonitrile (Fig. 5). Octahedral high spin Fe(III) complexes possess a 6A_1 ground state and generally exhibit a series of four ligand field transitions in the visible and near-infrared spectral region. These are assigned as ${}^6A_1 \rightarrow {}^4E$, ${}^6A_1 \rightarrow {}^4A_1$, ${}^6A_1 \rightarrow {}^4T_2$, and ${}^6A_1 \rightarrow {}^4T_1$ in order of decreasing energies [31]. Because of orbital singlet in the high spin Fe(III), there are no excited states of the same spin multiplicity and all the $d-d$ transitions are therefore spin-forbidden as well as Laporte forbidden. The strong charge-transfer bands which ‘tail-off’ into the visible region mask these spin-forbidden transitions [32]. Five intense bands in the 200–400 nm regions mainly characterize complex; it gives rise to intense broad band at 311 and 360 nm and three more intense sharp bands at 265, 240 and 210 nm. The relatively high extinction coefficient values for each band maxima are too high to be attributed as a ligand to metal charge transfer (LMCT) transitions. The high energy band at 311 nm implies LMCT ($N(p\pi) \rightarrow \text{Fe}^{III}(3d)$) mixed with intra ligand $\pi \rightarrow \pi^*$ (B band of 3-Clpy) transition. It has

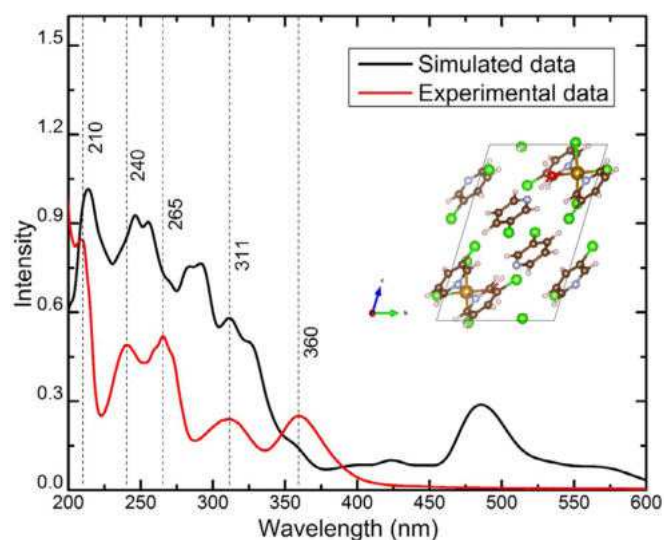


Fig. 5. UV-vis spectra of complex 1 measured in acetonitrile (32.8 μM) at room temperature.

been noted earlier that a dominant feature around 300 nm in the electronic spectra of some of the Fe(III) complexes containing chloride ion as a terminal ligand can be assigned to a predominantly $\text{Cl} \rightarrow \text{Fe}^{III}$ CT transition. However, charge transfer transitions involve primarily in the nitrogen donor atom of the 3-Clpy nucleus [31]. It is expected that for a d^5 configuration, the absorption spectrum gives no information because the tail of the intense charge-transfer ($\text{Cl} \rightarrow \text{Fe}^{III}(3d)$) absorption overlaps with the weak forbidden bands (${}^6A_1g \rightarrow {}^4Eg(G)$) at ~ 360 nm [33]. Hence, an electronic spectral result of the prepared complex shows LMCT transitions predominantly. Theoretically simulated optical absorption properties shown in the Fig. 5 also confirms the experimental results. The calculated absorption spectra of the complex 1 in the visible light region between 200 and 500 nm exhibits exact replica of the experimental results having characteristic peaks at 214, 245, 290, 311 and 360 nm.

3.3. Photoluminescence study

Fig. 6a shows the steady-state emission spectrum of complex 1 in acetonitrile. The excitation spectrum shows a broad band at ~ 312 nm, attributed to the LMCT transition of the Fe^{III} -ligand chromophore [34,35]. The designed iron(III) complex excited at 4.50

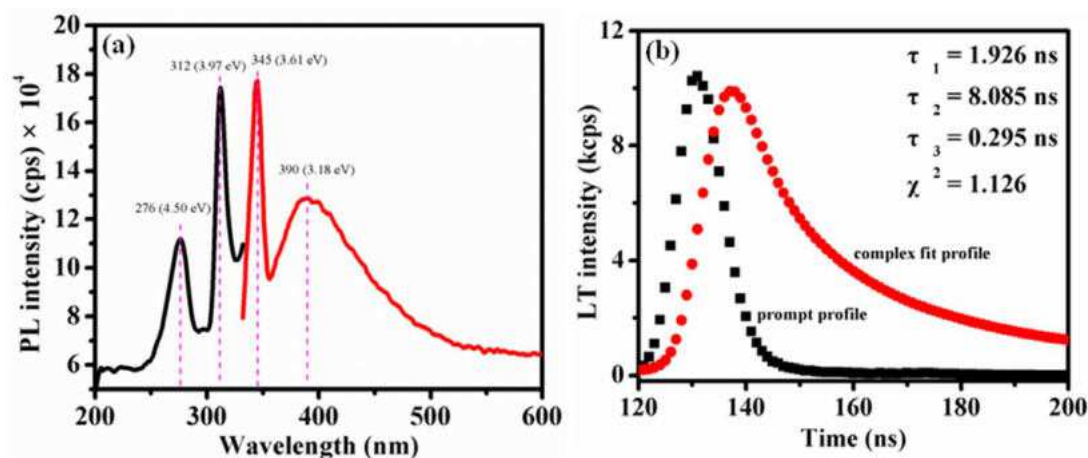


Fig. 6. (a) steady state excitation and emission spectra and (b) time-resolved luminescence profile of complex 1 at $\lambda_{exc} = 311$ nm in acetonitrile (32.8 μ M) at room temperature.

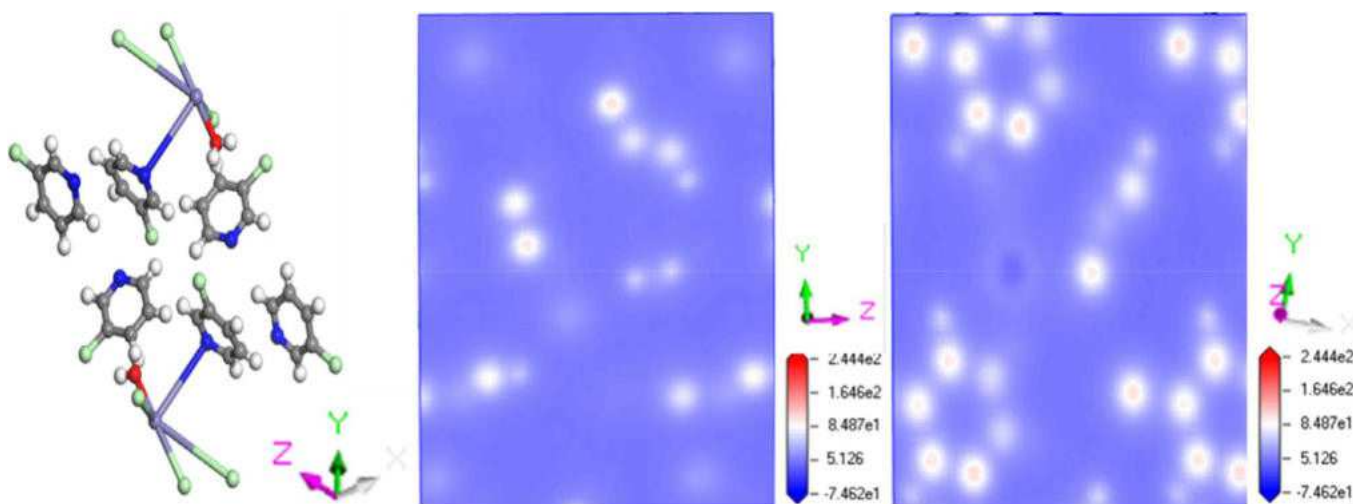


Fig. 7. Electronic charge density (in eV) change in the complex 1 the system simulated using CASTEP.

and 3.98 eV and the corresponding fluorescence signals recorded at 3.61 and 3.18 eV, respectively. However, the broad luminescence peaks observed at 4.35, 4.27 and 4.20 eV for pure 3-Clpy ligand (Fig. S2). Over and above the characteristic complex 1 exhibits intense luminescence. The origin of the emission band can be assigned from the LMCT transition, since it coincides with first maximum of the excitation spectrum.

Luminescence life time studies of as prepared complex clearly imply the character of excited state with charge transfer transitions such observation is well supported from broad band emission spectral analysis. The emission decay components are observed at $\tau_1 = 1.926$, $\tau_2 = 8.085$ and $\tau_3 = 0.295$ ns ($\chi^2 = 1.126$) as presented in Fig. 6b. In general, the decay curves are triphasic in nature, which is consistent with tri-exponential fitting [36]. This multi-exponential decay is explained on (i) different electronic coupling strengths existing in as prepared complex due to varied electron-rich coordinating ligand, and (ii) the fast and slow components due to thermally equilibrated emitting states. In this case the slow component τ_2 is more prominent in accounting the effect of coordination environment on the lifetime of emitting state. The PL and lifetime studies indicate that the as-prepared iron(III) complex has good emission behavior attributed due to LMCT transitions. The electronic charge density calculated from the CASTEP calculation shown in Fig. 7 stands as the evidence for the existence

of photoluminescence in the complex 1. It is clearly seen that the charge states around the Fe and Cl ligands are the main reason for the strong fluorescence signal emitted.

3.4. Electrochemistry

The electrochemical properties of iron(III) complex, particularly with mixed donor centers have been studied to monitor spectral and structural changes accompanying electron transfer. Measurements were made on the degassed (N_2 bubbling for 15 min.) solutions of complex 1 in DMF (1.48 mM), sweep rate (ν) from 50 $mV s^{-1}$ to 350 $mV s^{-1}$ containing 0.1 M tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte. At least 2-3 hrs elapsed between the preparation of DMF solutions of the iron(III) complex and the electrochemical measurements to allow the system to equilibrate. Typical current-potential curves are presented in Fig. 8a between $E_i = -2.0$ V and $E_f = 0.8$ V, which illustrate well-defined cathode waves (both metal-centered and ligand centered reduction) and weak / obscured anode waves (complementary oxidation). The compound displayed three reduction and two oxidation processes in DMF (Fig. 8a and Table S2). The reduction and oxidation processes with ($E_{1/2} = -0.547$ V, $\nu = 100 mV s^{-1}$) should be iron-based while the other process ($E_{1/2} = -1.367$ V, $\nu = 100 mV s^{-1}$) is ligand-based. It is notable (Fig. S3 and Ta-

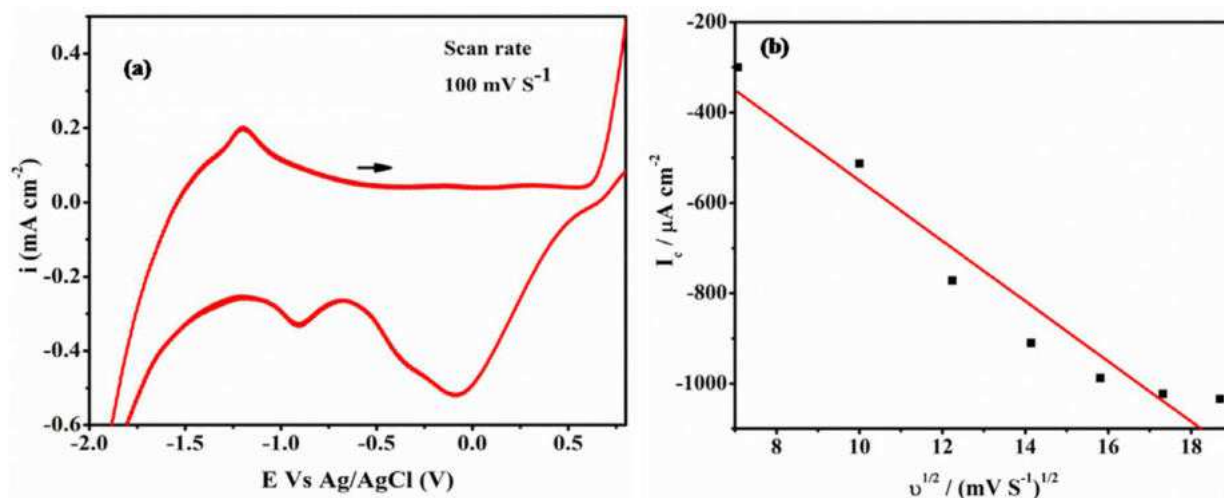


Fig. 8. (a) Cyclic voltammograms of complex 1 sweep rate at 100 mV S^{-1} in DMF (1.48 mM) solution at room temperature. Pt working electrodes and supporting electrolyte tetra-(n-butyl) ammonium perchlorate (0.1 M) and (b) plot of the I_c vs $v^{1/2}$ (sweep rate: 50 to 350 mV s^{-1}) for complex 1 in DMF.

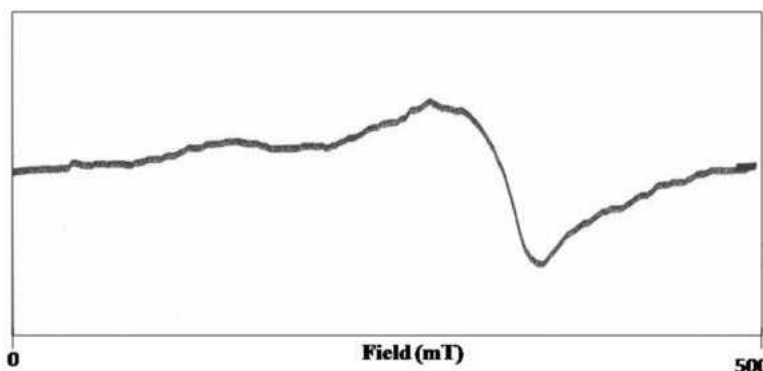


Fig. 9. EPR spectra for complex 1 at room temperature.

ble S3) that the peak position is sweep rate-independent. Thus the initial redox reaction is associated with a rapid heterogeneous electron transfer. The cathodic peak ($E_{pc} = -0.078 \text{ V}$) is due to a product from a fast chemical reaction; indeed, iron(III) coordinated to H_2O is the target for nucleophilic attack by solvent molecule. The cathodic peak ($E_{1/2} = -0.547 \text{ V}$, $v = 100 \text{ mV s}^{-1}$), stems from $\text{Fe}^{\text{III}}(\text{3-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3 + e^- \rightarrow [\text{Fe}^{\text{II}}(\text{3-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3]$ reduction and a nonreversible process. It is likely that this species undergoes a chemical reaction, since the respective re-oxidation was not observed. The complex is reduced at the lowest potential ($E_{1/2} = -0.547 \text{ V}$, $v = 100 \text{ mV s}^{-1}$), this can be attributed to the coordination of the 3-Clpy and Cl^- to the iron ion, which is a stronger Lewis base, consequently, diminishes the Lewis acidity of the metallic center.

In general, voltammograms represent quasi reversible behavior associated with slow electron transfer kinetics (Table S2, $\Delta E_p = 0.255 \text{ V}$ to 0.760 V). Cyclic voltammogram of complex 1 shows quasi-reversible oxidation and reduction processes for $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple allowing to a determination of half-wave potential at $E_{1/2} = -0.03 \text{ V}$ to 0.184 V with respect to the reference electrode. The actual site of electron transfer is difficult to predict in the oxidation step. However, the free ligand is reduced at 0.024 V to -0.323 V and -0.477 V to -1.170 V in DMF solution (Fig. S4 & Table S4).

The metal centered reduction is identified at 0.048 V to -0.400 V ($\Delta E_p = 0.272 \text{ V}$ to 0.741 V , sweep rate 50 mV s^{-1} to 350 mV s^{-1}), while two ligand centered reduction peaks are observed at from -0.839 V to -1.024 V and from -1.522 V to -1.664 V (Figure

S3). It is noteworthy that six-coordinate iron(III) complex with uninegative monodentate ligands, (Fig. 8b) in general, exhibit totally irreversible $\text{Fe}^{\text{III}} \rightarrow \text{Fe}^{\text{II}}$ reduction response with no evidence for well-defined anodic peaks [37–39]. However, in some cases during the reduction of iron(III) complex, the reoxidative anodic waves are observed but with large peak-to-peak separations (ΔE) [40]. This result indicates that the as-prepared complex is electrochemically active in DMF solution.

3.5. Magnetic measurements

X-band EPR spectra of complex 1 was measured at room temperature, which illustrates that the solid-state EPR spectra of the compounds are broad [41]. Room temperature EPR spectrum of the complex prepared from ferric chloride consists of a single very broad isotropic signal at $g_{\text{iso}} = 2.02$ with $\Delta H_{pp} = 43.62 \text{ mT}$ (Fig. 9). This explains the magnetic moment of $\mu_{\text{eff}} = 5.99 \text{ B.M.}$ In the present case, EPR spectrum shows a very intense width, characteristic for a paramagnetic, high-spin d^5 iron(III) complex [42,43].

Theoretical determination of the electronic density of states and the spin states in the complex 1 is shown in Fig. 10. The total density of states for the proposed crystal structure shows a dense population in the valence band region between -1.5 eV and -7 eV . The contribution of the Fe orbitals is seen closer to the edges of Fermi level region whereas the contribution of the Cl orbitals is widely distributed deeper into the valence band between -2 eV and -6 eV . As a result, the electronic structure of complex 1 is majorly contributed by the Fe and Cl orbitals. From the spin-enabled density

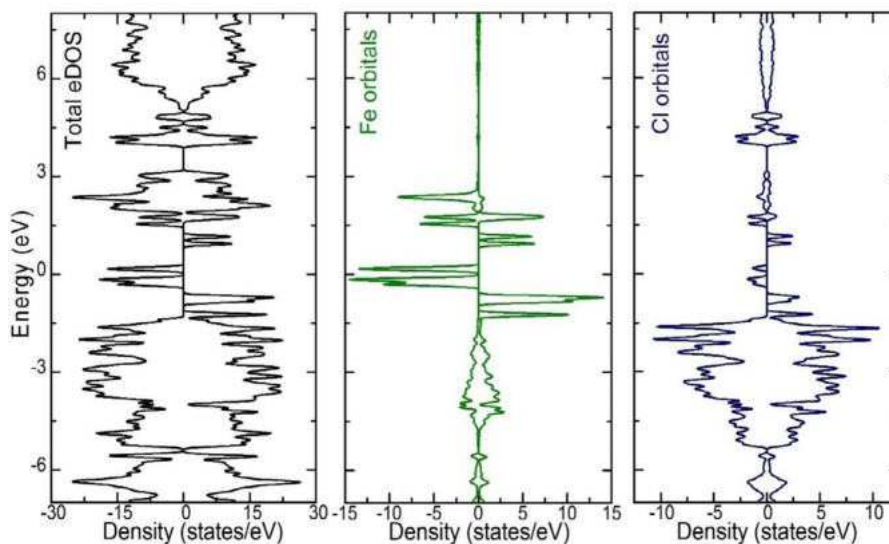


Fig. 10. Electronic density of states for complex 1 with the orbital split-up for Fe and Cl orbitals.

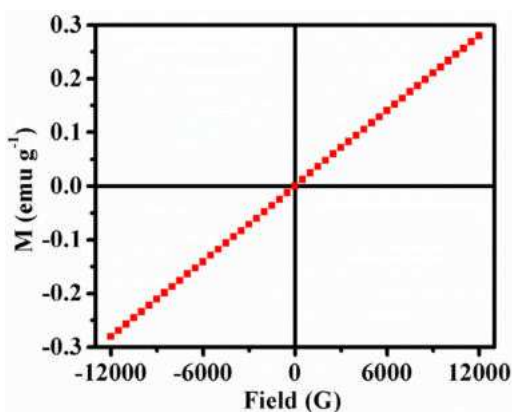


Fig. 11. Room temperature hysteresis curve for complex 1 in dc magnetic field.

magnetic moment change with the applied magnetic field depends on the spin states available, as shown in the density of states.

The variation of induced magnetic moment with respect to applied magnetic field would be identified by VSM for analysis of magnetic nature of metal complex. The complex is found to be paramagnetic with an effective magnetic moment is $5.856 \mu_B/\text{Fe}^{3+}$ at room temperature VSM measurement. It is observed that the intensity of magnetization varies linearly with the applied field, which confirms that the mononuclear iron(III) complex ($\text{Fe}^{\text{III}} (t_2g^3 eg^2, S = 5/2)$) with 3-Clpy, H_2O and Cl^- ligands show paramagnetic nature at room temperature (Fig. 11). Moreover, the temperature dependence of magnetization at an applied magnetic field (500 Oe) was done. Fig. 12 interprets that the complex exhibits paramagnetic nature in all the given temperature range. The temperature dependence magnetization plot confirms the existence of paramagnetic behavior for the iron(III) complex under study (Fig. 12a,b). The $\chi_{\text{mol}}T$ values decrease in the low temperature range (Fig. 12c), it could be either due to saturation effects / intermolecular ($S = 5/2 \rightarrow S = 3/2$) spin state interactions [44,45]. The structural data show that there are two units bridged by rather strong hydrogen bonds between the uncoordinated 3-Clpy molecule and two monomeric units of complex 1 molecules, it may induce spin coupling between iron(III) complex molecules. Therefore, the value of the $\chi_{\text{mol}}T$ is higher than that of reported

functional calculations, we observe the clear change in the spin orientations near the edge of the Fermi level. Fe orbitals exhibit a larger change in the spin orientations compared to the Cl orbitals. The theoretical existence and the intensity of the magnetic properties correlate well with the obtained experimental results. The

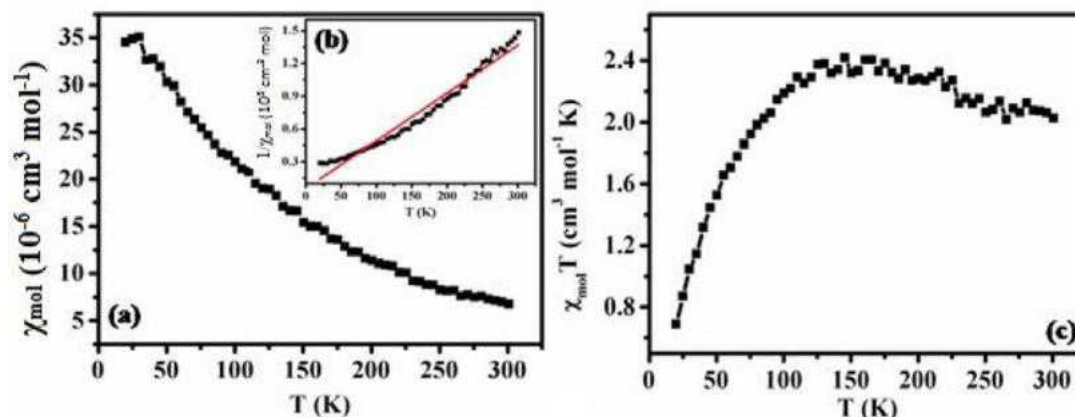


Fig. 12. Temperature dependent magnetization plots (a) χ_{mol} vs. T, (b) $1/\chi_{\text{mol}}$ vs. T, and (c) $\chi_{\text{mol}}T$ vs. T χ_{M}^{-1} of complex 1 at 500 Oe.

monomeric complexes [46]. It may be concluded that neutral complex 1 with uncoordinated 3-Clpy molecule remain paramagnetic with high-spin ($S = 5/2$ or $3/2$) over the whole temperature range.

4. Conclusions

The orange color prismatic crystals of monomeric complex 1 were grown by recrystallization of the respective microcrystals in 70% 3-Clpy/ethanol solution over a week. However, elemental analyses of the complex suggest the general formula corresponding to $[\text{Fe}^{\text{III}}(3\text{-Clpy})_2(\text{H}_2\text{O})\text{Cl}_3]\cdot 3\text{-Clpy}$. Spectral measurements such as FTIR, Raman, and UV-Vis were used for identifying the bonding and geometry in the complex. In this investigation, single-crystal X-ray diffraction technique was employed to refine the structure of the prepared complex. In addition, physicochemical features of optical, electrochemical and magnetic properties of the complex have been successfully investigated by PL, CV, EPR and VSM measurements. The theoretical electronic density of states explains the contribution of the atomic orbitals and their spin orientations. The compound is stable over a long period of time in crystalline form and is soluble in a wide range of common organic solvents such as acetonitrile, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO).

Supplementary data

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre Number, CCDC No: 2010152. Copies of this information can be had free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or <http://www.ccdc.cam.ac.uk>) on request quoting the deposition number.

Declaration of Competing Interest

I may wish to state that 'The author(s) declare(s) that there is no conflict of interest' in this article.

CRedit authorship contribution statement

Ganeshraja Ayyakannu Sundaram: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Karthikeyan Vaithinathan:** Data curation, Formal analysis, Methodology, Software, Visualization, Writing - review & editing. **Krishnamoorthy Anbalagan:** Conceptualization, Project administration, Supervision, Validation, Visualization, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.molstruc.2020.129160](https://doi.org/10.1016/j.molstruc.2020.129160).

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DNA BINDING, *IN VITRO* CYTOTOXICITY AND ANTICANCER DRUG MECHANISM OF COPPER(II) COMPLEX CONTAINING PYRIDYL-TRIAZINE LIGAND

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ABSTRACT

Mononuclear copper(II) complex $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**), where dppt is bidentate $\text{N}_{\text{py}}\text{N}_{\text{tz}}$ donor asymmetric ligand (pyridyl-triazine) has been isolated. The X-ray crystal structure of **1** possesses a CuN_4O_2 chromophore with elongated octahedral geometry. The electronic and EPR spectral properties demonstrate that the solvent molecules strongly interacted in the axial position thereby weakens the CuN_4 coordination plane. Absorption and emission spectral and electrochemical measurements clearly show the partial intercalative binding of **1** to calf thymus (CT) DNA. Remarkably, it exhibits potent cytotoxicity (IC_{50} , 3.73 μM) against human cervical carcinoma cells (HeLa), which is 4.5 times better than cisplatin and is non-toxic (IC_{50} , >500 μM) to normal mouse embryonic fibroblasts cells (NIH 3T3). It blocks cell cycle progression of HeLa cells in G1 phase. FACSverse analysis of **1** is suggestive of ROS (reactive oxygen species) generation and absolutely induces apoptotic cell death in HeLa cells.

Keywords: Copper(II) complex, DNA Binding, ROS, Apoptosis, Cytotoxicity.

1. INTRODUCTION

Currently, several reports were highlighting the use of transition metal complexes as anticancer agents [1, 2]. Probably the exception known of those is cisplatin [cis-diamminedichloroplatinum(II)]. It has been extensively used to treat a diffusion of cancers which include testicular, brain, ovarian, bladder, and breast cancer [3]. The scientific success of cisplatin is constrained by its considerable side effects, consisting of nausea, vomiting, and intense nephrotoxicity [3]. Using cisplatin and associated platinum complexes as anticancer agents have inspired a search for other energetic transition metal complexes which can be as powerful, but with lesser side effects. Many biological systems in nature make massive use of metal ions, inclusive of zinc and copper, which play essential roles within the regular functioning of organisms. Transition metals including copper, iron, and manganese, amongst others, are involved in a couple of biological processes from electron transfer to catalysis to structural roles and are often related to active sites of proteins and enzymes [4]. However, dysregulation of some of these vital metals in the course of normal biochemical processing has been implicated in

the development of many pathological disorders, together with cancer [5]. These cellular roles simply need the "trace metals" in miniscule however firmly regulated quantities. Through assessment, different metals including arsenic, cadmium, chromium, and nickel are much less useful in view that they produce an extensive variety of toxic facet consequences, which includes carcinogenesis [4,6]. Particularly, copper(II) cation can bind to negatively charged DNA and had been proven to play a crucial function within the nearby formation of hydroxyl radicals [7, 8]. One of the results of excessive copper levels inside the body is a growth within the rate of radical formation mainly to oxidative damage [8]. This results in a disruption of lipid bilayers because of oxidation and cleavage of inclined unsaturated fatty acid residues of phospholipids. Changes in protein features also are promoted via oxidation of thiol and probably amino groups. Gene expression may also be altered because of the oxidation of guanosine and adenosine residues in nucleic acids or altered transcription factor or growth factor activities [9, 10]. More anecdotally, in the human body, Cu binds to N7 of guanine residue of DNA and generates ROS

through the oxidation-reduction reaction resulting in DNA damage and cell apoptosis [11-13]. All these findings aid that the antitumor agent primarily based on Cu could be promising for the treatment of cancer. Therefore, a novel, green coloured copper(II) complex $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**), where dppt is 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine has been synthesized. Its ability to bind calf thymus (CT) DNA and cancer chemotherapeutic potential against human cervical carcinoma cells (HeLa) and normal mouse embryonic fibroblasts cells (NIH 3T3) has been studied. Also, interesting aspects of the anticancer drug mechanisms underlying the cytotoxic response were probed.

2. EXPERIMENTAL

2.1. Material and methods

Copper(II) acetate monohydrate, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine, NaClO_4 , *tetra-N*-butylammonium perchlorate (TBAP), ethanol, *N,N*-dimethyl formamide (DMF), anhydrous ether were of analytical grade and used as received from commercial sources. Calf thymus (CT) DNA was commercially purchased from Sigma Aldrich and stored at -20°C while tris (hydroxymethyl) aminomethane and ethidium bromide (EthBr) were obtained from Merck.

The cell lines HeLa and NIH 3T3 were procured from the NCCS, Pune, India. Cell culture media and reagents were purchased from Hi Media, India. 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) and Annexin V and Apoptosis Detection Kit were purchased from Sigma Aldrich, USA. All antibodies used in this study were procured from Cell Signaling Technology, USA. Ultra-pure Milli-Q water ($18.2 \mu\Omega$) was used for all experiments.

The elemental analyses (C, H, N) were carried out using a Perkin-Elmer 2400 series II analyzer. The electrical conductivity was obtained with a Systronic 305 conductivity bridge, using 1×10^{-3} M solution of complex in *N,N*-dimethyl formamide (DMF). FTIR spectra were recorded using a Perkin Elmer Spectrum RX1 FTIR spectrophotometer in the range $400\text{-}4000 \text{ cm}^{-1}$ with a sample prepared at KBr disc. The electronic spectra were recorded using Perkin Elmer Lambda 365 UV-VIS spectrophotometer using cuvettes of 1 cm length. X-band electron paramagnetic resonance (EPR) measurements were performed at room temperature in the solid state and at 77 K in the DMF solution on JEOL JES-FA200 ESR spectrometer. Emission intensity

measurements were carried out using a Shimadzu RF-5301PC spectrofluorophotometer equipped with a thermostatic bath. Solutions of DNA in the 5 mM Tris HCl/50 mM NaCl buffer gave a ratio of UV absorbances at 260 and 280 nm, A_{260}/A_{280} , of 1.9 [14], indicating that the DNA was sufficiently free of protein. Concentrated stock solutions of DNA (13.5 mol dm^{-3}) were prepared in buffer and sonicated for 25 cycles, where each cycle consisted of 30 s with 1 min intervals. The concentration of DNA in nucleotide phosphate (NP) was determined by UV absorbance at 260 nm after 1:100 dilutions. The extinction coefficient, ϵ_{260} , was taken as $6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. Stock solutions were stored at 4°C and used after no more than 4 days. Concentrated stock solutions of copper(II) complex was prepared by dissolving in 2% DMF 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 and diluting suitably with the corresponding buffer to required concentrations for all the experiments. For absorption and emission spectral experiments, the DNA solutions were pretreated with solutions of copper(II) complex to ensure no change in concentrations of the copper(II) complex. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed in a CHI 620C electrochemical analyzer at $25 \pm 0.2^\circ\text{C}$. The working electrode was a glassy carbon disk (0.0707 cm^2) and the reference electrode a saturated calomel electrode. A platinum wire was used as the counter electrode. The supporting electrolyte was *tetra-N*-butylammonium perchlorate (TBAP) or 2% DMF 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.1). Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements; during measurements a stream of N_2 gas was passed over them. The redox potential $E_{1/2}$ was calculated from the anodic (E_{pa}) and cathodic (E_{pc}) peak potentials of CV traces as $(E_{\text{pa}} + E_{\text{pc}})/2$ and also from the peak potential (E_{pa}) of DPV response as $E_{\text{p}} + \Delta E/2$ (ΔE is the pulse height).

2.2. Synthesis of complex, $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**)

An ethanolic solution (5 mL) of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (dppt: 0.62 g, 2 mmol) was added dropwise to an aqueous solution (10 mL) of copper(II) acetate monohydrate (0.20 g, 1 mmol). The resulting green coloured solution was stirred for 8 h at room temperature. The product was precipitated as the perchlorate salt by adding stoichiometric equivalent of NaClO_4 (0.12 g, 1 mmol) in water (3 mL). The bright

green product, $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$, was collected by suction filtration, washed with cold water and ether and then air-dried. Yield: 76%. Selected IR peaks (ν , cm^{-1}): 3447 b ($\nu_{\text{O-H}}$), 1500 m, 1527 s and 1600 w ($\nu_{\text{C=N}}$) and ($\nu_{\text{N=N}}$), 1103 and 1064 (ν_{ClO_4}). Anal. Calcd for $\text{C}_{40}\text{H}_{32}\text{N}_8\text{O}_{10}\text{Cl}_2\text{Cu}$: C, 52.27; H, 3.51; N, 12.19 %. Found: C, 52.32; H, 3.49; N, 12.24 %. Λ_{M} (DMF): $165 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$. μ_{eff} (27 °C): $1.78 \mu_{\text{B}}$. Electronic spectrum in DMF [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$): 278 (8240), 322 (4885), 494 (35) 688 (30). Electronic spectrum in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer solution [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$): 265 (8350), 292 (4900), 482(45), 676 (40). Room temperature polycrystalline EPR spectrum: $g_{\text{iso}} = 2.061$. EPR spectrum in DMF solution at 77 K: $g_{\parallel} = 2.292$, $g_{\perp} = 2.060$, $A_{\parallel} = 165 \times 10^{-4} \text{cm}^{-1}$, $g_{\parallel}/A_{\parallel} = 139 \text{cm}$, $G = 4.9$. Redox behaviour: $E_{1/2} = 0.243 \text{V}$ (CV) and 0.242V (DPV), $\Delta E_{\text{p}} = 81 \text{mV}$, $i_{\text{pa}}/i_{\text{pc}} = 1.1$, $D = 6.2 \times 10^6 \text{cm}^2 \text{s}^{-1}$.

The blocks of bright green single crystals of $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**) separated upon cooling a solution of **1** in MeOH:MeCN:Et₂O at 5 °C for seven days. The latter were found suitable for X-ray studies.

2.3. X-ray crystallography

A bright green needle-like single crystal of the complex $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ **1** with dimensions $0.35 \times 0.30 \times 0.30 \text{mm}^3$ was selected under the polarizing microscope and then mounted on glass fiber. The crystal data collections were performed on a Bruker AXS-KAPPA APEX II diffractometer equipped with a CCD area detector utilizing Mo-K α radiation ($\lambda = 0.71073 \text{Å}$) at 273 K. Data were collected and reduced by SMART and SAINT softwares in the Bruker packages [15]. The structure was solved by direct methods and subsequently refined by full-matrix least squares calculations with the SHELXL-2018/3 software package [16]. All non-hydrogen atoms were refined anisotropically while hydrogen atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. Also, hydrogens on water oxygen which is connected to Cu are located. The disorder in perchlorate anion is fixed and the ratio of occupancies of disordered moieties is found to be 53:47. The graphics interface package used was PLATON, and the figures were generated using the ORTEP 3.07 generation package [17]. Crystallographic data for the structural analysis of $[\text{Cu}(\text{dppt})_2$

$(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**) have been deposited with Cambridge Crystallographic Data Center, CCDC No. 1994856. Copies of this information may be obtained free of charge from <http://www.ccdc.ac.uk/const/retrieving.html> or from the CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (email: deposit@ccdc.cam.ac.uk).

2.4. Spectroscopic and electrochemical experiments of DNA interactions

The spectroscopic and electrochemical experiments of DNA interactions were carried out by employing the procedure reported by us previously [14].

2.5. Cell viability assays

The in vitro cytotoxicities of test complex **1** (HeLa, 0.1-100 μM ; NIH 3T3, 1-500 μM), free dppt ligand, $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ against HeLa and NIH 3T3 cell lines were assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The inhibitory effect of them on both cancer and normal cells were evaluated by means of their IC₅₀ values (concentration of compound required to inhibit 50% of cell proliferation). Cisplatin was chosen as a positive control.

2.6. Anticancer drug mechanistic studies

2.6.1. Cell cycle

The HeLa cells were seeded in 6 well plates with high-glucose DMEM media after the period of the time cells attained the growth and the cells were treated with **1** in its IC₅₀ concentration in the medium for 48 h. After 48 h of incubation, cells were trypsinized and resuspended with complete media. Cells were collected and centrifuged at 1000 rpm for 5 min. Then cell pellet was washed with PBS (Phosphate Buffer Saline) twice and subsequently fixed with 1 ml of 70% of ice cold ethanol overnight at 4°C. Following the ice cold ethanol, the cell pellet were washed twice with cold PBS and added 10 μl of RNase A at 10 g/ml concentration, which was then incubated for 30 min and washed with PBS at the end. Cells were then incubated in 1 ml of PBS with 50 μl of propidium iodide (1 mg/ml stock) for 30 min in darkness. Then cells were analysed to check the cell cycle phase using FACSverse flow cytometer (Becton-Dickinson).

2.6.2. ROS generation

The HeLa cells were seeded in 6 well plates with high glucose DMEM media for the treatment until cell

confluency after the cell growth treated with **1** in 3 h, 2 h and 1 h time interval. After the treatment, cells were trypsinized and 10 μM 2',7'-dichlorodihydrofluorescein diacetate (DCHF-DA, Sigma-Aldrich) dye was added to the pellet. This was kept in incubation under darkness for 10 min. The cells were then analyzed to determine ROS level using FACSverse flow cytometer (Becton-Dickinson).

2.6.3. Apoptosis

The HeLa cells were treated with **1** in high-glucose DMEM media for 48 h. After treatment, the cells were trypsinized, resuspended in PBS, washed twice and centrifuged to remove PBS. The cells were suspended in 100 μl of binding buffer containing 5 μl of Annexin V and propidium iodide (PI) and incubated for 15 mins under darkness. Stained cells were diluted using 450 μl of binding buffer. The cells were analyzed by using FACSverse flow cytometer (Becton-Dickinson) and the data were analyzed by FACSverse software.

2.6.4. DAPI staining

Cell nuclear morphology was evaluated by fluorescence microscopy following DAPI (4',6-diamidino-2-phenylindole) staining. The HeLa cells were treated with **1** for 48 h. The cells were washed with PBS (pH 7.4), fixed with ice cold paraformaldehyde and then cells were then washed with PBS followed by DAPI was added and incubated for 15 min at 37 $^{\circ}\text{C}$ wrapped in aluminium foil. The cells were then washed with PBS and examined under Zeiss Axio Observer fluorescence microscope.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of **1**

The bright green complex, $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ (**1**) was prepared in good yield (76%) and purity by the reaction of an aqueous solution of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$, ethanolic solution of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (dppt) and NaClO_4 in a stoichiometric ratio (1:2:1) at room temperature. The selected frequencies observed in the IR spectra of **1**, the broad band appeared at 3447 cm^{-1} represents the existence of H_2O [18]. The stretching vibrations of the $\text{C}=\text{N}$ and $\text{N}=\text{N}$ groups appear at considerable lower values with respect to the $\nu_{\text{C}=\text{N}}$ and $\nu_{\text{N}=\text{N}}$ of the free dppt ligand supporting the coordination of the pyridyl and triazine nitrogen donors to copper(II) ion [19]. It displays two well split bands at 1103 and 1064 cm^{-1} due to perchlorate anion. Such splitting normally arises due to coordination of or hydrogen bonding [20] of ClO_4^- ; however, none of these

is present in the crystal structure. Elemental analysis of **1** was entirely consistent with its determined composition by X-ray crystallography. This is substantiated by conductivity measurements in DMF solution, which is expected for a 1:2 electrolyte in solution.

In DMF solution, **1** exhibits only one broad band (λ_{max} , 688 nm) with very low extinction coefficient (ϵ_{max} , 30 $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$) value in the visible region, typical of ligand field (LF) absorption for Cu(II) located in a tetragonal field. The powder EPR spectrum at 298 K of **1** is isotropic while frozen DMF solution shows axial spectral features, typical of mononuclear Cu(II) species ($g_{\parallel} > g_{\perp} > 2.0$; $G = (g_{\parallel} - 2)/(g_{\perp} - 2) = 4.9$) suggesting the presence of $d_{x^2-y^2}$ ground state in copper(II) located in square-based geometries [21]. The observed g_{\parallel} (2.292) and A_{\parallel} ($165 \times 10^{-4} \text{cm}^{-1}$) values are consistent with the presence of a square based CuN_4 coordination plane with strong axial interaction by two oxygen atoms. The $g_{\parallel}/A_{\parallel}$ quotient (139 cm) is suggestive [22] of negligible distortion from the CuN_4 coordination plane. The electrochemical behaviour of **1** in DMF (TBAP as supporting electrolyte) shows a quasi-reversible redox couple, $E_{1/2}$ at 0.243 V versus SCE. The peak potentials separation, ΔE_p is 81 mV, the ratio of cathodic to anodic peak current ($i_{\text{pa}}/i_{\text{pc}}$) is close to unity and the reduction process is diffusion controlled. The weak σ bonding caused by the highly electron-withdrawing phenyl groups as well as strong π back bonding [23] involving the phenyl and pyridine rings, rather than the bulkiness of the ligand molecule, is responsible for the positive $E_{1/2}$ value.

In the complex (**1**), the copper atom is coordinated to two 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (dppt) in *trans* configuration via triazine nitrogen (N_{tz}) and pyridine nitrogen (N_{py}) and two water molecules. The crystallographic data (Table 1) and selected bond lengths and angles (Table 2) are provided.

The two $\text{Cu}-\text{N}_{\text{py}}$ (2.046(2) \AA) and $\text{Cu}-\text{N}_{\text{tz}}$ (2.030(2) \AA) bond distances are not appreciably different indicating that the donor strength of two nitrogens are equal while the two oxygen atoms of water molecules occupy axial position at longer distances (2.425(3) \AA), as a consequence of the Jahn-Teller effect. However, the structure of **1** (Fig. 1) at 273 K is almost the same as that already determined by Palaniandavar et al. [24] at 296 K and Takagi et al. [25] at 200 K and is hence a confirmation of the identity of **1**.

Table 1: Selected crystal data and structure refinement parameters for 1

Formula	C ₄₀ H ₃₂ N ₈ Cl ₂ O ₁₀ Cu
Formula weight	919.17
Temperature (K)	273(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	C2/c
a (Å)	12.1016(8)
b (Å)	11.3529(8)
c (Å)	29.8689(18)
α (°)	90
β (°)	92.782(4)
γ (°)	90
V (Å ³)	4098.8(5)
Z	4
D _{calc} (g cm ⁻³)	1.490
μ (mm ⁻¹)	0.732
F(000)	1884
Crystal size (mm)	0.35 × 0.30 × 0.30
θ (°)	2.461-28.159
Reflections collected	21988
Independent reflections	4940
Reflections observed [I > 2σ(I)]	3665
R _{int}	0.0324
GOOF	1.081
R ₁ [I > 2σ(I)]	0.0529
wR ₂ [I > 2σ(I)]	0.1414
R ₁ , wR ₂ all data	0.0965/0.1567

3.2. Spectroscopic and voltammetric studies on DNA interaction

The absorption spectra of **1** in the absence and presence of DNA at different concentrations ($R = [\text{DNA}]/[\text{complex}] = 1-25$) in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer (pH = 7.1) were recorded (Fig. 2) at 291 nm ($\pi-\pi^*$ transition). With an increase in concentration of CT DNA, the hypochromism of 61.0% and red-shift of 3 nm for **1** were observed indicating the partial intercalative interaction. The extent of binding was calculated [26] and the intrinsic equilibrium DNA binding constant, K_b , has been estimated to be $2.565 \pm 0.001 \times 10^5 \text{ M}^{-1}$, which suggests the enhanced DNA binding propensity of **1** possibly due to the involvement of partial intercalative interaction of the planar 5,6-diphenyltriazine moiety of coordinated dppt into the DNA base pairs leading to high hypochromism. In addition, there is no change in absorption spectral band of dppt upon increasing the CT DNA

concentration indicates that there is no interaction between dppt and the base pairs of DNA.

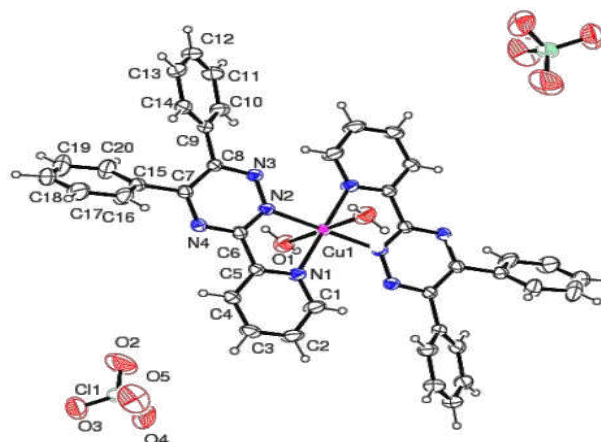


Fig. 1: An ORTEP drawing of [Cu(dppt)₂(H₂O)₂](ClO₄)₂ (1**) showing 30% probability thermal ellipsoids with atom labeling scheme**

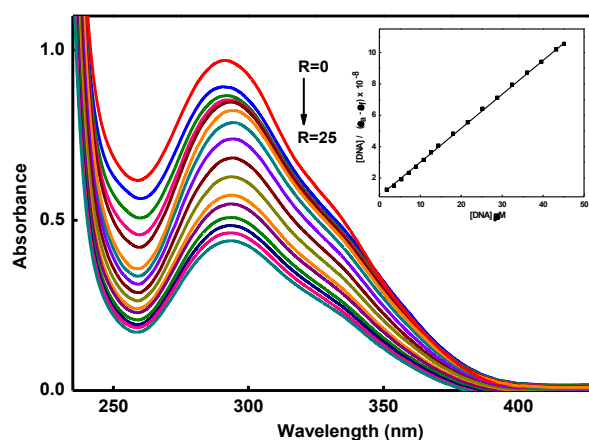


Fig. 2: Absorption spectra of 1 (concentration, $25 \times 10^{-6} \text{ M}$) in 2% DMF/5mM Tris- HCl/50 mM NaCl buffer at pH 7.1 in the absence ($R = 0$) and presence ($R = 25$) of increasing amounts of CT DNA. Inset: Plot of [DNA] vs [DNA]/($\epsilon_a - \epsilon_f$) at $R = 25$ of **1.**

The intrinsic fluorescence intensity of DNA and that of EthBr are low, while the fluorescence intensity of EthBr will be enhanced on addition of DNA due to its intercalation into the DNA. In our experiment, the fluorescence intensities of EthBr-DNA system show a decreasing trend with increasing concentration of **1** (Fig. 3), indicating that some EthBr molecules are

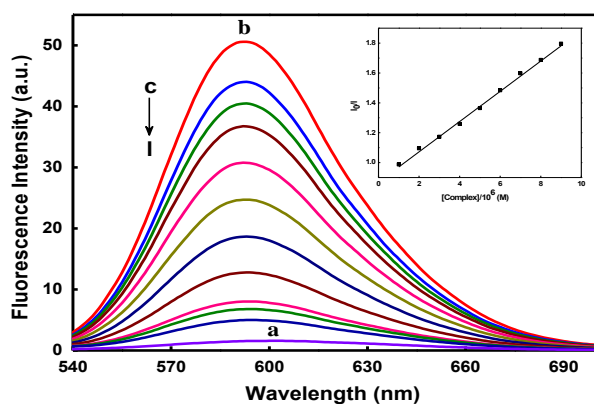
released from EthBr-DNA after an exchange with **1** which results in the fluorescence quenching of EthBr. This may be due to that **1** displaces the EthBr from its DNA-binding sites in the competitive manner. The plot (Fig. 3, inset) illustrates that the quenching of EthBr bound to DNA are in good agreement with the linear

Stern-Volmer equation [27]. The K_{SV} value ($1.60 \times 10^5 \text{ M}^{-1}$) of **1** indicates the quenching efficiency and especially significant degree of binding to DNA. The binding strength of **1** (only those samples caused a 50% decrease of fluorescence intensity) with DNA was estimated as apparent binding constant (K_{app}) [28].

Table 2: Selected bond lengths (Å) and bond angles (°) for **1**

N(2)-Cu(1)	2.030(2)	N(2)-Cu(1)#1	2.030(2)
N(1)-Cu(1)	2.046(2)	N(1)-Cu(1)#1	2.046(2)
O(1)-Cu(1)	2.425(3)	O(1)-Cu(1)#1	2.425(3)
N(2)-Cu(1)-N(2)#1	180.0 (9)	N(2)-Cu(1)-N(1)#1	100.14(9)
N(2)#1-Cu(1)-N(1)#1	79.86(9)	N(2)-Cu(1)-N(1)	79.86(9)
N(2)#1-Cu(1)-N(1)	100.14(9)	N(1)#1-Cu(1)-N(1)	180.0 (9)
N(2)-Cu(1)-O(1)#1	90.39(10)	N(2)#1-Cu(1)-O(1)#1	89.61(10)
N(1)#1-Cu(1)-O(1)#1	88.83(11)	N(1)-Cu(1)-O(1)#1	91.17(11)
N(2)-Cu(1)-O(1)	89.61(10)	N(2)#1-Cu(1)-O(1)	90.40(10)
N(1)#1-Cu(1)-O(1)	91.17(11)	N(1)-Cu(1)-O(1)	88.83(11)
O(1)#1-Cu(1)-O(1)	180.0 (9)		

Symmetry transformations used to generate equivalent atoms: #1 $-x+1/2, -y+1/2, -z+1$



Inset: Plot of $[complex \times 10^6]$ vs I_0/I of **1**.

Fig. 3: Fluorescence quenching curves of ethidium bromide bound to DNA in 2% DMF/5mM Tris-HCl/50 mM NaCl buffer at pH 7.1: (a) EthBr (1.25 μM); (b) EthBr + DNA (125 μM); (c-m) EthBr + DNA + **1 (0-9 μM).**

The K_{app} value ($2.82 \times 10^5 \text{ M}^{-1}$) supports a strong interaction of **1** with CT DNA and the mode of binding through partial intercalation [29]. The results are consistent with those obtained from electronic absorption titration studies. The cathodic (0.032 V) and anodic peak potential (0.132 V) values observed from the cyclic voltammetric (CV) responses for **1** in 2% DMF-5 mM Tris-HCl-50 mM NaCl buffer (pH = 7.1), which correspond to $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ redox couple [30]. Upon

addition of DNA, both the cathodic and anodic current decreases drastically (Fig. 4), this is expected of strong binding of the complex with DNA [31] via partial intercalation. The formal potentials of $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple (obtained from differential pulse voltammetry (DPV) studies) in the E_f^0 (0.116 V) and E_b^0 (0.032 V) forms shift negatively (-84 mV) after reacting with DNA. The ratio of equilibrium binding constants, K_+/K_{2+} , is calculated to be 0.04, which suggests that the B form of DNA tends to stabilize $\text{Cu}(\text{II})$ over $\text{Cu}(\text{I})$ state.

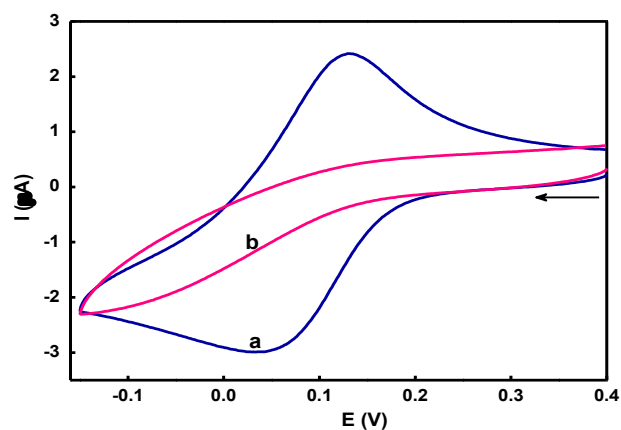


Fig. 4: Cyclic voltammograms of **1 (0.5 mM) in the absence (a) and presence (b) of CT DNA (R = 5) at $25.0 \pm 0.2 \text{ }^\circ\text{C}$ at 50 mV s^{-1} scan rate in 2% DMF/5mM Tris-HCl/50 mM NaCl buffer at pH 7.1.**

3.3. *In vitro* cytotoxic activity

HeLa cells were treated with increasing concentrations (0.1 to 100 μM) of **1** for 48 h inhibited the growth of HeLa cells in a dose-dependent manner (IC_{50} , $3.73 \pm 0.57 \mu\text{M}$) (Fig. 5).

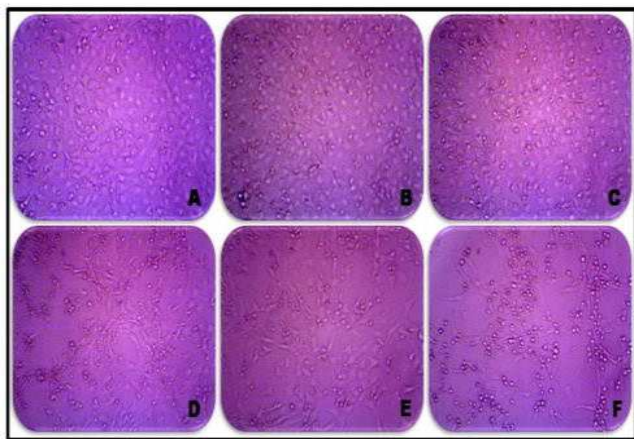


Fig. 5: Photomicrograph of human cervical carcinoma cells (HeLa) after 48 h exposure with **1 (A, control; B, 0.1 μM ; C, 1 μM ; D, 10 μM ; E, 50 μM ; F, 100 μM).**

From the IC_{50} of **1**, we can find that it is found to be highly active against the selected cancer cells [32] and 4.5 times better than cisplatin (IC_{50} , 16.4 μM). In comparison, free dppt ligand (IC_{50} , 112.48 μM) and

$\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (IC_{50} , 705.67 μM) showed no significant growth inhibition activities, which indicated that the chelation of dppt with copper ion was essential for anticancer activities of the copper(II) complex. In addition, the results indicated that the IC_{50} value of **1** against NIH 3T3 mouse embryonic fibroblasts (normal cells) is found to be above 500 μM , which confirmed that **1** is very specific on cancer cells. Finally, the cytotoxic behaviour of **1** is consistent with its ability to bind with DNA.

3.4. Anticancer drug mechanism

The profiles of propidium iodide stained HeLa cells treated with IC_{50} concentration of **1** for 48 h were analyzed by FACS [33]. As shown in Fig. 6, in the control, the percentage in the cell at G1 phase is 61.94% and the remarkable increase of 17.2% was found. The increase in G1 phase was accompanied by the corresponding reduction in G0, S and G2/M phases. The data mean that **1** induces cell cycle arrest at G1 phase in HeLa cells. The cells treated with **1** and DCFDA for 1 h show significant shift of the histogram towards the right, indicating an increase in the intensity of emission resulting from the generation of DCF from DCFDA [34]. As shown in Fig. 7, in the control, the DCF fluorescence intensity is 100% while the intensity of DCF fluorescence increases to 128% when HeLa cells were incubated with **1**.

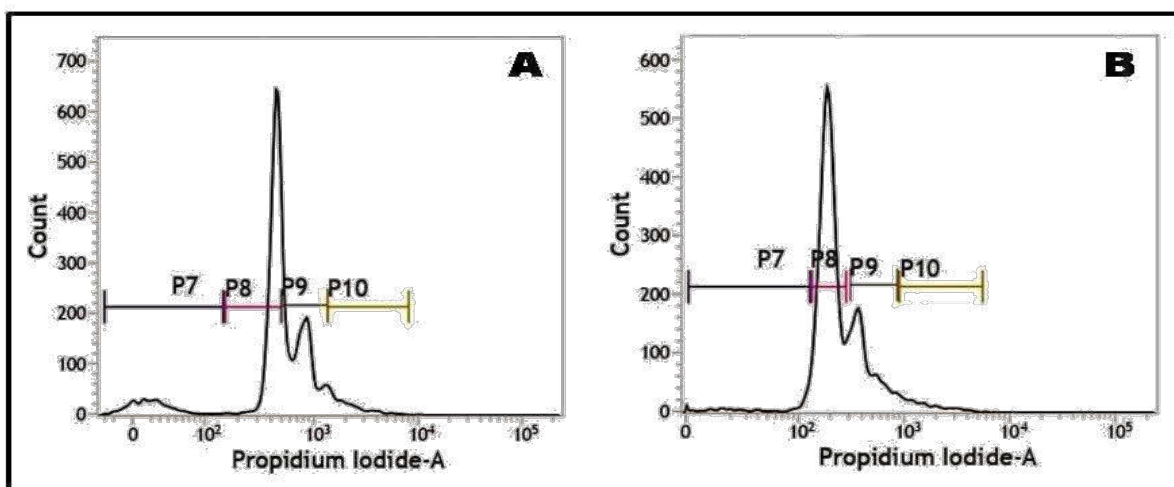


Fig. 6: Flow cytometric analysis showing the G1 phase cell cycle arrest by **1 in HeLa cells (P7, P8, P9 and P10 correspond to G0, G1, S and G2/M phases respectively). A, HeLa cells alone; B, HeLa cells treated with **1**.**

Greater shift implies higher fluorescence intensity resulting from higher amount of DCF formation and thus greater ROS generation, which is consistent with the apoptotic effect (cf. below) of **1**. Moreover, the ROS levels induced by **1** show a time-dependent manner, decreasing the fluorescence intensity for 2 h (119%) and 3 h (116%). The result demonstrates the generation of ROS and this reactive species possibly causing cell apoptosis. We then carried out apoptosis assay to further evaluate the possible mechanism of cell death induced by **1** [35]. In the control cells, the percentage of living, early apoptotic, late apoptotic and necrosis cells were 99.9, 0.0, 0.1 and 0.0%, respectively (Fig. 8).

However, **1** treated HeLa cells displayed a remarkable

average of 56.08% of early apoptotic cells and 18.33% of late apoptotic cells. The increased expression of Annexin V positive cells undoubtedly demonstrated the cells were in apoptosis stage and is consistent with its *in vitro* cell cytotoxicity. Interestingly, there is no sign of cell death via necrotic pathway. To understand the nuclear morphology and the nature of cell death mechanism, we have carried out DAPI (4',6-diamidino-2-phenylindole) staining with **1** (Fig. 9).

The control HeLa cells exhibited evenly stained nucleus with round and intact contours whereas the treated cells showed characteristic fragmentation of the nucleus or condensed nuclei which is supportive of the cell toxicity induced by **1** due to apoptotic mode of cell death.

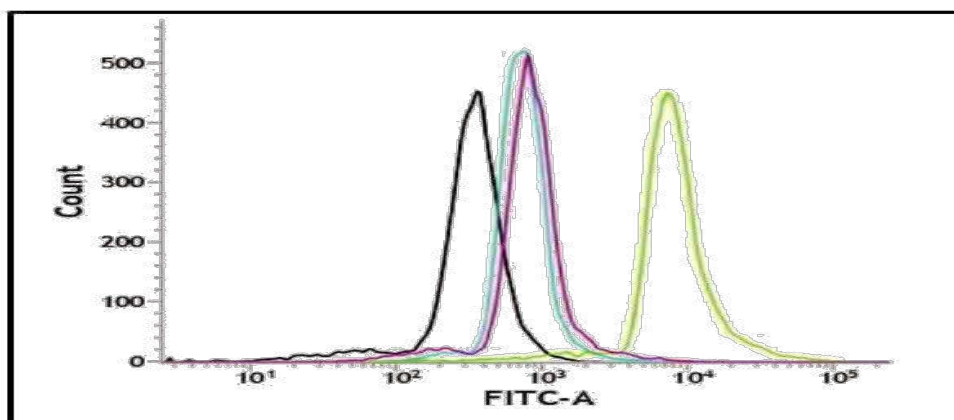


Fig. 7: DCFDA assay in HeLa cells for generation of ROS using the **1** in a time-dependent manner (control, black; 1 h, blue; 2 h, red; 3 h, green).

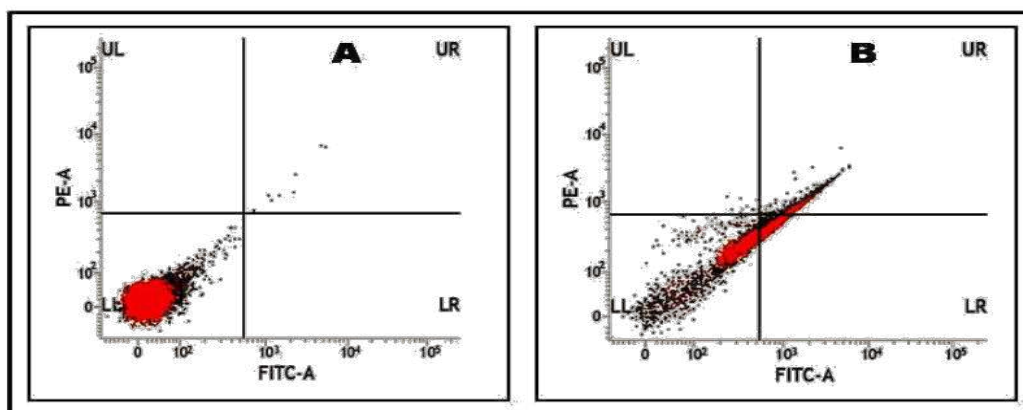


Fig. 8: Cellular apoptosis induced by **1** as determined from the annexin V-FITC/PI staining assay of the HeLa cells with four distinct phenotypes: viable cells (lower left quadrant, LL); cells at an early stage of apoptosis (lower right quadrant, LR); cells at a late stage of apoptosis (upper right quadrant, UR); and necrosis (upper left quadrant, UL). A, HeLa cells alone; B, HeLa cells treated with **1**; red dots, gated cells; black dots, ungated cells.

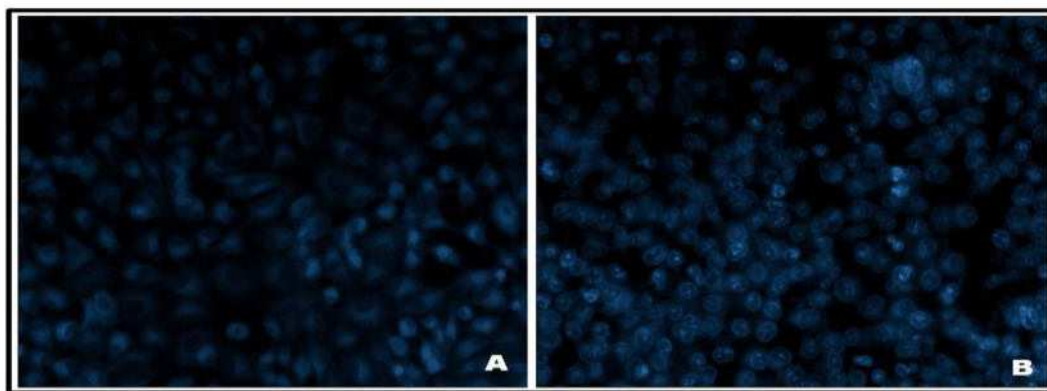


Fig. 9: HeLa cells stained with DAPI (A, HeLa cells alone; B, HeLa cells treated with 1) and visualized under a fluorescence microscope.

4. CONCLUSION

The copper(II) complex, $[\text{Cu}(\text{dppt})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$, has been synthesized and characterized. The complex binds to CT DNA through partial intercalative mode. It exhibits highly active inhibitory effect, which was higher than cisplatin and selective to cancer cells while non-toxic to healthy cells. The biological evaluation provides evidence that it blocked cell cycle at G1 phase and induced apoptosis alone along with the generation of ROS. The present lead complex is a novel therapeutic agent for the treatment of cervical cancer as well as encourages further exploration of non-platinum anticancer agents. It targets the mitochondria of cancer cells and induces apoptosis by a mechanism involving the formation of ROS.

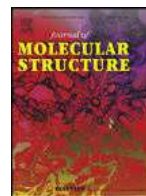
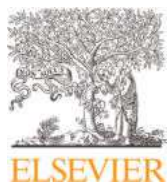
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Pyrene based Schiff bases: Synthesis, crystal structure, antibacterial and BSA binding studies

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ABSTRACT

Two novel pyrene-based Schiff base compounds 4-[(5-pyren-1-yl-thiophen-2-ylmethylene)-amino]-phenol (KSB-1) and 4-[(4-pyren-1-yl-benzylidene)-amino]-phenol (KSB-2) were designed and synthesized by a condensation reaction with a diminutive modification of π -spacers. The KSB-1 and KSB-2 molecules are thoroughly characterized by pivotal spectroscopic techniques. The prepared products were found to yield excellent quantity, and crystallographic data was also analyzed for KSB-1. Moreover, the antibacterial activities of the synthesized KSB-1 and KSB-2 were investigated against the two strains of *Pseudomonas aeruginosa*. BSA binding interaction with these pyrene-based Schiff bases was investigated by fluorescence quenching method, and the quenching of BSA followed a static type. The binding constant and number of binding modes were calculated.

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1. Introduction

Schiff bases are an essential category of organic compounds, and it could be prepared from the condensation of molecules contain aldehyde or ketone functional groups with an amine [1]. Schiff bases are named after Hugo Schiff [2], also known as imine or azomethine and have played an important role in the enrichment of chemical sciences. Intriguingly, the imine or azomethines group are found to be a versatile pharmacophore for design and development of various bioactive compounds and also could be tuned by varying the substituents. The Schiff bases possess with heterocyclic rings containing nitrogen, oxygen and sulfur donor atoms show the different pharmacological activities such as antibacterial, antifungal, antimalarial, anti-inflammatory and antipyretic [3–12]. On the other hand, the Schiff bases are effectively used to develop various chemosensors since the imine group acts as a strong binder with metal ions [13]. As a result, the Schiff base compounds utilized for diverse applications in various research fields [14]. For instance, Mesbah and co-workers developed different Schiff bases from heterocyclic moiety, and the compounds

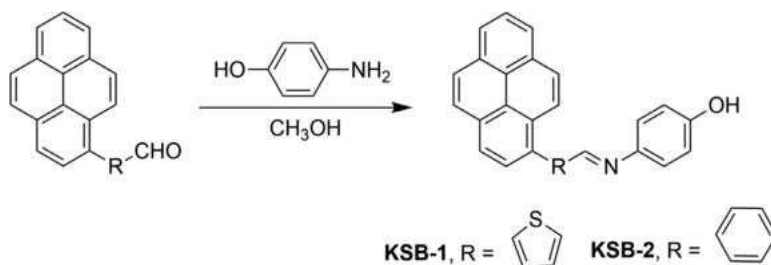
are investigated for their antimicrobial and antifungal activities against some pathogenic strains [15]. Raja et al. reported the Schiff base of isatin derivatives [16] for antibacterial activity. Antimicrobial activity of the derivatives was assessed by minimum inhibitory concentration (MIC) in comparison with standard antimicrobial drugs (ciprofloxacin and ketoconazole). Results show that the electron-donating groups present in the isatin derivatives plays a vital role in improving the antimicrobial activity. Other small molecules such as amino acids, coumarins, sulphonamides, 2-aminophenol and triazoles for the preparation of Schiff bases for antibacterial activities [17]. Previously, we have also reported the pyrene-based Schiff base with AIE property as a prospective biomaterial for in vitro bioimaging and BSA-binding studies [18,19]. Thus, the potent biological activity of pyrene based Schiff bases propels to develop new class of pyrene derivatives and to investigate the binding interaction with albumins. In this connection, bovine serum albumin (BSA) is considered by the researchers due to its availability and structural homology with human serum albumin (HSA) [20].

The design of the Schiff bases KSB-1 and KSB-2 are such that it can be advantageously used as a dual functional probe consisting of a hydrophobic pyrene group with phenyl and thiophene substituents and hydrophilic hydroxyl groups [21]. Hence it can be expected to show a high affinity for hydrophobic and hydrophilic sites of BSA protein. Since, π -conjugated pyrene derivatives

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Scheme 1. Synthetic scheme of KSB-1 and KSB-2.

have been successfully used as fluorophores [22], imaging [23], cytotoxicity [24], sensing [25] and antimicrobial activities [26]. Meng et al. investigated the interaction of pyrene with BSA under physiological condition [27]. Besides, Ali et al. had also reported phenanthrene-pyrene based fluorescent probe with BSA [28]. Here, we report the pyrene-based Schiff bases for biological applications. The 4-aminophenol is used for producing the Schiff base due to the presence of hydroxyl group. The hydroxyl group at para position has high electron releasing properties and it activates aromatic ring thereby increasing the biological activity of the compound. Hence, 4-aminophenol is used for synthesis of Schiff base as it increases the biological activity thereby upon treatment it reduces the bacterial growth by affecting its motility. On the other hand, the developed Schiff bases are only differing in π -spacers, such as thiophene and benzene. These molecules are characterized by ^1H NMR, ^{13}C NMR and mass spectroscopic techniques. In this manuscript, we targeted to explore the role of π -spacers on the BSA binding and antimicrobial studies. Therefore, the antibacterial activities of KSB-1 and KSB-2 compounds were tested against multi-drug resistant strains of *Pseudomonas aeruginosa*.

2. Experimental section

2.1. Materials and methods

4-aminophenol and bovine serum albumin were purchased from Sigma-Aldrich. Methanol, glacial acetic acid, petroleum ether, dichloromethane and dimethyl sulphoxide were purchased from Sisco Research Laboratories (SRL), India, and solvents of spectrometric grade were used without any further purification. The starting materials 5-(pyren-1-yl)thiophene-2-carbaldehyde, 4-(pyren-1-yl)benzaldehyde were prepared as reported previously [29]. PA9027 cells were obtained from the Microbial Type Culture Collection, and Gene Bank (MTCC), India, LB broth was obtained from Himedia, India.

2.2. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on a BRUKER AVANCE II spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C and collected at ambient probe temperature with spectra calibrated to either an internal tetramethyl silane (TMS) standard or a residual protio-solvent. Electrospray ionization mass spectra (ESI-MS) were collected on a Thermo Finnigan (San Jose, CA, USA) LCQTM Advantage MAX quadrupole ion trap instrument, by infusing samples directly into the source at 20 mL min^{-1} using a syringe pump. Absorption spectra were recorded by using Perkin Elmer Lambda 25 UV-visible spectrophotometer. The BSA quenching studies were carried out using a Hitachi F-4500 fluorescence spectrometer. Crystal data were collected at ambient temperature using an Oxford diffraction X-Calibur Eos Gemini diffractometer with graphite-monochromated Mo-K α radiation ($\lambda = 0.7107\text{ \AA}$).

The data were solved using direct methods with SHELXS and refined SHEXL-2013. The figures were generated using the ORTEP 3.07 generation package. The hydrogen atoms bound to the carbon were placed in geometrically constrained positions and refined with isotropic temperature factors, generally 1.2 Ueq of their parent atoms.

2.3. Synthesis

The pyrene containing Schiff base compounds were prepared by the following general procedure (Scheme 1).

2.3.1. 4-[(5-pyren-1-yl-thiophen-2-ylmethylene)-amino]-phenol (KSB-1)

To a hot methanolic solution (20 mL) of 5-pyren-1-yl-thiophene-2-carbaldehyde (0.156 g, 0.5 mmol), 4-aminophenol (0.06 g, 0.55 mmol) dissolved in 10 mL of methanol was added drop wise and catalytic amount of glacial acetic acid were added and the mixture was refluxed for 1 h. The yellow precipitate obtained after cooling to the ambient temperature was filtered, washed several times with cold methanol and dried yielding yellow solid (0.143 g, 71%). Single crystals of KSB-1, suitable for X-ray diffraction were obtained by slow evaporation of a DCM/hexane solution. ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 9.57 (s, 1H), 8.86 (s, 1H), 8.53–8.51 (d, 1H, $J = 10$ Hz), 8.38–8.34 (m, 3H), 8.30–8.20 (m, 4H), 8.14–8.11 (t, 1H, $J = 7.5$ Hz), 7.79–7.78 (d, 1H, $J = 5$ Hz), 7.57–7.56 (d, 1H, $J = 5$ Hz), 7.25–7.23 (t, 2H, $J = 5$ Hz), 6.82–6.80 (m, 2H); ^{13}C NMR (DMSO- d_6 , 500 MHz) δ ppm: 156.8, 150.8, 145.4, 144.3, 142.4, 133.5, 131.4, 131.3, 130.8, 129.7, 129.1, 129.0, 128.6, 128.4, 127.7, 127.1, 126.3, 125.9, 125.5, 124.6, 124.5, 124.3, 123.0, 116.2; ESI-MS: m/z calcd for $\text{C}_{27}\text{H}_{17}\text{NOS}$ (403.49), found 403.49. (Fig. S1-S3)

2.3.2. 4-[(4-pyren-1-yl-benzylidene)-amino]-phenol (KSB-2)

Pale yellow solid (0.127 g, 65%); ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: 9.57 (s, 1H), 8.76 (s, 1H), 8.39–8.38 (d, 1H, $J = 5$ Hz), 8.35–8.33 (d, 1H, $J = 10$ Hz), 8.31–8.29 (d, 1H, $J = 10$ Hz), 8.24 (s, 2H), 8.20–8.18 (d, 1H, $J = 10$ Hz), 8.15–8.0 (m, 5H), 7.77–7.75 (d, 2H, $J = 10$ Hz), 7.29–7.27 (d, 2H, $J = 10$ Hz), 6.85–6.84 (d, 2H, $J = 5$ Hz); ^{13}C NMR (DMSO- d_6 , 500 MHz) δ ppm: 157.1, 156.8, 143.1, 143.0, 136.8, 136.0, 131.4, 131.2, 130.8, 128.9, 128.4, 128.1, 128.0, 127.8, 126.9, 125.9, 125.6, 125.4, 124.9, 124.6, 124.4, 123.0, 116.2; ESI-MS: m/z calcd for $\text{C}_{27}\text{H}_{17}\text{NOS}$ (397.46), found 397.46. (Fig. S4-S6)

2.4. Molecular docking

We have retrieved the three-dimensional structure of bovine serum albumin (PDB ID: 4F5S) from the protein data bank (PDB) database. The binding site of bovine serum albumin was identified by PrankWeb (<https://academic.oup.com/nar/article/47/W1/W345/5494740>) web server. The predicted binding region consists of the following residues positions Tyr400, Asn404, Phe501, Phe506,

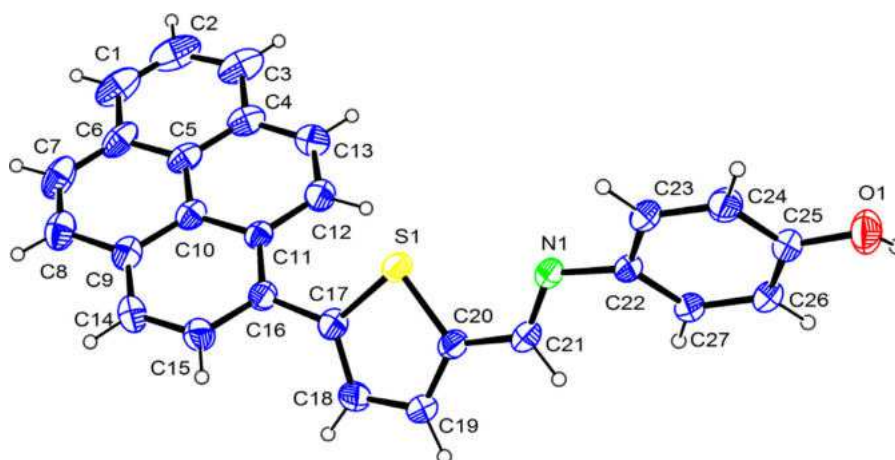


Fig. 1. Molecular structure of KSB-1 with crystallographic labeling (30% probability displacement ellipsoids).

Phe508, Lys524, Gln525, Ala527, Leu528, Leu531, His534, Val546, Met547, Phe550, Val551, Leu574, Val575, Thr578, Gln579 in the A chain. The semi-flexible docking was performed with the default parameters by using the AutoDock program (<https://pubmed.ncbi.nlm.nih.gov/19085980/>). During the docking process, Autodock uses the Lamarckian genetic algorithm for searching the free energy minimum of the BSA-pyrene derivatives. The interaction between BSA and pyrene derivatives was analyzed and visualized using LIGPLOT program (<https://pubmed.ncbi.nlm.nih.gov/7630882/>).

3. Results and discussion

3.1. Crystal structure of KSB-1

Single crystal of KSB-1 was grown from a mixture of petroleum ether and dichloromethane (2:1) mixture at room temperature. The crystallographic data and structural refinement details are given in Table 1. Additionally, the selected bond distances and bond angles are depicted in Table S1. The compound KSB-1 crystallizes

in the triclinic system with P-1 space group. Fig. 1 shows the ORTEP diagram of the KSB-1 with the atomic numbering scheme. The structure of KSB-1 is stabilized by intermolecular C-H...S, C-H...O, C-H... π (pyrene) and C15-H... π (ph) hydrogen bonding, responsible for producing a three-dimensional structure (Fig. 2). In KSB-1, the pyrene ring (atoms C1–C16) and the thiophene ring (S1/C17–C20) are twisted with respect to each other making a dihedral angle of 135.04°; the benzene ring (C22–C27) is nearly perpendicular to the thiophene ring (S1/C17–C20), with a dihedral angle of 174.15°. In this structure, the pyrene rings are widely separated with the closest centroid distance (C9–C11, C14–C16) of 4.626 Å, suggesting weak π - π interactions exists between them.

3.2. Optical properties

UV-Visible absorption spectra of KSB-1 and KSB-2 in DMSO are shown in Fig. 3. The KSB-1 and KSB-2 dyes show strong absorption bands at 388 nm and 358 nm, respectively. By comparing two dyes, KSB-1 absorbs at a longer wavelength with high molar absorption coefficient ($9.5 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$) than KSB-2 ($8.7 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$), which is due to thiophene ring present in the KSB-1 molecule, which increases the effective delocalization electron over the entire molecule (pyrene unit to the acceptor via thiophene π -bridge) [29]. Because, the insertion of different aromatic ring systems as a π -spacer is intended to improve the charge transfer character of the dye since benzene and thiophene can provide effective conjugation and reduce the charge-transfer energy transition due to the reduced resonance energy (thiophene, (29 kcal mol⁻¹) and benzene (36 kcal mol⁻¹)) [30].

3.3. Antibacterial activity

The KSB-1 and KSB-2 were screened for their antibacterial activity against the two strains of *Pseudomonas aeruginosa* ATCC 9027 and ATCC 27853, which are nosocomial pathogens as they are known to cause hospital-borne infections. *P. aeruginosa* cells were grown overnight in shaking at 37 °C in LB broth. From the overnight culture, an inoculum size of OD_{600nm} ~ 0.01 was used in each of the experiments. KSB-1 and KSB-2 were dissolved in DMSO to treat the cells for 24 hrs at 37 °C in a shaking incubator. In PA 9027 cells, KSB-1 treatment has shown to have minimum effect on the cell viability at concentration range from 100 to 500µg/ml. Only at higher concentration of KSB-1 i.e. 1000µg/ml was found to be cytotoxic where almost 70% cell growth inhibition was evident. PA9027 cells were also found to be

Table 1
Crystallographic data and structure refinement parameters for KSB-1.

CCDC Number	2000499
Formula	C ₂₈ H ₂₁ NO ₂ S
Formula weight	435.52
Crystal system	triclinic
Space group	P-1
Temperature (K)	293(2)
Wavelength (Å)	0.71073
a (Å)	8.8290(13)
b (Å)	11.1388(16)
c (Å)	12.3805(17)
α (deg)	75.415(12)
β (deg)	69.948(13)
γ (deg)	79.462(12)
V (Å ³)	1100.6(3)
Z	2
Radiation type	Mo K α
μ (mm ⁻¹)	0.173
Goodness-of-fit on F ²	1.098
R _{int}	0.030
ρ_{calc} mg/mm ³	1.314
final R indices	0.0969
	0.2581
R ₁ ^a	0.1405
wR ₂ ^b	0.2855

^a $R_1 = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$.

^b $wR_2 = \frac{\{\sum w[(F_o^2 - F_c^2)^2] / \sum w[(F_o^2)^2]\}^{1/2}}$.

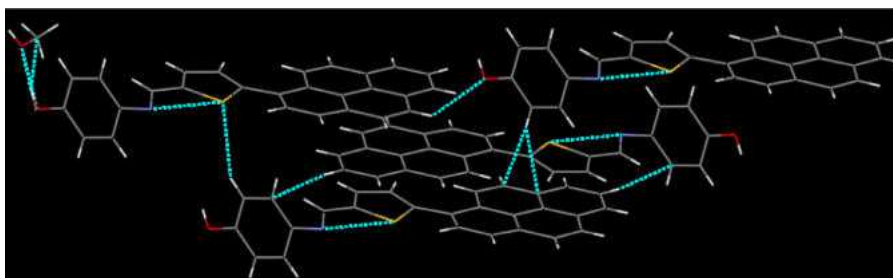


Fig. 2. Hydrogen-bonding network of KSB-1 with hydrogen bonds indicated by dashed lines.

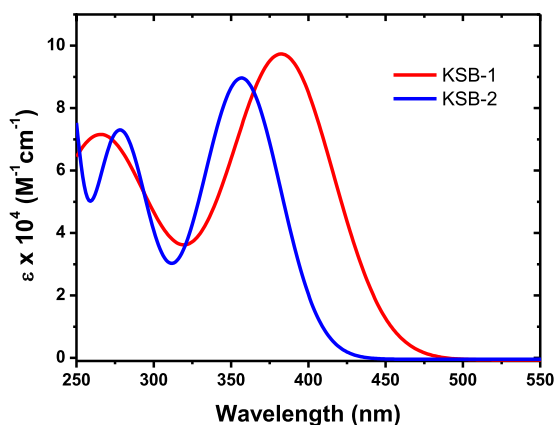


Fig. 3. Absorption spectra of KSB-1 and KSB-2 molecule in DMSO solvent.

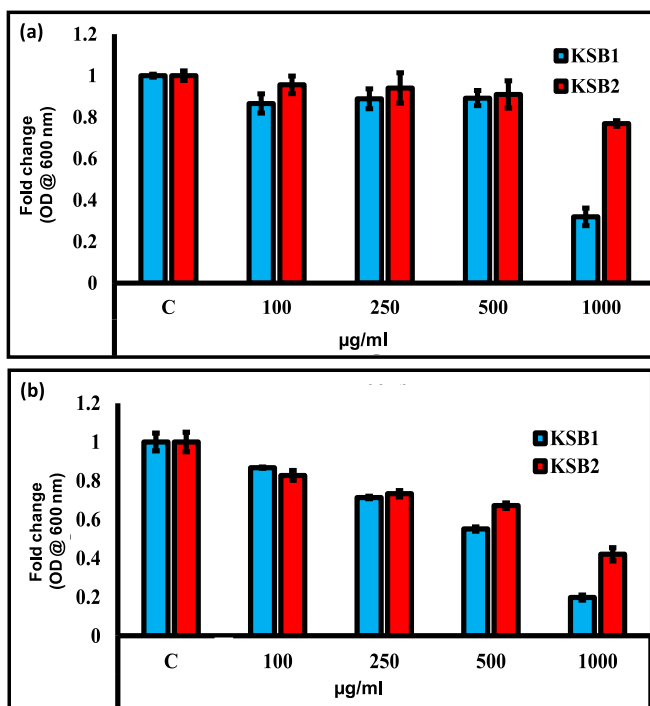


Fig. 4. Mic Broth dilution method effect of pyrene derivatives in DMSO (a) on PA 9027 planktonic cells and (b) on PA 27853 planktonic cells.

insensitive to KSB-2 with all the dosages used (100–1000 µg/ml) (Fig. 4a). On the contrary, the PA27853 cells were sensitive to both the KSB-1 and KSB-2 treatments and the effects were dose-dependent while KSB-1 is more effective in comparison to the KSB-2 (Fig. 4b). Schiff base developed from heterocyclic

compounds containing nitrogen, oxygen and sulfur atoms generally shows momentous antimicrobial activity [31–41].

3.4. BSA binding studies

Proteins are the most copious macromolecules in cells and are critical to maintaining healthy cell functions. Bovine serum albumin (BSA), one of the vital components in plasma protein, plays a vital role in transporting and metabolizing of many endogenous and exogenous compounds in metabolism [42]. In this work, BSA was selected as a target protein molecule for studying the interaction because of its medically significant, unusual ligand-binding properties, availability, and structural homology with human serum albumin (HSA) [43].

Fig. 5a & b shows the fluorescence spectra of BSA with increasing concentrations of pyrene derivatives. The results showed that the fluorescence intensity of BSA quenched in a regular pattern with increasing concentrations of KSB-1. The quenching results indicated that the occurrence of interactions between BSA and KSB-1. Moreover, no significant shift in the wavelength of BSA was observed, which indicates that KSB-1 did not make any conformational changes in the structure of albumin. A similar type of spectral behavior was observed for KSB-2.

In order to understand the quenching mechanism, the fluorescence quenching data were analyzed by the Stern–Volmer Eqs. (1) & (2):

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

$$K_{SV} = k_q \cdot \tau_0 \quad (2)$$

where I_0 and I are the fluorescence intensities of BSA in the absence and presence of quencher, respectively, k_q is the quenching rate constant, K_{SV} is the quenching constant, τ_0 is the lifetime (τ_0) of BSA without the quencher (6 ns) and $[Q]$ is the concentration of the quencher. According to Eq. (1), the plots were constructed between I_0/I vs $[Q]$ and shown in Fig. 6a. Both plots were linear indicates that the quenching may follow a dynamic type. However, in some cases, static type also shows such a type of linear plot. Hence, quenching rate constants were calculated using the slope of the linear plot and lifetime of BSA. The quenching constant and quenching rate constants values are shown in Table 2. Typically, the maximum limit of collisional (dynamic) quenching rate constant (k_q) in the water medium is $2.0 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ [44]. However, the rate constant ($\sim 10^{13} \text{ M}^{-1}\text{s}^{-1}$) we have calculated is much higher than the limited one. Thus, the higher k_q supports

Table 2

The parameters K_{SV} , k_q , K_b and n of BSA with the pyrene derivatives.

Molecules	$K_{SV}(\times 10^5 \text{ M}^{-1})$	$k_q(\times 10^{13} \text{ M}^{-1} \text{ s}^{-1})$	$K_b(\times 10^5 \text{ M}^{-1})$	n
KSB-1	1.64	2.73	3.81	0.78
KSB-2	1.04	1.73	4.53	0.93

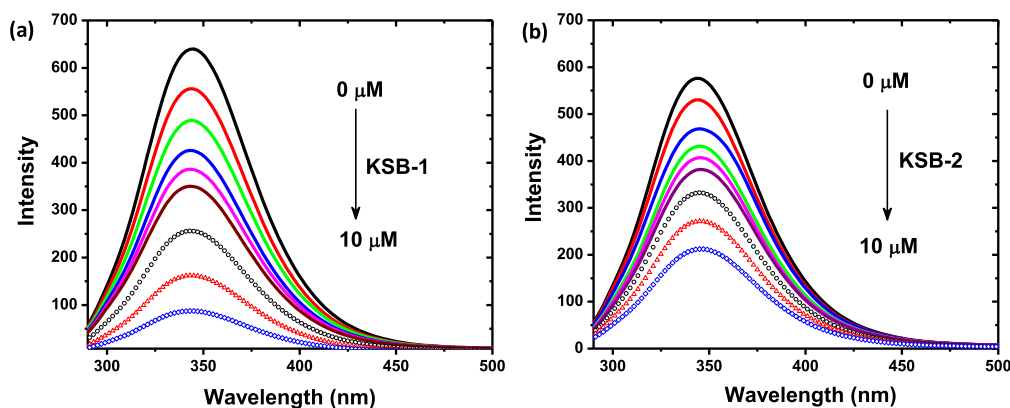


Fig. 5. (a) Fluorescence quenching of BSA with various concentrations of (a) KSB-1 and (b) KSB-2.

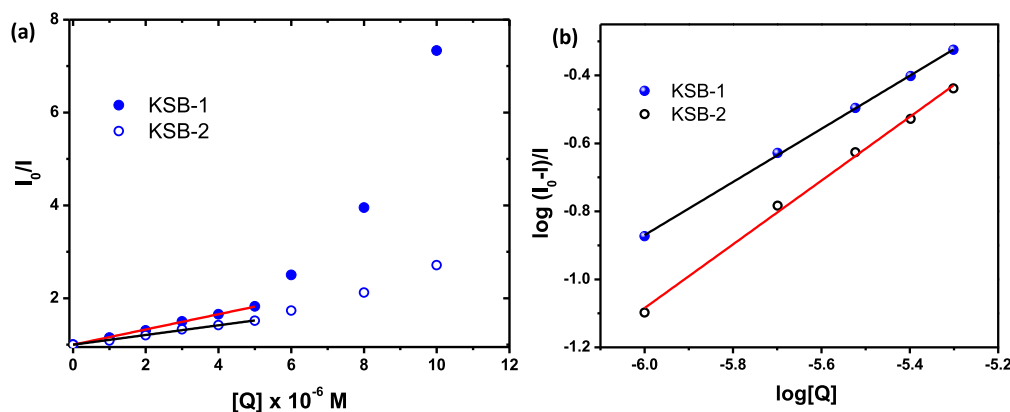


Fig. 6. (a) Stern-Volmer plot and (b) double-logarithmic plot of the fluorescence quenching of BSA by KSB-1 and KSB-2.

the quenching pattern followed by the static type [45–50] rather than the dynamic type.

As the quenching process is static, the binding constant (K_b) and the number of binding sites (n) can be calculated using the double-logarithmic Eq. (3)

$$\log(I_0 - I)/I = \log K_b + n \log [Q] \quad (3)$$

The values of K_b and n can be calculated from the intercept and the slope of the linear plot (Fig. 6b), respectively. Table 2 shows the calculated K_b and n values. The higher K_b value exposes the strong interaction between BSA and pyrene derivatives. On the other hand, the value of n was determined to be nearly 1, suggesting a 1:1 binding type of BSA with pyrene derivatives.

3.5. Free energy calculations

The standard Gibb's free energy change (ΔG_0) can be evaluated from the binding constant and using the following equation to understand the spontaneity of the reaction:

$$\Delta G_0 = -RT \ln K_b \quad (4)$$

where R and T refers to the gas constant and the room temperature, respectively. The calculated ΔG_0 values for the interaction of BSA with pyrene derivatives were -31.83 kcal/mol and -32.27 kcal/mol for KSB-1 and KSB-2, respectively. The obtained negative values were clearly highlighting that the binding between BSA and pyrene derivatives is highly favourable and spontaneous [51]. Intriguingly, the ΔG_0 value is higher for KSB-1 and it is nicely corroborate with antimicrobial results.

3.6. Molecular docking analysis

To understand the nature of molecular interactions between BSA and pyrene derivatives, molecular docking investigations were carried out. The two dimensional docking results were compiled in Fig. 7. As shown in this Fig. 7, the hydrophobic interactions are existing between pyrene derivatives and surround amino acids of BSA. In addition, the hydrogen bonding interactions also exist between hydroxyl group of pyrene derivatives and BSA. Intriguingly, both pyrene derivatives pose the hydrogen binding interactions with similar amino acid residues such as Gln579 and Val575. Although, both the hydrophobic and electrostatic interactions are existing, but the hydrophobic interactions are more vital for the interaction between pyrene derivatives and BSA. The binding free energy for KSB-1 and KSB-2 are -9.03 kcal/mol and -8.39 kcal/mol, respectively. The obtained negative value indicating that the interaction between pyrene derivatives and BSA is energetically favorable.

4. Conclusion

In this work, two new pyrene-based Schiff bases were synthesized by a diminutive modification of π -spacers and their structures were thoroughly characterized by NMR, mass and UV-Vis spectroscopic techniques. Absorption spectral studies indicated that the introduction of thiophene π -spacer ring increased the effective delocalization of electron over the entire molecule compared to benzene π -spacer. Besides, the synthesized KSB-1 and KSB-2 compounds were verified for their antibacterial activities against the drug sensitive and resistant strains

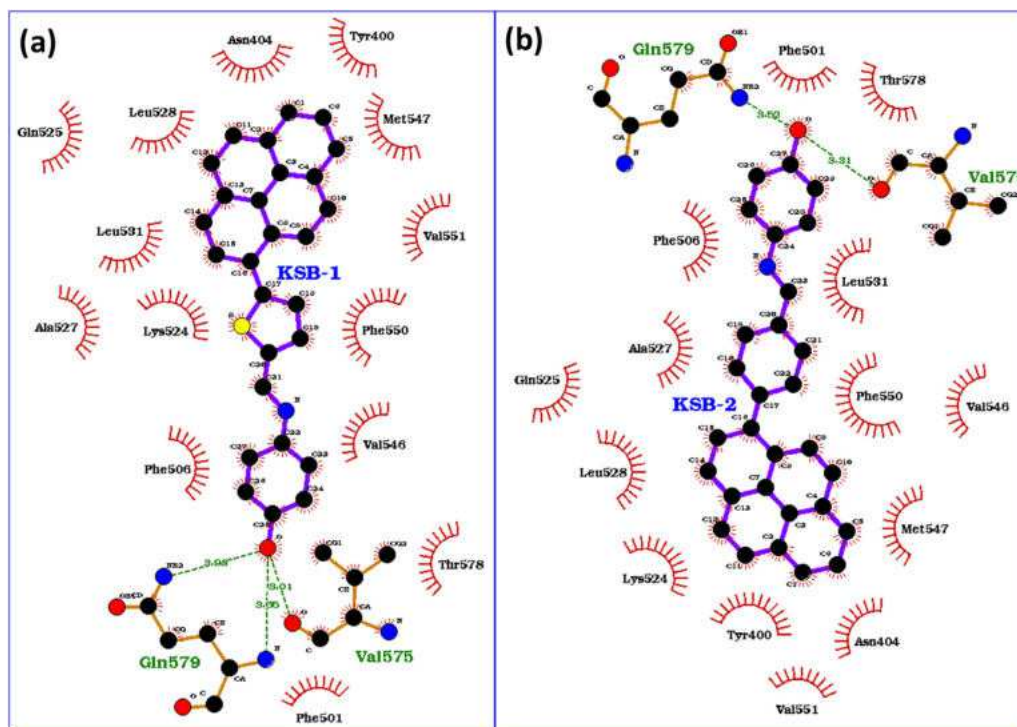


Fig. 7. The 2D schematic representation on nature of interactions between pyrene derivatives and BSA. Residues involved in the hydrophobic interactions are represents in red color sweeps. Hydrogen bond interacting residues are shown with their complete structure. color of the atoms: black – carbon, blue – nitrogen, yellow – sulfur and red – oxygen.

Pseudomonas aeruginosa ATCC 9027 and ATCC 27853. The results indicate that the KSB-1 was more active when compared with KSB-2, because of the introduction of a thiophene ring which alters the chemical structure and effectively affects the biologic activity. The KSB-1 and KSB-2 compounds showed excellent binding interaction to BSA, giving relatively high binding constants. Also, the fluorescence quenching results show that both the compounds were a potent quencher and higher quenching rate constant supports the quenching pattern followed by the static type rather than the dynamic type. From the molecular docking results, the hydrophobic interactions are played major role for the interaction between pyrene derivatives and BSA. Therefore, due to these properties, the synthesized pyrene-based Schiff bases KSB-1 and KSB-2 can further the used in therapeutic applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Venkatesan Srinivasan: Methodology, Data curation, Writing - original draft. **Themmila Khamrang:** Methodology, Data curation, Writing - original draft. **Chairman Ponraj:** Methodology, Data curation, Writing - original draft. **Dhandayutham Saravanan:** Conceptualization, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision, Project administration, Investigation, Writing - review & editing, Funding acquisition. **Rekha Yamini:** Methodology, Formal analysis. **Soumen Bera:** Methodology, Formal analysis. **Mariadoss Asha Jhonsi:** Conceptualization,

Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision, Project administration, Investigation, Writing - review & editing, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129153.

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Fate of model complexes with monocopper center towards the functional properties of type 2 and type 3 copper oxidases

Mariappan Murali¹ · Velusamy Sathya¹ · Balasubramaniam Selvakumaran¹

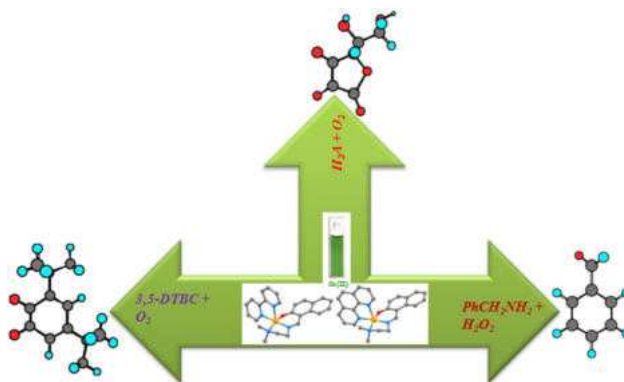
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Abstract

Green colored mononuclear copper(II) complexes viz. [Cu(L)(bpy)](ClO₄) (1) or [Cu(L)(phen)](ClO₄) (2) (where H(L) is 2-((2-dimethylamino)ethyliminomethyl)naphthol) show distorted square pyramidal (4 + 1) geometry with CuN₄O chromophore. The existence of self-assembled molecular associations indicates the formation of the dimer. Dimeric nature in solution is retained due to the binding of the substrate, encourages steric match between substrate and Cu(II) active site, which favors electron transfer. Interestingly, both the complexes exhibit high-positive redox potential. Therefore, the presence of self-assembled molecular association along with the positive redox potential enhances the catalytic oxidation of ascorbic acid to dehydroascorbic acid or benzylamine to benaldehyde or catechol to *o*-quinone thereby model the functional properties of type 2 and type 3 copper oxidases. Notably, catalytic activity is effective when compared with other reported mononuclear copper(II) complexes and even superior to many binuclear copper(II) complexes.

Graphic abstract

Existence of self-assembled molecular association in solution along with high-positive redox potential favors electron transfer process in mononuclear copper(II) complexes and models the functional properties of type 2 and type 3 copper oxidases.



Keyword Copper(II) complexes · Dimeric association · EPR spectra · Redox potential · Catalytic activity

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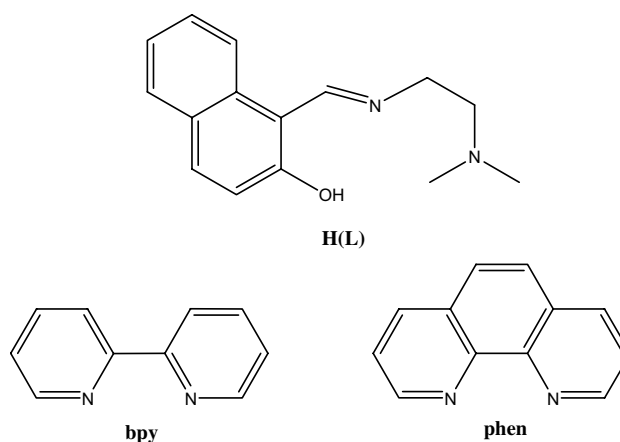
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Introduction

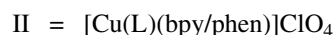
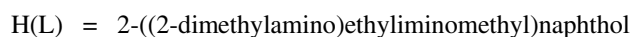
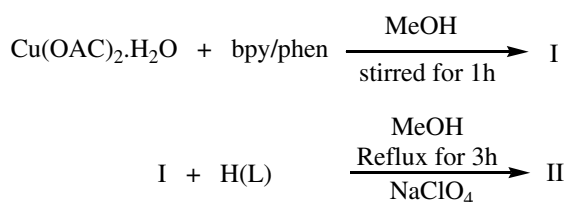
Copper containing metalloproteins in the living system play a fundamental role in revealing its catalytic role as dioxygen and/or substrates activators towards different bio-function [1]. In this context, chemists have significantly contributed in unveiling the mystery of nature, especially by designing

different metal complexes which can potentially mimic the active site and/or function of different copper proteins in nature [2, 3]. One of the important copper oxidases is ascorbate oxidase (AO), which catalyzes the aerobic oxidation of ascorbic acid to the dehydroascorbate [4]. Its active site consists of three types of copper centers in which the type 2 copper site ($A_{||} > 140 \times 10^{-4} \text{ cm}^{-1}$) belongs to the functional trinuclear cluster [5], coordinated to two histidine molecules and a water molecule. The amine oxidase (AmO) is also a type 2 copper oxidase, which undergoes deamination [6] that changes amine to aldehyde in a reaction $\text{RCH}_2\text{NH}_2 \rightarrow \text{RCHO} + \text{NH}_3$ due to two labile donor sites [7] in its active site as homodimers. Each subunit contains one copper center [8] coordinated to three histidine residues and two water molecules, and has a square pyramidal N_3O_2 coordination geometry. A ubiquitous enzyme, catechol oxidase (CO), is a type 3 copper protein containing dicopper center coordinated by six histidine nitrogen atoms and $\mu\text{-}\eta^2\text{:}\eta^2$ peroxo-bridged *oxy* form [9]. It catalyzes the oxidation of catechol to the highly reactive *o*-quinone [10], in the presence of dioxygen [11].

Many model complexes with dicopper center ($\text{Cu}\cdots\text{Cu} \leq 5 \text{ \AA}$) mimic the copper oxidases [12], both structurally and functionally, whereas the same for the monocopper center counterparts remain uncommon. Several works in the literature reveal that a number of factors influence the efficiency of a mononuclear copper(II) complex as catalyst in copper oxidase activity [13–16] such as degree of distortion in square pyramidal geometry, labile binding sites, chelate ring size, nature of the donor groups, electronic properties of the ligands, existence of self-assembled molecular association to form mononuclear pairs, $\text{Cu}\cdots\text{Cu}$ separation, electrochemical potentials, proper match between the substrate and catalyst, the pH of the medium and the nature of the solvent. These factors reflect that investigations of the chance of exhibiting copper oxidase activity by new varieties of compounds are very much demanding. Thus, we have isolated green colored monocopper center complexes, $[\text{Cu}(\text{L})(\text{bpy}/\text{phen})](\text{ClO}_4)$ (**1,2**), where H(L) is 2-((2-dimethylamino)ethylimino-methyl)naphthol (obtained from the condensation of 2-hydroxy-1-naphthaldehyde and *N,N*-dimethylethylenediamine), bpy is 2,2'-bipyridine and phen is 1,10-phenanthroline (Scheme 1). The single crystal X-ray structures of **1** and **2** have been determined. The spectroscopic and electrochemical properties of the complexes were investigated. Their potential catalytic activities toward the oxidation of ascorbic acid or benzylamine or 3,5-di-*tert*-butylcatechol have been tested with an aim to mimic functional properties of ascorbate, amine and catechol oxidases.



Scheme 1 Structure of primary H(L) and diimine (N–N) co-ligands



Scheme 2 Synthesis of copper(II) complexes

Results and discussion

Synthesis and general aspects

Complexes **1** and **2** were synthesized from 2,2'-bipyridine (bpy) or 1,10-phenanthroline (phen), 2-((2-dimethylamino)ethylimino-methyl)naphthol (H(L)), a Schiff base ligand derived from the condensation of 2-hydroxy-1-naphthaldehyde and *N,N*-dimethylethylenediamine) and $\text{Cu}(\text{O}_2\text{CMe})_2 \cdot \text{H}_2\text{O}$ and isolated as green colored crystals (Scheme 2) in good yield (~68–70%). The ESI–MS data in MeCN [m/z [$\text{M}^+ - \text{ClO}_4$], 460.09 (**1**) and 484.18 (**2**)] reveal that the complexes retain its identity even in solution and this is supported by the value of molar conductivity in methanol (**1**, 74; **2**, 73 $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$) for 1:1 electrolyte [14]. The complex exhibits infrared spectral band due to $\nu_{\text{imine}}(\text{C}=\text{N})$ (**1** and **2**, 1621 cm^{-1}), $\nu_{\text{py}}(\text{C}=\text{N})$ (**1**, 1592; **2**, 1599 cm^{-1}) and $\nu(\text{naph-O})$ (**1**, 1312; **2**, 1307 cm^{-1}), implying direct coordination of imine and pyridine nitrogen and naphtholate oxygen donors to copper(II). A broad intense band (**1**, 1088; **2**, 1098 cm^{-1}) and a strong sharp band (**1** and **2**, 621 cm^{-1}) are observed, which are the

characteristics of noncoordinated perchlorate ion. Based on the elemental analysis the complexes were formulated as $[\text{Cu}(\text{L})(\text{bpy}/\text{phen})](\text{ClO}_4)$, which was confirmed by single crystal X-ray determination of **1** and **2**.

Description of the structures

The details of the crystallographic data of **1** and **2** are presented in Table 1 and selected bond lengths and angles are given in Table 2. The Cu(II) in each complex cation, $[\text{Cu}(\text{L})(\text{bpy})/(\text{phen})]^+$ (**1,2**), is coordinated by imine N(1) and amine N(2) nitrogen atoms and the naphtholate oxygen atom O(1) of the meridionally coordinated H(L) ligand, and one of the imine nitrogen atom N(3) of bpy or phen occupy the corners of the CuN_3O square plane of this geometry (Figs. 1a and 2a). The copper is displaced by 0.154(8) Å (**1**) or 0.150(7) Å (**2**) from the N_3O plane towards the axially

Table 1 Selected crystal data and structure refinement parameters for complexes $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**), $[\text{Cu}(\text{L})(\text{phen})](\text{ClO}_4)$ (**2**)

	1	2
Formula	$\text{C}_{25}\text{H}_{25}\text{ClCu}\cdot\text{N}_4\text{O}_5$	$_{28}\text{H}_{25}\text{ClCu}\cdot\text{N}_4\text{O}_5$
Formula weight	560.48	603.52
Temperature (K)	296(2)	296(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Triclinic
Space group	$\text{P}2_1/c$	P-1
<i>a</i> (Å)	10.0857 (6)	10.6793 (4)
<i>b</i> (Å)	18.1383 (9)	11.2565 (5)
<i>c</i> (Å)	13.4892 (8)	12.7439 (5)
α (°)	90	70.283 (2)
β (°)	92.012 (2)	78.274 (2)
γ (°)	90	70.757 (2)
<i>V</i> (Å ³), <i>Z</i>	2466.2 (2), 4	1354.25 (9), 2
<i>D</i> _{calc} (mg m ⁻³)	1.510	1.480
μ (mm ⁻¹)	1.038	0.952
<i>F</i> (000)	1156	621
Crystal size (mm)	0.40 × 0.35 × 0.30	0.30 × 0.25 × 0.20
θ (°)	1.88–28.33	2.79–28.30
Index range	–13 ≤ <i>h</i> ≤ 13 –17 ≤ <i>k</i> ≤ 24 –17 ≤ <i>l</i> ≤ 17	14 ≤ <i>h</i> ≤ 13 –13 ≤ <i>k</i> ≤ 14 –17 ≤ <i>l</i> ≤ 16
Reflections collected	18,900	11,995
Independent reflections	6080	6557
Reflections observed	4537	5192
$[I > 2\sigma(I)]$		
<i>R</i> _{int}	0.0295	0.0179
GOOF	1.034	1.041
<i>R</i> ₁ [<i>I</i> > 2σ(<i>I</i>)]	0.0406	0.0377
<i>wR</i> ₂ [<i>I</i> > 2σ(<i>I</i>)]	0.1083	0.1012
<i>R</i> ₁ / <i>wR</i> ₂ all data	0.0605/0.1227	0.0522/0.1101

Table 2 Selected bond lengths (Å) and bond angles (°)

$[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (1)			
Cu(1)–N(1)	1.928(2)	Cu(1)–N(2)	2.065(2)
Cu(1)–N(3)	2.027(2)	Cu(1)–N(4)	2.204(2)
Cu(1)–O(1)	1.9162(18)	Cu(1)–N(2)	
O(1)–Cu(1)–N(1)	90.52(8)	O(1)–Cu(1)–N(2)	163.69(9)
O(1)–Cu(1)–N(3)	87.20(8)	O(1)–Cu(1)–N(4)	98.05(9)
N(1)–Cu(1)–N(2)	84.80(9)	N(1)–Cu(1)–N(3)	177.08(8)
N(1)–Cu(1)–N(4)	104.33(8)	N(2)–Cu(1)–N(3)	96.90(9)
N(2)–Cu(1)–N(4)	98.25(8)	N(3)–Cu(1)–N(4)	77.81(8)
$[\text{Cu}(\text{L})(\text{phen})](\text{ClO}_4)$ (2)			
Cu(1)–N(1)	1.9182(16)	Cu(1)–N(2)	2.1065(18)
Cu(1)–N(3)	2.0157(17)	Cu(1)–N(4)	2.2692(19)
Cu(1)–O(1)	1.9298(15)		
O(1)–Cu(1)–N(1)	91.29(7)	O(1)–Cu(1)–N(2)	167.19(7)
O(1)–Cu(1)–N(3)	89.67(6)	O(1)–Cu(1)–N(4)	95.25(7)
N(1)–Cu(1)–N(2)	84.50(7)	N(1)–Cu(1)–N(3)	174.83(7)
N(1)–Cu(1)–N(4)	106.74(7)	N(2)–Cu(1)–N(3)	93.48(7)
N(2)–Cu(1)–N(4)	97.55(7)	N(3)–Cu(1)–N(4)	78.22(7)

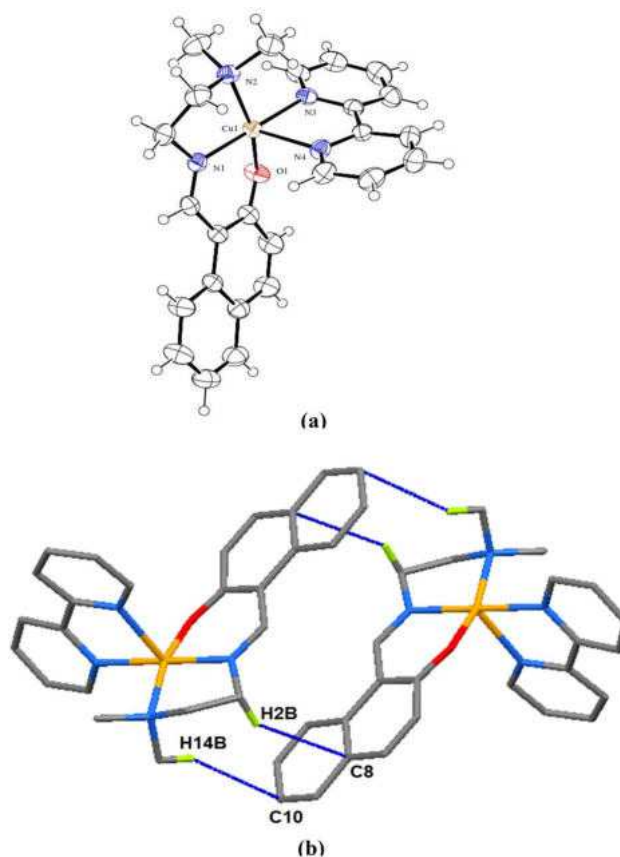


Fig. 1 a ORTEP diagram of $[\text{Cu}(\text{L})(\text{bpy})]^+$ (**1**) showing 40% probability thermal ellipsoids with the atom labeling scheme for the metal and heteroatoms and b Packing diagram viewed down the *a*-axis showing intermolecular interactions of $[\text{Cu}(\text{L})(\text{bpy})]^+$ (**1**) (blue, C–H...π)

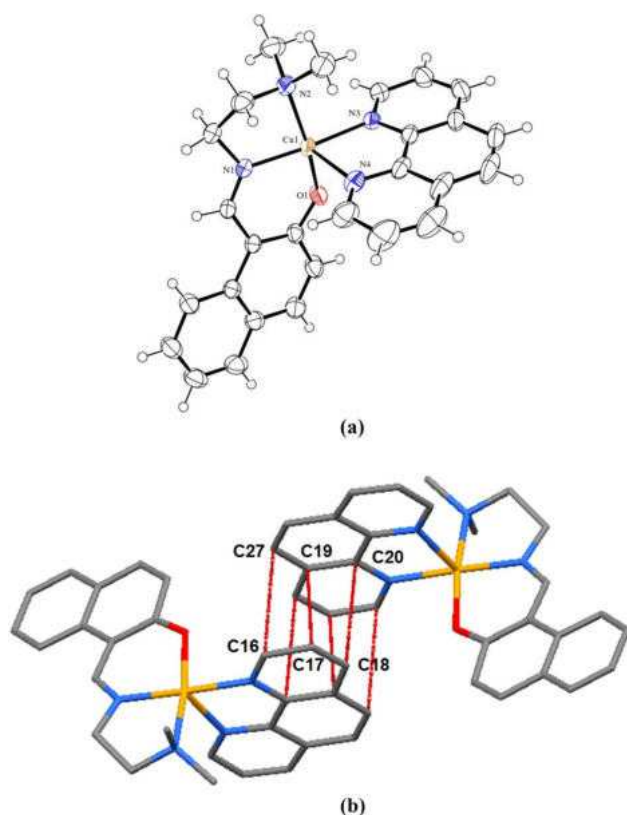


Fig. 2 a ORTEP diagram of $[\text{Cu}(\text{L})(\text{phen})]^+$ (**2**) showing 40% probability thermal ellipsoids with the atom labeling scheme for the metal and heteroatoms and (b) Packing diagram viewed down the a -axis showing intermolecular interactions of $[\text{Cu}(\text{L})(\text{phen})]^+$ (**2**) (red, π - π stacking)

coordinated nitrogen atom N(4) of bpy or phen. The value of the trigonality index [17], τ (**1**, 0.22; **2**, 0.13) reveals that the coordination geometry around copper(II) is best described as distorted square-based pyramidal. The axial nitrogen atom N(4) at a distance 2.204(2) Å (**1**) or 2.2692(19) Å (**2**) is longer than equatorial atoms [**1**: Cu–O, 1.9162(18) Å; Cu–N, 1.928(2)–2.065(2) Å and **2**: Cu–O, 1.9298(15) Å; Cu–N, 1.9182(16)–2.1065(18) Å] due to the presence of two electrons in d_{z^2} orbital of copper(II). The overlapping of the lone pair on N(2) nitrogen of $-\text{NMe}_2$ group with $d_{x^2-y^2}$ orbital is improper, which may also be responsible for the longer Cu(1)–N(2) bond distance. The Cu–N_{amine} bond [2.065(2)–2.1065(18) Å] is longer than the Cu–N_{imine} bond (1.9182(16)–1.928(2) Å) formed by the H(L) ligand, which is expected of sp^3 and sp^2 hybridizations of the amine N(2) and imine N(1) nitrogen atoms, respectively [18].

Crystal packing of **1** reveals that cations of adjacent molecules are arranged in dimeric association (Fig. 1b) and these pairs are held together by attractive C–H \cdots π noncovalent interaction. The strongest intermolecular interaction corresponds to H(2B) \cdots C_g(ph) and H(14B) \cdots C_g(ph) distance (2.918 Å, 3.289 Å) and $\angle\text{C}(2)\text{--H}(2\text{B})\cdots\text{C}_g(\text{ph})$ and

$\angle\text{C}(14)\text{--H}(14\text{B})\cdots\text{C}_g(\text{ph})$ angle (148.44°, 166.36°) [19]. It stabilizes the structure of **1**, and shows very closer approach and orientation of the adjacent molecules and displays the Cu \cdots Cu distance of 8.27 Å. In contrast, crystal packing of **2** shows dimeric association, which is built from pairs of adjacent molecules through interpair π – π interactions (Fig. 2b) brought on by intermolecular antiparallel stacking involving phen ligands. The π – π stacking between (1) C(16) of py and C(27) of phenyl (C(16) \cdots C(27), 3.308 Å), (2) C(18) of py and C(20) of py (C(18) \cdots C(20), 3.304 Å) and (3) C(17) of py and C(19) of py (C(17) \cdots C(19), 3.371 Å) of neighboring coordinated phen ligands. The py (C_g(p)) and phenyl (C_g(ph)) rings of adjacent molecules with average spacing of C_g(p) \cdots C_g(ph) (3.797 Å) and C_g(p) \cdots C_g(p) (3.525 Å) [19] is expected to stabilize the complex in the solid state. At a centroid contact of 3.525 Å and an angle of 20.51° between the ring normal and the centroid vector corresponds to a horizontal displacement of 1.344 Å reveals the shift of almost one C–C bond length face-to-face alignment (C_g(p)) [20]. Therefore, the Cu \cdots Cu separation through the stacked phen ligands is 8.49 Å.

Electronic, EPR, and redox properties

The electronic spectra of **1** and **2** in MeOH and MeOH:H₂O (4:1 v/v) are very similar and display a broad ligand-field band (**1**, 642; **2**, 617 nm) with a weak low-energy shoulder (**1**, 872; **2**, 853 nm) in the visible region (Figure S1–S4) is a characteristic of distorted square pyramidal copper(II) complexes [21, 22]. The band at 392 nm (**1** and **2**) is assigned to naphtholate anion to copper(II) ligand-to-metal charge-transfer (LMCT) transition. The diffuse reflectance spectra exhibit the broad ligand-field band (**1**, 657; **2**, 610 nm) suggesting that the position of the band remains almost unchanged (Figure S5 and S6) indicates that the complexes retain in solutions its solid-state coordination geometry (cf. above) [23]. The polycrystalline EPR spectra exhibit one broad singlet (g_{iso} : **1**, 2.083; **2**, 2.063) and an additional half-field signal ($S = 1/2$ system, $\Delta M_s = \pm 2$) is observed at 1602 G (**1**) consistent with the existence of weak dimeric intermolecular association (Figure S7 and S8). The frozen solutions in DMF, three of the four parallel hyperfine features were well separated while the fourth one overlapped with the perpendicular features (Fig. 3a and Figure S9a) typical of axial mononuclear Cu(II) species ($g_{\parallel} > g_{\perp} > 2.0$; $G = (g_{\parallel} - 2) / (g_{\perp} - 2) = 5.0\text{--}5.1$) suggesting the presence of $d_{x^2-y^2}$ ground state in copper(II) located in square-based geometries [14]. The g_{\parallel} (**1**, 2.223; **2**, 2.222) and A_{\parallel} (**1**, 186; **2**, $176 \times 10^{-4} \text{ cm}^{-1}$) values are found to increase (g_{\parallel} , 2.200) and decrease (A_{\parallel} , $180\text{--}200 \times 10^{-4} \text{ cm}^{-1}$), respectively, due to the replacement of nitrogen atom in square-based CuN_4 chromophore [24] by an oxygen atom to form CuN_3O plane with strong axial interaction, which is substantiated by the value

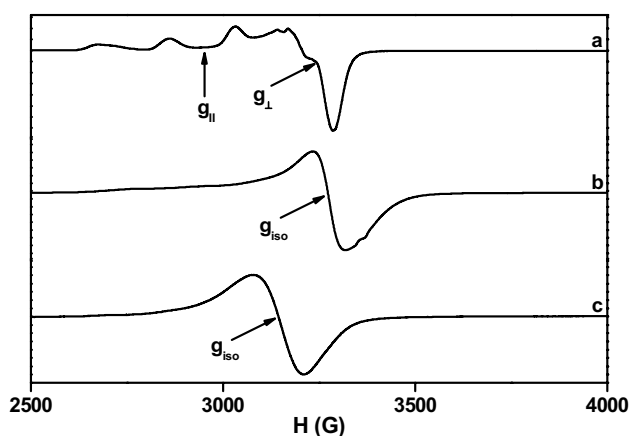


Fig. 3 EPR spectra for frozen solutions of $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) at 77 K: **a** DMF, **b** MeOH and **c** MeCN

of $g_{\parallel}/A_{\parallel}$ (**1**, 126; **2**, 119 cm^{-1}) quotient (cf. above). Molecular orbital coefficients [25], α^2 (**1**, 0.77; **2**, 0.79) and β^2 (**1**, 0.67; **2**, 0.68) values indicate that there is a substantial interaction in the in-plane σ -bonding whereas the in-plane π -bonding is almost covalent. Also, it is observed that the orbital reduction factors, K_{\parallel} (**1**, 0.72; **2**, 0.73) $>$ K_{\perp} (**1**, 0.64; **2**, 0.65) [26], show significant out-of-plane π -bonding. In contrast, the parallel feature was very weakly resolved and the perpendicular feature was moderately broader in frozen MeOH (g_{iso} : **1**, 2.126; **2**, 2.125. Figure 3b Figure S9b) and MeCN (g_{iso} : **1**, 2.091; **2**, 2.090. Figure 3c Figure S9c) and thus the signals are found to be isotropic. This suggests that the dimeric intermolecular association is retained in weak coordinating solvents like MeCN and MeOH [26] while strong coordinating solvent like DMF, the association is disturbed due to solvolysis. Further, electrospray ionization mass spectrometry (ESI-MS) and high resolution mass spectrometry (HRMS) measurements of mixture of **1** and 3,5-DTBC H_2 and **1** and H_2A in MeOH detected a peak at $m/z = 922.05$ and 924.70 (Figure S10 and S11), respectively, correspond to the existence of a dimer (cf. below), $[\text{Cu}^{\text{II}}(\text{L})(\text{bpy})]_2^{2+}$, in a weak coordinating solvents like MeOH. Cyclic (CV) and differential pulse voltammetric (DPV) responses of **1** and **2** obtained from MeOH (Figure S12 and S13) reveal the electrochemically and chemically reversible $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ redox behavior ($E_{1/2}$: **1**, 0.193; **2**, 0.139 V vs SCE and ΔE_p : **1**, 83; **2**, 88 mV). It indicates that the structural reorganization between their copper(II) and copper(I) species is minimal leading to a facile heterogeneous electron transfer [27] (cf. τ values: **1**, 0.22; **2**, 0.13).

Ascorbic acid oxidation

The addition of substrate (ascorbic acid, H_2A) to the solution of the catalysts (**1** and **2**; 3×10^{-3} M), the initial

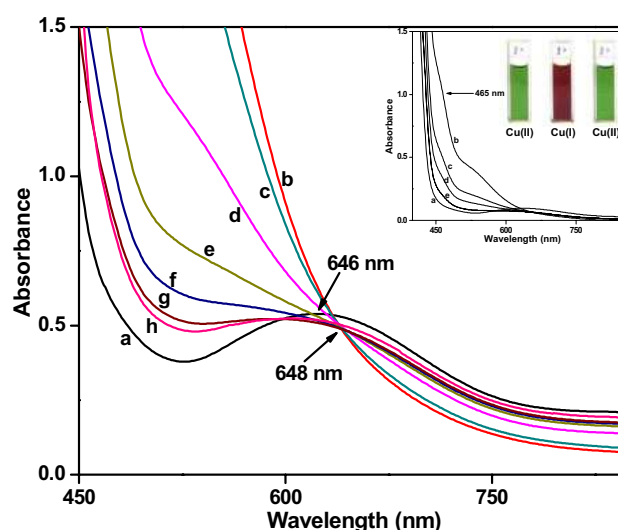


Fig. 4 Visible spectral traces showing the conversion of $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**; 3×10^{-3} M) (**a**) to $[\text{Cu}(\text{HL})(\text{bpy})](\text{ClO}_4)$ (**2**) on reduction with ascorbic acid (3×10^{-3} M) in MeOH:H $_2$ O (4:1 v/v) and the regeneration of the copper(II) species (c–h). Inset: conversion of the copper(I) species

green solution ascribed to the Cu(II) complexes (**1**, 646; **2**, 615 nm) immediately changed to give a brown solution and a new charge transfer (CT) band (**1**, 465; **2**, 461 nm) is formed due to the conversion of Cu(II) to Cu(I) species [13]. Brown color of the solution converts to the green color within a precise time on exposure to air coincidentally with the disappearance of CT band and reappearance of the d–d band (**1**, 640; **2**, 613 nm). It demonstrates the gradual conversion of Cu(I) to parent Cu(II) species, using dioxygen as an oxidant (Fig. 4 and Figure S14) and giving an isosbestic point (**1**, 648; **2**, 633 nm). The catalytic oxidation of H_2A to dehydroascorbic acid (dA) is highly effective at $[\text{H}_2\text{A}]: [\text{complex}]$ mole ratio of 80:1 (**1**) and 60:1 (**2**).

The kinetic data were determined by initial rates, monitoring the decrease in H_2A absorbance at 265 nm due to the oxidation of H_2A in the presence of copper(II) complexes produce dA. Solutions of **1** and **2** (1.667×10^{-6} M) in MeOH:H $_2$ O (4:1 v/v, pH 7.3) were treated with 60 equivalent of H_2A in the presence of air. The decrease in absorbance was continuously monitored at $\lambda = 265$ nm over the first 30 min (Fig. 5 and Figure S15). The average rate constant values (**1** and **2**) shows that the rate is first order at low concentration of H_2A while saturated at higher concentration. The rates of reaction obtained for various H_2A concentrations (20–140 equivalent) were fitted to the Michaelis–Menten equation (Figure S16 and S17) and linearized by means of Lineweaver–Burk plot to determine the various kinetic parameters (K_M , V_{max} and k_{cat}) as in Table 3 for copper(II) complexes. The higher k_{cat} ($1.10 \times 10^6 \text{ h}^{-1}$) value found for **1** suggest the best catalytic behavior for this

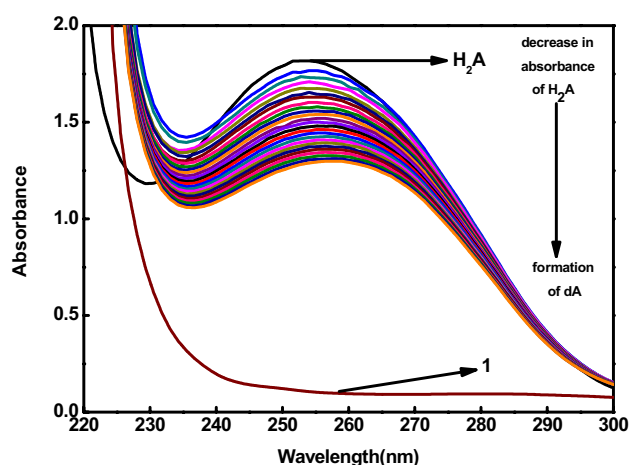


Fig. 5 Oxidation of ascorbic acid by $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) in MeOH monitored by UV–Vis spectroscopy

Table 3 Kinetic parameters of copper(II) complexes showing ascorbate oxidase activity

Complexes	K_M (mM)	V_{\max} (Ms^{-1})	k_{cat} (h^{-1})
1	7.60	31.0	1.10×10^6
2	4.08	27.1	9.81×10^5

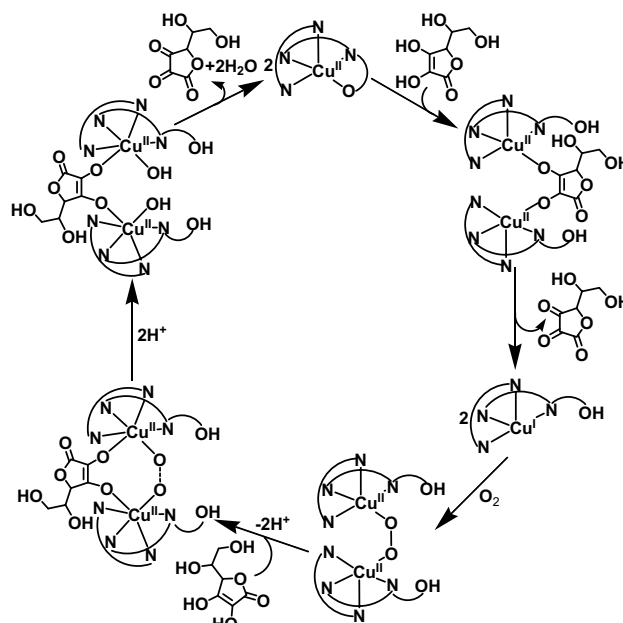
compound and comparable to the lower value that measured for various ascorbate oxidase enzyme in the range, k_{cat} , 2.45×10^6 – $3.1 \times 10^7 \text{ h}^{-1}$ [28, 29].

The possible mechanistic implications, whereby **1** and **2** catalyze H_2A to dA presumably implies the following interactions: (a) formation of a dicopper (II)–ascorbate intermediate, (b) this intermediate could be recognized under intermolecular electron-transfer to the oxidized dA and two copper(I) centers and (c) irreversible reaction of the generated copper(I) species with O_2 to give *cis*- μ - $\eta^1:\eta^1$ or *cis*- μ - $\eta^2:\eta^2$ peroxide adduct, $[\text{((H(L))}(\text{diimine})\text{Cu}^{\text{II}})_2\text{O}_2]$, in the rate-determining step. This process most likely involves the transfer of an electron from the copper(I) ion to the oxygen molecule, as the $\text{((H(L))}(\text{diimine})\text{Cu}^{\text{II}})_2\text{O}_2$ complex reacts more readily than $\text{((H(L))}(\text{diimine})\text{Cu}^{\text{I}})$ ion. Accordingly, the initiation step almost certainly involves the production of copper(I) by the interaction of copper(II) with ascorbic acid. In this situation, the $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple is involved as a redox center. According to the mechanistic hypothesis, the increase in Lewis acidity of a copper(II) center in the dicopper(II)–ascorbate intermediate increases the redox potential of the $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple [30] and thus, facilitates electron transfer from the lactone ring to the copper(II) center. The DPV studies reveal a positive redox potential ($E_{1/2}$) follow the order **1** (192 mV) > **2** (128 mV), which reflects that in the $[\text{((H(L))}(\text{bpy})\text{Cu}^{\text{II}})_2\text{-ascorbate}]$

intermediate (**1**), electron-transfer from the lactone ring to the copper(II) center is assumed to be more favored as compound with $\text{((H(L))}(\text{phen})\text{Cu}^{\text{II}})$ complex (**2**). (d) Finally, the interaction of $[\text{((H(L))}(\text{diimine})\text{Cu}^{\text{II}})_2\text{O}_2]$ complex with ascorbate leads to regeneration of the catalyst in its original active form and formation of dA in addition to two molecules of water (Scheme 3). The H_2A (m/z , 177.08), dA (m/z , 175.13), dimeric aggregate, $[\text{Cu}^{\text{II}}(\text{L})(\text{bpy})]_2^{2+}$ (m/z , 924.70), dicopper(II)–ascorbate intermediate, $[\text{((H(L))}(\text{bpy})\text{Cu}^{\text{II}})_2\text{-ascorbate}]$ (m/z , 1098.40) and dioxygen bound species, $[\text{((H(L))}(\text{bpy})\text{Cu}^{\text{II}})_2\text{O}_2]$ (m/z , 956.90) identified in the high-resolution mass spectrum (HRMS) of mixture of **1** and H_2A (Figure S11) in MeOH support the above catalytic process. Hence, both the complexes (**1** and **2**) mimic the ascorbate oxidation property of the type 2 sites in AO.

Benzylamine oxidation

The reaction of benzylamine ($100 \times 10^{-3} \text{ M}$) with catalysts (**1**, **2**; $1 \times 10^{-3} \text{ M}$) causes the complete oxidation of benzylamine to benzaldehyde (Fig. 6 and Figure S18) in the presence of H_2O_2 in MeOH:H₂O (4:1 v/v) [31]. The formation of benzaldehyde isolated from the reaction using catalyst **1** is evidenced by the ^1H NMR (PhCH_2NH_2 , δ 3.87 (– CH_2 –); PhCHO , δ 10.03 ppm (–CHO); Figure S19) and LC–MS (m/z , 105.9 (PhCO^+) and 107.0 (PhCHO); Fig. S20) spectral studies. The electronic spectral results (Fig. 6 and Figure S18) reveal that the initial step is the formation of an adduct showing a significant shift of the visible band



Scheme 3 Possible mechanism of oxidation of ascorbic acid (H_2A) in the presence of a catalyst, $[\text{Cu}(\text{L})(\text{bpy})]\text{ClO}_4$ (**1**) based on the experimental evidence

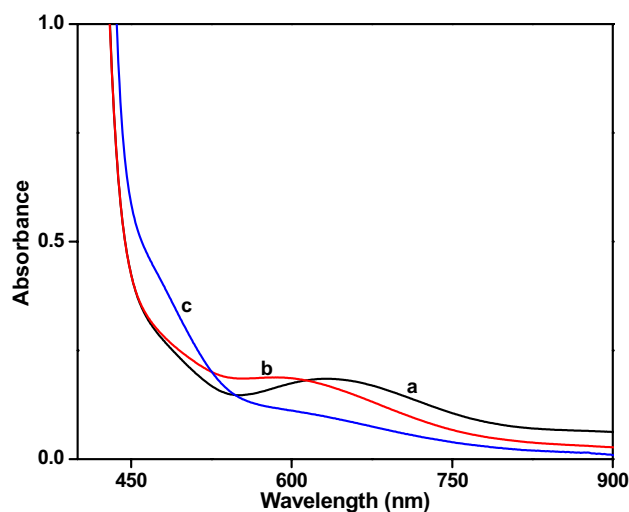


Fig. 6 Visible spectra of $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) in $\text{MeOH}:\text{H}_2\text{O}$ (4:1 v/v) (**a**), in presence of benzylamine (**b**) and after treatment with benzylamine and H_2O_2 (**c**)

(**1**, 631–586; **2**, 613–604 nm) to higher energy by the addition of benzylamine to the solution of **1** or **2**. In addition, the high resolution mass spectrum (HRMS) of mixture of **1** and benzylamine in MeOH shows the peak at $m/z = 585.55$, which is consistent with the formation an adduct, $[\text{Cu}(\text{L})(\text{bpy})(\text{benzylamine})]^+\cdot\text{H}_2\text{O}$ (Figure S21). This adducts species react with H_2O_2 to form an initial brown color solution that does not show d–d band. It indicates the one-electron reduction of copper along with the concomitant protonation of the L by benzylamine adduct with copper. Thus, the Cu(I) species is reactive towards benzylamine which undergoes oxidation to benzylimine and the presence of water hydrolyzes benzylimine to benzaldehyde. In an enzymatic process, the deamination reaction is significant, which converts amine to aldehyde in a reaction $\text{RCH}_2\text{NH}_2 \rightarrow \text{RCHO} + \text{NH}_3$ involves type 2 copper centers of AmO [6, 32].

Catechol oxidation

The methanolic solutions of **1** and **2** (2.9×10^{-5} M) were treated with 50 equivalents of 3,5-di-*tert*-butylcatechol (3,5-DTBCH₂) in the presence of air [33] result the disappearance of peak at 646 nm (**1**) or 615 nm (**2**) followed by the generation of new absorption band at 397 nm, characteristic of 3,5-di-*tert*-butyl-*o*-quinone (3,5-DTBQ; Fig. 7). The time-dependent spectral changes are monitored by UV/Vis spectroscopy for a period of 3 h. It discloses the development of a strong absorption band (Fig. 8 and Figure S22) at 397 nm, clearly proves the oxidation of 3,5-DTBCH₂ to 3,5-DTBQ. The 3,5-DTBQ was purified by column chromatography and isolated in significant yield (**1**, 75.1; **2**, 60.7%). The melting point of isolated *o*-quinone using **1** and **2** (~ 109 °C) is same

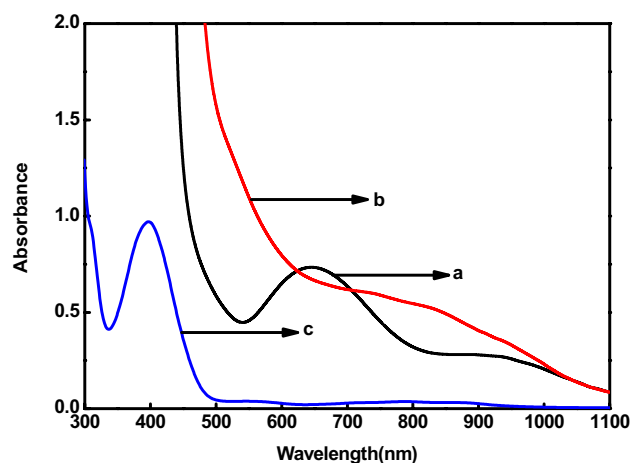


Fig. 7 Electronic spectral changes of $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) upon addition of 50 equivalents of 3,5-DTBCH₂ in MeOH . **a** electronic spectrum of **1**, **b** disappearance of d–d band immediately after the addition of 3,5-DTBCH₂ and **c** generation of 3,5-DTBQ

as that of the values reported in the literature (~ 110 °C) [34]. Also, the isolated *o*-quinone using **1** alone was identified by ¹H NMR spectra in CDCl_3 (Figure S23: $-\text{CH}_3$ (C), s, δ 1.229 ppm, 9H; $-\text{CH}_3$ (D), s, δ 1.272 ppm, 9H; H (B), s, δ 6.220 ppm, 1H; H (A), s, δ 6.936 ppm, 1H) and FTIR spectra (Figure S24: the bands at 3460 and 3260 cm^{-1} due to $\nu(\text{O}-\text{H})$ present in 3,5-DTBCH₂ is absent and a band present at 1661 cm^{-1} attributed to $\nu(\text{C}=\text{O})$ of the product). When the complexes were treated with varying concentrations of 3,5-DTBCH₂, the average rate constant values for **1** and **2** show that the rate is first order at low concentrations, but exhibit saturation kinetics at their higher concentrations. This type of saturation kinetics were fitted to the

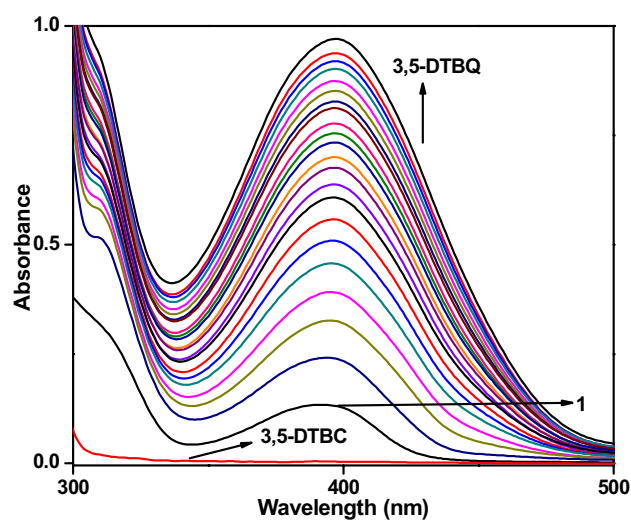


Fig. 8 Oxidation of 3,5-DTBCH₂ by $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) in MeOH monitored by UV–Vis spectroscopy

Michaelis–Menten model, which gives linear double reciprocal Lineweaver–Burk plot (Figure S25 and S26) to analyze the parameters, such as maximum velocity (V_{\max} : **1**, 2.82; **2**, $1.11 \times 10^{-5} \text{ Ms}^{-1}$), rate constant for the dissociation of the complex–substrate intermediate or turnover number (k_{cat} : **1**, 3.51; **2**, $1.38 \times 10^3 \text{ h}^{-1}$) and Michaelis binding constant (K_M : **1**, 35.8; **2**, 30.0 mM). Interestingly, the turnover rate of **1** and **2** is comparable to those reported by Monzoni et al. ($2.52 \times 10^3 \text{ h}^{-1}$) [35], Vittal et al. ($3.80 \times 10^3 \text{ h}^{-1}$) [36] and Biswas et al. ($3.91 \times 10^3 \text{ h}^{-1}$) [37] and to an order of hundred times more than the complexes reported by Neves et al. (12.4 h^{-1}) [38], Panja et al. (23.58 h^{-1}) [39] and Kerbs et al. ($10.7\text{--}40.0 \text{ h}^{-1}$) [40], but are remarkably lower than those reported by Terán et al. ($3.89 \times 10^6 \text{ h}^{-1}$) [41] Table 4. The latter is one of the best catalyst models for this oxidation process and that k_{cat} value is one of the highest reported, being comparable to that measured for catechol oxidase enzyme ($8.25 \times 10^6 \text{ h}^{-1}$) [42]. Also, we found the band at $\sim 353 \text{ nm}$ which is due to the existence of I_3^- [43], which provide evidence for the formation of H_2O_2 from the reaction mixture.

As **1** shows higher turnover rate on catalytic oxidation of 3,5-DTBCH₂ to 3,5-DTBQ, the electrospray ionization mass spectrum (ESI–MS positive) of a mixture of **1** and 3,5-DTBCH₂ (molar ratio, 1:10) was recorded after 30 min in methanol solution (Figure S10). The most abundant species found at $m/z = 461.00$ arises from monomeric unit of the complex and are assigned to $[\text{Cu}^{\text{II}}(\text{L})(\text{bpy})]^+$ while low intense peak at $m/z = 922.05$ appears due to the dimeric aggregate of the complex and this can be assigned to $[\text{Cu}^{\text{II}}(\text{L})(\text{bpy})]_2^{2+}$. The peak at 243.30 is well assignable to the $[\text{3,5-DTBQ} + \text{Na}]^+$. The remaining peak at $m/z = 706.35$ including two additional peaks at $m/z = 738.35$ and 1167.40 are observed, which clearly indicate that these peaks arise from complex–substrate aggregates and can be assigned to

$[\text{Na-Cu}^{\text{I}}(\text{HL})(\text{bpy})(3,5\text{-DTBCH})]^+$, $[\text{Na-Cu}^{\text{II}}(\text{HL})(\text{bpy})(\text{O}_2)(3,5\text{-DTBCH})]^+$ and $\{\text{Na-}[\text{Cu}^{\text{II}}(\text{HL})(\text{bpy})]^{2+}\cdot(3,5\text{-DTBCH})\cdot[\text{Cu}^{\text{II}}(\text{HL})(\text{bpy})]^{2+}\}^+$, respectively. The results reveal the presence of dimeric aggregate of **1**, which catalyzes the oxidation of catechol to o-quinone via reduced copper(I) intermediate and molecular oxygen, models the functional property of type 3 copper(II) centers in CO.

Experiment

Materials Copper(II) acetate monohydrate, 2,2'-bipyridine, 1,10-phenanthroline, 2-hydroxy-1-naphthaldehyde, sodium perchlorate, *N,N*-dimethylethylenediamine, 3,5-di-*tert*-butylcatechol, *tetra-N*-butylammonium bromide (Sigma-Aldrich), L-ascorbic acid (Fisher Scientific), benzylamine (Avra), KI, ammonium molybdate and hydrogen peroxide solution (30% w/v) (Merck) were used as received. HPLC grade methanol, *N,N*-dimethylformamide, ethyl acetate, hexane, dichloromethane and acetonitrile and reagent grade diethyl ether were purchased from Merck.

Physical measurements Microanalyses (C, H, and N) were carried out with a Vario EL elemental analyzer. The conductivity is measured using EQUIPTRONICS EQDCMP bridge with a solute concentration of $1 \times 10^{-3} \text{ M}$ in MeOH. Mass spectrometry was performed on a ZQ ESI–MS, ThermoFisher LTQXL and HRMS Exactive Plus EMR spectrometer. Magnetic susceptibility data at 27 °C were obtained for polycrystalline samples using George Associates Inc. FTIR spectra were recorded using a Perkin Elmer Spectrum RX1 FTIR spectrophotometer in the range 400–4000 cm^{-1} with a sample prepared as KBr disc. UV–visible spectroscopy of reflectance spectra were recorded using Shimadzu UV-2450 UV–Vis spectrophotometer and solution spectra were

Table 4 Kinetic parameters reported for different copper(II) complexes showing catechol oxidase activity

Complex	Solvent	K_M (mM)	V_{\max} (Ms^{-1})	k_{cat} (h^{-1})	References
$\text{Cu}_2(\text{EBA})(\text{OH})_2(\text{PF}_6)_2$	MeOH:H ₂ O	13.0	–	2.52×10^3	[35]
$[\text{Cu}_2(\text{Sab4})(\text{H}_2\text{O})_2]$	MeOH	8.7	193×10^6	3.80×10^3	[36]
$[\text{Cu}(\text{phen})](\text{OH})_2(\text{NO}_3)(\text{NO}_3)$	MeOH	2.7	1.08×10^{-3}	3.91×10^3	[37]
$[\text{Cu}_2(\mu\text{-OH})(\text{C}_{21}\text{H}_{33}\text{ON}_6)](\text{PF}_6)_2$	MeOH:H ₂ O	4.0	8.30×10^{-8}	12.45	[38]
$[\text{CuL}(\text{NCO})]$	MeOH:DMF	2.27	1.31×10^{-7}	23.58	[39]
$[\text{Cu}_2(\text{L1})(\mu\text{-OAc})_2](\text{BF}_4)$	MeOH	5.2	–	10.7	[40]
$[\text{Cu}_2(\text{L2})(\mu\text{-OAc})_2](\text{BF}_4)$	MeOH	0.67	–	28.9	[40]
$[\text{Cu}_2(\text{L3})(\mu\text{-OAc})_2](\text{BF}_4)$	MeOH	3.4	–	40.0	[40]
$[\text{Cu}_2(\text{L1})(\mu\text{-OAc})_2](\text{ClO}_4)_2$	MeCN	–	–	3.94	[40]
$[\text{Cu}_2(\text{L2})(\text{CH}_3\text{CN})](\text{ClO}_4)_4$	MeCN	–	–	No activity	[40]
$[\text{Cu}_2(\text{L3})_2(\text{CH}_3\text{CN})_2](\text{PF}_6)_2$	MeCN	–	–	31.64	[40]
$[\text{Cu}_2(\text{L4})_2](\text{PF}_6)_2$	MeCN	–	–	5.13	[40]
$[\text{Cu}_2\text{L}(\text{NO}_3)_2]$	MeOH	36.6	–	3.89×10^6	[41]
$[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$	MeOH	35.8	2.82×10^{-5}	3.51×10^3	This work
$[\text{Cu}(\text{L})(\text{phen})](\text{ClO}_4)$	MeOH	30.0	1.11×10^{-5}	1.38×10^3	This work

recorded using Perkin Elmer Lambda-365 UV–Vis spectrophotometer using cuvettes of 1 cm path length. X-band EPR spectra of the complex in DMF, MeOH and MeCN at liquid nitrogen temperature (77 K) and polycrystalline at room temperature (RT) were recorded on a JEOL JES-FA200 ESR spectrometer. ^1H NMR spectra were recorded on a Bruker 300 MHz with AVANCE II NMR spectrometer in CDCl_3 . Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on glassy carbon electrode were performed in MeOH 25 ± 0.2 °C. The voltammograms were generated using CH instruments 620C electrochemical analyzer. A three-electrode system has been used to study the electrochemical behavior of complexes (0.001 M) consisting of a glassy carbon working electrode ($A = 0.0707$ cm 2), a platinum wire auxiliary electrode and saturated calomel reference electrode and TBAP (0.1 M) is used as a supporting electrolyte. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to the measurements.

X-ray structure determination The green single crystals of $[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ (**1**) or $[\text{Cu}(\text{L})(\text{phen})](\text{ClO}_4)$ (**2**) appropriate for X-ray structural determination were obtained by keeping a solution of complex in MeOH:MeCN (2:1 v/v) at 5 °C for five to eight days. A green single crystal with dimensions $0.40 \times 0.35 \times 0.30$ (**1**) or $0.30 \times 0.25 \times 0.20$ (**2**) mm 3 , was selected under the polarizing microscope and then mounted on the glass fiber. The crystal data collections for **1** and **2** were performed through a Bruker AXS-KAPPA APEX II diffractometer using graphite monochromated Mo- K_α radiation ($\lambda = 0.71073$ Å). Cell parameters were recovered using Bruker SMART software and refined using Bruker SAINT on all experimental reflections. Absorption corrections were performed using SADABS. Structure solution was performed using direct methods with the program SIR-92 [44] and SHELXL version 2008 (**1**) [45] and 2012 (**2**) [45] was used to refine by full-matrix least squares on F^2 . All nonhydrogen atoms in **1** and **2** were refined anisotropically and the hydrogen atoms were refined isotropically as rigid atoms in their idealized locations. Disordered solvent molecules in **2** were squeezed before final refinement using the SQUEEZE option in PLATON. CCDC-1891294 (**1**) and CCDC-1891299 (**2**).

Synthesis of copper(II) complexes The complexes were prepared [46] by the reaction of $\text{Cu}(\text{O}_2\text{CMe})_2 \cdot \text{H}_2\text{O}$ (0.20 g, 1 mmol) with diimine (bpy, 0.16 or phen, 0.20 g; 1 mmol) in methanol (15 mL). The mixture was left stirring for 1 h at 25 °C at which point the yellow solution of H(L), obtained from the condensation of 2-hydroxy-1-naphthaldehyde (0.17 g, 1 mmol) and *N,N*-dimethylethylenediamine (0.09 g, 1 mmol) [47], was added. The resultant green colored solution was refluxed for 3 h. The green solids obtained after the addition of a methanolic solution of NaClO_4 (0.122 g, 1 mmol) were filtered, washed with cold methanol and dried in vacuo over P_4O_{10} . The

green single crystals of $[\text{Cu}(\text{L})(\text{bpy}/\text{phen})](\text{ClO}_4)$ (**1–2**) suitable for X-ray structural determination were obtained by cooling a solutions of **1** and **2** in MeOH:MeCN at 5 °C for 5 days.

$[\text{Cu}(\text{L})(\text{bpy})](\text{ClO}_4)$ **1.** Yield: 0.38 g (68%). Anal. Calcd. for $\text{C}_{25}\text{H}_{25}\text{N}_4\text{O}_5\text{ClCu}$: C, 53.57; H, 4.49; N, 9.99%. Found: C, 53.46; H, 4.54; N, 10.05%. Λ_{M} : 74 (MeOH) Ω^{-1} cm 2 mol $^{-1}$. ESI–MS (MeCN): m/z 460.09 [$\text{M}^+ - \text{ClO}_4$]. μ_{eff} (27 °C): 1.79 μB . FT-IR (KBr, cm $^{-1}$) selected bands: 1621 $\nu_{\text{imine}}(\text{C}=\text{N})$, 1592 $\nu_{\text{py}}(\text{C}=\text{N})$, 1312 $\nu(\text{naph-O})$, 1088, 621 $\nu(\text{ClO}_4^-)$. Electronic spectrum in solid/MeOH [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3$ mol $^{-1}$ cm $^{-1}$): 657/642 (186), 872 sh (75), 392 (6306), 315 (15,612), 285 (26,394). Electronic spectrum in MeOH:H $_2$ O (4:1 v/v) [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3$ mol $^{-1}$ cm $^{-1}$): 646 (184), 873 sh (80), 387 (6402), 318(12,264), 282 (26,487). Polycrystalline EPR spectrum at RT: $g_{\text{iso}} = 2.083$, $\Delta M_s = 1602$. EPR spectrum in DMF solution at 77 K: $g_{\parallel} = 2.223$, $g_{\perp} = 2.046$, $A_{\parallel} = 176 \times 10^{-4}$ cm $^{-1}$, $g_{\parallel}/A_{\parallel} = 126$ cm, $G = 5.0$, $\alpha^2 = 0.77$ (covalent in-plane σ -bonding), $\beta^2 = 0.67$ (covalent in-plane π -bonding), $\gamma^2 = 0.53$, $K_{\parallel} = 0.72$, $K_{\perp} = 0.64$. EPR spectrum in MeOH solution at 77 K: $g_{\text{iso}} = 2.126$. EPR spectrum in MeCN solution at 77 K: $g_{\text{iso}} = 2.090$. Redox behavior in MeOH (0.1 M TBAP): CV, $E_{1/2} = 0.193$ V, $\Delta E_p = 83$ mV, $i_{\text{pa}}/i_{\text{pc}} = 2.2$ (decreases towards unity with increase in scan rate), $D = 5.1 \times 10^{-6}$ cm 2 s $^{-1}$; DPV, $E_{1/2} = 0.192$ V.

$[\text{Cu}(\text{L})(\text{phen})](\text{ClO}_4)$ **2.** Yield: 0.41 g (70%). Anal. Calcd. for $\text{C}_{27}\text{H}_{25}\text{N}_4\text{O}_5\text{ClCu}$: C, 55.86; H, 4.34; N, 8.96%. Found: C, 55.94; H, 4.48; N, 8.99%. Λ_{M} : 73 (MeOH) Ω^{-1} cm 2 mol $^{-1}$. ESI–MS (MeCN): m/z 484.18 [$\text{M}^+ - \text{ClO}_4$]. μ_{eff} (27 °C): 1.81 μB . FT-IR (KBr, cm $^{-1}$) selected bands: 1621 $\nu_{\text{imine}}(\text{C}=\text{N})$, 1599 $\nu_{\text{py}}(\text{C}=\text{N})$, 1307 $\nu(\text{naph-O})$, 1098, 621 $\nu(\text{ClO}_4^-)$. Electronic spectrum in solid/MeOH [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3$ mol $^{-1}$ cm $^{-1}$): 610/617 (160), 853 sh (59), 392 (6168), 316 (15,901), 290 (23,780), 264 (51,171). Electronic spectrum in MeOH:H $_2$ O (4:1 v/v) [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{dm}^3$ mol $^{-1}$ cm $^{-1}$): 615 (172), 852 sh (70), 389 (6375), 316 (15,168), 289 (23,945), 265 (51,074). Polycrystalline EPR spectrum at RT: $g_{\text{iso}} = 2.063$. EPR spectrum in DMF solution at 77 K: $g_{\parallel} = 2.222$, $g_{\perp} = 2.045$, $A_{\parallel} = 186 \times 10^{-4}$ cm $^{-1}$, $g_{\parallel}/A_{\parallel} = 119$ cm, $G = 5.1$, $\alpha^2 = 0.79$ (covalent in-plane σ -bonding), $\beta^2 = 0.68$ (covalent in-plane π -bonding), $\gamma^2 = 0.53$, $K_{\parallel} = 0.73$, $K_{\perp} = 0.65$. EPR spectrum in MeOH solution at 77 K: $g_{\text{iso}} = 2.125$. EPR spectrum in MeCN solution at 77 K: $g_{\text{iso}} = 2.091$. Redox behavior in MeOH (0.1 M TBAP): CV, $E_{1/2} = 0.139$ V, $\Delta E_p = 88$ mV, $i_{\text{pa}}/i_{\text{pc}} = 1.6$ (decreases towards unity with increase in scan rate), $D = 5.3 \times 10^{-6}$ cm 2 s $^{-1}$; DPV, $E_{1/2} = 0.128$ V.

Ascorbic acid oxidation The ascorbate oxidase activity of the complexes is evaluated by reaction with **1** and **2** (3×10^{-3} M) and different concentration of ascorbic acid (H_2A). The experiments were run under aerobic conditions in MeOH:H $_2$ O (4:1 v/v) medium and monitored using UV–Vis spectroscopy [31, 42, 48].

Kinetic measurement To determine the ascorbate oxidase activity of the complexes **1** and **2**, 1.667×10^{-6} M solution of complex in MeOH:H₂O (4:1 v/v; pH 7.3) was treated with 60 equivalents of ascorbic acid (H₂A) under aerobic condition at 25 °C. The UV spectra of solution was recorded directly after the addition and subsequently after regular intervals of 1 min and the decrease in absorption value at 265 nm were measured as a function of time over a period of first 30 min. To determine the dependence of the rates on the substrate concentration and various kinetic parameters, 1.667×10^{-6} M solutions of copper complexes were treated with 20–140 equivalents of H₂A in MeOH:H₂O under aerobic condition [49].

Benzylamine oxidation

Catalytic action of **1** and **2** (1×10^{-3} M) in the H₂O₂ (1 ml of 30% w/v) dependent deamination of benzylamine (100×10^{-3} M) in 10 ml MeOH:H₂O (4:1 v/v) results in the oxidation of benzylamine to benzaldehyde [31, 50]. Reactions of benzylamine with H₂O₂ in absence of the complex or reactions of the complex with the only benzylamine do not demonstrate any detectable formation of benzaldehyde. The organic products were isolated from the reaction mixture by solvent extraction with diethyl ether after removal of methanol. Diethyl ether was removed by rotary evaporation and the residue was dissolved in CDCl₃ and MeOH for ¹H NMR and LC–MS measurements.

Kinetics of 3,5-di-*tert*-butylcatechol oxidation

To determine the catecholase activity of the complexes **1** and **2**, 2.9×10^{-5} M solution of complex in methanol was treated with 50 equivalents of 3,5-di-*tert*-butylcatechol (3,5-DTBC₂) in methanol under aerobic condition. The UV spectra of solution was recorded directly after the addition and subsequently after regular intervals of 30 min and absorption value at 397 nm (**1**–**2**) were measured as a function of time over a period of 3 h. The same reaction was done by scaling up the reactants and the desired 3,5-DTBQ was isolated column chromatographically using 10% mixture of ethyl acetate and hexane as eluent. The isolated *o*-quinone was identified by ¹H NMR spectra in CDCl₃ and FTIR Spectroscopy [37, 51, 52]. To determine the dependence of the rates on the substrate concentration and various kinetic parameters, 2.9×10^{-5} M solutions of copper complexes were treated with 5–45 equivalents of 3,5-DTBC₂ in methanol under aerobic condition [33, 53, 54]. To detect the formation of hydrogen peroxide during the catalytic reaction, reaction mixtures were prepared as in the kinetic experiments. During the course of the oxidation reaction, the solution was acidified with H₂SO₄ to pH 2 to stop further oxidation after a certain time and an equal volume of water was added.

The formed quinone was extracted three times with dichloromethane. To the aqueous layer were added 1 mL of a 10% solution of KI and three drops of a 3% solution of ammonium molybdate. The formation of I₃[−] could be monitored spectrophotometrically because of the development of the characteristic I₃[−] band ($\lambda = 353$ nm, $\epsilon = 26,000$ M^{−1} cm^{−1}) [55, 56].

Conclusions

The single crystal X-ray and electronic and EPR spectral studies of **1** and **2** reveal the distorted square pyramidal (4 + 1) geometry with a CuN₄O chromophore. The complex **2** displays the formation of the dimer due to antiparallel interpair π – π stacking interaction between diimine ligands (**2**, phen-phen), exhibiting rigid antiparallel orientation while **1** exists as a dimer through an attractive intermolecular C–H \cdots π interaction between the Schiff base ligands, showing flexible perpendicular orientation. Interestingly, according to frozen EPR studies in MeOH and MeCN, the complexes **1** and **2** exist as a dimer in solution due to self-assembled intermolecular associations [23, 55]. Therefore, the interpair association is retained due to the binding of the substrate in solution, which favors the electron transfer from the substrate to the Cu(II) active site. Notably, the copper(II) complexes (**1** and **2**) have been found to be catalytically active in the oxidation of ascorbic acid or benzylamine or catechol and mimic the functional property of type 2 and type 3 copper oxidases due to the easy formation of copper(II)–substrate intermediate, like ‘enzyme–substrate’ complex [43], which indicates the steric match between substrate and catalyst [57]. Therefore, it is concluded that these complexes are catalytically active to convert H₂A to dA by molecular oxygen involving reduced copper(I) intermediate, model the ascorbate oxidation property of the Cu(II) sites in AO (type 2). The greatest catalytic activity was found for the mononuclear copper(II) complexes with the turnover value (9.81×10^5 – 1.1×10^6 h^{−1}), compared to the AO enzyme itself (2.45×10^6 – 3.1×10^7 h^{−1}). Also, they are catalytically effective in the deamination reaction in the presence of hydrogen peroxide under aerobic conditions, analogous the functional property of AmO (type 2). The mononuclear copper(II) complexes exist as dimeric aggregates, which can act as potential catalysts for the oxidation of catechol to quinone, mimicking the catecholase activity of CO (type 3) with high turnover number. Further, **1** has been found to show significantly higher activity than **2** due to flexible perpendicular orientation with C–H \cdots π interaction and high-positive redox potential.

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Compliance with ethical standards

Conflicts of interest There are no conflicts to declare.

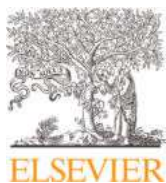
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Y-shaped fluorophore: Synthesis, crystal structure and picric acid detection

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ABSTRACT

Design and synthesis of a novel fluorescent probe for the detection of picric acid is a topic of an incessant research interest. In this context, we have developed a novel 'Y' shaped D-A-D type chromophore (TP171). Phenothiazine and methoxy phenyl groups are acting as donor whilst N-substituted imidazole as acceptor unit. As synthesized TP171 show an excellent fluorescent property with a quantum yield of 24%. Intriguingly, the TP171 fluorescent probe instantaneously responded towards the detection of picric acid with a concentration of 12 μ M. The detection limit is found to be 7.95 nM.

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1. Introduction

Detection of explosives mainly nitroaromatic compounds is one of the pivotal research topics due to their toxicity and fiery nature [1,2]. Generally, picric acid, 2,4,6-trinitrotoluene and dinitrotoluene are consumed as prime constituents of many explosive compositions [3,4]. The currently available explosive detection methods rely on a wide variety of instrument techniques and the real-time uses of these methods is being limited due to requirement of pre-treatment of samples, are vulnerable to interference and suffer from low sensitivity [5]. To address these issues, the attention has been directed in the recent years towards the design of a novel and efficient turn-off fluorescence probes for explosive detection [6]. The fluorescence quenching method [7] is preferred for sensing due to the non-fluorescent nature of explosives. Theoretically, the alteration of fluorescence intensity or lifetime and wavelength shift (either bathochromic or hypsochromic) upon expose to the explosives was considered to be a sensing method. There are varieties of nanomaterials, inorganic complexes and organic molecules were reported as fluorescent probes for explosive sensing [8–12] and however the searching of a new and efficient fluorescent probe is not exhausted yet. In this juncture, a 'Y' shaped donor–acceptor–donor (D–A–D) type fluorophore

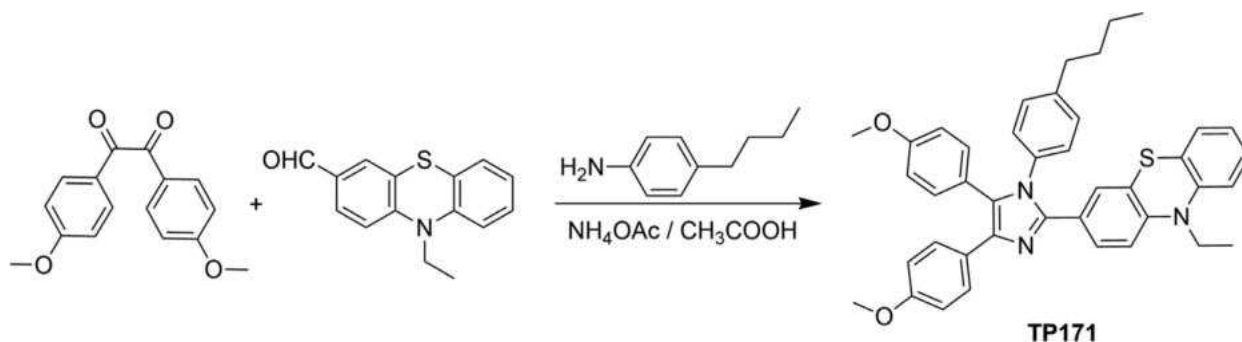
based on N1-substituted imidazole as acceptor unit, (C2) phenothiazine and (C4 and C5) methoxy phenyl groups act as donor groups namely 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1H-imidazol-2-yl]-10-ethyl-10H-phenothiazine (TP171) was successfully synthesized and meticulously characterized. The targeted fluorophore was synthesized by four component cyclocondensation reaction. The condensation occurs between 1,2-diketone, aryl amine and aryl aldehyde in the presence of ammonium acetate and glacial acetic acid. The targeted fluorescent molecule was successfully characterized and contented results were obtained. The crystal structure is also presented to attest the 'Y' shaped structure. Further, the fully characterized fluorescent probe is successfully employed for picric acid detection through fluorescence quenching method.

2. Experimental section

The starting materials 10-ethyl-10H-phenothiazine-3-carbaldehyde was prepared as reported in the literature [13]. The ¹H and ¹³C NMR spectra of TP171 were measured in 400 MHz and 100 MHz respectively. The NMR and mass spectra were depicted in the supporting information (Figure S1, S2 and S3). Absorption and fluorescence spectra of TP171 were recorded in a Shimadzu UV-1800 spectrophotometer and Hitachi make fluorescence spectrofluorimeter (Model: F-4500), respectively. X-ray diffraction data for TP171 was collected by using an Oxford Diffraction Xcalibur Eos Gemini diffractometer. Crystal data were

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Scheme 1. Synthesis and structure of TP171.

collected at ambient temperature using graphite-monochromated Mo-K α radiation ($\lambda = 0.7107 \text{ \AA}$).

3. Results and discussion

The title compound 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1H-imidazol-2-yl]-10-ethyl-10H-phenothiazine (TP171) was prepared by the Debus-Radziszewski multi-component reaction as illustrated in Scheme 1.

3.1. Synthesis of 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1H-imidazol-2-yl]-10-ethyl-10H-phenothiazine (TP171)

To the stirring mixture of 4,4'-dimethoxybenzil (0.97 g, 3.6 mmol), 10-ethyl-10H-phenothiazine-3-carbaldehyde (1.02 g, 4 mmol), ammonium acetate (3.6 g, 86.4 mmol) in 40 ml of glacial acetic acid, 4-n-butylaniline (1.07 g, 7.2 mmol) was added dropwise. The resulting mixture was placed under reflux for 12h in the inert atmosphere. On completion of reaction, the mixture was cooled to room temperature. Further, the mixture was poured into ice cold water and extracted from dichloromethane solvent. The organic layer was washed with brine and the volatile solvent was removed to obtain crude product. The residue was then purified on column chromatography using silica gel and ethyl acetate: hexane (1:3) yielding greenish yellow solid. Yield 76 %; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ : 7.54-7.52 (d, 2H, $J = 8 \text{ Hz}$), 7.14-7.12 (d, 2H, $J = 8 \text{ Hz}$), 7.10-7.08 (d, 3H, $J = 8 \text{ Hz}$), 7.06-7.01 (m, 3H), 6.95-6.93 (d, 3H, $J = 8 \text{ Hz}$), 6.82-6.80 (d, 3H, $J = 8 \text{ Hz}$), 6.76-6.74 (d, 3H, $J = 8 \text{ Hz}$), 3.89-3.84 (m, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.62-2.59 (t, 2H, $J = 6 \text{ Hz}$), 1.63-1.55 (m, 2H), 1.40-1.36 (t, 3H, $J = 8 \text{ Hz}$), 1.33-1.26 (m, 2H), 0.95-0.91 (t, 3H, $J = 8 \text{ Hz}$); $^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ : 159.16, 158.48, 143.24, 137.59, 134.81, 132.41, 129.21, 128.60, 128.27, 127.93, 127.47, 127.25, 122.97, 122.36, 115.02, 114.36, 113.79, 113.60, 55.25, 55.18, 41.82, 35.23, 33.34, 22.14, 14.11, 12.88. Element. Anal.: Calc. for $\text{C}_{41}\text{H}_{39}\text{N}_3\text{O}_2\text{S}$: C, 77.21; H, 6.16; N, 6.59; found: C, 77.17; H, 6.22; N, 6.62. ESI-MS: m/z Calc. for ($\text{C}_{41}\text{H}_{39}\text{N}_3\text{O}_2\text{S}$): 637.28; Found [$\text{M}+\text{H}$] 638.30.

3.2. Crystal structure description of TP171

An appropriate crystal of TP171 for crystallographic study was obtained from the mixture of petroleum ether and DCM (1:1) at room temperature. The crystallographic data and structural refinement details are given in Table 1 and selected bond distances and bond angles are given in table S1. The molecule crystallized in monoclinic system with P21/n space group. Fig. 1 shows the ORTEP diagram of the TP171 with the atomic numbering scheme and Fig. 2 shows the packing diagram of the compound along the c -axis. The phenothiazine, p -butylbenzene and two p -anisole moieties are connected to the middle imidazole ring with non-planar

Table 1
Crystallographic data and structure refinement parameters for TP171.

Formula	$\text{C}_{41}\text{H}_{39}\text{N}_3\text{O}_2\text{S}$
crystal system	Monoclinic
space group	P 21/n
a (\AA)	17.2506(18)
b (\AA)	9.5049(9)
c (\AA)	22.073(2)
α (deg)	90
β (deg)	104.135(10)
γ (deg)	90
V (\AA^3)	3509.7(6)
Temperature (K)	293(2)
Wavelength (\AA)	0.71073
Radiation type	Mo $K\alpha$
Z	4
μ (mm^{-1})	0.131
ρ_{calc} (mg/mm^3)	1.207
Goodness-of-fit on F^2	1.016
final R indices	$R_1 = 0.0649$, $wR_2 = 0.1407$
R_1^a	0.1123
wR_2^b	0.1666
CCDC Number	2031794

$$^a R_1 = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}, \quad ^b wR_2 = \left\{ \frac{\sum w[(F_o^2 - F_c^2)^2]}{\sum w(F_o^2)^2} \right\}^{1/2}$$

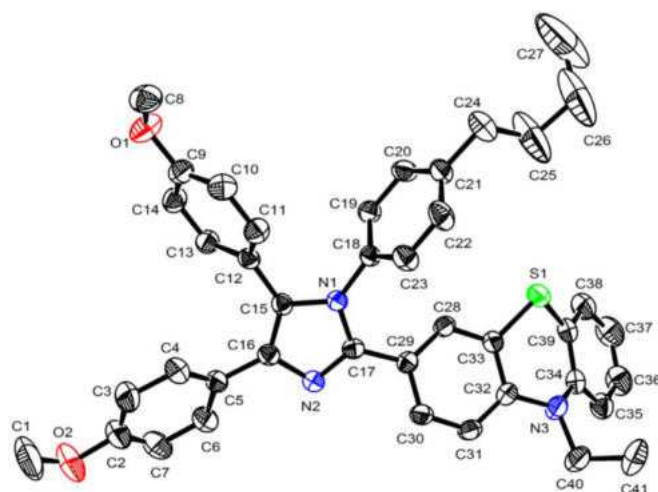


Fig. 1. ORTEP diagram of compound TP171. Hydrogen atoms are omitted for clarity.

conformation. The two p -anisole rings bonded to the imidazole ring at the position of C15 and C16 are inclined to the imidazole ring at the dihedral angle $151.0(3)$, $121.8(3)$ respectively. The 4-butylbenzene ring (atoms C18–C23) and the imidazole ring (C15–C17/N1/N2) are twisted with respect to each other making a dihedral angle of 106.37° . However, the central six-membered ring

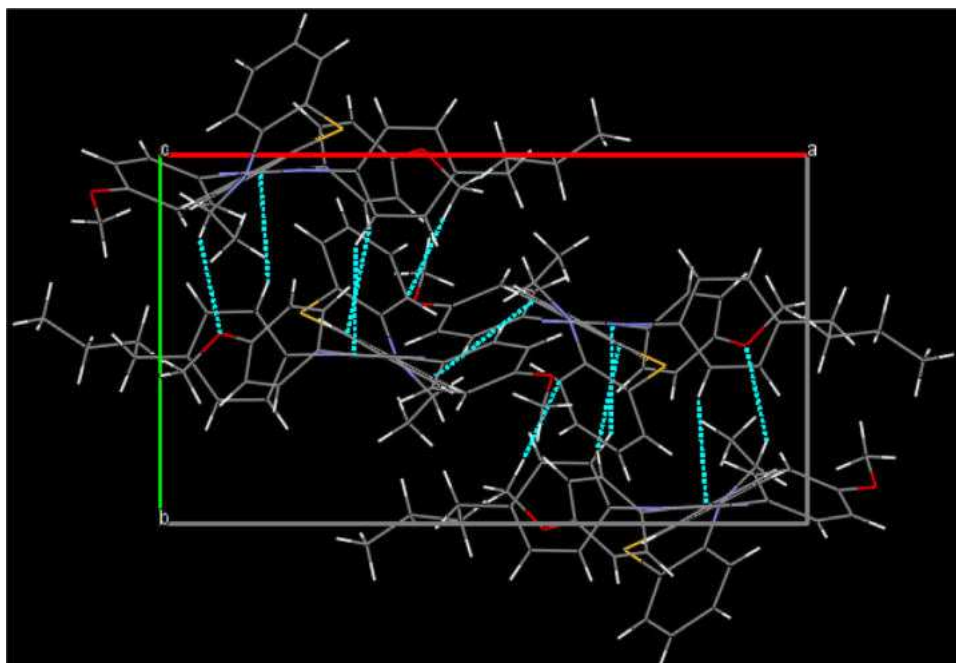


Fig. 2. Packing diagram of TP171 along the *c* axis.

(N3/C32/C33/S1/C34/C39) adopts a boat conformation. The structure of TP171 is stabilized by intermolecular C–H...O and C–H... π hydrogen bondings and are responsible for producing a three dimensional structure (Fig. 2).

3.3. Thermal properties

The thermal character of TP171 was examined by thermogravimetric (TGA) and differential scanning calorimetry (DSC) measurements under nitrogen atmosphere at a heating rate of 10 °C/min. The TGA curve of TP171 is presented in **figure S4a**. As shown in this figure, the first and second inflection temperatures of TP171 are observed at ca. 380 °C and 520 °C, respectively. This result clearly demonstrates that TP171 has good thermal stability. The DSC curve (**Figure S4b**) displays a glass transition temperature (T_g) is around 173 °C. The high T_g of TP171 designates the good morphological stability and it could be used for assorted applications.

3.4. Fluorescence quantum yield and lifetime

Under UV light illumination the TP171 exhibits bright fluorescence in both solution and powder forms (**Figure S5**). Therefore, the fluorescence quantum yield (ϕ_F) measurements are conducted in THF solvent. The value of ϕ_F is calculated using the following Eq. (1)

$$\phi_F/\phi_R = (I_S/I_R) \times (1 - 10^{-AR}/1 - 10^{-AS}) \times (\eta_S/\eta_R)^2 \quad (1)$$

where, the S and R refers to the sample (TP171) and the reference (Quinine sulphate), respectively. *A* is the fraction of light absorbed at the excitation wavelength (366 nm), *I* is the integrated area of the fluorescence spectrum, and η is refractive index of the solvent. Quinine sulphate ($\phi_R = 0.54$) was used as a standard for the TP171. Intriguingly, the measured quantum yield is of 24% and this propels us to employ the TP171 as fluorescent probe for sensing applications.

The fluorescence decay of TP171 in THF solvent is measured using TCSPC technique. The excitation and emission wavelengths are 375 nm and 450 nm, respectively. The measured fluorescence decay of TP171 is fitted with single exponential function (Fig. 3).

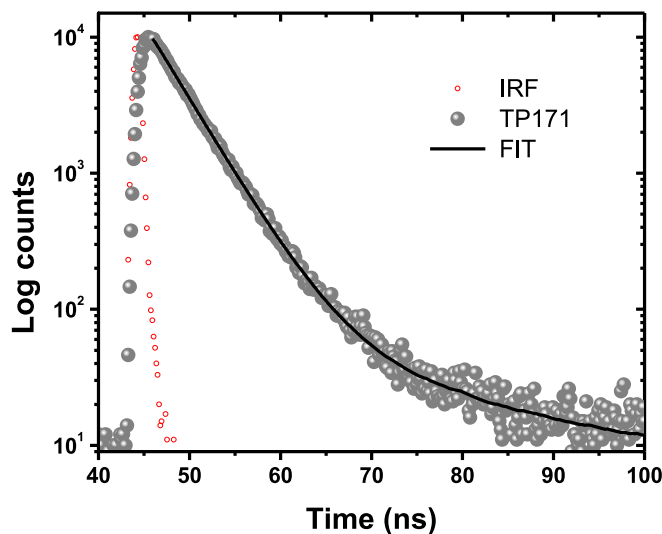


Fig. 3. Time resolved fluorescence decay of TP171 in THF solvent.

The calculated fluorescence lifetime of TP171 is 3.9 ns. Further, to verify the fluorescence properties of TP171 in solution, the radiative [$k_r = \phi_F/\tau$] and the non-radiative [$k_{nr} = (1 - \phi_F)/\tau$] rate constants were calculated using the fluorescence lifetime and the quantum yield. The calculated k_r and k_{nr} values are $6.15 \times 10^7 \text{ s}^{-1}$ and $1.94 \times 10^8 \text{ s}^{-1}$, respectively. The k_{nr} value was higher than k_r value. The obtained higher k_{nr} value is the major reason for the lower quantum yield in solution.

3.5. Detection of picric acid

To investigate the photoinduced interaction between TP171 and PA, the UV-visible absorption and fluorescence spectroscopic technique have been employed. Fig. 4a shows the absorption spectrum of TP171 with various concentrations of PA. Upon titration of TP171 with increasing concentrations of PA, the absorption spectrum of

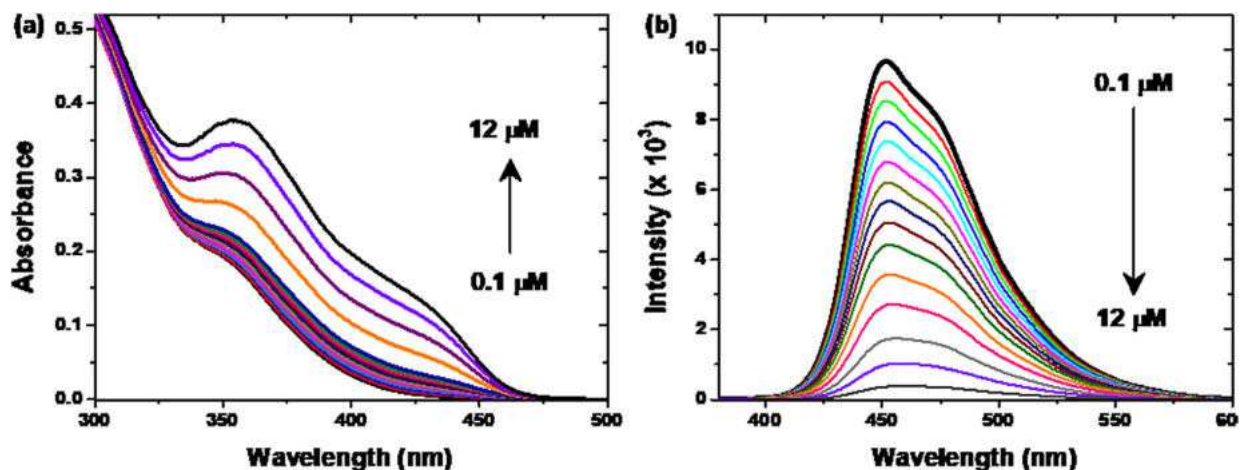


Fig. 4. (a) UV-visible absorption (b) fluorescence quenching spectra of TP171 with varying concentration of PA.

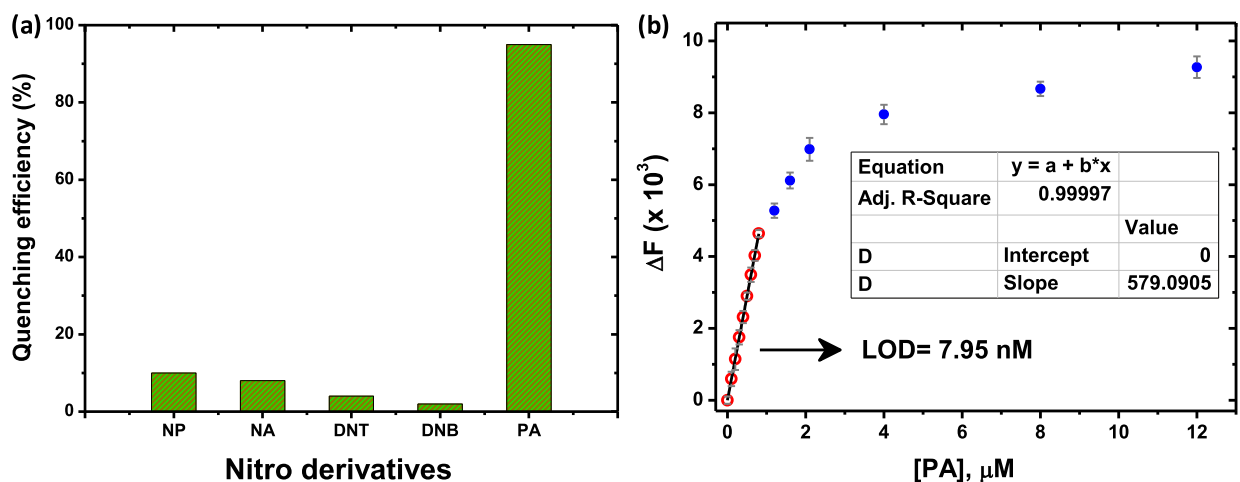


Fig. 5. (a) Selectivity and (b) fluorescence intensity responses of TP171 with varying concentration of PA.

TP171 is regularly increased without any noticeable spectral shift. This result clearly infers that there is no ground state charge transfer complex formation between TP171 and PA. With this setting, the fluorescence titration measurements also conducted to understand excited state interactions. Fig. 4b shows the fluorescence quenching results of TP171 exposed with various concentrations of PA.

The fluorescence of TP171 is quenched with regular intervals and completely quenched with the PA concentration of 12 μM . On the other hand, the other relevant interferences such as 4-nitrophenol (NP), 4-nitroaniline (NA), 2,4-dinitrotoluene (DNT) and dinitrobenzene (DNB), have did not interfere the fluorescence of TP171 (Fig. 5a). To evaluate the interference of other analytes, fluorescence intensity titration measurements of TP171 with PA were investigated in the presence of metal ions, anions and organic acids. No interference from external analytes was perceived (Figure S6a-c). These results unambiguously demonstrate that the PA selectively quenches the fluorescence of TP171. From the quenching results the detection limit (LOD) could be calculated using $3\sigma/k$ relationship. The plot between changes in fluorescence intensity and PA concentration yielded an upward curvature. From the linear portion of the plot, the LOD was calculated and is found to be 7.95 nM (Fig. 5b). When compared to relevant fluorescent probes [14–19], the obtained LOD values are consistent to the reported values as shown in Table 2. The obtained higher sensitivity of TP171 fluorescent probe infers that this could be a prospective

Table 2

Comparison of LOD values for PA detection with various fluorescent probes.

Fluorescent probes	LOD (nM)	Ref.
Imidazole derivative	170	[14]
Benzimidazole derivative	21	[15]
Coumaryl linked imidazolium salts	87	[16]
Pyrene-appended imidazolium probe	10	[17]
Imidazole-appended anthracene	20	[18]
Y-shaped D- π -A compounds	61	[19]
Y-shaped Fluorophore	7.95	This work

candidate to detect PA even at very low concentrations. The mechanism behind the quenching could be operated by many photo-physical channels such as photoinduced electron transfer [20], energy transfer and inner filter effect (IFE). However, here we have considered IFE quenching mechanism [21] which is clearly evident from the spectral overlap between absorption spectra of PA and TP171 (Figure S6). Further, to provide evidence for the role of IFE mechanism, the observed fluorescence intensity was corrected by using the following Eq. (2):

$$F_{\text{cor}} = F_{\text{obs}} e^{(A_{\text{ex}} + A_{\text{em}})/2} \quad (2)$$

where, F_{cor} is the corrected fluorescence intensity of TP171 and F_{obs} is the observed fluorescence intensities of TP171 in the presence of PA, A_{ex} and A_{em} are the absorbance at the excitation and the emission wavelengths (λ), respectively. According to the

Table 3
Recovery of PA spiked with different real water samples by TP171.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Lake water	0.5	0.4856	97	1.89
	1	0.9975	99	1.36
	2	2.0004	100	0.27
	4	4.0802	102	1.29
Sea water	0.5	0.0782	16	3.09
	1	0.8082	81	2.01
	2	1.8827	94	1.36
	4	4.0050	100	1.88
Pond water	0.5	0.4826	96	1.46
	1	0.9732	97	1.36
	2	1.9877	99	1.17
	4	4.0302	101	1.43
Tap water	0.5	0.4954	99	1.40
	1	0.9847	98	1.25
	2	1.9952	99	1.08
	4	4.0102	100	1.08
Distilled water	0.5	0.4989	99	1.04
	1	0.9947	99	1.24
	2	2.0047	100	1.17
	4	4.0052	100	1.03

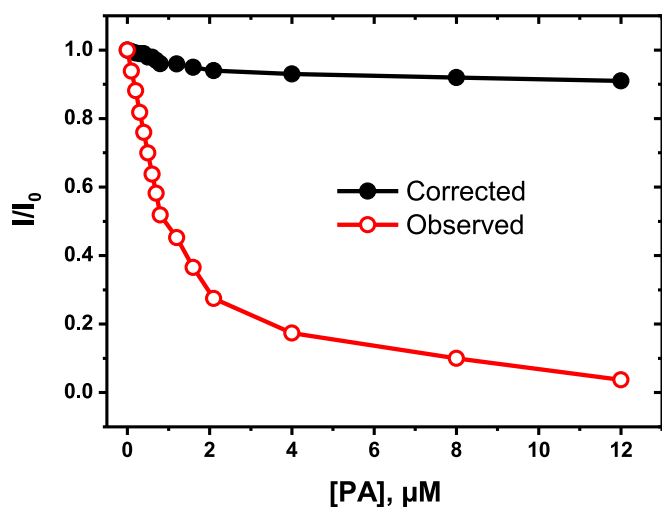


Fig. 6. A plot of observed and corrected fluorescence intensities with various PA concentrations.

Eq. (2), the fluorescence intensities were corrected and plotted with respect to the various PA concentrations (Fig. 6). The obtained plot unambiguously validated the fluorescence quenching mainly originated from IFE mechanism only. Similar type of IFE based sensors have been reported for the detection of analytes [22–24].

3.6. Detection in aqueous environment

To investigate the sensitivity of TP171 in aqueous environment, the detection of PA in various water samples was also conducted. The water samples were collected from the nearby lake, sea, pond and the tap water and double distilled water were used from the source of our laboratory. The solution of TP171 was prepared in the above said water samples followed by spiking of PA with different concentrations (0.5, 1, 2 and 4 μM) and the fluorescence intensity measurements were performed. Table 3 depicts the average recovery of PA and relative standard deviations (RSD) for three replicates of quenching experiments. Except sea water, the percentage of recovery at low level PA concentration (1 μM) was nearly 100% indicating the reliability of the TP171 for PA determination in various water samples.

4. Conclusion

A novel Y-shaped fluorescent probe was synthesized and an adequate characterization results were obtained. The fluorescent probe showed a high selectivity and sensitivity toward picric acid over other relevant nitroaromatics. The prominent fluorescence quenching upon interaction with picric acid was observed and achieved a detection limit of 7.95 nM. The inner filter effect is the main photophysical channel for detection of picric acid. Intriguingly, the fluorescent probe is successfully applied for the on-site detection of PA in real water samples. Thus, the synthesized Y-shaped fluorescent probe is a prospective material for the detection picric acid in the local environment. Further, the deployment of this material for on-site analysis is under progress with the help of 3D printed portable device.

Author statement

All the authors have participated sufficiently in the design, synthesis, and spectroscopic studies along with writing of the manuscript.

Declaration of Competing Interest

There are no conflicts of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130442.

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Copper(II) complexes of 2-methyl-8-hydroxyquinoline and tri/diimine co-ligand: DFT calculations, DNA and BSA binding, DNA cleavage, cytotoxicity and induction of apoptosis†‡

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The mixed ligand copper(II) complexes [Cu(terpy)(mq)]ClO₄ (**1**) and [Cu(phen)(mq)]ClO₄ (**2**), in which terpy = 2,2':6',2''-terpyridine, phen = 1,10-phenanthroline and H(mq) = 2-methyl-8-hydroxyquinoline, have been synthesized. The crystal structure of **1** discloses a distorted square-pyramidal geometry ($\tau = 0.16$). The coordination geometry around Cu(II) in the density functional theory optimized structures of **1** and **2** has been assessed and found to show a distorted square pyramidal and square planar respectively. They exhibit a ligand field band and their frozen solution EPR spectra are axial ($g_{\parallel} > g_{\perp} > 2.0$) suggesting a square-based geometry around Cu(II) with a $d_{x^2-y^2}$ ground state. The complexes interacted with calf thymus (CT) DNA and were found to be groove binding, as determined by various spectral and redox techniques. Interestingly, **2** cleaves supercoiled ϕ X174 DNA without a reductant while **1** cleaves with the reductant. Furthermore, the quenching of the tryptophan emission of BSA in the presence of the complexes is static. The binding is mainly entropy-driven and hydrophobic forces played a major role. The DNA and protein binding affinity and the DNA cleavage activity is in the order **2** > **1**, and the higher DNA and protein binding affinity and DNA cleavage activity of **2** illustrates the strong involvement of the 2-methyl group on the mq ring in the hydrophobic interaction with the DNA and protein. Furthermore, cytotoxicity studies of **1** and **2** on MCF7 and SiHa cell lines show that the IC₅₀ values are lower than those of cisplatin and illustrate a lower cytotoxicity against the Jurkat T4 normal cell line. AO/EB and Hoechst 33258 staining assays reveal the higher induction of apoptosis for **1** and **2**. The alkaline single-cell electrophoresis (comet assay) indicates that the complexes induce DNA fragmentation, which provides additional proof of the apoptosis.

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Introduction

Cisplatin and its derivatives are the most widely used metal-based drugs for cancer therapy, but their use is restricted by serious side effects, acquired resistance in cancer cells, and a high general toxicity to normal cells.^{1–4} These drawbacks have

sparked an ever-increasing search for transition metal complexes that incorporate metals other than platinum,^{3,5,6} which are less toxic, more target-specific, and have more cytotoxic properties towards cancer cells. Copper, as a bio-essential element, plays a key role in the endogenous oxidative DNA damage associated with aging and cancer. It has been shown that copper accumulates in tumors owing to the selective permeability of the cancer cell membranes to copper compounds⁷ and it is thought to be less toxic than nonessential metals such as platinum.⁸ Copper(II) complexes have been synthesized in large numbers, such as the synthetic bleomycin models Cu(3-Clip-Phen) and derivatives.⁹ Most of them exhibit a high anticancer activity towards a variety of cancer cell lines, including copper(II) complexes with thiosemicarbazone, quinoline derivatives, and nitrogen-containing heterocyclic ligands.^{10,11}

The copper concentration inside cells treated with [Cu(phen)₂Cl]Cl is about 10-fold higher than those treated with standard copper salts, suggesting that the lipophilic phen

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† Dedicated to Professor Ramaswamy Murugavel, Department of Chemistry, Indian Institute of Technology Bombay on the occasion of his 57th birthday.

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moiety helps the copper to pass through the cell membrane.¹² Following this observation, the anticancer properties of a wide range of copper complexes containing 1,10-phenanthroline (phen) and related ligands have been intensively investigated. For instance, Ruiz-Azuara and co-workers reported and patented a series of novel antineoplastic agents based on mixed chelate copper(II) complexes named Casionpeínas[®] with the general formulae $[\text{Cu}(\text{phen}/\text{bpy})(\alpha\text{-L-aminoacidato})]\text{NO}_3$ and $[\text{Cu}(\text{phen}/\text{bpy})(\text{acac}/\text{sala})]\text{NO}_3$ (acac is the acetylacetonate anion), they showed a satisfactory cytotoxicity, with a significantly lower IC₅₀ value (concentration (μM) of drug required to inhibit the growth of 50% of cancer or normal cells).^{13,14}

Many mixed ligand copper(II) complexes have been found to exhibit prominent cytotoxicity by inducing apoptosis and, interestingly, they have also been found to strongly bind and cleave DNA.^{15–19} Sadler *et al.*²⁰ have shown that mixed ligand bis(salicylato)copper(II) complexes with diimine as a co-ligand exhibit cytotoxic and antiviral activities. Neves and coworkers reported that the mononuclear phenolate copper(II) complexes $[\text{Cu}(\text{HL})\text{Cl}_2]$, in which HL is 2-[bis(pyridylmethyl)amino]methyl-4-methyl-6-formylphenol, effects double-strand DNA cleavage and amide bond cleavage.²¹ Also, Reedijk *et al.* found that the complex $[\text{Cu}(\text{pyrimol})\text{Cl}]$ brings about efficient self-activated DNA cleavage and cytotoxic effects toward the A2780 human ovarian and L1210 murine leukemia carcinoma cell lines.^{22,23}

In this work we have isolated the two different mixed-ligand copper(II) complexes *viz.*, mononuclear five-coordinate $[\text{Cu}(\text{terpy})(\text{mq})]\text{ClO}_4$ (**1**) and four-coordinate $[\text{Cu}(\text{phen})(\text{mq})](\text{ClO}_4)$ (**2**), in which terpy = 2,2':6',2''-terpyridine, phen = 1,10-phenanthroline and H(mq) = 2-methyl-8-hydroxyquinoline (Scheme 1) and explored their ability to bind and cleave DNA. The H(mq) (N,O-donor) primary ligand (A) was chosen to provide the donor elements, such as pyridine and phenolate groups, and thus tune the DNA cleaving properties of the complexes. The 2-methyl group on the pyridine moiety of H(mq) provides a hydrophobic recognition element. On the other hand, trimine (terpy, B) and

diimine (phen, C) are used as co-ligands in a ternary structure "A-Cu-B or A-Cu-C", in which the planar phen bases act as binders to double-stranded DNA and could facilitate the hydrogen atom abstraction from the sugar unit, and the terpy can provide structural stability to the DNA complex adduct as the platinum(II) complexes of terpy derivatives are reported to show a good antitumor activity *via* stabilization of the G-quadruplex.^{24,25} If a cytotoxic metal-containing compound is administered intravenously, it may interact with macromolecular blood components such as proteins and accumulate in the tumor tissue. Therefore, we have investigated the protein binding ability of the complexes using a serum protein such as bovine serum albumin (BSA), which may act as a transport function for drug molecules. Also, such interactions will determine the overall drug distribution, excretion and differences in the efficacy, activity, and toxicity.^{26–28} Breast and cervical cancer are the most prevalent types of cancer in women worldwide, 500 000 new cases are diagnosed every year and the currently used drug, cisplatin, is limited by its side effects. Thus, this study details the synthesis and characterization of mixed ligand copper(II) complexes, together with their DNA binding, protein binding, and DNA cleavage activities. We have also scrutinized their effect on cancerous (breast cancer cell line, MCF7 and human cervical carcinoma cell line, SiHa) and non-cancerous (Jurkat T4) cell lines along with their ability to induce apoptosis.

Experimental methods

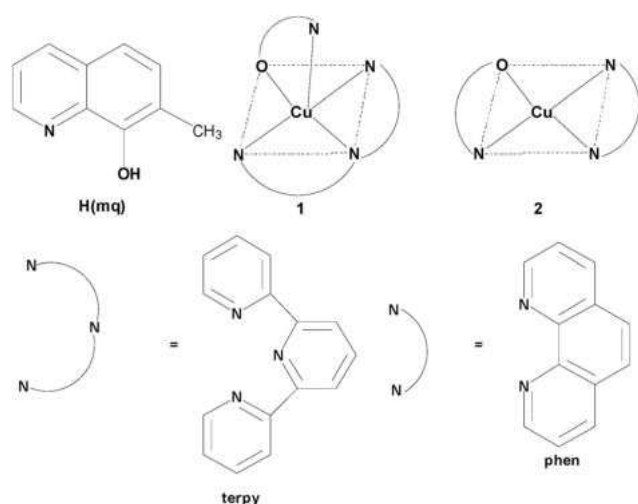
Materials

Copper(II) perchlorate hexahydrate, tris-(hydroxymethyl)amino-methane, ethidium bromide (EthBr), sodium azide (NaN₃), ascorbic acid (Merck), 1,10-phenanthroline, 2,2':6',2''-terpyridine, 2-methyl-8-hydroxyquinoline, sodium perchlorate (Sigma-Aldrich), φX174 RF supercoiled phage DNA (Invitrogen Life Technologies), Hoechst 33258 (stored at –20 °C), calf thymus (CT) DNA (highly polymerized; stored at 4 °C), superoxide dismutase (SOD) (stored at 4 °C), and bovine serum albumin (BSA) (stored at 4 °C), (Sigma-Aldrich) were obtained.

The human cervical carcinoma cell line (SiHa), breast cancer cell line (MCF7), and Jurkat T4 normal cells used in the study were obtained from the National Center for Cell Science (NCCS), Pune, India. The cells were cultured in RPMI 1640 medium (Biochrom AG, Berlin, Germany), supplemented with 15% heat-inactivated fetal bovine serum, 4 mM glutamine (Sigma-Aldrich), 100 U per mL penicillin, and 100 μg per mL streptomycin (Himedia, Mumbai, India) at a density of 2.5×10^5 cells per mL in 96-well culture plates at 37 °C in a humidified atmosphere of 5% CO₂ in a CO₂ incubator (Heraeus, Hanau, Germany).

Physical measurements

The elemental analyses (C, H, N) were carried out using a PerkinElmer 2400 series II analyzer. The electrical conductivity was obtained with a Systronic 305 conductivity bridge, using a 1×10^{-3} M solution of the complex in dimethylformamide (DMF). Fourier transform infrared (FTIR) spectra were recorded using a PerkinElmer Paragon 1000 FTIR spectrophotometer,



Scheme 1 Schematic representation of the copper(II) complexes (**1** and **2**), and the primary and tri/diimine ligands.

equipped with a Golden Gate Diamond attenuated total reflectance (ATR) device, using the reflectance technique ($4000\text{--}300\text{ cm}^{-1}$) and the peaks are reported in cm^{-1} . The electronic spectra were recorded using a PerkinElmer Lambda 35 UV-visible spectrophotometer. X-band electron paramagnetic resonance (EPR) measurements were performed at room temperature in the solid-state and at 77 K in the DMF solution using a Bruker-EMXplus (2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a standard, $g = 2.0036$). The emission intensity measurements were carried out using a Shimadzu RF-5301PC spectrofluorophotometer equipped with a thermostatic bath. A circular dichroic (CD) spectrum of DNA or BSA was obtained by using a JASCO J-716 spectropolarimeter.

Synthesis of copper(II) complexes

Preparation of [Cu(terpy)(mq)](ClO₄) 1 or [Cu(phen)(mq)](ClO₄) 2. A methanol solution (10 mL) of Cu₂(O₂CMe)₄(H₂O)₂ (0.2 g, 0.5 mmol) was reacted with 2,2':6',2''-terpyridine (terpy: 0.23 g, 1 mmol) or 1,10-phenanthroline (phen: 0.18 g, 1 mmol) in 5 mL methanol under stirring at 25 °C for 0.5 h. The resulting solution was then reacted with 2-methyl-8-hydroxyquinoline (H(mq): 0.17 g, 1.2 mmol) dissolved in 10 mL methanol. The mixture was stirred for 1 h and the complex was precipitated as a green solid with a yield of approximately 65% (**1**) (0.36 g) or approximately 67% (**2**) (0.31 g) with the addition of a methanolic solution of NaClO₄ (0.12 g, 1 mmol) into the reaction mixture. The solid was separated, washed with cold methanol and dried *in vacuo* over P₄O₁₀. Analytically calculated (Anal. calcd) for C₂₅H₁₉N₄O₅ClCu (**1**): C, 54.16; H, 3.45; N, 10.11. Found: C, 54.11; H, 3.24; N, 10.24%. A_M in DMF at 25 °C: $70\ \Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$. Electrospray ionization mass spectrometry (ESI-MS) in DMF solution: [Cu(terpy)(mq)]⁺ displays a peak at m/z 455.3 (calcd 455.0). μ_{eff} (27 °C): $1.81\ \mu_B$. Selected infrared (IR) vibrational peaks (ν , cm^{-1}): $\nu(\text{C}=\text{C})$ and $\nu(\text{C}=\text{N})$ (1602, 1558, 1505, 1476, 1447), $\nu(\text{C}_{\text{ph}}-\text{O})$ (1334, 1280), $\nu(\text{Cl}-\text{O})$ (1081, 621), $\nu(\text{C}-\text{H})$ (776, 741), $\nu(\text{Cu}-\text{N})$ (558), $\nu(\text{Cu}-\text{O})$ (440). Electronic spectrum in DMF [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{ cm}^{-1}$): 263 (41 430), 281 (17 150), 325 (13 500), 339 (13 430), 367 (2810), 816 (170). Electronic spectrum in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer solution [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{ cm}^{-1}$): 263 (41 430), 281 (17 150), 326 (13 540), 339 (13 420), 369 (2830), 814 (160). Room temperature polycrystalline EPR spectrum: $g_{\text{iso}} = 2.084$. EPR spectrum in DMF solution at 77 K: $g_{\parallel} = 2.250$, $g_{\perp} = 2.061$, $A_{\parallel} = 160 \times 10^{-4}\text{ cm}^{-1}$, $g_{\parallel}/A_{\parallel} = 141\text{ cm}$, $G = 4.2$. Single crystals of **1**, suitable for X-ray analysis were obtained by dissolving the complex in DMF (0.5 mL), adding MeCN:MeOH (1:1 V/V, 3 mL each) and then maintaining the conditions for slow evaporation at room temperature for 15 d. Anal. calcd For C₂₂H₁₆N₃O₅ClCu (**2**): C, 51.27; H, 3.13; N, 10.87. Found: C, 51.31, H, 3.14, N, 10.92%. A_M in DMF at 25 °C: $76\ \Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$. ESI-MS in DMF solution: [Cu(phen)(mq)]⁺ displays a peak at m/z 416.2 (calcd 415.9). μ_{eff} (27 °C): $1.85\ \mu_B$. Selected IR vibrational peaks (ν , cm^{-1}): $\nu(\text{C}=\text{C})$ and $\nu(\text{C}=\text{N})$ (1568, 1520, 1428, 1386, 1335), $\nu(\text{C}_{\text{ph}}-\text{O})$ (1282), $\nu(\text{Cl}-\text{O})$ (1081, 621), $\nu(\text{C}-\text{H})$ (773, 748), $\nu(\text{Cu}-\text{N})$ (514), $\nu(\text{Cu}-\text{O})$ (428). Electronic spectrum in DMF [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{ cm}^{-1}$): 266 (40 950), 291 (23 030), 325sh, 341sh, 391 (2890), 649 (240). Electronic spectrum in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer solution [$\lambda_{\text{max}}/\text{nm}$ ($\epsilon_{\text{max}}/\text{M}^{-1}\text{ cm}^{-1}$): 268 (41 260), 297 (23 500), 325sh, 340sh, 392 (2890),

647 (230). Room temperature polycrystalline electron spin resonance (EPR) spectrum: $g_{\text{iso}} = 2.136$. EPR spectrum in DMF solution at 77 K: $g_{\parallel} = 2.267$, $g_{\perp} = 2.062$, $A_{\parallel} = 172 \times 10^{-4}\text{ cm}^{-1}$, $g_{\parallel}/A_{\parallel} = 132\text{ cm}$, $G = 4.2$.

X-ray crystal structure determinations

A crystal of [Cu(terpy)(mq)](ClO₄) **1** of a suitable size was selected from the mother-liquor and immersed in paraffin oil, then mounted on the tip of a glass fiber and cemented using epoxy resin. Intensity data for the crystal was collected on a Bruker AXS SMART 1000 diffractometer equipped with a charge coupled device (CCD) area detector at 293 K using Mo-K α ($\lambda = 0.71069\ \text{\AA}$) radiation. The ω scan technique was used to record the intensity data. SMART software was used for data acquisition and SAINT²⁹ for data extraction. Empirical absorption correction was applied to the collected reflections using SADABS software.³⁰ The space group of **1** was assigned as $P\bar{1}$ using systematic absences and the E statistics of the data set. The space group was later confirmed using successful structural determination and refinement. The details of data collection and structural analysis are given in Table S1 (ESI \ddagger). The SIR97 program³¹ was used to solve the structure of **1** using direct methods and the SHELXL 97 program³² was used to refine the structure using the full-matrix least-squares method on F^2 . All non-hydrogen atoms were refined anisotropically until convergence was reached. Hydrogen atoms attached to the ligand moieties were stereochemically fixed. The structure of **1** was refined with a goodness-of-fit (GoF) value of 1.006. Selected crystal data and structure refinement parameters are given in Table S1 (ESI \ddagger).

Computational studies

The coordination geometries of the copper(II) mixed ligand complexes in the ground state with a doublet spin state were optimized by using density functional theory (DFT) at the B3LYP level of theory by employing the Gaussian 09 program package.³³ The calculations were administrated with a mixed basis set (B1) of LANL2DZ for the copper metal atom, which contains a relativistic effective core potential with a valence basis set and 6-31G* for the remaining atoms.³⁴ The normal mode analyses were performed to examine the minimal energy nature of the geometry. The copper(II) complexes **1** and **2** were considered as mono-positive cations as the copper metal was considered in the +2 oxidation state and the doublet spin state, whereas the ligand H(mq) was considered as a mono-negative ligand. The salvation of the complexes was applied by employing the conductor-like polarizable continuum model (CPCM) method using methanol as the solvent.

DNA binding experiments

The ratio of the UV absorbances of the solutions of DNA (5 mM Tris HCl/50 mM NaCl buffer) at 260 and 280 nm (A_{260}/A_{280}) was 1.9,³⁵ which indicates that the DNA was sufficiently free of protein. Concentrated stock solutions of DNA (13.5 mol dm⁻³) were prepared in the buffer and sonicated for 25 cycles, in which each cycle consisted of 30 s with 1 min intervals. The DNA solutions were pretreated with solutions of copper(II) complex to

ensure there were no amendments in the concentrations of the copper(II) complex for the absorption and emission spectral experiments.

Absorption spectral titration experiments were performed by maintaining a constant concentration of the complex and varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the metal complex and DNA stock solutions while maintaining the total volume constant (1 mL). This resulted in a series of solutions with varying concentrations of DNA, but with a constant concentration of the complex. The absorbance (A) of the most red-shifted band of the complex was recorded after successive additions of CT DNA.

The CD spectral experiments were performed using a cylindrical 0.1 cm path length quartz cell. Each CD spectrum was collected after averaging at least four accumulations using a scan speed of 100 nm min^{-1} and 1 s response time. Machine plus cuvette baselines were subtracted and the resultant spectrum was zeroed outside the absorption bands.

For the emission intensity measurements, a 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer was used as a blank to perform the preliminary adjustments. The excitation wavelength was preset and the emission range was adjusted prior to the measurements. The DNA was pretreated with ethidium bromide in the ratio of [NP]:[EthBr] = 1:1 for 30 min at 27°C . The effect on the emission intensity was measured after the addition of a metal complex to this mixture.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed in a CHI 620C electrochemical analyzer at $25 \pm 0.2^\circ\text{C}$. The working electrode was a glassy carbon disk (0.0707 cm^2), the reference electrode was a saturated calomel electrode, and the counter electrode was a platinum wire. The supporting electrolyte used was 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer (pH 7.1). Solutions were deoxygenated by purging with nitrogen gas for 15 min before the measurements; during the measurements, a stream of N_2 gas was passed over them. The redox potential $E_{1/2}$ was calculated from the anodic (E_{pa}) and cathodic (E_{pc}) peak potentials of the CV traces as $(E_{\text{pa}} + E_{\text{pc}})/2$ and also from the peak potential (E_{p}) of the DPV response as $E_{\text{p}} + \Delta E/2$ (ΔE is the pulse height).

Protein binding experiments

The stock solution of protein ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) was prepared by dissolving the solid BSA in 0.05 M phosphate buffer at pH 7.4 and this was stored at $0-4^\circ\text{C}$ in the dark for about a week before being diluted to $1.0 \times 10^{-6} \text{ mol L}^{-1}$ using a phosphate buffer (pH 7.4, 0.05 M), if used. The concentration of BSA was determined from the optical density measurements, using the value of the molar absorptivity ($\epsilon_{280} = 44720 \text{ M}^{-1} \text{ cm}^{-1}$).³⁶ All fluorescence measurements were performed using a 10 mm quartz cuvette at two different temperatures (300 and 310 K).

Quantitative analyses of the interaction between the complex and BSA were performed using fluorimetric titration (0.05 M phosphate buffer, pH 7.4). A 3.0 mL portion of an aqueous solution of BSA was titrated with successive additions of the complex. The titrations were performed manually using

an Eppendorf micropipette. For every addition, the mixture solution was shaken and allowed to stand for 20 min at the corresponding temperature (300 and 310 K) and then the fluorescence intensities were measured with an excitation wavelength of 280 nm and emission wavelengths in the interval 290–500 nm. No correction for the inner filter effect was applied as the complex represented a very low absorbance (less than 0.1) at the excitation and emission wavelengths. The excitation and emission slit width (each 5.0 nm) and scan rate (fast) were maintained throughout the experiments. The UV-visible absorption spectra of $1.0 \mu\text{M}$ free BSA, as well as the BSA/complex (equal molar ratio) in a 0.05 M phosphate buffer of pH 7.4, were recorded from 200–500 nm.

DNA cleavage experiments

The cleavage of DNA in the absence and presence of activating agents such as ascorbic acid ($10 \mu\text{M}$) was monitored using agarose gel electrophoresis. A typical reaction mixture, containing ϕX174 RF supercoiled phage DNA (form I, $20 \mu\text{M}$) and copper(II) complex in 0.1 M phosphate buffer (pH 7.2) was incubated at 37°C for 1 h. After the incubation period, the reaction was quenched by maintaining the samples at -20°C , followed by the addition of a loading buffer (0.025 mg bromophenol blue, 1 mL glycerol, and 1 mL MilliQ water). This was then loaded onto 1% agarose gel containing ethidium bromide ($2.54 \mu\text{M}$ in the gel, as well as in the buffer). The gels were run at a constant voltage of 40 V for 3 h in $1 \times$ TBE buffer (TBE = tris-borate-EDTA buffer) containing ethidium bromide. After washing with distilled water, the gels were visualized under a UV transilluminator and the bands were documented and quantified using a BioRad Gel Doc 1000 apparatus interfaced with a computer. The ability of the complex to convert the supercoiled DNA (SC) to the nicked circular form (NC) and linear form (LC) was determined in order to measure the cleavage potency. To identify the reactive oxygen species (ROS) involved in the cleavage reaction, radical scavengers, such as a hydroxyl radical (DMSO, $20 \mu\text{M}$), singlet oxygen (NaN_3 , $100 \mu\text{M}$), and superoxide (SOD, 0.5 units), were introduced.

Cytotoxicity assay

The cytotoxicity was estimated using an MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) assay.³⁷ For the cytotoxicity evaluation in human cancer cell lines, SiHa and MCF7, and the normal cell line, Jurkat T4, the ligands (phen, terpy, H(mq)), $\text{Cu}_2(\text{O}_2\text{CMe})_4(\text{H}_2\text{O})_2$, **1**, **2**, and cisplatin, at a concentration of 0.05–100 μM , were dissolved in 2% DMSO. 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 (Sigma-Aldrich), were added to the wells 24 h after they were seeded with 5×10^3 cells per well in 200 μL of freshly prepared culture medium and the maximum percentage of DMSO present in all the wells was 0.2% (v/v). A phosphate-buffered saline (PBS) (Sigma-Aldrich) solution of MTT ($20 \mu\text{L}$, 5 mg mL^{-1}), was added to each well after 24 and 48 h, aluminum foil was used to wrap the plates and they were incubated for 4 h at 37°C . DMSO ($100 \mu\text{L}$) was added to each well to dissolve the formazan crystals. A 96-well plate reader (Bio-Rad, Hercules, CA) was used to monitor the absorbance at 570 nm

(measurement) and 630 nm (reference). To calculate the mean, the data were calculated for three replicates each. This data was used to calculate the percentage inhibition using the formula:

$$= \frac{\text{mean OD of untreated cells (control)} - \text{mean OD of treated cells}}{\text{mean OD of untreated cells (control)}} \times 100$$

Table Curve 2D (version 5.01) was used to calculate the IC_{50} values.

Cell observation using an inverted microscope

The MCF7 cell lines were grown in 6-well plates and treated with complexes dissolved in DMSO. The cells were then washed with $1 \times$ PBS. The morphological and confluency changes in the cells in both the control and the treated group were observed using an inverted microscope (Nikon, TMS).

Acridine orange/ethidium bromide staining. For both the suspension and adherent cells, 96-well plates were centrifuged at 1000 rpm (129 g) for 5 min using a Beckman Model TJ-6 centrifuge with inserts for 96-well plates. An acridine orange/ethidium bromide (AO/EB) dye mix was added to each well and the cells were viewed using the same microscope as described above. The tests were performed by counting a minimum of 100 total cells, each in triplicate.

Giemsa staining. Briefly, the MCF7 cell line was seeded at 2.5×10^5 cells per well in 6-well plates and the plates were then incubated overnight at 37°C . After incubation, the complexes were added, at their IC_{50} concentrations, and incubated for an additional 24 and 48 h. The plates were washed with $1 \times$ PBS and the cells were stained with May-Grunwald (BDH Chemical Ltd) for 4 min. The slides were then rinsed with sterile water and flooded with freshly prepared Giemsa's stain solution (BDH Chemical Ltd) for 6 min. The dyestuff was discarded and rinsed again three times with sterile water. Morphological changes were examined using an inverted microscope (Nikon, TMS) with $100\times$ actual magnifications.

Hoechst 33258 staining. The cellular pathology was detected by staining the nuclear chromatin of the trypsinized cells ($4.0 \times 10^4 \text{ mL}^{-1}$) with $1 \mu\text{L}$ of Hoechst 33258 (1 mg mL^{-1}) for 10 min at 37°C . Staining of the suspension cells with Hoechst 33258 was used to detect apoptosis.³⁸ A drop of the cell suspension was placed on a glass slide and a coverslip was placed over to diminish the light diffraction. At random, 300 cells were observed in a fluorescent microscope (Carl Zeiss, Jena, Germany) fitted with a 377–355 nm filter and observed at $400\times$ magnification, and the percentage of cells reflecting the pathological changes were calculated. The data obtained from four replicates were used to measure the mean and the standard deviation.

Comet assay. The DNA damage was quantified using the comet assay as described.^{39,40} Assays were performed under red light at 4°C . Cells were sampled from a monolayer during the growing phase 24 h after seeding and used for the comet assay. Cells were treated with copper(II) complexes at an IC_{50} dose and the cells were harvested using a trypsinization process at 24 and 48 h. The normal agarose (1%) in PBS at 65°C ($200 \mu\text{L}$) was

dropped gently onto a fully frosted micro slide, which was covered immediately with a coverslip and placed over an ice pack for 5 min. The coverslip was removed after the gel had been set. The cell suspension from one fraction was mixed with 1% low-melting agarose at 37°C in a 1:3 ratio. This mixture ($100 \mu\text{L}$) was applied quickly on top of the gel that was coated over the micro slide and allowed to set as before. The third coating of $100 \mu\text{L}$ of 1% low-melting agarose was placed on the gel containing the cell suspension and allowed to set. Similarly, slides were prepared (in duplicate) for each cell fraction. After solidification of the agarose, the coverslips were removed and the slides were immersed in an ice-cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA , 10 mM Tris, NaOH; pH 10, 0.1% Triton X-100) and placed in a refrigerator at 4°C for 16 h. All of the above operations were performed in low-lighting conditions to avoid additional DNA damage. Slides, after removal from the lysis solution, were placed horizontally in an electrophoresis tank. The reservoirs were filled with an electrophoresis buffer (300 mM NaOH and 1 mM Na_2EDTA , pH > 13) until the slides were just immersed within it. The slides were allowed to stand in the buffer for approximately 20 min (to allow DNA unwinding), after which electrophoresis was carried out at 0.8 V cm^{-1} for 15 min. After electrophoresis, the slides were removed, washed thrice in a neutralization buffer (0.4 M Tris, pH 7.5), and gently dabbed to dry. Nuclear DNA was stained with $20 \mu\text{L}$ of EthBr ($50 \mu\text{g mL}^{-1}$). Photographs were taken using an epifluorescence microscope (Carl Zeiss). A total of 200 cells from each treatment were digitized and analyzed using image analysis (CASP Software). The images were used to estimate the DNA content of the individual nuclei and to evaluate the degree of DNA damage representing a fraction of the total DNA in the tail.

Results and discussion

Synthesis and general properties

The mixed ligand copper(II) complexes were readily prepared in a good yield by the reaction of copper(II) acetate monohydrate with terpy or phen and N,O-donor H(mq) by adding a methanol solution. The stoichiometries of the complexes were determined and found to be $[\text{Cu}(\text{terpy})(\text{mq})](\text{ClO}_4)$ (**1**) and $[\text{Cu}(\text{phen})(\text{mq})](\text{ClO}_4)$ (**2**) based on the elemental analysis and confirmed by determining the X-ray crystal structure of **1**. Conductivity experiments revealed that both the complexes act as 1:1 electrolytes in DMF solution. The ESI mass spectrum of complexes **1** and **2** show base peaks at m/z 455 and 416 respectively, which can be attributed to the complex cations $[\text{Cu}(\text{terpy})(\text{mq})]^+$ and $[\text{Cu}(\text{phen})(\text{mq})]^+$, respectively. The formation of the monocationic complexes was evidenced by the presence of an intense absorption for the ClO_4^- stretches at 1081 and 621 cm^{-1} in the IR spectrum of the complexes. As expected, the free H(mq) phenolic $\nu(\text{O}-\text{H})$ stretching ($\sim 3400 \text{ cm}^{-1}$) was not observed and the band belonging to $\nu(\text{C}_{\text{ph}}-\text{O})$ (1195 cm^{-1}) was shifted to $1280\text{--}1335 \text{ cm}^{-1}$. The $\nu(\text{C}=\text{N})$ frequencies of the ligands show changes upon complexation and appear at 1602 and 1558 cm^{-1} (**1**) and 1520 and 1428 cm^{-1} (**2**),

indicating the involvement of nitrogen in the coordination of the metal ion. The sharp bands observed at $428\text{--}440\text{ cm}^{-1}$ and $514\text{--}558\text{ cm}^{-1}$ can be assigned to $\nu(\text{Cu-O})$ and $\nu(\text{Cu-N})$ respectively, probably originating from the coordination of the terpy (1) or phen (2) and H(mq) (1 and 2) ligands.

Molecular structure of $[\text{Cu}(\text{terpy})(\text{mq})](\text{ClO}_4)$ **1**

An ORTEP view of the coordination environment around copper(II) in the complex cation and non-coordinated perchlorate anion is shown in Fig. 1A and the selected bond lengths and angles are listed in Table 1. The crystal structure of **1** reveals the presence of a ternary structure consisting of a bidentate N,O-donor of H(mq), a tridentate 3N-donor of terpy, and the copper(II) center in the discrete monomeric species. Considering the $\text{Cu}_1\text{N}_3\text{N}_2\text{N}_4\text{N}_1\text{O}_1$ chelate, the axial $\text{Cu}_1\text{--N}_1$ bond is indeed longer than that of the other four bonds. The long $\text{Cu}_1\text{--N}_1$ bond was selected by the criterion, which defines the two largest angles, α and β ($\alpha = \text{N}_3\text{--Cu}_1\text{--N}_4 = 157.64^\circ$ and $\beta = \text{N}_2\text{--Cu}_1\text{--O}_1 = 167.35^\circ$). Thus, the value of the structural index,⁴¹ τ (0.16) reveals that it possesses a square pyramidal geometry with a phenolate (O1) oxygen atom of H(mq) and three pyridine (N2, N3, N4) nitrogen atoms of terpy occupy the corners of the basal plane. The axial position is occupied by the pyridine nitrogen of H(mq) at a distance ($\text{Cu}_1\text{--N}_1 = 2.256\text{ \AA}$) that is longer than that observed for the equatorial pyridine nitrogen atoms ($\text{Cu}_1\text{--N}_3 = 2.044\text{ \AA}$ and $\text{Cu}_1\text{--N}_4 = 2.064\text{ \AA}$) of the terpy ligand. The third central pyridine nitrogen atom of terpy at a distance of $\text{Cu}_1\text{--N}_2 = 1.942\text{ \AA}$ is shorter than that observed for the other two-terminal pyridine nitrogen atoms of the terpy ligand coordinated equatorially and the equatorial phenolate oxygen atom of H(mq) at a distance of $\text{Cu}_1\text{--O}_1 = 1.898\text{ \AA}$ is very much shorter in the CuN_3O basal plane, this is a consequence of the tetragonal distortion caused by the Jahn–Teller effect (one electron in $d_{x^2-y^2}$ orbital). The geometry around copper(II) is best described as distorted square pyramidal owing to the values of the bond angles ($\text{N}_2\text{--Cu}_1\text{--N}_3 = 79.26^\circ$, $\text{N}_2\text{--Cu}_1\text{--N}_4 = 79.27^\circ$, $\text{N}_3\text{--Cu}_1\text{--N}_4 = 157.64^\circ$, $\text{O}_1\text{--Cu}_1\text{--N}_2 = 167.35^\circ$, $\text{O}_1\text{--Cu}_1\text{--N}_4 = 102.26^\circ$), that deviate from those (90 and 180°) expected for a perfect square pyramidal geometry. Another significant structural observation is the inter-pair $\text{C--H} \cdots \pi$ anti-parallel,

Table 1 Selected bond lengths (Å) and bond angles ($^\circ$) for **1**

N(1)–Cu(1)	2.256(15)	N(2)–Cu(1)	1.942(14)
N(3)–Cu(1)	2.044(16)	N(4)–Cu(1)	2.064(16)
O(1)–Cu(1)	1.898(13)		
O(1)–Cu(1)–N(1)	80.95(5)	O(1)–Cu(1)–N(2)	167.35(6)
O(1)–Cu(1)–N(3)	97.35(6)	O(1)–Cu(1)–N(4)	102.26(7)
N(1)–Cu(1)–N(2)	111.66(6)	N(1)–Cu(1)–N(3)	102.99(6)
N(1)–Cu(1)–N(4)	90.70(6)	N(2)–Cu(1)–N(3)	79.26(6)
N(2)–Cu(1)–N(4)	79.27(6)	N(3)–Cu(1)–N(4)	157.64(6)

non-covalent interactions (Fig. 2b) involving C(2)–H of H(mq) and the centroid (C_g) of one of the terminal pyridine rings of terpy ($\text{C}(2)\text{--H} \cdots C_g(\text{pyridine}) = 3.496\text{ \AA}$), which is probably responsible for the stabilization of the distorted square pyramidal geometry.⁴²

Structure of copper(II) complexes: DFT calculations

Geometry optimization for **1** and **2** was carried out using DFT calculations. The initial coordinates for **1** were taken from the single-crystal X-ray data of $[\text{Cu}(\text{terpy})(\text{mq})]^+$ **1** and the structure was subjected to optimization. The optimized structures of **1** and **2** are shown in Fig. 2 and the geometrical parameters *viz.* bond lengths, optimized energies, frontier molecular orbitals (MOs), and singly occupied molecular orbital (SOMO)–SOMO+1 energy gap have been calculated (Table 2, Fig. 3 and Fig. S1, ESI \ddagger) at the B3LYP 6-31G*/LANL2DZ levels using the Gaussian 09 program package. The computed structural parameters for **1** agree well with those observed in their X-ray crystal structure; however, the computed Cu--N_1 , Cu--N_2 , Cu--N_3 , and Cu--N_4 bond length of **1** is about $0.01\text{--}1\text{ \AA}$ longer, and the computed Cu--O_1 bond length is about 0.05 \AA longer than those found in the X-ray structure, which is attributed to the overestimation of the covalency of Cu(II) by the established exchange–correlation function, which is well-recognized.⁴³ The trigonality index for **1** ($\tau = 0.11$) calculated from the computed bond angles, is closer to that of the experimental values (*cf.* above). Apart from these, there are no significant differences in the bond length and spin densities of **1** and **2**. Thus, as the method of calculation adopted could reproduce the distorted square pyramidal structure of **1**, the computed structure of **2** is reliable and is suitable

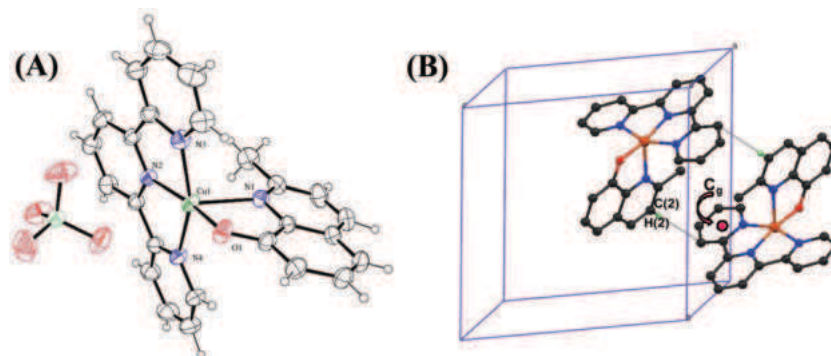


Fig. 1 (A) An ORTEP view of $[\text{Cu}(\text{terpy})(\text{mq})](\text{ClO}_4)$ **1** with atom numbering of the complex cation and thermal ellipsoids at 40% probability. (B) The inter-pair $\text{C--H} \cdots \pi$ interaction in $[\text{Cu}(\text{terpy})(\text{mq})]^+$ **1** involving the π -ring of the terminal pyridyl ring of terpy and the hydrogen atom [C(2)–H(2)] of the pyridyl moiety of H(mq).

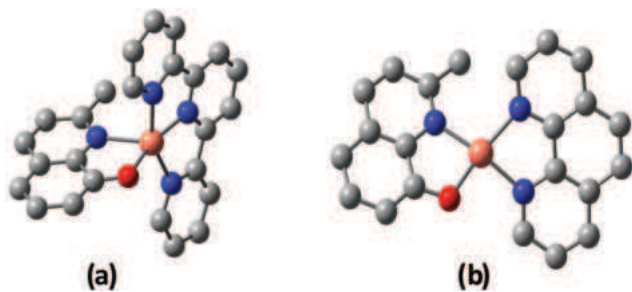


Fig. 2 DFT optimized structures of complexes $[\text{Cu}(\text{terpy})(\text{mq})]^+$ **1** (a) and $[\text{Cu}(\text{phen})(\text{mq})]^+$ **2** (b).

for structural discussions. In addition, the significant difference in the twist angle between the O–Cu–N and N–Cu–N planes, estimated for **1** and **2** (**1**, 1.7; **2**, 2.7) is due to the higher aromatic ring delocalization in the phen ring (**2**) compared to the terpy ring (**1**), which enforces the planarity around the Cu(II) center in **2**. This has a beneficial consequence on the DNA binding ability and mode of binding, as well as the DNA cleavage efficiency of the complex (*vide infra*).

Interestingly, the SOMO/ α spin is localized entirely on the H(mq) ligand in **1** and **2** and the β spin density of SOMO is always localized on the H(mq) ligand, irrespective of the co-ligand. As the frontier molecular orbital approximation indicates that SOMO determines the electron-donating ability of the ligand, the highest value of the SOMO energy of **1** (-5.6341) > **2** (-5.9870) reveals that the terpy ligand releases more electrons, and hence these are involved in the strongest σ -bonding to Cu(II). Thus, the σ -donor capability of the primary ligand H(mq) is tuned by the variation in the terpy or phen co-ligand, which reflects the presence of synergy between the primary and secondary ligands when bound to the metal. The energy of the SOMO+1 orbitals is localized largely on the terpy or phen and corresponds to the ability of the co-ligand to accept electrons, and the observed trend in the energy of SOMO+1, **1** (-2.9899) < **2** (-2.8885) indicates that the π^* orbital of phen is delocalized more than that in terpy (**1**). Therefore, the more delocalized π^* orbital of phen would be involved in a stronger π back-bonding with Cu(II) to stabilize the Cu(I) oxidation state more than that observed with the terpy co-ligand. This is in good agreement with the observed trend in $E_{1/2}$ values and illustrates that delocalization of the π^* orbital of the co-ligand is important in stabilizing the lower oxidation of Cu(I)

Table 2 Computed structural parameters and values of the HOMO–LUMO energy gap for complexes **1** and **2**

Parameters	1	2
Cu–N(1)	2.074	2.013
Cu–N(2)	1.984	2.057
Cu–N(3)	2.075	
Cu–N(4)	2.265	2.025
Cu–O(1)	1.946	1.938
Optimized energy (eV)	-3.96×10^4	-3.49×10^4
SOMO (HOMO) (eV)	-5.6341	-5.9870
SOMO+1 (LUMO) (eV)	-2.9899	-2.8885
HOMO–LUMO energy gap (eV)	-2.6441	-3.0986

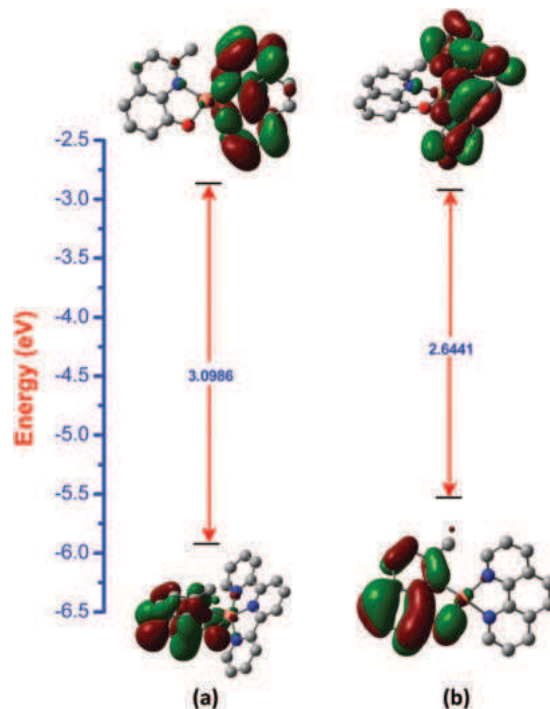


Fig. 3 Energy profile diagram of complexes $[\text{Cu}(\text{terpy})(\text{mq})]^+$ **1** (a) and $[\text{Cu}(\text{phen})(\text{mq})]^+$ **2** (b). The doublet spin state: highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) in the restricted spin calculations were calculated using time dependent (TD)-DFT using the B3LYP dispersion corrected functional level with the 6-31G/LANL2DZ basis set as implemented in the Gaussian 09 package.

(*cf.* below). The variation in the SOMO–SOMO+1 energy gap calculated, *viz.* **1** > **2**, reveals that the metal to ligand charge transfer (MLCT) band energy is expected to vary in this order.

Electronic and EPR spectra

The electronic absorption spectrum of **1** in DMF exhibits only one well-defined ligand field band at 816 nm (ϵ_{max} , $170 \text{ M}^{-1} \text{ cm}^{-1}$) suggesting a square-derived geometry with a strong axial interaction.⁴⁴ However, **2** shows a ligand field band at 649 nm with a high absorptivity (ϵ_{max} , $240 \text{ M}^{-1} \text{ cm}^{-1}$), revealing a slightly distorted square-planar coordination geometry. The ligand field band energy of **2** is greater than that of **1**, suggesting that the π back-bonding of the diimine phen ring confers a stronger ligand field than the σ -bonding of the triimine terpy nitrogen atoms (*cf.* above). The observation of the $\text{PhO}^- \rightarrow \text{Cu}(\text{II})$ LMCT transition,⁴⁵ as an intense band in the range 367–391 nm (ϵ_{max} , $2810\text{--}2890 \text{ M}^{-1} \text{ cm}^{-1}$), reveals the coordination of the phenolate oxygen atom. An intense band or shoulder (339–341 nm) is assigned to the $\text{N}(\pi) \rightarrow \text{Cu}(\text{II})$ LMCT transition.⁴⁶ The high intense bands between 281–325 nm are assigned to the $\pi\text{--}\pi^*$ transitions of H(mq), whereas the bands in the range 263–266 nm are due to the intraligand transitions of terpy or phen. The UV-visible spectral studies of the complexes in 2% DMF/5 mM Tris–HCl/50 mM NaCl buffer solutions reveal no change in the coordination sphere, thus, the complexes retain their identity in buffer solution (24 h of incubation at 37 °C).

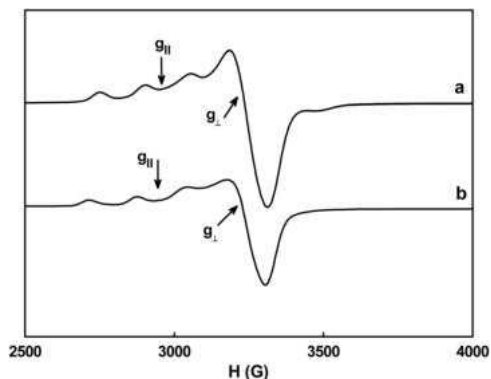


Fig. 4 EPR spectrum of [Cu(terpy)(mq)](ClO₄) **1** (a) and [Cu(phen)(mq)](ClO₄) **2** (b) in DMF solution at 77 K (microwave frequency: **1**, 9.377; **2**, 9.360 GHz).

The polycrystalline EPR spectra of **1** and **2** are isotropic. The frozen DMF solution spectra of the complexes are axial [$g_{\parallel} > g_{\perp} > 2.0$, $G = (g_{\parallel} - 2)/(g_{\perp} - 2) = 4.2$],⁴⁵ which is usual for mononuclear square-based mixed ligand copper(II) complexes with a $d_{x^2-y^2}$ ground state (Fig. 4).³⁶ A square-based CuN₄ chromophore shows an A_{\parallel} value of $180\text{--}200 \times 10^{-4} \text{ cm}^{-1}$ and a g_{\parallel} value of around 2.200 and the substitution of a nitrogen atom in this chromophore by an oxygen atom decreases the A_{\parallel} value and increases the g_{\parallel} value.⁴⁷ Also, A_{\parallel} decreases and g_{\parallel} increases owing to distortion or incorporation of a strong axial interaction of the square planar coordination geometry. Thus, the experimental values for **1** (g_{\parallel} , 2.250; A_{\parallel} , $160 \times 10^{-4} \text{ cm}^{-1}$) and **2** (g_{\parallel} , 2.267; A_{\parallel} , $172 \times 10^{-4} \text{ cm}^{-1}$) reveal the presence of a square-based CuN₃O chromophore involving the strong axial interaction in **1** (*cf.* above) and a square-based CuN₃O chromophore with a slight distortion from planarity ($g_{\parallel}/A_{\parallel} = 132 \text{ cm}$; $g_{\parallel}/A_{\parallel}$ quotient for perfect square planar Cu(II) complexes, 105 to 135 cm)⁴⁸ in **2**. The higher g_{\parallel} , A_{\parallel} and $g_{\parallel}/A_{\parallel}$ quotient for **2** suggests that the steric bulk of the 2-methyl group in H(mq) induces a relatively higher tetrahedral distortion. This is consistent with the absence of nitrogen superhyperfine lines and the relatively high ϵ value (*cf.* above) of the ligand field band.⁴⁹

DNA binding studies

DNA binding is a crucial step in DNA cleavage⁵⁰ in most cases and is very important to understanding the tumor inhibition mechanism for cancer treatments.⁵¹ Therefore, the mode and the propensity of binding of the mixed ligand copper(II) complexes to DNA were analyzed with the aid of different spectral and electrochemical techniques. The absorption band resulting from the ligand-centered $\pi \rightarrow \pi^*$ transitions at 326 (**1**) and 268 nm (**2**) exhibits hypochromism (**1**, 15; **2**, 45%) with red-shifts (**1**, 1; **2**, 4 nm) in the band position (Fig. 5) upon the incremental addition of CT DNA to complexes at $R = 25$ ($R = [\text{DNA}]/[\text{Cu}]$). As the extent of hypochromism is commonly associated with the strength of the DNA binding affinity, the observed order of decrease in hypochromism was **2** > **1**, and hence **2** possesses a higher propensity for DNA binding (*cf.* below). However, the hypochromism is in the range of 15–45%, which is due to the small (**1**) or medium (**2**)

partial filling of the empty π^* orbital of the co-ligand with the π electrons of the DNA base pair or distortions in DNA upon binding of the complex, and the very small red-shift reveals that the complexes do not partially intercalate into DNA base pairs. The intrinsic binding constants, K_b , obtained for the complexes follow the order **2** ($2.2 \times 10^5 \text{ M}^{-1}$) > **1** ($0.2 \times 10^5 \text{ M}^{-1}$), which reveals that the diimine (**2**) or triimine (**1**) co-ligand determines the DNA binding affinity. The K_b value obtained for the four-coordinate complex (**2**) is eleven times higher than that of the five-coordinate complex (**1**). These observations suggest that the four-coordinate square planar complex (**2**), rather than the five-coordinate square-based pyramidal complex (**1**), would fit well with the DNA groove resulting in stronger DNA binding. The hydrophobic DNA interaction of the electron-releasing 2-methyl group on the primary ligand would also enhance the DNA binding affinity of **2** compared to **1**. This is interesting because the 2-methyl group enhances the DNA binding affinity of the phen ring owing to the size and shape of the complex, which is tuned to closely fit into the groove leading to a strong hydrophobic interaction with the DNA surface, while the steric clash of the terpy rings weakens the hydrophobic interaction of the 2-methyl group with DNA. Thus, the alteration in the coordination geometry resulting from the bonding of the nitrogens from the phen to the terpy co-ligand to copper(II) dictate the DNA binding structure and the hydrophobic propensity of the primary ligand depends upon the coordination geometry further increasing the DNA binding affinity. It has already been established that copper(II) complexes, which possess a higher DNA binding affinity display a higher cytotoxicity.⁵² Thus, the phen (**2**) complex with a higher DNA binding affinity is expected to demonstrate a higher cytotoxicity compared to that of the terpy (**1**) complex.

The emission intensity of DNA-bound EthBr decreases (Fig. 6), upon adding **1–2** (0–10 μM) to CT DNA pretreated with EthBr ($[\text{EthBr}]/[\text{DNA}] = 0.01$) in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1, revealing the presence of the paramagnetic copper(II) complexes in the DNA-bound form.⁵³ The plot of the relative intensity of the fluorescence (λ_{em} , 595 nm) versus the concentration of the copper(II) complex agrees with the linear Stern-Volmer equation⁵⁴ (fluorescence quenching constant, K_{SV} : **1**, 3.6×10^5 ; **2**, $5.0 \times 10^5 \text{ M}^{-1}$). The apparent DNA binding constant (K_{app} , assumed to be 50% displacement of EthBr) decreases in the order, **1** ($5.9 \times 10^4 \text{ M}^{-1}$) < **2** ($1.5 \times 10^5 \text{ M}^{-1}$), supporting the strong DNA binding propensity of the phen ring⁵⁵ compared to the terpy ring *via* the groove binding mode of the interaction (*cf.* above).

The CD spectrum of CT DNA ($2 \times 10^{-5} \text{ M}$) consists of a positive band at 278 nm that may result in base stacking and a negative band at 247 nm owing to the helicity (Fig. S2, ESI \ddagger) that is also characteristic of DNA in the right-handed B form.⁵⁶ The CD spectrum of CT DNA was monitored in the presence of **1** and **2** at $1/R = ([\text{Cu complex}]/[\text{DNA}])$ at value of 3 (Fig. S2, ESI \ddagger). The positive band showed an enhancement in the molar ellipticity (**1**, 53; **2**, 58%) with a small red-shift (**1**, 3; **2**, 5 nm) in the band maxima, whereas the negative band showed a decrease in the intensity (**1**, 27; **2**, 30%), which indicates that the groove binding mode of interaction is occurring in the

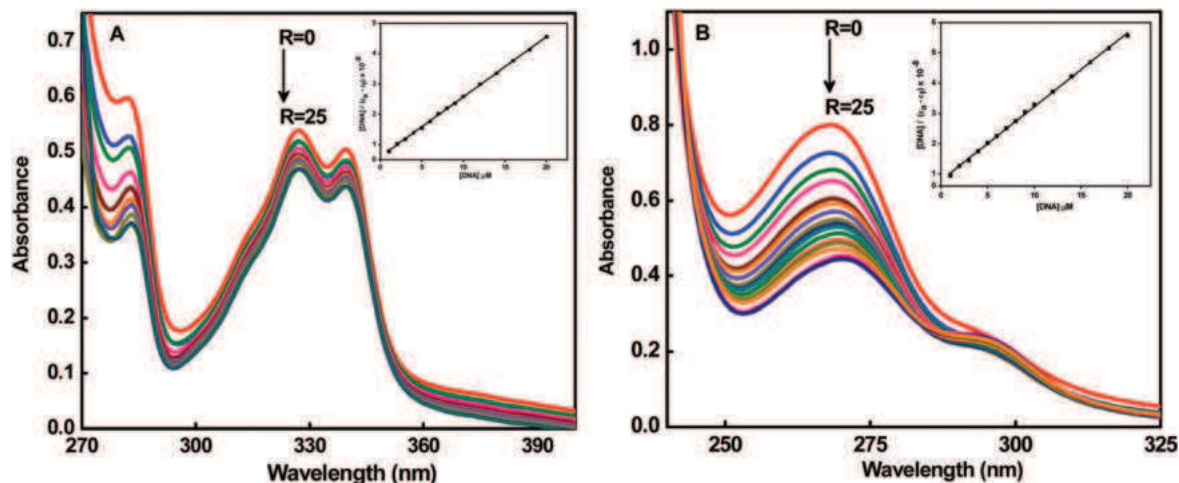


Fig. 5 Absorption spectra of **1** (left, A) and **2** (right, B) (3.0×10^{-5} M) in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 in the absence ($R = 0$) and presence ($R = 25$) of increasing amounts of CT DNA. Inset: Plot of $[DNA]/([DNA] + [Cu])$ versus $[DNA]$ at $R = 25$ of **1** (left, A) and **2** (right, B).

complexes and the stabilization of the right-handed *B* conformation of the CT DNA.⁵⁷

Both the complexes display a well-defined cathodic wave, but the corresponding anodic waves were drawn out, revealing that the very unstable Cu(I) species are not discernible (Fig. S3, ESI†) within the time scale of the CV experiments (2% DMF/5 mM Tris-HCl/50 mM NaCl buffer; pH 7.1). The $E_{1/2}$ values of the complexes (DPV: **1**, -0.805 ; **2**, -0.413 V) follow the trend $2 > 1$. The more π -delocalized phen ring in **2** is expected to stabilize Cu(I), while the σ -donating terpy rings in **1** (*cf.* above) are expected to stabilize Cu(II), conferring a more negative Cu(II)/Cu(I) redox potential on **1** and hence the Cu(II)/Cu(I) redox potential of **2** is more positive (~ 400 mV) than that of **1**. Upon adding CT DNA ($R = [DNA]/[Cu] = 5$) the peak currents of the reduction waves in **1** and **2** decrease significantly, suggesting the binding of the complexes to the large slowly diffusing DNA molecule and the decrease is more significant for **2** than for **1** (Fig. 7). The $E_{1/2}$ values (DPV: **1**, -0.799 ; **2**, -0.390 V) for the

Cu(II)-Cu(I) redox couple of the complexes are shifted (**1**, 6; **2**, 23 mV) to positive values (Fig. 7) and the positive shift follows the order $2 > 1$, revealing the stronger DNA binding affinity of **2** compared to that of **1** (*cf.* above).⁵⁸ The shift in the value of the formal potential (ΔE^0) can be used to estimate the ratio of the equilibrium binding constant (K^+/K^{2+}) values of the Cu(I) and Cu(II) form to DNA, which are calculated to be 1.3 (**1**) and 2.3 (**2**), suggesting their stabilization in the Cu(I) oxidation state upon binding to CT DNA.⁵⁹

BSA interaction

Tryptophan, tyrosine, and phenylalanine are the three intrinsic characteristics of the protein, which are responsible for the fluorescence of BSA. Among them, tryptophan and tyrosine are the dominant intrinsic fluorophores. The quenching of fluorescence corresponds to any process that decreases the intensity of fluorescence from a fluorophore and is attributable to a range of molecular interactions, such as molecular rearrangements,

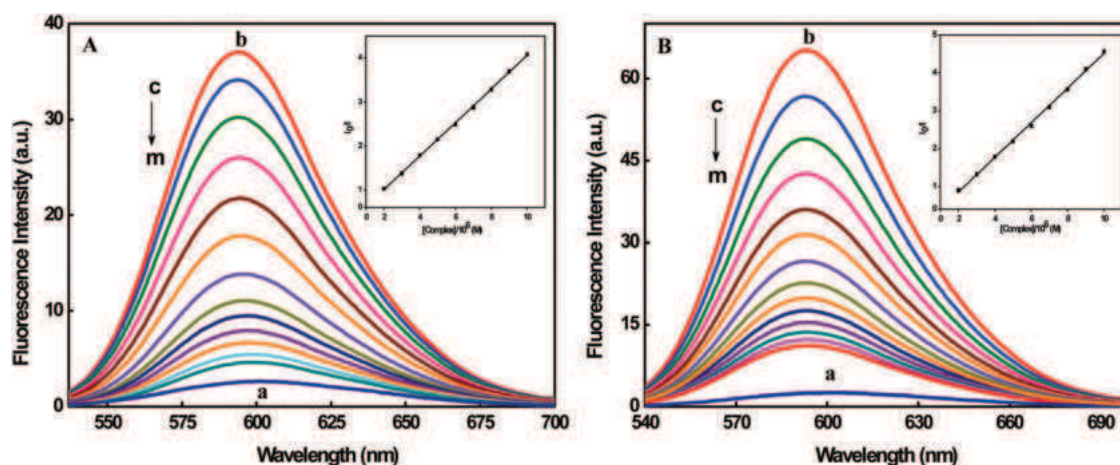


Fig. 6 Fluorescence quenching curves of ethidium bromide bound to DNA in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1: (a) EthBr (1.25 μ M); (b) EthBr + DNA (125 μ M); (c)-(m) EthBr + DNA + **1** (A) and **2** (B) (0–10 μ M). Inset: Plot of I_0/I vs. $[complex]$ of **1** (A) and **2** (B).

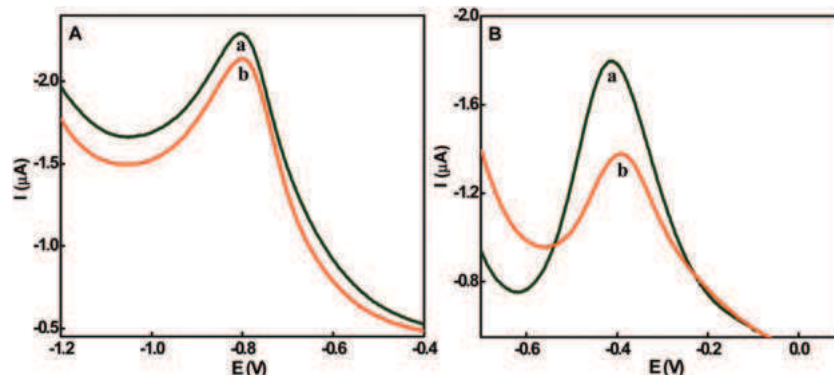


Fig. 7 Differential pulse voltammograms of **1** (left, A) and **2** (right, B) (0.5 mM) in the absence (a) and presence (b) of CT DNA ($R = 5$) at 25.0 ± 0.2 °C at a 2 mV s^{-1} scan rate in 2% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1.

energy transfer ground-state complex formation, excited-state reactions, and collisional quenching. Therefore, the fluorescence spectra of BSA ($1 \mu\text{M}$; λ_{em} , 340 nm; λ_{ex} , 280 nm) in the presence of increasing concentrations (0–4 μM) of **1** and **2** were recorded at 300 and 310 K. The fluorescence intensity of BSA decreases significantly up to 67.9 (**1**), 68.2% (**2**) at 300 K and 68.3 (**1**), 70.7% (**2**) at 310 K accompanied by a hypsochromic

shift of 2–3 nm (Fig. 8). The observed Stern–Volmer plots (Fig. S4, ESI†) represent a good linear relationship,⁶⁰ in which, K_{SV} (Table 3) increases with the increasing temperature (300 K: **1**, 2.16; **2**, $2.58 \times 10^5 \text{ M}^{-1}$ and 310 K: **1**, 2.43; **2**, $2.76 \times 10^5 \text{ M}^{-1}$), indicating that the fluorescence quenching probably occurs *via* a dynamic quenching mechanism. The obtained bimolecular quenching rate constant (k_q) is in the order of $10^{13} \text{ M}^{-1} \text{ s}^{-1}$,

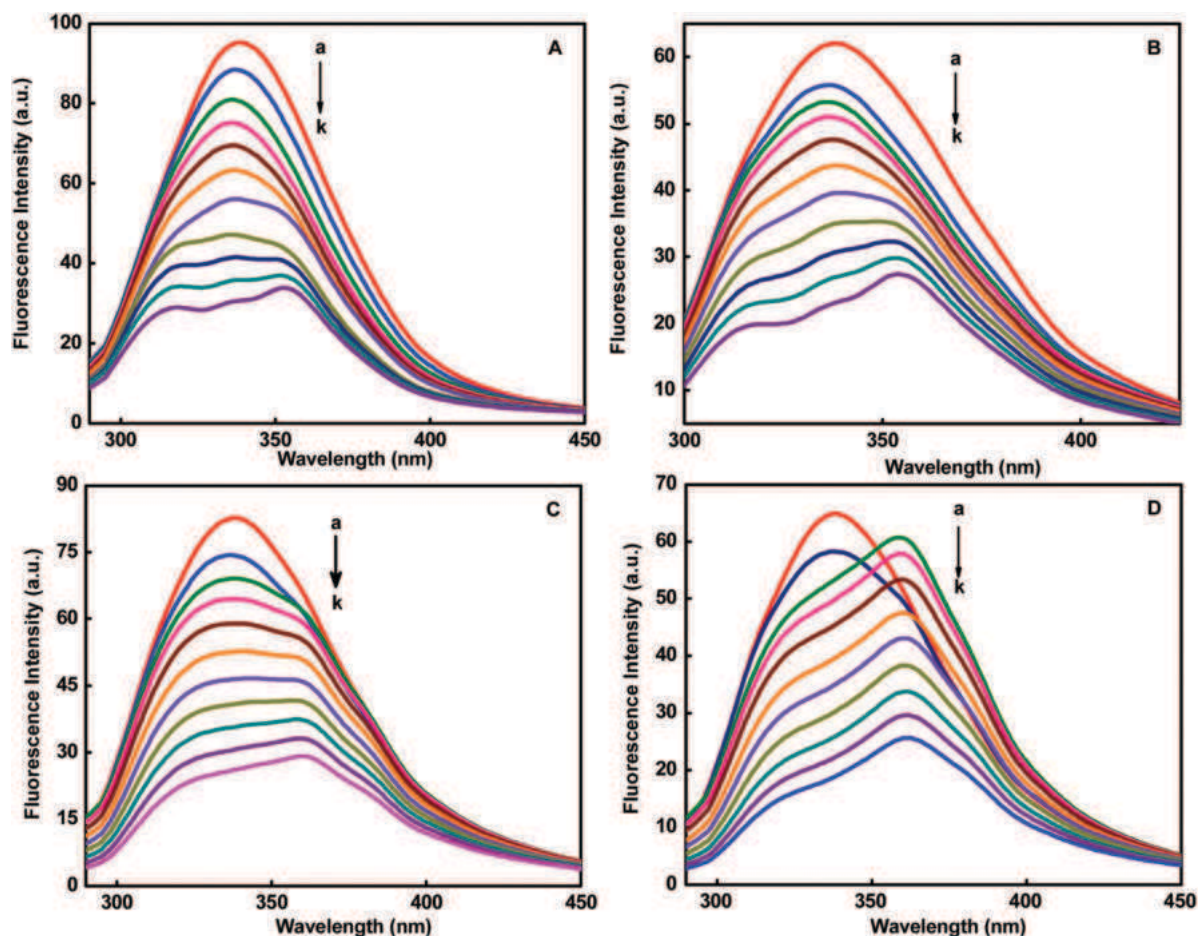


Fig. 8 Changes in the fluorescence spectra of BSA through titration with **1** and **2** at 300 K (left, A, and C) and 310 K (right, B and D). The concentration of BSA is $1 \times 10^{-6} \text{ mol L}^{-1}$, and the concentration of **1** and **2** was varied from (a) 0.0 to (k) $4.0 \times 10^{-6} \text{ mol L}^{-1}$; pH 7.4 and λ_{ex} 280 nm.

Table 3 Quenching, association, binding and thermodynamic parameters of the interaction of **1** and **2** with BSA at different temperatures^a

Parameters	300 K	<i>R</i>	310 K	<i>R</i>
[Cu(terpy)(mq)](ClO₄) 1				
<i>K</i> _{sv} (10 ⁵ M ⁻¹) ± SD	2.161 ± 0.003	0.9996	2.431 ± 0.003	0.9992
<i>k</i> _q (10 ¹³ M ⁻¹ s ⁻¹)	2.161		2.431	
<i>K</i> _a (10 ⁵ M ⁻¹) ± SD	4.597 ± 0.003	0.9995	5.016 ± 0.003	0.9993
<i>K</i> _b (10 ⁵ M ⁻¹) ± SD	3.620 ± 0.031	0.9994	4.230 ± 0.062	0.9994
<i>n</i> ± SD	1.02 ± 0.01		1.04 ± 0.01	
Δ <i>H</i> ^o (kJ mol ⁻¹)	77.899			
Δ <i>S</i> ^o (J mol ⁻¹ K ⁻¹)	108.606		109.414	
Δ <i>G</i> ^o (kJ mol ⁻¹)	-32.504		-33.840	
[Cu(phen)(mq)](ClO₄) 2				
<i>K</i> _{sv} (10 ⁵ M ⁻¹) ± SD	2.584 ± 0.002	0.9952	2.760 ± 0.003	0.9995
<i>k</i> _q (10 ¹³ M ⁻¹ s ⁻¹)	2.584		2.760	
<i>K</i> _a (10 ⁵ M ⁻¹) ± SD	5.416 ± 0.004	0.9996	5.777 ± 0.004	0.9996
<i>K</i> _b (10 ⁵ M ⁻¹) ± SD	4.925 ± 0.079	0.9996	5.447 ± 0.008	0.9996
<i>n</i> ± SD	1.039 ± 0.014		1.046 ± 0.007	
Δ <i>H</i> ^o (kJ mol ⁻¹)	77.696			
Δ <i>S</i> ^o (J mol ⁻¹ K ⁻¹)	110.023		111.047	
Δ <i>G</i> ^o (kJ mol ⁻¹)	-33.023		-33.346	

^a *R* is the linear correlated coefficient.

which is 1000-fold higher than the maximum limit ($2.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$)⁶¹ possible for diffusion-controlled quenching. Also, the effective quenching constant, *K*_a (Table 3) obtained using the modified Stern–Volmer equation (Fig. S5, ESI[†]),⁶² increases upon raising the temperature (300 K, **1**, 4.60; **2**, $5.42 \times 10^5 \text{ M}^{-1}$ and 310 K, **1**, 5.02; **2**, $5.78 \times 10^5 \text{ M}^{-1}$). The absorption spectra of BSA⁶³ possesses two absorption peaks at 210 and 280 nm (Fig. S6, ESI[†]), in which 210 nm represents the content of the α-helix in the BSA.⁶⁴

A dramatic decrease in the 210 nm absorbance peak of BSA with a red-shift (**1**, from 210 to 230 nm; **2**, from 210 to 231 nm) is observed upon the addition of the complexes. Meanwhile, the absorption intensity of the 280 nm peak is increased (Fig. S6, ESI[†]) indicating that more aromatic acid residues were extended into the aqueous environment and the tertiary structure of BSA was destroyed.⁶⁴ These results show that the interaction between the copper(II) complexes and BSA is mainly a static quenching process resulting from the formation of a ground-state complex (BSA-**1** or BSA-**2**). The binding constant,⁶⁵ *K*_b (Fig. S7, ESI[†]) increases with the increasing temperature (300 K: **1**, 3.62; **2**, $4.93 \times 10^5 \text{ M}^{-1}$ and 310 K: **1**, 4.23; **2**, $5.45 \times 10^5 \text{ M}^{-1}$), which substantiates the formation of stable BSA-**1** or BSA-**2** and the number of binding sites is one indication of the availability of the single binding site. These values indicate that

the complexes bind to the hydrophobic pocket located in the sub-domain IIA.⁶⁶ As shown in Table 3, a negative Δ*G*^o value supports the assertion that all binding processes are spontaneous. The Δ*H*^o and Δ*S*^o values are positive, which indicates that the binding is especially entropy-driven and enthalpy is unfavorable to it, the hydrophobic forces played a major role in the reaction.^{67,68} In addition to the hydrophobic interaction, covalent bonding could also be considered. However, the obtained value for Δ*H*^o (78 kJ mol⁻¹) is considerably below what would be expected for covalent bond formation, which should be $\geq 120 \text{ kJ mol}^{-1}$.⁶⁹

Oxidative cleavage of φX174 RF DNA

The reaction of **1** with supercoiled φX174 RF DNA (20 μM, in base pairs) in phosphate buffer at pH 7.2 with increasing concentrations of 10–100 μM and incubation at 37 °C for 1 h results in an electropherogram pattern for lanes 3–7 (Fig. S8, ESI[†]), which is very similar to that of the control (lane 1) indicating that a supercoiled phage DNA is not cleaved. In contrast, **1** exhibits 70% cleavage (100 μM complex concentration; incubation, 2 h) in the presence of ascorbic acid (Fig. 9). The appearance of well-defined electrophoresis band characteristic of NC (form II) and the absence of a band corresponding to the LC form (form III) suggest that only

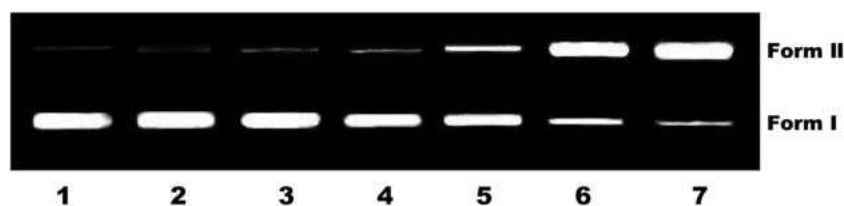


Fig. 9 Agarose gel showing cleavage of 20 μM SC φX174 RF DNA incubated with **1** in 0.1 M phosphate buffer (pH 7.2) at 37 °C for 1 h in the presence of ascorbic acid (ascorbic acid, 20 μM). Lane 1, DNA; lane 2, DNA + ascorbic acid; lanes 3–7, DNA + ascorbic acid + **1** (20, 40, 60, 80, and 100 μM respectively). Form I and II are supercoiled and NC forms of DNA respectively.

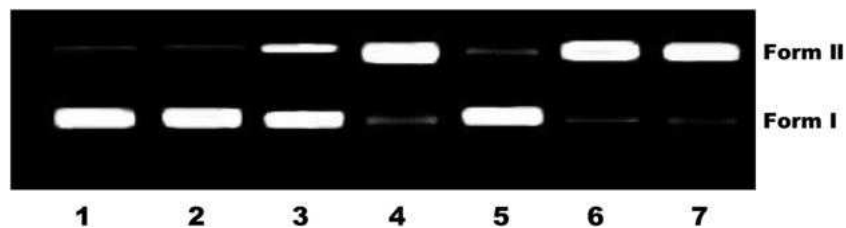


Fig. 10 Agarose gel showing cleavage of 20 μM SC ϕX174 RF DNA incubated with **1** in 0.1 M phosphate buffer (pH 7.2) at 37 $^{\circ}\text{C}$ for 1 h in the presence of ascorbic acid (ascorbic acid, 20 μM). Lane 1, DNA; lane 2, DNA + ascorbic acid; lane 3: DNA + **1** (100 μM); lane 4: DNA + ascorbic acid + **1** (100 μM); lane 5: DNA + ascorbic acid + **1** (100 μM) + SOD (0.5 units); lane 6: DNA + ascorbic acid + **1** (100 μM) + NaN_3 (100 μM); and lane 7: DNA + ascorbic acid + **1** (100 μM) + DMSO (20 μM).



Fig. 11 Agarose gel showing cleavage of 20 μM SC ϕX174 RF DNA incubated with **2** in 0.1 M phosphate buffer (pH 7.2) at 37 $^{\circ}\text{C}$ for 1 h. Lane 1: DNA; lanes 2–6: DNA + **2** (20, 40, 60, 80, and 100 μM , respectively). Form I and II are the SC and NC forms of DNA, respectively.

single-strand DNA cleavage occurs in **1** with a non-random strand scission event. The reaction of **2** at 37 $^{\circ}\text{C}$ with ϕX174 RF DNA (20 μM , in base pairs) resulted in vital amounts of form II, in more than stoichiometric amounts (around 1–10 fold). About 80–90% of the SC DNA (form I) was nicked to produce relaxed or open circular DNA (form II) when incubated with an increasing concentration of the complex from 20 to 100 μM (Fig. 10). Although this efficient DNA cleavage was promising, only a very small proportion of the DNA was linearized by **2**. The cleavage reactions were carried out by the addition of a variety of radical scavengers such as DMSO (hydroxyl radical), NaN_3 (singlet oxygen) and SOD (superoxide). The DNA cleavage of **1** is inhibited by SOD (Fig. 11) while **2** is inhibited by DMSO (Fig. 12), suggesting the involvement of superoxide radicals (**1**) and the potential involvement of hydroxyl radical intermediates (**2**) in the oxidative DNA cleavage. Interestingly, lane 4 of **2** (Fig. 12) is the reaction of 20 μM ϕX174 RF DNA with 100 μM of the complex and 20 μM ascorbic acid, the SC form (form I) is

discernible as a faint band with smearing compared to the DNA control. Thus, the DNA cleavage is suggested to be oxidative with probable alterations in the sugar and/or base units.

The more intense nuclease activity of **2** is apparently a result of the enhanced stabilization of its Cu(I)-phen species, compared to the Cu(I)-terpy species upon binding to DNA. The Cu(I) species stabilized through strong groove binding with DNA close to the deoxyribose rings⁷⁰ is the one responsible for the DNA cleavage activity and the free hydroxy radicals generated by the interaction of the Cu(I) species with molecular oxygen are then involved in DNA cleavage to produce a deoxyribose-centered radical by C-1 hydrogen abstraction. The reduced ability of **1** to enable oxidative DNA cleavage, despite its higher ability to stabilize the Cu(I) oxidation state (*cf.* above) and hence Cu(I)-oxo species responsible for the oxidative DNA cleavage,⁷¹ may be attributed to the inability of the terpy ligand to locate its Cu(I) form close to the site of the cleavage reaction and to its weak surface binding with DNA.⁷⁰ However, **2**, with a high value

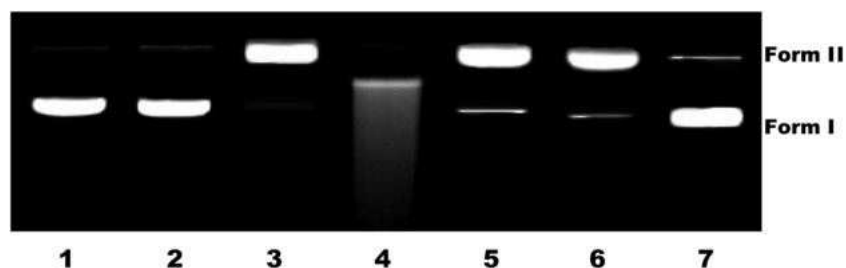


Fig. 12 Agarose gel showing cleavage of 20 μM SC ϕX174 RF DNA incubated with **2** in 0.1 M phosphate buffer (pH 7.2) at 37 $^{\circ}\text{C}$ for 1 h in the presence of ascorbic acid (ascorbic acid, 20 μM). Lane 1: DNA; lane 2: DNA + ascorbic acid; lane 3: DNA + **2** (100 μM); lane 4: DNA + ascorbic acid + **2** (100 μM); lane 5: DNA + ascorbic acid + **2** (100 μM) + SOD (0.5 units); lane 6: DNA + ascorbic acid + **2** (100 μM) + NaN_3 (100 μM); and lane 7: DNA + ascorbic acid + **2** (100 μM) + DMSO (20 μM).

Table 4 *In vitro* cytotoxicity assays for complexes [Cu(terpy)(mq)](ClO₄) **1** and [Cu(phen)(mq)](ClO₄) **2** and cisplatin against MCF7 breast cancer cell line, SiHa cervical cancer cell line and Jurkat T4 normal cell line

Compounds	MCF7		SiHa		Jurkat T4	
	IC ₅₀ (μM) ± SD		IC ₅₀ (μM) ± SD		IC ₅₀ (μM) ± SD	
	24 h	48 h	24 h	48 h	24 h	48 h
H(mq)	48.4 ± 1.2	46.5 ± 1.2	52.5 ± 1.3	49.4 ± 1.3	—	—
terpy	20.4 ± 1.2	19.2 ± 1.2	22.5 ± 1.3	20.4 ± 1.3	—	—
phen	64.4 ± 1.3	58.2 ± 1.1	67.5 ± 1.2	60.4 ± 1.0	—	—
Cu-acetate	> 100	> 100	> 100	> 100	—	—
1	18.5 ± 1.2	17.3 ± 1.0	22.4 ± 1.3	20.2 ± 1.2	40.5 ± 1.3	38.4 ± 1.3
2	4.6 ± 0.8	3.2 ± 0.6	10.3 ± 1.0	8.8 ± 1.3	102.6 ± 1.1	101.2 ± 1.1
Cisplatin	24.4 ± 1.3	22.2 ± 1.2	33.6 ± 0.5	31.3 ± 0.8	27.3 ± 1.0	25.5 ± 1.0

of K^+/K^{2+} (**2.3**) stabilizes the Cu(i) species, acts as a better DNA cleaving agent and displays a higher cytotoxicity than **1** with a low value of K^+/K^{2+} (**1.3**).

Anticancer activity studies

The anticancer activities of the strongly binding copper(II) complexes (**1** and **2**) towards the breast cancer cell line (MCF7), human cervical carcinoma cell line (SiHa), and normal cell line (Jurkat T4) were examined compared with the presently used drug cisplatin under identical conditions using the MTT assay (Table 4). IC₅₀ values obtained for the free ligands and copper-acetate were also obtained for the sake of comparison. The activities of **1** and **2** are higher than those of the corresponding free ligands, which demonstrates that copper coordination enhances the cytotoxicity and they exhibit a promising and time-dependent anti-proliferative activity against MCF7 and SiHa cancer cell lines. The IC₅₀ values (24 and 48 h incubation times) reveal that the cytotoxicity of **1** and **2** is higher than cisplatin. The ability of the complexes to exhibit cytotoxicity follows the order **2** > **1** and both the complexes display cytotoxicity values approximately 1.3–1.5 (**1**) and 3.6–6.9 (**2**) times more prominent than cisplatin (IC₅₀: MCF7, 22.2; SiHa, 31.3 μM). Interestingly, the cytotoxic activity of **2** shows an excellent activity for MCF7 (IC₅₀: 3.2 μM) compared with SiHa (IC₅₀: 8.8 μM). It was already established that copper(II) complexes, which exhibit a higher DNA binding affinity and prominent DNA cleavage activity, display a higher cytotoxicity and efficient anticancer properties.^{72,73} Thus, interestingly, **1** shows a reduced cell killing activity compared to **2**, which binds more strongly and cleaves DNA. Furthermore, the enhanced hydrophobicity, and hence lipophilicity, of H(mq) in **2** compared to H(mq) in **1** is expected to facilitate the transport of **2** across the cell membrane into the cell and contribute to its higher cytotoxicity. Remarkably, the IC₅₀ value of **2** is higher compared to the Jurkat T4 normal cell line at incubation times of 24 and 48 h which indicates a lower cytotoxicity compared to **1** and cisplatin. As the cell-killing activity of the copper(II) complexes is higher than that of cisplatin,⁷⁴ the present complexes are suggested to be suitable candidates for potential applications as cytotoxic drugs. The apoptosis-inducing ability of drugs seems to be a primary factor in determining their efficacy.⁷⁵ Therefore, to identify the possible involvement of apoptosis, MCF7 cells treated with copper(II)

complexes were stained with Giemsa, AO/EB, and Hoechst 33258. The following characteristic morphological changes were found in Giemsa (Fig. S9, ESI†) stained cells: (i) chromatin fragmentation, (ii) bi- and multinucleation, (iii) cytoplasmic vacuolation, (iv) nuclear swelling, (v) cytoplasmic blebbing, and (vi) late apoptosis indication of dot-like chromatin condensation. The following cytological changes were observed in the AO/EB stained nuclei: (i) viable cells that have uniformly green fluorescing nuclei with a highly organized structure; (ii) early apoptotic cells (which still have intact membranes, but have started undergoing DNA fragmentation) that have green fluorescing nuclei, but in which the perinuclear chromatin condensation is visible as bright green patches or fragments; (iii) late apoptotic cells that

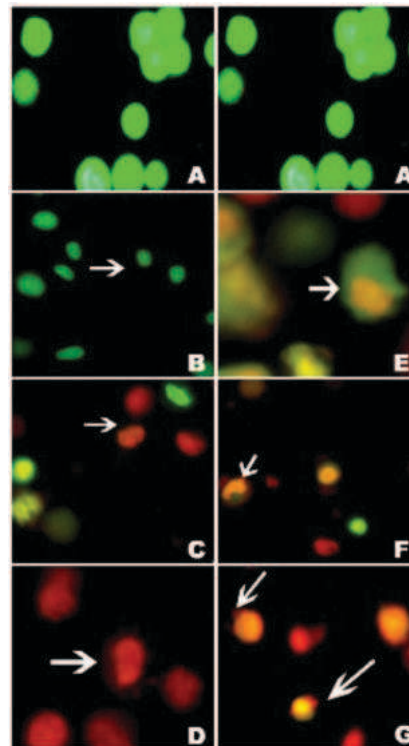


Fig. 13 AO/EB staining of MCF7 breast cancer cells untreated with **2** (A), treated with **2** at 24 (B)–(D) and 48 h (E)–(G) of incubation (arrowheads indicate chromatin fragmentation, chromatin condensation and late apoptosis indication of apoptotic bodies).

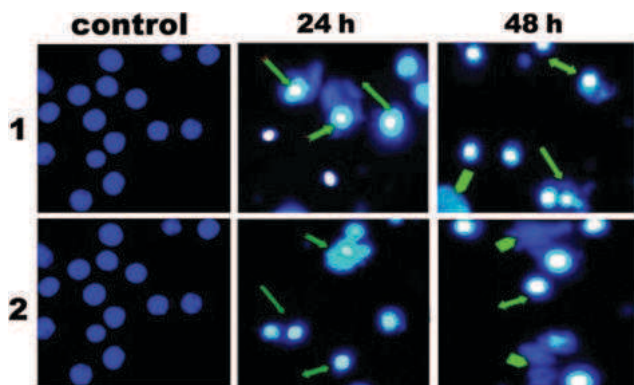


Fig. 14 Hoechst 33258 staining of MCF7 breast cancer cells untreated with **1** and **2**, treated with **1** and **2** at 24 and 48 h of incubation (arrowheads indicates chromatin marginalization (\leftarrow), cytoplasmic blebbing (\rightarrow), Binucleation (\rightarrow), apoptotic body formation (\rightarrow)).

have orange to red fluorescing nuclei with condensed or fragmented chromatin; and (iv) necrotic cells, swollen to large sizes, that have uniformly orange to red fluorescing nuclei with no indication of chromatin fragmentation. The apoptotic morphologies induced by the complexes were confirmed by AO/EB staining using fluorescence microscopy, which reveals the apoptotic cell death from the perspective of the fluorescence (Fig. S10, ESI \ddagger and Fig. 13). Inverted microscopic analysis of the cell morphology (Fig. S11, ESI \ddagger) shows the decrease in the number of cells and the induction of morphological changes, with the typical fragmentation of apoptotic cells. Furthermore, marked apoptotic morphological changes (*e.g.*, membrane blebbing and cell shrinkage) are observed, and the number of rounded-up cells with plasma membrane blebs increases as the treatment duration increases to 48 h. Moreover, to observe the characteristic changes in nuclear morphology, cells were stained with Hoechst 33258 (Fig. 14).

The control cells show homogeneous nuclear staining. After treatment with **1** and **2** the number of apoptotic cells increases and typical changes are displayed, including bright staining, condensed chromatin, fragmented nuclei, and apoptotic bodies.^{76,77}

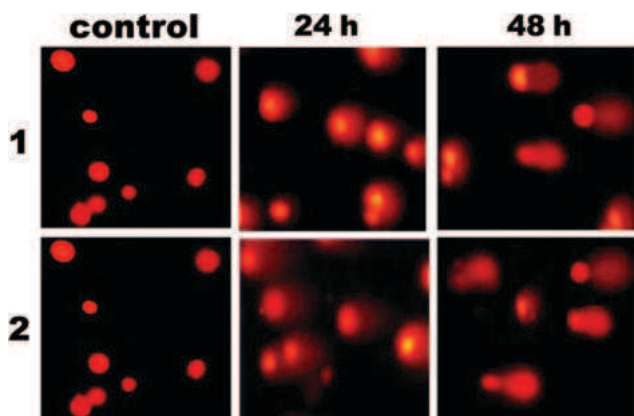


Fig. 15 Comet assay of EthBr-stained MCF7 breast cancer cells untreated with **1** and **2** (control) and treated with **1** and **2** at 24 h and 48 h of incubation.

If a cell with damaged DNA is subjected to electrophoresis and then stained with EthBr, it appears as a comet, and the length of the comet tail represents the extent of DNA damage.⁷⁸ Cells treated with **1** and **2** show statistically significant well-formed comets, whereas the control (untreated) cells do not demonstrate a comet-like appearance (Fig. 15). Also, the tail length observed in **2** is longer than that for **1**, which is consistent with the higher cytotoxicity observed for **2** (*cf.* above). This indicates that the complexes induce DNA fragmentation, which is further proof of apoptosis.

Conclusions

Two mixed ligand copper(II) complexes with five-coordinate [Cu(terpy)(mq)](ClO₄) (**1**) and four-coordinate [Cu(phen)(mq)](ClO₄) (**2**) in a distorted CuN₄O square pyramidal (**1**) and CuN₃O square planar (**2**) coordination geometry have been synthesized and **1** has been structurally characterized using X-ray crystallography. The complexes are stable in the solid and solution phases and are avid binders to CT DNA. The mode of DNA binding was found to be groove binding and this type of binding is enhanced by the strong hydrophobic interaction of the 2-methyl group on the H(mq) ligand with the DNA surface. The BSA fluorescence is statically quenched by copper(II) complexes, which implies the formation of a non-fluorescent ground state complex. It is estimated that BSA has one binding site and the positive values of ΔH° and ΔS° indicate that the binding is entropy-driven owing to the hydrophobic forces. Thus, both the complexes could bind to BSA and be effectively transported and eliminated from the body. It is worth noting that, of both the complexes, **2** alone alters the DNA superhelicity in the absence of any external reagent and causes smearing of the supercoiled ϕ X174 RF DNA in the presence of ascorbic acid. Notably, the close availability of **2** with DNA leads to a very efficient cleavage of the supercoiled plasmid DNA, reflecting the dynamic, as well as stronger, binding of **2** with the biopolymer. It is remarkable to find that this complex exhibits a higher cytotoxicity than that of **1** and the currently used drug cisplatin against breast cancer (MCF7) cell lines and human cervical carcinoma (SiHa) cell lines, and was also four times less toxic towards Jurkat T4 normal cells than cisplatin. Both the complexes bring about the condensation and breakage of chromatin into clumps typical of apoptosis cell death. Comet experiments also confirm the ability of **1** and **2** to induce apoptosis in breast cancer cells. The results are of importance as 3d-metal complexes containing bio-essential constituents such as copper have the potential for use in the design and development of complexes for cellular applications in cancer therapy.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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GREEN MARKETING: IT'S INFLUENCE ON BUYING BEHAVIOR AND ATTITUDES OF THE PURCHASERS TOWARDS ECO-FRIENDLY PRODUCTS IN TIRUCHIRAPPALLI DISTRICT

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ABSTRACT

Today's consumer, price difference in environment-friendly products has disappeared to be a negative factor now and promotion has become important for consumers. When companies take these into consideration and determine marketing strategies accordingly, they can reach their goals by considering needs and demands of the consumers and by responding them in the most appropriate way. Therefore, tendency to environmental-friendly product shall exhibit gradual increase. Marketing managers should pay also attention to demographic features in separation of consumers in the target mass to the segments. Green marketing is a phenomenon which has developed particular important in the modern market. This concept has enabled for the re-marketing and packaging of existing products which already adhere to such guidelines. In fact, consumers' concerns about the environment have encouraged the marketers to change their strategies and adopt a marketing strategy called "Green marketing". So this research focuses on what makes green marketing successful, consumers' behavior and their perceptions towards green products, the reason why marketers decided to adopt green marketing and how marketers can implement the green marketing mix. The paper also examines the present trends of green marketing in and describes the reason why companies are adopting it and future of green marketing and concludes that green marketing is something that will continuously grow in both practice and demand.

Keywords: Green Marketing, Green Consumer, Green Marketing Mix, Consumer Behavior, Green Product, Recyclable, Environmentally Safe, Eco Friendly.

INTRODUCTION

Green marketing term was first discussed in a seminar on "ecological marketing" organized by American Marketing Association (AMA) in 1975 and took its place in the literature. In this seminar where the impact of marketing on natural environment was analyzed with the contribution of academicians, bureaucrats and other participants, ecological marketing concept was defined as follows: Studies regarding adverse or positive impacts on environmental pollution, energy consumption and consumption of other resources as result of marketing (Cevreorman, 2010).

GREEN MARKETING

It is really scary to read these pieces of information as reported in the Times recently: "Air pollution damage to people, crops and wildlife in US. Total tens of billions of dollars each year". "More than 12 other studies in the US, Brazil Europe, Mexico, South Korea and Taiwan have established links between air pollutants and low birth weight premature birth still birth and infant death". As resources are limited and human wants are unlimited, it is important for the marketers to utilize the resources efficiently without waste as well as to achieve the organization's objective. So green marketing is inevitable.

There is growing interest among the consumers all over the world regarding protection of environment. Worldwide evidence indicates people are concerned about the environment and are changing their behavior. As a result of this, green marketing has emerged which speaks for growing market for sustainable and socially responsible products and services. Thus the growing awareness among the

consumers all over the world regarding protection of the environment in which they live, People do want to bequeath a clean earth to their offspring. Various studies by environmentalists indicate that people are concerned about the environment and are changing their behavior pattern so as to be less hostile towards it. Now we see that most of the consumers, both individual and industrial, are becoming more concerned about environment friendly products.

REVIEW OF LITERATURE

Sujith TS (2017) has suggested that Successful marketing has always been about recognizing trends and positioning products, services and brand in a manner that supports buyer intentions. Today, many companies have accepted their responsibility to protect our environment. So, products and production process become cleaner. More companies introduce green products and it helps to change the polluted world. “Go green”, because they realize that they can reduce pollution and increase profits at the same time. Green marketing is a creative opportunity to innovate in ways that make a difference and at the same time achieve business success. This paper investigates consumer perception and attitude of purchasing eco-friendly products.

Aman Diglel & Dr. Rashad Yazdanifard (2014) has argued that Ecological issues are still the major concern toward the whole world and people. Air contamination, deforestation and greenhouse impacts are the major natural issues that have happened till now alongside the activities of a person. In fact, consumers’ concerns about the environment have encouraged the marketers to change their strategies and adopt a marketing strategy called “Green marketing”. So this research focuses on what makes green marketing successful, consumers’ behavior and their perceptions towards green products, the reason why marketers decided to adopt green marketing and how marketers can implement the green marketing mix.

Jeevarathnam P. Govender and Tushya L. Govender (2016), A survey was conducted on a sample of 100 consumers using a quantitative, exploratory and descriptive design. The results indicate that South African citizens have high knowledge levels on the issues facing the environment. Elements of the green marketing mix, specifically, green promotion, were found to raise awareness and encourage positive change in consumption behavior. A large proportion of respondents preferred to patronize socially responsible retailers. Furthermore, respondents preferred green products over standard alternatives. However, they were price sensitive which affected their purchasing decisions.

Lavanya. K & Dr.P. MadhanKumar (2019), the result in the study have high implication for manufacturer of consumer durables, as their consumption is highest compare with other products category. Hence it is important for marketers to be in top of mind of consumers. When it comes brand of recall of green consumer durables

Shruti P Maheshwari (2014) This paper identifies that consumers are not exposed enough to green product marketing communication and suggests the greater use of marketing and brands to promote and sell products that are environmentally friendly and function effectively. The paper suggests that the Indian market for greener products could be exploited more within consumer groups that have pro environmental values.

GREEN PRODUCTS AND ITS CHARACTERISTICS

The products those are manufactured through green technology and that caused no environmental hazards are called green products. Promotion of green technology and green products is necessary for conservation of natural resources and sustainable development. We can define green products by following measures:

- Products those are originally grown
- Products those are recyclable, reusable and biodegradable
- Products with natural ingredients

- Products containing recycled contents, non-toxic chemical
- Products contents under approved chemical
- Products that do not harm or pollute the environment
- Products that will not be tested on animals Products that have eco-friendly packaging i.e. reusable, refillable containers etc.

CHALLENGES IN GREEN MARKETING

1. Need for Standardization:

It is found that only 5% of the marketing messages from “Green” campaigns are entirely true and there is a lack of standardization to authenticate these claims. There is no standardization to authenticate these claims. There is no standardization currently in place to certify a product as organic. Unless some regulatory bodies are involved in providing the certifications there will not be any verifiable means. A standard quality control board needs to be in place for such labeling and licensing.

2. New Concept:

Indian literate and urban consumer is getting more aware about the merits of Green products. But it is still a new concept for the masses. The consumer needs to be educated and made aware of the environmental threats. The new green movements need to reach the masses and that will take a lot of time and effort. By India's ayurvedic heritage, Indian consumers do appreciate the importance of using natural and herbal beauty products. Indian consumer is exposed to healthy living lifestyles such as yoga and natural food consumption. In those aspects the consumer is already aware and will be inclined to accept the green products.

3. Patience and Perseverance:

The investors and corporate need to view the environment as a major long-term investment opportunity, the marketer's need to look at the long-term benefits from this new green movement. It will require a lot of patience and no immediate results. Since it is a new concept and idea, it will have its own acceptance period.

4. Avoiding Green Myopia:

The first rule of green marketing is focusing on customer benefits i.e. the primary reason why consumers buy certain products in the first place. Do this right, and motivate consumers to switch brands or even pay a premium for the greener alternative? It is not going to help if a product is developed which is absolutely green in various aspects but does not pass the customer satisfaction criteria. This will lead to green myopia. Also if the green products are priced very high then again it will lose its market acceptability.

GREEN MARKETING SERVES TWO PURPOSES

In order to develop goods that can appeal to the consumer, reasonably affordable prices and environment-friendly products causing minimal damage are required. In order to reflect an image of high quality, environmental sensitivity and hence production of products compatible with environment are required.

Businesses and Green Marketing There are serious changes for awakening in the business world regarding the responsibility towards the environment and the society. Strategies targeting not only making a profit for the day but also for long-term profitability and environmentally friendly sustainability have started to become agendas of the companies. Corporate ethical code of the 21st century is being green. The ideology of “always me, always me” of profit-making companies has no longer any effect. Of course, the primary objective of companies is profitability but it is too hard for companies with the sole objective of making profit to obtain sustainability.

Companies should be aware of their responsibilities towards the environment and the society in the same way as towards clients, shareholders and employees. Climate change, environmental issues and social problems will challenge the leaders of future generation for taking efficient and comprehensive

decisions. In the process of taking these decisions, the priority of business people should be based on the principal of protecting the environment rather than profitability of the business. The concept of creative capitalism defined by Bill Gates underlines that the leaders of future generations are responsible not only for obtaining desired results but also for the impacts of their decisions on elements other than their own companies and markets (Businews, 2010).

GOLDEN RULES OF GREEN MARKETING

1. **Know you're Customer:** Make sure that the consumer is aware of and concerned about the issues that your product attempts to address, (Whirlpool learned the hard way that consumers wouldn't pay a premium for a CFC-free refrigerator because consumers didn't know what CFCs were.).
2. **Educating your customers:** isn't just a matter of letting people know you're doing whatever you're doing to protect the environment, but also a matter of letting them know why it matters. Otherwise, for a significant portion of your target market, it's a case of "So what?" and your green marketing campaign goes nowhere.
3. **Being Genuine & Transparent:** means that a) you are actually doing what you claim to be doing in your green marketing campaign and b) the rest of your business policies are consistent with whatever you are doing that's environmentally friendly. Both these conditions have to be met for your business to establish the kind of environmental credentials that will allow a green marketing campaign to succeed.
4. **Reassure the Buyer:** Consumers must be made to believe that the product performs the job it's supposed to do—they won't forego product quality in the name of the environment.
5. **Consider Your Pricing:** If you're charging a premium for your product—and many environmentally preferable products cost more due to economies of scale and use of higher-quality ingredients—make sure those consumers can afford the premium and feel it's worth it.
6. **Giving your customers an opportunity to participate:** means personalizing the benefits of your environmentally friendly actions, normally through letting the customer take part in positive environmental action.
7. **Thus leading brands should recognize that consumer expectations have changed:** It is not enough for a company to green its products; consumers expect the products that they purchase to be pocket friendly and also to help reduce the environmental impact in their own lives too.

BENEFIT OF GREEN MARKETING

Nowadays as technology improves so does the mind of consumers about the environment. And the fact that consumers have started to build concern about the environment. Hence, organizations could only share their consumers' concern and go by respecting their values by reducing the production of goods that are considered to be harmful towards the environment.

The Shortcut is what many companies are looking forward to have as they will ultimately step forward to become green. There are various numbers of advantages that companies face if they ever decide to go green, those advantages are:

- Employees would proudly and happily work for companies that are environmentally responsible, in other words workers would be motivated to put their potential in any tasks they do
- At first the expenses are more, but it tends to save money down the road in the long term.
- It helps companies in getting into a new market and produce goods and services while keeping the environment concerns into consideration.
- It guarantees supported long term development alongside profitability

GREENMARKETING MIX

At the point when organizations think of new innovations like green products, they can get to new markets, not to mention that organizations are usually picky, hence companies have a marketing mix that is considered to be their favorite and, this basically leads to expanding benefits and market

shares. Pretty much as we have 4ps product, price, place and promotion, we have 4ps in green marketing as well, however they are a bit distinctive. Nevertheless, the four Ps in the green marketing mix are concisely detailed in this paper and it mentioned how challenging it actually is for the market managers to use the green marketing mix in a way that is considered to be creative.

According to Darling, Heller, & Tablada, (2009), as Cited by (Awan & Raza, 2010)

1. Product: companies innovate their products according to the needs and preferences of their consumers and usually consumers tend to be concerned about the environment, so therefore companies prefer to produce products that are less harmful towards the environment. Environment friendly products tend to save money, water and other natural resources. As the products can be manufactured from reused materials or products that has been used before. The marketer's role in product management includes providing product designers with market-driven trends and customer requests for green product attributes such as energy saving, organic, green chemicals, local sourcing, etc., For example, Nike is the first among the shoe companies to market itself as green. It is marketing its Air Jordan shoes as environment-friendly, as it has significantly reduced the usage of harmful glue adhesives. It has designed this variety of shoes to emphasize that it has reduced wastage and used environment-friendly materials (Dua, 2013).

2. Price: All among all the green marketing mix "price" is a standout, it is considered to be the factor in the green marketing mix that is most imperative and critical compared to the rest of the other factors in the green marketing mix. Not to mention that consumers are very sensitive to price changes and it easily affect consumers purchasing decision (Morel & Kwakye, 2012). Most purchasers might be willing to pay extra value if there is a self-impression of additional item value. Green marketing ought to look into visual appeal, taste, design, and performance while charging a premium price from the clients (Sharma, 2011). According to Dua, (2013) Green marketing contemplates the profit, individuals and planet in a manner that deals with the strength of workers and groups and guarantees proficient productivity.

3. Place: This is related to distribution gates use that deal with green products, which are proper for customers, as far as encouraging their conveyance, and to secure cycling methods leading inside natural conditions and prerequisites (N. Hashem, 2011). According to K. Sudhalakshmi & Chinnadorai, (2014) green distribution consists of two different aspects which are defined as "inner and outer", by inner viewpoint, we mean the internal environment of the company that must be a spot in which supervisors and representatives have a feeling of peacefulness other than watching the natural issues in inward techniques of the corporation and the proportionality between the inside space and the planned item (Hashem & Rifai, 2011). Because of the agreeable and acceptable behavior of the employees, consumers seem to be gravitated by it. And on the other side outer aspect is defined as the place where environmentally-friendly goods and services are placed for sale.

4. GreenPromotion: There are numerous concerns among the buyers about environmental advertisement. According to N. Hashem, (2011) This refers to giving true data about the items in a manner that does not harm the materialistic and good buyers' investments. Companies should give it a critical thought before they advertise their products, it's very important for companies to list the functions, design or uses of their products before they advertise them, this will help to avoid any misleading information about the products. According to Dua, (2012) designing the tools of promotion is what the green promotion includes. For instance, web sites, signage, material for marketing and white papers by keeping planet and individuals in mind as their main aim is to make profit. After carrying out empirical research Morel & Kwakye, (2012) concluded their results by stating that "there is a positive relationship between advertisement (publicizing) and state of mind towards green items (Arora, 2014).

GREEN CONSUMER

The green consumer is generally defined as one who adopts environmentally-friendly behaviors and/or who purchases green products over the standard alternatives. Green consumers are more

internally-controlled as they believe that an individual consumer can be effective in environmental protection. Thus, they feel that the job of environmental protection should not be left to the government, business, environmentalists and scientists only; they as consumers can also play a part. They are also less dogmatic and more open-minded or tolerant toward new products and ideas. Their open-mindedness helps them to accept green products and behaviors, more readily (Shamdasani at al. 1993:491).

OBJECTIVE OF THE RESEARCH

- To investigate the impact of Green Marketing on Consumer Purchasing Patterns and Decision Making in Tiruchirapalli District.
- To investigate the direction and strength of the relationship between the six distinct environmental belief factors and environmental behaviour, while controlling for key socio-demographic factors.
- To give recommendations and suggestions to increase the uptake of green products in Tiruchirapalli District.

PROPOSED HYPOTHESIS

- There is a positive relationship between green branding and packaging and the environmental behaviour of consumers.
- There is a positive relationship between green pricing and the environmental behaviour of consumers.
- There is a positive relationship between embedding and eco-image and the environmental behaviour of consumers.
- There is a positive relationship between environmental concerns and beliefs and the environmental behaviour of consumers.

DATA ANALYSIS

- Data analysis of the filled questionnaire has been done using SPSS. Primarily a Correlation was done on the questionnaire to identify the important factors affecting Green Marketing among Tiruchirapalli District Consumers.

Table: 01 Relationship between gender of the respondents and their green branding and packaging and the environmental behaviour of consumers

Research Hypothesis: There is no significant relationship between gender of the respondents and their green branding and packaging and the environmental behaviour of consumers.

Null Hypothesis: There is a significant relationship between gender of the respondents and their green branding and packaging and the environmental behaviour of consumers.

Correlations

		Gender	Green Branding And Packaging And The Environmental Behaviour Of Consumers
Gender	Pearson Correlation	1	.770**
	Sig. (2-tailed)		.000
	N	100	100
Environmental Behaviour Of Consumers	Pearson Correlation	.770**	1
	Sig. (2-tailed)	.000	
	N	100	100

** . Correlation is significant at the 0.01 level (2-tailed).

Interpretation

From the above table we infer that the correlation coefficient is $.000 > 0.05$. So relationship between gender of the respondents and their green branding and packaging and the environmental behaviour of consumers are positively correlated.

Table: 02 Relationship between Gender of the Respondents and Their Overall Satisfaction with Green Pricing and the Environmental Behaviour of Consumers

Research Hypothesis: There is no significant relationship between gender of the respondents and their overall satisfaction with green pricing and the environmental behaviour of consumers.

Null Hypothesis: There is a significant relationship between gender of the respondents and their overall satisfaction with green pricing and the environmental behaviour of consumers.

Correlations

	Gender	Green Pricing And The Environmental Behaviour Of Consumers
Gender		
Pearson Correlation	1	.847**
Sig. (2-tailed)		.000
N	100	100
Green Pricing And The Environmental Behaviour Of Consumers		
Pearson Correlation	.847**	1
Sig. (2-tailed)	.000	
N	100	100

** . Correlation is significant at the 0.01 level (2-tailed).

Interpretation

From the above table we infer that the correlation coefficient is $.000 > 0.05$. So relationship between gender of the respondents and their overall satisfaction with green pricing and the environmental behaviour of consumers are positively correlated.

Table: 03 Relationship between gender of the respondents and their overall satisfaction with the embedding and eco-image and the environmental behaviour of consumers.

Research Hypothesis: There is no significant relationship between gender of the respondents and their overall satisfaction with embedding and eco-image and the environmental behaviour of consumers.

Null Hypothesis: There is a significant relationship between gender of the respondents and their overall satisfaction with embedding and eco-image and the environmental behaviour of consumers.

Correlations

		Gender	Embedding And Eco-Image And The Environmental Behaviour Of Consumers
Gender	Pearson Correlation	1	.828**
	Sig. (2-tailed)		.000
	N	100	100
Embedding and eco-image and the environmental behaviour of consumers	Pearson Correlation	.828**	1
	Sig. (2-tailed)	.000	
	N	100	100

** . Correlation is significant at the 0.01 level (2-tailed).

Interpretation

From the above table we infer that the correlation coefficient is $.000 > 0.05$. So gender of the respondents and their overall satisfaction with embedding and eco-image and the environmental behaviour of consumers are positively correlated.

Finding and Suggestions

- Marketers get access to new markets and gain an advantage over competitors that are not advocating “greenness.”
- Marketers can charge a premium on products that are seen as more eco-responsible.
- Organizations that adopt green marketing are perceived to be more socially responsible.
- Green marketing builds brand equity and wins brand loyalty among customers.
- Most customers choose to satisfy their personal needs before caring for the environment.
- Overemphasizing greenness rather than customer needs can prove devastating for a product.
- Many customers keep away from products labeled “green” because they see such labeling as a marketing gimmick, and they may lose trust in an organization that suddenly claims to be green.
- Green marketers need to find out the value their customers place on green benefits. It is important that they position the product on the basis of the functional need it caters to and then talk about the additional benefits of greenness.

Conclusion

Nowadays, with a rapid advancement and improvement of technology, consumers are having great access to various information no matter in what geographical location they could be located and that has led to a great expansion in human needs and wants for instance, the demand for cosmetic products has intensively increased due to development in technology like "social networks". The fact that we live in a scars planet with limited resources that cannot be used to satisfy all the needs and wants of individuals on this earth, we have to utilize it effectively while paying concern for the environment. As a result of our purchasing decision, expansion in our needs and wants, and business strategy, the world is facing pollution and destruction as its resource is negatively used by the human species. Nowadays people are more concerned about the environment and they are worried whether the natural resource could be sustained for the upcoming generation. Their concern has helped in protecting the environment as they have initiated to become green consumers where they can only buy products

that are considered to be eco-friendly and the fact that they are considered to be expensive consumers are willing to spend on it. As consumers are becoming more and more concern about the environment, organizations have begun to change how they produce their products and what are more they have begun to adopt new marketing strategy "green marketing".

Now this is the right time to select "Green Marketing" globally. It will come with drastic change in the world of business if all nations will make strict roles because green marketing is essential to save world from pollution. From the business point of view because a clever marketer is one who not only convinces the consumer, but also involves the consumer in marketing his product. Green marketing should not be considered as just one more approach to marketing, but has to be pursued with much greater vigor, as it has an environmental and social dimension to it. With the threat of global warming looming large, it is extremely important that green marketing becomes the norm rather than an exception or just a fad. Recycling of paper, metals, plastics, etc., in a safe and environmentally harmless manner should become much more systematized and universal.

It has to become the general norm to use energy-efficient lamps and other electrical goods. Marketers also have the responsibility to make the consumers understand the need for and benefits of green products as compared to non-green ones. In green marketing, consumers are willing to pay more to maintain a cleaner and greener environment. Finally, consumers, industrial buyers and suppliers need to pressurize effects on minimize the negative effects on the environment-friendly. Green marketing assumes even more importance and relevance in developing countries like India.

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GREEN MARKETING PREDICTED BY MEDIATOR CORPORATE SOCIAL RESPONSIBILITY TOWARDS CONSUMER PURCHASE INTENTIONS OUTCOME

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Abstract

This study examines the mediating roles of corporate social responsibility on the links between green marketing awareness and consumer purchase intentions. Data was analyzed using the partial least squares (PLS) approach for the analysis of structural equation models. Results revealed that corporate social responsibility partially mediated the link between green marketing awareness and purchase intentions of the product. Consumers develop positive green marketing awareness based on the growing environmental knowledge. They were aware of the green marketing program of the retail store when they noticed that the store allocated specified space to sell eco-friendly products. Furthermore, the companies make their green marketing activities known to the public by distributing eco-friendly fliers which helps to increase sales revenue, raise consumer awareness, and develop greater intention to purchase the products. The outcomes of the mediating effects of this study add a new momentum to the growing literature and preceding discoveries on consumer green marketing awareness, which is inadequately researched in the Tiruchirappalli setting.

Key Words: Green Marketing, Corporate Social Responsibility, Consumer, Partial least squares

Introduction

Green marketing is defined as “the effort by a company to design, promote, price and distribute products in a manner which promotes environmental protection” (Polonsky, 2011, p. 1311). Green marketing is part of the key movements in modern business sustainability though their primary concern has always been revenues and profits (Akenji, 2014; Maniatis, 2015; Yang et al., 2015). Companies focusing on the natural ecological balance in their entire operation are more environmentally friendly while maximizing profits; they reduce environmental pollution, conserve natural resources and protect the environment. They gain a unique competitive advantage and develop new markets as they improve their corporate image their reputation and their product image from the consumer perspective (Chen, 2008). Consumer green marketing awareness is materialized when customers have confidence in eco-label and eco-brand which influences their green product purchase behaviour (Norazah, 2013a, 2013b; Rahbar & Abdul Wahid, 2011).

Numerous studies have been conducted to establish the influence of consumer attitude towards green products and its impact on customer satisfaction in the Western countries but few studies of consumer green marketing awareness and purchase intention have been done in Asian countries, including Tiruchirappalli (Haytko & Matulich, 2008; Menichelli et al., 2014). Hence, this research aims to examine the mediating roles of corporate social responsibility on the links from green marketing awareness to consumer purchase intention. Research output on the existence of mediating effects would add a new impetus to the emergent literature and preceding studies on consumer green marketing awareness, which has been so far inadequately researched in the Tiruchirappalli setting.

Following this introduction, the ensuing section reviews the literature on green marketing awareness, purchase intention, and corporate social responsibility with the key hypotheses. Next, a clarification of the research method is presented. The subsequent section presents the data analysis, path analysis and hypotheses testing based on the development of structural equation models via the partial least square (PLS) approach. The final section is the discussion and conclusion of the research findings.

Literature Review

Green marketing awareness is related to companies' understanding of their accountability for the quality of the environment while meeting customer needs, demands and satisfaction (Chan et al., 2012; Soonthonsmai, 2007). Scholars like Boztepe (2012), Haws et al. (2010), Kai et al. (2013), Maniatis (2015), Tseng and Hung (2013), Thøgersen et al. (2012), Yang et al. (2015) have investigated about customers' attitudes and behaviours concerning green consumerism. The key influencers of consumers' selection of a green product are green features of the product, and environmental awareness related to the particular product (Boztepe, 2012; Thøgersen et al., 2012). Consumers trust green labeling as an indicator of green features in the product that they otherwise might have known through some research and study (Thøgersen et al., 2012).

Prior research like Wheeler et al. (2013) noted that rejection of 'green' brands is due to a lack of awareness where a 'green' message is not sufficient to influence the shoppers' consideration set. Moreover, Haws et al. (2010) identified five factors contributing to environmental consciousness while buying products - consciousness about impact of products on environment, considering impacts of personal actions on environment, linking purchase habits with environmental protection, concerned about waste, commitment to environmental protection, and willing to be inconvenienced for taking environment friendly actions.

Green purchase intention is related to an individual's inclination to buy and use products with eco-friendly features when purchase considerations are based on the product features and source country of the product (Nik Abdul Rashid, 2009). Indeed, green product quality such as clear product ingredients information, eco-labeling, product appearance, and general assurance of user friendliness affected consumer green purchase decision making (Maniatis, 2015; Tseng & Hung, 2013). Producers position the environmental benefits of green products in consumers' minds to evoke their purchasing decision (Rex & Baumann, 2007, p. 567). Consumers tend to mix their green knowledge and attitudes with green brand awareness while choosing a green product (Matthes et al., 2013; Zhao et al., 2014).

Corporate social responsibility (CSR) is in a "pre-paradigmatic phase where there is scant agreement on definitions and terms and no consensus has been reached about what it includes and does not include in its boundaries" (Googins et al., 2007, p. 29). Companies which participate in ecologically sound activities provides social value to customers and stakeholders and project an image that they are responsive to the environment while operating business transactions (Lingreen et al., 2009; Susniene & Sargunas, 2009). CSR positively impacts product image, quality attributes, corporate image, and consumer purchase intention (Ko et al., 2008; Lee et al., 2010).

In accordance to the above reasoning, the following hypotheses are posited.

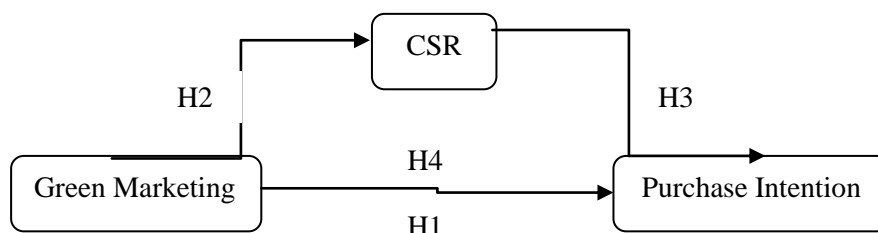
H1: Green marketing awareness has positive impact on consumer purchase intention.

H2: Green marketing awareness has positive impact on corporate social responsibility.

H3: Corporate social responsibility has positive impact on consumer purchase intention.

H4: Corporate social responsibility mediates the links between green marketing awareness and consumer purchase intention.

Table 1



Methodology

Respondents were engaged from the public who practiced a green lifestyle with green products purchasing experience such as buying organic products within Tiruchirappalli. Convenience sampling method was adopted for this purpose. The questionnaires were distributed to 300 respondents in October 2020 over a period of two weeks. There is 83% response rate with 200

questionnaires were found to be usable and valid for the data analysis. The questionnaires were designed into three sections. Section A presented socio-demographic questions. Section B of the questionnaire raised questions on the consumers' experience of green marketing. Section C confined questions on consumers' perception of green marketing. Five items emphasized green marketing awareness which was adapted from Kim (2002); four items of corporate social responsibility, and three items of purchase intention were jointly borrowed from Ko et al. (2008) and Winter (1986). These items were measured on a five-point Likert scale, stretching from 1 (strongly disagree) to 5 (strongly agree). Data was analyzed using the partial least squares (PLS) method, which is a variance-based technique for the analysis of structural equation models via SmartPLS computer program version 2.0.

Data analysis

Detailed socio-demographic characteristics of the respondents are offered. A total of 200 respondents were included in the sample. Respondents comprised 53 percent females and 47 percent males. Three-quarters of the respondents were 21 years old and above. The respondents' highest level of education completed varied, with 7 percent having a high school certificate, 33 percent a college degree, 10 percent a diploma, 32 percent a university graduate degree, and 18 a university postgraduate degree. The responses revealed a high level of polarization regarding occupation distribution (i.e., the majority of responses were received from students, followed by professionals). Almost 36 percent of the respondents were earning more than Tiruchirappalli Rs. 12,000 per month. The highest frequency of retail store visits per month was 3 times (29 percent), while only 18 percent visited more than 5 times. Almost 85 percent of the respondents consumed green items less than 10 times per month, 10 percent 11-15 times a month and 5 percent 16 times and above. Only 6 percent spent Rs.8,000 and above.

Partial least squares

PLS was performed through two stages of data analysis, the measurement and structural models. Assessments of the internal consistency, convergent validity, and discriminant validity of the construct measures were examined at the measurement model stage. Results are presented in Table 2.

Factors	Items	Standardized Loadings	Cronbach's alpha	Composite Reliability (CR)	Average Variance Extracted (AVE)
Green Marketing Awareness	GMA1	0.819	0.873	0.899	0.654
	GMA2	0.676			
	GMA3	0.873			
	GMA4	0.716			
	GMA5	0.843			
Corporate Social Responsibility	CSR1	0.785	0.788	0.882	0.670
	CSR2	0.759			
	CSR3	0.807			
	CSR4	0.873			
Purchase Intentions	PI1	0.872	0.782	0.863	0.718
	PI2	0.893			
	PI3	0.848			

Reliability and validity analysis

Reliability of the measurement items was inspected using Cronbach's alpha and composite reliability whereby results for all constructs exceeded the threshold value of 0.70 (see Table 2), indicating strong reliability among the measures. Besides, the convergent validity is achieved when

the AVE values are above 0.50 set by Fornell and Larcker (1981). All shared variances between factors were below the square root of the individual factors AVE, endorsing adequate discriminant validity.

Structural model

Based on the path coefficients of the PLS approach, with all direct relationships were supported, it point towards a comprehensive model specification. The explanatory power (R²) of the predictor construct (i.e. purchase intention) is 33 percent (see Table 3). Consumer awareness of green marketing had a significant and positive relationship with purchase intention ($\beta_1=0.203$) and corporate social responsibility ($\beta_2=0.573$). Thus, H1 and H2 were supported. Likewise, corporate social responsibility had a significant and positive association on purchase intention ($\beta_3=0.153$), meaning that H3 was also retained. Next, corporate social responsibility partially mediates the relationship between consumer awareness of green marketing and retail purchase intentions of the product, implying that H4 was sustained, as estimated. The variance accounted for (VAF) value was calculated in order to estimate the ratio of the indirect effect to the total effect. In this research model, the VAF value indicates that 50.5% of the total effect (i.e. consumer awareness of green marketing on retail purchase intentions of the product) is explained by the indirect effect (i.e. corporate social responsibility).

Table 3 Statistical results of the structural model

Hypothesized Paths	β	t-value	R ²	Results	
Direct Effects					
Green Marketing Awareness → Purchase Intention	0.203	2.991*	0.328	H1	Supported
Green Marketing Awareness → Corporate Social Responsibility	0.573	8.229*		H2	Supported
Corporate Social Responsibility → Purchase Intention	0.153	2.414*		H3	Supported
Mediation Effects					
Green Marketing Awareness → Corporate Social Responsibility → Purchase Intention		2.328*		H4	Partially Supported
Note: * Statistically significant at $p < 0.05$ (for t-value > 1.960)					

Discussion and conclusion

PLS results discovered that corporate social responsibility partially mediated the effect of green marketing awareness on consumer purchase intentions of the retail product. Research by Dinnie et al. (2006), Hartmann and Ibañez (2006), Norazah (2013a, 2013b) have highlighted that consumers develop positive green marketing awareness based on the growing environmental knowledge. They were aware of the green marketing program of the retail store when they noticed that the store allocated specified space to sell eco-friendly products. Marketing managers should optimize the budget allocation for resources in corporate social responsibility activities, consumerism, and community relations programs and engage in voluntary programs for positive return on investment through increased business profitability and long run business sustainability (Shim, 2009). The company could receive greater prominence and experience high media publicity from consumer viewpoints with high attention given for being socially responsive. They need to show that their business operations are in line with the rules and regulations of government environmental standards and other related bodies (Smith & Perks, 2012). Opportunities exist to further advance this research by examining the effect of moderating variables, like demographics and culture.

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CORPORATE SOCIAL RESPONSIBILITY OF BHEL IN TIRUCHIRAPPALLI DISTRICT

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Abstract: Corporate social responsibility is one of the responsibility concept in all over the developed business enterprises. Now-a-days the government of India regulate the business aspect to contribute some of the CSR activities in all over the country. It is divided into the Navaratna status companies and Navaratna status companies. In this type of business forums to full fill the CSR activity throughout underdeveloped villages. In Bharat Heavy Electrical Limited (BHEL) is one of the famous business venture in Tiruchirappalli district, it is contributed some of the csr activities between these district people. In particularly in this company to provide village well wisher activities and running schools, hospitals, and other service sectors to very successfully running under the CSR activities in Tiruchirappalli district. In this paper will explain the corporate social responsibility of BHEL in Tiruchirappalli district.

Keywords: CSR, Navaratna status, Maharatna status, Karmayog Rating, Social Responsibility, CSR Policy Statement

Introduction

Corporate social responsibility is a reality of recent decades. It is the commitment to improve community wellbeing through discretionary business practices and contributions of corporate resources. It is a form of corporate self-regulation integrated into a business model. It is also called corporate citizenship, corporate philanthropy, corporate giving, corporate community involvement, community relations, community affairs, community development, corporate responsibility, global citizenship and corporate social marketing. It is the continuing commitment business to behave ethically and contribute to economic development while improving the quality of life of the workforce and their families as well as of the local community and society. It represents one of the pillars of an organization's business excellence and is a concept whereby companies decide voluntarily to contribute to a better society and a cleaner environment.

Research relating to the corporate social responsibility is one of the emerging aspects, which help to understand the commitment of corporate sector towards the society. Public sector undertakings are more responsible to render services to the society because they are established by the public money. BHEL is one of the leading public sector undertakings, which commenced its plant in Tiruchirappalli in 1964. BHEL in Tiruchirappalli is one of the

landmarks of Tiruchirappalli, which provides huge employment, assists ancillary industries and is contributing to the progress of the society through corporate social responsibility initiatives. Hence, this research would be very useful and relevant to measure the performance of corporate social responsibility activities of BHEL and its effectiveness.

Objective

1. To derive an overview of corporate social responsibility in general.
2. To examine the trend and progress of corporate social responsibility by central public sector enterprises with respect to Maharatna and Navaratna companies.
3. To measure the performance of corporate social responsibility by BHEL.

Review of Literature

Corporate social responsibility is an important business strategy because, wherever possible consumers want to buy products from companies they trust; Suppliers want to form business partnerships with companies they can rely on; Employees want to work for companies they respect and Non Government Organizations, increasingly want to work together with companies seeking feasible solutions and innovations in areas of common concern. Corporations should consider three dimensions of corporate social responsibility for running successfully in their competitive world.¹ This part deals with the general studies related to the corporate social responsibility and its implementation.

S. No.	Author & Year	Focused Area	Results Found
1.	Alexander Dahlsrud. (2008)	Developed five dimensions of the corporate social responsibility through a content analysis	Corporate social responsibility is socially constructed in a specific context
2.	Anupam Sharma and Ravi Kiran. (2013)	Changing developments of corporate social responsibility practices	Implicit and explicit corporate social responsibility factors should be considered for a holistic approach
3.	Anupama Mohan. (2001)	Current practice of both indigenous and foreign businesses in India in order to examine the trends of corporate citizenship	The traditional philanthropy or merchant charity of business in India has changed over time to become social responsibility of business and corporate citizenship

¹ Mohammed Belal Uddin, Md. Riad Hssan and Kazi Md. Tarique. (2008). Three Dimensional Aspects of Corporate Social Responsibility. Daffodil International University Journal of Business and Economics, Vol. 3, No. 1, pp 199-212.

4.	Archie B. Carroll. (1999)	Evolution of the corporate social responsibility construct beginning in the 1950s	Corporate social responsibility concept will remain as an essential part of business language and practice
5.	Ariane Berthoin Antal and Andre Sobezak. (2007)	How the cultural, socio-economic, and legal traditions influence the corporate social responsibility	Because of global influences such as the international academic discourse, the international practices of multinational companies, non government organizations and trade unions and initiatives of supranational organizations
6.	Arno Kourula and Minna Halme. (2008)	Different corporate responsibility actions into three types of philanthropy, corporate responsibility integration and corporate responsibility innovation	Develop and improve indicators for business and societal outcomes
7.	Arun Maira. (2013)	Mandatory expenditure of two percent of profit to corporate social responsibility	Several formats for reporting the impact of businesses have been developed
8.	Chetan D. Lakhani. (2011)	Leading corporations across the world promoting their brands of corporate image	Education, health and the environment are the top priorities and they will continue
9.	Chong Wei Nurn and Gilbert Tan. (2010)	How corporate social responsibility leads to the tangible benefits	Attracting better employees, reduced turnover rate, greater efficiency and reduced operating
10.	David A Waldman, Mary Sully de Luque, Nathan Washburn, and Robert J House. (2006)	Cultural and leadership variables associated with corporate social responsibility	Cultural dimension of institutional collectivism and power distance predict social responsibility values on the part of top management team members

Methodology

The present research study is quantitative in nature by using secondary data, which are collected mainly from the published reports of the concerned authorities. The relevance and originality of the data lie with the responsible agencies. Secondary data relating to BHEL, Tiruchirappalli unit has been collected with the help of Right to Information Act 2005.

Maharatna Status

In 2009 the government established the Maharatna status, which raises a company's investment ceiling from Rs. 1,000 crores to Rs. 5,000 crores. The Maharatna firms would now be free to decide on investments up to 15 percent of their net worth in a project. In order

to qualify as a Maharatna, a company must have, three years with an annual net profit of over Rs. 5000 crores, Net worth of Rs. 15,000 crores and turnover of Rs. 25,000 crores.

Navratna Status

The Navratna status is offered to central public sector enterprises, which gives a company enhanced financial and operational autonomy and empowers it to invest up to Rs. 1000 crores or 15 percent of their net worth on a single project without seeking government approval. In a year, these companies can spend up to 30 percent of their net worth not exceeding Rs. 1000 crores.

Karmayog Rating

Karmayog is an organization based out of Mumbai, India. It has evolved as an internet platform for non-profits and supporters to inform others about themselves and their needs and offers. The Karmayog corporate social responsibility ratings of Indian companies were undertaken to explore and understand the role that corporate are playing and can play in finding meaningful solutions to the problems facing India today.

Social Responsibility

Social responsibility is an ethical framework which suggests that an entity, be it an organization or individual, has an obligation to act for the benefit of society. It pertains not only to business organizations but also to everyone whose action impacts the environment.

Table No. 1
The World's Best Companies for CSR 2013

S.No.	Company Name	CSR Rep Trak Score	Category
1.	Microsoft	72.97	I
2.	The Walt Disney Company	72.83	
3.	Google	72.71	
4.	BMW	72.14	
5.	Daimler (Mercedes-Benz)	70.65	
6.	Sony	69.49	II
7.	Intel	69.32	
8.	Volkswagen	69.29	
9.	Apple	69.21	
10.	Nestle	69.00	
11.	Lego Group	68.77	III
12.	Rolex	68.45	
13.	Canon	68.02	
14.	Kellogg Company	67.90	IV
15.	Johnson & Johnson	67.80	
16.	Colgate-Palmolive	67.62	
17.	DANONE	67.25	

18.	IBM	67.09	V
19.	Philips Electronics	67.03	
20.	Honda Motor	67.03	
21.	Toyota	66.96	
22.	Adidas Group	66.90	
23.	Michelin	66.75	
24.	L'Oreal	66.66	
25.	Hewlett-Packard	66.51	
26.	Samsung Electronics	66.50	
27.	The Coca-Cola Company	66.43	
28.	Amazon.com	66.26	
29.	Procter & Gamble	66.16	
30.	Ferrero	66.15	

Table No. 2
Maharatna companies and their corporate social responsibility spent during the years
2009-10 to 2013-14

(Rs. in Crores)

S.No	Maharatna Companies	Year	Gross Turn over	Profit After Tax	CSR Spending	Percentage to PAT
1.	Coal India Limited	2009-10	25794	9622	43.81	0.46
2.		2010-11	33305	10867	262.28	2.41
3.		2011-12	40441	14788	553.33	3.74
4.		2013-14	48461	17356	595.74	3.43
5.		2014-15	42392	15112	409.37	2.71
6.	Indian Oil Corporation Limited	2009-10	271095	10221	37.69	0.37
7.		2010-11	328652	7445	131.11	1.76
8.		2011-12	373926	3955	95.60	2.42
9.		2012-13	414909	5005	78.97	1.58
10.		2013-14	457553	7019	81.91	1.17
11.	National Thermal Power Corporation Limited	2009-10	46450	8728	16.74	0.19
12.		2010-11	55063	9103	72.37	0.79
13.		2011-12	62052	9224	45.52	0.49
14.		2012-13	65674	12619	69.24	0.55
15.		2013-14	72019	10975	109.77	1.00
16.	Oil and Natural Gas Corporation Limited	2009-10	61983	16768	322.52	1.92
17.		2010-11	68649	18924	335.35	1.77
18.		2011-12	76887	25123	378.48	1.51
19.		2012-13	83309	20926	261.57	1.25
20.		2013-14	84203	22095	341.25	1.54
21.	Steel Authority of India Limited	2009-10	43935	6754	80.00	1.18
22.		2010-11	47041	4905	94.00	1.92
23.		2011-12	50348	3543	64.00	1.81
24.		2012-13	49350	2170	42.00	1.94
25.		2013-14	51866	2616	40.00	1.53
26.	Gail India	2009-10	24996	3140	55.91	1.78

27.	Limited	2010-11	32459	3561	59.90	1.68
28.		2011-12	40281	3654	82.77	2.27
29.		2012-13	47333	4022	92.00	2.29
30.		2013-14	57245	4375	91.00	2.08
31.	Bharat Heavy Electricals Limited	2009-10	34154	4311	4.01	0.09
32.		2010-11	43337	6011	21.55	0.36
33.		2011-12	49510	7040	36.47	0.52
34.		2012-13	50156	6615	37.96	0.57
35.		2013-14	40338	3461	46.54	1.35

Source: annual report 2009-10 to 2013-2014

Table No. 3

Navratna Companies and their corporate social responsibility spent during the years 2009-10 to 2013-14

(Rs. in Crores)

S.No	Navratna Companies	Year	Gross Turn over	Profit After Tax	CSR Spending	Percentage to PAT
1.	Bharat Electronics Limited	2009-10	5219	721	2.59	0.36
2.		2010-11	5529	862	2.08	0.24
3.		2011-12	5704	829	2.36	0.29
4.		2012-13	6012	889	4.21	0.47
5.		2013-14	6174	932	10.55	1.13
6.	Bharat Petroleum Corporation Limited	2009-10	127884	1538	14.72	0.96
7.		2010-11	154886	1547	22.00	1.42
8.		2011-12	203866	1311	7.73	0.59
9.		2012-13	229796	2643	17.88	0.68
10.		2013-14	253492	4061	34.38	0.85
11.	Container Corporation of India Limited	2009-10	3886	787	-	-
12.		2010-11	4032	876	2.93	0.34
13.		2011-12	4377	878	2.18	0.25
14.		2012-13	4743	940	4.38	0.47
15.		2013-14	5356	985	10.38	1.05
16.	Engineers India Limited	2009-10	1994	435	6.88	1.58
17.		2010-11	2823	522	8.70	1.67
18.		2011-12	3699	636	15.36	2.42
19.		2012-13	2506	628	5.97	0.95
20.		2013-14	1824	479	6.48	1.35
21.	Hindustan Aeronautical Limited	2009-10	13489	1967	No specific allocation for CSR	
22.		2010-11	16451	2114	1.79	0.09
23.		2011-12	12693	2539	5.81	0.23
24.		2012-13	14202	2997	11.01	0.37
25.		2013-14	15867	2692	9.19	0.34
26.	Hindustan Petroleum Corporation Limited	2009-10	114889	1301	15.00	1.15
27.		2010-11	142396	1539	15.00	0.98
28.		2011-12	188131	911	30.78	3.38
29.		2012-13	215666	905	21.76	2.40
30.		2013-14	232188	1734	23.74	1.37
31.	Mahanagar Telephone Nigam Limited	2009-10	36561	2611	Since MTNL is in losses, no specific allotment is made under CSR Head	
32.		2010-11	36739	2802		
33.		2011-12	33732	4109		
34.		2012-13	34287	5321		
35.		2013-14	33917	7825		
36.	National	2009-10	5311	814	12.72	1.56

37.	Aluminum Company Limited	2010-11	6370	1069	26.77	2.50
38.		2011-12	6500	850	34.22	4.30
39.		2012-13	7247	593	30.09	5.23
40.		2013-14	7024	642	29.00	4.52
41.	National Building Construction Corporation Limited	2009-10	2982	116	1.65	1.42
42.		2010-11	3127	140	1.72	1.23
43.		2011-12	3429	190	2.21	1.16
44.		2012-13	3187	207	1.83	0.88
45.		2013-14	4009	247	5.75	2.33
46.	National Mineral Development Corporation Limited	2009-10	6239	3447	80.00	2.32
47.		2010-11	11369	6499	81.56	1.26
48.		2011-12	11262	7265	80.13	1.10
49.		2012-13	10704	6342	72.38	1.14
50.		2013-14	12058	6420	152.85	2.38
51.	Neyveli Lignite Corporation Limited	2009-10	4121	1247	5.90	0.47
52.		2010-11	4296	1298	12.47	0.46
53.		2011-12	4867	1411	13.00	0.92
54.		2012-13	5590	1460	14.26	0.98
55.		2013-14	5967	1502	26.30	1.75
56.	Oil Limited India	2009-10	8860	2611	20.00	0.77
57.		2010-11	9194	2888	25.00	0.87
58.		2011-12	11309	3447	50.00	1.45
59.		2012-13	9948	3589	49.63	1.38
60.		2013-14	9613	2981	43.90	1.47
61.	Power Finance Corporation Limited	2009-10	8077	2357	-	-
62.		2010-11	10161	2620	11.89	0.45
63.		2011-12	13037	3032	13.24	0.44
64.		2012-13	17272	4420	16.30	0.37
65.		2013-14	21537	5418	63.23	1.17
66.	Power Grid Corporation of India Limited	2009-10	7504	2041	12.67	0.62
67.		2010-11	9099	2697	20.41	0.76
68.		2011-12	10785	3255	24.93	0.77
69.		2012-13	13329	4235	21.84	0.52
70.		2013-14	15721	4497	21.66	0.48
71.	Rashtriya Ispat Nigam Limited	2009-10	10765	797	12.75	1.60
72.		2010-11	11616	658	15.40	2.34
73.		2011-12	14570	751	12.00	1.60
74.		2012-13	13565	353	15.99	4.53
75.		2013-14	13431	366	20.31	5.55
76.	Rural Electrification Corporation Limited	2009-10	6708	2001	3.18	0.16
77.		2010-11	8495	2569	5.00	0.20
78.		2011-12	10509	2817	12.85	0.46
79.		2012-13	13599	3818	14.19	0.37
80.		2013-14	17121	4684	38.18	0.82
81.	The Shipping Corporation of India Limited	2009-10	3896	377	9.41	2.50
82.		2010-11	4020	567	3.77	0.67
83.		2011-12	4500	-428	5.84	1.36
84.		2012-13	4496	-114	3.12	2.74
85.		2013-14	4539	-275	1.24	0.45

Source: Annual Report 2009-10 to 2013-14

In the year 2012-13, gross turnover amounted to Rs. 4539 crores of which profit after tax was Rs. -275 crores. Rs. 1.24 crores was spent for corporate social responsibility

activities, which shows 0.45 percent of profit after tax.

Providing financial and other assistance to students who belong to socially weaker sections supporting efforts for community health in slums and area inhabited by weaker sections supporting the programmes and efforts for environment protection and enhancement promoting, encouraging and supporting the social and cultural heritage and traditions of our society taking proactive measures for the well being of society, as per needs are the activities undertaken.

Central public sector enterprises are playing a major role in the field of socio economic development of the country with some as employment generation as well as industrial employment. Both Maharatna and Navaratna companies are significantly known through their efficiency. These companies are contributing funds to the corporate social responsibility activities liberally and concentrating on key area in corporate social responsibility according to the needs of the local conditions. This chapter discusses the central public sector enterprises, eligibility or criteria for granting Ratna status, top public sector undertakings' spending on corporate social responsibility, and Maharatna companies and Navratna companies.

History of Bharath Heavy Electricals Limited

BHEL was one of the largest engineering and manufacturing enterprises in India. It was one of the leading international companies in the field of power equipment measures. BHEL owned by government of India is a power plant equipment manufacturer and operates as engineering and manufacturing company based in New Delhi, India. The planning board felt the requirement for electrical machinery in India in the year 1947. In the year 1948, J.C. Ghosh was the first to set up heavy electrical generating equipment factory. In January 1955, S.A Gadkary committee restated the need for heavy electrical factory. In the year 1956, Heavy Electrical private limited was incorporated which was later renamed as Heavy Electrical (India) Limited.²

The first plant of BHEL was set up at Bhopal in 1956. In November 1964, three more BHEL were established and plants at Haridwar, Hyderabad and Tiruchirappalli were set up. The unique speciality of BHEL was that it had a well-recognized track record of performance making profits continuously since 1971-72 and paying dividends since 1976-77. BHEL has added to its high pressure boiler plant, a seamless steel tube plant at

² [www.history of bhel](http://www.historyofbhel.com)

Tiruchirappalli, boiler auxiliaries' plant at Ranipet in the state of Tamil Nadu, a piping centre at Chennai in Tamil Nadu and an industrial valve plant at Goindwal in the northern state of Punjab.

Corporate Social Responsibility policy in BHEL

Corporate social responsibility in BHEL is a continuing commitment to behave ethically and contribute to harmonious and sustainable development of society and planet through business, while improving the quality of life of the community and the society. Corporate social responsibility is therefore the organization's commitment to operate in an economically, socially and environmentally sustainable manner while recognizing the interest of its stakeholders.³

CSR Policy Statement

BHEL, a global engineering enterprise providing solutions for a better tomorrow is committed towards holistic welfare of the society by undertaking corporate social responsibility activities within the ambit of schedule-VII of the companies Act 2013 as amended from time to time. However thrust areas for corporate social responsibility activities will be:

Inclusive India: Mitigation of hunger and poverty through livelihood promotion or augmenting income generation, imparting vocational skills

Healthy India: Promoting health care and sports

Clean India: Sanitation and making available safe drinking water, cleaning and preserving the rivers, clean toilets in schools especially for girls

Educated India: Promoting education with thrust on informal education to reduce dropouts at primary school level, value education and digital education

Responsible India: Women Empowerment, setting up old age homes, day care centres and such other facilities for senior citizens, rural development projects and slum development projects

Green India: Ensuring environmental suitability with emphasis on projects based on solar energy

Heritage India: Protection of national heritage, art and culture

³ Bharat heavy electrical limited, new delhi (december,2014)corporate social responsibility csr policy

Table No. 4
Completed CSR Projects of BHEL

S.No	Thrust Area	Coordinating BHEL Unit/ Region Division	Sanctioned Amount in Rs. Lakhs
1.	Community Development	Bhopal	13.58
2.		HERP, Varanasi	37.94
3.		PSER, Kolkata	145.00
Total			196.52
4.	Education	HPBP Trichy	17.00
5.		HPBP Trichy	200.00
6.		ISH Bangalore	104.62
7.		PEM, Noida	28.33
8.		PEM Noida	17.31
9.		PSER Kolkata	34.00
10.		SSBG Noida	32.69
11.		SSBG Noida	16.59
12.		R&D Hyderabad	7.44
Total			457.98
13.	Environment Protection	HPBP, Trichy	107.20
14.		HPBP, Trichy	20.00
Total			127.20
15.	Health Management	EMRP, Mumbai	336.81
16.		Jhansi	35.50
17.		PSER Kolkata	100.00
18.		PSNR, New Delhi	59.42
19.		PSSR, Chennai.	50.00
20.		TBG, New Delhi	72.00
Total			653.73
21.	Infrastructure Development	PSSR Chennai	19.00
Total			19.00
22.	Vocational Training	Jhansi	5.00
23.		Jhansi	4.00
24.		PEM Noida	5.97
25.		Jhansi	4.30
26.		Jhansi	5.70
Total			24.97

Source: www.bhel csr thrust area, bhel hr department

Suggestions

- Corporate social responsibility is one of the vibrant parts of the corporate sector in the 20th century which is not only giving back to the society but also it lies with building the reputation of the corporate sectors. Therefore, corporate social responsibility becomes

popular in the recent corporate filed which is also considered as a mandatory mechanism as per the new companies Act 2013.

- The present study attempts to understand the basic concept of the corporate social responsibility with respect to its historical perspectives, in the Indian environment.
- This study appeals to business enterprises to contribute something to the stakeholders in the form of business responsibility. With the impact of new economic policy in India, corporate sector transforms the existing business ethics practices into corporate social responsibility.
- Government has given a restriction to implement the corporate social responsibility by corporate sectors through its legal amendments in companies Act and then it is treated as mandatory provisions of the corporate social responsibility by corporate sectors through its legal amendments in companies Act and then it is treated as mandatory provisions of the corporate sectors.
- With these aspects, every company is responsible to concentrate on corporate social responsibility.
- In India, central public sector undertakings are maintaining their own provisions regarding corporate social responsibility of which BHEL is one of the leading central public sector undertakings, which spends adequate amount to its corporate social responsibility activities.
- As for BHEL, it has achieved and contributed significantly to the society. But in the real sense, there is a need of more concentration in their adopted areas.
- As for BHEL, Tiruchirappalli unit corporate social responsibility activities are successfully implemented but when compared to its profitability, the amount spent for corporate social responsibility is not sufficient.
- In donating the equipment to the society, there is a need of wealth creation through corporate social responsibility, which is an emerging need of the society, which will make a sustainable growth in their targeted area.

Conclusion

In the early period, business was restricted with a particular community, which was treated as a traditional occupation and it became a service-oriented activity with reasonable margin. People believe in the businesses that are truly rendering services at an affordable price and business men also trust the value of money which is paid by the people. Concept of the business ethics had been slowly reducing its identity in the business activities. Again,

the attitude of the business men from profitability to sustainability is remaining evolved. Hence, they realize that sustainability is more valuable than profitability. Therefore, this study concludes that the performance of BHEL with respect to corporate social responsibility is significant on the whole. With respect to the particular unit of BHEL, Tiruchirappalli, its corporate social responsibility activities are more in the fields of infrastructure facilities to educational institutions, community development, skill development, sports activities, women empowerment and health care activities. Efficiency of the corporate social responsibility activities of the BHEL, Tiruchirappalli unit during the study period is significantly good but there should be a detailed evaluation study to measure the outreach of corporate social responsibility activities. Therefore, BHEL should take necessary steps to assess the effectiveness of corporate social responsibility in a systematic manner and that is recommended through this research study.

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THE ILLUSTRATION OF MATERIALIZING WOMAN IN SELECT NOVELS OF FLORA NWAPA

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ABSTRACT

Nwapa's greatest achievement, however, remains her forth right challenge to the distortion and a historicity of the racist anthropological writings on the position and role of women and on the nature of relationship between men and women in Igbo land/Africa prior to the European conquest. She is best known for recreating Igbo (Ibo) life and customs from a woman's view point. Feminist heroine Her heroine's revolts against, and condemns circumcision in no uncertain terms. She also questions repetitively her secondary status and makes an impassioned plea for the right that women make their own choice. She is an illiterate rural woman whose feminism is not theoretical or derived from text books. She experiences her unequal status in her everyday life as a woman.

Key words: Nwapa, Igbo woman

Efuru is a portrayal of life in Igbo culture, especially women's life. Set in the village of Oguta, where Nwapa herself lived, the novel tells the story of an independent minded woman named Efuru. She is a woman who becomes a paradigm and a compound for change in her own society. Her honesty, nobility and her success in trading are appreciated that empowers her to give her husband, Adizua, the money required pay her own bride price.

Efuru's growing assertiveness in deciding when her first marriage is over, in pursuing her trading career-for which she gains communal recognition, "She is a good woman. Her hands make money. Anything she touches is money" (125) and Idu is also very successful in her palm oil trade. Gay Wilentz states in her book *Binding Culture*, that Nwapa's *Efuru* was the first novel published in English by a Black African woman:

With the publication of *Efuru*, Flora Nwapa brought a fresh perspective to traditional West African Culture and modern Nigeria in literary works by exploring a woman's point of view and exposing a society close to its pre-colonial roots Nwapa tells us that she writes these stories about women because they are familiar to her. (3)

By providing a symbol of female transcendence and empowerment, the novel furnishes a basis for resistance to images that render woman powerless. The focus however is not on rivalry between the sexes, but rather on societal form.

Efuru is the story of the tragedy and triumph of a young and beautiful woman, Efuru the only daughter of Nwashike Ogene, a distinguished and noble personality and his wife, a beautiful woman who took several titles herself. She is no less distinguished. Thus, Efuru not only comes from a distinguished family but herself is a remarkable woman as the novelist clearly states in the opening sentences of the novel. She has chosen a novel way of getting married. Efuru is a typical Igbo village girl, but highly individualistic. Born and brought up in a typical Igbo village, Efuru sums up in her character the best characteristics of African womanhood. She is meek and gentle, loving and generous and respectful to elders. She is an out-standing character, a popular figure everywhere, liked and loved by the entire village for her beauty, pleasing manners and speech. In the novel, people say to each other that One could not help liking Efuru after one had any close association with her.

This courageous girl's odyssey in life starts with this challenging decision of hers to seek fulfilment for herself in her marriage with a man of her own choice. Adizua was poor and in no way distinguished. But Efuru's love for Adizua gives her that defiant courage to take up her stand against

the native custom, as in her view bride-price is only of secondary importance and living with the man of her choice is of primary importance. She earns and saves money and sets in motion the due processes of matrimonial negotiations and payment of the customary bride-price. Adizua's men visit Efuru's father, pay the bride-price and obtain his and his family's formal approval for her marriage. Unflinchingly standing up to face every fierce campaign of slander directed against her and Adizua, Efuru ultimately proves herself instrumental in bringing about a happy reconciliation between the two estranged families.

Adizua is a farmer and he naturally expect his wife to follow him in his work in the fields. Efuru blandly refuses to be a farmer for she knows that she has a talent for trade and it is in trade that she could make good money. Her refusal to go to work in the farm and her preference for trade prove once again the independence of mind and self-assertive choice of Efuru. Adizua is a wastrel and socially a 'nobody' being the son of an unsteady man who deserted his wife for another woman. In every way he proves himself unworthy of the honour of being the husband of this brave, beautiful and intelligent girl, Efuru. It must be one of the quirks of fate or blind love that Efuru chooses to be Adizua's wife for Adizua is not a match either for Efuru's intelligence, nobility of character or enterprise. She becomes a figure of sorrow in spite of outward success. She is racked by an inward grief which none of her obvious gifts could redress. Her marriage remains fruitless and she dreads the consequences of a tragically sterile life,

Barrenness is a curse, a slur on femininity, and a flaw in womanhood. To become a wife and mother of a number of children is the highest ideal and aspiration of every African woman. Marriage and consequent motherhood being the focal centre in a woman's traditional role in African society, an African woman feels herself fulfilled and contented only in the attainment of these twin goals. A barren woman is a contemptible creature, an affront to her community and an offence in the eye of God for tribal ethos cast the woman in the role of creative and protective force in life. These restrictive norms militated against the full flowering of African womanhood and the barrenwife's life was miserable. The only escape she has is to convert herself as a devotee of Uhamiri, the beautiful, merciful, rich but barren Goddess of the Lake and wife of Okita the God of the River and live the pious and saintly life of a recluse who has renounced all worldly or mundane pleasures.

Nwapa's *Efuru* is a bold venture on the part of the young author seeking to strike out into a new, untrodden path of projecting the image of highly intelligent and beautiful female protagonist who wills her way with indomitable courage and stands her ground firmly when confronted with vehement and often vitriolic social opposition and protest and an adverse fate. Efuru, the heroine, is the embodiment of every feminine virtue and grace. With courage and grim determination, she pursues the path of her own choice and with patience and fortitude bears the sorrows that come her way.

Efuru shows by her life that she is no escapist 'sissy' female who meekly surrenders herself to the dictates of tribal traditions, village gossip, envious scandal-mongering or threats of excommunication. Twice she marries and twice she is cheated of the happiness she sought in marriage and yet survives these shocks to attain a higher bliss in attaining integration and complete identity with the Goddess of the Lake, Uhamiri whom she worships, thereby transcending the earthly considerations of pleasure and happiness. Both male and female characters play their role in a significant way as wife, husband, father, mother, brothers and sisters etc. Thus, Nwapa's *Efuru* is the first and most bold departure from traditional image of a characters in African literature.

Nwapa's second novel *Idu* confirms the arrival of the truly heroic woman character in African creative writing. Idu, of the novel is a more penetrating portrait and her ideal love reminds us of the pativratas of Indian tales and arrests our attention. Nwapa's conception of love and the marital bond is rooted in the African world view. Deep love does not contemplate the possibility of separation. The heroine Idu is a beautiful, kind-hearted loving and industrious woman admired by all in her community. She is the wife of Adiwere, a trader. They have everything, prosperity, plenty of good trade, contentment in personal lives, but theirs is a fruitless marriage. Although Adiwere shows no unhappiness, Idu has not forgotten the tribal tradition to choose for her man another wife so that the family line does not grow extinct.

The domestic tensions and unease caused by the entry of the young wife who does not easily fit into the pattern of life Idu and Adiwere have evolved for themselves come to an end soon. Idu becomes pregnant and gives birth to a bouncing baby boy Ijoma. When the child is nearly four years old Idu becomes pregnant again but before the second child is born, Adiwere dies of some mysterious disease which is a shattering blow to Idu. She prefers death and through it reunion with her Adiwere, Neither children nor prosperity counts for anything with her now. On the day she chooses to die she eats food as she has never eaten and tells her sister that she is going to 'sleep' and dies. She succeeds in her death-wish by her renunciation of worldly pleasures and by her tapas, and attains a kind of swachanda marana, voluntary death.

Nwapa's another famous novel is *One is Enough*, in this novel a woman who is harassed to find a self-governing and fulfilling life of her own. Amaka, the heroine of the novel starts off in a state of female submission and dependence. We find Amakan who has failed to produce a child in six years of marriage, grovelling at the feet of her mother-in-law, begging not to be 'thrown away'. "What is important is not marriage as such but children...a marriage is no marriage without children." (p.10)

Amaka reflects on her fate, with negative thoughts which she later successfully rejects. "God had deprived her of the greatest blessing bestowed on a woman, the joy of being a mother". (p.11) But then she also asks herself, "Was that really the end of the world? Was she useless to the world if she were unmarried?" (p.11) Her pursuit of economic freedom and her success in business releases her from this burden of proving her womanhood or self only through motherhood. Ekwensi's glamorous and sexually liberated heroine Jagua Nana revels in her power over men but feels humiliated each time it is viciously pointed out to her that she is barren. Her rehabilitation from urban prostitute to rural entrepreneur takes place only after she is able to conceive and deliver a child. The death of the child does not deprive her of her proven status of motherhood. "Thank God my womb carried a baby for nine months. Thank God I had this baby and she was a normal baby. It would have been dreadful if I had been denied the joy of motherhood." (p.32) She sighs with relief. After six years of happy, though childless, marriage, Amaka, at thirty, is shattered to discover that her husband plans to take another wife-a woman who has already borne him two sons in secret. She makes a brave decision. Rather than stay in the comfort and security of her marital home, she will go to Lagos and try to make a fresh start in life. In order to become a successful and wealthy businesswoman, Amaka finds she has to use methods as corrupt as the society in which she finds herself. Then she becomes involved with a Catholic priest. Finally, Amaka has to decide whether she has the strength to continue alone, in the face of criticism from her family and friends. Should she take another husband and find respectability, or should she decide that 'one is enough'? The heroines of Flora Nwapa rise head and shoulders above the women characters other African novelists have presented.

Flora Nwapa's *Efuru* and *Idu* are the beginning of a new breed of heroines in African literature. In effect they proclaim to the world the rise of the self-assertive African woman whose willing subordination of her 'self' can no longer be taken for granted by the African male. They are a radical departure from the traditionally subordinate role assigned to them. Through the sheer force of the high level of their intelligence, integrity of character and competence they carve out a place for themselves in the African social framework and demonstrate to men that woman is at least equal, if not superior, to them in every field of activity. In fact, they show that a woman is potentially the superior of man in every way. They suffer, they bend but their will and determination were not broken. Tragic but triumphant, they emerge as towering examples of the new, awakened African womanhood. Woman is the pivotal centre of the drama of human life in Flora Nwapa's major novels,

Efuru, Idu and One is Enough.

A close study of Nwapa's plots reveals that the central theme with which she is continuously preoccupied is childlessness and the consequent misery women face in African society, particularly among Igbo women. Her novels, *Efuru* and *Idu*, primarily illustrate the social and psychological consequences of sterility in women. It takes a woman to understand the problems of women and do

her best to redress the underserved suffering cast on women, Where her male counterparts in African creative writing tended to dress their women characters in the strait jacket of the traditional role assigned to the African woman, Flora Nwapa, herself being an educated and liberated woman, is chagrined to find so much that is deplorable and disparaging to the honour of womanhood and felt called upon to project the image of an African woman who is daring and defiant, self-assertive and self-reliant and who grows beyond the narrow considerations of mere wifehood and motherhood and rises towards true womanhood. Her novels and portrayals are so true to life and so authentic that Ernest N. Emenyonu's *Who Does Flora Nwapa Write For*, in his acknowledgement of Nwapa's true-to-life portrayals, comments:

Flora Nwapa writes with a peculiar realism. She brings a feminine closeness and intuition into a theme which has been repeatedly treated by such male Igbo authors as Onuora Nzekwe, John Munonye, Cyprian Ekwensi, among others, but which is best understood in all its ramifications by a woman (6).

Flora Nwapa's intentions in the portrayal of the character of Efuru are made abundantly clear right from the start of the narrative. Efuru's life is Flora Nwapa's manifesto to African womanhood, a pathway for them to free themselves from the stranglehold of tribal traditions. Her heroines, Efuru, Idu and Amaka are unbendingly engaged in a struggle against cruel fate that casts her shadow over their lives and marks them out for her own. Brave, beautiful, gentle, kind, resourceful, patient and forgiving and bearing their adversities with fortitude, Nwapa's heroines emerge triumphant through their personal misfortunes They are the trend-setters and are the progenitors of a new generation of African woman who rebel against the restrictive tribal norms of conduct, and assert their individuality and freedom the course of life prompted by their conscience and would not yield to the dictates and pressures of tribal traditions and practices which do not fall in line with their own thinking and conviction. As Nigerian, and as well as belonging to Ibo community, Nwapa is spokeswoman in literature for the rights of women to function as human beings. And having lost power to women, men become the other losers at the margin. Thus, even in societies where the African male is the dominant patriarch one comes across admirably strong and independent women.

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**EXISTENCE PRECEDES ESSENCE: AN INSIGHTFUL EVALUATION OF AMITAV
GHOSH'S NOVEL FLOOD OF FIRE.**

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Abstract

We live in a world where societies are filled with epidemics, poverty, economical greed, political aggression, women oppression, racism, suicide, etc. As necessity is the mother of inventions and the human mind is afraid of encountering problems, it tries to surpass the upcoming hardship through an existential thought. The existential vision encourages human to transcend the prevalent situations. This transcendental thought of existence that precedes essence in both the fields of science and moral is due to the freedom, which the human mind recognizes leaving behind the essence of the past. The truths which the human mind approves are born of his/her consciousness. In this modern world each and every individual are highly responsible for their own decisions. Ghosh in his novel *Flood of Fire* with the assistance of factual descriptions of the world creates an appealing life picture before the eyes of the readers, which presents various sorrowful, unaddressed issues that is continuing from the distant past and is causing distraught in the present world. His concentration on human suffering elevates the existential thought with which man tries to frame meaning in the discourse of life. The present paper focuses on the element of existentialism, where the human existence precedes essence with freedom. The novel clearly delineates the misconception of this notion in the present modern world. Ghosh cautions on human freedom bound with autonomy and insists on responsibility and authenticity which at instances is replaced by selfishness and greed.

Key Words: responsibility, authenticity, existential vision, situations, existence precedes essence, freedom.

Amitav Ghosh's novel *Flood of Fire* written in 2015 which is the last volume of the *Ibis Trilogy* is a thought-provoking novel that registers a good understanding towards life. The opium war fought against China for free trade is the background of the novel and the characters and their lives are connected with it. As Flynn in his book *Existentialism: A very Short Introduction* states that existentialism has infused itself right from the Hellenistic period "on ethical questions and discerning the proper way to live one's life" (1), likewise, in this novel Ghosh's characters too suffer to find meaning in this everchanging world for life. The story not only brings out the consequences of war fought demanding free trade but also registers the consequences of it in human life. Ghosh, who cautions of the changing climatic conditions and its risk factors in his book *The Great Derrangement* has also cautioned the emerging world where human mind does not exhibit much concern on responsibility.

In the novel *Flood of fire* some of his characters struggle with dissatisfaction and dilemma, which is a continuing malady in human survival. Ghosh's plot and narration reveal the consequence of irresponsible and unauthentic decisions taken in haste. He clearly depicts that life which has lost its order due to the mounting difficulties has paved way to individualistic decisions. Sometimes the existence that precedes essence mounts challenge in human life and the consequence of it is presented graphically by Ghosh. This individualistic decision made by human for better survival to satisfy both physical and psychological needs at times, lands the individual in dilemma and existential crisis.

The ibis Trilogy which starts with the novel *Sea of Poppies* and narrates the painful life of Deeti, ends in the final volume *Flood of Fire*. As Nietzsche states in his *Birth of Tragedy* that "the experience of tragedy forces a culture to reconsider or revalue its values" (Spinks 14) similarly,

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Abstract

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soldier like Kesari escaping with the price money of Mee, without sticking to the rules and regulations of the army and losing the money to people who are deceivers and nothing more, Ghosh reveals how the existence of human surpasses the essence.

Zachery Reid who travels in the *Hind* with Kesari is portrayed as a modern subject of the emerging world and he tends to change himself as per the time demands. In the novel Zachary is the character, who is a typical example of "double consciousness" (APA 333). He is persuaded by people who impress him and he cannot be termed as good or bad. Taking the advice of his mother to praise at least two things in a day and by the encouragement of the lascar Serang Ali, he learns to benefit himself from the surroundings. His life takes a twist when his attraction to Paulette makes him help in the escape of Jodu and other convicts and is held up in the case.

Zachary suffers in India until he is acknowledged as innocent by the court. His difficulty mounts when he is asked to settle a sum of one hundred rupees and warned "that your mate's license will not be restored until the bills are cleared" (FF 10). His suffering continues until he gets an opportunity to work as a maistry in the Budgerow of Mr. Burnham. He feels very happy when Mr. Doughty puts before him the proposal. His calm life in the budgerow is triggered by Mrs. Burnham. Mrs. Burnham the Memsahib, who thinks of finding the reason behind Paulette's run away from home, brings him under her control and charges him of Onatism and asks him to meet her in the pretext of suggesting treatment to him. She frames an illegal relationship with him and plays with his consciousness. Zachery who was suffering from loneliness due to the death of his mother and who had fixed his mind on Paulette is moved by the care and concern which Mrs. Burnham showers on him. His ideology changes totally due to Mrs. Burnham. A normal man in his youth connects himself with Mrs. Burnham for pleasure and distances himself from the remembrance of Paulette.

Zachery who has immense faith that his relationship with Mrs. Burnham will continue, gets shocked when she ends it abruptly. He is filled with grief when Mrs. Burnham reveals to him that she needs Mr. Burnham and his money to continue her luxurious life. As Cathy puts forth about the idea of traumatic neurosis explained by Freud as "the experience that emerges as the unwitting reenactment of an event that one cannot simply leave behind" (Cathy 2) this traumatic incident creates a big change in him and he tries to acquire wealth at any cost. He is stuck with dilemma when Mrs. Burnham rejects their relationship and counsels him to move on with his life. After hearing to her, his action takes a selfish turn and he thinks to rise high at any cost. He understands the power of money and he finds "a great disgust for the life he had led before – a life of profligacy and poverty, in which he has wasted his mind and body in pointless pursuits.... He longed to leave that life behind him but was again confounded by that hateful query: how" (FF 251)? A man who argues in length with Mr. Burnham on the matter of the Abolition of Slavery by the British and the continuing transport of coolies; a person who feels bad for the injustices done in the schooner *Ibis* to Jodu, Ah Fatt and Neel and an empathetic person who argues on whipping Kalua when he was caught for the death of a Silhadar, changes himself to live his life. By leaving behind the tenderness and morals he determines to raise high without caring about the ethics of life.

Ghosh gives evidences of how Zachary sheds humanity to come up in life. He gains motivation when he hears from Doughty about Mr. Burnham and his past. Mr. Burnham's metamorphosis from a country born boy of a Liverpool timber merchant to a big business man widens his thought and provokes his interest for a better survival. Ghosh's narrative reveals the impact of situations that changes a man and his approach. He states how Zachary uses the money and the advice of Mrs. Burnham as his investment to frame a better life. His narration explains how with the help of Nob Kissin, Zachary gets the accent of Mr. Burnham to travel in the *Hind* and is permitted to carry twenty chests of opium to sell it at his own liberty for profit. There starts Zachary's life which is much different from its past. But the authenticity which he gives to his decisions helps him to lead a life without any remorse. Through the character of Zachary, Ghosh presents how acknowledgement of the decision makes a man to continue without any dilemma at any point of time.

When he detects the relationship of Mrs. Burnham with Captain Mee, he threatens them of revealing their old relationship. Its Mrs. Burnham's letter, which he reads after her death, that

presents him some insights towards life and he turns back to Paulette and thinks of reframing their relationship. But Ghosh leaves the readers in dilemma when Zachary at the end of the novel stands joining his hands with Merchant Chan, buys land along with Mr. Burnham and is set to venture a new career with them as his partners.

In the novel Ghosh's women characters such as Paulette, Mrs. Burnham and Shireen carry out their life with good modernistic tendencies and freedom. With these characters Ghosh once again stresses the importance of authenticity. When Paulette finds dissatisfaction in Bethel and wants to live a life of dignity she flees from there courageously to an unknown place, Mauritius. Her courage to escape in the *Ibis*, her lonely accommodation in the ruins of 'Mon Plaisir' shows the courage with which she masters the pains of her existence. Ghosh brings out the power of sufferance that connects her to the life of Fitcher. She does not accept the offer of Fitcher until he reveals to her about his daughter Emile. She accepts to join Fitcher as a caring daughter in the *Redruth* and starts helping him in his endeavor of tending plants and preserving precious species.

Ghosh's female characters are bestowed with courage where their life is led by their own determination and is not governed by the rules of the society. Mrs. Burnham and her dissatisfaction in life encourage her to break the ethics of marriage and relate herself with Zachary and later with Captain Mee. Shireen being a woman brought up in a prosperous and orthodox environment is subjected to change herself as the family suffers because of the unpredictable loss in Bahram's business. When her brothers discuss on the decision made by the authorities of London and a better chance to get back Bahram's investment, Shireen a housewife who has not crossed the borders of her home town gathers her courage and says, "But what about me? She said, blurting out the first words that came to mind: What if I were to go myself?" (FF 43).

Shireen's determination to move abroad intensifies when she hears of the other life of her husband and his son in Canton. When she knows more about the boy and his sufferance in a tender age from Zadig Bey, her concern towards him intensifies. She becomes curious to see him and ponders over the matter very practically and also decides to move to Canton to meet him. In spite of various hindrances from the family, which has never allowed its women folk step out of their houses, she emerges out as a courageous woman to face the situation without any dilemma. She determines to move out without any hesitation to a distant land. She thinks of getting back the share to return the debts of the business men and to provide some benefits to her daughters. Without any hesitation she moves to a place of which she has not even imagined in her dreams.

Ghosh portrays Shireen as a confident woman, who decides sensibly during times of neurosis and angst. Her disappointment is reduced when she hears from Zadig Bey about the concern that her husband carried on her and their family. She surpasses the old dogmatic thought as Simon de Beauvoir reveals that "One is not born, but rather becomes a woman" (Tidd 51). In spite of being brought up in a strict patriarchal background she emerges to the requirement without holding the old dogma of the Parsi Culture. With this Ghosh reveals acutely, that finding meaning at adverse conditions is in the hands of the individual.

Ghosh presents the new understanding in Shireen enhanced by her will. When Freddie dies, Shireen suggests burying him in the 'happy valley' next to his father and when puzzled Zadig questions, "But what about Dinyar and the other Parsi Seths?" Her stern answer, "Let's not worry about the Seth's. What matters is what Bahram would have wanted. And in death at least I think he would have wanted to give Freddie the acceptance he could not give in life. It's only right that Freddie should be buried beside him" (FF 548) reveals the sternness and authenticity which she gives to her decision.

When Shireen determines to continue her remaining life in Hong Kong marrying Zadig, Dinyar threatens her, "You can't do that! It's impossible. You will be cut off by all of us. None of us will ever speak to you again". But Shireen sternly replies, "you will... if I am driven away from here... you can be sure that many Parsi families are going to find out that they have unknown relatives in China. And yours is the first" (RR 566). When Dinyar remarks on the loneliness which men experience when they come to distant lands for trade, she voices out the condition of women and questions why she could not take such a decision when many men are practicing it. Ghosh writes

about the utilitarian aspect of life in the character Zadig, where one human can benefit the other by supporting them at the time of their loneliness. Ghosh presents a productive factor of life when Shireen decides to buy land in the 'Peaceful Mountain' and build a public hospital in remembrance of her late husband, Mr. Bahram Modi.

Ghosh places Mrs. Burnham and her hasty decisions in parallel to that of Shireen to bring about an understanding on authenticity in life. When Shireen sticks to her decision, Mrs. Burnham suffers with existential crisis. Her condition becomes worse when Zachary compels her to agree to his intensions. Her requests to Zachary remain unheard and he replies sternly, "Oh but it is you who deserve all the credit, Mrs. Burnham. It was you who taught me cruelty – and as you know I am a quick learner" (FF 581). She feels hard when Zachary accuses her as the tutor of his reframed life from which turning back is impossible for him. She is afraid when he threatens her of revealing her relationship with Captain Mee. As Sartre in his *Existentialism and Marxism* talks of Mallarme and, "his sudden discovery on the reason the reason why suicide was efficacious was that it replaced the abstract, fruitless negation of the whole of being and the negativity of self-consciousness becomes real negativity" (174) similarly, Mrs. Burnham who could not find any escape from her own creation decides to meet death, before she is made shameful amidst the surrounding.

Life and its illogical flow and human decision towards it are evident at every mode in Ghosh's Plot. Neel the Raja of Raskali is left in a miserable condition at a distant land for survival, after the death of Bahram. He visits Compton's workshop to meet Zhong Lou-si who was in charge of a bureau to translate and gather information about India and East India Company. When Zhong Lou-si asks help in translating the information about India and the East India Company, Neel without any hesitation accepts the offer. He acknowledges that once he and his family were the supporters of the company but, at the end the company has left them with nothing. Being an Indian he is not hesitant to translate the Indian scenario to the Chinese. He understands the imperialistic attitude of the British and wishes to go against them without any hesitation.

The character Captain Mee, a sincere officer, in the Pacheesi Batallion who has several years of sincere service, feels his first defeat when Zachary comes and compels him to support the new business of selling essential things to Indian soldiers at high cost. His intention to live his lost life with Catherine transcends the ethics of the society and problems mount for both of them. A man, who has not succumbed to any situation, could not resist the threat of Zachary because of his secret relationship with Catherine, wife of Mr. Burnham. Finally, when Mrs. Burnham dies due to the threat of Zachary, he is unable to withstand the pain of her loss and shoots himself and dies.

Nietzsche talks of his interest in tragedy because it offers the supreme example of an art form that provides insights into the strength and weakness of a culture (Spinks 14). Like Nietzsche, Ghosh in his tragic story, presents the good and bad consequences of freedom in thought and recommends a deep understanding to it. His direct characterization provides enough details to understand the psychological behavior of his characters, when caught amidst the circumstances of the family and the society. Ghosh through his comprehensive plot presents how the traumatic incidents in the lives of Mrs. Burnham, Zachary and Captain Mee lead them to take decisions for their existence, ostracizing the rules of the society. He also presents how Kesari, Zachary, Neel, Paulette and Shireen authenticate their decisions and their approval of it from their consciousness makes them stick to it without any dilemma. Sartre in his *Being and Nothingness* claims that we are aware to some extent of our freedom and the responsibility that comes with it, but we try to hide this from ourselves (74).

It is factual that human experiences and inner anguish build an intension to resist and transcend suffering. But how can the human mind overcome the traumatic sufferance that unknowingly presents itself during decision making? When the Lacanian psychoanalysis terms that, "the unconscious is the ground of all being"; Freud and his hope suggests, "for a return to the humanist model that placed consciousness and rationality at the center...by bringing the contents of the unconscious into consciousness... could minimize repression and neurosis" (Klages 74) which in turn will result in humanism that does not find a hasty remedy for problems in life.

Accordingly, the first thing one need to do is to acknowledge the traumatic incident and understand the change it instills. As a caution towards this condition, Petit in his *Theory of freedom*

insists that the individual is, "held responsible for what he or she did" and he is the master of his or her "own thinking". He suggests that the choice should not descend from any, "hypnotic suggestion or an unconscious complex or childhood conditioning" (6). Hence Ghosh's novel *Flood of Fire* stresses on freedom and responsibility when one thinks to transcend the essence for existence. Thus, one can overcome the dilemma due to existential despair and anxiety which leads to existential crisis by knowing the limitations of Freedom and responsibility.

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Transformation of Female Immigrant Identity in Bharati Mukherjee's *The Tiger's Daughter*

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Abstract: - Mukherjee lays claim to an America that is both constantly transforming, and transformed by, the new immigrant. However, it is clear that Mukherjee's representation of a fluid American (trans)national identity influenced by diversity is ultimately predicated on the foregrounding of differences. Despite Mukherjee's call for America to go beyond multiculturalism in its treatment of new immigrants, her own postcolonial immigrant subjectivity-inevitably shaped by her elite British and American educational background-remains aligned with white hegemony, which continues to hierarchize its immigrants on the bases of ethnicity, class and gender. So this paper attempts to show the sense of assimilation and assertion of female protagonist Tara in Bharati Mukherjee's *The Tiger's Daughter* (1971), the main causes of the suffering of her women characters are therefore the hostile situations and the unfavourable social conditions which obstruct the path of their lives for a meaningful existence. Feelings of loneliness and homelessness contribute to their existential predicament where as Bharati Mukherjee's women experience these feelings abroad. Being an expatriate herself, Mukherjee often deals with the plight and conditions of the immigrants and expatriates. In her novels the problems of displacement and cultural crises are the main causes of the suffering of women. This article intends to study the problems of existentialism and its subsequent effects on the women protagonists in the selected novels of both the writers.

Keywords- Migration, Displacement, Assertion, Assimilation, Identity crisis, Predicament

Introduction

Bharati Mukherjee, an India born Canadian/American novelist, has made a deep impression on the literary canvass. She is an investigative pioneer--of innovative terrains, practices, and literatures-co-existent with her wide-ranging mission to discover new worlds. Her novels, honestly, depict the issues of her own cultural location in West Bengal in India, her displacement (alienation) from her land of origin to Canada where she was simultaneously invisible as a writer and over exposed as a racial minority and her final re-location (assimilation) to USA as a naturalized citizen. Acculturation is the depressing upshot of post-modern scenario, which Mukherjee had comprehended much early in her life. That is why, as a postmodern writer, her foremost concern has been the life of South-Asian expatriates and the dilemma of Assertion and Assimilation.

Mark Shackleton has stated that in his book *Diasporic Literature and Theory --Where Now?* The theoretical innovations of Edward Said, Homi Bhabha, Gayatri Spivak, Stuart Hall, Paul Gilroy, James Clifford and others have in recent years vitalized postcolonial and diaspora studies, challenging ways in which we understand 'culture' and developing new ways of thinking beyond the confines of the nation state. The notion of diaspora in particular has been productive in its attention to the real-life movement of peoples throughout the world, whether these migrations have been through choice or compulsion. But perhaps of even greater significance to postcolonial theory has been the consideration of the epistemological implications of the term - diaspora as theory.

Diasporic writings also known as 'expatriate writings' give voice to the traumatic experiences of the writers when they are on the rack owing to the clash of two cultures or the racial discrimination they undergo.

Immigration proves a pleasant experience only to a few immigrants who succeed in assimilating themselves with new geographical, cultural, social and psychological environment. To most of the diasporic writers, immigration is not a delectable experience. They often find themselves sandwiched between two cultures. The feelings of nostalgia, a sense of loss and anxiety to reinvent home obsess them, consciously or unconsciously. They all voice the anguish of the people, living far away from their native land and being discriminated on the grounds of race, colour or creed.

Through her female characters who are autobiographical projections of her experience as an expatriate she represents in her novels the contemporary woman's struggle to define herself and attain an autonomous selfhood, especially in cross-cultural crisis, a subject which has assumed a great significance in the present world of globalization. She endeavors to dive deep into the distorted psyche of those immigrant women who have been surviving in the conflict of traditional Indian values; inherent in their personality and their fascination for western mode of living.

In Mukherjee's first novel *The Tiger's Daughter*, the story about Tara, a convent educated Calcutta Brahmin girl, who goes to America for higher education, and is married to an American, David Cartwright. She returns to Calcutta after seven years of stay in America. This is similar to Mukherjee's own trip back to India with her Canadian husband, Clark Blaise. Tara feels more alienated on her return, as she encounters the clash of cultures and values in Calcutta. Hence, the westernized Tara feels like an alien in her own country. Therefore in the end, she decides to return to her husband David in America.

Aparajita Ray rightly comments:

"The protagonist Tara Banerjee Cartwright makes a trip home to India to soothe her ruffled feathers but becomes painfully aware that her memories of a genteel Brahmin lifestyle are usurped by her westernization" (84).

Instead of being comforted by middle-class Bengali Brahmin traditions, Tara is now struck by great impressions of poverty, hunger, disease, and political turmoil. Tara's father sends Tara to New York for higher studies at the age of fifteen. Though she confronts discrimination in the foreign land, she faces it boldly and even reacts aggressively to defend her family and her native country when her friends try to ridicule it. Whenever she feels broken, she prays to goddess Kali for strength. She hangs silk scarves around her apartment to make it more Indian at times of her loneliness.

She thinks on her own and gives importance to her desires. When she comes across cultural conflicts in America, she tries to resolve it by herself. In the meantime, she meets David Cartwright an American, falls in love with him and takes a bold decision to marry him overlooking her family customs and traditions. Tara who had defended her Indian heritage breaks it with courage by marrying an American who is considered an outcaste by her family. She believes that her marriage with an American will give her new meaning to her American life. Tara's American attitude to life is easily sensed by her relatives in India. The dullness, emptiness and desperation are evoked by her American life:

New York, she thought now, had been exotic. . . . there were policemen with dogs prowling the underground tunnels. Because girls like her...were being knifed in elevators in their own apartment buildings.... The only pollution she had been warned against in Calcutta had been caste pollution. New York was certainly extraordinary and it had driven her to despair.
(33-34)

Mukherjee's protagonists are all sensitive and are differently trained in the new ethnic imagination. They are tossed in an environment of the multicultural reality in the process of cultural differentiation and assimilation. The multiculturalism ethos with which they are confronted leads to the struggle for a new life and a near break with the past. They are shown at an emotional transit point and from their dual and bicultural perception they attempt to measure the disjuncture and persecutory paranoia.

Conclusion

Mukherjee, female characters are faced many problems in this postmodern world. Here this paper has examined Indian migrated women are search for their identity in the multicultural land of America is excellently revealed through the spaces of tradition, personal memories, different places and new ways of

life style in the altered socio-cultural constrains. They tried to reconstruct their own identity against the traditions to which they belong. While doing so, they also maintain their Indian identity of which they feel proud. Hence the efforts of maintaining both identities – partly Indian, partly American – make them the hybrid of new culture that again poses the question of their real identity.

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20-21

**PORTRAYAL OF WOMEN AND TRANSGENDERS IN SELECT PLAYS OF
MAHESH DATTANI**

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ABSTRACT:

Drama is literature that "walks and talks." In the literary arena, it has a long presence since the times of classical antiquity. In this context, it is a fact that the concepts conceived by the playwrights come in the form of texts and later they find articulation on the stage through the mouth of the characters. The play requires an actor to perform using his/her eyes, ears, emotions and intellect. It creates an opportunity for the actors to establish direct communication with the audience. When composing a play, a playwright needs to establish coordination among several rudiments so as to cobble an authentic and entertaining work. This research article delves into understanding the relationship between all genders in the plays of Mahesh Dattani. In his works, he ventures into comparing the life of women's society with the society of the third gender. In this, both 'new women' and 'suppressed women' constitute one group and they come under women's society. In contrast, Lesbians, Gays, Bisexuals and Transgenders constitute the third society. The logic behind this analysis is to understand relationship between groups.

Keywords: Trans genders, oppression, aesthetic needs, gender relations, humiliation.

The term gender indicates sexual difference. Gender relations are both intertwined and interrelated. It talks about the attributes and qualities given to the members of the society as well as the cultural practices that determine the gender roles. Hence, it is considered as social label. Gender is based on attributes which are considered inherent to the male or the female of the human species. The emergence of the third gender which bends the gender rules poses the problem of categorization, but in the process becomes a category in itself. Mahesh Dattani breaks the traditional stereotyping and portraying of the female characters and presents before us confident individuals who are capable of reacting to any injustice meted out to them in ways that are insurrectionist. Mahesh Dattani has been at his creative best in portraying women characters without any bias against them.

In the opinion of Lakshmi Subramanyam:

"They are humans. They want something. They face obstacles. They will do anything in their power to get it. All I focus is the powerlessness of these people....And I am not going to change my sensibilities for political correctness either. My only defense is to change my sensibilities for political correctness either. My only defense is to say that I am not biased against women.

(Subramanyam).

The way Dattani projects women characters is endowed with enormous meaning. Oppression of the female is an accepted and unchallenged way of life in both rich and poor families. For generations patriarchy has organized a hierarchical society and treated women as the second sex; on their part women have allowed themselves to be manipulated by men. It requires political will and intellectual understanding to bring about changes in a society that has seen women as no better than slaves within the household, by colonizing their mind and body. To liberate the minds of men from diehard traditional thinking and enabling them to understand the new reality wherein women are treated on a par with them requires artistic finesse and courage to present the truth, Dattani has them plenty and he has authored plays such as *Dance Like A Man*, *Bravely Fought the Queen*, *Tara*, *Thirty Days in September*, *Where There is a Will* and similar plays, targeting the urban audience. As a medium drama allows female characters on stage to connect immediately with the audience; in his case mostly sophisticated

urban audience who behind this thin veneer harbors age old prejudices. The critics need to bear in their minds that most Indian women possess qualities like grace, elegance and poise. They don the roles such as mother, daughter and wife in the most willful manner. In every role they play, they take decisive steps to exert control over the financial position of the family, household management as well as maintain a harmonious relationship in the family. Their qualities and behavior are based on the culture in which they grow up.

Though several women characters involve themselves in several kinds of activities like shopping, partying, dancing, doing household works, they are disappointed and distraught due to their inability to find lasting comfort and solace. Women struggle hard to establish their identity at home as well as in the society. At the start of the play, the viewers could find the women to be puppets in the hands of men. The same women would progress from a state of servitude to establish their personal freedom after a great struggle. Their grit, indomitable courage, extraordinary will and sometimes insurrectionist spirit show them emerge as self-dependent characters. Powerful dialogues with incisive words and the modern day lingo add to the rich texture of the play. Dattani believes in the magic of the spoken words and therefore lays emphasis on the performance of his characters. While acting on the stage, Dattani's characters are aware of the contemporary reality that stares in the audience's face. He says, "If you look at my plays, you would find that each and every character has, you know his or her space in the play, which an actor can develop" (Chaudhuri 104). He says so, for the simple reason much stress is not made on the printed words but on the utterance of words through the mouth of characters

In the play *Dance like a Man*, Ratna comes as a dominating and ruthlessly ambitious woman. She bravely stands up to her autocratic father-in-law Amritlal Parekh in her youth and also gives no room for her husband to control her throughout the play. Ratna is an emotionally high-strung woman who is always on the verge of a nervous breakdown and like a typical Indian woman she is anxious about her husband's heavy drinking and at one stage she bursts out into the declaration of her failure in her career:

"yes, your father was right. Dance has brought us nowhere. It's his curse on us. Nothing seems to be worth it anymore. Oh, It's all so worthless. You should have listened to your father. He was right. We were never anything great, but an average human being. (Collected Plays 391)

Ratna is a woman of many parts. She is a partly devoted wife, loving mother, and defiant daughter-in-law. She is a very ambitious lady who could go to any extent to fulfill her dreams and desires. It is evident through the conversation between Ratna and Jairaj that Ratna has destroyed Jairaj's dancing career only to assure her success in the same. She never accepts that she is responsible for the ruin of Jairaj's dancing career. On the other hand, Jairaj nurtures this belief that his wife is solely responsible for the death of their son, Shankar:

"Jairaj : No matter how clever an actress you are, you can't convince me that you are playing the part of devoted mother very well. You wouldn't even know where to start.

Ratna : I can start by ending this sick talk with you and feeding the baby. If you have nothing else to say, good night." (Collected Plays 394).

Through his characters, Dattani is able to exhibit the extent to which modern women would dare to go to fulfill their ambitions. These women are societal representatives who are radical in their views and path breakers in their roles as wives and mothers. Also, the reader may notice a strong identity assertion on the part of these women and every action of theirs is oriented towards self-actualization. In the same play, there is ample space for other characters, time periods and varied locales. He makes effective usage of lighting and it enables him to move from one frame to another. In this context, it is pertinent to point out the remarks of C.K.Meena that, " this distribution of the action among different levels on stage ... not only makes his plays visually exciting , but makes them move at a snappy space. " (Meena 143).

His next play *Tara* talks about both physical and emotional separation which has taken place between two conjoined twins namely: Tara and Chandan. Tara happens to be not only an independent character, but also turns out to be an icon among Indian girls who are forced to undergo humiliation because of traditions and societal compulsions. Even in her casual talks with others, she expresses her pitiable condition in a society that reminds her of male supremacy on every occasion. Indeed there comes an opportunity for Tara to express her feelings to Dr.Thakkar who talks about the details of surgery to sever both of them. This impels Tara to comment that :

“ Oh, what a waste! A waste of money. Why spend all the money to keep me alive? It cannot matter whether I live or die. There are thousands of poor sick people on the roads who could be given care and attention, and I think I know what I will make of myself. I will be a career of those people. I. I will spend the rest of my life feeding and clothing those... starving naked millions everyone is talking about .” (*Collected Plays* 362)

Through a character named Roopa, the playwright brings to light the evil practice of female infanticide that is followed among certain Gujarathis. Here the female baby is often killed by feeding it with excessive milk. Tara is portrayed in the play as a girl who speaks and acts tough in spite of her handicap. Still, her behavior is very much advanced for a girl of her age and she exhibits a kind of stoicism that is rare. She maintains a very cordial relationship with her family members and more so with her sibilant Chandan. She is more focused about her brother's future than her own. She states that, “ we women mature fast. Speaking of maturity , you better not skip any physiotherapy sessions. Daddy wants you to be big and sturdy.” (*Collected Plays* 374). She conveys her excitement and anguish by saying that, “ maybe we still are. Like we've always been inseparable. The way we started life. Two lives one body, in one comfortable womb. Till we were formed out.”... (*Collected Plays* 375).

Bharathi, the mother of Tara, is yet another character who plays a prominent role in the play. Although Bharathi hails from a modern Kannadiga family, her husband is given to patriarchal way of life. She is like any other woman in her social milieu caught up between her choices under societal and familial pressures. Her prejudices are the outcome of her helplessness in an andocentric society. Her sense of guilt drives her to the brink of insanity and hysteria. She resembles many other women who are torn between pressures emanating from the family and the society. Over a period of time, she becomes a split personality because of her role as a mother and a woman. She is left with no free will to assert her own choices to craft her own destiny. She continues to suffer from pangs of guilt for having denied Tara her legs. Bharati plays her psychological game of becoming over protective of Tara. She quenches the fire of her guilt with love and care and tells Chandan of her plan for Tara, “Yes I plan for her happiness. I mean to give her all love and affection which I can give. It's what she...deserves. Love can make up for a lot” (*Collected Plays* 371). Tara and Bharati, in their own way, perform their typical role of being an Indian daughter and mother as portrayed by Dattani through intense dialogues and action. Both of them suppress and raise their voice when the situation demands.

Transgendered individuals are the unacknowledged beings since the origin of human race. The social forces since time immemorial demand conformity to notions of sexual and gender identity. Judith Butler argues in her *Gender Trouble* that human subjectivity is always already variable and various. While discussing gender the focus is mostly on the biological functions and the contours of the human body. Further, the idea of desire itself becomes complicated because of the insistence of biological difference as the basis. Sexual difference has always been treated as essentially binary and fundamentally natural. And oppression on the basis of sexual identity is all too common and women are victims in every patriarchal society. The transgendered person, who is degradedly designated as 'eunuch' or 'gender-bender,' belongs to a unique category of individuals who are not “othered” or regarded as the significant other either to the male or the female of the species. In the absence of a dialogic relationship between an imaginary or generalized 'other' discussion of identity and desire of the transgenders has become

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problematic to those given to traditional thinking. Psychic difference between sexes is also cited as the reason for not treating the other as equal. On account of these differences, physical and psychic, the transgendered individuals suffer discrimination in many ways.

In the tradition bound Indian society, the transgenders are treated as social outcasts and are marginalized to such an extent that they have to live in colonies on the periphery of towns and cities. Abandoned by family, friends and relatives they have been denied education and livelihood for a long time. There is also this false belief that they are subhumans or abnormal beings who are given to 'queer sex.' To overcome such irrational mindset and to find social acceptance and living space, the transgenders have to fight a long battle. Dattani has been vociferous in his demand for equal respect and treatment in the society. Saraswathi in her article titled "Unmasking Societal Hypocrisy: In the Plays of Mahesh Dattani" states that "Dattani tackles what he calls the invisible issues " of the Indian society, issues not known to us, but of which we would rather not talk about; issues we would conveniently cover up with a rug and act as though they do not exist." (Saraswathi 274-275).

Whenever someone performs the role of a transgender on the stage, there is this need to focus more on words, silence, sound effects, background music, facial expression, gestures and so on. Dattani has beautifully depicted the sociological, sexual, psychological and cultural violence faced by the transgender community in the play *Seven Steps around the Fire*. He gives his full effort to the language and characterization to touch the heart, mind and soul of the audience. When the living conditions of the transgenders are compared and contrasted with women and homosexuals, one could understand the pitiable condition of them. In the play *Seven Steps around the Fire*, Dattani states that, "perceived as lowest of the low, they yearn for family and love." The pitiable condition of the Hajira is displayed through the maiden appearance of Anarkali on the stage.

It is understood from the play that Anarkali has been kept in a male cell and treated shabbily. She could not stand false sympathy anymore and restrains herself from talking to Uma. After learning that Uma belongs to the family of Deputy Commissioner of Police, Anarkali pleads Uma to release her. Munuswamy behaves rudely with her and asks the prisoners to attack her. Uma gets deeply disturbed by the turn of these events that she begins to think about their identity and present condition. She says, "Nobody seems to know anything about them. Neither do they. Did they come to this country with Islam, or are they a part of our glorious Hindu tradition? Why they are so much obsessed with weddings and ceremonies of childbirth" (*Collected Plays 27*).

In the very beginning of the play itself we see how the *Hijras* feel lonely and develop a sense of frustration and isolation and think that they are not a part of the society and are sinners who are always kept aloof from others. Their inner feeling of being the member of a society is seen in the laconic speech of Champa:

Champa: Oh! So you are a social worker. Say that.

Uma: Yes.... I am a social worker.

Champa: Please excuse me, madam. I didn't know that... you see us also as society, no? (*Collected Plays 19*)

These words of Champa express the innermost longing of transgenders who are considered lower than animals and not as a part of the society. The male characters in the play address the transgenders after their common name "*Hijra*". The prejudice against the transgenders is reflected even in the language used to refer to them. 'She' or 'He' is the pronoun used for female and male, but there is no pronoun for the transgender. Although they have their own name, they are addressed using third person singular that is 'it'- the term which is used for an inanimate object or a thing. Such traces are found in the play:

Munuswamy: You may see the *Hijra* now if you wish, madam.

Uma: Will she talk to me.

Munuswamy: (chuckling). She! Of course, it will talk to you. We will beat it up if it doesn't. (*Collected Plays 9*)

Though facial expressions and dress code of the transgenders enable others to understand that they have undergone change in their gender and behave more like women. Yet, the society is not yet ready to give them a deserving treatment. There is an element of dilemma in treating them as male or female and purely for this reason they have become a laughing stock of the society. The fact is that all transgenders are very much willing to accept the others and want to be accepted in return. Yet there are some vicious elements in the society who perpetuate the belief that they are 'sinners' and 'violent creatures' and it is safe to maintain a distance from them.

From the study carried out, it is evident that Mahesh Dattani has been ruthless in exhibiting the foibles prevailing in the society. It is understood that the Indian women had been under oppression for centuries and were treated as subservient to men folk. In comparison, the transgenders simply fade into oblivion. They are not even treated as human beings. Social iniquity, lack of access to education, health service, legal redress and even public space are some of the concerns that are addressed spiritedly in all earnestness. In this fast-paced world, the social changes are however slow to come by. But, Mahesh Dattani addresses the issues boldly and in a way his plays have become strong social satires.

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**RUMMAGE OF ROOTS IN BHARATI MUKHERJEE'S *DESIRABLE DAUGHTERS* AND
THE TREE BRIDE.**

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ABSTRACT

Bharati Mukherjee's fiction is centered around the question of borderline existence. Her novels speak about the woman protagonists who are in longing for their identity in the brave new world. Their displacement from one country to another country made them to lose their roots in the native land. When they come and search for their roots after many years, they found some astonishing truths and miracles. Mukherjee's sixth novel, *Desirable Daughters* is about Tara Chatterjee, an immigrant who comes back to her native land in finding the roots of her ancestors. The novel *The Tree Bride*, is a companion novel written by Mukherjee, were the protagonist Tara Lata, the great grand aunt of Tara, died mysteriously in the hands of the British Raj. The truth behind the death of Tara Lata made Tara to think about the value of life and also realizes how important family is. The rummage of the roots helps one to understand not only about family but also about oneself.

Keywords: Borderline existence, Displacement, Immigrant, Rummage.

Bharati Mukherjee, an Indian born American writer was one of the most famous celebrated writers around the world. Her works are mostly considered to be as an autobiographical one. As an immigrant writer, Mukherjee's themes are mostly based on expatriation, displacement, identity crisis, racial discriminations and so on. Mukherjee wants to incorporate the discrimination she faced in her novels. She wants her novel to be an eye opener for the people who believed that the life in foreign countries is not made up of bed of roses but with much hurdles. In an interview she states:

We immigrants have fascinating tales to relate. Many of us have lived in newly independent or emerging countries which are plagued by civil and religious conflicts. When we uproot ourselves from those countries and come here, either by choice or out of necessity, we suddenly must absorb 200 years of American history and learn to adapt to American society...I attempt to illustrate this in my novels and short stories. My aim is to expose Americans to the energetic voices of new settlers in this country. (01)

Having undergone from the situation of "Melting pot" condition to the situation in a "Lifeboat", Mukherjee's protagonists reflects the "home" for thousands of migrant women who are victims of dramatic identity crisis. They move towards acceptance and exuberance of a new culture and emerge as fighters and survivors with a proper meaning to their life. They strain themselves to become a self-assertive individual, who are free from the shackles of conservative existence and give a tight slap for the patriarchal society. Mukherjee is an exponent in following the traditions of myth and Hindu mythology and it plays an integral part in her literary world. Her attention focuses towards the protagonists, who struggle hard to come out from those Cross-cultural encounters. Hence, she is referred as "the clear eyed but affectionate immigrant in American Society" (Pandey 82).

Mukherjee's female protagonists play a major role in psychological studies. Their continuous urge has made them to build up their fragmented life and it expresses their affirmation to life. Her novel captivates the progression of women from one stage of evolution to the other depicting them as ebullient and courageous character. Her novels are somehow distinctly resembled to her own life and it carries an autobiographical note which can be observed invariably. She has made an endeavor to demonstrate a steady progression of a woman from 'feminine to female' and made a clear understanding of what life is.

Mukherjee in her novels *Desirable Daughters* (2002) and *The Tree Bride* (2004) has made her protagonist to travel all her way from America to her home town in search of her ancestral history.

Both these novels are published as sequel to one another and the main protagonist of both the novels are Tara Chatterjee and Tara Lata, the great grand aunt of Tara Chatterjee. In both the novels, Mukherjee beautifully explains the plight of an Indian woman who is totally distraught by her original self and was found completely numb about her ancestral history. Tara from *Desirable Daughters* is completely bounded in the situation of anagnorisis, when she has discovered a truth about one of her family members. Until that discovery, Tara acts as a normal woman who showers so much love to her sisters and find comfort with the family. That discovery made her to be totally alienated from the family and made her to go in search for the roots of her family.

In *Desirable Daughters*, Mukherjee have portrayed Tara as a woman, who is totally uprooted from her native town and gets settled in America. Tara, in the novel, comments on how the identity of a person is strongly connected with one's home, culture and community. She believes that the things which she is going to do, may be in her own conscience and she never blames others for her decision. By this decision, she also challenges the social and cultural identity which is one of the greatest possessions for a married Indian woman.

Mukherjee portrayed Tara as an Indian princess who is born and brought in a well to do family. Her father gets her married to Bish, a Silicon valley tycoon in California. Tara who is bounded by the traditional customs and ideas, wants to find a job for in the new land. But her husband, Bish was a traditional man, and does not allow Tara to achieve her dream. The broken Tara, divorced Bish and moved out from her husband's life along with her son, Rabi. She started to live as a single mother in the new town and enjoys the freedom provided by the alien land. She believed that she was completely caught up by the American way of living and was amused by its beauty.

When she discovers the truth about her sister, in the form an illegitimate son, Tara was not able to digest the real fact. She was astonished to see that the Indian traditions are still running inside her body and only her outlook changes. She believes that her inner Bengali consciousness has not gone away from her. When she met Dr. Victoria Treadwell, another phase of her Indian consciousness comes to the surface. It was Dr. Victoria who unlocks the secret of *The Tree Bride* to Tara. When she read the ledgers of Dr. Victoria, Tara was quite amazed to know that the tree bride is her namesake, Tara Lata.

To know more about the tree bride, Tara comes to India. With the help of the ledgers, she found about their ancestral home in Mishtigunj, where Tara Lata spends her entire lifetime as a tree bride. Tara Lata was married to a tree at the age of five, when her human husband was died on a snake bite on his way to the marriage. Tara Lata's father does not want her daughter to be called as a widow. So he made her daughter to get married to the tree and placed her inside the Mist Mahal. Tara Lata, like tree husband, stayed inside the house all her lifetime and turns out to be as a Tara Ma for the people of Mishtigunj. She started to teach the people of Mishtigunj and made them to participate in the freedom movement. Tara- Lata understands that her duty in life is to get freedom from the British Raj. British people do not like the attitude of Tara-Lata, and so they arrested her without any prior notice.

People of Mishtigunj do not know the cruel side of the British Raj. Only the news spreads that Tara Ma, the tree bride is died of heart attack inside the prison. When Tara goes on in search of this background news, she comes to have in contact with the spirit of Tara Lata. The spirit says that inside the prison she murdered cruelly and her body does not get a proper cremation. She also says that she was wandering in the mortal world to get a proper burial, so that she can enters the world of heaven. By understanding the real pain of Tara Lata, Tara asked her son Rabi to perform the rituals for his great grandmother.

In the mean -time, Tara, understands the love of her husband, when he saved his wife and son from a bomb blast. Then on, Tara takes care of her husband and believes that the love she had with others was only a temporary one and the love and support provided by Bish is everlasting. Tara on her root- search feels a mysterious connection to the tree bride. She has been married, borne a son, and has travelled all over the world. Yet, she asserts that she has never changed the world. In locating empowerment within Tara-Lata's narrative of extreme restriction, Mukherjee offers a feminist projection of such uninspiring and vapid ethnicity. Tara Lata, as portrayed earlier, plays a retrieving role to save her father from disgrace by accepting her fictional wed-lock to a lifeless tree. She in one

way helps in preserving the triumph of patriarchy, where her father plays a lead role in a macabre exercise of the marriage in woods. When Tara starts to write a book on her namesake in India, she is completely reminiscences by the glorious past memories she has with Calcutta. She remembers the good old days she has spent with her sisters, Padma and Parvati, and at the same time she has sensed “something new and strange”(22) that is happening in the world. The unearthing of the mystery of her great grand aunt feels like she was at “a point of light from the remotest, darkest galaxy of life.” (22) She believes that the truth behind the mystery may help her broaden up her perspectives about life and identity and will found a meaning for her existence.

Mukherjee in this novel connects both the past and present with the new element called mysticism. Tara’s curiosity for the tree bride and her association with the ghost seems to proclaim the strength of roots that binds generation after generations. Though Tara has lived in America, she has always felt crazy for her Bengali tradition. Like many other Bengalis, Tara too has crossed her path with the black waters and she felt that she had lost her cast. As a myth, she has gone and mingled with the casteless people in America, and has eaten red and white meat. Unlike the traditional Bengali woman, she has been divorced, has lovers and has tasted wine. When she thinks of her native town, she immediately remembers the tradition which is running in their roots for many generations. In case of Tara Lata, she too is a typical Brahmin woman who does not get away from her roots and became a role model for the future generation. When Tara has recalled about the tree bride, she has immediately thinks “When we dream or perhaps I should limit such a broad declaration only to myself, I dream of the past” (252). In an interview with Dave Weich, Mukherjee says “the authentic strategy for this book (*Desirable Daughters*) was also using the width of the field of history, geography, Diaspora gender, ethnicity, language-rather than the old fashioned, long clean throw” (29).

Mukherjee in these novels considers different pattern of belonging in the global perspectives from in-between temporality to assimilative permanence with hyphenated and unmixed nationess. The two novels, *Desirable Daughters* and *The Tree Bride*, has been written in the form of blending the family says of generations which is handed down to the younger generations. Though Tara, decides to walk down in Tara Lata’s footsteps in searching of her ancestral roots, she firmly denied to stay afloat and partially submerged in the glittering American ethos. Mukherjee portrays Tara, as a woman, who voices for her belief in the individual’s liberty and freedom to mould herself, to reconstruct and reshape her identity. “Life was of all a matter of shaping up and hitting one’s mark, satisfying expectations, achieving a quota.

Tara is not able to believe reality that represses and stifles one’s self-expression, expectations and one’s liberty. She keeps on asserts in the process of splitting and cultural dislocation which seems to lose her meaning and purpose in life. But after identifying the history behind Tara Lata, Tara has found a meaning for the dislocation and creates a new identity. Charles Taylor in his *The Ethics of Authenticity* writes:

Authenticity is a certain way of being human that is *my way*. I am called upon to live my life in this way, and not in imitation of anyone else’s. But this gives a new importance to being true to myself. If I am not, I miss the point of my life, I miss what being human is for *me* (29).

Hence, Tara finds her own way to lead her life by coming back to her native land and her quest makes her to find herself.

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**EXISTENTIAL DILEMMA AND A CAUTION TOWARDS AUTHENTICITY: AN
OVERVIEW FROM AMITAV GHOSH'S NOVEL *RIVER OF SMOKE***

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ABSTRACT

Life, an unpredictable game with human beings as participants, must accept both the pleasure and the pain it brings along with it for existence. When we ponder over the history of human suffering it reveals how the ancient people endured it with reliance to absolutism. The prevalent world filled with situations such as poverty, loneliness, absurdity, colonial syndrome, rootlessness, boredom, dissatisfaction, vagueness, patriarchy, empathy less society etc. presents crucial conditions for human survival. The ideological move towards freedom stresses on the sense of self reasoning and individualism. The shift towards the knowledge of freedom has encouraged the human mind to take decisions by their own will. This freedom of thought rejects the old beliefs as bad faith and the decisions made are derived from the traces of the traumatic life experience of the past. Human existence which precedes essence, at certain instances, result in existential dilemma. Existentialism, according to Cromwell in the present era demands to analyze it with the norm of authenticity "what is often taken to be its 'heart'" (Kaufmann, 12). Accepting this Ghosh lays stress on Thomas Flynn's concept of existentialism that insists on a way of life, where one is much accountable for his own existence. By giving a keen notice to human suffering and its implications, Ghosh's novel *River of Smoke* imparts a good understanding towards human existence and its relationship with authenticity. The paper attempts to analyze how authenticity becomes an essential factor in human life and how the characters who fail to provide it to their decisions suffer from existential dilemma which in turn results in existential crisis, spiritual crisis and suicides. Amitav Ghosh, an experimentalist, a historical and a comprehensive writer who has proven his excellence by winning the Jnanpith Award in 2018 writes on the changes that happen in human mind and behavior due to natural, social and political interference and cautions on the results towards lack of authenticity in human decisions.

Key words: situations, freedom, existential dilemma, existentialism.

In the novel *River of Smoke* written in 2012 Ghosh writes about the crisis which individuals faced in the first half of the eighteenth century, a crucial period before the opium war. His characters stay affected by both the external and internal factors and they try to resolve their problems by adopting freedom of thought. His writing which carries the effect of his anthropological research not only brings the history before the eyes of the readers, but also portrays human life and its changing standards. Mary Klages in her *Literary theory: A guide for the perplexed* recommends, "human investigation and thought" (klages10- 11) to frame sensible analysis. Likewise, Ghosh's realistic portrayal presents the hardship of the human life led amidst the norms of the family, society and their sufferance both physical and psychological, caused by both natural and artificial forces for sensible analysis. His plot unfurls how the endured hardships unknowingly impart many changes in individual. By writing this novel Amitav Ghosh reveals the hardships in human mind and soul and records the results of human existence that precedes essence in situations.

Amitav Ghosh's novel *River of Smoke* brings before our eyes the life in Canton and Mauritius in the first half of the nineteenth century. The historical background in which Ghosh's characters live, are imbibed with their life which makes the novel more realistic. The novel clearly reveals how the cultivation of opium, tea, sugar and many other commodities were wisely planned and planted by the British in their various colonies and how it was traded by the East India Company for making profits.

The novel starts with the life of the grimitiyas taken to Mauritius and were let free because of the anti-slavery act. This novel being the second part of the trilogy continues with the life of Deeti and her Clan in a distant land. The novel presents characters from various categories of life such as Bahram who hails from a poor background and tries to find his own identity in the society, Neel the Raja who joins as munsiji under Bahram, the pitiful condition of Bahram's illegal son, AhFatt, who stays unrecognized by Bahram, Paulette who escapes from the clutches of Mr. Burnham from Bethel and joins herself with Fitcher, the life of Fitcher the botanist, Robin Chinnery the painter, other merchants from various parts of the world who accumulate in Canton for trade, the Chinese merchants, lascars and many others who struggle for life in this critical period of history. It elaborates the quest of Seth Barham Modi and his intension to make handsome profit by selling opium in China during the restricted period and to buy the export division from his brothers-in-law by the profits gained from it.

The human struggle for existence has changed the approach towards life and Ghosh makes it evident through his characters. His characters start adopting freedom in their attitude which is the important characteristic of an existentialist. Existentialism as a theory according to Thomas Flynn not only talks about the free will, the choices, the neurosis or angst as an underlying reason behind transcendence but also lays concern over the personal responsibility and discipline in a society where traditions and religion are considered arbitrary. But the despair and the ambiguity of the individuals in this post-modern world have compelled some among them to think as an existentialist without providing emphasis on authenticity and responsibility to their decisions. Many philosophers such as Kierkegaard, a religious philosopher, Nietzsche, an anti-Christian, Sartre and Camus an atheist, are credited for their works and writings about existentialism. Among them Sartre is much acknowledged for bringing out the ideas of existentialism and integrating it by finding its traces in various fields in the 20th century.

Ghosh symbolically reveals the fallen condition of the world in the novel *River of Smoke* by statistically revealing the three vessels that are sailing in the sea during the storm. Among them, the Redruth, a two masted brig, was the only ship that was sailing with the intention of plant hunting without carrying any destruction to the cosmos. But the other two, the *Ibis* loaded with sorrowful slaves and convicts and the *Anahita*, with a great deal of harmful opium were taken to be traded in China. With this Ghosh keenly symbolizes the rising human greed and intension to gain living by harming others. The character Deeti, is set as a perfect example of detribalization. According to Walsh,

Detribalization is the process by which we escape from some of the distorting, constricted, erroneous beliefs of our cultural world view (Levinson, 1978). Through detribalization we are able to step back from these beliefs so that we no longer look through and identify with them; but rather begin to look at them, and in looking at them, disidentify from them, and in disidentifying from them, are able to work to transform both them and ourselves.

The novel starts with Deeti, a pious house wife from Bihar, and her transformation into an old matriarch leader of the La Fami Clan. Her annual intentional plan to visit the shrine, the place in which she and her son Girin stopped for a night during the storm long ago when she was gathering bananas for her son, is undertaken insisently to record the pain that life exhibits in the young minds of the Clan. She enlightens the young members of the Clan by treading over the path in which she has walked previously. She repeats and elaborates the traumatic incidents that happened in ibis and about the violent storm to the young members of the clan showing the figures drawn in the shrine. The figures that represent the characteristics of the members who travelled along with her stresses how people from different parts of the world lay connected in their path of life and how determination and toughness has become an essential element of life.

The existentialist talks of life in which the meaning is gained by their self effort. Chistopher Panza in his book *Existentialism for Dummies* defines

For the existentialists, life is about creation, about creating the ground for your own meaning, creating value and making your world, and making yourself into something... If God is dead,

the existentialist proclaims, life is not meaningless. It has the meaning you choose to give it.

(Panza 45)

Ghosh's characters reveal the fact that life takes its form which an individual tries to give it. In his plot, Ghosh uses the technique of duality in his characterization and provides a better understanding towards this essential concept. With his well-planned narrative and by presenting the characters, Seth Bahram Modi and Fitcher he denotes the consequences of individualistic decision. His characters make it evident that the economic struggle in a young age registers as a trauma in the heart and the intension of never to come across such experience creates anxiety in both Bahram and Fitcher to gain wealth for a better survival.

A comparison between these two characters and their way of survival in the world presents how choices differ from person to person to determine their living. Bahram who marries Shireen could not connect himself with the house of his father-in-law and his quest for identity lands him to choose a life by collecting and selling opium in a distant land, China. But Frederick 'Fitcher' Penrose, becomes a noted nurseryman, who has made a great deal of money by marketing seeds and saplings. His great enterprise Penrose & Sons was based in Falmouth in Cornwall and has gained enormous popularity in the British Isles especially for its Chinese importations. The alterity in the decision of these two characters, Fitcher and Bahram, who have risen in their life from a poor family background changes their approach to life. The choice of Fitcher to earn money by sharing and collecting species of plants and the greed of Bahram to earn money by selling the restricted opium reveals how their bitter past culminate with the individualistic instincts and reigns the human mind.

The economic condition of Bahram Modi demands him to marry Shireen the daughter of the Mistries and through a flashback the author intentionally reveals how in Navsari, in coastal Gujarat, Bahram's own family was reduced from prosperity to enormous burden of debt due to the rash investment of his grandfather and the father's consumptive habit. Ghosh's realistic narration presents the hardship of the family that mounts after the sale of their beautiful haveli by the grandfather and their tattered condition when they occupy a room at the edge of the town. Their survival was only by his mother's skilled needlework that fetched orders and helped to feed them and educate them. This economic situation becomes unforgettable in Bahram's heart and his aim to reach heights is supplemented by their old connection with the mistries that emerged when the Parsi businesses man Seth Rustamjee, ordered wedding shawl to his daughter's wedding mainly because of his lineage with the Modi's family who has supported the business of the Navsari's initially when they started their business with a small furniture workshop.

Bahram's quest for identity and his ambition of earning money inspite of his father-in-law's advice against starting an opium trading unit was intense because of the inferiority he experiences during his stay in Navsari. Even his establishment as a great Seth in Canton did not appease him and after his father-in-law's death, when his brother -in -laws insist on stopping the trade denoting the tensions that were mounting on opium trade in Canton; he sticks to his own decision and insists that "he had given the rumors and gossip much more attention than they had. The conclusion he had reached was exactly the opposite of theirs; it seemed to him that the present conditions offered an unmatched commercial opportunity, the like of which came only once or twice in a life time" (RS 55).

"Existentialism which could be summarized as 'freedom', 'responsibility' and 'authenticity' (Tidd 14)" differs in the perception of Ghosh's characters. Their freedom and rejection of responsibility and failure to provide authenticity to their decisions brings out dilemma in life. Bahram at a point of time suffers due to existential crisis. His journey towards Canton starts with the external storm that uproots and damages many chests of opium and ends with the storm of his inner mind that costs his life. A man who carries great privilege on becoming a Seth is struck with sorrow when he notices the growing strictness towards the trade of opium. His childhood traumatic experience due to economical suffering and his anxiety never appeases his yearning for money and he fears of the history getting repeated back in his life as it has happened previously in the case of his grandfather.

The author presents many instances in his narrative to prove how the childhood suffering conditions stays back in Bahram's mind and how he relates it with his present condition. One such

occasion is when Bahram is privileged and gets a letter from the committee to join as a member in Canton and Vico praises him saying, "Arre Patro! See what you have become now? You are a Seth of Seths" he replies to Vico with pride, "Bahramji Nauroji Modi, whose mother had made ends by embroidering shawls, has become a leader amongst a group that inclined some of the world's richest men" (RS 198).

Basically, a man who is loved by his servants for his kindness suffers with dilemma when Zadig questions him on continuing with the trade and asks, "Is it right to carry on trading opium when Chinese are against it" (RS 211)? Ghosh systematically reveals how people carry differed views over the same concept. The crisis in Bahram's life starts when he agrees to sell a small portion of opium to Allow to whom he was personally related because of Chi-Mei. But when his consignments are seized and when Allow is caught and assassinated for it by the mandarins, he feels guilty for the death of Allow. He also feels hard as he was the person who has sown the habit of opium in Allow when he was a small boy who helped him as a messenger for his meeting with Chi Mei.

Ghosh presents how freedom in thought creates the difference in opinion among the merchants in the matter of Free Trade. When merchants like Charlie King, Wetmore and Eliot were against trading opium: merchants like Mr. Burnham, Bahram, Dent and Innes were persistent in continuing opium trade with China. When he was nominated in the committee to decide on behalf of all the Indians about the opium trade, he did not think of the Chinese people who were suffering due to addiction. Instead, he thinks very subjectively and decides to be persistent with the decisions taken by the British merchants and Mr. Burnham. He justifies it saying, "Because it is not my hand that passes sentence upon those who choose the indulgence of opium. It is the work of another, invisible, omnipotent: it is the hand of freedom, of the market, of the spirit of liberty itself, which is none other than the breath of God" (RS 463).

When the strictness towards Free Trade worsens his condition and he is called on a meeting to surrender all his cargo and Captain Eliot assures him that "we will get back everything we give up and more. Our investors stand to make handsome profits. It is just a matter of waiting" (RS 518) he remembers the angry letters that were accumulating in his office. When he hears from Eliot that the investors have to wait for two or three years to get back their investment; the situation throws him into extreme grief and his guilt keeps growing and when Zadig consoles him saying, "It is only money, Bahram-bhai. Soon you will recover your loses" (RS 520). He reveals his extreme dilemma painfully regretting, "I gave my soul to Ahriman.... And it was all for nothing. Nothing" (RS 520).

The dilemma of Bahram on his own decisions is revealed by Ghosh and his existential dilemma is presented by the sleepless nights and his bothersome condition when he stays annoyed by the shadows thrown back by the Maidan and he could not digest the voices of the soldiers who trooped past the Fungtai Hong. Even laudnam does not work to bring peace to his mind but it made the sounds louder and dreams more vivid. "The thought of food made Bahram nauseous" (RS 525) and with days and nights moving hard, he refuses to move outside with Zadig. His existential dilemma pushes him to the verge to end his life by climbing down the sea, using a rope ladder through the window. A man who does not wish to acknowledge Chi Mei in his life and who says to Zadig that there is no intense bondage with her dies imagining of getting united with her. Bahram's mechanical relationship with Shireen which made Chi Mei enter his life has pinned an unacknowledged relationship in his heart which is revealed at the time of his death.

Ghosh through his well-constructed plot of Bahram and utilizing the technique of back story reveals how existential dilemma can be caused due to thoughtless, unauthentic decisions. He precisely presents how the once approved factor, creates a dilemma in Bahram's future life. Bahram's story is a parody of many recent incidents all around the world where people suffer and encounter tragedy because of their own decisions. On the other hand, Fitcher a man of unusual accomplishment and considerable wealth has raised himself by marketing seeds, saplings, cuttings and horticulture implements- and his patented materials were of great demand in England. By narrating about *the Redruth*, and by the character of Fitcher, Ghosh denotes the ethical way of livelihood, where one can make his living and lead a passionate life by adoring nature and finding fulfilment by tracing the hidden treasures which has been stacked in different parts of the world.

When Burnham approves to make money by selling opium, Fitcher frames his life by transporting and preserving sampling. When Burnham tries to embrace prosperity by selling the evil drug opium, Fitcher decides to make his fate by finding the golden Chamelion which can create magic in human health. This alterity in decision creates a big difference in their life. When Fitcher leads a prestigious life until his death, Bahram dies of remorse on his own choice.

Although existential crisis creates sufferings, it also provides way for new possibilities that open up when one faces the situation honestly. It is also assisted by the anxiety and determination which one carry due to the intensity and traumatic fear towards the situation. The question 'What's next?' that occupies human mind makes them to think of the Other and new truths are framed by transcending the old beliefs as bad faith. The failure to provide authenticity to the new truths that they frame to proceed in their life, at circumstances results in dilemma from where their return to the previous stage becomes impossible. Thus, Ghosh clearly specifies that existentialism demands to find meaning to one's life through free will and choice where personal responsibility and authenticity will help to lead a remorseless life.

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FOOD AS A METAPHOR IN GITHA HARIHARAN'S SHORT STORY THE REMAINS OF THE FEAST.

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Abstract

Food is closely associated with our culture and emotion. Among the basic necessities which are essential for human life, food occupies prime position. In Indian culture, eating food together is our tradition to strengthen familial relationships. Lord Krishna clearly explains in Chapter 17 that the purpose of food is to increase the duration of life, purify the mind and aid bodily strength. Food could be considered as a symbol to showcase one's class, kinship, religion and many. Incorporating food as "metaphor" in any work of literature creates verbal and emotional impacts within the minds of the readers. In Githa Hariharan's short story *The Remains of the Feast*, food plays a vital role to understand the existing complexities. This paper is an attempt to examine how food is used as a metaphor of subversion.

Keywords: morality, hygiene, resistance, clandestine, liminal space, embodiment, agency.

Githa Hariharan, one of the prolific writers in India is known for her care and concern with women. As a Social Activist, editor and columnist, and renowned writer has written many novels, short stories and critical essays on different topics. *The Thousand Faces of Night* (1992), *The Ghost of Vasu Master* (1994), *Times of Siege* (2003) are some of her notable works. Her collection of short stories includes, *The Art of Dying* (1993), *The Winning Team* (2004), *A Southern Harvest* (1993) etc. Her works are translated into many regional languages.

The Remains of the Feast, is one of the inspiring short stories in the collection entitled, *The Art of Dying*. This striking story has a remarkable plot, which combines humour and a tinge of sadness. The story is about an old woman, who is at the deathbed and her urges to taste all the food, which were forbidden to bring inside a brahmin's house. Mary Anne points the use of food in literature as, "writers often use food as a rhetorical device to represent the abstract internal and difficult to express concepts." (192)

Lord Krishna clearly explains in chapter 17 of *the Mahabharat* through the following verses:

ayuh-sattva-balarogya-sukha-priti-vivardhanah
rasyah snigdham sthira hradya aharah sattvika-priyah
katv-amla-lavanaty-usna-tiksha-ruksha-vidahinah
ahara rajasyesta dukkha-sokamaya-pradah
yata-yamam gata-rasam puti paryusitam ca yat
ucchistam api carmedhyam bhojanam tamasa-priyam

Foods in the mode of goodness increase the duration of life, purify one's existence and give strength, health, happiness and satisfaction. Such nourishing foods are sweet, juicy, fatty and palatable. Foods that are too bitter, too sour, salty, pungent, dry and hot, are liked by people in the modes of passion. Such foods cause pain, distress, and disease. Food cooked more than three hours before being eaten, which is tasteless, stale, putrid, decomposed and unclean, is food liked by people in the mode of ignorance.

The purpose of food is to increase the duration of life, purify the mind and aid bodily strength. This is its only purpose. In the past, great authorities selected those foods that best aid health and increase life's duration, such as milk products, sugar, rice, wheat, fruits and vegetables. These foods are very dear to those in the mode of goodness. Some other foods, such as baked corn and molasses, while not very palatable in themselves, can be made pleasant when mixed with milk or other foods. They are then in the mode of goodness. All these foods are pure by nature. They are quite distinct from untouchable things like meat and liquor. Fatty foods, as mentioned in the eighth verse, have no connection with animal fat obtained by slaughter. Animal fat is available in the form of milk, which is the most wonderful of all foods. Milk, butter, cheese and similar products give animal fat in a form which rules out any need for the killing of innocent creatures. It is only through brute mentality that this killing goes on. The civilized method of obtaining needed fat is by milk. Slaughter is the way of sub humans. Protein is amply available through split peas, dal, whole wheat, etc.

Foods in the mode of passion, which are bitter, too salty, or too hot or overly mixed with red pepper, cause misery by producing mucus in the stomach, leading to disease. Foods in the mode of ignorance or darkness are essentially those that are not fresh. Any food cooked more than three hours before it is eaten (except 'prasadam,' food offered to the Lord) is considered to be in the mode of darkness. Because they are decomposing, such foods give a bad odour, which often attracts people in this mode but repulses those in the mode of goodness.

Remnants of food may be eaten only when they are part of a meal that was first offered to the Supreme Lord or first eaten by saintly persons, especially the spiritual master. Otherwise, the remnants of food are considered to be in the mode of darkness, and they increase infection or disease. Such foodstuffs, although very palatable to persons in the mode of darkness, are neither liked nor even touched by those in the mode of goodness. The best food is the remnants of what is offered to the Supreme Personality of Godhead. In *Bhagavad Gita* the Supreme Lord says that He accepts preparations of vegetables, flour and milk when offered with devotion. "Patram puspam phalam toyam" (*Ibid.*, Chapter 17). Of course, devotion and love are the chief things which the Supreme Personality of Godhead accepts. But it is also mentioned that the prasadam should be prepared in a particular way. Any food prepared by the injunctions of the scripture and offered to the Supreme Personality of Godhead can be taken even if prepared long, long ago, because such food is transcendental. Therefore, to make food antiseptic, eatable and palatable for all persons, one should offer food to the Supreme Personality of Godhead.

In "The Remains of the Feast," the old woman Rukmini and her abnormal activities, challenges the norms and values which are already existing. The dying grand mother's relationship with the narrator of the story seems to be painful. The use of imagery, symbols in narrative technique makes the story more interesting. Even a small object in the used by the character could convey certain sense of meaning to the plot. Both Ratna and Rukmini, shares equal space in the story. The writer herself admits it in one of her interviews as, "there is no one single authority in my stories."

Ratna, the narrator of the story is the granddaughter of ninety-years old woman Rukmini. Her memories with her great grandmother were expressed well in the adjectives she shared, such as "ignorant village - bred woman, faded rose, hilarious, ambitious and a giggling little girl." (12) Ratna is preparing for Medical Entrance Exams. Her grandmother, who has become a widow at a very young age, sacrifices her life for others in the family. A huge limb at Rukmini's throat indicates the growth of cancer cells. Being an orthodox woman, she is adamant and ignores the advice of others to take treatment. She is in her terminal phase, where her death is fast approaching, is forcibly taken to the hospital. Rukmini ignores the advices, concerns and even threats from the doctors. Ratna and her parents conceal the degrading health condition of Rukmini from relatives. A sudden change in the behaviour of the old lady surprises everyone. Ignoring her critical condition, she develops a craze to eat outside foods. All the food items, which she mentions are neglected to bring inside Brahmin's house. The concept of hygiene and morality is questioned in the house now. To hide Rukmini's desires from others, Ratna secretly brings spicy food items and egg-cakes, which Rukmini tastes during the night times. Slowly her act of consuming food during midnight becomes clandestine. Her strange partnership with Ratna, despite the massive differences in their age, is made to fulfil the desires of Rukmini, who is going to face death soon. She eats ice-creams, samosas, cakes from the street bazaars. The vegetarian house loses its morality slowly by intaking the foods prepared by non-brahmin hands. It has become an adventurous incident for Ratna to get outside food from the bazaars secretly and to have it along with grandmother during midnight. Rukmini's unpredictable cravings to consume different varieties of food is outside their moral parameters. Ratna becomes tired of these midnight feasts.

The old woman's strong desire to eat cakes made of eggs is humorously expressed by Ratna as, "her pink ton darted out and licked the frosting. Her toothless mouth worked its way, steadily, munching, making happy sucking noises." (13) Day by day, her intention to taste unhygienic food from streets becomes stronger, which often puts herself in trouble. Her urgency to swallow food or sucking aerated drinks like coco-cola in a steel tumbler shows her contentment after consuming them. Like a small child, she opens the bottles enjoys the popping sounds of the bubbles shows her path to the second childhood - old age. The family members worry about her health condition, because it becomes worse every day. The symbolic desire to consume foods shows her longingness, Ratna mentions it as, "she had tasted, by, now, lemon tarts, garlic, three types of aerated drinks, fruit laced cakes with brandy, bhel puri from the fly infested bazaar nearby." (13) The intake of unhygienic foods made an adverse effect in her health which is actually in poor condition. Her uncontrollable desires result her to get hospitalised for more than ten days. Ratna and her mother lends moral support to the old woman, the former shares Rukmini's losing of mind as:

It burns, it burns, she would yell then, but she pursed her lips tightly together when my mother spooned a thin gruel into her mouth. No, no, she screamed deliriously. Get me something from the bazaar, Raw onions, Fried Bread. Chickens and Goats. (14)

The next ten days in the hospital is miserable. Sitting beside the old woman day and night onlooking her sufferings is painful for both Ratna and her mother. She refuses to intake medications prescribed. Her hands clenched and at last she becomes quiet. The last minute on the deathbed is not peaceful. She pulled the tubes and crashed the I.V poles on the floor, her voice cracked and she sweats. Her last words convey her furiousness, she screams, "bring me a red sari, a red one with big border of gold. And bring me peanuts with chilli powder from the corner shop. Onion and green chilli bondas deep fried in oil." (15) While Ratna is seeking the blessings of the dying lady, she rejects, and at last she died desiring street foods. Her desires and cravings remain unfulfilled during her death.

The death of the old woman creates a great impact on the minds of Ratna. The former experienced patriarchal oppressions inside her tradition. Her husband died leaving her as a widow at a very early age. The only child of her is no more now. The cancer cells inside her body are slowly eating herself. As a resistance to all her sufferings, she started to consume non-vegetarian foods too. Starting from food to dress, she asserts her rights to fulfil her hidden desires. The miserable loss of the great grandmother is felt everywhere, Ratna shares her pain as,

I search her, my sweet great grandmother, in plate after plate of stale confections, in needle like sharp green chillies, deep fried in rancid oil. I plot her revenge for her, I give myself diarrhoea for a week," (16)

FOOD AS A METAPHOR IN GITHA HARIHARAN'S SHORT STORY THE REMAINS OF THE FEAST.

Food here is mentioned as a "symbol of remembrance." Dr. Roopa Philip in her study, makes a clear statement that, " food becomes the source of assertion of self and agency, even if it is short lived and temporary."(47)

Food is used as a metaphor to represent the "re-silence". The longingness of an old woman is a permanent activity to fight against her miseries. Her wilful consumption of "foods which are considered as taboo in a brahmin's house" shows her as a symbol of subversion. Food symbolises the discursive link between hygiene and morality. On the other hand, the text highlights the room of a woman's desire in the conservative societal structure. The liberalistic attitude of city-bred younger generations to consume non-hygienic foods and its adverse effects on their hearth are clearly revealed in the story.

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HERZOG AND THE FOREIGNER - A COMPARATIVE STUDY FROM EXISTENTIAL PERSPECTIVE

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ABSTRACT

Saul Bellow's popularity reached new heights after writing *Herzog*. Bellow dealt with a complicated theme on an experimental basis by probing into the protagonist's mind through his unpublished letters. As soon as it got published in 1964, the *Herzog* novel earned a rousing reception everywhere. The novel is all about five days in the life span of Professor Herzog, it includes concept pertaining to philosophy, politics, morality, psychology and literature. Professor Herzog is on the point of becoming insane due to his unexpected divorce of his second marriage. The novel is a tell tale record of development from depression to positive view towards life.

The *Foreigner* is the maiden novel of Arun Joshi that gave him instant popularity among Indian novelists. The theme of the novel is about the conflict between the East and the West and the novel bears ample testimony to Arun Joshi's keen perception of society and time in the cross cultural background. The novel discusses an individual who experiences alienation from his society, environment and personal identity. Joshi plunges deep into the dark recess of a human mind and the novel is littered with the personal experiences of the author. This research article makes an attempt to carry out a comparative study between these two novels based on existential perspective.

Key words: romanticism, existentialism, physical intimacy, human suffering, innocent sufferer.

The novel *Herzog* initiates an intellectual discussion on romanticism and existentialism. Romanticism took off in Europe during the eighteenth and nineteenth centuries and it talked about the good aspects of human beings. It presented the human soul as a captive of society. In the novel *Herzog*, the protagonist is a romantic sufferer to begin with and finally he becomes an enlightened individual. He is a middle aged person who works as a professor in a university. He suffers from insanity after his second divorce. His mind is teased with some uneasy thoughts and he looks for solution to his unsolved puzzles regarding existence. He is over intellectual and this quality lands him in a chaotic frame of mind. Hence, *Herzog* abodes in the realm of unsolved questions. *Herzog*'s mind turned to be a cauldron of boiling questions. He desperately looks for answers but they are hard to come by. His personal agony culminated into a highly dissatisfied and complaining self. Though he is blessed with a scholarly wisdom, he nurtures personal, literary, historical and political complaints. However, he is not worried about writing letters to anyone and instead keeps them in a valise. These letters carry enormous information about his past, his reaction to it and his thinking process. In this manner, these letters are necessary part of the novel's structure. His letters express his psychological disintegration and it is an effort to move back and forth and identify reason behind suffering and death.

Herzog is rendered helpless because of the idea of being done in by his wife and his best friend. His psyche is imbalanced when he becomes aware of the fact that Valentine and Madeleine have fooled him tactfully. They wished to call him insane.

Many theories can be presented pertaining to *Herzog*'s madness. According to one critic, *Herzog*'s experience in second marriage is treated as a transition from state of innocence to state of experience. However, this belief can be ridiculed based on *Herzog*'s self initiated divorce in the first marriage. There is no logic in using the term innocent to a scholarly wizard like *Herzog*. *Herzog* can be read as experience of an individual who is a victim of self doubt pertaining to his social relevance. For a brief period, *Herzog* gets alienated from his society. He remembers his past incidents so as to figure out his share of guilt. Temporarily, he dislikes himself. His mind is burdened with doubts regarding his individuality. He had problems in following a monotonous life in his first marriage.

The novel has to be examined from existential perspective. To begin with, Herzog was thought to be an innocent sufferer. Slowly, he takes responsibility for becoming what he is. He admits that all his hardships are because of his own decision. He maintained an amorous relationship with a Japanese girl named Sono earlier. Later, he divorces Daisy to lead an independent life. He enters into a wedlock with Madeline knowing very well that she is conceited, self-centered and unpredictable. He quits his attractive job and shifts his abode to Ludeville. Madeleine finds fault with him for being a despot and insensitive. It was an error of judgment that he deserted his first wife. If the married life had continued, he would have led a contented life as a university professor. Instead, he plunges himself into deep struggles of second marriage. He, finally, decides not to kill Madeleine and Valentine. Had he killed them, he would have become a murderer and invite punishment for the same. He understands that he has no right to take away anybody's life. One day, He finds Valentine bathing his daughter. It dawns into his head that Valentine is also a distinct individual who has a right to live. He also finds Valentine treating his daughter affectionately. Hence, he discards the thought of killing Valentine and Madeleine.

Hence, suffering is very much essential to move out of an illusionary world. Although there is no romantic notion attached to suffering, it is considered as an essential part of human life. To add more, it is a kind of Quixotic suffering experienced by an individual's mind. Mind always aspires for dizzy heights and hence failure is essential to bring it back reality. Being an intellectual, it is safely assumed that his perception about human suffering is also very narrow. He opines that mind is the cause of all sufferings. If it can create a suffering, it can also refuse the same. His mind does the same. He stops wasting time on pointless arguments. He severs himself from his egoist self. He considers Madeleine as his past and Ramona as his future.

The *Foreigner* is the maiden novel of Arun Joshi that gave him instant popularity among Indian novelists. The theme of the novel is about the conflict between the East and the West and the novel bears ample testimony to Arun Joshi's keen perception of society and time in the cross-cultural background. The novel discusses an individual who experiences alienation from his society, environment and personal identity. Joshi plunges deep into the dark recesses of a human mind and the novel is littered with the personal experiences of the author.

The novel focuses on the life of Surrinder Oberai. He is affectionately called Sindi. He is an orphan who suffers from loneliness in this world and "wants to do something meaningful." (Joshi : *The Foreigner* 14). He goes in pursuit of solace and the actual meaning of the term existence. He does not experience a sense of belonging anywhere at Kenya, Uganda, London, America and India. He suffers from detachment, possession and involvement. In the climatic stage of the novel, the readers find his life being anchored in India. Here, he understands to live not for himself and his narrow selfishness. He starts living for those who really need him and this change of attitude is affirmed through detached action which has been explained by Lord Krishna in *Bhagavat Gita* through *anasaktikarmayoga* theory (detached action). The novel brings forth the sufferings of Sindi who is denied love and cultural identity in the din and bustle of his city life. In the opening scene of the novel, Sindi visits a morgue at Boston to identify the dead body of Babu Rao Khemka. Babu Rao Khemka happened to be an engineering student who died in a motor accident. Sindi conveys this tragic information to June Blyth who was his fiancée as well as carrying his child. The story goes back and forth regarding past and present events. In the present, Sindi visits the house of Mr. Khemka after two months his son's death. Mr. Khemka takes pity on him and places him in his own company. However, Sindi loathes pity from outside and so he considers the business run by Khemka to be like "carving out a little niche" (15) for him still he cannot resist the temptation of joining in his company so that he does something meaningful in his life. During his sojourn at Delhi, Sindi is troubled with some uncomfortable questions regarding circumstances that lead to Babu's death.

Sindi completed his studies and obtained a project in New York. He keeps going to Boston once in a while. He meets June and has a detailed discussion with her regarding love and marriage. After the discussion, they experience physical intimacy. When June comes back home, she explains

everything to her husband. This flabbergasts Babu and makes him drive his car madly and get killed in an accident. Sindi took the responsibility of informing Babu's father about the accident. Sindi returns to India to find answers for his existential concerns. Just before his departure to India, he receives a letter from June asking him to visit her. From the letter Sindi understands that June has been bearing Babu's child in her stomach. Sindi goes to Boston after a week. However, it became too late as June had died in an attempted abortion. Sindi's guilty consciousness touches new heights and he reels under the impact of it. He comes to India to get rid of this guilty consciousness. He gets placed in Mr.Khemka's business. But he feels uneasy in cooperating with Khemka in his illegal business practices. He thinks of moving to Bombay. But the plight of workers like Muthu dissuades him from doing it. The novel concludes with Sindi's readiness to take over the mantle of Mr.Khemka during his period of imprisonment. The novel finally offers a solution for Sindi's existential problem by saying that to exist for other's welfare is the authentic existence and the true meaning of detachment.

Both Saul Bellow and Arun Joshi are similar in their approach in depicting human existence. Bellow mostly depicts individuals who indulge in introspection to identify a solution for settling a conflict between the old world and the new world values while managing with personal anxieties and aspirations." (99). The novel Herzog is about a middle aged professor of history who abodes temporarily in his home at Berkshire named Moses Herzog. The protagonist Herzog suffers from an internal conflict when the novel starts. He practices the habit of drafting letters to his family members, friends, acquaintances, scholars, writers and the dead. However, he seldom posts them. In the early part of the novel, the readers find Herzog shortly after his divorce from second wife Madeline. The divorce is the result of his second wife Madeleine taking up his bosom friend Valentine Gersbach. He has problems in comprehending that mis-happenings in his life. He becomes a paranoid and is certain that several figures in his life his doctor, his lawyer, his therapist and his aunt conspired in wrecking his married life. He writes letters to them but seldom dispatches them. His letter writing is a method employed by him to recover from the crisis he is faced with. Both Mushtag Abdulhaleem Mohammed and Abdulkarim Musir Hamadallah opine that:

"Bellow emphasizes the role of the individual who is overwhelmed and overburdened by personal and interpersonal crisis in a perplexed society." (180)

Bellow has exhibited that Herzog is an individual who has taken up writing to anoint his rumbled feelings. His married life has failed twice. Bellow tactfully unfolds the miseries of the protagonist Herzog through a series of letters that he writes.

The novel Herzog talks about fragmentation of human identity due to modernization and the era where moral values are considered as needless virtues and a woman such as Madeleine never feels worried about deceiving her husband. Infact Madeleine is a symbol of the extreme condition of a man-woman relationship in a married life.

The novel is a tell tale presentation of the plight of modern man who appears", to be very much pinned into the labyrinth maze of fast paced, ambitions, material and morally debased world. Quiato Fan observes in his article that Herzog symbolizes the realistic views of Saul Bellow pertaining to modern society and man's predicament especially the problems faced by intellectuals. He is a literary product who represents the historical background of the United States in the 1960s. It was a time when utilitarianism was quite rampant. With the advent of new technologies, the post war America very much acquired abundance in material wealth. On the contrary, spirituality and moral values started dwindling. Modern scientific technology made a significant presence and at the same time , the increasing pressure of the Cold war brought about a series of problems; unemployment, poverty, crime, violence, social unrest and so forth.

This novel by Saul Bellow is an outcome of several significant events like The Great Depression, World War II, the Cold War and the Vietnam war which caused disillusionment. Any way, Moses wishes to get liberated from the crisis in his life. In this connection, Saul Bellow displays some positive thinking in Moses at the end of the novel where he stays for a night with his friend Lucas Asphalter. Through Lucas, he manages to meet his daughter June. It is literally through this father and daughter relationship, Bellow has emphasized the importance of one's crisis moment. Next day morning, Moses accompanies June to an aquarium. When they leave the place, Moses meets an accident. Because of the accident, Moses becomes unconscious whereas June remains unhurt. Later on, Moses lands up in prison for charges of possessing weapon with the help of which he has extracted revenge on his wife Madeleine and Valentine, her loves. Moses is taken to prison. However his brother gets bail for him. After his release, Moses comes across Ramona. It is through Ramona that Bellow answers all questions of Herzog. Now Herzog is able to relate woman in a new manner. He no longer thinks of marriage as an easy solution. On the contrary, he views sex as a means to cure himself. At the end of the novel, he is well contented with his contrary home and pleasant weather. He no longer wants to write letters to anyone as he is able to maintain his mental balance.

Likewise, Arun Joshi pictures the modern man's dilemma of human existence in his *The Foreigner* novel. The novel makes an attempt to describe the essential nature of human existence. One striking difference between Saul Bellow's *Herzog* and Arun Joshi's *The Foreigner* is that in *Herzog*, the author shows the identity crisis experienced by one individual. On the contrary, in *The Foreigner*, there are clashes between two different cultures which result in fragmentation of identity. The protagonist Sindi's identity is torn apart as he does not know himself. He lives without any purpose quite similar to Herzog.

The novel *The Foreigner* is all about the life of Sindi oberoi. He is the son of a Kenyan – Indian father and English mother. He lost his parents in an air crash and becomes an orphan at a tender age. There is a similarity between Herzog and Sindi. Both of them are denied love and care. In the early part of the novel, the readers find Herzog being tormented by the betrayal of his spouse Madeleine. Likewise, Sindi is in short of emotional nourishment which is the cause of his identity crisis. His life shows him as a struggler. He happens to be a dish washer and a barman at Saho. Later on, he moves to Scotland where he finds employment in a small village library and starts conversing religion, God and mysticism with a Catholic priest. He studies on Boston. There he happens to meet June and Babu. He comes back to India and settles at Delhi. But he finds his existence to be meaningless in India. The novelist very tactfully brings out the alienation of Sindi Oberoi which causes existential concerns and sufferings Sindi says:

“My foreign background stood against me.”

It exhibits his psychological trauma in an alien land. Joshi has described Sindi as an individual who has faith in the theory of detachment. However, in the presence of female companions, his belief in detachment gets shattered. His detachment ruins his relationship with June and she turns to Babu, a young Indian student. Both decide to get married. However, it does not take place due to the erotic relationship between Sindi and June. Babu is unable to withstand this disappointment and he commits suicide. June also passes away as neither Babu nor Sindi render her any stable support. It is Sindi's false belief of in detachment which causes the death of Babu and June. Sindi leaves America and comes to India. Here, he settles down in Delhi. This transition is very significant as far as self realization is concerned.

In describing existential concerns, there is a similarity between Bellow and Joshi. Both Herzog and Sindi are sufferers of existential concerns and their life is thick with conflict, anxieties and depression. Quite like Joshi's June, Bellow's Herzog is also rendered helpless.

In Arun Joshi's *The Foreigner*, Sindi, Babu and June are constantly in a state of mental trauma. Sindi takes efforts to identify his roots as he does not belong to anywhere. The novel talks about the existential dilemma of people who are the sufferers of identity crisis. Due to his detached policy, Sindi suffers from mental trauma in his relationship with his females friend Junes. She feels being exploited in their relationship. Their physical intimacy makes her guilty and causes existential anxiety. However she finds a thin ray of hope in Babu who wishes to marry her. Very sadly, Babu becomes a vision of the unholy relationship between Sindi and June. June also follows suit. Hence, both Saul Bellow and Arun Joshi have made a fantastic effort in portraying identity crisis in their respective novels. These novels gain importance even in 21st century where human existence has become much more challenging. Existential problems are very much rampant. There are several people who live without any purpose in their lives similar to Bellow's Herzog, Daisy, Madeleine, Valentine Gersback and Joshi's Sindi, June and Babu. They do not understand the value of their life. Both Bellow and Joshi bring out the identity crisis in human beings so that these problems are rectified and people live in peace.

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HOMELAND THROUGH DISRUPTION IN THE NOVELS OF AMITAV GHOSH

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Abstract:

Diaspora may be defined as a group or groups of people dislocated from homeland and then, getting re-rooted in numerous homes on the unfamiliar soil, being emotionally involved to each of them through some harmony and constant crave for their roots back 'home'. Diaspora can be understood as consequences of imperial dominance, the displacement of people through slavery, indenture and settlement. It not only involves geographical dispersal of significant number of people, but also the "identity, memory and home which such displacement produces" (Ashcroft, Griffiths, and Tiffin *The Empire* 218). The various forms of displacement, such as exile, diaspora, relocation, have been forcefully investigated in both postcolonial theory and Indian literary scenario. The offshoot of colonialism has centred its focus on postcolonial contemplation. Diaspora narrative brought about copious change in the cultures, epistemologies and politics of the post-colonial world. In due course, Diaspora shed off its particular phenomenon assuming dissimilar and heterogeneous aspects in its features. The issues of Diaspora writers such as his affiliation to homeland, culture and his identification captured my notion for investigation. It is pertinent to quote the opinions of some experts to bring my point at home: Diaspora refers to legal or illegal practice of border crossings; and after being dispersed diasporas remains transitionally linked with a real or symbolic homeland (Clifford 304-05). Diaspora studies are, generally, concerned with cultural dislocation, examining the effect of displacement in relation to a new constitution of cultural meanings (Ashcroft, Griffiths, and Tiffin *Key Concepts* 75).

Keywords: Diaspora, nostalgia, cultural dislocation, homeland, identity

Diaspora literature comes from the margins, entering the arenas that it is allowed to occupy. There has been steady flow of novels and short stories from Indian writers residing abroad while their hyphenated identities and longing sense of homeless, rootless personality emerge from their works. It is a normal tendency among the writers initially about their own experiences riding on the wings of their creative acumen, the works of diaspora focus on the issues of dislocation. Their writings reflect their nostalgia as they write about their existing home embracing their roots and their past that is homeland. Extremely sensitive about Indian cultural background, they connect to Indian history, culture and identity. V. S. Naipaul, Salman Rushdie, Rohinton Mistry, Pico Iyer, Amit Chaudhari, Vikram Chandra, Anita Desai, Kiran Desai, Upamanyu Chatterjee, Bharati Mukherjee, Jhumpa Lahiri, Hanif Kureishi are some of the writers contributing to Diaspora Literature. The texts of diaspora writers reflect journey between source culture to target cultures, between homelands and diaspora until the two overlap and merge.

Although Amitav Ghosh's diasporic confederation are not as clear or straightforward as those of the other novelists. All migrants who have made their homes in other geopolitical spaces, Ghosh's embrace of the diaspora aesthetic is evident in his fundamental rejection of the purity of cultural identities and the idea of the coherence of 'home'. Ghosh himself has argued that the Indian diaspora is not so much predisposed to 'roots', the desire to return to the 'homeland', as it is to 'routes', the ability to recreate a distinct culture in discrepant locations ('The Diaspora in Indian Culture' 73-78). Life odyssey of Amitav Ghosh, his affiliation to multiple locations, humanitarianism and cosmic psyche, have worked as an inspiring force for exploring the perceptions of homeland in dislocation. Like Rushdie, Ghosh's concern for historical affiliation with homeland stories shows his deep-rooted bonds with Indian ethos. The wide canvas of Indian life has been sketched successfully through writing. The native culture, predominantly Bengali, has often found centrality in his fiction. In other words, Ghosh's life, experiences, observation and inner psyche has been reflected in his writing. The

nostalgic feeling for the homeland exhibits in the telling of its stories can be seen as function to (re)claim India as homeland. Homi Bhabha in *The Location of Culture* states that the belonging is affiliated to these locations but the longing for the homeland reflects through memory and nostalgia. Amitav Ghosh chooses natural themes such as occurrences in an individual's life, conflict between nation and people, the role of memory in one's recovery of identity in the march of time, the feeling of nostalgia, the problems of migration, the sense of longing, love towards native land and the nature of adaptability in the place of settlement. Ghosh's novels reflect the spontaneous act of crossing boundaries — travels, migration, exile due to various reasons. The rooted characters immigrated in new socio-political, cultural and economic milieu continue their perennial quest for home. Dislocation results in the fate of homelessness, or constructing homes in memory.

Ghosh's novels foreground the longing sense of homeless, rootless persona; reflect protagonists' nostalgia about their existing home embracing their roots and their past that is homeland. One of the major concerns of the characters is the journey between source culture to target cultures, between homelands and diaspora until the two overlap and merge. His characters put themselves in an everlasting quest for selfhood, as they move from almost all familiar Indian ambiances to a completely different kind of locale in which they have sometimes to be a compulsive life. The characters upholding the idea of distinct homeland unsettled themselves relegating to the sense of insecurity—uncanny. The displaced individuals lounge on two tirades, either they could acculturate themselves or trapped in a hostile ambience.

The characters' homeland lingers in their inner psyche even if they get displaced in a new land. The memory and longing for home in a shifting cultural paradigm rings a sense of rootlessness. *The Circle of Reason, The Shadow Lines, The Glass Palace, The Hungry Tide and Sea of Poppies* are interposed with travel motif with generations and individual's sense of longing for home. Diasporic fate of characters brimmed with problems of home revealed through rootlessness, nostalgia, memory and alienation. The characters get reprieve or a sense of relief from tense situation generated out of displacement in host land through the memory of past home. The flashback technique has been adopted by Ghosh very brilliantly to intensify the problems of home. However, the aggressive imperialism, colonialism and capitalistic fervour threatened the idea of fixed, distinct home for individuals. Some immigrants showed the sense of unavoidable dislocation who withdraw into themselves in isolation and derive a sense of release recollecting the past walking down the memory lane. Prof. Makarand Paranjape in his seminal work *In Diaspora: Theories, Histories, Texts*, succinctly depicts consequence of physical and psychological alienation saying: "the homeland becomes so far that the motherland remains frozen in the diasporic imagination as a sort of sacred symbol, almost like an idol of memory and imagination" (9). As this homeplace occupies a vital and dominant place in one's livelihood, great writers like Salman Rushdie, V.S. Naipaul, Amitav Ghosh, Bharati Mukherjee, Jhumpa Lahiri, Kiran Desai have taken this complex issue of 'belongingness' and search for home in their writings.

Ghosh delineates an array of victims of diaspora who stand basically detached, yet strongly attached to the nostalgic past. Consequently, they develop a sense of insecurity and as they encounter a counter culture, they attempt to resurrect their nostalgic homes. Ghosh's characters never hold on with attachment or to be called a home, there exists only movement from one place to another in search for security and love. Ghosh himself has argued that the Indian diaspora is not so much predisposed to 'roots', the desire to return to the 'homeland', as it is to 'routes', the ability to recreate a distinct culture in discrepant locations. The novel, *The Circle of Reason* offers nothing, which we would normally call home. Initially located in a refugee village, the story refers back to Bangladesh and Calcutta, finally moving to the Middle East via Kerala where it reaches its denouement in a desert of shifting sand-dunes. In addition to it, Ghosh's characters derive their sense of consolation from home memories. When characters attempt to make their living; they are drawn towards their homeland, an image of the form that condenses the character's desire. For Ghosh's characters like Alu, Jyoti Das, Rakesh and Prof. Samuels, home in a form of domesticity are undeniably alluring for these characters long for a perfect home. He was much aware that the attractions of domestic security could pull characters back from the realm of very different – potentially more adventurous or

exciting life. But at the beginning of the novel, Ghosh points out that the secure home proved entrapping and oppressing for those characters.

Ghosh has presented in his fiction many instances of cropping up a deep longing for homeland. While drawing instances of this nostalgic intricacy, he attempts to establish the point that the reminiscences of the past help immigrants to reprieve from the present miseries. Home memories are generated only in those immigrants who refuse to acculturate themselves in the existing cultural space. In a realistic framework, they kindle the home memories, which naturally help them overcome all obstacles and strengthen the individual to extremely difficult circumstances. The instances of search for roots-home through memory, longing and nostalgia amply pervades in the novel, *The Shadow Lines*. The structural division of the novel in two parts "Going Away" and "Coming Home" symbolizes displacement as the ambivalent meaning of home. The glorious memories of Calcutta and Dhaka are beautifully pictured by the characters longing for their homelands. The past home memories act as a recurring background throughout the novel. In this novel, Tha'mma and Ila are physically and culturally dislocated due to partition and globalization and they recover their home through memory, fantasy, narrative and myth. In addition to the deep-rooted attachment and longing, one could see the depth of his past always peeping in the present. The novel shows that memory of every individual is measured against the intensity of how much one shares his past experiences with others.

When the home gets shifted with colonial and political circumstances, the characters suffer a sense of loss and isolation. In spite of their attempts to accept new place as their home, their heart longs to get associated with their land of birth. Ghosh has made his narratives a vehicle to delineate this fact. The *Shadow Lines* highlights nostalgia which is an intrinsic part of history. In the novel, one could trace the longing of 'home' from the character Tha'mma, the grandmother of the narrator. Reminiscence of her childhood in Dhaka keeps haunting Tha'mma, who has been living in Calcutta for about two decades. Even though she makes conscious efforts to accept Calcutta as her home, her heart remains in the land of her birth. The engrossment of the protagonists in the idea of the imagined home and the ultimate survival in the new place is the chief concern of the novel that the novelist wishes to bring home.

The characters displaced in the new lands are naturally haunted by some sense of desperation and loss and eventually develop an urge to walk down the memory lane, the homes where they once lived becomes their 'imaginary homelands' (Rushdie) which provide succour and respite. The novel *The Glass Palace* vividly underscores the need for the reconstruction of more than one home for being a successful immigrant. The protagonist, Rajkumar, stresses that home is an original stable establishment, which will help to reconfigure new home as one move across cultures. He generates from his home memory the origin to continuity and a sense of preparedness to negotiate new cultures. This sense of nostalgia helps the other characters to form a new community. While some of them experience comfort there while the others are not.

Ghosh shows through his novels how political upheavals make people refugees or immigrants and its resultant diasporic living with a longing for imaginary homeland. Home is identified with the affiliation of language and surrounding environment. The intense sense of exile arouses the deep pangs of homelessness. Ghosh's fictional characters in *The Hungry Tide* are genuinely dispossessed who long to find a home to gain the rest and security. Home in this perfect form, nostalgically reminiscent for Ghosh is a refuge from the unhappy realities of the outside world. Refugees from Khulna district of Bangladesh could not accommodate themselves in resettlement camp in Central India because there was nothing which could bind them there. Their destiny had turned them rootless, homeless so they preferred to settle in tide country, which binds them with its eco-culture and dialect. The uprising of Morichjhapi is the principal result of the event of the partition, which is reflected in *The Hungry Tide*. Consequently, the refugees jostle along the border of India and Bangladesh. To live outside homeland is a kind of exile, which Kusum and the settlers of Morichjhapi experience. Amitav Ghosh has shown that home can become a tool for the reconstruction and reinterpretation of a person's experiences. The reflection of a globalised world in the novel, *The Hungry Tide*, where migrations, whether anchored in individual choice or not, does

not diminish the desire to locate the 'self' in a 'home' is a fine example. At the end of the narrative, both Piya and Kanai are situated at the threshold of their respective search for home vis-a vis the tide country. Therefore, home, for both Kanai and Piya, becomes a medium to restructure and reinterpret their experiences in the Sundarbans. In order to facilitate their respective endeavours, both Kanai and Piya decide to move their base away from New Delhi and Seattle respectively.

The writer wishes to bring to the fore the fact that sometimes original home may be oppressive and full of hateful and uncanny experiences and the diasporic exile and migration provides an opportunity to reconfigure the oppressive and restricted original home in new adopted land. In *Sea of Poppies* the *girmitiyas* on the ship are victimised by the sociocultural, political, economic and racial factors. These factors forced them to migrate to escape from the 'uncanny' homeland hoping to live a free life more egalitarian sans constrictive decayed socio-political system. On the ship the *Ibis*, passengers from various sections had the story of exploitation, torment and deprivation at the back. The place of their origin had never been the place of their self-satisfaction. The diaspora place that is ship becomes the place of their self-satisfaction and self-development. It is also to be noted that the memory and nostalgic feeling of lost homeland never desert them and prove boon to cope with the new dilemma of life. The characters try to connect the past with the present memory wilfully, old ties with new association and moves to their destinations with a strong degree of ambivalence. They create their imaginary home in the journey on the *Ibis* – the relationship patterns, stores, rituals, cultural rite, nuptial songs etc. The old home was replaced with new world but more egalitarian and humanistic. The sea becomes their new nation (home), the shipmates form a new bond of empathy and understanding. They leave behind the structure of caste, community and religion rename themselves as '*jahaz-bhais*' and '*jahaz-bahens*' is suggestive of new adoptive homeland. Exile proved a kind of reprieve in their lives. However, the past home memories could not be easily snapped even it is uncanny experience. The novel represents the notion of floating home with memory in a nostalgic way. Home as a memory cropping is not brimming from the clash with the present dilemma. It is not a kind of escape from the harsh realities of present life. Nevertheless, it takes a safe refuge in some ties in the form of belongingness with homeland. Ghosh himself admits in *The Hungry Tide* that he had been greatly occupied with his earliest linkage of memory with the tide country. Ghosh himself webbed the memory of homeland in *The Hungry Tide*. The nostalgic feelings might have inspired him to reprieve in penning down the story and its vivid memory.

In Ghosh's novels, a nostalgic pattern beautifully linked to the psyche of those characters who, either happen to stay abroad or travel for a while. He generates a sense of displacement and disillusionment by making use of a ship or the river imagery. Like the movement of the ship, the psyche of displaced characters is shown to be tossed a while and especially during those moments, they drift off into nostalgic memories. These memories emanate from their homes and the landscapes in which they were born and brought up. Hence, these immigrant characters are helplessly linked to the home memories, which eventually give them respite. Ghosh, perhaps, wishes to establish the fact that the moment the characters encounter a hybrid culture, they find themselves very difficult to acculturate or reprieve. This pattern of home memories stands pivotal in his novels. The past home memories practically nourish and sustain a hopeful, if not a successful living.

It is concluded that in terms of home, Ghosh's characters cherish the longing for homeland hoping to eventual return to original home. Significantly, like other diasporic writers, Ghosh's characters are sensitive towards their homeland. Homelessness doesn't affect the ties with original culture; even in the alien land. The root culture contributes to visualise life as a successful immigrant. Ghosh reflects not only his awareness of re-construction of multiple homes in alien land and blurring of boundaries of distinctions.

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It is concluded that in terms of home, Ghosh's characters cherish the longing for homeland hoping to eventual return to original home. Significantly, like other diasporic writers, Ghosh's characters are sensitive towards their homeland. Homelessness doesn't affect the ties with original culture; even in the alien land. The root culture contributes to visualise life as a successful immigrant. Ghosh reflects not only his awareness of re-construction of multiple homes in alien land and blurring of boundaries of distinctions.

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A STUDY OF LOCALES IN THE SELECT NOVELS OF CHETAN BHAGAT

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Abstract:

Chetan Bhagat, the icon of popular fiction stories, beguiles millions of readers with his humorous and sparkling dialogues. His stealthy success in the publishing field brings him into the lime-light. Bhagat's art of storytelling and characterisation finds him an unique place in the contemporary literature. The writer has a story message to the readers in all his novels. The setting of the story plays an important role for his success in the publishing field. From metropolitan cities to rural Bihar, he has covered every landscape with its minute details. Through his locales, one can easily identify the culture, social setup in the novels. This paper is an attempt to study on the locales in the select novels of Chetan Bhagat.

Keywords: Passion, Peaceful coexistence, livelihood, nationalism, cultural significance, urban.

Chetan Bhagat's novels convincingly captures the refreshing and noticeable changes happening in the country. The social milieu presented in his fictions primarily focusses on the urban society. While the rest of the contemporary writers focussed on the past, Bhagat chooses his story to narrate the contemporary scenario. This emerging 'Best-Seller' throws light on small towns as well as metropolitan cities in India. Reena Sablok observes the significance of his settings, "his novels are not set in the laid-back milieu of small-town India; they are right in the hustle and bustle of metropolitan Indian cities where life moves at a fast pace and change is preferred to continuity." (vii)

Chetan Bhagat's phenomenal success in the publishing field helps him to change out as a vibrant writer. The writer has caught the pulse of the younger generations. The Bhagat's characters are ambitious, young and energetic. Being it a call centre employee or an entrepreneur or a public relations officer, they provide new dimensions and new scope. The success of the writer not only depends on the portrayal of the characters but also on the choice of the setting. Bhagat's first novel *Five Point Someone* is set in IIT, Delhi. Beauty of the campus, Delhi City and Kumoan hostel are clearly inter woven in the plot. The second novel *One Night @ the Call Centre* is set in Gurgaon, the financial and technology hub in Southwest of New Delhi. The entire story happened in one night, within the limited space. *The Three Mistakes of my Life* is set in Gujarat. The writer has given ample details about Gujarat and Business. The characters are Ambavadis, who sweat blood for business. The Bhagat has paid much care in choosing locales for his story. His fourth novel *2 States* is yet another classy

novel. The entire story clearly depicted the cultural significance and traditions of Punjabis and Tamilians. The novel is a typical example of National Integration. Bhagat's fifth novel *Revolution 2020*, has made a dare attempt to reveal the darker side of the holy city Varanasi. The city is a hotspot of political unrest. Amidst historical and religious sentiments, the writer captures the minute details of the setting along with the scams done by some of the corrupt politicians. Kota, the city of Rajasthan, its crowded and dusty streets, overloaded coaching classes. The writer throws light on the darker sides of coaching centres, which charged a heavy price from the aspirants. On the other hand, the beautiful scenery of Varanasi city, the boat ride in river Ganga, evening Aarti in the temples, adds flavour to the novel. *Half Girlfriend*, Bhagat's sixth novel is shifting its setting from Delhi to rural Bihar and to USA. Of all the novels of Chetan Bhagat, *Half Girlfriend* captures the sufferings of young adults in abroad. The protagonist of the novel Madhav Jha, is a typical representative of Bihar. His Bhojpuri laced Hindi, Dumraon Palace, poor streets of Buxar brings the readers closer towards the story. Bhagat captures the royal spirit of Raja Sahib of Dumraon and Rani Sahiba Durga Jha. Bhagat's choice of locales add more relevant to the plot.

Five Point Someone:

The semi-autobiographical novel admirably depicts the struggles of the middle-class youth Ryan, Alok and Hari. The trio failed to cope with the tough grading system in the extremely draconian-educational scenario. Bhagat vituperatively represents the uninspiring educational system that stifles creativity of the students. The plot is set in the campus and the Kumaon Hostel. The novel holds all the salient features to claim it as a 'campus novel'. The young technocrats manage academics and hostel life concurrently. Ryan, Alok and Hari find hard times in the hostel. To get rid off the monotonous academics, they prefer to spend time in theatres and dhabhas. Hari's desire to hangout is well expressed in the following lines, well, they have this cheap dhabha there with the best butter chicken and we can catch a good Hindi movie. And then may be check out some girls in the market FPS 36. Alok and Hari visited Ryan's room to have group study. Bhagat's humourously depicted bachelors room as:

That was not all. Nude women adorned the walls, posters extracted from US porn magazines which made their way to Kumaon through ex-seniors in innocuous US University admission brochures via mail. Blondes, brunettes, red-heads, tin, voluptuous and petite, posed on Ryan's wall, uniformly wanton. (FPS 97)

Both Alok and Hari love scooter rides, offered freely by Ryan. The writer points out the pathetic roads of the suburbs, Hari pointed it as, Delhi roads are a nightmare and I couldn't dream of driving as fast as Ryan. Alok and I couldn't go beyond fifty and Alok keep talking as I navigated the cows and the cops to the suburbs. (FPS 121) Apart from the hostel room, the trio spend time on the 'insti-roof'. Bhagat clearly depicted the infrastructure of one of the India's premier educational institution. The top floor of the insti building keep them away from tedious grading system. The nine-storey giant building, old lock guarding entrance, 'insti-bell' tower, few dish antennas, campus roads and other hostels were clearly visible from the top of the 'insti-roof'. When the trio mess up with plans like operation pendulum and cooperate to dominate, the senior professors filed severe actions against them. As soon as they caught red handed while stealing question paper, the 'insti-roof' helps them to keep them away from others. Hari pointed out it as, 'I liked the idea of the 'insti-roof'. It was the one place where

we felt secure now, as even Kumaon was difficult to be in right now, with a million eyes on us. (FPS 205)

The same night, Alok took a bold decision to jump from the roof. Alok's emotional outburst reveals his failures and fears about his future. The entire story begins with ragging in the hostel and finally ends with a happy convocation day. Bhagat has captured the nuances of the campus life in his debut novel within the limited setting.

One Night @ the call centre:

'Connexions' call centre, in Gurgaon, is the predominant locale in the novel. Located at the outskirts of the city, the call centre employees need to book quails to reach the office. Needless to say, the call centre employees have regular night shifts with break in-between. Dealing with American customers in a small cabin, speaking American accent put them in trouble. To relax and refresh, they seek bars and discotheque to 'de-stress'. Weekend parties, fast food, speedy bike ride on NH8 becomes their routine. Sometimes after the night shifts, there are bars and lounge with beds to relax. Vroom explains the interior design of the 'New Lounge Bar Bed' as:

The interior design of bed was a cross between star Trek and a debauched King's harem. Ultraviolet bulbs and candles were the only sources of light. As my eyes adjusted to the semi-darkness, I noticed two rows of six beds each. Only five beds were occupied, so I couldn't understand the big fuss at the entrance. I guess it never easy to get people into bed. (ON@CC, 184)

The writer has set the story within the call centre cabin but some portions were set in hotels, museums and discotheques, where the characters adjust themselves to get rid-off the tiresome job. Gurgaon, being the technological hub of Delhi, is celebrated for MNC's through the call centre employees in the BPO's, the writer clearly depicts the life style modifications of young technocrats in the capital of India.

The 3 Mistakes of my Life:

Ahmedabad is the city of business and businessmen. Bhagat's choice of Ahmedabad as setting for *The 3 Mistakes of My Life* is ideal and perfect. The protagonist Govind has strong bond with his city and to me more specific with his pol-Belrampur. He is emotionally attached with the pride and historical significance of his city. He adds:

There is something about Gujaratis, we love business. And Ambavadis love it more than anything else. Gujarat is the only state in India where people tend to respect you more if you have business than if you are in service. The rest of the country dreams about a cushy job that gives a steady salary and provides stability. In Ahmedabad, service is for the weak. (12)

Bhagat captures inevitable details about Gujarat, snacks shop, multiplexes, nicer roads, better restaurants and busy businessman in Ahmedabad. The writer makes use of Bhuj earthquake to show the scattered hopes and aspirations of Gujarati people. The traumatic experiences of Gujaratis, post-earthquake gained the attention of the whole India. The novelist rightly projected the business spirit of Navaldharis in his novel, through Dr. Verma, he says, 'Navaldharis is a hard-core entrepreneur community in Gujarat. Everyone there dos business.'

And they say, a true Navaldhari businessman is one who can rise after being razed to the ground nine times.' (3MML 110)

The state has given renowned cricket players like Bumrah, Parthiv Patel, Ravindra Jadeja and many others. Similarly, the role of cricket in the story is vital. The protagonist Govind explains, how the victory of Indian cricket team is celebrated with crackers, sweets and delicious foods in Gujarat. From kids to elders, all love cricket. In fact, Govind and his friends Ishaan and Omi finds a descent income from their cricket shop, for which they have given a name, 'Team India Cricket Shop'.

Gujarat holds a rich cultural and political heritage. As the birthplace of the Father of our Nation, Mahatma Gandhi, the state has more delight. The novel vividly portrays the details about Gujarati food and culture. Bhagat has cautiously followed certain principles of Gandhi. Govind tastes mutton curry at Qazi restaurant and shares his experience as, 'The owner assured us small mutton' implying goat and not beef. I believed him, as he would not have survived in the neighbourhood is he served beef.' (3MML 7)

Bhagat's choice of Gujarat for his novel is befitting as it is the epicentre of numerous historical, political and economic activities to shape the modern India. The state has witnessed many communal riots, massacres and various changes in the political scene. The communal riot in 2002, has become a strong evidence of the clash between the Hindus and Muslims in Gujarat. Bhagat has used this riot as an important theme which disturbs peaceful coexistence. Parekji's Hindu party and Secular party decides to contest in the upcoming elections. Parekji and his gangs tries to mobilize young people towards their party. Ali's dad views about religion and Politics, once again stressed the role of Gujarat in reshaping the modern India. He clearly states, "The Gods we pray to, stayed away from politics in their time. If we truly want to follow our Gods, we must keep our religion separate from politics. Religion is private, politics is Public." (3MML, 152) The youth in the novel is very clear to cooperate for the development of the Nation, unminding the bias in caste, culture and politics.

2 States:

The novel deals with the love affair between a Punjabi boy and a Tamil girl, their struggles to convince the parents. The encounter between North and South is an interesting theme in the novel. Bhagat has given innumerable details about the cultural practices followed in Punjab and Tamilnadu. Krish's first visit to Chennai is humorously revealed as:

The first thing I noticed, excuse my shallowness, was that almost ninety percent of the people were dark complexioned. Of these ninety percent, eighty percent had dabbed talcum that gave them a grey skin tone. I understood why Fair & Lovely was invented. I couldn't understand why people wanted to be fair so bad. (2 States, 77)

To convince Ananya's parents, Krish visits Swaminathan's house. His first experience in a Tamil Brahmin house ended up in veil. Ananya's mother engaged herself in cooking and practising Carnatic music. As an alien in the house, Krish is unable to follow the conversation in the regional language. Amidst all the discriminations in language, culture and complexion, Krish tried to maintain cordial relationship with the Madrasis. The wedding of Krish and Ananya is planned in Madras. Their struggle to get the families united attained success. On his way from Delhi to Madras in Rajdhani Express, Krish mentions his struggles to cross the barriers for his love life as:

I came outside to stand at the compartment door. The train whizzed past Agra, Gwalior and Jhansi over the next few hours. I still had a day to go as the train traversed through this huge country, cutting through the states I had battled for the last year. These states make up our nation. These states also divide our nation. And in some cases, these states play havoc in our love lives (2 States, 248)

Chetan Bhagat has made a cautious attempt to explore the differences in our culture and tradition. Though the characters are from Punjab, Delhi and Tamilnadu, they are united as 'Indians'. A typical Tamil Brahmin wedding includes Punjabi Dancers and Punjabi dishes. The elders see the happy faces of the young ones, neglecting the bias in culture. The huge crowd seems to be a 'human museum' living as a community. The notion of Pan Indian identity is emphasised in the epilogue, in which Ananya delivered twins. The doctor asks, "you are from two different states, right? So, what will be their state?" Krish proudly announces, "They'll be from a state called India". (2 States, 269)

Revolution 2020:

Bhagat dedicates the novel *Revolution 2020* 'to his mother, the city Varanasi, the holy river and the Indian student.' The writer shifts his attention from metropolitan cities to one of the spiritual cities in India, Varanasi. The city is also famous for muslins and silk fabrics too. It is believed that Varanasi has played a large role in the founding of Sikhism, when Guru Nanak visited it in 1507. Buddha gave his first sermon at Saranath, near Banaras. This celebrated city is also known for temples and educational institutions such as Banaras Hindu University. The novel's choice of the city as a setting in the novel is ideal, in which he explored its religious significance and political impact it has created. Gopal Misra proudly mentions his city as:

... I simply call Varanasi my home. I stay near Gadholia, a place so noisy, you need to put cotton balls in your ears if you want to sleep. Gadholia is near to ghats, along the river Ganga. Some call my city beautiful, holy and spiritual – especially when we call it filthy and a dump. I don't think my city is dirty. It is the people who make it dirty. (*Revolution 2020*, 13)

On the other hand, the city is crowded with tourists and pilgrims. The beauty of morning Aarti in Dashaswamedh ghat, where Brahma performed ten ashwamedha yajnas, situated on the banks of Ganga, attracts more visitors. In addition to that, hundreds of priests holding giant lamps, enchanting mantras on the river bank mesmerise everyone in the city. Aarti and Gopal spends time in the happy boat ride during the day dawn. Gopal spends about a year at Kota, in Rajasthan to attend coaching for IIT entrance. Gopal's lack of interest results in boredom, however he manages to attend the classes without fail. His inner psyche is longing for his native city, which he mentions as:

Even the filthy and crowded streets of Gadholia seemed beautiful to me. No place like your hometown. More than anything, I wanted to meet Aarti. Every inch of Varanasi reminded me of her. People come to my city to feel the presence of God, but I could feel her presence everywhere. (*Revolution 2020*, 95)

Gopal shares equal love for the city and his partner Aarti. Due to his poor financial condition, Gopal join hands with MLA Shukla, opened a new college without completing the infrastructure. Gopal offers a 'huge sum' as bribe to get the approval. This scandalous activity

of Gopal and the party members are explored by the young journalist Raghav. Though Raghav and Gopal are childhood friends, Raghav never hesitates to accentuate the scams in 'Ganga action plan', investing corrupt money in 'Gangatech College', scams in 'Dimnapura sewage plant' in his magazine "Revolution 2020." Raghav wants to build a corrupt free country. As an angry young man, Raghav expresses the pathetic condition of his city as, "what about the things around us? The food being cooked in an unhygienic manner. Labs with outdated machines. Look at our city why is Varanasi so dirty? who is going to clean our rivers?" (*Revolution 2020*, 100)

Raghav also made a significant attempt to throw light on the demands in the Education Sector. As the population in the city is rapidly increasing, the people are requesting for more colleges. Raghav in his article, 'Varanasi needs more colleges', emphasised the fact to give priority to education. On the other hand, Bhagat focusses his attention towards the fast emerging 'coaching class culture' in the educational field. Kota, a city in Rajasthan is offering coaching to IIT 'repeaters' or 'beginners' to clear the entrance exams. Based upon the percentile of the students, the coaching centres offer discounts too. They also provide uniform to show equality. Gopal's first-hand experience in Kota as an alienated human being evokes sympathy. He describes the city as:

The auto drove down the dirty streets of Kota. It looked like any other small town in India, with too much traffic and pollution and too many telecom, underwear and coaching class hoardings. I wondered what was so special about this place. How could it make thousands of students clear the most competitive exam in the world? (*Revolution 2020*, 50)

The youth of today, in the novels of Chetan Bhagat, are dutiful. They are trend setters. Raghav's spirit of nationalism is clearly revealed in his efforts. He never minds settling with a cushy job which offers a decent income. The real hero, Raghav turns to be a 'social reformer' for the sake of his city and people. Being born and raised up in Varanasi, Raghav is very curious to set a Revolution in the Year 2020, to view a 'Corrupt Free Nation'. Nevertheless, Gopal, has realised his faults. Gopal stood amidst the unhappy incidents happened between Raghav and him. Gopal loves Aarti, he sacrifices his love for Raghav because, Gopal has more concern for Varanasi than for Aarti. Both Aarti and Raghav has started a new life. The 'loss of innocence' in Gopal's mind is realised by him in one of his encounters with Raghav and Keshav. The writer before leaving Varanasi sends a text message to Gopal, appreciating him as, 'you are a good person' (R 2020 - 296)

Half Girlfriend:

Chetan Bhagat's *Half Girlfriend* deals with multiple settings. The story begins with Madhav Jha's college days in St. Stephen's College in Delhi, later on he decides to settle in his native city, Dumraon, in Bihar. The final part of the story takes place at Mexico, in USA. Madhav's English is not good, which he himself admits as a mixture of 90 percent Bihari Hindi mixed with 10 percent really bad English. While the senior professors and other students are mocking at his language, his badminton coach Piyush motivates Madhav to learn English. Madhav, being a state level Badminton player, he gets admission in one of the prestigious colleges in Delhi. During the personal interview, Madhav illustrates the real Bihar as:

It's mostly rural sir. 'People don't have any exposure to modernity and hold on to backward values. There's poor education. Nobody invests in my state. The government is in bed with criminals and together they exploit the state and its people.' (HGF, 12)

Madhav feels inferior in front of others. His inadequate language proficiency restricts him to have cordial relationship with others. Riya, his Badminton teammate helps Madhav to maintain his self-esteem despite the mocking of others. Riya hails from a wealthy family in Delhi. Her parents and brothers plan a grand party on the view of Riya's birthday. Madhav is invited to attend the same. On seeing the luxurious houses, expensive cars and other members from royal family, Madhav feels isolated. His sense of loneliness explains, how terribly he missed his mother. The wedding between Riya and Rohan is planned instantly. Being a passionate girl, Riya aspires to achieve a lot. But her wedding is decided all of a sudden, which ruins all her dreams. Though Madhav proposes her, she is very clear about her passions. She regrets and wants to settle with Rohan in London. Madhav completes his studies in London, never minding Riya's absence. He also refuses the job offered by HSBC Bank, Delhi, saying, 'No Sir. I am done serving rich people.' (HGF, 94)

Madhav's concern for his people and state is evident in his plans. He undertakes the school run by his mother. With the help of politicians and government, he tries to renovate the school. The administration in Bihar is poor, as Madhav finds it difficult to enrol the kids in his school. The roads in poor condition, frequent power cut, water scarcity is some of the major issues in the state. Due to these complexities, people in rural Bihar never permit their kids to have education. A farmer desperately says, 'he has no future. Like his father, he will also work in the fields and try to survive. Schools are for rich people'. (HGF, 108) Water scarcity ruins the future generation in the state. An old man demands Madhav, 'help us get water. Kids in the village walk two kilometres for it every day. If that ends, we will send them to school'. (HGF, 108)

The behaviour and makeup of fictional characters in Bhagat's novels depends on the locale. The environment decides their character's future. Madhav's childhood days in Bihar moves with frustrations and failures. His ancestors were great landlords, whose acres of land were taken away by the government after independence. Madhav's parents Raja Shahib and Rani Dhurga Jha, however manages to lead a simple life. After the death of Raja, Madhav becomes the Prince of Dumraon. His 'coronation ceremony' proves grandeur of his beginnings, but it seems to me meaningless, because of his poverty. Madhav and his mother love Bihar and Biharis. To achieve the ultimate goal to give education to the Bihari kinds, they need financial assistance. Bill Gates' visit to India seems to be a golden opportunity. The Microsoft teammates plan to offer grants to the needy. Madhav makes attempts to invite Bill Gates to his school. In front of hundreds of Biharis and other delegates, Madhav gives a wonderful speech in fluent English. He explains the existing complexities in the state, which denies good education to the needy. Inspired greatly by Madhav's speech, the Gates Foundation agreed to offer them grants with a dozen of computers, they also offer twenty lakhs rupees. Madhav feels a sense of contentment, as he succeeds in uplifting the lives of ordinary people. He gives up his achievements and sacrifices will definitely change the future of thousands of students of Dumraon.

On the other hand, Madhav receives a letter informing Riya departure to US. The denouement of the story happens in US. Riya, who confines herself as 'half girlfriend' to

Madhav, in in love with him once again. The achievements of Madhav and his sincere love gains Riya's attention to fulfil her dreams and to get rid-off her past anxieties, Riya joins as a vocalist in Café Wha, New York. Madhav's first experience outside India is filled with excitements and expectations. As an outsider in US, he compares his mother country with the country which he has visited. The sky seems to be clear as crystal. One could enjoy ultimate silence. The roads filled with traffic. Nevertheless, nobody horned. Thousands of skyscrapers, tall buildings, early morning joggers, criss-crossing streets and avenues of Manhattan gives wonderful experience to Madhav. To adopt with the new culture is not easy, as Madhav agrees:

New York city looked beautiful and clean. The first day you spend out of India in a developed country takes a while to sink in. the swanky buildings, the smooth roads, the gleaming shops and the lack of noise make you feel like you have entered a fairy tale where nothing can ever go wrong. (HGF, 218)

The reconciliation between Madhav Jha and Riya Somani happens after three months of severe investigations by Madhav and his friends.

Chetan Bhagat through the characters ad incidents highlights the predicaments of the protagonists. The readers can find the locale gets integrated into the theme and narration. The themes and stories closely knit with the locale of his writings. Especially the cities in India. Bhagat captures the beauty of Indian landscapes, its vegetation, historical significance etc., in a very elaborate manner. His interest in portraying the locale is not limited. Whereas, his involvement with the locale, culture and lifestyle of the people seems to be so deep. Bhagat's novels carry acute descriptions about the locale beginning from metropolitan cities to rural Bihar. The writer's rich and varied experiences is depicted lucidly in the subject matter. Through his novels, Bhagat entertains and inspires the readers.

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**INTERPLAY OF NOSTALGIA AND ALIENATION IN CHITRA BANERJEE
DIVAKARUNI'S *SISTER OF MY HEART* AND *THE VINE OF DESIRE***

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Right from the days of colonization, India has been experiencing a sort of cultural crisis. As it was a colony of the British for two centuries, it reeled under tremendous western influence in administration. Further, the British imposed their language which brought along with it a huge cultural baggage. Due to this trend, the nation experienced a decline in its traditional cultural practices. It made several writers and activists to express their concern about it. Before India became a colony of British, it had been following several cultures and religious practices. But during colonial days, the public felt their culture to be much inferior to the one followed in European countries. They discarded their traditional culture and adopted the alien one so as to project themselves as civilized individuals. This adoption of foreign culture created a crisis for the native culture. Homi Bhaba opined in this context that, "the political moment of cultural difference emerges within the problem of colonial government mentality and eclipses the transparency between legibility and legitimate rule" (Homi K. Bhaba 134). He opined further in the work named *The Location of Culture* thus:

"Yacabo! Yacabo! It is finished... finished': these words stand out not for the platitudinous place of cultural diversity, but at the point of culture's fading".

They display the alienation between the transformational myths of culture as a language of universality and social generalization, and its tropic function as a repeated 'translation' of incommensurable levels of living and meaning."

(Homi K Bhaba 178)

The advent of globalization and its concomitant socio-economic practices have forced India to face yet another cultural crisis. These are issues pertaining to politics, economy, history, media and education. Among them, culture and immigration are right at the top. At present, the public in India are obsessed with capitalist formations that include individualism, consumerism and free market economy. The individuals are overly ambitious and they are into a rat race trying to fulfill their materialistic needs so as to lead a sophisticated life. This middleclass mind set has forced them to embrace an alien material culture at the expense of our spiritual traditional one. Cultural materialism has been the main focus of writers of the 20th and 21st centuries. They bring to limelight the political and cultural history of a particular period. Also, they delve into the decay of traditional culture and the space it enjoyed in their works.

Chitra Banerjee Divakaruni is a novelist who focuses on our cultural values and the lasting impacts of western culture. There are several other writers like her who focus on the same themes with marginal difference in their approaches. Since she is an immigrant, her works concentrate on the cultural crisis experienced by Indian immigrants. All her writings compare and contrast the life style of second generation settlers with the first generation migrants. It has to be understood that the second generation migrants were born and raised in an alien land and the characters in her novels are caught between the traditional values of their home country and the culture of the host nation. In fact, the second generation immigrants have a desire to follow their own culture as they have little or no desire to perpetuate the culture of their forefathers. When they discover parental imposition of native culture and denial of liberty to follow their own culture, they find themselves a confused lot caught between two extremes namely: Indian ethos and Western culture. Divakaruni places her characters in such a situation and ventilates her feelings about her nation and culture through her writings. Bill Ashcroft expresses about her writing as follows: "the diasporic production of cultural meanings occurs in many years, such as contemporary music, film and dance, but writing is one of the most interesting and strategic ways in which Diaspora might disrupt the binary of local and global and problematize national, racial and ethnic formulation of identity" (*The Empire Writes Back* 218).

It is germane to find a definition for the word 'culture' here as it has many social connotations. Raymond Williams rightly opines it as one of the two or three most complicated words in English language. Andrew Milner says, "after all most of the work I was doing was in an area which people called culture', even in the narrower sense, so that the term had a certain obviousness. But, you know the number of times I've wished that I had never heard of that damn word" (*Cultural Materialism* 3). Both the critics took efforts to study the cultural history and came out with different ideas. Andrew Milner defines culture as something that "runs between a generality and a particular general public sphere and a singular subculture" (Milner 4). In the words of Raymond Williams "... it ran between two generalities, the arts and the whole way of life" (Milner 4). In short, whenever individuals move out of their home culture, they find themselves caught between pulls and pressures of the competing cultures. It is a dilemma faced by diasporic communities in all parts of the globe.

This paper attempts to trace the influences made by Divakaruni's experiences in her writing and the manner in which she has portrayed cultural crisis in her works. For carrying out this study, it is imperative to have a deeper understanding of Indian diaspora. It is understood that there are 1.7 million people from south Asia living in the USA. The census carried out in USA in 2010 states that the Asian American population is expected to reach 8.6 million in July 2050. From the census report, it is evident that it has been one of the rapidly growing communities in the USA.

The voyages of discovery during the end of the 15th century and throughout the 16th century accelerated geographical mobility of people worldwide. The first wave of migration started to European colonies because of mercantile economy, religious persecution and large scale unemployment back home in the 17th and the 18th centuries. The second wave of migration included slaves, refugees, exiles and indented labourers in the 18th and the 19th centuries. The end of two World Wars and the independence of colonies spurred the third wave with many intellectuals, chiefly those who went as students, making the nations of their former colonizers as their own homes. This produced brain drain and robbed the former colonies of their intellectual wealth. The fourth wave of migration took place during the last decade of the 20th century because of globalization of trade and commerce and revolution in information and communication technology. This dislocation from their native places is both mandatory and voluntary. Critics such as Gareth Griffiths, Bill Ashcroft and Helen Tiffin define diaspora as, "... the voluntary or forcible movement of the people from their homelands into new regions" (*The Empires Writes Back* 68). As a matter of fact, living in a foreign land is not living in actual sense, but it is only a mere existence trying to get assimilated with newer relations.

People who have migrated to foreign land always have this strong nostalgia for their homeland and its culture and traditions that were once integral to their daily life. This particular quality of longing for a way of life that is no more there has paved way for diasporic writing. The cultural alienation that they undergo in their uprooted state because of their racial, religious and linguistic differences has a telling effect on their everyday life. Any diasporic writing can be examined through these two distinctive frameworks. Chitra Banerjee Divakaruni belongs to the group of diasporic writers and in her novels she gives a telling account of the miseries of the immigrants and the hostile atmosphere they face through her protagonists. Her focus has become more intense on women immigrants the moment she left India. From there on, she started writing about the status of Indian women who live abroad. Chitra Banerjee Divakaruni is very much aware of the Indian cultural legacy and she gloats about the fact that she has inherited the customs of the most consistent human advancement. She is very smart in maintaining her cultural heritage. Though the present crop of Indian diasporic writers have been viewed as transcultural, transnational writers and are treated as novelists of both the countries, her desire to give a verbal expression about her diasporic identity has been clear.

Chitra Banerjee Divakaruni's novels are mostly set in the USA and they focus on day today life of Indian immigrants. In the novel *Sister of My Heart*, there are two women who share their challenges in life with each other and help each other in solving them. This helping tendency between them becomes a stumbling block in the married life of them. The novel delves into the life

of Anju and Sudha, who are cousins. The entire novel is narrated in their own voices revealing their childhood, adolescence and early adulthood. Although the early part of the novel is set in the USA, subsequently the novel revolves around India and talks about the pressure felt by mothers who value Indian culture and the sisters who adapt to western culture. The novelist very diligently develops the characters' life in both the countries and makes minute changes in the plot exhibiting the values of human relationships in India. The narrative style enables an individual to understand the diasporic realities of locations as well as the attachment the novelist has towards her motherland. While studying the reason behind her choice of the subject matter, it is very well understood that she has been making attempt to educate and reconstruct the Indian society by helping individuals acquire knowledge about Indian culture. This attempt can be very well understood in the words of Homi Bhaba. He states, "... that it is from those who have suffered the sentence of history - subjugation, domination, diaspora, displacement- that we learn our most enduring lessons for living and thinking" (*The Location of Culture* 172). A major part of the novel is set in south Asia and is burdened with diasporic awareness.

As Divakaruni's novels have been situated both in India and America, it is imperative to have a discussion on the differences between cultures in these countries. American society is distinct and different from India as it is not as old as India. On the contrary, India has a long history and is blessed with several social reformers like Swami Vivekanandha who was one of the stalwarts of social renaissance in the 19th century. In the World Religious Parliament conducted at Chicago, he made the westerners sit up and listen with rapt attention to his discourse on religion. The listeners of his discourse carried an impressive opinion about India and its religions. In addition to it, it is well known that there is a whale of difference between eastern and western societies in terms of religious beliefs and practices followed. Say for example, Hinduism has immense belief in 'karma' and 'rebirth'. This belief has instilled fear in the minds of Indians regarding things evil. The faith in multiple births, transmigration of the soul or metempsychosis is something unique to Hindu faith. Unlike the monotheistic religion of the West and the Islamic countries, Hindus worship a pantheon of gods and goddesses. In fact the deities are countless. Practices like worshipping the sun, doing yoga, chanting slokas from the Vedas, conducting marriage with the god of fire as witness, and ceremonies right from the birth to the death, including puberty rites are unique to the Hindu way of life. Veneration of animals, particularly the cow is very common in India. Taking annual pilgrimage to the abodes of Gods and ritual baths on sacred days in tanks and rivers that are marked as holy are part of religious duties. Days, weeks and months and even certain hours are regarded as auspicious to perform rites or start some good work. For someone steeped in Hindu beliefs and practices, the Western culture comes as a shock during the initial years of migration.

The novel *Sister of My Heart* makes a vivid discussion on opportunities and threats faced by women in a traditional Indian culture and it is compared with the modern world. The novel talks about a family dwelling in West Bengal and it is in a dilapidated state. Both Anju and Sudha are from a poorer background and the novelist focuses on the world around them. Both of them are clever, self-sufficient and practical. Being a master story teller, the novelist gives a pictorial account of the ordinary life and the dreams nurtured by them. All female characters in the novel work along with other characters so as to attain legitimate, equivalent and autonomous status. As a matter of fact, it carries an objective controlled action to liberate womenfolk from their dependence mindset. The bondage between the two siblings namely Anju and Sudha has been the crux of the novel and it is based on the novelist's personal experience as a migrant.

The challenges before all womenfolk have been to find fulfillment for their longings, to find satisfaction in their life and career and to find equity in all situations and circumstances. Moreover, the marital discord between Sudha and Ramesh is of serious nature. Sudha even entertains the idea of parting ways with him along with her child Dayita. Her attitude is simply different from that of other Indian women who meekly submit to societal compulsions and patriarchal prescriptions. To break free from a sacred institution like marriage, she needs courage and hope to charter her life

independently. The entire social framework embodies religion, myth, instruction and other social standards. The females need to get liberated from these shackles and maintain their status as individuals. Sudha is fortunate to have the complete backing of Ashok who happens to be her first love and he stands by her during her troubled times. Sudha's miseries do not end and Ashok offers unstinted support during her marriage, pregnancy and separation. America is expressed through the eyes of the sisters as follows:

"America has its own problems, she said, but at least it would give me the advantage of anonymity. No one in America would care that I was a daughter of the Chatterjees, or that I was divorced. I could design a new life, earn my own living, and give everything she needed. (*Sister of My Heart* 294).

The works of Divakaruni portray America as a comfortable place offering peace and prosperity to the migrants. She describes America in the most exalted terms as possible. In her perspective, it is a land of people with progressive thoughts whereas India is regressive needing redemption. She considers people moving from India to America to be fortunate as they are moving into a land of much promise.

While depicting her mother land, she gives importance to its culture and myth. In her opinion, the Indian women can gain prominence in America amidst the white population only by respecting her traditions. In an alien land like the USA, the Indian woman needs to reestablish her identity. This observation is very well found in her novels titled *Sister of My Heart* and *The Vine of Desire*. Between these two novels, *Sister of My Heart* carries several plots woven together in an artful manner. The novel brings out in a forceful manner the pressures experienced by Indian mothers who are sworn to the traditional Indian society and Indianism. The western theory has been the major focus of the novel. The novel talks about the circumstances leading to the birth of Anju and Sudha as well as their love relationship. As the novel moves, the readers have a very good understanding about the sister's euphoria, distresses, desire, misfortune, sadness and the vicissitudes. The novel *Sister of My Heart* enables the readers to acquire a thorough understanding of Indian family system. The novelist gives utmost importance to the specific nature of the females. In earlier days, women were brought up in an orthodox surrounding. They were forbidden from doing things like meeting strangers. The conservative and orthodox family values and practices were discussed threadbare in this work. However, things changed due to western education. In the novel, both the sisters retain their connection with their mother land. The novelist expresses her mentality in her writings. Epics like *The Ramayana* and *The Mahabharata* were the sources very well used in *Sister of My Heart*. Divakaruni gives a pictorial description about Indian cultural heritage. She states that, "when a child is born, Bidhata Purush comes down to earth himself to decide, what its fate and fortune is to be religious ceremonials had a great attempt in describing Indian Phenomenon" (*Sister of My Heart* 15).

The plot is very simple and straight forward and concentrates on female characters. Through the life of Anju and Sudha, the novel very well depicts the traditional Indian life. The novel also talks about Dayita, the daughter of Sudha who becomes an orphan and it exhibits the will power of womanhood. In the novel, the union of the sisters is tested. The relationship between Sudha and Sunil become quarrelsome. Sudha experiences a nightmare and moves out of USA. In the life of Anju, her companion's duplicity happens to be the dark drama. Her married life runs into rough weather and she decides to lead her life alone. Their affection surpasses all complications and Sunil has problems in prioritizing the needs of Sudha. In the opinion of Simon de Beauvoir, "once a woman is self-sufficient and ceases to be a parasite, the system based on her dependence crumbles; between her and universe there is no longer any need for a masculine mediator" (*The Second Sex* 689). The interesting part of the novel is in the way both Anju and Sudha get acclimatized to the western influence. The separation between them is explained in a subtle tone.

In all her novels, Chitra Banerjee Divakaruni does not say whether her characters are Indians or Americans. She goes on disseminating generalizations. Her novels probe into the genuine feelings of Indianness. The Indians, though naturalized as Americans, always remember their motherland and

have fond memories about it. The novelist exhibits this attitude through Sunil in the novel. In a social gathering, Sunil comes across an American who talks ill of India. Unable to withstand this mudslinging, Sunil slaps him flat on his face. There are characters like Lalit, Trideep and Sara who also nurture the Indian idea of living. From her novels, the readers are able to understand the Indian style of living abroad. Sunil, Anju and Sudha are found to have these traits in them. Indianess and Indian sensibility find an incredible arrangement while Divakaruni exults in describing Indian traditions, customs and cultural goods. Even, Indian nourishments such as dal, parota, and pickle along with costumes like saree, kurta and pyjama are explained in detail. Flowers like jasmine, bangles, bindi and sindhur which are very common in India find detailed accounting in the novel.

Her next novel *The Vine of Desire* unravels the story of two youngsters who establish female freedom. The novelist considers the Indian female migration to the United States of America to be a kind of pleasant trip made and learning to lead a westernized life. Through the eyes of the characters, Divakaruni tells the story and gives vivid details about the country they have left behind. The reader is left to wonder whether the novelist has yielded to the temptation of giving a pictorial description about India and its public. *The Vine of Desire* is the sequel to Chitra Banerjee Divakaruni's *Sister of my Heart*. The protagonists in the story are the two sisters Anju and Sudha born on the same day opening their eyes to the ill-fated death of their fathers also brothers' of the same family Gopal and Bijoy respectively, on a ruby exploration journey. The story revolves around the two women caught between hard core family traditions and the evolving modern thoughts of the 1980s. Anju migrates to America with her husband Sunil and Sudha stays in India with Ramesh. The distance does not separate them emotionally but only physically as the communication goes on through letters. The novel depicts the reunion of the two sisters in America. The sequel begins with a tragedy of miscarriage, emotion and trauma of the separation of son 'Prem' from her womb which ends in an abortion leaving Anju in bouts of depression. Sudha flees from her family to America. Anju feels the need for her sisterly support and feels obliged to stand by Sudha during her tormenting divorce. She decides "I want to bring Sudha to America" (*The Vine of Desire* 18). Sudha visits America with a hope to make a life for herself and her daughter Dayita. Divakaruni follows the trend, shows America as the land of opportunities and a refuge for all emotionally broken and mentally distressed women.

Divakaruni brings about the contrasting cultures of India and the US. The novel constantly focuses on the transculture; the characters seem to be shuttling between two worlds. Loss, alienation, rootlessness and dislocation are experienced by every immigrant. The expatriates initially try to adjust with the new culture and society into which they have joined. But something holds them back in the form of nostalgia for a way of life that is no longer possible. The sense of nostalgia is often seen among the dislocated and displaced people in most of the diaspora writings. In *Brick Lane*, *An American Brat*, *The Namesake*, *Disappearing Moon Café* and in *The Vine of Desire* one can find women characters leave their home to foreign countries after their marriage in order to settle in a new land with their unknown husbands. Anju in *The Vine of Desire* migrates to America as Ashima in *The Namesake*. Both share a lonely life though the situations and problems differ. Anju, during her miscarriage yearns for someone to console her and liberate her from her intense loneliness. Anju's isolation reveals her-acute nostalgia as well. The immigrants find themselves put out of place from the home society, they are upset emotionally and strive to remember and place themselves in a nostalgic past. There are frequent musings of the past memories, especially of the childhood days, people and surroundings. All through the story there are sentimental attachments well displayed by different characters. Anju in this novel often finds talking to her unborn son; she names him "Prem" and to him she conveys the reminiscences of her childhood. She tells 'Prem' who is attached to the warmth of her womb. She journeys back in time and narrates instances of homesickness at the very memory of the old house, the white elephant of a mansion that had been in the Chatterjee family for generations: its

crumbling marble façade, its peeling walls, the dark knots of its corridors, the brick terrace where she and Sudha went secretly at night to watch for falling stars to wish on. She senses pain as things change. She remembers even the smallest of the incidents in the past which she used to loath and hate as a child, but as an immigrant there is this terrible longing to retrieve the past.

In an interview to *The Telegraph* (13th March 2005), she says that women in particular responds to her work because she writes about them; women in love, women in difficulty, women in relationship. She wants people to relate to her characters so that they can feel their joy and pain, since it will be harder to be prejudiced when they meet them in real life. Though Anju dreams of America from the time she reads books, it becomes the promised land for her "as amazing as the fairy kingdom of Pishi's tales" (*The Vine of Desire* 179).

'So in my teenage years, I read things like *Anna Karenina* and *Sons and Lovers* and *The Great Gatsby* and *A Room of One's Own*. I'm glad I did, but maybe Aunt Nalini—that's Sudha's mom—was right. They were no good for me. They filled me with a dissatisfaction with my own life, and a longing for distant places. I believed that, if I could only get out of Calcutta to one of those exotic countries I read about, it would transform me. But transformation isn't so easy, is it?" (*The Vine of Desire* 14).

Divakaruni portrays remarkable characters who build hope within themselves in an alien country. Here in the midst of different cultures the immigrants venture to set an identity battling against loneliness and overcoming tormenting emotions. They carefully discard their initial cultural shock and exhibit remarkable resilience. Divakaruni's narration in *The Vine of Desire* is adroitly tailored from first person to second person and also to third person narration. The emotional communication through letters also expresses the family bonds, responsibilities and yearning for home.

In fact, both the novels analyse and contrast the prevailing Indian mindset, attitude, prejudice and pride among the immigrants in a foreign country. Divakaruni, in short, 'gives an authentic pictorial description about the Indian community in an alien land and its ways of coming to terms with a newfound reality.

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POSTMODERNIST MYTHICAL CHARACTER DRAUPADI IN CHITRA BANERJEE DIVAKARUNI'S THE PALACE OF ILLUSIONS

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ABSTRACT

This paper explores the concept of postmodernist character Draupadi in the novel *The Palace of Illusions* by Chitra Banerjee Divakaruni. The term Postmodernism has been associated on purpose to this particular myth character, since Draupadi doesn't want to bind herself with the rules already laid by men. This novel is particularly chosen with the consideration of this novel being the paradigm shift in the time in which it supposed not to be so. Divakaruni has included the women from the myth background to extricate themselves from the rules that has been drawn to put them under control. She has produced the novel from the Draupadi's point of view, that clearly has stated the novel is women centred and their view of the world they have live in, is really the appreciable negative capability of Chitra Banerjee Divakaruni and that has

been really the drive to stretch this paper and sketch the paper with the postmodernist perspective.

KEYWORDS: MYTH, DRAUPADI, PANDAVAS, KAURAVAS, POSTMODERNISM, VYASA, THE THREE MOMENTS,

FULL PAPER

Chitra Banerjee Divakaruni is a multifaceted author of novels, poems, short stories, activist and Professor of creative writing programme. Her books have been translated into many languages which means her world-wide reach of her writings. Her theme has been mainly focused and focusing on the problems of South Asian women in the domestic, physical and psychological level. Chitra Banerjee Divakaruni was very normal person when she was staying and doing her P.G. course and Doctorate in USA until she had joined in the writers' group. Moreover, she had been motivated by the librarian when she had decided to leave USA as the unsuccessful woman. She has uplifted herself only through reading a lot and has spent a lot of years practicing writing along with her writers' group. In addition to it, she has raised herself to this position by doing many odd jobs like babysitting, having worked sometime in the bakery etc. As she has raised herself as a writer only through a lot of practice, she has understood, many has a lot of aims to become a writers but could not know how to broach themselves as writers. With a view to the future writers in mind and has acted as a practical guide and Professor of Creative Writing programme and has totally devoted herself in achieving her aim from being the author herself for multifarious writing varieties. Chitra Banerjee Divakaruni has even taken a challenge in voicing out the characters of Draupadi in *Mahabharatha*, Sita in *Ramayana*, the mythical characters to share their view through her writings in their point of view.

One of the mythical characters has been taken to explore in this paper about her contradictions of her ideology of the world where she has been resided by God Himself through the yagna done by Drupad, the king of Panchaal, in invoking God to offer him a son to avenge his old friend and his present enemy Drona. Surely the character has been taken to exhibit her own thoughts is Draupadi, the surprising daughter of Drupad, who had not expected a daughter but only a son Dhristadyumna. In her birth itself, Draupadi herself has chosen to born there for some purpose, may be as a boon to Drupad otherwise called a blessing in disguise, that has been proved when she has won the hand of Arjun, with whose support Drona has done the act of having dragged Drupad to remind of his promise to share his Kingdom when they both were in tutelage but having forgotten it with the years have gone by, thereby she has married the Pandavas who are really being considered as arch-enemies for the Kauravas especially by Duryodhana in whose court Drona also the member, so definitely the connection has been made by Him the God in the correct way to correlate the situation in the future where in the confirmed way, both Kauravas and Pandavas are to stand against each other for the same cause of Drona and her father Drupad, but as cousins. Draupadi is really a postmodernist woman, actually has an idea to explore the world in a different way, where in her own world is expected to behave in a disciplined and in the exemplified way like Savitiri, Dhamayanthi, and Sita, etc.

Draupadi, though the mythical character, wants to explore the world as a postmodernist as it is stated Barry (2014) has clearly stated about Postmodernist as "For the postmodernist, by contrast, fragmentation is an exhilarating, liberating phenomenon, symptomatic of our escape from the claustrophobic embrace of fixed systems of belief" (p. 81). Draupadi, in her life, she has been an unexpected one in Drupad's kingdom where the king Drupad has done a yagna to get a son with a blessings of God through the fire, unfortunately

a son Dhristadyumna has come along with a sister holding his hand has been named Draupadi, has been known as the twin sister of Dhristadyumna. Even though Drupad has a lineage for his kingdom he invoked God for having a child to avenge for his insult by Drona his former friend and present enemy due to his disowning of his own words. Since Drona is in his desperate need to feed his own poor son Aswattama, because of his poverty.

The novel *The Palace of Illusions* has been taken to examine the character Draupadi, her composition of character has been sculpted out by Chitra Banerjee Divakaruni with her view to the perception in the feministic point of view. Divakaruni has always has an idea of experimenting the possibilities of characterization with the mythical characters like Draupadi and Sita, whom everyone expects to live a model life for the progeny where every man is expecting his own consort to behave naturally in the orderly way of men being the designer of the order. This novel *The Palace of Illusions* is from the point of view of Draupadi, otherwise called Panchaali, where her own thoughts, actions, views are given prominence. It has been stated in the novel that Draupadi is the designer of the palace of Illusions to the Engineer Maya, who has accepted to construct a magical palace in appreciation of the assistance done by Arjun in excepting Maya's family by the fire in the forest.

Draupadi has exceptional character with qualities that has brought her to be the witness of Kurukshetra, through the vision has been given to her by the sage Vyasa who is a great grandfather of both Kauravas and Pandavas and as a writer of Mahabharatha as the world has known about him. The responsibility has been given to her by the sage Vyasa is great, due to her firm and strong mentality to prove her own self at any cost. Her won real self is to have competed with men in all their actions and learning the crafts that have been involved in war.

Draupadi is definitely the myth character in which the roles of women are put as examples for the present women to follow it up exactly. N. S. Ravi's *Different Shades of Women*, in a chapter called *Savitri The Wife*, in which Savitri a character in Myth who is always expected to save a husband's life Satyavan but a character in N. S. Ravi's work has killed her husband Girdhar whom she has had loved and married, has been suspected and confirmed in the crime of blood trading and trafficking in the Laboratory, and even after the transferred from the old place due to the suspicion on him and even in the new place where they have got the chance to run a crèche and earn a money for their living. In crèche also, Girdhar has taken an advantage in his crime and earned a lot of money to settle in a life without any disturbance in money and other matters. For his crime, he has been killed by his wife unlike the myth character Savitri, who had risked her life in struggling with Mrithyu, the God of death, in bringing back her own husband Satyavan.

On the contrary, Devdutt Pattanaik in his *Indian Mythology: Tales, Symbols, and Rituals from the Heart of the Subcontinent* has given the idea of myth as the one which transfer culture specific information to be passed on generation after generation to lead their life successful. In myth it is expected that subservient woman is deified always one who is totally yielded to her husband's wishes. Like Nalayini, to satisfy her husband's wishes even take him to the prostitute's house to satisfy his sensual pleasures. Unlike mythological women, Draupadi has come out of fire means to say born out of fire. From her birth onwards she has been yearning to learn, what her twin brother Dhristadyumna has had learnt as a man to win the war as the warrior has come to know to defend his kingdom and his father's respect. Since, Draupadi is a female she has been sent to the women's quarters, has asked to learn to dance, mehendi drawing and even the cookery classes have been assigned and has been asked to learn a lot of moral stories to behave like the women in the stories. She personally

always has felt herself not to be shown interest towards womanly qualities that is why she has wanted the connection with Sikhandi the eunuch, and Krishna, the Lord with whom she loves to maintain the connection. From this it has been revealed that though she is a woman, she does not want of her woman kind to bind them with any specific qualities like womanly qualities, and manly qualities with reference to one's own sex.

When Dhai Ma, Draupadi's maid, her assistant, companion, informant and like a mother to her has told Draupadi regarding the king's life personally in association with more than a wife and number of wives. Draupadi in her reply has said that she would not allow her husband to have any wives other than her. On the other hand, after claiming Draupadi to be Arjun's wife having won the swayamvar, when this has been informed to Arjun's mother Kunti, being the mother without noticing what he has brought Kunti has told Arjun to share with every one of his brothers. This is really shocking for Draupadi to be shared among Arjun's brothers like the commodity, for which she has immediately brought back her memory with having asked Lord Shiva a boon to have a husband with five good qualities, then it has been analysed clearly that without listening properly of Draupadi's boon, Lord Shiva in a hurry and excitement to offer her a boon to have five husbands with the qualities she has been asked for and these things has been clearly stated with Devdutt Pattanaik's *Book Indian Mythology: Tales, Symbols, and Rituals from the Heart of the Subcontinent*.

Draupadi's polyandry has been done due to the mistake done by the miscommunication happened between the disciple and devotee Draupadi and Lord Shiva. Like these days women, when she has come to know that her husbands have been married separately after having married to her all at once immediately after the swayamvar, she has thrown tantrums, everyone has started fearing of her because of her ferocious

behavior of throwing things at them in anger and in connection with her marriage the sage Vyasa has given the boon to have virginity every time she has to face a new husband among the pandavas, this has increased her anger that through the boon the sage Vyasa, the renounced sage as the world has known about him himself being partial in his offering of boon toward women in favour of the men community in giving the virginity to Draupadi instead of forgetfulness for her to face every husband as a new bride all the time possibly.

After the Pandavas' marriage to Draupadi, Bheeshma has come to invite them to Hastinapur where they are the lineage of them. When they have come to their proper heritage, Duryodhan has been very furious in their coming, so he has joined with Sakuni, Duryodhan's maternal uncle in gambling away the property of whatever they have, including Draupadi along with her palace of illusions. As an impact, She has been called for in the court through the servant, when she has refused to come, Dussasan himself, the brother of Duryodhan, has dragged Draupadi along to the Kauravas' court and has started humiliating her with the insulting words of Duryodhan and with the instruction of Duryodhan to disrobe Draupadi in spite of her defending herself at the court in front of everyone including her husbands, Bheeshma, the Grandfather; Drona, the teacher; and even with the witness of the blind king Dhritarashtra, Duryodhan's father. She even has recouped the courage for raising questions for which everyone has been made to shut their mouth due to Duryodhan's arrogant tendency.

The sage Vyasa has come to aware of what would happen to Draupadi, so he has really warned her not to do three things to escape her fate as well as to protect the world from bloodshed, due to her being the cause of the war Kurukshetra, which has been designed as per Vyasa's writing. The sage Vyasa has played two roles one is Grandfather of the Kauravas and Pandavas as well as the sage in telling the future

only to Draupadi in this novel. The novel has been drafted in the way that the sage Vyasa himself has a close contact with Draupadi and even has shaped Draupadi as the witness of the entire event that's how Chitra Banerjee Divakaruni has given prominence towards women, in shaping the retelling of Mahabharath through Draupadi's point of view, who once has been the victim in Kauravas' court, now has become the cause of Kurukshetra and at last would be a witness of every single event in the Kurukshetra.

To avoid the future war and its impact the sage Vyasa has told and warned Draupadi in her three dangerous moments to be quiet:

"... Three dangerous moments will come to you. The first will be just before your wedding: at that time, hold back your question. The second will be when your husbands are at the height of their power: at that time, hold back your laughter. The third will be when you're shamed as you'd never imagined possible: at that time, hold back your curse. Maybe it will mitigate the catastrophes to come." (Divakaruni, 2009, p. 40)

The first moment during the swayamvar of Draupadi, when Karna has come forward and compete with other Kshatriya, he has been denied for being raised in a low caste in spite of his statement being the defence with a view to Parasuram, his guru's vision of Karna belongs to the Kshatriya community. Draupadi's question has been related with Karna's origin as,

Divakaruni has put forth as a paradigm for every woman to know her husband thoroughly through Draupadi to Karna:

Before you attempt to win my hand, king of Anga, it said, tell me your father's name. For surely a wife-to-be, who must sever herself from her family and attach herself to her husband's line, has the right to know this. (Divakaruni, 2009, p. 95)

After listening to this question the sage Vyasa has frowned at her for having been the cause of her brother's death in the near future. This states that Draupadi is the one who could not be controlled by any fear or promise, whatever she has in her mind; she would accomplish it with fervour.

The second moment during the stay of Duryodhan and his friends including Karna in the palace of illusions after the Rajasuya yagna, when Sisupal has been given his punishment due to his crossing of his limits of having reached his hundred and more sins. In his stay Duryodhan has toured the palace and has been surprised with wonders of it and having reached the "illusory bridge" and fell down with a upright comment from her maid, being motivated by the smile of Draupadi, has uttered these words in the upright way, in Divakaruni (2009) "It seems the blind king's son is also blind" (p. 173)! Draupadi would have stopped and punished her maid to prevent the future collapse but she could not because she herself has some idea to have insulted Duryodhan, in her absence of doing it, her palace along with her maid themselves have done it in her stead.

The third moment, when Draupadi has been insulted at the Kaurava court, in front of every ministers, her own husbands and also with the blind king's presence, she has begged everyone to stop this non-sensical attitude of Dussasan but no one has initiated to put paid to it from Dussasan's disrobing Draupadi's sari, at last with the help of Lord Krishna, her robes have never met an end and finally ends Dussasan with a swoon. Then she has started cursing which has been warned by the sage Vyasa to stop but she couldn't since she has faced the greatest insult she has ever had in her lifetime the curse as follows,

As Draupadi's nature in doing things in the natural flow with the annoyance:

"All of you will die in the battle that will be spawned from this day's work. Your mothers and wives will weep far more piteously than I've wept. This entire kingdom will become a charnel house. Not one Kaurava heir will be left to offer prayers for the dead. All that will remain is the shameful memory of today, what you tried to do to a defenseless woman." (Divakaruni, 2009, p. 194)

Draupadi, the woman of myth has really lived a life of modernity, she has lived her entire life according to her wish, except her own marriage that has been a great miss due to the community's clash, otherwise would have selected Karna as her suitor, since he is a very great warrior. She does not want to be the one, overwhelmed with motherly affection. She always loves to have companions like Lord Krishna with Dhristadyumna her brother and Lord Krishna with Arjun her husband, with whom she could possibly share about everything if only, provided with a companion, she would have possibly shared her love toward Karna. Draupadi is the lover of warcraft where men are given importance in the mythical period, if only she has been given a chance, she would have proved herself definitely as a warrior, but instead she has been shown only as the cause of the destruction of the Kaurava clan totally during the Kurukshetra war.

Draupadi is the composer of poems and along with that she is the designer of her own palace which is otherwise called the palace of illusions, the palace and its beauty has really attracted jealousy and vengeance which she has really enraged hatred in Duryodhan's blood rather than the insult he has had received in that palace. She is the very good adviser of the Pandavas and she is worth the respect she has received from Pandavas. She herself has accepted that she is filled with anger and desire, and insisted that it is her long time companions since she has been ignored due to her unwanted presence and her dusky complexion. Though she is dusky she has attracted everyone in the swayamvar and could not tolerate their defeat

in it and has started joining as an accomplice through joining hands with Duryodhan in the Kurukshetra that's how her beauty has ended the entire venomous clan as it has been weeded out to bring back the prosperity in this world. Draupadi has been the main role in making the good ones to live in peace without the harassment of the Kauravas and the palace of illusions as the conduit to attain her success over her humiliation and has been stood out steadfast in her promises. This character of Draupadi is really being the inspiring role that has been carved out very well among the other mythical main characters of women like Kunti, the mother of Pandavas; and Gandhari, the mother of Kauravas and Dhritarashtra, the blind king's wife, by Chitra Banerjee Divakaruni in the possibly excellent way.

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Flipped Classroom, a room with a difference

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Abstract

In the age of speeding jets and missiles, no one has got time to look at the faces of their partners. Speeding with the fleeting time makes one to run around the set targets and destinations. Completing the curricular task would become a herculean task while planned activities remain silent spectators. How to achieve the target syllabus with activities? The solution is 'Flipped Class'. The present paper aims at exploring the advantages of flipped classroom.

Flipped classroom is the right solution for making the students to involve and utilize the class time qualitatively. When time is the constraint in semester system to speed up with the syllabus and accomplish the task of fulfilling the academic activities, the classroom has to be substantiated by videos, pictures and handouts. A video before a class will leave a cue to the students to catch a lead to the next day's lecture in the classroom. The present paper deals with flipped class, its advantages and a few possible activities.

What is flipped Teaching?

Flipped teaching is the process of moving lecture content from face-to-face class time to before class by assigning it as homework. This allows for more interactive forms of learning taking place during class. Flipped teaching often involves

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ASSERTIVE NATURE OF HODA IN ADELE WISEMAN'S *CRACKPOT*

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Abstract

Adele Wiseman is one of the well-known Jewish immigrant woman novelists of the 20th Century. She is aware of the victimization and sufferings of the Jewish community in Canada, an alien land. The non-Jewish world that existed outside the walls their sheltered upbringing offered social and economic opportunities previously unknown to the Jewish community and they faced a choice of 'assimilation' or 'assertion'. Adele Wiseman deals with the theme of alienation and isolation in her two novels *The Sacrifice* and *Crackpot*. Her protagonists are typical Jewish youth who are governed either by the principle of assimilation or assertion who yearn for recognition in the Canadian society. This paper analyses the character of Hoda in Adele Wiseman's *Crackpot* who establishes her strong Jewish identity amidst Jewish and non-Jewish communities through direct affirmation of her distinctive ness as a Jew.

Key words: immigrant; victimization; assertion; assimilation; distinctiveness

As a woman who was raised in a society where it is implied that women should be agreeable and amenable, where speaking up for yourself can label you "difficult," I personally have found it difficult to do that very thing. Why is it important to have personal boundaries and make sure they are not crossed? More importantly, how can we keep them while coming off strong and not strident? (Dr. Karen Binder-Brynes, <https://goop.com/wellness/career-money/female-assertiveness/>)

The Canadian literary canon contains few writers of diversity as Adele Wiseman. Although she is chiefly remembered for her two novels – *The Sacrifice* and *Crackpot*, her canon extends well beyond the fiction for which she is predominantly reminisced. In fact, Adele Wiseman is skilled practitioner of numerous genres including drama, the essay, the short story, and the memoir. Her works illustrate her evolving interests; and each work is indicative of Wiseman's particular interests at the time of its compositions.

Adele Wiseman's second novel *Crackpot* is the story of Hoda, a woman who "lives her life backwards" and begins in "a graveyard with a plague" (Panofsky, 8) and ends in the hope of renewal. Indeed, as she dons her wedding dress at the end of the novel, it appears that Hoda has weathered the storms of poverty and sexual promiscuity to begin a new life. *Crackpot* documents a woman moving backwards from death and destruction to childbirth to marriage with the promise that doing things in reverse offers its own rewards.

Every act of migration reflects some wider social, political and economic developments, such as modernization process. Thus, one may wish to draw on immigrant literature with reference to fiction as social evidence. On the other hand, literature is individual, subjective and diverse. It may reflect, but may also exaggerate, challenge or invert the social experience that informs it. Throughout the century, Canada has used the experiences of immigrants and ethnic-group members

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as an important element in their depictions of life in Canada. They have frequently explored questions about the adjustment, adaptation and assimilation of migrants as a way of probing culture, inter-group relations and individual psychic fatigue in Canadian society. This is especially true of writers who themselves are migrants.

The theme of alienation forms the warp and woof of immigrant writers in Canada, and it can be depicted on a more comprehensive scale in an epic or in a novel. Adele Wiseman takes up a recurrent pattern of a theme in her novels. She brilliantly and vividly weaves the motif of social forces and her interplay with stark realities of life through the portrayal of her protagonists – Abraham in *The Sacrifice* and Hoda in *Crackpot*, who are mostly immigrants. Sociologically, alienation is the act of alienating or the state of being alienated, and anxious or resentful feeling of not belonging to or having a fit place in society.

Wiseman's Hoda, an immigrant child during the nativistic years of World War I, does not internalize the mainstream ethnic and class hierarchy that devalue both her North End neighbourhood and her cultural origins. Hoda constructs childish fantasies about receiving some sign of acceptance into 'the empire,' her dream of being single out as his future queen by the Prince of Wales is based on her own sense of self-worth – on her faith in her own special qualities, which she may be discerning enough to recognize.

In this recurring fantasy, as in her interactions throughout the novel, Hoda accepts herself, and assumes her value. In sharp, Hoda is obsessed not with concealment, but with revelation. She wants desperately to tell her story, to break the silence about the nature and significance of the personal, familial and cultural odyssey that has brought her to the new world. As a child, she is sure that telling her story will win her the respect and affection she knows and she deserves.

If they only knew! Well, they ought to know ... And Hoda began to wonder whether she shouldn't perhaps tell Miss Flake and the rest of the class how much more there was to it than they could possibly imagine. (*Crackpot*, 59)

When Hoda attempts to tell her story in her school class, her teacher Miss. Boltholmsup asks her to cut short and thoroughly rejects hers. Miss Boltholmsup, her name suggesting the inadequacy of the values and sensibilities she represents. However, Hoda does not internalize that rejection, rather, she is simply astonished and hurt by the stupidity of it. After the death of her mother, Hoda longs for affection and love, the real thing. But she finds in fumbling sex with the neighbourhood boys. Her sense of shame at her own appearance, her loud-mouthed bravado as a young teenager, her tenderness towards the boys, such as a big dumb Morgan who initiates her into sex – these are shown with an intricate ambiguity. Later, when she has become a kind of parodic embodiment of elemental female capacities, both whore and dutiful Jewish daughter, she continues to value herself and to act confidently despite her amplified, almost larger-than-life marginality: as a poor Jewish fat woman and a prostitute, she is vulnerable to being seen as an outsider in a number of ways. Despite this, Hoda confidently assumes her place within the Jewish community, attending every wedding and later in her life very funeral, gradually earning a kind of place, not only at the wedding table, where eventually "to be expected is to be invited" (124), but also in the stories and gossip that Wiseman uses to create and sustain the Canadian Jewish community. While as a prostitute, she of course often figures negatively in that lore, over time she earns a sort of grudging respect and a definite place, suggested in the phrase Wiseman uses repeatedly, "legend has it"

(168). By the time WWII breaks out, she has even become “good old mamma Hoda” (270). Hoda does not flee the ghetto, either physically or psychologically. Wiseman’s Hoda remains there, attempting to reconcile past, present and future within it. Hoda equates happiness, safety, and her own sense of self-worth with her private and communal home in new world. As a child: “Hoda was rather proud of her tumble up and down porch and the tree that was giving it a ride ... She showed it off to her friends” (26).

Hoda misses the sense of community that she feels, even as a prostitute, in the ethnic ghetto, and decides that “it would be a long time before she tried to work downtown again” (135). She takes measures to protect her home from city authorities whom she fears might condemn it, particularly since the growth of the tree in front yard has finally ripped the verandah completely off. She develops a similar strategy with the public health officials at City Hall, where she goes periodically checking herself. This strategy involves using words skillfully to undermine through parody and iron the false sense of superiority of those in power. She declares loudly that she too “is a citizen and she had her rights and they ought to be glad she had a sense of responsibility” (203).

Hoda is trusting and naïve; she is also learning that not everyone is to be trusted, and, yet because of her father Danile’s early teaching, she does not easily give up that faith in human creatures. In fact, she never gives it up, even though she ultimately come to see the fact of evil in the world. Life and society hurt her a great deal. She reacts with puzzlement, anger, pain, humour and ultimately with determination to survive and to retain a fair its life itself. In the early stages of her life, she is not provided with the necessary knowledge to live a moral life. Ignorant of the conventions of the old society and in her new world, she is forced to define a personal morality that is based on what she knows on her individual needs and the demands of her new society in which she grows up. She develops her own verbal system.

Conclusion:

This article has discussed the responses to the nature of ‘assertion’ created by Hoda, the Jewish immigrant. The novel *Crackpot* highlights the theme of identity as it is connected to the process of assertion that the dominant majority required of the protagonist Hoda, to the demarcated spaces in which she has to live, and to her standing in peripheral communities. The attitude of the protagonist seems to end with assertive in nature. For instance, on the last page of the novel, Hoda dreams of drawing a circle around those whom she loves – her fellow marginalized friends – Pipick, Lazar and her father. This vision suggests that Hoda is no long willing to accept her marginalized status, recognizing instead her central place within the world around her.

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PANACHE OF J. M. COETZEE: A STUDY*

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Abstract

The intention of this paper is not so much to examine how much Coetzee and his characters harmonised, but to effort to read his effects and the light they bring to his novels. During modern eras Coetzee measured English and Mathematics at the University of Cape Town. Maybe one of the most significant features of Coetzee's writing lies in small sentences that shape a house, so to speak, brick by brick. He digs for deep meaning in a leisurely fashion. His style is very problematic to define. Possibly for this cause, some critics say that his novels lack enthusiasm and excitements even though the opposite is true.

Keywords Racism, discrimination

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If the literary works of a novelist are a representation of his lifetime, the works of the author J.M. Coetzee capture an existing mirror image. From this life he originates the spirit of his thoughts and interrogations connected to the world, presence and persons. The intention of this paper is not so much to examine how much Coetzee and his characters coordinated, but to effort to read his effects and the light they bring to his novels. During modern eras Coetzee measured English and Mathematics at the University of Cape Town. In 1960 and 1961 he progressed with honours in both majors. After that he spent three years in Britain (1962- 1965) employed as a computer programmer. In the meantime, he accomplished research on the English writer Ford Madox Ford. In 1968, at the age of twenty-eight, he awarded a PhD in English, Linguistics and German languages at the graduate school at the University of Texas at Austin. His in-depth, ongoing academic and practical experience did not prevent him from pursuing writing.

J.M. Coetzee employed very hard to break into the world of literature. He was thirty-four years old when he published his first book in 1974. In 1977 he has written and published his second novel *In the Heart of the Country*. Rapidly he increased consideration in his own nation, South Africa, and also overseas. The novel was published in Britain and the U.S.A.

Coetzee was born in Cape Town, South Africa, on 9 February 1940. His father was a lawyer and his mother a primary school teacher. In 1963 he married Philippa Jubber and fathered her two sons, Nicholas in 1966 and Gisela in 1968. In 1980 Coetzee separated. She died in 1991, two years after Nicholas deceased in an accident at twenty-three. Coetzee finished his primary, secondary and university education in Cape Town. He journeyed to Britain and America to follow his higher education and turn out to be a professor conquering several positions from the beginning of 1968 until now. He survives with his partner Dorothy Driver in the Australian city of Adelaide, which he drifted to in 2002. He embraces an honorary position at its university. In 2006 he acquired Australian nationality. He is a novelist and an academic who is involved with local and global subjects. In 1972 he resumed to South Africa after the United States declined to grant him permanent dwelling because of his participation in the anti-Vietnam War undertakings, even though he was an assistant professor of English at New York University in Buffalo.

Coetzee has published sixteen novels, together with *Dusklands* (1974), *In the Heart of the Country* (1977), *Life and Times of Michael K* (1983), *Age of Iron* (1990), *Disgrace* (1999), *Elizabeth Costello* (2003), *Slow Man* (2005), *The Childhood of Jesus* (2013), and other writings extending

between memoir, literary criticism and essays. In 1984, his novel *Life and Times of Michael K* won the British Booker Prize. Later in 1999 he gained the Booker Prize again for his novel *Disgrace*, which established international applause. He was the first novelist to win the Booker Prize two times. After a year of living in Australia, Coetzee won the Nobel Prize for Literature in 2003.

In appreciation of the prominence of his novels, the Harry Ransom Centre at the University of Texas library accepted Coetzee's document for \$ US 1.5 million in 2011. The collection contains 155 packets of articles and texts, notes, letters and lectures by Coetzee since 1965. The document also comprises nine drafts of the novel *The Life and Times of Michael K*, which turns about a simple gardener and his wife living in Cape Town when violent happenings start. What gives Coetzee's novels energy and uncertainty? He regularly inclines to address events in small sentences, unlike the lengthy sentences full of facts in the novels of Colombian writer Gabriel Garcia Marquez. Maybe one of the most significant features of Coetzee's writing lies in small sentences that shape a house, so to speak, brick by brick. He digs for deep meaning in a leisurely fashion. His style is very problematic to define. Possibly for this cause, some critics say that his novels lack enthusiasm and excitements even though the opposite is true. The simple sentences disguise the fact that he is far from direct, and the reader has to work to find simplicity.

Places, life involvements and ideas are an essential portion of the writer's career. Through one system or another the writer makes use of raw ingredients in the literary work he creates for his characters, and gives free rein to them to go toward their unpredicted purposes. Coetzee's life is exciting privately and publicly. Being a South African has shaped very amusing material for his themes and concepts. He saw and lived the history of the apartheid carefully. His novel *Disgrace*, for example, discourses and condemns this issue without dropping into the trap of didacticism. He has learnt the narrative creation by economising his script style, which supports the current of events and paints characters impulsively in difficult human circumstances. In this novel, David Lurie, the professor of Modern Languages, loses his job at the university after seducing and assaulting one of his students. He then leaves the city and goes to visit his daughter living in the countryside. While he is there, three Africans attack his daughter's house, attack him, rape his daughter and set the house on fire. Different to what he imagines, his daughter declines to demand sentence of the attackers, but rather she pursues to cut her relationship with her father and remains to live as if nothing has occurred!

Coetzee wrote *Disgrace* in 1994, in the rouse of the downfall of the apartheid rule in South Africa. There are those who understand the daughter's approval of what has happened to her as an acknowledgment of the alteration of the condition in South Africa. Is the daughter's rape accurately equal to her father's violating the sacredness of education and endeavouring to seduce his pupil? Again, Coetzee's novels seem unclear and want more than one reading. Time has altered and this novel endorses that the condition in South Africa is no longer as it used to be. Discrimination is ultimately condemned as events develop.

There are not many characters in *Disgrace*, which is not a bad thing. Too many characters could have interfered on the novel's sense of loneliness. Maybe this is a different control forced by the isolated nature of Coetzee himself. Obviously no one has the right to judge the writer's life and take up his creative work resembles to his experiences. However, one cannot overlook the fact that there are always some personal qualities combined in one's writing. Coetzee is well known for his love of loneliness. To avoid any misperception it must be said that the bottomless roots of a genuine writer are artistically revealed in his writing as the result of persistent excavating into the depths of the inner self. Literature, isolation, introspection and distance are a set of influences demonstrating qualities that cannot be overlooked in many of Coetzee's novels such as *Disgrace*, *Slow Man*, and *Elizabeth Costello*.

In 1984 and 1999, the years when Coetzee won the British Booker Prize twice, he did not go to obtain the two awards! He himself is as unclear as his novels' characters. He is often labelled as an introverted, ascetic, recluse and reluctant to appear public events. These potentials do apply to most of his characters peculiarly. Even at the level of literary events, Coetzee hardly blends with communities of writers and does not seek to get closer to critics, but his works have ultimately stood on their own merits and become highly distinguished. He does not drink, smoke or eat meat and he maintains his capability by bike riding every day in the city of Adelaide. One cannot manage the fact that Paul Rayment, the solitary protagonist in *Slow Man*, lives in Adelaide, too.

The main incident in this novel is a bicycle accident, which rapidly happens to Paul Rayment and leads to the elimination of his leg. In this story, Coetzee appeals on his own involvement as a cyclist to make the character of Paul Rayment more credible. The character of the cyclist is the core and basis of the novel. From that point on, Coetzee used his fancy to set Paul Rayment into a sovereign character with his own unique qualities. Paul Rayment left Coetzee and advanced his own life. In this

context lying is associated with extensive imagination contrary from rigid reality and the ordinariness of daily life that does not essentially signify objectivity, namely morality or truth. The phrase is also open to another clarification, in the sense that human souls tend to overstate and colour facts. So they wear masks to hide their authenticity, which results in the change and misrepresentation of the human principle, which in turn leads to the final existential puzzle: human's continuous search of truth through the formation of another genuineness. This philosophical and existential battle is a projecting theme in the works of Coetzee.

Therefore, the confidentiality of the characters' deeds is a leading psychological element in some of Coetzee's novels. Uncertainty, misperception and inwardness are cautious appliances required by characters to conscious their lives. Thus, we find that Paul Rayment in *Slow Man* is anti-social and does not permit his friends to get closer to him, particularly after suffering a shocking involvement that altered his life and forces him to review his past. He is in an indescribable melancholy: blankness, wilt and unimportance. In short, he gradually expires. Chasing Coetzee's characters pulls the reader's attention to how they interconnect with his own life experiences. In the meantime, it surely sheds light on the unique capability of the writer to distinct his characters from himself and give them freewill and their own human potentials. Coetzee is able to review the innermost thoughts of an unhappy unsocial human who suffers from the insignificance and illogicality of life. Coetzee's works present literature as a crucial element of his life. It is renowned that although Coetzee is a university professor, he does not talk! But he makes Elizabeth Costello, his novel's epic protagonist, travel around the world, lecturing and lecturing gibberish to the point of nausea.

Elizabeth Costello is almost empty of events and emphasizes on the internal battle going on in the complexities of its characters: a male novelist and Costello, a critic. It is about the battle of the thoughts and the boundless misperception that arises from inconsistencies and lack of confidence in anything. This novel is a good example of the connection of the life of the writer and his characters, because one of its main heroes is a writer, whom Elizabeth Costello attacks and powerfully criticizes for writing about human sinful, oppression and domination. Sometimes it is hard to distinct Coetzee's characters from his authenticity. His life and literature are a cheerful testament of the refusal of oppression, prejudice and oppressive governments. His novels discloses human confusion, weakness,

evil and flaws and have allowed him to take a noticeable place in literature and become one of the greatest novelists in the world.

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IMPACT OF MULTICULTURALISM IN VIKRAM SETH'S A SUITABLE BOY

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Abstract: Vikram Seth's outstanding and admirable literary journey so far has comprehended several books of poetry, a travelogue, a verse novel, an epic novel, modernist fiction and a memoir-cum biography. Each book is set in a different ethnic setting and background in terms of form and genre. Every new book of Seth creates a fresh form and theme. His first novel, *The Golden Gate* (1986) in verse about the lives of young professionals in San Francisco, established him commercially as one of the promising Indian English writers. *A Suitable Boy* (1993), his 1474 page voluminous second novel as a postcolonial narrative deals with the socio-political issues that covers the issues of national politics, elections in 1952, disaffection, the status of lower caste people, land reforms and the eclipse of feudal princes and landlords amidst four family saga. It is an attempt to reassess Vikram Seth's *A Suitable Boy* as a pen-picture of multicultural Crisscross of post independent India and its varied consequences in the socio-political realms. The many different themes and episodes discussed here present an all-inclusive idea of socio-cultural crisscross with all its plurality, challenges and shortcomings to present my views and reading of *A Suitable Boy* as an authentic picture of post-independence India.

Key Words: Multiculturalism, Cultural semiotics, Ethnicity, socio-cultural issues, political back drop, Assimilation of multicultural discourses.

Introduction:

Introduction Vikram Seth is one of the most prominent and promising writers of recent times with a powerful expressive flare in almost all the genres of literature. With the complexity and depth of his work in prose as well as verse, Vikram Seth has achieved an unflinching top place in both the genres in Indian English writings. His many themes and concerns, from his interest in land-ceiling in Post-Independence India to western classical music, all were handled with equal ease and aplomb. Vikram Seth, born in Kolkata, lived in many cities including the Bata Shoe Company town of Batanagar, near Kolkata, Patna, near Danapur and London. His father Premnath Seth was an executive with the Bata India Limited, a shoe Company and his mother, Leila Seth, was the first woman judge on the Delhi High Court as well as the first woman to become Chief Justice of a State High Court at Simla. He is the first Indian English Novelist to get a remarkable amount of rupees two corers as advance for his epoch-making novel *A Suitable Boy* (1993). He has received the Commonwealth Writer's Prize and WH Smith Literary Award for *A Suitable Boy* in 1994. This novel was short-listed for the Irish Times International Fiction Prize as well. *A Suitable Boy* presents a cogent view of the post-independent India through realistic and symbolic narratives of the making of a nation. Seth approaches post-independent India from a secular point of view with a strong influence of Nehruvian ideology. The aim of the present research is to find out the multicultural perspectives of men and matters in the current literary world this paper also enlighten as to do many research work on this mark.

As a land of piebald cultural diversity, India is the biggest democratic country keeping its unity in diversity. The unification of diverse and distinct practices and cultures of several religions and communities constitute the kaleidoscopic trait of the nation. This multifaceted feature of Indian society explains the speckled aspects of Indian life and culture. The basic unit of the family forms the foundation of the social layers where the individual gets familiar to the cultural life in a particular society through his/her exposure. Family is one of the most important social institutions that play an essential role in building up one's behavioural traits at equality with the established traditional norms of the society. *A Suitable Boy* narrates the story of four families that durations over a period of eighteen months, set in the post-independence, post-partition India. The panoramic vision into the multiplicity of Indian family structure in the novel, sharp-sighted the current politics, changing racial norms, generation conflicts, family status, arranged marriages and other socio economic factors throws a suspicious question into the holiness of the institution of marriages in India.

Assimilation of Multicultural Issues:

The concept of multiculturalism recommends the idea of difference and heterogeneity that is embodied in the concept of diversity. The concept of multiculturalism contributes to this agenda of democratization and non-discrimination. First it locates cultural identity as a source of discrimination in society. While earlier theories focused on discrimination that occurs on account of one's religion, race and gender, multiculturalism points to discrimination of minority cultures within the nation state. Second it argues that equality for diverse cultures requires a system of special, group differentiated rights. Although in its discussion of diverse communities, it distinguishes

between the majority community and the minorities. That is the diverse cultural communities are categorized as majority and minorities. *A Suitable Boy* brings to lime light the various reactions of the ideology of Multiculturalism. India has always been the most culturally, linguistically and genetically diverse geographical entity after the African continent. India's democratic republic is premised on a national belief in pluralism and not the standard nationalist invocation of a shared history, a single language and an assimilationist culture.

Perspectives of Varied Characters:

Seth shows the India of fading idealism, tainted corruption, pestering communal disharmony, parasitical intrigue of politicians, the perpetual fight between the forces of progress and maternity and the forces of tradition and obscurantism through his characters. The characters of *A Suitable Boy* present a certain 'double vision' (Bhabha 5) in the wake of the process of decolonization in India. Seth has illustrated the modern upper class society who is inbred by the western culture through the Chatterjees. Justice Chatterji, who takes pride of being a Bengali as much as an Indian prefers Subhash Chandra Bose, a Bengali than Gandhiji and considers English as a colonial language. Justice Chatterjee has five children, Amit, Meenakshi, Kakoli, Dipankar and Tapan. Seth writes, "None of them worked, but each had an occupation." (Seth 135) Amit writes poetry, a native of Calcutta considers Bengali as a refined language than Hindi. His glamorous sister Meenakshi is a social butterfly, married to Arun Mehra who was neither a Brahmo, nor of Brahmin stock, nor even a Bengali. Arun, Mehra's eldest son, is an example of a hybrid postcolonial product that visits London periodically and follows English customs. He mimics the English and admires English style, an Anglicized Indian. Dipankar seeks the Meaning of life and spends his time reading the poets Sri Aurobindo, Kakoli is always on the phone with has string of admirers and Tapan, only twelve or thirteen years old, attends a prestigious boarding school at Jheel. This family has a dog called Cuddles and a cat Pillow and hosts three or four grand parties in the course of the year. The Chatterjees have employed two cooks, one for Bengali and the other for western food. Rupa Mehra a widow with four children assumes freedom for her to have things organized for the family. Mrs. Mehra does not like their hospitality she was afraid lest her daughter Lata should contract this disease, though she enjoyed their company. She says to Lata "I do know what is best. I am doing it all for you" (Seth 3). The advances made by Amit Chatterjee towards Lata were abruptly stopped mid-way by her mother who had instantly taken a dislike for the Chatterjees. Mrs. Mehra says to Lata; "I have no intention of accepting things as they come, said Mrs. Rupa Mehra, the unsavoury vision of scarifying yet another of her children on the altar of the Chatterjees making her flush with indignation" (Seth 146) On the other hand, Mrs. Mehra immediately liked Haresh but Lata thinks that Haresh was shorter than she had expected. When Haresh opened his mouth to speak he had been chewing paan. If Mahesh Kapoor is progressive and secular, his cabinet colleague, L.N. Agarwal, is obscurantist and communalist. Lata's choice of Haresh instead of Kabir later presents a case of how characters are portrayed, Kabir, who comes from a family which has a history of madness, is a pretense, introvert and self-agonizing being but Haresh is a "generous, robust, optimistic...responsible" (Seth 1291), composed and gentle personality. Lata imagines her mother's reaction of her falling in love with a Muslim boy: "she could see her mother's tears as she faced the horror of her beloved daughter being given over to the nameless 'them'" (Seth 168)

Socio-Cultural Complications:

The Hindu and Muslim characters are frequently kept in disparity with each other. Almond observes that in Seth's presentation Muslims "are more serious, more melancholy, more self-disciplined, and with the kinds of goals and expectations which self-discipline connotes somehow more repressed, more unhappy, more replete with possibilities for the tragic" (Almond 46-7). Characters from Hindu and Muslim families symbolise traditions and the emergent transitions that relates to cultural and individual liberty and the making of identity. Seth uses Hindu and Muslim characters in pairs—Kabir and Haresh, Maan and Rasheed, Kapoor and the Nawab. Maan as a witty and carefree person is a foil to a very sensitive Rasheed. Maan's company always brings in cheer into the novel but Rasheed seems to counteract life. Maan's Muslim friend Firoz though depressed is made happy with the disappointments of Maan. Characters like Rasheed, Durrani and the Nawab are depictions of 'remote' human beings detached from the social. Durrani's total apathy to fellow beings and lack of social skills are evident when he says "What did young people have to do with anything? He wondered." (Seth 213). However, one might see in his portrayal of Muslims a delicate tragic thread inherently involved in the making of the characters and the incidents. Moreover, Seth's portrayal of Muslims, Almond suggests, reflects a sense of inhumanity: "and inhumanity not to be understood as cruelty or monstrosity, but rather a simple indifference to the world of human beings, a carefully-kept distance from the society of Brahmapur" (Seth 44). Suicides, disillusionment, and madness are interwoven with an otherwise cheerful novel. The insanity in Kabir's mother blocks her to identify her own son. The novel illuminates the social implications of the zamindari system, the impact and the manner in which the agrarian structure took shape in the post-Independent India through Mahesh Kapoor who recommends the reform and the Nawab Sahib of Baitar, a zamindar. Mahesh Kapoor is presented as a foil to those who are tied to the position of the zamindar. Seth presents a discussion on the land property of peasants and the impact of the closure of the zamindari system that was brought in by the Mughals as part of collecting land taxes from the peasants and maintained during the imperial period. However, the system was abolished after Indian independence and the lands were given back to the peasants. While the British view the elimination of the Zamindari system as a mark of 'real' freedom, the farmers in the independent India continue to suffer and this reflected in the increasing rate of farmer suicides. The issue with the zamindari system was mainly related to the land registration. As

the land was owned by few landlords, the British protected this semi-feudal agrarian system by taking the side of the landlords. Taxes were collected from the people through the landlords who were endorsed by the British.

Conclusion:

After having the thorough investigation of multicultural perspectives in Vikramseth's *A Suitable Boy*, it can be concluded that it establishes Seth, not as an Indian emigrant but as an artist interested in socio-political, multicultural and historical affairs of India. Every minute detail of the cultural diversity has been shown by Seth. In a globalized world the co-existence of varied cultures has become a common phenomenon and the biggest question remains whether all these diverse cultures can remain within a single nation. Seth's India as presented in *A Suitable Boy* answers this question as all ethnic groups diverse in terms of religion, caste, creed, colour, gender, economy live under one umbrella i.e. a single nation would brought harmony in this country.

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THE BAROQUE AS A STYLISTIC DEVICE IN THE MOOR'S LAST SIGH

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Abstract

An entire literary-ideological controversy has sprung up around Salman Rushdie since the fatwa was pronounced against him in 1989. Following the fatwa, Rushdie became a widely discussed author, and while the literary establishment unanimously defended his right to continue living and writing, it also frequently voiced its criticism of certain aspects of his work. Referring to Rushdie's *Midnight's Children* as an example of the extravagant use of language and the "baroque mode of overextension" that characterize magic realist fiction, Faris remarks that "it is appropriate... to invoke Scheherazade... with her number of 1001 - a numeral of excess, emblematic of the notion that there is always one more" (185). It is therefore not surprising that Scheherazade is invoked in *The Moor's Last Sigh*, too, and quite appropriately so for a text that is marked by as extravagant a use of language as any of Rushdie's earlier novels. Rushdie's style is characterized by long, broken sentences, and a frequent use of dashes, brackets and italics. There are also digressions and repeated short thrusts into the future, all of which gives his prose a quality of breathlessness. Elaborate descriptions, which often turn into virtual catalogues, abound. So do all kinds of wordplay, rhymes, songs, puns, jingles, mottoes and neologisms, as well as so many funny nicknames that they become the book's mannerism.

Key Words: Baroque; magical-realism; emblematic; extravagant; digression.

Introduction

An entire literary-ideological controversy has sprung up around Salman Rushdie since the fatwa was pronounced against him in 1989. Following the fatwa, Rushdie became a widely discussed author, and while the literary establishment unanimously defended his right to continue living and writing, it also frequently voiced its criticism of certain aspects of his work. Accusing him of "magic lampism", a too facile use of magic realism, critics have claimed that Rushdie's novels are politically successful at the formal level only, because they present a vision of the world that is too pessimistic, disinterested, and devoid of the sound ideological foundation expected of a post-colonial author. However, these critics generally do not venture beyond *The Satanic Verses* (1988) in their discussion. Although the usual objections have also been made with regards to *The Moor's Last Sigh* (1995), the majority of critics agree that, perhaps as a result of Rushdie's predicament, a more personal tone marks much of this novel, allowing a greater degree of identification with its characters. J.M. Coetzee believes that the novel marks "a crisis in Rushdie's thinking", in which his life "has been overtaken by an increasingly political conception of personal identity" (13-14). Contrary to what some other critics claim, it argues that the ideological tensions of Rushdie's earlier books are resolved in *The Moor's Last Sigh*, since in this novel the political message is stated frequently and quite openly. Rushdie has in this respect managed to overcome what critics regard as the limitations of his writing.

The Baroque

Excess, overabundance, profusion, variety: these qualities mark the baroque, not only in Rushdie and most other magic realists, but as a general tendency in art and literature of various periods. Severo Sarduy notes:

Baroque space is that of superabundance and overflow. In contrast to language which is communicative, economic, austere, and reduced to its function - to serving as a vehicle for information - baroque language takes pleasure in the supplement, in the excess (129).

As a prose style, the baroque is often associated, via Bakhtin, with Rabelais and even the Menippean satire (Durix 106, 115, 143, Engblom 296-304), as it involves carnivalization, polyphony, intertextuality and the mixing of genres (Sarduy 123). While these devices are admittedly employed in many contemporary novels, not all of which are magic realist, they are nevertheless a common trait of the works that belong within this category. The baroque was associated with magic realism from its inception; it was Carpentier who "describe[d] the New World as baroque in its architecture but also in the complexity of its vegetation, in the polychromic around, in the telluric pulsion to which the people are submitted" (Durix 105). Opposing academism, Carpentier favoured the baroque, as a style "which corresponds to periods of transformation and innovation" and "provides an alternative system of values which integrates only one part of European culture (that which is judged most lively and creative) and relates to other world civilizations" (Durix 106). The revised canon that he proposed thus included, significantly for my discussion, "Hindu and Iranian literature" in addition to Rabelais, Shakespeare and selected works from the Spanish "Golden Age". So, the baroque, alongside with magic realism, becomes in this view the appropriate means for depicting the non-European reality. Latin American authors as diverse as Lezama Lima (*Paradiso*, 1969) and Isabel Allende (*The House of the Spirits*, 1982) have used this style, and Durix believes that

Rushdie's discourse belongs to the rhetoric of excess also characteristic of the works of Carpentier, Asturias and Garcia Marquez. Details are plentiful; the fictional world swarms with different characters, scenes and locales which are so many opportunities for the narration to digress, almost for its own sake. The world to be described is too large, too profuse in various colours, smells, emotions and life to be circumscribed by classical rhetoric (142).

Referring to Rushdie's *Midnight's Children* as an example of the extravagant use of language and the "baroque mode of overextension" that characterize magic realist fiction, Faris remarks that "it is appropriate... to invoke Scheherazade... with her number of 1001 - a numeral of excess, emblematic of the notion that there is always one more" (185). It is therefore not surprising that Scheherazade is invoked in *The Moor's Last Sigh*, too, and quite appropriately so for a text that is marked by as extravagant a use of language as any of Rushdie's earlier novels.

Rushdie's Style

Rushdie's style is characterized by long, broken sentences, and a frequent use of dashes, brackets and italics. There are also digressions and repeated short thrusts into the future, all of which gives his prose a quality of breathlessness. Elaborate descriptions, which often turn into virtual catalogues, abound. So do all kinds of wordplay, rhymes, songs, puns, jingles, mottoes and neologisms, as well as so many funny nicknames that they become the book's mannerism. The wordplay sometimes seems to have allegorical connotations, as in the case of the Anglican priest Oliver D'Aeth. When Aurora turns his name into "Allover Death" (94), it is intimated that as a character he represents the "death" of English colonial rule in India. "[O]n such hooks hang my tales", concludes the narrator self-reflexively after an outburst of similar punning (145). Michael Gorra vividly describes Rushdie's flamboyant style as a maze of clauses, almost impossible to parse; a portmanteau stuffed to bursting, with question marks and dashes serving as luggage straps that barely hold it all together... Rushdie makes English prose an omnium-gatherum of whatever seems to work... unbound by any grammatical straitjacket. The very structure of the sentence seems to open possibilities, to recut the borrowed clothes of English until they've become those of that new Indian language Angrezi (133-34).

Heteroglossia is another feature; in addition to the free use of Hindi that marks his entire opus, in *The Moor's Last Sigh* Rushdie throws in phrases in Latin, French, German, Italian and Spanish. He even invents idiosyncratic and exceedingly comic idiolects, attributing them to certain characters. In all of this, Rushdie's purpose is ultimately "to create a literary language and literary forms in which the experience of formerly colonized, still-disadvantaged peoples might find full expression" (IH 394). The use of phrases in foreign languages gains additional significance within *The Moor's Last Sigh*, as a symbolic opposition to the monoculturalism of the fundamentalists. To quote Gorra again,

The inventive impurity of Rushdie's heteroglot style provides a challenge to the idea of proper English, the King's English, and therefore to English colonialism... Yet if the hybridity of Angrezi marks the postcolonial 'separation' of English from its 'origins and essences', the same hybridity challenges any notion of the authentically Indian as well... it deals... with a particular conception of India's 'national culture' (137-38).

The baroque in *The Moor's Last Sigh* is more than just a quality of style. This book is an "encyclopedic fiction", as Patricia Merivale calls *Midnight's Children* (331), and just like the earlier novel. *The Moor's Last Sigh* is also "the family chronicle of an extended, claustrophobic, ingrown, quasi-incestuous, matriarchal, and doomed family", a Bildungsroman and a Künstlerroman, as well as a genealogical allegory "of historical and metatextual particularities" (Merivale 332). In other words, it is a mixture of several genres, which perhaps copies the Bombay "masalas", a genre in Indian cinema that appeared in the 1970s - "films with a smattering of everything, including romance, crime, comedy sub-plots, action, melodrama and music" (CEI 487). It is indicative that the second Book of *The Moor's Last Sigh* is entitled "Malabar Masala". Octavio Paz remarks that a similar mixture characterizes Indian literature: In Indian stories, genres that are separate in our tradition combine in surprising ways: the fairy tale and the picaresque novel, didacticism and libertinage. It is a characteristic of the Indian people: frank realism allied with delirious fantasy, a refined astuteness with an innocent credulity (32-33).

Although not the conclusion of an Indologist, Paz's opinion is relevant for my discussion inasmuch as it comes from a keen-spirited Latin American intellectual, who witnessed the emergence of the magic realist tradition in his own continent and culture? Apart from any suggestions that the mixing of genres is something typically Indian, we have seen that it is a tendency present in contemporary literature at large. In Rushdie, this mixture has symbolic ideological significance as another way of expressing in the form of his novels his major political and ethical concerns, a way of challenging "the absolutism of the Pure" (IH 394).

The consequence sometimes looks like an attempt to "encapsulate the whole of reality", to use Rushdie's own phrase (MC 75). As Durix observes,

What many 'magic realistic' works have in common is this mixture of 'fantasy' and a clear concern with reference, historical allegory and social protest... The scope of these books largely transcends the individual fate of a few characters in order to constitute an imaginary re-telling of a whole nation through several decades (116).

Rushdie's *Midnight's Children* was already an expression of "a national longing for form" (MC 291). The world of *The Moor's Last Sigh* sometimes seems to represent the whole of India too, if not even more than that. This tendency is most strikingly suggested by the exuberant description of Aurora's first painting, done while she was locked up in a room for a week. Although this is a rather long passage, it is worth quoting in full, as it offers the best example of Rushdie's baroque imagery and style:

Every inch of the walls and even the ceiling of the room pululated with figures, human and animal, real and imaginary, drawn in a sweeping black line that transformed itself constantly, that filled here and there into huge blocks of colour, the red of the earth, the purple and vermilion of the sky, the forty shades of green; a line so muscular and free, so teeming, so violent, ... versions of erotic temple-carvings, whose explicit details made Camoens blanch, and of the building of the Taj Mahal, after which, as she unflinchingly showed, its great masons were mutilated, their hands cut off, so that they could never build anything finer; and from her own South she had chosen the battle of Srirangapatnam and the sword of Tipu Sultan and the magic fortress of Golconda where a man speaking normally in the gatehouse may be heard clearly in the citadel and the coming long ago of the Jews. ... In an honoured place was Vasco da Gama himself, setting his first foot on Indian soil, sniffing the air, and seeking out whatever was spicy and hot and made money. ... Carmen having her bottom pinched, Epifania's rump being kicked by a drunken gardener; but then the rapid rush of the composition drew him onwards, away from the personal and into the throng, for beyond and around and above and below and amongst the family was the crowd itself, the dense crowd, the crowd without boundaries; Aurora had composed her giant work in such a way that the images of her own family had to fight their way through this hyper abundance of imagery, ... (59-60).

Amazed, Camoens finally concludes that it is actually a representation of

Mother India herself Mother India with her garishness and her inexhaustible motion, Mother India who loved and betrayed and ate and destroyed and again loved her children, and with whom the children's passionate conjoining and eternal quarrel stretched long beyond the grave; ... her cranes on treetops with necks like coat-hangers, and high circling kites and the mimicry of mynahs and the yellow-beaked brutality of crows, a protean Mother India who could turn monstrous... who could turn murderous, dancing cross-eyed and Kali-tongued while thousands died (60-61).

Both the image of this gargantuan painting and the language in which it is described are exceedingly baroque. Moreover, it is stated that "the truth" is revealed through the depiction of "the great swarm of being", "the crowd without boundaries", "the hyperabundance of imagery" and "the endlessly metamorphic line of humanity"; finally, the symbolism is rendered explicit through the image of "Mother India". The scope of the novel matches to a certain extent that of the painting; read in this key, the above passage is, in fact, a striking instance of self-referentiality. I shall return presently both to Rushdie's use of self-referentiality and the concept of Mother India; at this point I just want to note that similar baroque images of overabundance recur time and again in the novel. The "multitudinous" form, Rushdie explains, is a way of "hinting at the infinite possibilities of the country"; it is meant to be a source of optimism (IH 16).

It is worth noting that Rushdie already called attention to maximalism as an artistic strategy in *Midnight's Children*, in the story about Nadir Khan's roommate: "As a young man he [Nadir Khan] had shared a room with a painter whose paintings had grown larger and larger as he tried to get the whole of life into his art. 'Look at me', he said before he killed himself, 'I wanted to be a miniaturist and I've got elephantiasis instead!'" (MC 48-49). This brand of "elephantiasis" is strikingly reminiscent of Aurora's paintings and Rushdie's own manner of writing. *Midnight's Children* also contains the story of one Lifafa Das, a street entertainer who was trying to put "everything" into his peepshow box with postcards and wonders. The narrator comments:

The hyperbolic formula began, after a time, to prey upon his mind; more and more picture postcards went into his peepshow as he tried, desperately, to deliver what he promised, to put everything into his box. (I am suddenly reminded of Nadir Khan's friend the painter: is this an Indian disease, this urge to encapsulate the whole of reality? Worse: am I infected, too?) (MC 75).

Keith Wilson observes that Das's attempt "stands as ironic comment on the worthlessness and inevitable failure of exercises in complete and contained mimesis" (57). And while this is true, the claim that the urge for maximalism is an Indian idiosyncrasy could be backed, for example, by the fact that Mahabharata is the world's longest epic poem, containing the declaration that "What is here is nowhere else; what is not here, is nowhere." Noting this, Tharoor points out that

Few other works in world literature could make such an extravagant claim, but in doing so, the two-thousand-year-old Indian epic poem is not defending a closed structure: rather, the Mahabharata has had so many accretions over the years in constant retellings that there is practically no subject it does not cover (366).

Rushdie is certainly not someone who would defend any kind of closed structure; it is the very form of his novels rather than mimetics that attempts to depict the complex, baroque, multi-layered, "torrential reality" (45) of India. A novel may work as a metaphor and a metonymy of this reality through its extravagant language, labyrinthine plot and a mixture of realism, myth, allegory, and the fantastic. According to Afzal-Khan, such a mixture "reveals the ludicrousness of the Orientalist notion that Third World societies are 'whole', 'unified', and 'simple' structures, which, once understood, can be either dismissed or placed at a safe distance and labeled other" (177). This is precisely the image of India that Rushdie opposes. Afzal-Khan concludes that "the narrative must be a mishmash of conflicting genres and modes, a narrative in which the comic and the tragic, the real, the surreal, and the mythic all 'defuse' one another, so no one genre can predominate and 'unify' the others" (154).

Brennan calls attention to another interesting aspect of Rushdie's mixed style. In *Midnight's Children*, the narrator Saleem is compared with the god Ganesha, to whom Vyasa, according to the legend, dictated The Mahabharata. In this way Rushdie partly accounts for his own mixture of genres, which is similar to the one in the Indian epic. Brennan concludes:

The formal assault on orderly fictions provides the only way out of Saleem-Shiva duality. It is precisely their rivalry that produces a third element - Ganesha, whose style amounts to the chaotic 'sum total of everything' ... 'Everything' means not just India. If neither Saleem nor Padma create 'true' national images, it is because the truth of postwar nationalism is international (117).

Conclusion

Brennan's remark throws a new light on Rushdie's use of baroque style and mixture of genres, which thus extends beyond a stylistic idiosyncrasy and acquires political connotations. In my opinion, by placing the history of minorities on the imaginative map of the country, Rushdie does not wish to claim "This is India", but rather "This is India too". Moreover, the narrator of *The Moor's Last Sigh* ascribes the "gaudily colourful" (4) quality of his life-story to the reality of Bombay, "that super-epic motion picture of a city" (129), a "great cosmopolis" which was and is the Central Junction of all such tamashas, and the hottest tales, the juiciest-bitchiest yarns, the most garish and lurid not-penny-but-paisa-dreadful, are the ones walking our streets. In Bombay you live crushed in this crazy crowd, you are deafened by its blaring horns of plenty, and - like the figures of family members in Aurora's Cabral Island mural - your own story has to shove its way through the throng (128). At another point the narrator refers to the city as "my inexhaustible Bombay of excess" (193). The use of baroque style and maximalism is thus justified by the nature of reality that the novel is trying to represent, which closely resembles Carpentier's views outlined above.

After a vivid description of his first walk through Bombay, which made him feel completely exhausted, Octavio Paz relates how he sat at the foot of a huge tree... and tried to make an inventory of all I had seen, heard, smelled, and felt: dizziness, horror, stupor, astonishment, joy, enthusiasm, nausea, inescapable attraction. What had attracted me? It was difficult to say: Human kind cannot bear much reality... (12). Paz's remark that an "excess of reality" easily becomes "an unreality" for us may be used as yet another, this time psychological, and in my opinion felicitous, explanation of the recurrence of baroque multiplicity in magic realism. It would follow from Paz's observation that it may add to the magical effect of the narrative.

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ALICE WALKER'S MERIDIAN AS A REFLECTION OF IDENTITY CRISIS

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Abstract

Alice Walker's *Meridian* (1976) is considered an autobiographical Work. The title character was born in rural south, like walker and uses Education as a means of escape. She got married and conceived to a high school dropout. Meridian struggles with the thought of suicide and killing her child but decides to give up education at the College. After graduation, she enters an organization of Black Militants in Mississippi, but realizes that she is not willing to kill for the Cause. With this idea, she determines to return to rural Mississippi to help its residents' struggle against oppressions. One of the basic principles of Walker's "Womanism" is based on the concept of identity in Gender, racial, sexual oppressions and crisis. This paper attempts to explore the writer's identity crisis in the African American Society with reference to Walker's *Meridian*.

Key Words: Autobiography; suicide; killing; oppressions; Gender-identity.

Alice Walker has been a 'Womanist' in the American Fictional Scene for more than two decades. She prefers to call herself a "Womanist" because Womanism is better than feminism which appreciates "Women's culture, women's emotional flexibility and women's strength". Walker explores the relationship between black women's past tradition and Social changes. In the aspects of Racial, Gender and Sexual discriminations, the writer wants to attain spiritual wholeness in the same. Novelist seeks to transform "Suspended women" in to "emergent" black women. Mary Helen Washington analyses that walker's personal construct of History of Black women shows how the "Suspended" black women characters in literature emerge as the Emergent Women".

Walker's pre occupations are "the spiritual survival of her gender and race. But she committed to explore the oppressions, the insanities, the loyalties, and the triumph of black women". Certainly, these pre occupations are evident in Walker's Second novel *Meridian*. This novel has been chosen for exploration of the process of personal and social growth through the character Meridian. The Novel concerns a black women's life as it unfolds itself for self realization and freedom.

Walker brought out black people's struggle, the relationship between parents and children in order to set cultural identity to meet triumphs of black women. The Protagonist of the novel, Meridian searches out her tradition, and her family heritage in about Meridian hills. She faces the struggle against the oppression of Black women to reclaim her past, and past relationship to the present black community to gain internal strength to endure hardships.

Meridian has three major parts: the first part focuses on Meridian's initiation into adulthood and her preparation for a journey; the second part describes Meridian's active participation in the Civil Rights Movement after her renunciation of her child; and the third part "Ending" concentrates on atonement and release. The novel opens with Meridian's encounter with Truman, her old comrade in the Civil Rights Movement. He observes her leading the black children of the town of Chicokema to see marilene O'Shay, a mummy of a dead white woman, and tells her: "when things are finished it is best to leave." Meridian's replayed "And pretends they were never started?" (27) is the prelude to a journey back in time. The author moves backward in time to meridian's recent past and her mother's past to introduce the theme of her grown up. In a flashback Walker briefly mentions Meridian's experience with the revolutionary group in New York. They forced her to answer the question "Will you kill for the Revolution?" with a positive yes. As they were waiting for her to speak, she recalled her past experience by remembered her mother and the day of her death. Her mother's love was withdrawn when she was thirteen. Her sense of alienation and isolation had deepened. Knowing that she was not whole, because at thirteen she had not come to grips with the whole truth about herself, she began a search for freedom. Coming back to the present, she replies like a true revolutionary that she would reject violence as the approach to change. She opines

... With pre consumed notion of the past; by the memory of the old black men in the South... and the sight of the young girls singing in the country choir, their voices the voices of angels (28).

The authorial comments: "And so she had left North and come back South... remaining close to the people to see them, to be with them, to understand them and herself" (31) foreshadows the direction of Meridian's pilgrimage in search for genuine values. Walker arranges the narrative material in the novel in "a crazy-quilt story" form. They work on many different levels and form a complex structure. The personal histories of Anne-Marion Coles, the Wild Child, Meridian's father's grandmother, Feather Mae and the legend of the sacred tree Sojourner are combined with the past of Meridian's parents, Mr. and Mrs. Hill, provide the insight into the various layers of black experience. The chapter "Indian and Ecstasy" focuses on Meridian's loving relationship with her father and her spiritual

communion with him. This spiritual experience down the Serpent's side which gives Meridian "the feeling of flying" (58) is their tangible connection to the past. It is through her relationship with her father that the seeds of her spiritual growth are sown.

Meridian's experience in the initial stage is more painful. No one in her family had taught her what to expect from men, from sex. The lascivious Daxter, the in charge of the funeral home, pursues Meridian when she was only twelve. She sees his assistant's seduction of another school girl. Still, she is unaware of her physical vulnerability and acquires a young boyfriend, Eddie. She marries her lover and awaits the birth of her son. Her whole life is changed by an experience but she did not enjoy.

Meridian sees sex as a "sanctuary." Once in her sanctuary 'Meridian wonders if she could "look out at the male world with something approaching calmness, even charity; even friendship" (62). Her marriage with Eddie falls apart because she feels that as a wife her life will always be empty and she cannot diminish her "Self" Besides, Eddie, like his name, "would never be grown up" (70).

Now the focus of Meridian's story is her motherhood. Walker presents a cultural context in which motherhood becomes a vehicle for rebellion for Meridian. She employs two frames: the outer frame demonstrates how the culture gives women few alternatives to the suffocation and sacrifice of traditional wifedom and motherhood. The inner frame is the family life of the Meridian Hills. She discovers from the example of her own mother that motherhood is "being buried alive, enclosed away from her own life, brick by brick. "Her mother makes her feel guilty for "shattering her mother's emerging self" (51). Her girlhood and young adulthood represent periods of emotional impoverishment.

Meridian's process of initiation into this new responsibility of motherhood, her pregnancy came as a total shock. She knew she did not want the child. After the birth of her son, he did not feel like anything to her but "a ball and chain" (p.69). to know the needs of the child was, "Slavery" (ibid). She craved for freedom and felt as something perched inside her brain was about to fly. She does not want to raise her child in a society "where children are not particularly valued" (114)

Walker suggests that it is not easy for Meridian to break outer frame and to free herself from the mythic image of motherhood in which culture and society has imposed upon her. The chapter "Battle Fatigue" analyses Meridian's confrontation with her mother and her inner conflict. The seventeen-year-old Meridian, a deserted wife and a mother, becomes aware of the past and present of the larger world in 1960 she decides to offer Education to her child at Saxon College to save the life of the same. Meridian has confrontation with a degraded self-image because she could not live up to "the standard-of motherhood that has gone before" (91) which results in her illness and the "spiritual degeneration" in herself (92). She can study at Saxon and actively participate in the Civil Rights Movement. Meridian gets rid of her illness, her recurring dream of death and her own feelings of inadequacy and "primeval guilt" (125). Miss Winter treats Meridian as if she was her own child, forgives her and makes it possible for her to encounter the hostile world with renewed strength.

Though marriage and motherhood had given bitter experience, she attempts to transform herself. Meridian's journey through myth and legend, which caused by the dream of her mother, take her back to the time and space. When she admits her child and leaves Mississippi to attend college, the first journey of Meridian toward Saxon College symbolizes white values that have been seeped into the thinking of middle-class blacks. The college was a training ground for capitalists and for "ladies". Meridian, Anne Marion and other likeminded friends decide that they have two enemies: "Saxon, which wanted them to become something. Ladies that was obsolete, and the larger, more deadly enemy, white racist society" (95) Meridian hates capitalism and by her involvement in the Civil Rights Movement and the Atlanta Movement she wants to seek social justice, and her black women to be "accepted" as equal as white.

In the movement she meets "the vain, pretentious" (99) activist and artist, Truman Held. While demonstrating against segregated facilities both Meridian and Truman are arrested and beaten. During this struggle for their rights Meridian realizes that she loves Truman and that "they were at a time and place in History that forces the trivial to fall away and they were absolutely together" (84) But such experience of union with Truman is changed when she conceives his child and has an abortion because Truman becomes involved with a white exchange student, Lynne Rabinowitz. To Meridian it seemed "doubly unfair that after all her sexual experience and after one baby and one abortion she had not once been completely fulfilled by sex" (115) she realizes that in order to retain her wholeness she must rise above bodily claims.

Abortion and sterilization symbolize her anger and frustration against Truman's cause for pregnancy and motherhood. It is a metaphor for rooting out sexual weakness because Meridian wants to meet Truman at an equal level. It is a key event that pushes Meridian forward to act on her will. In fact, Meridian's "pilgrimage" cannot be complete until she surpasses sexual, maternal and racial categories through her participation in the revolution and her commitment to "recreate" the world where black children may thrive without thorns of guilt. Walker focuses on the complex relationship of Meridian, Truman and Lynne. She analyzes how sexism and racism work in to influence black woman-black man-white woman relationships. Truman marries Lynne because he wants to marry a white woman; because the white woman is the closest thing to power, he can get in white America. But the other black revolutionaries, like Tommy Odds, view Lynne as a white "bitch and Truman suffers under the "pressure of Ostracism from the group" (138). He considers whether Lynne is guilty of "whiteness" or he is guilty of marrying a white bitch. Truman finally returns to Meridian three years after he married Lynne and confesses that loving Meridian makes him feel "healthy, purposeful" (140) Meridian's love for Truman is "purged." It was not sexual, "it was forgiveness" (173) Lynne gives him back to Meridian and returns to the South.

The novelist takes a visionary step into the finally section of the novel. Meridian stands as a witness to the common lot and a survivor of the movement. She has not wanted to kill people in the movement and convert them to a new approach to revolution. She has reached a point in her life where she is no longer evasive. Listening to the old music, she is moved by the beauty of the black church.

Her contribution to the revolution will be her "memory songs". For its "the song of the people, that transformed by the experiences of each generation, that holds them together, and if any part of is lost, the people suffer and feel like are without soul" (201). in order to transform their society, the black people must understand their own heritage and transform themselves. It is the process of attempting social change through: the movement that Meridian discovers her own personal path. This discovery is itself the core of the novel. Truman apologizes for hurting Lynne's feeling. When Truman asks Meridian if her love for his is changed, her response "No, I see you free. ..." (216) shows that she has released herself from the sexual bonds and she intends to pursue her own wholeness. Meridian's search for identity in all respects can be defined as her attempt to express the totality of self and how that self is related to the world. It is a search for freedom, Joy and contentment in being a woman, and for self-love. In keeping with the black literary tradition, it is a search for escape from the body and freedom for the soul by discovering "the truth" in the darkness. Walker suggests that Meridian is "free at last" Her ties are not with a man, a family or with a specific community. Motherhood for her includes not only rearing of children but nurturing life, the continuity of life. She sees her existence as inseparable from all black people and writes. There is water in the world for us brought by our friends though the rock of mother and god vanishes into sand and us, cast out alone to heal and re-create ourselves (213)

It is in this sense that Meridian's search for self-affirmation and wholeness acquires a mythic dimension. Unlike Nella Larsen's Helga Crane in *Quicksand* whose anguish is existential because she is bound first by race and then by sex, Meridian embraces her black heritage, her woman's heritage and reaches out her people. She is a liberated black woman who knows what she should take from the past to create a new future. Truman knows that in her "pilgrimage" Meridian would return to the world "cleansed of sickness." He would never see "his" Meridian. "The new part had grown out of the old" (p.219) Thus Meridian's incorporation into the community is, in essence, a new birth into spiritual wholeness in Race, Gender and Sex.

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Abstract

Much of Coetzee's writing reflects either directly or indirectly on current measures describing within South African society, though critics have cautioned against frank allegorical interpretations of his work. For Coetzee the post-colonial does not sign the formal breakdown of empire, but rather a new, and in many respects more treacherous phase of colonisation. Coetzee has fought writing straight works of fiction and non-fiction, favouring instead to work across classes and genres in ways that produce ontological and epistemological questions for his readers.

Key Words: South African Society, post-colonial, oppressed, community

One of a number of youthful, rebellious literary expressions communicating against the apartheid administration in the 1970s and 1980s, Coetzee's typical prose was recognised early on as both expressive/indefinable and as politically crucial. His work has been associated positively with Nabokov, Kafka and Conrad, and by the time of established works such as *Foe* (1986) he had already attained international approval.

Much of Coetzee's writing reflects either directly or indirectly on current measures describing within South African society, though critics have cautioned against frank allegorical interpretations of his work. More effectively we might think of Coetzee's writing as curious any easy communication between fictional representation and the fast, shocking changes that have altered and continue to make over South Africa. As the story of his recent Man Booker Prize-winning novel *Disgrace* (1999) establishes (with its met fictional essentials, its suspension in the present tense and its generation of critical vagueness) accuracy is somewhat Coetzee pursues to problematize rather than produce.

In Lurie's decrease from Romantic Professor to Professor of Communications we see the wider decrease of art and language to the dominion of the literal, the functional, and the practical. Within this new world academics have become, as Lurie goes on to put it 'clerks in a post-religious age'. The limitation of imagination inferred here is paradoxically taken in the translucent literalism of the new courses Lurie imparts, and in the numbers used to tag them (which suggest justification and mechanical development). The literary critic Derek Attridge argues that moments such as these notify

the reader against decreasing *Disgrace* to an active political function. That to do so is to ignore crucial sections of the text that are hard to 'read off' as conventional messages or communication acts, such as the puzzling role of dogs and animals in the novel, or David's unfinished opera, or the significance of the central (but absent) rape scene in the novel.

When Lurie is disgraced by his university following an affair with a student, the professor retreats to his daughter's isolated smallholding. The personal differences between David and his daughter unfold against this backdrop as tensions rise within the recently emancipated local community. Coetzee's unforgiving vision of South Africa exposes the insecurities of a floundering, but still dominant white culture.

Disgrace lights two of the key concerns of Coetzee's work: the historical inspirations behind colonialism and its inheritances in the post-colonial era. For Coetzee the post-colonial does not sign the formal breakdown of empire, but rather a new, and in many respects more treacherous phase of colonisation. For example, his debut novel, *Dusklands* (1974) includes two novellas that suggest seemingly discrete historical events, one colonial and the other post-colonial, in a way that obviously asks us to reflect upon their relationship to one another and to present-day South Africa more commonly. The first handles America's part in Vietnam. The second is set 200 years earlier and emphasizes on a Boer settler in the 1700s. The very different protagonists of these narratives: Eugene Dawn (an expert in psychological warfare) and Coetzee (an adventurer and pioneer), turn out to be involved in strikingly similar forms of oppression. It is this kind of connection between oppressors and oppressed in the second part of *Dusklands* that also structures one of Coetzee's most influential, worrying and fruitful works to date: *Waiting for the Barbarians* (1980). The novel, which is on one level an examination of the relationship between barbarity and culture, takes its title from a poem by the Greek poet Constantine Cavafy. Winning the James Tait Black Memorial Prize, the spare, razor sharp prose renowned in *Waiting for the Barbarians* has become a trademark of Coetzee's later fictions.

Set in an unspecified boundary, a desert landscape at unspecified point in time, the novel is an allegorical examination of the relationship between coloniser and colonised. The Magistrate, who is in charge of the frontier settlement, finds himself caught between the empire that pays him and the barbarians for whom he feels cumulative compassion. Through the disputed viewpoint of the Magistrate it becomes seeming that the barbarians are not simply a population 'out there' beyond the frontier occupied by the empire. The dreadful, barbaric violence that Colonel Joll deals out to an elderly barbarian and a young child in the opening pages works to draw into question the very difference between civilised and unrestrained. The barbarians, it would seem, lie at the heart of the very empire that builds them as other. *Waiting for the Barbarians* was followed by the brilliant Booker Prize winning *Life & Times of Michael K* (1983).

The allegorical concepts of Coetzee's *Barbarians* are exchanged here for a moving, close account of Michael K and his mother. The dilemma of these two characters, both of whom are

physically incapacitated, gets worse as they find themselves without a safe home or income in a South Africa uncertain apart by civil war. A dream of a better life in the country inspires their choice to leave the city behind. Their twisting journey out of Cape Town (Michael pushes his mother in a wheelbarrow) offers little sign of freedom or escape. Michael's mum dies, along with the dream they shared, long before they reach the dreamed of destination. Like *Disgrace*, the novel arouses a rural departure, an idyllic setting that eventually fails to materialise and resolve the problems of the central character. (Escape, incidentally, is also the organising theme of Coetzee's most recent novel, *Youth: Scenes from Provincial Life II* (2002)). These are often bleak inflexible works of fiction in which determinations tend to replace solutions.

Coetzee's critically much-admired novel *Foe* hints a provisional leaving from the South African countryside. A short, influential book, it reinvents the story of Defoe's *Robinson Crusoe* from within the city of London. Re-imagining a recognised novel of British imperialism, it adopts and adapts a separate policy within postcolonial fiction (including Jean Rhys' *Wide Sargasso Sea* and Morag Gunn's *Prospero's Child*), as it writes back to the culture of the coloniser. *Foe* is eventually a tale about tale telling: the female narrator, Susan Barton, expresses her story in order to find an important person who will publish it. Yet for all its fruitfulness and diversity of voice, *Foe* is most particularly a novel about muteness, the silence of Friday, whose voice Coetzee refuses to signify. Through the silent centre of this text, Coetzee manages to expose the extent to which language, too, is a key device of colonisation. More recently, in work like *The Master of Petersburg* (1994), Coetzee signs his obligation to other literary figures and traditions notably the work of Dostoevsky and *Crime and Punishment*. Coetzee's various inspirations can also be found within his critical writing, of which *Stranger Shores: Literary Essays 1986-1999* (2001) is an excellent recent example. Bringing together 29 essays, including pieces on T. S. Eliot, Defoe, Turgenev, Kafka, Rushdie, Gordimer and Lessing (not to mention an account of the 1995 Rugby World Cup) this collection is an important companion volume to Coetzee's earlier collection, *Doubling the Point: Essays and Interviews* (1992).

If *Doubling the Point* and *Stranger Shores* help brighten the characteristically sloping fictional work of a scandalously isolated and reserved writer, Coetzee's 'memoirs', *Boyhood* (1997) and *Youth* (2002), assure even greater insight. Nevertheless, *Boyhood* elects to speak of the young Coetzee in the third person and its brief oblique narratives ('scenes', the subtitle tells us) serve to keep the reader at arm's length. Both *Boyhood* and *Youth* can be read either as novels or memoirs and their combination of fiction and biography serves to frustrate any authoritative understanding of the author's formative years. Coetzee's genre-bending work continues in text like *Elizabeth Costello: Eight Lessons* (2003), a book defined in *The Guardian* as 'non-non-fiction', and by David Lodge in *The New York Review of Books* as a work 'which begins like a cross between a campus novel and a Platonic dialogue, segues into thoughtful memoir and imaginary musing, and ends with a Kafkaesque bad dream of the next world.' Some of the so-called 'lessons' of *Elizabeth Costello* are in fact lectures Coetzee delivered at Princeton and published under the heading *The Lives of Animals* in 1999.

Coetzee's next novel, *Slow Man* (2005), received mixed criticisms. It concerns Paul Rayment, a 60 year old Australian who loses a leg after being hit by a car. Paul is cared for by a Croatian migrant until he announces his love for her and she escapes. At this point (and this is the bit that dissatisfied some reviewers) the reader discovers Paul is in fact a fictional character in the literary fancy of Elizabeth Costello (the protagonist of *Elizabeth Costello*). The metafictional narrative that follows, in which the text searches and abandons various fictional potentials for Paul, brings the reader closer than either *Boyhood* or *Youth* to the artistic impasses of Coetzee the artist. If Coetzee emboldens his readers to identify Costello as Coetzee's alter ego, his latest novel, *Diary of a Bad Year* (2007), signifies a more fundamental misperception of the boundary between character and author. Its central figure is an ageing author who shares Coetzee's initials, has newly moved to Australia, and has even written some of the same books. The book takes the form of a series of essays on real subjects, from terrorism and Tony Blair to Tolstoy. But this is not a simply a gathering of essays and the books protagonist is not (quite) Coetzee. Since the publication of *Disgrace* in the late 1990s, Coetzee has fought writing straight works of fiction and non-fiction, favouring instead to work across classes and genres in ways that produce ontological and epistemological questions for his readers.

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ROLE OF WOMEN IN THE SELECT NOVELS OF CHETAN BHAGAT: A STUDY

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Abstract:

Chetan Bhagat's novels mirror the problems of youth in his novels. In the fast moving world, both men and women get various opportunities to showcase their talents. The novels of Chetan Bhagat break the social norms by providing equal importance to female protagonists. He portrays them as noble, independent and perfect. This new 'Indian Postmodern Women' are ever ready to face the frustrations and catches the choices with dignity. This paper is an attempt to study the role played by women protagonists in the novels of Bhagat.

Keywords: liberation, empowerment, physical violence, culture shock, passions.

Born in a middle class family, Chetan Bhagat has composed well known novels like Five Point Someone (2004), One Night @ the Call Centre (2005), The 3 Mistakes of My Life (2008), 2 States (2009), Revolution 2020 (2011), Half Girlfriend (2014), One Indian Girl (2016) and One Arranged Murderer (2020). Bhagat focuses his attention on the passions and problems of the youth. His characters are neither subtle nor violent. The female protagonists in the novels of Chetan Bhagat are preferably courageous and outspoken. In the contemporary world, women are fighting for equal rights. The status of women in some countries is in worse condition. On the other hand, some women are educated well. Their progress and empowerment are surely the impacts of globalization and modernised thinking. In his novels, it is evident that Bhagat shows his concern for the female leads. From a college student to life partner, the female characters are presented with dynamism. Bhagat acknowledges his gratitude to this editor, friend and first reader Shinie Antony, who plays a vital role in the success of his novels.

One night @ the Call Centre:

Esha, Radhika and Priyanka in the novel are portrayed with intellect and abilities, equal to men. As call centre employees, they earn equal as the men. The tedious night shift hours shows their choices to choose a profession. Esha's dream to become a model isn't possible without money. She is busy in visiting advertising agencies during the day time. For Esha, the call centre job offers salary to fulfil her wishes. She is independent and courageous and run away from home to pursue her dreams. As the fast icon of the call centre, she carries herself well. Esha wins the title of 'the hottest chick at connexions' (ON@CC 19)

Radhika, the married woman in the group is the victim of patriarchy. After revealing the secret of her husband's extramarital affair, she took a bold decision. As a typical daughter-in-law, she cooks three meals per day, completes household chores and get ready for night shifts. For Radhika, the call centre cabin is the only place where she can rest for a while. This middle class woman is dealing a tough time between domestic violence and pressure in the work place. As a result, she consumes 'anti-depressants', which she admits as 'yes it's legal addiction' I can't live without it. But it is still better than having to

face my life (ON@CC 79). With the help of her teammates, Radhika confirms the extramarital affair of her husband. She gives divorce to her Anuj neglecting the apologies of him. She chooses a right decision in her life to put an end to the never ending tortures.

Priyanka in the novel is clenched between mother and her boyfriend Shyam. She is unable to refuse the choices made by her mother. She decides to marry an NRI guy Ganesh to fulfil the wishes of her family. Women are ready to do sacrifices to uphold the values of the family. Priyanka's attitude is neither subtle nor dominant. Being a single parent, her mother interferes in the life of Priyanka. She never admits Priyanka to act independently. Most of the women in India are discriminated to take part in family related decisions. Shyam and Vroom help Priyanka to reveal the secrets of Ganesh. After realising the wrong decision took by her mother, Priyanka boldly refuses the wedding. The power of education in the life of a woman is infinite, which helps Priyanka a lot. Understanding the uncertainties in the call centre job, she quits and joins in a B.Ed college to continue her studies.

The 3 M'stokes of My Life:

Vidya, the female lead in the novel is young, energetic and beautiful. Hiding her dreams to become a PRO, she is unwillingly preparing for the medical entrance examinations, to fulfil the dreams of her parents. She settles herself inside her house like a caged bird. Her parents are very strict and they never permit her to go out. Ishaan, her brother arranged home tuition for Vidya. Govind, the tuition teacher is very cautious about her studies and rejected the love proposals of Vidya. However Govind reveals his love for Vidya on her eighteenth birthday. The intimacy between them has gone beyond the limits. Vidya and Govind have sex with each other many times. Her bold nature is revealed when she says, 'wow, I am an adult and am no longer a virgin, so cool. Thank God.' (3 Mistakes 201). Govind feels guilty of having sexual affair with his friend's sister. Teenage is a crucial stage in everyone's life. Such is the case of Vidya, who never listens to others. Govind's role as a tuition teacher is quiet tough. She shares her personal life experiences with Govind, which shows the restricted communication inside the house. She begs Govind to listen to her passions. She loves to fly like a bird, which she admits as, 'yeah, what is your role as my teacher? Teach me how to reach my dreams or how to be a drone?' (3 Mistakes 102)

In the novel, Vidya represents culture shock in the society. She violates the traditional beliefs by having pre-marital sex. Her wavering mind shows the lack of maturity to handle the situation, Bhagat's portrayal of Vidya, could be taken as an example for teachers and parents, to handle the teenagers with love and care.

2 States: The Story of my Marriage:

The novel depicts the insurmountable odds faced by Krish and Ananya to convince their parents to get success in love. The wedding between the Punjabi boy and a Tamil girl seems to be a great adventure. At last, the dream wedding comes true with the efforts made by both Krish and Ananya. Ananya's journey from Madras to Indian Institute of Management, Ahmedabad shows the extraordinary achievements. She is bold, brilliant and beautiful. Her positive approach towards the Punjabi people, helps her to overcome the problems in the wedding between Duke and Minti. Both Krish and Ananya hold responsibility to convince their parents for the wedding. As the elder daughter, she respects the values of her parents. She never lets them to humiliate in front of the Punjabis. On the other hand, she goes to any extent to fulfil her wishes. This typical Brahmin girl craves a lot for chicken, unminding the orthodoxical norms. Ananya's mind is in helpless position between the expectations of her parents and Krish. Her adjustment with the customary practices of the Punjabis and modernity is well portrayed in the novel. Bhagat pays more attention to Ananya to depict herself as autonomous and savvy. Ananya never compromise the dignity of her parents in front of her love. Her questions to Krish to accept a South Indian

bride is an eye opener in the novel. Ananya asks, will your mother change? Will her bias towards me, towards South Indians, towards the girl's side, change? (2 States 233) shows the strong approval of bride's side.

Bhagat pays attention to the encounter between North Vs South in the novel. The South Indians are discriminated in the novel for their dark complexioned skin. Many references in the novel, clearly depicts the variations between the Punjabis and Tamilians which is a great threat to National Integrity. Amidst all these differences, India is standing as 'One Nation' upholding the motto 'Unity in Diversity'. Both Krish and Ananya in the novel are very strong to identify themselves as 'Indians' and not as 'Punjabi' or 'Tamilian'. The importance of cordial relationship for a healthier society is unavoidable. Ananya's strong desire to see the happy faces of her parents on the wedding is fulfilled. When Krish opens up a choice to elope, Ananya refuses to do so. Her control in actions and modernised thinking puts her in a unique place. As a daughter-in-law in a Punjabi family, she steals the hearts of the millions of the readers.

Half Girlfriend:

Chetan Bhagat's portrayal of women in his novels show the status of women in the contemporary world. More than an author, he voices out the liberation of women in his motivational speeches and columns. The women in his fictions strongly opposed against patriarchal system. To establish equal rights in political, economic and social status for them, he uses his fictions and non-fictions as a tool. Riya Somani in Half Girlfriend is one such typical representation, born in an affluent Marwari family, she leads a luxurious life. The underlying patriarchal norms in her family is revealed when she says, 'the problem is my family, they're obsessed with money. I'm not' (HGF 32) while the male members in the family are running behind money, the females are spending it lavishly.

Riya's relationship with Madhav fills the affection, which is neglected to her in her family. Her dream to ring in a bar in New York surprises the readers. The longing of her inner consciousness to chase her dreams is just like an escapist fantasy. She admits it as:

"Not really. May be it's just an escapist fantasy. But I have had it since I was twelve. We had gone to New York. The city blew me away. I saw people who loved what they did. They weren't rich, but happy. And there was this lady in a bar. She sang from her heart, unaware of everything around her." (HGF 33)

Bhagat's choice of words to depict the character traits of Riya as 'silent Riya', 'shy Riya' is astonishing. The real reason behind her silence is a painful story. Riya experienced physical violence by her father once, which she mentions in one of her journals. Many children and women in India suffer from physical violence. Sandra Martin's article "Domestic Violence in Northern India" reveals it as, 'domestic violence in India includes any form of violence suffered by a person from a biological relative, but typically is the violence suffered by a woman by male members of her family or relatives. (HGF 421). Her relationship with Madhav ended up in vain when Madhav make attempts to have sexual affair in the hostel room. Out of the compulsion of her parents, she marries Rohan. Her in-laws and husband tortured her of being useless at home. The level of violence goes beyond the limit when Riya finds the secret affair between Christine and Rohan. Riya's complaints about Rohan and in-laws to her mother seems to be worthless.

Riya's bitter experiences with the male members in the society reminded about her liberation. She gets divorce from her husband and flies to New York to pursue her dreams. Her love for Madhav is kept secret as her journal because of the convincing advice by Rani Sahiba. Riya's longingness to be free from male chauvinism is very clear in the novel.

The role of women in the novels of Chetan Bhagat is vital. Being a girlfriend or wife or sister or mother, they play a significant role. Sometimes, they seem to be more courageous than the male. The moral support given by them suggests the necessity of them. The strong appearance of any woman in Bhagat's novel is a clear representation of a bold human being. Bhagat adds value to Simone de Beauvoir's distinction, "One is not born, but rather becomes, a woman." (The Second Sex 301). The challenges and the hardships overcome by the female in the novel help them to live happily.

Chetan Bhagat's female protagonists are in no way inferior to the male. The fight for equal, liberty and empowerment. Their elegance and modernised thinking give them courage to face the difficulties. They are bold, brilliant and optimistic. Their approach towards the society questions the patriarchy and existing traditional norms in the society. Women in his novels boldly share certain things like sex, periods etc., which were considered as taboo. Either as a path breaker or as a trendsetter, they show their vitality in all the aspects.

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ABSTRACT

Every culture in India is unique because of its distinct tradition, rituals, beliefs and cuisine. Since time immemorial, food plays an important role in Literature. Food often represents the basic understanding of social order and civilization. Chetan Bhagat's 2States exemplifies food as an important metaphor to symbolise emotions, resistance, acceptance of a new culture, hierarchy and so on. Food in any artistic work provides both visual and verbal depiction. This paper is an attempt to analyse the use of food as a symbol of identity in the novel.

Keywords: cuisine, maternal rights, tradition, orthodox, food choice, health, life style, ethnocentrism.

INTRODUCTION

Chetan Bhagat's *2 States: The Story of my Marriage (2009)* depicts the love episodes of a Punjabi boy Krish Malhotra and a Tamil girl Ananya Swaminathan. The couple plans to convince the parents for the marriage. They never have the intention of eloping like other youngster do. Although they are aware of the odds in front of convincing the elders, their strong love gave them the spirit to face it. Krish settles in Chennai for a while, finds opportunity to mingle with Ananya's parents and finally succeeds in his task. Ananya on the other hand experiences a tough battle to convince her in-laws. North vs South encounters and racial discrimination in the novel is clearly depicted as an important symbol to understand the cultural, religious and domestic space. To convey the human emotions, food plays an inconvincible role. Many incidents in the novel make references to food and eating habits.

Krish and Ananya's first meeting happens in college canteen, where Ananya mess up with the canteen worker. The tasteless food in the hostel spoils the health of the students. Ananya bravely files complaint about the poor quality of food. Krish understands the situation and convinces Ananya with a painful note, "hunger or tasteless food, hostel life is about whatever is easy to deal with." (6) Food provided in many hostels are in poor quality. The novel gives a special attention to the sufferings of the hostel mates in Indian Institute of Management, Ahmedabad and nobody cares about it. Both Krish and Ananya spends leisure time in restaurants, craving for junk foods. The air-conditioned restaurants near the campus provides spicy foods with soulful music which is comparatively better than the hostel food. Being a Tamil brahmin girl, Ananya has certain restrictions in food habits. The absence of her strict parents gives her the courage to taste to taste non-vegetarian foods. She craves a huge desire for tandoori chicken, romalirotis, rasagullas and beers. Krish is shocked to know her desire to have alcohol in Gandhiji's birth place. Ananya casually mocks his attitude as, "what's the point of getting people free only to put restrictions on them?" (6) Ananya's wavering young mind doesn't allow her to confine within a limited space. For her, food becomes an important tool to fulfil her tastebuds.

The traditional Hindu brahmin family in Chennai is much concerned about the orthodoxies and beliefs. The vegetarian dishes in her own family becomes tasteless for Ananya. When she returns from Ahmedabad, she hides her non-veg eating habits from the strict elders. Krish secretly arranges the chicken dishes and beer for her in his mansion. Bala and other room mates astonishes on seeing the arrangements made by Krish for a typical brahmin girl. Sendil, one of the room mates shockingly asks, "and chicken? What kind of Brahmin is this? And dude, don't get non-veg in this house." (100) The youth of today are born with infinite choices and there is no restrictions even in the choices of

food they wish to have. Rules are just ruling for them, but when it comes to eating habits, they dare to break the restrictions.

Chetan Bhagat's use of food items to depict the conservative attitude of middle-class parents is interesting in the novel. After graduation in IIM, Ahmedabad, both the protagonists earn handful of salary. Though they are financially strong, the choice to marry someone from outside their state is denied to them, as it hurts the beliefs of the parents. Krish painfully shares, "because they are parents. From biscuits to brides, if there is anything their children wants, they have a problem." (39) This conservative approach of some Indian parents annoys the youth of today. Such interruptions inside an individual's desire would spoil the individuality of his or her. Krish and Ananya invite their parents for the convocation day celebration. They find a chance to arrange a casual talk between the elders regarding the wedding. To create a good impression about the Tamil people within the mind of Krish's mother isn't easy for him. However, he tries to impress her telling the talents of the girl, but Kavitha, mother of Krish never lends ears to them, which upsets all. With extreme anger and hot dialogues, the elder's exchanges antartic glances. The writer once again made food as a tool to convince the mother. Krish calms down Kavitha with soothing words and took her to Law Garden, one of the tastiest snack shops in Ahmedabad, to offer Pao bhaji with paneer. To make a good impression about Ananya and her family, Krish should convince his mother initially. His choice to offer desserts with extra butter on his words is ideal, and it helps him a lot. Krish admits his success as, "Nothing soothes an upset Punjabi like dairy products." (44)

Kavitha's restrictions in private and public spheres is revealed in many incidents in the novel. Though she married a military man with the consent of her parents, she is unable to cope with her husband in many ways. He never permits Kavitha to invite her relatives to her house. This made a deep wound in her mind and now she is double conscious to choose a bride for her son. Kavitha's restrictions to have a fruitful communication with fellow human beings in a public sphere shows her preconceived judgements about the people in other cultures. Being a Punjabi, she concerns primarily about her culture and food habits. To mingle with others is however tough for the elders. Culturally biased judgement about other people, food, religion is common in country like India, where people refer one's culture as superior to the other. Kavitha's greatest obstacle is her prejudices about people outside her Punjabi traditions, which is revealed when she packs limited mithai boxes and meals for her son and her. She restricts Gujarati food and says, "I almost brought packed meals. I don't want to eat the Gujarati daal with sugar. Is it really sweet?" (42) Her ethnocentric attitude limits the communication with the Tamilians. William G. Sumner in his book *Folkways* (1906) analysed the characteristic features of ethnocentric people as, "ethnocentrism as often leading to pride, vanity, the belief in one's own group's superiority and contempt for outsiders." (13)

Food in the novel often restricts the interpersonal communication in a public sphere. Kavitha is always busy in eating biscuits, paneer tikka masala rather than making a cordial relationship with the south Indians. A packet of biscuits silences the conversation between the elders during the eight kilometres travel to Sabarnathi Ashram from the campus. While Ananya's parents are busy in watching the scenery, Kavitha ignores others eating Nice biscuits. She never offers anything to them, which Krish admits as, "she took one biscuit and put it in my mouth, to assert maternal rights on me. Of course, I couldn't refuse." (50) The novel throws light on the racial discrimination of the dark skinned people in the country. Kavitha convinces Krish about fair complexioned girls in Punjab as, "I can show you Punjabi girls as fair as milk." (57)

Bhagat uses food as a reference to show the priority, superiority and as an opportunity to depict the wealthy life style of the people. Pammi aunty, Kavitha's neighbour in Pitambura lives in a luxurious villa. She serves heavy snacks like cashews, Dubai dates, milk cakes, samosas, chole bhature, kachoris, jalebis and many varieties of chutneys to display her affluent life style. Krish understands the reason for her obesity and he worries much about his diet and calculates the calories by just looking at the dishes, he adds, "twenty thousand calories were plonked on the table." (63) The youth of today are aware of health and psychology. To maintain good health, one should consume healthy food. In a big country like India, people develop food habits according to the socioeconomic status, culture, geographical location, climatic conditions and convenience. To provide a common

diet for all is a challenge in India which cannot be achieved easily. Ogden explains the importance of health as, "health psychologists understand health to be the product not only of biological processes but also of psychological, behavioural and social processes." (*Health Psychology* 52) The novel portrays necessary details about the character's choice of food to understand the emotions and needs of them.

Eating and serving food supplies a good chance for advancement in progress and to share our feelings. A special bond between parents and children occur while serving food with love and care. Kirsh reveals his love for mother when he experiences a different cuisine in a strange culture. Being an alien, he develops poor eating habits when he is Madras. The vegetarian dishes like upma, sambhar, tomato rice, rasam, idly podis which he mentions as 'gun powder' seems to be tasteless in front of the hot paranthas prepared by his mom. Missing mother's love is clearly expressing in his choice of food, he says, "I dipped my idly's into coconut chutney and ate it. I missed my mother's hot paranthas at breakfast." (98)

The wedding between Ananya and Krish finally happens after many tough battles. The basic understanding between the two families is necessary for the wedding to happen. Preparations are made in Madras and special arrangements are done to invite the Punjabi relatives. In spite of that, the cousins of Krish enjoy the free meals offered in Rajdhani Express announcing the bitter truth, "take non-veg, the madrasis won't give you any." (249) The silly advises from Shipra masi shows her concerns for the food rather than meeting the new in-laws. Chetan Bhagat tries to sort out the differences between the Punjabis and the Tamilians through the food they prefer. The South Indian cuisine is limited with rice, dhal and curds according to them. Krish explains, "for Punjabis food triggers an emotional response, like say music. And the array of dishes available in a buffet is akin to the Philharmonic orchestra. The idea is to load as many as calories as possible onto one plate, as most party caterers charge based on the number of plates used..." (222)

Ananya tries to create a good impression among the Punjabis by her cooking talent. Her maiden attempts to prepare Punjabi style dinner end up in turmoil. She is upset thinking her inability to satisfy the desires of her mother-in-law. Almost in every Indian family, there is mother or daughter or daughter-in-law to cook food. This kind of basic expectation is found in Krish's family. To the surprise of every one, Ananya fails in the contest. Krish consoles her and renders his support to fulfil the desires of Kavitha. The typical South Indian girl is unaware of the spices predominantly used in Punjabi houses. Krish explains her about them as, "Remember the five constant spices in every Punjabi dish – salt, turmeric, red chillies, coriander powder and garam masala." (193)

Food, whether served in restaurant or house gives refreshment, nourishment and happiness to the consumers. Hotels and restaurants offer variety of dishes, some of them are very hard to try at home. In huge cities, people find these places are apt to spend time with the family members during the weekends. The importance of hospitality along with good food can be experienced in hotels. According to Christine Pohl, "In ancient cultures hospitality involved welcoming the stranger and offering him food, shelter and safety." (4) (*Making Room: Recovering Hospitality as a Christian Tradition*) In the novel, such hotels play an important role to understand the inner psyche of the elders. Krish takes a bold decision to seek the permission of his in-laws while they dine at Taj Connemara hotel. The cuisine is known for spicy food, desserts and cocktail. Krish orders the favourite dishes of all of them to show his familiarity in understanding their desires. To inspire them, he tries to read the menu card which is in Tamil language. However, he finally succeeds in his task, wins the hearts of Ananya's parents. Krish experiences more emotional responses from Ananya's family. For the first time, he finds food filling both his stomach and soul. With delighted feeling, Krish answers the waiter, when he asks:

'Sir, did you enjoy your meal?'

You bet, I did, tipping him more than the bill that night. (184)

The elders in the family join hands in the fairy tale wedding. Mr. Swaminathan surprises everyone by serving food with love and affection. His ego and hatred towards the north Indians fade out in front of the care and support. The relatives of Krish wonder about the way they are treated by

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the girl's side. Both the family members seem to be united as one family in the wedding forgetting all the discriminations, differences and the bias.

The choice of food is another important aspect focussed in the novel to portray the memories and aspirations of the characters. Either Krish or Ananya never demand the food preferred in their culture. Both of them assimilate with the food and cultural practices followed in the particular state wherever they move. Only the elders in the family concern much about the choice of food. Compared to men, women in the novel worry about the food choice. The preferred food includes taste, convenience, satisfaction and cultural familiarity. To satisfy the needs of the Punjabis and to make them feel closer, the wedding day dinner includes north Indian choices. A recent study in *European Journal of Clinical Nutrition* analyses the gender differences in selecting food as, "when it comes to selecting food, women are more likely than men to choose and consume foods based on health concerns or food contents."(1258)

CONCLUSION

Food is used as a tool to mark the identity of the people in different cultures in the novel. All the activities related to food including serving, preparing and consuming explores the importance of human association with the fellow human beings. Food is one of the basic necessities, which provides meaning to the intangible cultural heritage in the world. *The Meaning of Food*, according to Patricia Harris is, "our attitude practices rituals around food are a window onto our most basic beliefs about the world and ourselves." (8) Chetan Bhagat's *2 States* examines the importance of cordial relationship among the people in India by reflecting the role of food. Through the food habits, the writer has made a clear understanding about the underlying values of life. One cannot deny the fact that our social, economic, political and cultural aspects are being shaping up by the food.

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Ostracoda Morphometry in Deciphering the Paleoenvironment of Epipelagic to Bathypelagic Zone, off Visakhapatnam, Bay of Bengal

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Abstract

An account of the Ostracoda morphometric measurement from the epi to bathypelagic zone of offshore Visakhapatnam and thereafter, the impact of paleoenvironmental changes are discussed. Traditional morphometry was done in carapace of *Actinocythereis scutigera*, *Bradleya andamanae*, *Cytheropteron* sp., *Lankacythere coralloides*, *Ruggieriaindo pacifica*, *Bythoceraina* sp., and *Bairidoppilata (Bairidoppilata) alcyonicola*. *Krithe* sp. Antero-posterior length and dorso-ventral height (dimensions) have been marked, measured and analysed for these dominant species. Different species exhibit shape variations with respect to bathymetry. Relative warp analysis reveals that Ostracoda can also thrive on deep waters by adapting the shells to environmental necessities. The results show that shallow dwelling species having greater dorso-ventral readings, than deep water forms. Observed antero-posterior length and dorso-ventral height ratio is 3:1 for deep water *Krithe* sp., but for shallow water forms the ratio is 2:1. However, the deep water taxa *Cytheropteron* sp. exhibits a thicker shell by accumulating maximum calcium percentage in the shell, which is absent in shallow water *Cytheropteron* sp. and this clarifies that different species of Ostracoda reacts differently to environmental changes for survival. Valve area, valve perimeter, shell thickness, roundness and elongation of Ostracoda valves vary with respect to the habitat of the species. The valve area of the carapace decreases in the agitated environment and cool water temperatures. Species with well-developed ornamentation maintain a thicker shell.

Keywords: Ostracoda, Morphometry, Relative warp analysis, Paleoenvironment, Bay of Bengal

Introduction

Morphometry typify the general characteristics of an organism. To be precise, sensitivity of an organism to the changes in environment will be reflected in the morphometry of an organism. Morphometrics, according to Mark and David (2010), "is the quantitative study of biological shape, shape variation and co-variation of shape with biotic or abiotic variables or factors". To be specific, morphometric measurement is combined analytical work associating statistics and biology. The shape variation account for the environmental changes could use to access ecological as well as paleoecological characters (Claudia *et al.*, 2018). Morphometrical applications can suite to systematic, evolutionary biology, genetic and developmental biology (Dan Danielopol *et al.*, 2002).

Morphometry and orientation of Ostracoda shells have intrigued micropaleontologists across the globe. Microcrustacean group, Ostracoda are very useful organisms

in paleoenvironmental reconstruction and are very sensitive to feeble environmental changes during their lifetime. The shells of Ostracoda which are known as 'carapace' exhibits the composition of the water in which they thrive on. Ostracoda identification relies on the morphological and ornamentation structures. The carapace exhibits different morphological structures like hinge, muscle scars, pore canals, inner lamella and ornamentation (Moore and Pitrat 1961; Van Morkhoven 1962). Ostracoda valve length and height measurements are used for determining the ontogenetic stages, sexual dimorphism and species variation and diversity (Smith and Martens 2000; Baltanás *et al.*, 2004). Morphometric calculations on Ostracoda from Monte San Nicola section was used to decipher the shape variations (Aiello *et al.*, 2007). The shape variations in Ostracoda accounts for the environmental changes and could use to access ecological as well as paleoecological characters (Claudia *et al.*, 2018).

Landmark based morphometric study is an effective proxy in measuring the biological shape as well as for the

shape variation (Cabuga *et al.*, 2016). However, the behaviour of the shells varies between the environmental fluctuations. This is because of the different growth stages in Ostracoda shells. The shell size is larger with the variation in temperature, which changes with depth. Many have tried to study the impact of temperature and salinity and their influence on Ostracoda shells.

Unlike many other microforms, the morphological traits of Ostracoda are dealt with the shell ornamentation, structure, muscle scars, pore patterns, hinge structure, eye placement and anterior-posterior shell valve studies. Morphometric studies are useful in tracing accurate, objective numerical information on the carapace due to environmental and genetic changes (Baltanas and Danielopol, 2011). Morphometry is not morphology but a school of morphological analysis, a multidimensional study on carapace/valve is required to bring out the implications on the environment. The morphometric calculations are to be studied under 2 divisions; the first type is known as traditional or conventional morphometrics and the second division modern morphometrics. The traditional methods are based on point to point measurements from anterior to posterior or dorsal to ventral regions. Thus, traditional morphometric studies highly dependent on two measurements; the length and the height of the carapace. Usually, the thickness is not considered as a parameter because the parameter value is not unique even in the same Ostracoda shell. The growth of the shell and the size is understood in studying the length and height of the species.

The traditional morphometrics deals with a point to point measurement, which reflects on the morphological traits (Baltanas and Danielopol, 2011). Traditional morphometric analysis purely relies on distance between 2 points in the Ostracoda carapace; the antero-posterior length and dorso-ventral height. Variation in the attributes, length and height of

the shell with the environmental fluctuation, are commonly measured as a surrogate for the morphometrical variation in the Ostracoda (Dan Danielopol *et al.*, 2002). Conventional morphometry is purely relying upon the size variations, irrespective of the shape disparities. For the past three decades, the technique of morphometrical understanding was evolving from traditional to geometric morphometrics. Geometric morphometry is characterised by landmarks which are used as a marker for measuring the biological shape, shape variation and covariation both for biotic and abiotic parameters (Cabuga *et al.*, 2016). Traditional morphometry focuses more on the size. However, the recent morphometric measurements advocate on the shape of the carapace.

Study Area

Geographically, to the north-eastern region of Indian Ocean the Bay of Bengal is located. The land bordering to the west is India and to the north is the Bangladesh. To the eastern Bay of Bengal, Andaman and Nicobar Islands are located. Based on the geographical co-ordinates the sampling locations are marked in the study area map (Fig.1). The expanse of the Bay of Bengal is ~2090 m lengthwise and ~1610 m in width (Mohanty *et al.*, 2008). The physiography and geology of the region are complex as the sedimentation is from dominant rivers, cyclones and storms, water influx to the bay during monsoon which influences the region. Echo sounding studies in the continental shelf from Bhemunipatnam to Kutukonda, Bay of Bengal revealed that topographic features like terraces, karstic and reef structures are recorded in between 70 m water depth and continental shelf edge in the area (Rao *et al.*, 1980). The Bay nurtures the world's largest mangrove forest Sundarbans, a home for endangered species. Annually the bay receives a freshwater influx of $1.5 \times 10^{12} m^3$. (Mohanty *et al.*, 2008).

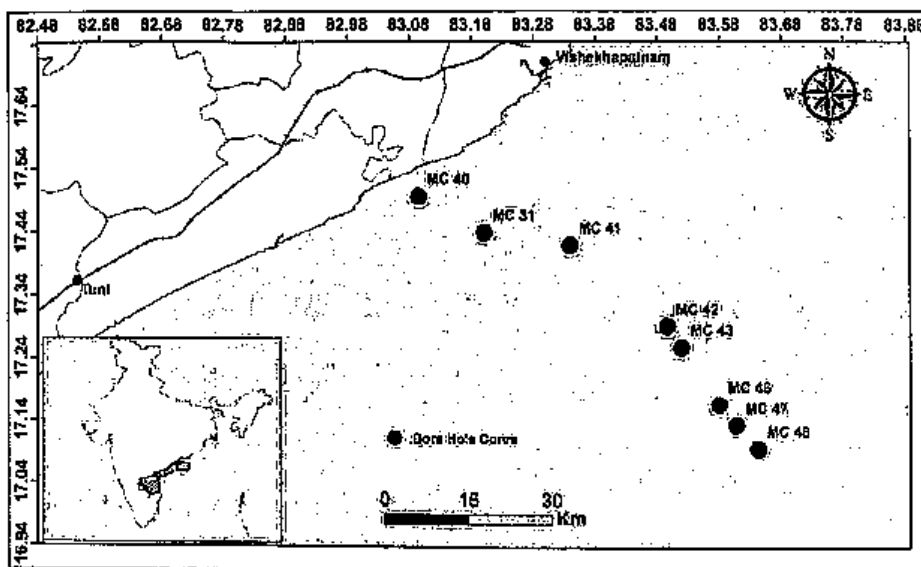


Fig. 1. Map of the study area

The samples were collected at a water column depth varying from 29 metres to 2540 metres. The research vessel was Sagar Kanya, owned and operated by India's National Centre for Polar and Ocean Research (NCPOR). Eight short core samples {MC 40 (29 m), MC 39 (55 m), MC 41 (73 m), MC 42 (280 m), MC 43 (320 m), MC 46 (1511 m), MC 47 (2030 m) and MC 48 (2540 m)} were collected at different bathymetric locations from continental shelf to upper abyssal floor. The sediment core was subsampled at 1cm interval for sedimentological studies and 3cm interval for micropaleontological analysis which are preserved in coded zip lock bags.

Materials and Methods

Ostracoda Shell Identification and Separation from the Sediment Matrix

Ostracoda species identification was done using the classification catalogue of Hartmann and Puri (1974). Separation techniques of the fauna from the sediments are chosen after observing the nature and characteristics of the sediments and can be done with ease in loose sediments. For the present study, the texture observed was muddy except for shallow core MC 40. 30 grams of each dried sub-sample was taken which are soaked in 30 Normality Hydrogen Peroxide aqueous solution for 4 hours and was sieve washed through sieves ASTM 230 mesh. Fractions retained on the sieve were oven-dried at 50° C. Ostracoda specimens were handpicked from all the sub-samples using a 0.00 sharp haired brush. The handpicked faunal specimens from each sample were arranged according to the generic and specific levels. It was then transferred to 24-chambered picking slides.

Relative Warp Analysis and Principal Component Analysis

Relative warp analysis of seven species from off Visakhapatnam area are analysed to avail the pore pattern variation. The landmark based geometric morphometry has been opted to plot out the shape variations in the shell. 14 points are marked in each of the shell. The softwares used for plotting and observing the relative warps are tpsdig and

tpsutil32. Principal component analysis was software done using PAST3 (Paleontological Statistics). The following fauna were abundant through the off Vishakhapatnam transect and analysed for morphometric studies, relative warp and principal component analysis. The checklist of 7 dominant Ostracoda taxa selected for morphometric analysis is as follows.

- Actinocythereis scutigera* (Brady, 1868).
- Bairidoppilatata (Bairidoppilatata) alcyonicola* (Maddocks, 1969).
- Bradleya andamanae* (Benson, 1972).
- Cytherella semitalis* (Brady, 1868).
- Krithe* sp.
- Lankacythere coralloides* (Brady, 1886).
- Loxoconcha cericinata* (Sars, 1866).

Results and Discussion

Ostracoda marks ecologically important and the implications on distribution and diversity are with respect to salinity, temperature, substrate and depth (Hussain *et al.*, 2009). Carapace from shallow depth shows diversified shell shape, because of the multiple processes acting upon the area like riverine influx, terrestrial precipitation influx, ion supply, organic matter accumulation, presence of reefs and the occurrence of reef affiliated Ostracoda forms, changes in the chemistry of water. For deep water fauna, the carapace is thicker in *Cytheropteron* sp. or having a greater length as in *Krithe* sp. Such modifications are naturally adapted for the survival by the organism.

Towards deeper depth of the bay, the height of the species decreases and the antero-posterior length increases for *Krithe* sp. The observed value for length is 692 µm, 803 µm and 704 µm respectively for *Actinocythere isscutigera* (Brady, 1868), *Krithe* sp., and *Bairidoppilatata (Bairidoppilatata) alcyonicola* (Maddocks, 1969). The height is 389 µm, 272 µm and 416 µm for *Actinocythere isscutigera*, *Krithe* sp. and *Bairidoppilatata (Bairidoppilatata) alcyonicola* (Fig. 2.). The species *Bradleya andamanae* have an average length of 697µm and height 342 µm. Average values for length of *Bythoceratina* sp. 672µm and the average height is 332 µm.

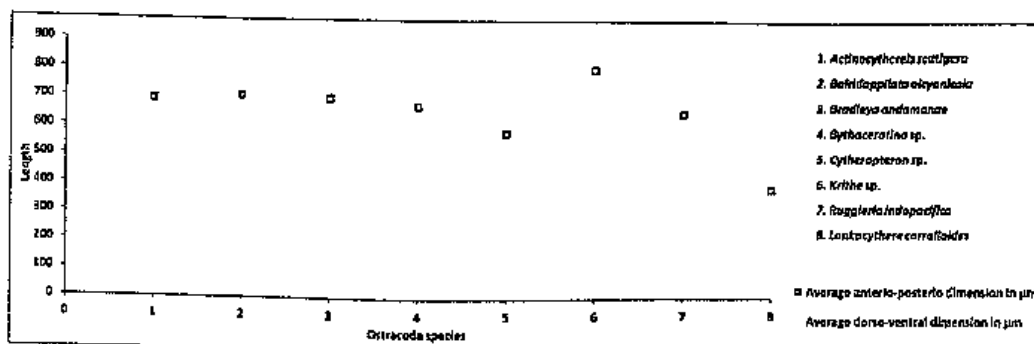


Fig. 2. Traditional morphometric measurements of Ostracoda shells from off Visakhapatnam

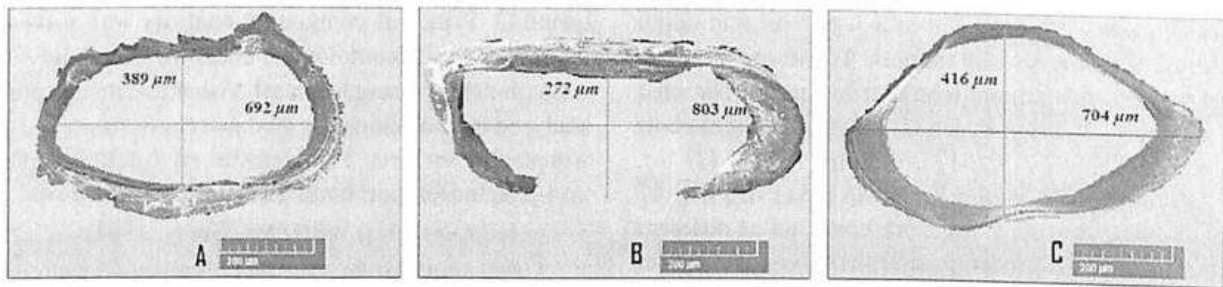


Fig.3. A: *Actinocythereis scutigera* (Brady, 1868), B: *Krithe* sp., and C: *Bairidoppilata (Bairidoppilata) alcyonicola* (Maddocks, 1969)

Point to point measurement identifies the average antero-posterior length of *Cytheropteron* sp., *Ruggieriaindo pacifica*, *Lankacythere coralloides* are 582 μ m, 649 μ m and 386 μ m, respectively. The dorso-ventral breadth of the species *Cytheropteron* sp., *Ruggieriaindo pacifica*, *Lankacythere coralloides* are 272 μ m, 324 μ m and 104 μ m, respectively.

Lankacythere coralloides are recovered from MC 40 only and all species from the core MC 40 are juvenile forms. Deeper taxa *Krithe* sp. has greater length as compared to the other shallow forms. *Cytheropteron* sp., a deep water assemblage has normal antero-posterior measurements like shallow forms but the deep water form can be distinguished from the others by the thickness the taxa possess at the depth of 2540 m. It is observed that the length and height ratio of the carapace is 3:1 for deep water form *Krithe* sp. and for the shallow water forms the ratio is 2:1 (Fig.3).

Relative warp analysis are analysed for the shell terminus and to avail the pore pattern variation for seven Ostracoda taxa. It is noted that different species exhibits shape variations. 14 landmarks are pointed in the carapace for the shell boundary. Relative warp analysis reveals that the deeper taxa can thrive on deep waters by adapting the shells to environmental necessities. Shells of shallow water taxa show a greater variation in shell shape. This is because of the multiple processes acting upon the area like riverine influx, terrestrial precipitation influx, ion supply, organic matter accumulation, presence of reefs and the occurrence of reef affiliated Ostracoda forms, changes in the chemistry of water. For deep water Ostracoda, in the present study, the shell is thicker in *Cytheropteron* sp. or having a greater length as like in *Krithe* sp. Such modifications are naturally adapted for the survival by the organism. The relative warp studies identify the shape of shallow marine forms are near ovular and deep sea forms are more of linear in nature. The shape variation in Ostracoda shell using relative warp analysis is illustrated (Fig.4).

The principal component analysis (PCA) is useful in discussing the relation between morphometric variables such as valve area, valve perimeter, major axis length, minor axis length, height, valve elongation and roundness (Roberts et al., 2002). Variation patterns in the shells can be described using PCA. The principal component analysis also reveals the shape variations and correlation of congruency in the shells. The PCA analysis identifies that deep sea forms *Krithe* and

Cytheropteron having an irregularly arranged distantly spaced pore pattern, to have a vibrant interaction with the environment. Shallow marine forms are dominantly having a close pore arrangement.

Seven species of Ostracoda were analysed for 40 point landmarks are chosen for identifying the pore characteristics for PCA. The pore pattern is different for different species. *Cytherella semitalis* (Brady, 1868) is having a kind of pattern that has larger pores in every region of the shells except for the central part of the shell. *Bradleya andamanae* (Benson, 1972) has the pore arrangement in the muri. *Loxoconchaceri cinata* (Sars, 1866) and *Lankacythere coralloides* (Brady, 1886) have

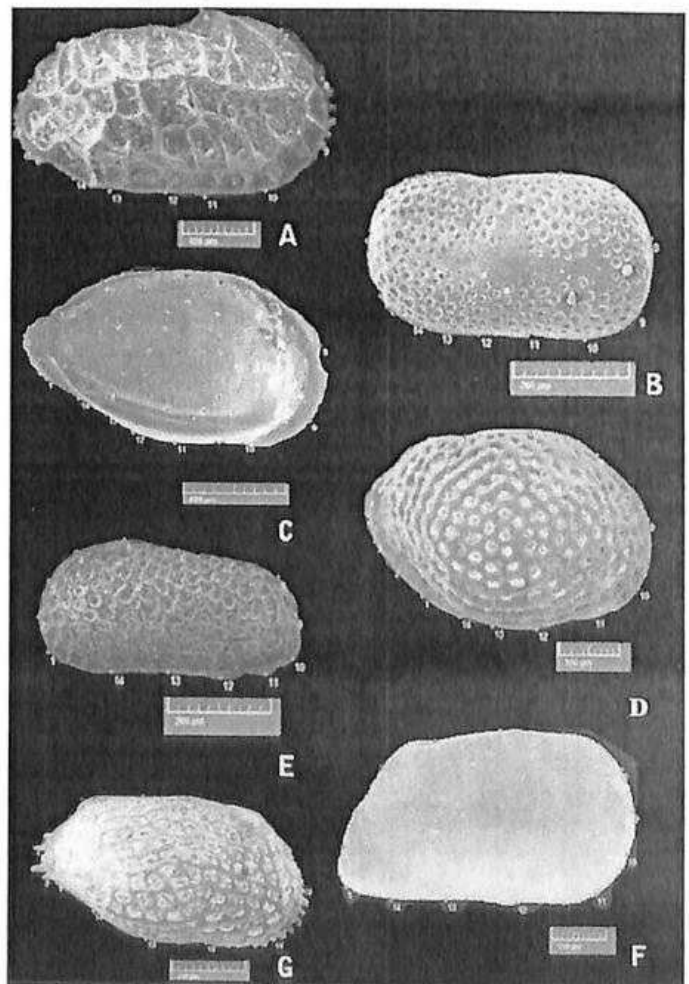


Fig.4. Relative warp points in Ostracoda for shape variations

pores and ornamentations that run through all their valve. Deepwater form *Cytheropteron* sp. has some large secondary pores in the shells but, dominantly minute pores which are covered by a small layer of calcite. *Krihe* sp. has notably small-sized pores running all over the valve but with a distant pore spacing is observed.

The PCA analysis categorises the fauna in three different groups (Fig.5). Four of the seven species taken for PCA are grouped together with a near ovular outline. Linear shaped *Krihe* sp. is grouped as single species with unique characteristics and pore pattern. The ridged shaped forms *Cytheropteron* sp. and *Bythoceratina* sp. are grouped together in PCA analysis. Component 1 in PCA diagram marks the antero-posterior length and component 2 denotes the pores location.

Valve Perimeter

The term valve perimeter is the path that encompasses the outline of the carapace. Hence, the perimeter of the valve is attributed to the shell shape. The valve perimeter of deep water assemblage is higher than the shallow water forms. *Krihe* sp. is having a higher valve perimeter than *Cytheropteron* sp. The form *Lankacythere coralloidesis* having least valve perimeter since the specimens recovered from the juvenile stock. Zonal

studies show that the forms in MC 40, which is the shallowest of all the cores from the area, is having less valve perimeter of the fauna. The taxa in core MC 39 and MC 41 (*Bairidoppilata* (*Bairidoppilata*) *alcyonicola*, *Actinocythere isscutigera* and *Bradleya andamanae*) which is also found in the epipelagic zone is having an increased valve perimeter. The higher valve perimeter observed is due to the vivid ornamentation pattern in the shell terminus. In an agitated environment, the Ostracoda possesses a smaller valve perimeter. To the mesopelagic zone, only taxa recovered were *Bythoceratina* sp. and the species have a slightly greater valve perimeter than the shallow occurring forms.

Ostracoda Valve Area

Valve area of Ostracoda is the extent of the measurement of valve surface. Species in MC 40 as like valve perimeter the valve area is also less. Valve area of all the species in core MC 40 is nearly less than $680 \mu m^2$. In epipelagic sediments, (MC 39 and MC 41) valve area of the forms are increasing than MC 40. In mesopelagic zone, the valve area is grater and due to the alar projection. The core MC 43, MC 46 and MC 47 are not yielding fossils. In MC 48, the form *Cytheropteron* sp. have a comparatively higher value of valve area of $2642 \mu m^2$ than *Krihe* sp. *Krihe* sp. is a cooler water form at a water depth of

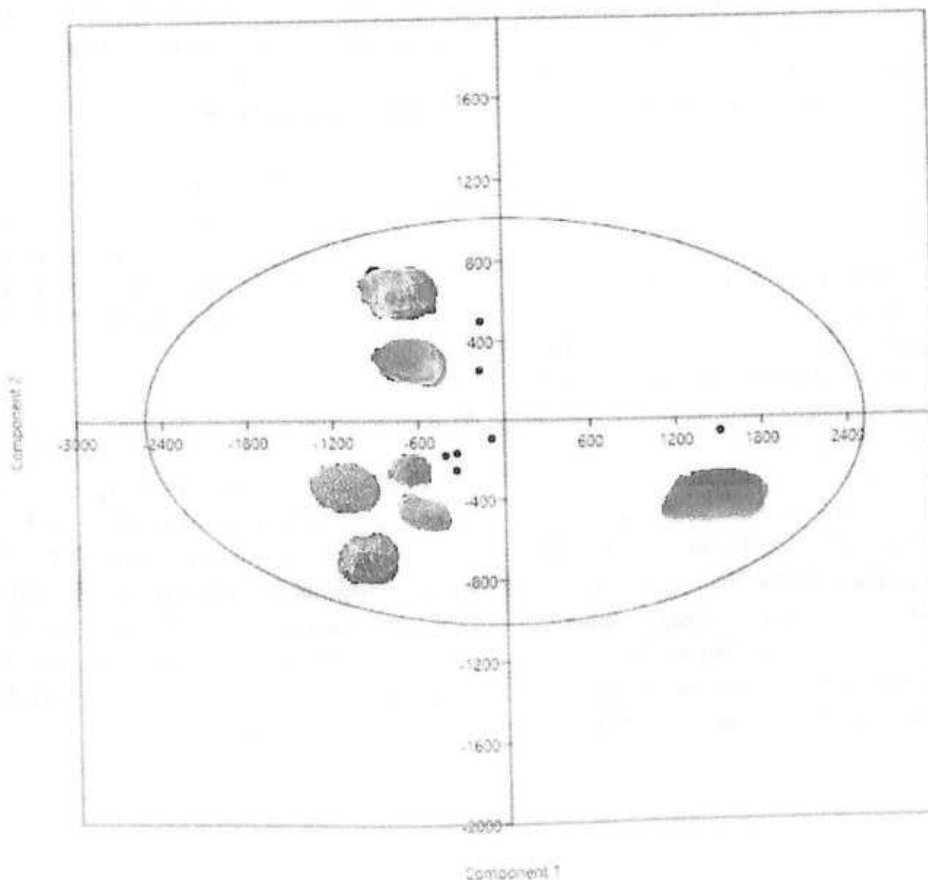


Fig.5. Principal Component analysis in Ostracoda carapace

2540 m and having smaller valve area of $1902 \mu\text{m}^2$. Evident from the study that the valve area of the shells decreases in agitated environment and cool water temperatures.

Valve Thickness

Ostracoda valve thickness means the measure of the valve layer. The thickness of the valve is a complex attribute in morphometrics. The thickness of the valve in different species shows complexities. Species with well-developed ornamentation are having thicker valves. Smooth carapaces, however, are fragile forms. In the deeper ocean sediments, Ostracoda shells show a bizarre behaviour. *Krithe* sp. is extremely fragile with less valve thickness, but the species *Cytheropteron* sp. occurring in the same are thicker and possess ridged ornamentation.

Carapace Roundness and Elongation

Roundness in Ostracoda morphometry is the shape of the carapace matches with the general oval outline. The shape of carapace in a linear manner rather than the traditional oval characteristics is termed as elongation. The degree of roundness is higher in shallow marine forms which are found occurring in the epipelagic zone except core MC 40. In MC 40 all the taxa derived are juvenile forms and anterior-posterior growth is more. The adjacent cores MC 39 and MC 41 are having more rounded to semi-rounded forms. The deep-sea Ostracoda from core MC 48 (2540 m), *Krithe* sp., is an elongated form. *Cytheropteron* sp. is also a deep water form and showing roundness, rather than an elongated growth pattern (Fig.6)

Conclusions

Morphometrical measurements mark a near oval shape for the shallow benthic Ostracoda from the area and to the deeper water region, *Krithe* sp. exhibits a linear valve outline. *Cytheropteron* sp., from MC 48 (2540 m) has a thicker shell. Similarly for each species, the behaviour according to the temperature fluctuation is different. The valve area of the species decreases in cool waters. Shallow marine forms possess higher valve area. Valve perimeter for deepwater *Krithe* sp. is maximum, because of the linear growth of the shell. Morphometrically, along with the body size of Ostracoda, the shell thickness, valve area, shape variation, pore characteristics, presence of appendages and ornamentation in shells can be used as a marker indicator for paleoenvironmental studies. Most important morphological

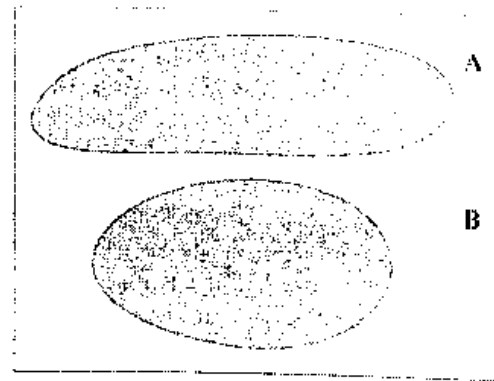


Fig.6. A: Linear deep marine *Krithe* sp. from deep waters, off Visakhapatnam, Bay of Bengal B: Near oval shape shallow marine *Actinocytheris scutigera* shallow water off Visakhapatnam, Bay of Bengal

feature in Ostracoda shell is the pore pattern. It is observed that fauna occurring in the shallow marine sediments is having large-sized pores that run through the valve surface.

It is evident from the studies that Ostracoda arrange their shell structure in such a way to adopt to the environmental changes. Morphometric measurements are observed for the size and shape variations occurring in the Ostracoda shell. However, Ostracoda morphology identifies the form and features in the shell interior, as well as, exterior. Significantly, both morphometrical measurements and morphological traits in the Ostracoda shells can be influenced by the climatic changes happening in their lifetime. These characteristics differ in different species of Ostracoda.

Authors' Contributions

Mohammed Noohu Nazeer: Conceptualization, Investigation, Writing - original draft, reviewing and editing. S.M. Hussain: Visualization, Supervision, Editing. S.S Salaj: Methodology, Investigation. Razi Sadath: Investigation, formal analysis. N. Mohammed Nishath: Methodology, Investigation

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Paleoproductivity shifts since the last 130 ka off Lakshadweep, Southeastern Arabian Sea



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ABSTRACT

Marine sediment deposited on the ocean floor and near coastal areas, the western coastal regions of India provide records of monsoonal shifts and productivity. To understand the paleoproductivity in the northern Indian Ocean, we analyzed carbon, hydrogen, nitrogen, sulfur, total organic carbon (TOC) and calcium carbonate (CaCO₃) in a deep-sea sediment core collected using gravity corer (GC-01) off Lakshadweep, Southeastern Arabian Sea. The results were then compared with the previous data generated from the surrounding area sediment cores to understand the productivity variations since the last 130 ka. The CaCO₃ content in the sediment core varies from 40.82% to 62.48% (with a mean value of 51.96%) and it is noted that these values were lower during the glacial episodes (Marine Isotope Stages-2 and 4) than the interglacial episodes (MIS-1, 3 and 5). The C/N ratio varied from 0.14 to 34.25, but was less than 9, since ~74 ka to recent, suggesting a marine origin for the organic carbon. The C/N ratio fluctuated significantly during MIS-5, and relatively the highest C/N ratio was observed at 5e ~127, 5d ~110 and 5b ~85 ka, corresponding to stadials 5b and 5d, (except 5e) indicating terrestrial OC from C₃ plants. The low C/N ratios during ~128, ~102, ~76 and ~32 ka match with the interstadials especially during MIS 5 (5a, 5c and 5e), correspondingly, and are marine OC in the source. This suggests that the MIS-5 stadial was interrupted via land source signifying higher productivity owing to the strong southwest monsoon during these periods. Further, high productivity was also observed during the Last Glacial Maximum (LGM) and Holocene in the Southeastern Arabian Sea since the 130 ka.

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1. Introduction

The Arabian Sea is well known for its high productivity due to the summer monsoon precipitation during the southwest (SW) monsoon (August to September). The cloud bearing winds blow from the Arabian Sea towards the Indian subcontinent and bring strong rain to India and neighboring countries (Barber et al., 2001). Terrigenous matter supply and biological productivity in the Arabian Sea is based on the monsoon intensity (Gupta and

Anderson, 2005). Intense SW wind occur during the summer monsoon cause upwelling along the Somalian coast, Oman and along SW coast of India by which transport nutrient rich sub-surface waters to the photic zone arise in increased productivity (Banse, 1987). High concentration surface productivity was reported towards the end of the SW (August–September) and northeast (February) monsoon (Marra and Barber, 2005). Different parts of the Arabian Sea, though, react in a different way to the seasonal wind forcing, most important to a massive regional difference in productivity during different seasons (Wiggert et al., 2005). Also regional variations in surface productivity has been inferred in the oligotrophic eastern and mesotrophic northern eastern Arabian Sea, and relatively weaker variations in productivity between interglacial and glacial intervals are noticed in the

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Table 1
List of research work done from present study region.

Authors	Area	Proxy used	Significances
Nagoji and Tiwari (2017)	Southeastern Arabian Sea	Multiple geochemical records	South Asian Summer Monsoon variability since mid-Holocene to the present past 4772 yr BP
Naik et al. (2017) Avinash et al. (2016)	Southeastern Arabian Sea Eastern Arabian Sea	Planktic foraminifera records Environmental magnetism, geochemistry and clay mineralogy	Last glacial–interglacial productivity past 32 ka Sedimentary sources and processes (surficial sediment samples used).
Das et al. (2013) Farooqui et al. (2014)	Southeastern Arabian Sea Southeastern Arabian Sea	Clay mineral assemblages Palynological record	Paleoenvironmental significance last 30 ka Rainforest vegetation and sea level fluctuations last 140 ka
Kessarkar et al. (2010)	Southeastern Arabian Sea	Sedimentological and stable isotopic	Fluctuations in productivity and denitrification last 70 ka
Pattan and Pearce (2009)	Southeastern Arabian Sea	Redox-sensitive elements record	Bottom water oxygenation history past 140 ka
Pattan et al. (2003)	Southeastern Arabian Sea	Multiple geochemical record	Productivity Fluctuations history past 140 ka

eutrophic upwelling region off Oman in the western Arabian Sea (Ivanova et al., 2003). Based on the modeling studies forecast a huge variations in monsoon patterns in a warming world, suggesting a probable deference in productivity in the Arabian Sea (Christensen et al., 2007). Variations in productivity through a longer time periods also the Holocene and interglacial–last glacial transition have been reconstructed from various regions of the Arabian Sea (Agnihotri et al., 2003; Singh et al., 2011).

The monsoon variability has been recorded in the marine sediments, lake sediments, speleothems, and tree rings in south Asian monsoonal regime. Previous studies found that the sediment cores from the Arabian Sea are widely used to study the past climate variability and its forcing factors with continuous, high resolution and longer records (Kroon et al., 1991; Clift et al., 2008; Gupta et al., 2015). However, understanding the mechanisms controlling the amplitude and frequency of climate changes requires the comparison of various proxies in many well-dated sediment records from this region.

Numerous studies have been carried out in the eastern Arabian Sea (Table 1) (Singh et al., 2011; Ishfaq et al., 2013; Naik et al., 2017), northwestern Arabian Sea (Kroon et al., 1991; Gupta et al., 2015) northeastern (Clift et al., 2008), and southeastern Arabian Sea (Agnihotri et al., 2003; Pattan and Pearce, 2009; Kessarkar et al., 2010; Saravanan et al., 2019, 2020) to understand the Late Quaternary terrigenous input and paleoproductivity shifts in relation with the SW monsoon strength (Sirocko and Sarneath, 1989; Shimmield et al., 1990; Clemens et al., 1991; Anderson and Prell, 1993; Sirocko et al., 1993; Naidu and Malmgren, 1996). In the western continental margin of India, research work was carried out with a primary focus on the OC and CaCO₃ variations (Naidu, 1991; Paropkari et al., 1991; Thamban et al., 1997; Sarkar et al., 2000; Bhushan et al., 2001; Thamban and Rao, 2000; Pattan et al., 2003; Govil and Naidu, 2010). However, paleoproductivity variations related to the intensity of SW monsoon in the southeastern Arabian Sea covering the Late Quaternary period using multiproxy records is still lagging. Therefore, the present study is aimed to investigate the paleoproductivity changes and linkage with the SW monsoon variations from the southeastern Arabian Sea sediment core.

2. Oceanography settings and sediment flux

The Arabian Sea is bounded by deserts such as Thar (India)-Makran (Iran) in the northern region, arid lands such as Arabian Peninsula and Africa in the western region, and eastern region covered by coastal highlands of the west coast, India (Nair et al., 1989). It is geographically surrounded by Chagos Laccadive Ridge (Carlsberg Ridge) in the southern region, and the Murray Ridge (Owen Ridge) in the western region (Avinash et al., 2016). Eastern Arabian Sea covers mountain ranges of Western Ghats of India,

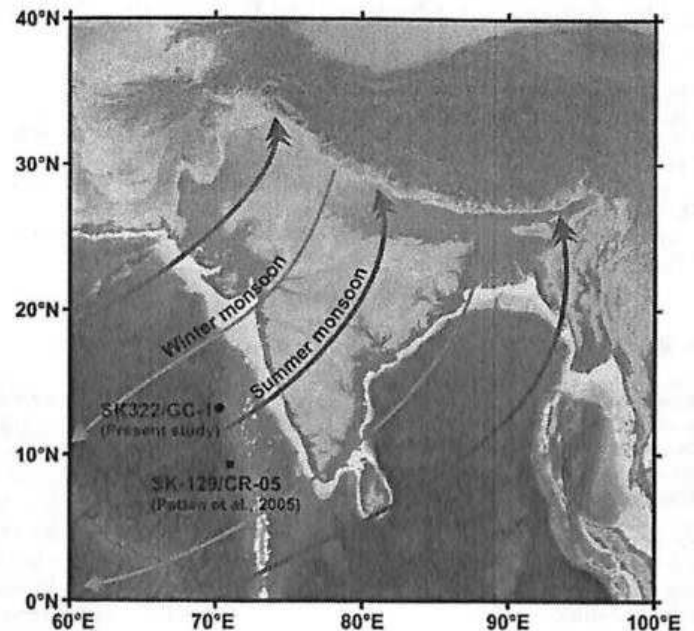


Fig. 1. Indian monsoon system surface wind pattern: red color show summer monsoon and blue color show winter monsoon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

present all along the coast in the western peninsular. A few medium and small rivers drain these regions into the Arabian Sea (Rao and Wagle, 1997). The Arabian Sea is influenced by two monsoon seasons (Fig. 1): the dominant by the SW or the summer monsoon (June to September) and moderately by the northeast (December to February) (Olsen, 1990; Schott et al., 1990; Tindale and Pease, 1999; Avinash et al., 2016). The west India coastal current brings comparatively huge saline water towards southeastern Arabian Sea equatorward from the northern Arabian Sea during SW monsoon, while winter monsoon current transports poleward the low salinity water from the western Bay of Bengal into the southeastern Arabian Sea during boreal winter (Prasanna Kumar et al., 2004). During the pre-summer monsoon season in southeastern Arabian Sea the sea surface temperature increases and form as a mini warm pool that peaks in April. The mini warm pool dissipates starts the summer monsoon rain, as a result of upwelling (Shenoi et al., 1999). The upwelling causes a numerous double increase in productivity during summer monsoon period in the southeastern Arabian Sea (Lévy et al., 2007). The southeastern Arabian Sea marked by perennial intermediate water depth oxygen minimum zone which is ranging from ~150 m to ~1200 m (Naqvi et al., 2003).

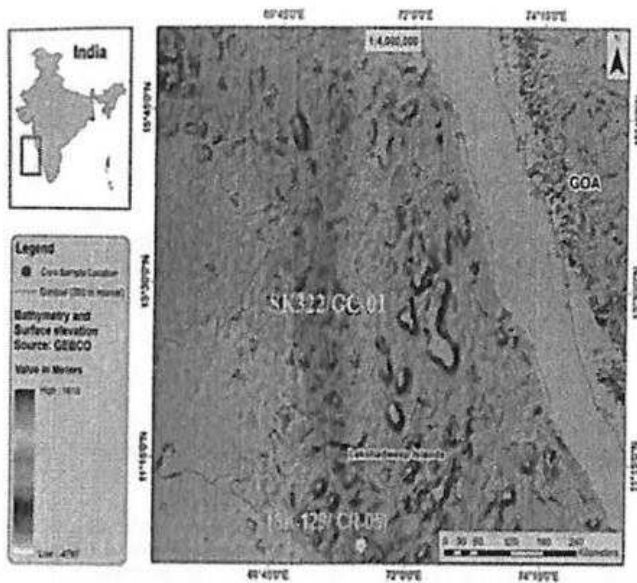


Fig. 2. Location and bathymetry map of GC-01 in the Southeastern Arabian Sea.

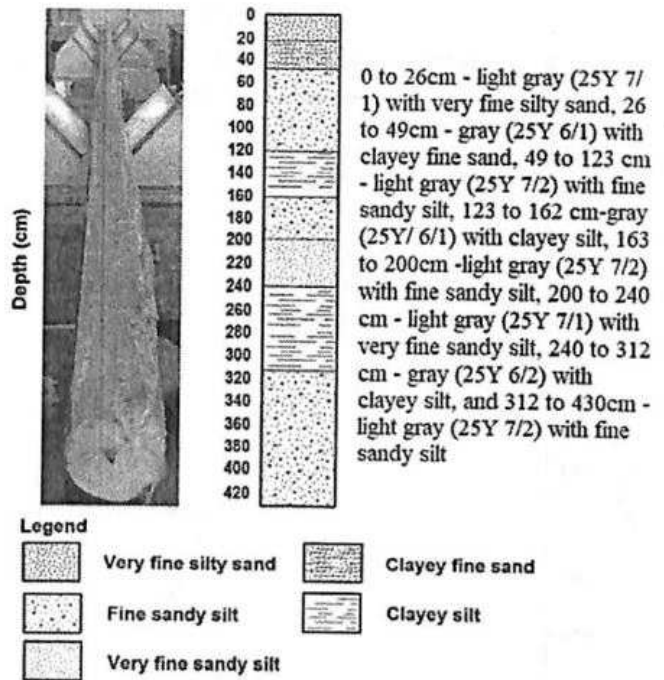


Fig. 3. Lithology of the studied core GC-01, SE Arabian Sea.

Sediment and dust influx into the Arabian Sea is received via several sources like the aeolian dust deposited from the Arabian Peninsula, Northern India (mainly from the Thar Desert), Pakistan and Eastern Africa (Dahl and Oppo, 2006). The major fluvial sediment sources are from the Indus, Narmada and Tapi Rivers and the Makran margin. Indus River has the largest drainage area in the NW Himalaya (2900 km length, basin area: $\sim 966,000$ km²) and carry a huge amount of sediments to the Arabian Sea (Haq and Milliman, 1984) and drains several thousand tonnes of sediment flux from the Himalayas and forms the second largest deep-sea fan in the world (Prins et al., 2000). The Sabarmati River flows from the Aravali mountain range and through the Quaternary formations before reaching the Gulf (Sharma et al., 1994). The Narmada River with its elongated drainage basin area around 1300 km long (basin area: $\sim 93,000$ km²) flows through a complex lithology comprising the Precambrian gneisses, Vindhyan sedimentary formation, and Deccan basalts in the upper and middle reaches, and the Quaternary alluvium in the lower reaches. The Western Ghats due to its orographic effect receives a maximum annual rainfall of ~ 3000 mm and are drains several small and short flowing rivers that transport sediments into the Arabian Sea coastal regions. Extensive lateralization of the parent rocks is a characteristic feature of the Western Ghats. Common rocks found in the coastal Kachchh and Saurashtra regions are bauxite and laterite and their derived sedimentary facies (Valeton and Wilke, 1993; Kessarkar et al., 2003). The Deccan trap basalts are present in the northern part of Goa, Charnokites, Precambrian gneisses and schists are the main rock type in between Goa and Cochin (Thamban et al., 2002).

3. Materials and methods

In this study, a 4.3 m long sediment gravity core (GC-1) was retrieved from off the Lakshadweep islands (Latitude: 13°04'5.50" N, Longitude: 70°56'10.21" E) using a gravity corer by R/V Sagar Kanya-322 expedition (National Center for Polar and Ocean Research, Goa, India) during August 2015. The water depth of the sampling location was 2057 m (Fig. 2). The sediment core was later sub-sampled at every 2 cm interval from top to 2 m, and 5 cm interval for the remaining portion of the core until the depth of 4.3 m.

3.1. Radiocarbon dating

¹⁴C dating was performed by using the Accelerator Mass Spectrometry (AMS) facility at Inter-University Accelerator Center (IUAC), New Delhi. For this purpose, two bulk sediment samples were selected from different depths for the AMS ¹⁴C dating. The selected sediment samples were dried and examined under the microscope for careful removal of microfossils, shells and other impurities. Thus, cleaned sediments were pretreated using the ultrapure acid (HCl), base (NaOH), and acid (HCl) protocol. Furthermore, the sediment samples were neutralized by frequent washing and then freeze-dried for graphitization. For graphitization, the samples were combusted and thus CO₂ produced was reduced to graphite powder form using Automated Graphitization Equipment (AGE) (Wacker et al., 2010; Sharma et al., 2019). The graphitized samples were measured for different isotopes of carbon 12, 13 and 14 with a 500 kV tandem ion accelerator (Kumar et al., 2015; Rajendran et al., 2018; Raja et al., 2019). The ¹⁴C ages obtained from AMS measurement were corrected with Arabian Sea reservoir age (400 years) on the basis of findings of Southon et al. (2002) in the Indian Ocean and finally calibrated by using Calib 6.0 software (Stuiver and Reimer, 1993; Stuiver et al., 2010).

3.2. CHNS (Carbon, Hydrogen, Nitrogen, and Sulfur) analysis

The sub-sampled sediments were analyzed for Total Carbon (TC), Total Nitrogen (TN), Total Hydrogen (TH) and Total Sulfur (TS) were analyzed using an Elemental analyzer (Vario EL cube; Make Elementary) at Pondicherry University, Puducherry. In this method, the powdered samples were weighed (~ 10 mg) in the aluminum tin sample boats and placed in an auto-sampler. The accuracy of the measurements was determined in triplicate by sulfanilamide test standard, and the error was observed to be $<1\%$.

3.3. Total Inorganic Carbon (TIC) and CaCO₃

Total Inorganic Carbon (TIC) was determined by using a coulometer (UIC model 5030) at the National Center for Polar

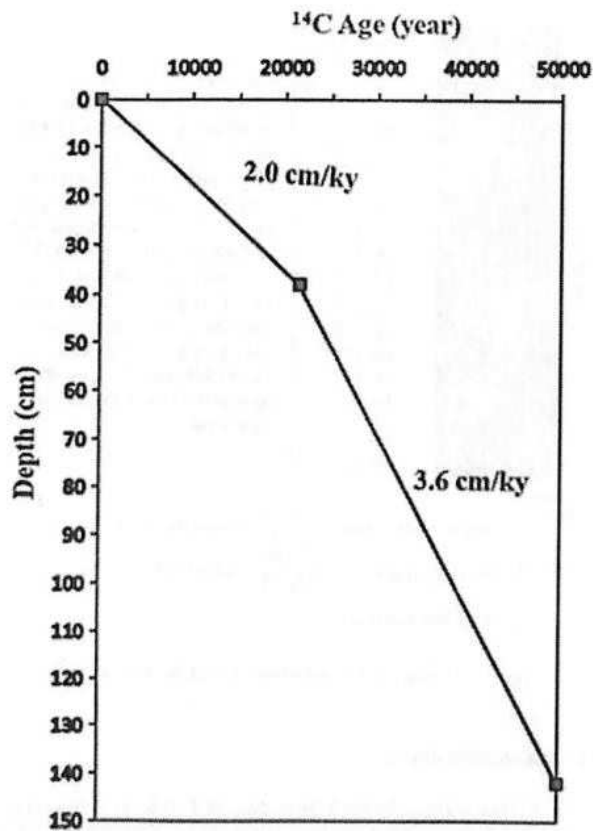


Fig. 4. Chronology and sedimentation rate of the marine sediment core GC-01, SE Arabian Sea.

and Ocean Research (NCPOR), Goa, India. 10 mg of homogenized sediment samples were treated with 5 mL of 4N HCl acid at 50 °C (Bhushan et al., 2001). Pure and dried CaCO_3 (supra pure grade Merck) was used as a standard for calibration. The precision of CaCO_3 analysis was found to be <3% based on repeated standard and sample analysis. The percentage of CaCO_3 was calculated using the formula of O'Connell et al. (1996).

$$\text{CaCO}_3(\text{wt}\%) = \%C_{\text{inorg.}} \times 8.334$$

3.4. Trace elements

The sediment samples were analyzed for trace element contents using Jarvis and Jarvis (1985) method. A 0.2 g of the homogenized sample was transferred to cleaned Teflon beakers with a mixture of hydrofluoric acid: perchloric acid: nitric acid in the ratio of 7:3:1. The digested solutions were then analyzed for significant element as Cd using an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, AA 800 model). During the analysis, blank corrections were applied, wherever needed. The average recoveries obtained were 90%–97%. The accuracy and precision were monitored by standard reference material MESS-2 and triplicate analysis of the selective sediment samples within the batches.

3.5. Data analysis

Past software (V#3.2.6b) has been used for principal component analysis (PCA) and spectral analysis (Hammer et al., 2001) of all the proxies for the multivariate study to get organized

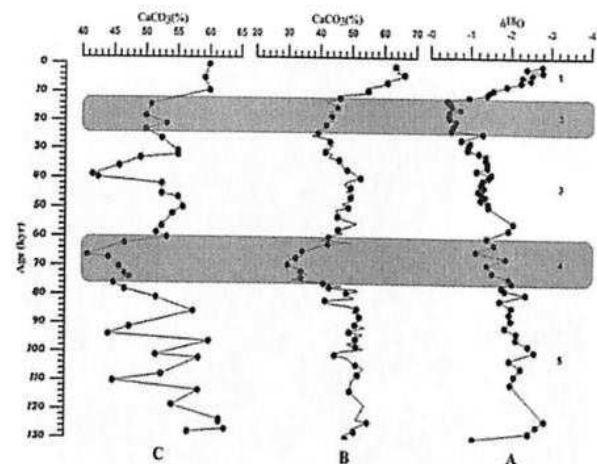


Fig. 5. A, Oxygen isotope composition of *Globigerinoides ruber* in a sediment core (SK-129/GC-05) versus age (ka). Numbers 1–6 indicate marine isotope stages and shaded blocks are glacial (Pattan et al., 2003). B, Distribution of calcium carbonate content in a sediment core (Pattan et al., 2005). C, Present core SK322/GC-01 calcium carbonate distribution.

covariations between a collection of variables. REDFIT spectral analysis (Schulz and Mudelsee, 2002) of unevenly spaced time series were conducted on the CaCO_3 data of Site SK322/GC-1 to examine the cyclicity of summer monsoonal records and its potential driving forces during the last 130 ka. For preforming spectral analysis, we have used a Hanning window of 2 segments and an oversampling value of 3 with the Monte Carlo method. Significance lines of 90%, and 80% levels of chi-square test applied.

4. Results and discussion

4.1. Lithology of the sediment core

The sediment core exhibited sediment layers in shades of light gray and the sediment lithology was classified based on sediment color following Munsell color chart, sedimentary structures and grain texture (Fig. 3).

4.2. Chronology of the core

Based on reservoir corrected age, the average sediment age for sub-samples of 38 and 142 cm were calculated as 21583 ± 87 and 49953 ± 827 years, respectively. The sedimentation rate varied from 2.0 to 3.6 cm/kyr at the depth of 38 to 142 cm, respectively (Fig. 4). Below 142 cm depth, interpolation of ages was calculated by considering the sedimentation rates between the obtained calibrated radiocarbon ages. Distribution of CaCO_3 values was compared with the nearby site SK-129/GC-05 (Latitude $9^{\circ}21'$ N, Longitude $71^{\circ}59'$ E) oxygen isotope and CaCO_3 proxy studied (Pattan et al., 2003, 2005). Oxygen isotope values of *Globigerinoides ruber* and CaCO_3 distribution were found to be matching well with the present core (Fig. 5). Further, the highest cadmium and sulfur contents noticed ~ 75 ka BP (Fig. 6) was inferred as the result of the Youngest Toba Tuff effusion (Pattan et al., 2003). Traces of the Toba eruption have been found in deep-sea cores of the northeastern Indian Ocean, the Bay of Bengal, and the Andaman Sea. Similar deposits have also been reported from the Arabian Sea, the South China Sea (Song et al., 2000), the Indian subcontinent and the Central Indian Basin (Pattan et al., 1999).

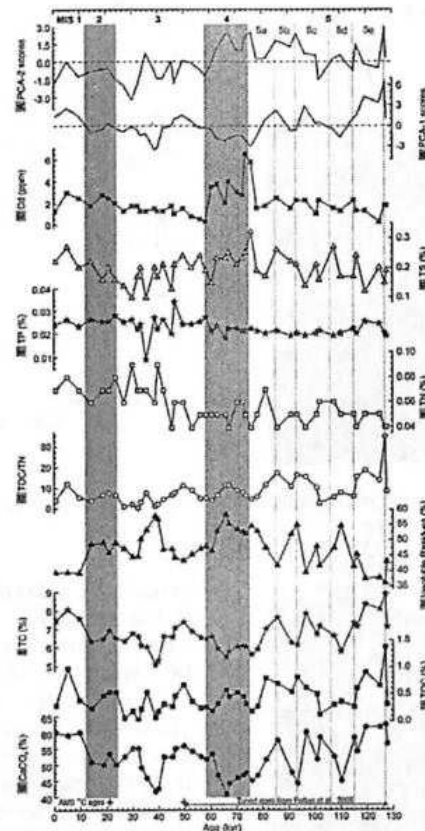


Fig. 6. Down core variations of organic and inorganic proxies in the studied core.

4.3. Calcium Carbonate

The CaCO_3 content in the sediment core ranged from 40.82 to 62.48% (mean 51.96%) (Fig. 6). The carbonate content was observed to be higher during MIS-5e, c, a, 3, and 1, and lower during MIS-5d, b, 4 and 2. During the MIS 4 and 2 glacial periods, values of CaCO_3 were lower (40.82%) than the interglacial periods, MIS-5 (62.48%) (Fig. 6), which could be due to the rate of dissolution of carbonates and dilution by the non-carbonate portion in the area. During the SW monsoon, more terrestrial input and higher biological productivity were noticed by sediment trap studies (Haake et al., 1993). Earlier, Gupta and Anderson (2005) found that the terrigenous matter and biological productivity supplied by the effect of monsoon during Late Quaternary into the Arabian Sea.

Many researchers observed that CaCO_3 and the OC contents contribute to the productivity in the marine sediment (Jaccard et al., 2013; Cartapanis et al., 2018). It was also noticed that the persevered CaCO_3 is generally affected by terrigenous dilution in the Arabian Sea (Pattan et al., 2003). During the summer monsoon, there are fluctuations in the lithogenic terrigenous flux derived from the Somalian peninsula and the Arabian Sea. The CaCO_3 dilution was mainly due to the terrigenous fluctuation derived from the SW coast of India or due to the detrital contribution, or both (Sarkar et al., 1993; Pattan et al., 2003). Nagoji and Tiwari (2017) reported that Chagos-Laccadive Ridge and the shelf sediments are characterized by a high CaCO_3 content when compared to deep-sea sediments.

4.4. Total Phosphorus (TP) and total Sulfur (TS)

The phosphorus content varied from 0.01 to 0.03% (Fig. 6). During MIS-5e, 3, 2, and 1, a higher value (0.034%) was noticed

and the lower value (0.018%) was found during MIS-5d, c, b, a, and 4. Based on phosphorus concentration it can be noted that high value indicated higher productivity and the low value indicated lower productivity. Sulfur content in sediment core ranged between 0.10 and 0.32% (Fig. 6), and relatively higher values (0.32%) were noticed during the glacial than the interglacial period. Except for MIS-1, the highest TS noticed at sharp ~ 75 ka (MIS-4) (0.32%) might be due to the effect of the Youngest Toba Tuff origin.

4.5. Organic Carbon and total Nitrogen

In the marine environment, the primary sources of OC are terrestrial as well as marine origins. The OC content in the marine sediments was strongly associated with surface water productivity. Hence, variations of the OC in marine sediments were used as a productivity proxy (Ausín et al., 2019). Total organic carbon (TOC) content in sediment core varied from 0.01 to 1.37% (mean 0.42%) (Fig. 6), whereas the Total Nitrogen (TN) content in the core varied from 0.04 to 0.09% (mean 0.06%). TN values in MIS-1, 2, and 3 showed the same trend to those of TOC and with higher values compared to the sediments below MIS-3 (only in the case of TN). The distribution pattern of the TN and OC in the sediment core was almost similar, which could be the plankton incorporates in both nitrogen and carbon in a defined molar ratio (Paulsen et al., 2018).

The lower average TOC content (0.31%) was found in MIS-1, whereas those values in MIS-2 increased gradually (0.43%). Within MIS-2, a higher OC value (0.52%) was noticed during the Last Glacial Maximum (LGM, Fig. 6). Higher OC was noticed during MIS-1 than LGM. The obtained results in this study were similar to some previously published research articles (Sarkar et al., 1993; Pattan et al., 2003) and inferred that more suboxic

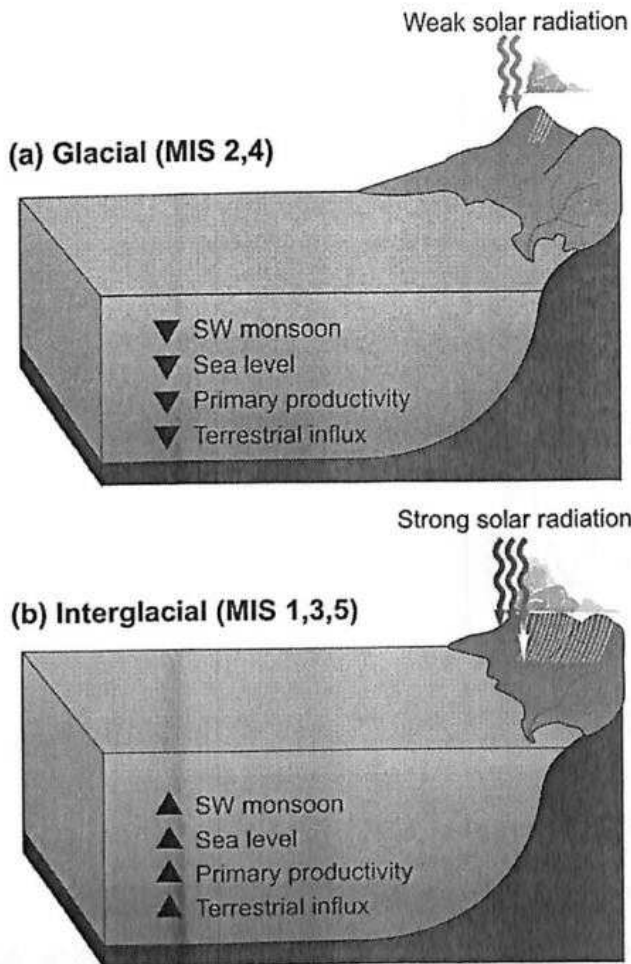


Fig. 7. A conceptual model representing glacial and interglacial depositional conditions.

conditions existed in MIS-2 compared to MIS-1. Likewise, higher OC was observed in other parts of the world during the LGM, for example, the Argentine Basin (Stevenson and Cheng, 1972) and the Pacific Ocean (Pedersen, 1983). The higher TOC observed during the interglacial (5a and 5e) of MIS-5 and in the stadials (5b and 5d), which might be due to the result of suboxic conditions. An oxic environment existed in MIS-1 (Holocene) due to oxidation of OC, and the resulting sediments contained less OC contents (Fig. 6). During MIS-5 higher TOC was observed during stadials 5b and 5d (93 and 111 ka) and similar results were observed by Pattan et al. (2003), Pattan and Pearce (2009). The interstadials and stadials of the OC ranged from low (~0.5%) to high (~1.5%). Carbon sources were influenced by both marine and land originated OC by (C/N) ratios of ~7 and 25% (Pattan et al., 2003).

The low values of TOC content fluctuated from ~24 to 58 ka; however, from ~62 to ~75 ka it gradually increased and again decreased. Significant fluctuation in the OC (0.17 to 1.37%) was observed during MIS-5. The TOC reached a minimum of 0.17%, 0.11%, and 0.31% at ~76, ~102 and ~128 ka, respectively which may correspond to interstadials 5.a, 5.c, and 5.e, whereas the carbon content was high 0.8% and 0.35% at ~93 and ~110 ka, may correspond to stadials 5.b and 5.d, respectively (Fig. 6). A higher TOC (0.46%) was noted during the LGM (~18 ka) in the sediment core, supporting suboxic bottom water conditions (Pattan et al., 2003; Pattan and Pearce, 2009). OC variation has not shown any trend regarding core depth and the glacial and interglacial

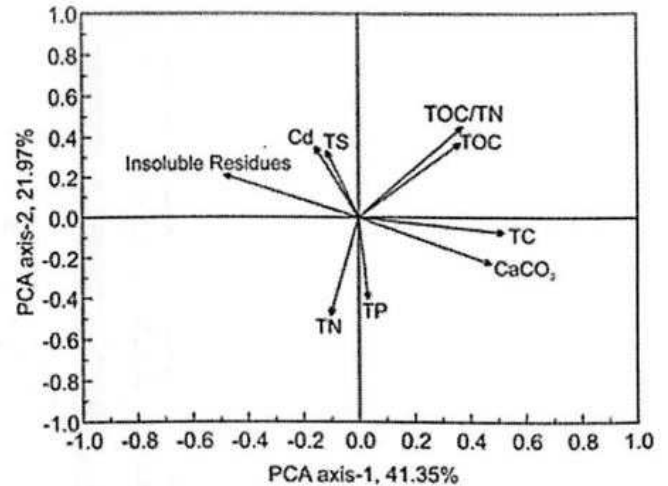


Fig. 8. Biplot of the principal component analysis (PCA) of productivity proxies.

periods. A similar observation was noticed from the Oman margin of the Arabian Sea (ODP site 724) (Zahn and Pedersen, 1991). Further, compared to the glacial period the Holocene had higher OC, which can be attributed to the higher productivity during Holocene in the northeastern, western, and northern Arabian Sea, and the southern continental margin of India (Sirocko et al., 2000). This suggests that the OC preservation pattern in the southeastern Arabian Sea could be different from these areas.

In marine sediments, carbon/nitrogen (C/N) ratio has been widely used to trace the source of OC. In core GC-01, the C/N ratio differed from 0.14 to 34.25. However, C/N values less than 9 were observed from ~74 ka to recent. This suggested that organic carbon has derived from the marine origin (Fig. 6). In the marine sources, TOC is originated via phytoplankton, whereas that in terrestrial is originated from plant remains and dissolved humic substances through the river (Emerson and Hedges, 1988). The C/N ratio fluctuated significantly during MIS-5, and relatively highest C/N ratio was noticed at 5e (127 ka), 5d (110 ka) and 5b (85 ka), which may correspond to stadials 5.b and 5.d, (except 5e), suggesting a land originated OC from C₃ continental plants (Meyers, 1994). The low C/N ratios at (~32) at ~76, ~102 and ~128 ka matched to interstadials 5.a, 5.c and 5.e within MIS-5, respectively, and the OC sources were from in situ marine origin (Fig. 7). This suggests that the MIS-5 stadials were interrupted via land-based OC. Land originated OC decreased the contribution as the distance from the coast to the deep ocean increased (Fontugne and Duplessy, 1986). Even though the studied core is around 450 km away from the coast, land originated OC found during ~110 and ~85 ka, possibly indicated that the Indian subcontinent had abundant rainfall and more vegetation during these periods. The land originated OC was brought through rivers; later it got transported to the southeastern Arabian Sea by the bottom water current. During MIS-5, corresponding to the stadials and interstadials with the variation of the C/N ratio, and the TOC needs to be confirmed by studying long sediment cores closer to the coastal region of the southeastern Arabian Sea (see Fig. 6).

4.6. Computational analysis

The principal component analysis was carried out based on eight geochemical parameters, having two components PC1 (41.35%) and PC2 (21.97%) with 63.32% of the total variation (Fig. 8). CaCO₃, TOC/TN, TC, TOC, and TP were dominated in the positive side of the PC1, whereas TN was located on the negative side. In PC2, TOC/TN, TOC, insoluble residues, Cd and TS were

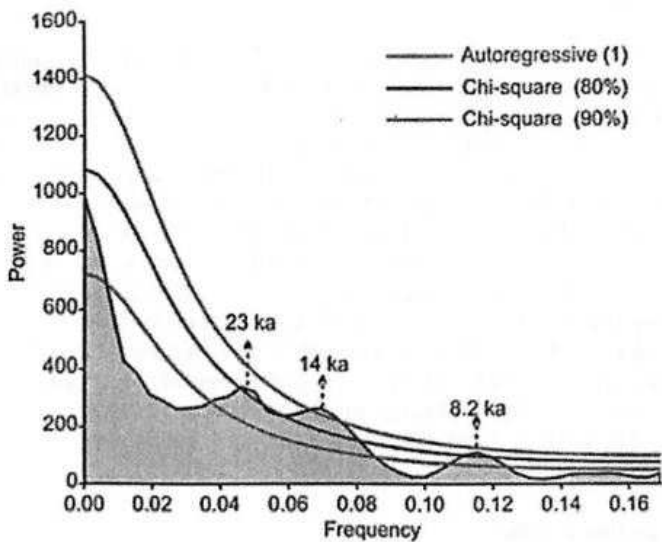


Fig. 9. Spectral analysis of calcium carbonate percentage during the last 130 ka.

located on the positive side and TN was located on the negative side. MIS-1, 3 and 5 had higher PC1 scores, corresponding to the interglacial period with higher productivity, which could be due to the deposition of higher CaCO_3 and OC in the sediments (Pattan et al., 2003).

Spectral analyses shows periodicities at 23 kyr, 14 kyr and 8.2 kyr (Fig. 9). The occurrence of 23 and 14 kyr periodicities in the CaCO_3 record confirms the dynamic connection of the primary productivity changes with the summer monsoon to glacial–interglacial and orbital forcings. This periodicity has been studied by Clemens et al. (2010) in the Indian summer monsoon regime. The 23 kyr and 14 kyr cycles are linked with the precession of the Earth's orbit (with a phase of -125°) inferred from the Summer Monsoon proxies of the northern Indian Ocean (Clemens and Prell, 1991; Wang et al., 2005).

The observed 8.2 kyr spectral frequency is close to the 6 kyr cycle, which is probably associated with the Heinrich rhythm cycles (~ 6 kyr). This cycle was documented in the North Atlantic sediments and Greenland ice core (Bond et al., 1993; Heinrich, 1988). It illustrates the teleconnection between the Indian summer monsoon and the North Atlantic Sea surface temperature changes. These periodicities suggesting that the low latitude summer insolation and Atlantic teleconnection may play a major role in shaping the Indian summer monsoon variability and primary productivity shift in this region.

4.7. The last 130 ka paleoproductivity shifts

The paleoproductivity has fluctuated since the past geological record as it is defined by the monsoon in the southeastern Arabian Sea. Rostek et al. (1997), based on alkenones and coccoliths reconstructed the paleoproductivity changes. The primary productivity during the periods increased and decreased during the beginning of interglacial periods. The higher productivity at glacial periods had been credited due to the strong NE monsoon wind pressure, and this was accountable for bringing nutrient-rich water to break the water column stratification into the photic zone, inducing higher productivity. According to Thamban et al. (2001), during the early Holocene period productivity was low. Sarkar et al. (1993) showed maximum productivity during the (LGM) period using the productivity indicator of CaCO_3 content of sediments. Fontugne and Duplessy (1986) reported

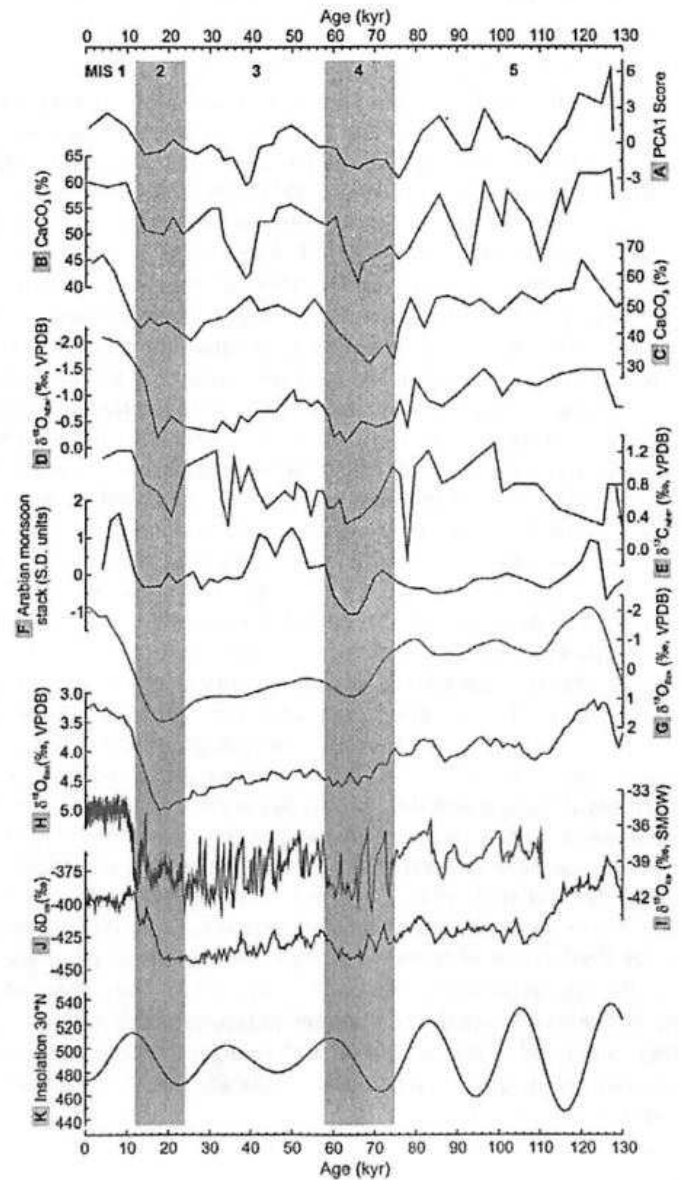


Fig. 10. Paleoclimatic and paleoproductivity changes during the last 130 ka. (A) PCA1 Score (present study), (B) Percentage of CaCO_3 (Present study), (C) Percentage of CaCO_3 from SE Arabian Sea (Pattan and Pearce, 2009), (D) & (E) oxygen and carbon isotope record from SE Arabian Sea (Ivanova et al., 2003), (F) Arabian Monsoon Stack (Clemens and Prell, 2003), (G) SPECMAP (Imbrie et al., 1984), (H) LR04 global benthic foraminifera isotope stack (Lisiecki and Raymo, 2005), (I) GISP2 ice core data from Greenland (Grootes et al., 1993), (J) EPICA ice core data from Antarctica (Augustin et al., 2004) and (K) Insolation 30°N (June) (Berger and Loutre, 1991).

higher primary productivity during the glacial periods than the interglacials. A similar observation was reported in the Arabian Sea paleoproductivity based on various geochemical productivity proxies such as biogenic barium, calcium carbonate, and biogenic opal on the sediments suggested that higher productivity was observed during major interglacials and low during the glacial periods (Pattan et al., 2003; Prabhu et al., 2004; Banakar et al., 2005; Prabhu and Shankar, 2005; Ishfaq et al., 2013; Naik et al., 2017). A few more researchers also made similar observations from northwestern, eastern, and northern parts of the Arabian Sea, Atlantic Ocean, and Antarctica regions (Shimmield et al., 1990; Clemens and Prell, 1991; Paropkari et al., 1991; Howard and Prell, 1994; Shimmield et al., 1994; Frank et al., 1995; Bonn et al., 1998; Reichert et al., 1998).

4.8. Monsoon link with global Climate: Glacial-interglacial episodes

Long-term changes in solar insolation 30 °N seem to overlay the millennial-scale calcium carbonate fluctuations during 130 ka in the southern Arabian Sea (Fig. 10). The present data were compared with other regional and global data such as LR04 Global isotope data (Lisiecki and Raymo, 2005), SPECMAP (Imbrie et al., 1984), Arabian Monsoon Stack (Clemens and Prell, 2003), and oxygen isotope record of ($\delta^{18}\text{O}$) SE Arabian Sea (Ivanova et al., 2003), and percentage of CaCO_3 from SE Arabian Sea (Pattan and Pearce, 2009). The Quaternary climate phase showed huge latitudinal changes, and incoming solar radiation was the main energy source to the earth. The solar insolation changes are cyclic with a periodicity of 100, 41, and 23 ka and are jointly defined as Milankovitch cycles (Hays et al., 1976). Efforts have been made to understand the effect of Milankovitch equilibrium changes in solar radiation on the intensity of the monsoon. Based on terrestrial and marine proxies, monsoon records had inferred a strong summer monsoon on obliquity maxima and 8 ka after precession minima (Clemens et al., 2010). As long-standing records imply during the interglacial-glacial period, the variability of monsoon was distinct during the Pleistocene (Clemens and Prell, 1991). During the late interglacial-glacial transition as reconstructed widely and easily using gravity cores (< 6 m length) from the lakes as well as continental shelf regions, out of numerous interglacial-glacial cycles (Banakar et al., 2010). Various proxy records from both Bay of Bengal and the Arabian Sea suggest that the summer monsoon is weakening accompanied with a probable intensification of winter monsoon at the glacial period (Jain and Tandon, 2003; Banakar et al., 2010; Govil and Naidu, 2011; Saraswat et al., 2013). The weedy monsoon interval period was interrupted by a short-lived phase of intense monsoon, which was marked during the last deglaciation (Saraswat et al., 2013). Throughout the Bølling-Allerød period, the summer monsoon precipitation was high compared to the last glacial and Younger Dryas, probably in reaction to changes in North Atlantic climate (Sinha et al., 2005; Rashid et al., 2007).

5. Conclusions

In this study, CaCO_3 , TOC, trace metals, Carbon, Hydrogen, Nitrogen, and Sulfur in the present core GC-1 Southern Arabian Sea were used as a paleoproductivity proxy and these results were compared with previous studies carried out in this region. CaCO_3 content during Marine Isotope Stages (MIS) 1, 3, and 5a, c, and d were higher compared to that during MIS-2, 4, 5b, and d, which could be due to higher interglacial productivity than in the glacial. Likewise, OC also showed increased productivity during MIS-1, 3, and 5a, c, and d. The C/N ratio revealed that the source of OC is marine-based from ~62 ka to recent. However, the C/N ratio has fluctuated considerably during MIS-5, which could be due to the OC derived from both marine and fluvial origins. The SW monsoon also plays a significant role in controlling the primary productivity changes in this region.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A Stochastic Model on Time to Recruitment in a Single Grade Manpower System with Cluster of Exits

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Abstract — In this paper, a single grade marketing organization is considered in which exits of personnel occur in clusters at random epochs. A suitable univariate recruitment policy based on cumulative damage process and shock model approach in reliability theory is used. Analytical result for variance of time to recruitment and expected total number of exits up to time to recruitment are obtained when (i) the number of clusters of exits forms a homogeneous Poisson process (ii) number of exits in each cluster forms a sequence of independent and identically distributed geometric random variables and (iii) the mandatory breakdown threshold is a positive integer valued random variable. The influence of nodal parameters on the performance measures is studied and relevant conclusions are presented.

Keywords—Single grade marketing organization, Cluster process, breakdown threshold, univariate recruitment policy, variance of time to recruitment and expected total number of exits up to time to recruitment.

AMS Subject Classification: 60H30, 60K05, 90B70, 91D35

1. INTRODUCTION

In [2] and [3], the author, a pioneer in the study of manpower planning, has used appropriate statistical techniques and studied renewal theoretical models in manpower planning. Many researchers have studied the problem of time to recruitment for a single grade marketing organization, where exits occur as the effect of policy decisions such as revision of pay, targets etc. taken by this organization, by considering different assumptions on loss of manpower [[6], [11]], mandatory threshold for loss of manpower [[8], [13]] and inter-policy decision times [[5], [7]] using univariate policy of recruitment. In [12] the

authors have obtained long run average cost for single grade marketing organization by assuming the threshold as a constant and survival time process as a geometric process using univariate policy of recruitment. In the above cited work involving policy decisions, separate epochs for policy decisions and exits are not considered, in spite of the fact that such separate epochs are more realistic. In this context, the author in [4] has studied the problem of time to recruitment for a single grade manpower system with different epochs for decisions and exits when the system has a mandatory breakdown threshold using a method different from the conventional method which uses Laplace transform. In [10] the author has studied the work in [4] when the system has optional and mandatory breakdown thresholds for different wastages. In [1] the author has obtained the variance of time to recruitment for a single grade manpower system, where depletion of manpower occurs due to (i) voluntary and involuntary exits of personnel from the organization and (ii) breaks taken by personnel working in the organization, under different assumptions on inter-exit times and inter-breaking decision times using univariate policy of recruitment. Recently, in [9] the authors have studied the problem of time to recruitment for a three graded marketing organization by considering two different cluster processes due to exits and transfers with new extended threshold for loss of manpower. In the present paper, the problem of time to recruitment for a single grade marketing organization in which exits of personnel occur in clusters at random epochs is discussed. A stochastic model is constructed with suitable assumptions on the cluster process, number of exits in any cluster and the mandatory breakdown threshold. A univariate recruitment policy is used and variance of time to recruitment, average number of clusters of exits up to time to recruitment and the total number of exits up to time to recruitment are determined for the present model. A numerical illustration with findings and conclusion are presented for a better understanding.

2. MODEL DESCRIPTION AND ANALYSIS

Consider a marketing organization consisting of one grade (referred as a system) in which clusters of exits of personnel takes place at random epochs in $(0, \infty)$. Let $B(t)$ be the number of clusters of exits in $(0, t]$. It is assumed that $\{B(t); t \geq 0\}$ is a Poisson process with rate $b, b > 0$. Let X_i be the number of exits in the i^{th} cluster, $i = 1, 2, 3, \dots$. It is assumed that $\{X_i\}_{i=1}^{\infty}$ form a sequence of independent random variables following deapctivated geometric distribution with parameter $p, 0 < p < 1$.

Let $C(t)$ be the total number of exits in $B(t)$ clusters in $(0, t]$. Note that $C(t) = \sum_{i=1}^{B(t)} X_i$. Let Y be a positive integer valued random variable representing the mandatory random breakdown threshold level for the cumulative number of exits in the system with mean $E(Y)$ and variance $V(Y)$. It is assumed that Y is independent of $B(t)$, for all $t \geq 0$ and $X_i, i = 1, 2, 3, \dots$. The univariate recruitment policy states that recruitment is done when the total number of exits exceeds the mandatory breakdown threshold. Let T be the random variable denoting the time to recruitment with mean $E(T)$ and variance $V(T)$.

3. MAIN RESULT

From the recruitment policy, the tail distribution of time to recruitment is given by

$$P[T > t] = P[C(t) \leq Y]. \tag{1}$$

Conditioning upon the event $[Y=m]$ and noting that $C(t)$ and Y are independent, we get

$$P[T > t] = \sum_{m=1}^{\infty} P[Y = m] \{P[C(t) = 0] + \sum_{n=1}^m P[C(t) = n]\} \quad (2)$$

Since $C(t)$ is a randomly indexed partial sum indexed by the Poisson process $\{B(t); t \geq 0\}$ with independent and identically distributed deactivating geometric random variables as summands, we note that

$$P[C(t) = 0] = e^{-bt}$$

and

$$P[C(t) = n] = e^{-bt} \sum_{r=1}^n \frac{1}{r!} \binom{n-1}{r-1} (btp)^r (1-p)^{n-r}, \quad n = 1, 2, 3, \dots \quad (3)$$

From (2) and (3), it can be shown that

$$P[T > t] = e^{-bt} + \sum_{m=1}^{\infty} P[Y = m] \sum_{n=1}^m (1-p)^n \sum_{r=1}^n \frac{1}{r!} \binom{n-1}{r-1} \left(\frac{bp}{1-p}\right)^r t^r e^{-bt}. \quad (4)$$

We now determine $E(T)$ and $V(T)$ from (4).

We know that

$$E(T^r) = r \int_0^{\infty} t^{r-1} P[T > t] dt, \quad r = 1, 2, 3, \dots \quad (5)$$

From (4), (5) and taking $r - 1 = l$, it can be shown that

$$\begin{aligned} E(T) &= \frac{1}{b} + \frac{1}{b} \sum_{m=1}^{\infty} P[Y = m] \sum_{n=1}^m (1-p)^n \left(\frac{p}{1-p}\right) \sum_{l=0}^{n-1} \binom{n-1}{l} \left(\frac{p}{1-p}\right)^l \\ &= \frac{1}{b} + \frac{1}{b} \sum_{m=1}^{\infty} P[Y = m] \sum_{n=1}^m (1-p)^n \left(\frac{p}{1-p}\right) \left(1 + \frac{p}{1-p}\right)^{n-1} \\ E(T) &= \frac{1}{b} + \frac{p}{b} \sum_{m=1}^{\infty} m P[Y = m] \\ \text{i.e } E(T) &= \frac{1}{b} \{1 + pE[Y]\}. \end{aligned} \quad (6)$$

We next determine $E(T^2)$.

From (4) and (5), it can be shown that

$$E(T^2) = \frac{2}{b^2} + \frac{2}{b^2} \sum_{m=1}^{\infty} P[Y = m] \sum_{n=1}^m (1-p)^n \sum_{r=1}^n (r+1) \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^r. \quad (7)$$

Consider $\sum_{r=1}^n (r+1) \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^r$.

Since $(r+1) = (r-1) + 2$ and $\binom{n}{r} = \frac{n}{r} \binom{n-1}{r-1}$, we get

$$\begin{aligned} \sum_{r=1}^n (r+1) \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^r &= \sum_{r=1}^n (n-1) \binom{n-2}{r-2} \left(\frac{p}{1-p}\right)^r + \\ &2 \left(\frac{p}{1-p}\right) \sum_{r=1}^n \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^{r-1}. \end{aligned}$$

Consider $\sum_{r=1}^n (n-1) \binom{n-2}{r-2} \left(\frac{p}{1-p}\right)^r$. (8)

Taking $r - 2 = j$, it can be shown that

$$\begin{aligned} \sum_{r=1}^n (n-1) \binom{n-2}{r-2} \left(\frac{p}{1-p}\right)^r &= (n-1) \left(\frac{p}{1-p}\right)^2 \sum_{j=0}^{n-2} \binom{n-2}{j} \left(\frac{p}{1-p}\right)^j \\ \text{i.e } \sum_{r=1}^n (n-1) \binom{n-2}{r-2} \left(\frac{p}{1-p}\right)^r &= \frac{(n-1)p^2}{(1-p)^n}. \end{aligned} \quad (9)$$

Since $\sum_{r=1}^n \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^{r-1} = \left[1 + \frac{p}{1-p}\right]^{n-1}$,

$$2 \left(\frac{p}{1-p}\right) \sum_{r=1}^n \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^{r-1} = \frac{2p}{(1-p)^n}. \quad (10)$$

From (8), (9) and (10), we get

$$\sum_{r=1}^n (r+1) \binom{n-1}{r-1} \left(\frac{p}{1-p}\right)^r = \frac{(n-1)p^2 + 2p}{(1-p)^n}. \quad (11)$$

From (7), (11) and on simplification, we get

$$E(T^2) = \frac{2}{b^2} + \frac{p^2}{b^2} \sum_{m=1}^{\infty} (m-1)m P[Y=m] + \frac{4p}{b^2} \sum_{m=1}^{\infty} m P[Y=m]$$

i.e $E(T^2) = \frac{1}{b^2} \{2 + p^2[E(Y^2) - E(Y)] + 4pE(Y)\}. \quad (12)$

We now find $V(T)$.

From (6) and (12), it can be shown that

$$V(T) = \frac{1}{b^2} + \frac{p^2}{b^2} \{E(Y^2) - [E(Y)]^2\} + \left\{\frac{2p-p^2}{b^2}\right\} E(Y)$$

i.e $V(T) = \frac{1}{b^2} \{1 + p^2V(Y) + p(2-p)E(Y)\}. \quad (13)$

Equations (6) and (13) give the mean and variance of time to recruitment for the present model.

We next determine $E[C(T)]$, expected total number of exits up to time to recruitment.

By Wald's Equation, expected total number of exits up to time to recruitment is given by

$$E[C(T)] = E(X)E[B(T)]. \quad (14)$$

where $E(X) = \frac{1}{p}$ is the common mean of decapitated geometric random variables $X_i, i = 1, 2, 3, \dots$

Since $\{B(t); t \geq 0\}$ is a Poisson process with rate b , we know that $E[B(t)] = bt$.

By conditioning upon T and using law of total probability we find that

$$E[B(T)] = b E[T]. \quad (15)$$

From (6), (14) and (15), we get

$$E[C(T)] = \frac{1}{p} + E[Y]. \quad (16)$$

Remark:

When the mandatory breakdown threshold Y is a constant threshold, analogous results for the mean and variance of time to recruitment can be obtained.

In fact, if $Y = c, c > 0$, it can be shown that

$$P[T > t] = e^{-bt} + \sum_{m=1}^c (1-p)^m \sum_{r=1}^m \frac{1}{r!} \binom{m-1}{r-1} \left(\frac{bp}{1-p}\right)^r t^r e^{-bt}.$$

$$E(T) = \frac{1}{b} \{1 + pc\}.$$

$$E(T^2) = \frac{1}{b^2} \{2 + 4pc + c(c-1)p^2\}.$$

and

$$V(T) = \frac{1}{b^2} \{1 + 2pc - p^2c\}.$$

4. NUMERICAL ILLUSTRATION AND FINDING

'p' and 'b' are the nodal parameters in the performance measures $E(T)$ and $V(T)$. 'p' is the only nodal parameter in the performance measure $E[C(T)]$. It is palpable that $E(T)$ and $V(T)$ increase with 'p', keeping 'b', $E(Y)$ and $V(Y)$ fixed. But $E[C(T)]$ decreases when p increases, keeping $E(Y)$ fixed. These are also true logically. In fact, if 'p' increases, then $1/p$, the average number of exits in each cluster decreases, which implies, the average time taken for exceeding the threshold level increases, but the average total number of exits decreases.

It is also clear that $E(T)$ and $V(T)$ decrease when 'b' increases, keeping 'p', $E(Y)$ and $V(Y)$ fixed. In fact, if 'b', the average number of clusters per unit time increases, then the average time taken for exceeding the threshold level decreases and hence the mean time to recruitment decreases.

The following table gives the effect of simultaneous variation of 'p' and 'b' on the performance measures $E(T)$ and $V(T)$ when $E(Y) = 150$ and $V(Y) = 250$.

Tab. 1

<i>b</i>	<i>p</i>	$E(T)$	$V(T)$
5	0.1	3.2000	1.2800
6	0.3	7.6667	2.7778
7	0.5	10.8571	3.5918
8	0.7	13.2500	4.0625
9	0.9	15.1111	4.3457

Since $E[C(T)]$ is independent of 'b', the following table gives effect of variation of 'p' on the performance measure $E[C(T)]$ when $E(Y) = 150$.

Tab. 2

<i>p</i>	$E[C(T)]$
0.1	160.0000
0.3	153.3333
0.5	152.0000
0.7	151.4286
0.9	151.1111

FINDING:

Tables (1) and (2) reflect the logical conclusions on the monotonicity of $E(T)$, $V(T)$ and $E[C(T)]$ when the respective nodal parameters increase.

5. CONCLUSION

The present work contributes to the existing literature in the sense that the model discussed in this paper is new in the context of considering (i) the number of clusters of exits forms a homogeneous Poisson process (ii) the number of exits in each cluster forms a sequence of independent and identically distributed geometric random variables and (iii) the mandatory random breakdown threshold as a positive integer valued random variable.

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ON THE GAUSSIAN INTEGER SOLUTIONS FOR AN ELLIPTIC DIOPHANTINE EQUATION

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Abstract

The quadratic Diophantine equation with two unknowns represented by an elliptic curve $DE : 65J^2 + 225K^2 - 230JK = 1600$ is analyzed for its non-zero separate solutions in $Z[i]$. We also gain a few formulae and reappearance relations on the Gaussian integer solutions (J_n, K_n) of DE .

1. Introduction

The Diophantine equation $x^4 \pm y^4 = z^2$ where x, y and z being Gaussian integer were examined by Hilbert. It was proved that there exist only inconsequential solutions in $Z[i]$. Elliptic curves have also been used in [3] to prove that the Diophantine equation $x^3 + y^3 = z^3$ has only trivial solutions in Gaussian integers. These outcomes have motivated us to find non-zero distinct Gaussian integer solutions to a homogenous quadratic Diophantine equation in three variables. Convinced Diophantine problems

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move towards beginning problems or immediate arithmetical generalizations and others come from geometry in a variety of ways. Swayed Diophantine problems are neither inconsequential nor complicated to analyze [1, 2, 4, 5, 6].

In this manuscript, we look into Gaussian integer solutions of the Diophantine equation $65J^2 + 225K^2 - 230JK = 1600$ which is malformed into a Pell's equation and is solved by a variety of methods.

2. The Diophantine Equation

Think about the Diophantine equation

$$DE : 65J^2 + 225K^2 - 230JK = 1600 \quad (1)$$

to be solved over $Z[i]$. It is not trouble-free to work out and discover the nature and properties of the solutions of (1). So we concern a linear conversion Trs to (1) to shift to a simpler form for which we can find out the integral solutions.

Let

$$Trs : \begin{cases} J = ha + ib \\ K = a + ikb \end{cases} \quad (2)$$

be the shift where $a, b, h, k \in Z$.

Applying to Trs to DE , we get

$$\begin{aligned} Trs(DE) = \tilde{DE} : 65(ha + ib)^2 + 225(a + ikb)^2 \\ - 230((ha + ib)(a + ikb)) = 1600. \end{aligned} \quad (3)$$

Equating the imaginary part to zero and the coefficient of a^2 and b^2 to the smallest amount numeral, we get $h = 2$ and $k = 3$.

Hence for $J = 2a + ib$ and $K = a + i3b$, we have the Diophantine equation

$$\tilde{DE} : a^2 - 56b^2 = 64 \quad (4)$$

which is a Pell equation. Now we try to find all integer solutions (a_n, b_n) of

\tilde{D} and then we can retransfer all outcome as of \tilde{D} to by using the converse of *Trs.*

Theorem 2.1. *Let $\tilde{D}E$ be the Diophantine equation in (4). Then*

(i) *The continued fraction expansion of $\sqrt{56}$ is*

$$\sqrt{56} = [7; \overline{2, 14}]$$

(ii) *The primary result of $a^2 - 56b^2 = 1$ is $(u_1, v_1) = (15, 2)$*

(iii) *For $n \geq 4$,*

$$u_n = 31(u_{n-1} - u_{n-2}) + u_{n-3}$$

$$v_n = 31(v_{n-1} - v_{n-2}) + v_{n-3}$$

Proof.

(i) The continued fraction expansion of $\sqrt{56} = 7 + (\sqrt{56} - 7)$

$$\begin{aligned} &= 7 + \frac{1}{\frac{1}{\sqrt{56} - 7}} \\ &= 7 + \frac{1}{\frac{\sqrt{56} + 7}{7}} \\ &= 7 + \frac{1}{2 + \frac{\sqrt{56} - 7}{7}} \\ &= 7 + \frac{1}{2 + \frac{1}{\sqrt{56} + 7}} \\ &= 7 + \frac{1}{2 + \frac{1}{14 + (\sqrt{56} - 7)}}. \end{aligned}$$

Therefore the continued fraction expansion of $\sqrt{56}$ is

$$[7; \overline{2, 14}]$$

(ii) Note that by (3), if $(u_1, v_1) = (15, 2)$ is the primary result of $a^2 - 56b^2 = 1$, then the supplementary solutions (u_n, v_n) of $a^2 - 56b^2 = 1$ can be consequently employing the equalities

$(u_n + v_n\sqrt{56}) = (u_1 + \sqrt{56}v_1)^n$ for $n \geq 2$, in supplementary expressions,

$$\begin{pmatrix} u_n \\ v_n \end{pmatrix} = \begin{pmatrix} u_1 & 56v_1 \\ 2 & u_1 \end{pmatrix}^n \begin{pmatrix} 1 \\ 0 \end{pmatrix}.$$

For $n \geq 2$.

Hence it can be given away by generation on n that

$$u_n = 31(u_{n-1} - u_{n-2}) + u_{n-3}$$

and also

$$v_n = 31(v_{n-1} - v_{n-2}) + v_{n-3}$$

for $n \geq 4$.

Now we think about the problem

$$a^2 - 56b^2 = 64.$$

Make a note of that we stand for the integer solutions of $a^2 - 56b^2 = 64$ by (a_n, b_n) , and represent the integer solutions of $a^2 - 56b^2 = 1$ by (u_n, v_n) . Then we have the subsequent theorem.

Theorem 2.2. *Describe a progression $\{(a_n, b_n)\}$ of positive integers by $(a_1, b_1) = (120, 16)$ and*

$$a_n = 120u_{n-1} + 896v_{n-1}$$

$$b_n = 16u_{n-1} + 120v_{n-1}.$$

Somewhere $\{(u_n, v_n)\}$ is a progression of constructive solutions of $a^2 - 56b^2 = 1$. Then

(1) (a_n, b_n) is a solution of $a^2 - 56b^2 = 64$ for any integer $n \geq 1$.

(2) For $n \geq 2$,

$$a_{n+1} = 15a_n + 112b_n$$

$$b_{n+1} = 2a_n + 15b_n.$$

(3) For $n \geq 4$

$$a_n = 31(a_{n-1} - a_{n-2}) + a_{n-3}$$

$$b_n = 31(b_{n-1} - b_{n-2}) + b_{n-3}.$$

Proof.

(1) It is without a doubt seen to

$$(a_1, b_1) = (120, 16)$$

is a way out of $a^2 - 56b^2 = 64$ since

$$\begin{aligned} a_1^2 - 56b_1^2 &= (120)^2 - 56(16)^2 \\ &= 64(15^2 - 56(2^2)) = 64(1) \\ &= 64. \end{aligned}$$

A reminder that by description (u_{n-1}, v_{n-1}) is a method out $a^2 - 56b^2 = 1$, of that is,

$$u_{n-1}^2 - 56v_{n-1}^2 = 1. \tag{5}$$

Moreover, we observe as greater than that (a_1, b_1) is a resolution of $a^2 - 42b^2 = 16$, to is,

$$a_1^2 - 56b_1^2 = 64. \tag{6}$$

Applying (5) and (6), we get

$$\begin{aligned} a_n^2 - 56b_n^2 &= (120u_{n-1} + 896v_{n-1})^2 - 56(16u_{n-1} + 120v_{n-1})^2 \\ &= u_{n-1}^2(2^6) - v_{n-1}^2(2^6(56)) \end{aligned}$$

$$\begin{aligned}
&= 2^6(u_{n-1}^2 - 56v_{n-1}^2) \\
&= 2^6.
\end{aligned}$$

Consequently (a_n, b_n) is a way out of $\alpha^2 - 56b^2 = 2^6$.

(2) Bear in mind that

$$a_{n+1} + b_{n+1}\sqrt{d} = (u_1 + v_1\sqrt{d})(a_n + b_n\sqrt{d})$$

Consequently $a_{n+1} = u_1 a_n + v_1 b_n d$ and $b_{n+1} = v_1 a_n + u_1 b_n$.

So

$$a_{n+1} = 15a_n + 112b_n \text{ and } b_{n+1} = 2a_n + 15b_n. \quad (7)$$

Since $u_1 = 15$ and $v_1 = 2$.

(3) Applying the equalities

$$a_n = 2^2(15)u_{n-1} + 2^3(56)v_{n-1} \text{ and } a_{n+1} = 15a_n + 112b_n.$$

We find by generation on n that

$$a_n = 31(a_{n-1} - a_{n-2}) + a_{n-3}$$

for $n \geq 4$. In the same way, it knows how to be given away that

$$b_n = 31(b_{n-1} - b_{n-2}) + b_{n-3}.$$

We saw as on top of that the Diophantine equation DE could be malformed into the $D\tilde{E}$ via the conversion Trs . Also, we showed that $J = 2a + ib$ and $K = a + i3b$. So we can retransfer all results from $\tilde{D}E$ to by using the converse of Trs . Thus we can give the subsequent major theorem.

Theorem 2.3. *Let DE be the Diophantine equation in (1). Subsequently*

(1) *The primary solution of DE is $(J_1, K_1) = (240 + 16i, 120 + 48i)$.*

(2) *Describe the progression $\{(J_n, K_n)\}_{n \geq 1} = \{(2a_n + ib_n, a_n + i3b_n)\}$,*

wherever $\{(a_n, b_n)\}$ defined in (7).

Then (J_n, K_n) is a solution of DE .

(3) *The explanation (J_n, K_n) convince*

$$J_n = 2(15a_{n-1} + 112b_{n-1}) + i(2a_{n-1} + 15b_{n-1})$$

$$K_n = (15a_{n-1} + 112b_{n-1}) + i3(2a_{n-1} + 15b_{n-1}).$$

(4) *The solutions (J_n, K_n) satisfy the reappearance relations*

$$J_n = (62(a_{n-1} - a_{n-2}) + 2a_{n-3}) + i(31(b_{n-1} - b_{n-2}) + b_{n-3})$$

$$K_n = (31(a_{n-1} - a_{n-2}) + a_{n-3}) + i(93(b_{n-1} - b_{n-2}) + 3b_{n-3}).$$

Conclusion

Diophantine equations are prosperous in diversity. There is no general method for discovering the entire possible Gaussian integer solutions (if it exists) for Diophantine equations one possibly will explore for additional choices of Diophantine equations to find their equivalent Gaussian integer solutions.

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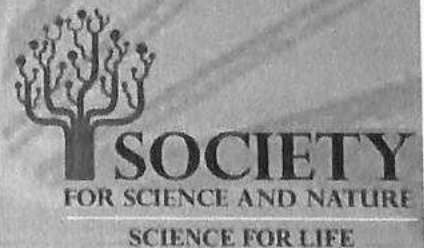
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On Non - Homogeneous Cubic Equation With Four Unknowns $x^2 + y^2 + 4(35z^2 - 4 - 35w^2) = 6xyz$

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ABSTRACT

This paper is devoted to obtain non-zero distinct integer solutions to non-homogeneous cubic equation with four unknowns represented by $x^2 + y^2 + 4(35z^2 - 4 - 35w^2) = 6xyz$ along with few observations.

KEY WORDS: NON-HOMOGENEOUS, CUBIC WITH FOUR UNKNOWN, INTEGER SOLUTIONS 2010 MATHEMATICS SUBJECT CLASSIFICATION: 11D09.

INTRODUCTION

The cubic Diophantine equations are rich in variety and offer an unlimited field for research. This paper concerns with another interesting cubic Diophantine equation with four unknowns $x^2 + y^2 + 4(35z^2 - 4 - 35w^2) = 6xyz$ for determining its infinitely many non-zero integral solutions.

Notations Used:

1. Regular Polygonal Number of rank n with sides m : $P_{m,n} = n[1 + \frac{(m-1)(n-1)}{2}]$
2. Pyramidal Number of rank n with sides m : $P_n^m = \frac{1}{6}[n(n+1)](m-2)n + (5-m)$
3. Pronic Number of rank n : $pn = n(n+1)$
4. Stella Octangular Number of rank n : $SO_n = n(2n^2 - 1)$
5. Octahedral Numbers of rank n : $OH_n = \frac{1}{3}n(2n^2 + 1)$
6. Star Number of rank n : $S_n = 6n(n-1) + 1$
7. Pentatope Number of rank n : $P_n^5 = \frac{n(n+1)(n+2)(n+3)}{24}$

Method of Analysis: The homogeneous cubic equation with four unknowns to be solved is

$$X^2 + Y^2 + 4(35Z^2 - 4 - 35W^2) = 6XYZ \quad (1)$$

Introducing the linear transformations

$$x = 2X + 12Z, y = 4 \quad (2)$$

in (1), it leads to

$$X^2 = Z^2 + 35W^2 \quad (3)$$

We present below different methods of solving (3) and thus, obtain different patterns of integral solutions to (1).

Pattern-I

It is observed that (3) is satisfied by

$$w = 2rs, z = 35r^2 - s^2, X = 35r^2 + s^2 \quad (4)$$

Hence, in view of (2) and (4), the non-zero integral solutions of (1) are given by

$$\begin{aligned} x &= x(r, s) = 490r^2 - 10s^2 \\ y &= y(r, s) = 4 \\ z &= z(r, s) = 35r^2 - s^2 \\ w &= 2rs \end{aligned}$$

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Properties:

1. $x(r(r+1),r) - 35z(r(r+1),r) + w(r(r+1),r) = 25t_{r+1} - 4P^2$
2. $x(r(r+1),r+2) - 35z(r(r+1),r+2) + y(r(r+1),r+2) = 25(P_r)^2 + 12P^2 + 4$
3. $35\{x(r,r) - 10z(r,r)\}$ is a perfect square.
4. $6\{x(r,s) - 35z(r,s)\}$ is a Nasty Number.
5. $x(r,3(r-1)) - 35z(r,3(r-1)) + y(r,3(r-1)) + w(r,3(r-1)) = 75t_{r-1} + S_r + 3$

**Pattern-II
Method of factorization**

Write (3) as $z^2 + 35w^2 - X^2 = X^2 + 1$ (5)

Assume $X = a^2 + 35b^2$ (6)

where a and b are non-zero integers.

Write 1 as $1 = \frac{(1+i\sqrt{35})(1-i\sqrt{35})}{6^2}$ (7)

Using (5), (6) in (5) and applying the method of factorization, define

$$(z + i\sqrt{35}w) = \frac{1}{6} (1 + i\sqrt{35})(a + i\sqrt{35}b)$$

Equating the real and imaginary parts, we have

$$z = \frac{1}{6} [a^2 - 70ab - 35b^2]$$

$$w = \frac{1}{6} [a^2 + 2ab - 35b^2]$$

As our interest centres on finding integer solutions, it seen that the values of z, w, X are integers, when a= 7A, B = 7B

Thus, we have

$$\left. \begin{aligned} X &= X(A, B) = 36A^2 + 1260 AB \\ z &= z(A, B) = 6A^2 - 210B^2 - 420 AB \\ w &= z(A, B) = 6A^2 - 210B^2 + 12 AB \end{aligned} \right\} \quad (8)$$

Hence, in view of (2) and (8), the non- zero integral solutions of (1) are found to be

$$x = x(A, B) = 144A^2 - 5040AB$$

$$y = y(A, B) = 4$$

$$z = z(A, B) = 6A^2 - 210B^2 - 420AB$$

$$w = z(A, B) = 6A^2 - 210B^2 + 12AB$$

Properties:

1. $x(A, A+1) - 24z(A, A+1) = 1260t_{A+1} + 10080t_{1,A}$
2. $w(A(A+1), (A+2)(A+2)) - z(A(A+1), (A+2)(A+2)) = 129P_A$
3. $w(A, A) - Z(A, A)$ is a Nasty Number.
4. $z(A, B) + w(A, B) = 0 \pmod{12}$
5. $x(A, (A+1)) + 12[z(A, (A+1)) + w(A, (A+1))] + 420t_{A+1} + 9936 P_1$

Note: It is worth mentioning here that, 1 may also be represent as the product of complex conjugates as shown below:

$$1 = \frac{(17 + i\sqrt{35})(17 - i\sqrt{35})}{324}$$

$$1 = \frac{(13 + i3\sqrt{35})(13 - i3\sqrt{35})}{484}$$

$$1 = \frac{(35r^2 - s^2 + 2i\sqrt{35}rs)(35r^2 - s^2 - 2i\sqrt{35}rs)}{(35r^2 + s^2)^2}$$

**Pattern-III
Method of factorization**

Rewrite (3) as

$$X^2 - 35w^2 = z^2 + 1 \quad (9)$$

$$\text{Let } z = p^2 - 35q^2 \quad (10)$$

where p and q are non-zero integers.

$$\text{Write 1 as } 1 = (6 + \sqrt{35})(6 - \sqrt{35}) \quad (11)$$

Substituting (10) and (11) in (9) and employing the method of factorization, define

Table 1. System of double equations

System	1	2	3	4	5	6
$X + z$	$35w^2$	$7w^2$	$5w^2$	w^2	$35w$	$7w$
$X - z$	1	5	7	35	w	$5w$

$(X + \sqrt{35}w) = (6 + \sqrt{35})(p + i\sqrt{35}q)$ Equating the rational and irrational parts, we have

$$X = X(p, q) = 6p^2 + 210q^2 - 70pq$$

$$w = w(p, q) = p^2 + 35q^2 - 12pq$$

Thus, the corresponding non-zero distinct integral solutions of (1) are

$$x = x(p, q) = 24p^2 - 140pq$$

$$y = y(p, q) = 4$$

$$z = z(p, q) = p^2 - 35q^2$$

$$w = w(p, q) = p^2 + 35q^2 - 12pq$$

Properties:

1. $x(p(p+1), 2(p+1)) - 24x(p(p+1), 2(p+1)) + 840P^2 = 840t_{2p+1}$
2. $2\{x(p, (2p^2-1)) + w(p, (2p^2-1)) + 24SO_p\}$ is a perfect square.
3. $35\{w(p, (2p^2+1)) - x(p, (2p^2+1)) + 36OH_p\}$ is a Nasty Number.
4. $x(p, (p+1)(p+2)) - 12z(p, (p+1)(p+2)) + 12w(p, (p+1)(p+2)) = 24P^2$

Pattern-IV System of double equations

Observe that (3) as $X^2 - z^2 = 35w^2$

It can be represented as the system of double equation as shown below:

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System :1	System:2	System:3
$x = 980T^2 + 980T + 240$	$x = 196T^2 + 196T + 24$	$x = 140T^2 + 140T$
$y = 4$	$y = 4$	$y = 4$
$z = 70T^2 + 70T + 17$	$z = 14T^2 + 14T + 1$	$z = 10T^2 + 10T - 1$
$w = 2T + 1$	$w = 2T + 1$	$w = 2T + 1$
System :4	System:5	System:5
$x = 28T^2 + 28T - 168$	$x = 240T$	$x = 24T$
$y = 4$	$y = 4$	$y = 4$
$z = 2T^2 + 2T - 17$	$z = 17T$	$z = T$
$w = 2T + 1$	$w = T$	$w = T$

- If the non-zero integer quadrup $(x_0, 4, z_0, w_0)$ is any solution of (1), then, each of the following three quadruples of integers based on x_0, z_0 and w_0 also satisfies (1).

Quadruple:1 (x_n, y_n, z_n, w_n)

$$x_n = \frac{1}{2}[-38 \times 4^{n-1} + 54(-4)^{n-1}]x_0 + [114 \times 4^{n-1} + 18(-4)^{n-1}]z_0$$

$$y_n = 4$$

$$z_n = \frac{1}{2}[-3 \times 4^{n-1} + (-4)^{n-1}]x_0 + [9 \times 4^{n-1} + (-4)^{n-1}]z_0$$

$$w_n = 4^n w_0$$

Quadruple:2 (x_n, y_n, z_n, w_n)

$$x_n = 2(6)^{2n} x_0 + 6[70 \times 36^{n-1} - 2(-36)^{n-1}]z_0 + [-70 \times 36^{n-1} + (-36)^{n-1}]w_0$$

$$y_n = 4$$

$$z_n = [35 \times 36^{n-1} - (-36)^{n-1}]z_0 + [-35(36^{n-1} + (-36)^{n-1})]w_0$$

$$w_n = [-36^{n-1} - (-36)^{n-1}]z_0 + [36^{n-1} - 35(-36)^{n-1}]w_0$$

Quadruple:3 (x_n, y_n, z_n, w_n)

$$x_n = [-70 + 72(-1)^n]x_0 + [420(1 - (-1)^n)]w_0 + 12z_0$$

$$y_n = 4$$

$$z_n = z_0$$

$$w_n = -6[1 - (-1)^n]x_0 + [36 - 35(-1)^n]w_0$$

CONCLUSION

In this paper, we have made an attempt to determine different patterns of non-zero distinct integer solutions to the non - homogeneous cubic equation with four unknowns. As the cubic equations are rich in variety, one may search for other forms of cubic equations with multi-variables to obtain their corresponding solutions.

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Observations on the Pell Equation $x^2 = 3(y^2 + y) + 1$

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ABSTRACT

This paper concerns with the problem of obtaining non-zero distinct integer solutions to the positive pell equation represented by the binary quadratic equation $x^2 = 3(y^2 + y) + 1$. A few interesting relations among the solutions are presented. Further, by considering suitable linear combinations among the solutions of the considered hyperbola, the other choices of hyperbolas, parabolas, 2nd order Ramanujan numbers, sequence of diophantine 3-tuples with suitable property are presented. A general formula for generating sequence of integer solutions based on the given solution is illustrated.

Keywords— Positive pell equation, binary quadratic, hyperbola, parabola, 2nd order Ramanujan numbers, sequence of diophantine 3-tuples

1. INTRODUCTION

One of the areas of Number theory that has attracted many mathematicians since antiquity is the subject of diophantine equations. A diophantine equation is a polynomial equation in two or more unknowns such that only the integer solutions are determined. No doubt that diophantine equation possess supreme beauty and it is the most powerful creation of the human spirit. A Pell equation is a type of non-linear diophantine equation in the form $y^2 - Dx^2 = \pm 1$ where $D > 0$ and square-free. The above equation is also called the Pell-Fermat equation. In Cartesian co-ordinates, this equation has the form of a hyperbola. The binary quadratic diophantine equation having the form

$$y^2 = Dx^2 + N \quad (N > 0 ; D > 0, \text{ a non-square integer})$$

is referred to as the positive form of the Pell equation and one may refer [1-10] for a few illustrations on Pell equations. As quadratic Diophantine equations are rich in variety, the above references motivated us for determining integer solutions to other choices of positive Pell equation.

In this communication, the Pell equation $x^2 = 3(y^2 + y) + 1$ is considered for obtaining non-zero distinct integer solutions. Further, by considering suitable linear combinations among the solutions of the considered hyperbola, the other choices of hyperbolas, parabolas, 2nd order Ramanujan numbers, sequence of diophantine 3-tuples with suitable property are presented. A general formula for generating sequence of integer solutions based on the given solution is illustrated.

2. METHOD OF ANALYSIS

The hyperbola represented by the non-homogeneous quadratic equation under consideration is

$$x^2 = 3(y^2 + y) + 1 \tag{1}$$

Treating (1) as a quadratic in y and solving for y , we get

$$y = \frac{-3 \pm \sqrt{12x^2 - 3}}{6} \tag{2}$$

Let

$$Y^2 = 12x^2 - 3 \tag{3}$$

The smallest positive integer solution to (3) is $x_0=1, Y_0=3$

To find the other solutions to (1), consider the corresponding Pellian equation given by

$$Y^2 = 12x^2 + 1 \tag{4}$$

Where the general solution \tilde{x}_n, \tilde{Y}_n is

$$\begin{aligned} \tilde{Y}_n &= \frac{1}{2} f_n \\ \tilde{x}_n &= \frac{1}{4\sqrt{3}} g_n \end{aligned}$$

where

$$\begin{aligned} f_n &= (7 + 4\sqrt{3})^{n+1} + (7 - 4\sqrt{3})^{n+1} \\ g_n &= (7 + 4\sqrt{3})^{n+1} - (7 - 4\sqrt{3})^{n+1}, \quad n = 0, 1, 2, \dots \end{aligned}$$

Employing the lemma of Brahmagupta between the solutions (x_0, Y_0) & $(\tilde{x}_n, \tilde{Y}_n)$, the general solution (x_{n+1}, Y_{n+1}) to (3) is given by

$$\begin{aligned} x_{n+1} &= x_0 \tilde{Y}_n + Y_0 \tilde{x}_n \\ &= \frac{1}{2} f_n + \frac{\sqrt{3}}{4} g_n \\ Y_{n+1} &= Y_0 \tilde{Y}_n + D x_0 \tilde{x}_n \\ &= 3 * \frac{1}{2} f_n + \sqrt{3} * g_n \end{aligned} \tag{5}$$

In view of (2) and taking the positive sign before the square-root on the R.H.S. of (2), we have

$$y_{n+1} = \frac{1}{12} (3f_n + 2\sqrt{3}g_n - 6) \tag{6}$$

Thus, (5) and (6) represented the integer solutions to (1).

A few numerical solutions to (1) are presented in Table below:

Table: Numerical solutions

N	x_{n+1}	y_{n+1}
-1	1	0
0	13	7
1	181	104
2	2521	1455
3	35113	20272

3. OBSERVATIONS

The x-values are odd primes whereas y-values are alternatively odd and even.

A few interesting relations among the solutions are given below:

- $x_{n+3} - 14x_{n+2} + x_{n+1} = 0$
- $y_{n+3} - 14y_{n+2} + y_{n+1} = 6$
- $12y_{n+1} + 6 = x_{n+2} - 7x_{n+1}$
- $12y_{n+2} + 6 = -x_{n+1} + 7x_{n+2}$
- $12y_{n+3} + 6 = -7x_{n+1} + 97x_{n+2}$
- $168y_{n+1} + 84 = x_{n+3} - 97x_{n+1}$
- $24y_{n+2} + 12 = x_{n+3} - x_{n+1}$
- $168y_{n+3} + 84 = 97x_{n+3} - x_{n+1}$
- $4x_{n+1} = y_{n+2} - 7y_{n+1} - 3$
- $4x_{n+2} = 7y_{n+2} - y_{n+1} + 3$
- $4x_{n+3} = 97y_{n+2} - 7y_{n+1} + 45$
- $56x_{n+1} = y_{n+3} - 97y_{n+1} - 48$

- $8x_{n+2} = y_{n+3} - y_{n+1}$
- $56x_{n+3} = 97y_{n+3} - y_{n+1} + 48$
- $x_{n+1} = 7x_{n+2} - 12y_{n+2} - 6$
- $x_{n+3} = 7x_{n+2} + 12y_{n+2} + 6$
- $y_{n+1} = 7y_{n+2} - 4x_{n+2} + 3$
- $y_{n+3} = 7y_{n+2} + 4x_{n+2} + 3$

Expressions representing square integers:

- $[15x_{2n+2} - x_{2n+3} + 2]$
- $\frac{1}{14}[209x_{2n+2} - x_{2n+4} + 28]$
- $[2y_{2n+3} - 26y_{2n+2} - 10]$
- $\frac{1}{7}[y_{2n+4} - 181y_{2n+2} - 76]$

Expressions representing cubical integers:

- $[15x_{3n+3} - x_{3n+4} + 3(15x_{n+1} - x_{n+2})]$
- $\frac{1}{14}[209x_{3n+3} - x_{3n+5} + 3(209x_{n+1} - x_{n+3})]$
- $[2y_{3n+4} - 26y_{3n+3} + 6y_{n+2} - 78y_{n+1} - 48]$
- $\frac{1}{7}[y_{3n+5} - 181y_{3n+3} + 3y_{n+3} - 543y_{n+1} - 360]$

Expressions representing biquadratic integers:

- $(15x_{4n+4} - x_{4n+5}) + 4(15x_{n+1} - x_{n+2})^2 - 2$
- $(15x_{4n+4} - x_{4n+5}) + 4(15x_{2n+2} - x_{2n+3} + 2) - 2$
- $\frac{1}{14}(209x_{4n+4} - x_{4n+6}) + \frac{1}{49}(209x_{n+1} - x_{n+3})^2 - 2$
- $\frac{1}{14}(209x_{4n+4} - x_{4n+6}) + \frac{2}{7}(209x_{2n+2} - x_{2n+4} + 28) - 2$
- $(2y_{4n+5} - 26y_{4n+4} - 14) + 16(y_{n+2} - 13y_{n+1} - 6)^2 - 2$
- $(2y_{4n+5} - 26y_{4n+4} - 14) + 8(y_{2n+3} - 13y_{2n+2} - 5) - 2$
- $\frac{1}{7}(y_{4n+6} - 181y_{4n+4} - 90) + \frac{4}{49}(y_{n+3} - 181y_{n+1} - 90)^2 - 2$
- $\frac{1}{7}(y_{4n+6} - 181y_{4n+4} - 90) + \frac{4}{7}(y_{2n+4} - 181y_{2n+2} - 76) - 2$

Employing linear combinations among the solutions, one obtains solutions to other choices of hyperbolas

Choice1: Let $Y = x_{n+2} - 13x_{n+1}$, $X = 15x_{n+1} - x_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$3X^2 - 4Y^2 = 12$$

Choice2: Let $Y = x_{n+3} - 181x_{n+1}$, $X = 209x_{n+1} - x_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$3X^2 - 4Y^2 = 48 * 49$$

Choice3: Let $Y = 15y_{n+1} - y_{n+2} + 7$, $X = 2y_{n+2} - 26y_{n+1} - 12$

Note that (X, Y) satisfies the hyperbola

$$4X^2 - 3Y^2 = 4$$

Choice4: Let $Y = 209y_{n+1} - y_{n+3} + 104$, $X = y_{n+3} - 181y_{n+1} - 90$

Note that (X, Y) satisfies the hyperbola

$$4X^2 - 3Y^2 = 49 * 16$$

Employing linear combinations among the solutions, one obtains solutions to other choices of parabolas

Choice1: Let $Y = x_{n+2} - 13x_{n+1}, X_1 = 15x_{2n+2} - x_{2n+3} + 2$

Note that (Y, X_1) satisfies the parabola

$$3X_1 - 4Y^2 = 12$$

Choice2: Let $Y = x_{n+3} - 181x_{n+1}, X_1 = 209x_{2n+2} - x_{2n+4} + 28$

Note that (Y, X_1) satisfies the parabola

$$21X_1 - 2Y^2 = 21 * 56$$

Choice3: Let $Y = 209y_{n+1} - y_{n+3} + 104, X_1 = y_{2n+4} - 181y_{2n+2} - 76$

Note that (Y, X_1) satisfies the parabola

$$28X_1 - 3Y^2 = 4 * 196$$

Choice4: Let $Y = 15y_{n+1} - y_{n+2} + 7, X_1 = y_{2n+3} - 13y_{2n+2} - 5$

Note that (Y, X_1) satisfies the parabola

$$2X_1 - 3Y^2 = 4$$

Considering suitable values of x_{n+1} and y_{n+1} , one generates 2^{nd} order Ramanujan numbers with base integers as real integers

For illustration, consider

$$y_2 = 104 = 1 \times 104 = 2 \times 52 = 4 \times 26 = 8 \times 13 \quad (*)$$

Now, $1 \times 104 = 2 \times 52$

$$\rightarrow (104+1)^2 + (52-2)^2 = (104-1)^2 + (52+2)^2$$

$$\rightarrow 105^2 + (50)^2 = (103)^2 + 54^2 = 13525$$

$1 \times 104 = 4 \times 26$

$$\rightarrow (104+1)^2 + (26-4)^2 = (104-1)^2 + (26+4)^2 = 11509$$

$1 \times 104 = 8 \times 13$

$$\rightarrow (104+1)^2 + (13-8)^2 = (104-1)^2 + (13+8)^2 = 11050$$

$2 \times 52 = 4 \times 26$

$$\rightarrow (52+2)^2 + (26-4)^2 = (52-2)^2 + (26+4)^2 = 3400$$

$2 \times 52 = 8 \times 13$

$$\rightarrow (52+2)^2 + (13-8)^2 = (52-2)^2 + (13+8)^2 = 2941$$

$4 \times 26 = 8 \times 13$

$$\rightarrow (26+4)^2 + (13-8)^2 = (26-4)^2 + (13+8)^2 = 925$$

Also,

$$2 \times 52 = 4 \times 26 \rightarrow 27^2 - 25^2 = 15^2 - 11^2$$

$$\rightarrow 27^2 + 11^2 = 15^2 + 25^2 = 850$$

Thus, 13525, 11509, 11050, 3400, 2941, 925, 850 represent 2^{nd} order Ramanujan numbers with base integers as real integers.

Considering suitable values of x_{n+1} & y_{n+1} , one generates 2^{nd} order Ramanujan numbers with base integers as Gaussian integers.

For illustration, consider again y_2 represented by (*)

$$\text{Now, } 1 \times 104 = 2 \times 52 \rightarrow (1+i104)^2 + (2-i52)^2 = (1-i104)^2 + (2+i52)^2 = -13520$$

$$\text{Also, } 1 \times 104 = 2 \times 52 \rightarrow (104+i)^2 + (52-i2)^2 = (104-i)^2 + (52+i2)^2 = 13520$$

Note that -13520 & 13520 represent 2^{nd} order Ramanujan numbers with base integers as Gaussian integers.

In a similar manner, other 2^{nd} order Ramanujan numbers are obtained

Formation of sequence of Diophantine 3-tuples:

Consider the solution to (1) given by

$$x_1 = 13 = a \text{ (say)}, y_1 = 7 = c_0 \text{ (say)}$$

It is observed that

$$ac_0 + k^2 - 91 = k^2, \text{ a perfect square}$$

The pair (a, c_0) represents diophantine 2-tuple with property $D(k^2 - 91)$.

If c_1 is the 3rd tuple, then it satisfies the system of double equations

$$13c_1 + k^2 - 91 = p^2 \tag{1*}$$

$$7c_1 + k^2 - 91 = q^2 \tag{2*}$$

Eliminating c_1 between (1*) and (2*), we have

$$6(k^2 - 91) = 13q^2 - 7p^2 \tag{3*}$$

Taking

$$p = X + 13T, q = X + 7T \tag{4*}$$

in (3*) and simplifying, we get

$$X^2 = 91T^2 + k^2 - 91$$

which is satisfied by

$$X = k, T = 1$$

In view of (4*) and (1*), it is seen that

$$c_1 = 2k + 20$$

Note that $(13, 7, 2k + 20)$ represents diophantine 3-tuple with property $D(k^2 - 91)$.

The process of obtaining sequences of diophantine 3-tuples with property $D(k^2 - 91)$ is illustrated below:

Let M be a 3*3 square matrix given by

$$M = \begin{pmatrix} 1 & 0 & 2 \\ 0 & 0 & -1 \\ 0 & 1 & 2 \end{pmatrix}$$

Now

$$(13, 7, 2k + 20)M = (13, 2k + 20, 4k + 59)$$

Note that

$$13 * (2k + 20) + (k^2 - 91) = \text{perfect square}$$

$$13 * (4k + 59) + (k^2 - 91) = \text{perfect square}$$

$$(2k + 20) * (4k + 59) + (k^2 - 91) = \text{perfect square}$$

Therefore, the triple $(13, 2k + 20, 4k + 59)$ represents diophantine 3-tuple with property $D(k^2 - 91)$. The repetition of the above process leads to sequences of diophantine 3-tuples whose general form (a, c_{s-1}, c_s) is given by

$$(13, 13s^2 + (2k - 26)s - 2k + 20, 13s^2 + 2ks + 7), s=1, 2, 3, \dots$$

A few numerical illustrations are given in Table below:

Table: Numerical illustrations

K	(a, c ₀ , c ₁)	(a, c ₁ , c ₂)	(a, c ₂ , c ₃)	D(k ² - 91)
0	(13, 7, 20)	(13, 20, 59)	(13, 59, 124)	D(-91)
1	(13, 7, 22)	(13, 22, 63)	(13, 63, 130)	D(-90)
2	(13, 7, 24)	(13, 24, 67)	(16, 114, 136)	D(-87)

It is noted that the triple $(c_{s-1}, c_s + 13, c_{s+1}), s=1, 2, 3, \dots$

forms an arithmetic progression.

In a similar way, one may generate sequences of diophantine 3-tuples with suitable property through the other solutions to (1).

Generation of solutions:

Let (x_0, y_0) represents any given solution to (1).

Consider the second solution (x_1, y_1) to (1) given by

$$x_1 = 2h - x_0, y_1 = y_0 + h \tag{7}$$

Substituting (7) in (1) and simplifying, one obtains

$$h = 4x_0 + 6y_0 + 3$$

In view of (7), we have

$$x_1 = 7x_0 + 12y_0 + 6, y_1 = 4x_0 + 7y_0 + 3$$

which is written in the form of matrix as

$$(x_1, y_1, 1)^t = \begin{pmatrix} 7 & 12 & 6 \\ 4 & 7 & 3 \\ 0 & 1 & 1 \end{pmatrix} (x_0, y_0, 1)^t$$

where t is the transpose. The repetition of the above process leads to the general solution to (1) as

$$(x_{n+1}, y_{n+1}, 1)^t = \begin{pmatrix} Y_n & 3X_n & \frac{3X_n}{2} \\ X_n & Y_n & \frac{Y_n-1}{2} \\ 0 & 0 & 1 \end{pmatrix} (x_0, y_0, 1)^t, n = 0, 1, 2, \dots$$

where

$$Y_n = \frac{1}{2} \left((7 + 4\sqrt{3})^{n+1} + (7 - 4\sqrt{3})^{n+1} \right)$$

$$X_n = \frac{1}{2\sqrt{3}} \left((7 + 4\sqrt{3})^{n+1} - (7 - 4\sqrt{3})^{n+1} \right)$$

4. CONCLUSION

As quadratic Diophantine equations are rich in variety, the readers may attempt for finding integer solutions to quadratic Diophantine equations with two or more variables along with suitable properties.

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PELL EQUATION SOLUTION BY SOPHIE GERMAIN PRIMES

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ABSTRACT - We bring forth one of the most sought after and intriguing space related to Number Theory; and our attempts to unearth the continuing developments to find solutions for different aspects of the Pell's equation. In this paper, we attempt to find the solutions for the Pell's equation $x^2 = 41y^2 - 5^t$ for all choice of $t \in \mathbb{N}$. In this paper, we focused primarily on Pell's equations involving the Sophie Germain primes and present to you another mysterious series and pattern typically associated with the Pell's equation. Subsequently, the recurrence relations among the solutions are also obtained.

Index Terms - Pell equation, Integer Solutions, Diophantine equations, Sophie Germain Primes, Recurrence relation

I. INTRODUCTION

The Pell's equation is a Diophantine equation $x^2 - dy^2 = 1$, where d is a given positive non-square integer and integer solutions are sought for x and y . As an illustration, for d having value 5; one of the integer solutions is $x = 9, y = 4$. As long as d is not a perfect square, Pell's equation has infinitely many distinct integer solutions.

The Pell's equation discussed here is a negative Pell's equation given as $x^2 - dy^2 = -N$ to be solved in positive integers x and y . As indicated here forth, we are using the Sophie Germain prime in negative Pell's equation in finding positive integer solutions. In number theory, a prime number p is a Sophie Germain prime if $2p + 1$ is also prime. The number $2p + 1$ associated with a Sophie Germain prime is called a safe prime. In the Pell's equation $x^2 = 41y^2 - 5^t, t \in \mathbb{N}$; we are using the Sophie Germain primes 41 and 5 and search for its non-trivial integer solutions. In order to derive the solutions, we approached it with the choices of t given by (i) $t = 1$ (ii) $t = 3$ (iii) $t = 5$ (iv) $t = 2k$ and (v) $t = 2k + 5$.

Applying Brahma Gupta lemma, the sequence of non-zero distinct integer solutions are obtained. This solution addresses the many positive integer solutions obtained thence. A few research driven relations with respect to the solutions are presented. Furthermore, the process is taken a bit ahead to derive the recurrence relations that addresses such types of Pell's equations.

II. PRELIMINARIES

THEOREM 2.1 [2]

If x_1, y_1 is the fundamental solution of $x^2 - dy^2 = 1$. Then every positive solution of the equation is given by x_n, y_n where x_n and y_n are the integers determined from

$$x_n + y_n\sqrt{d} = (x_1 + y_1\sqrt{d})^n, \text{ for } n = 1, 2, 3, \dots$$

a. [12] Testing the solubility of the negative Pell equation

Suppose D is a positive integer, not a perfect square. Then the negative Pell equation $x^2 - Dy^2 = -1$ is soluble if and only if D is expressible as $D = a^2 + b^2, \gcd(a, b) = 1, a$ and b positive, b is odd and the Diophantine equation $-bV^2 + 2aVW + bW^2 = 1$ has a solution. (The case of solubility occurs for exactly one such (a, b)).

The Algorithm

- (i) Find all expressions of D as a sum of two relatively prime squares using Cornacchia's method. If none, exist - the negative Pell equation is not soluble.

- (ii) For each representation $D = a^2 + b^2$, $\gcd(a, b) = 1$, a and b positive, b odd, test the solubility of $-bV^2 + 2aVW + bW^2 = 1$ using the Lagrange-Matthews algorithm. If soluble, exist - the negative Pell equation is soluble.
- (iii) If each representation yields no solution, then the negative Pell equation is insoluble.

THEOREM 2.2[8]

Let p be a prime. The negative Pell's equation $x^2 - py^2 = -1$ is solvable if and only if $p = 2$ or $p \equiv 1 \pmod{4}$.

This paper concerns with a negative Pell equation

$$x^2 = 41y^2 - 5^t, t \in \mathbb{N}$$

For this particular equation, we consider the prime $p = 41$, which satisfies the conditions of Theorem 2.2. Therefore, we can substantiate the proof that the negative Pell's equation $x^2 = 41y^2 - 5^t, t \in \mathbb{N}$ is solvable in integers.

Using the Algorithm as in 2.1 and testing $(a, b) = (4, 5)$:

$$-bV^2 + 2aVW + bW^2 = 1 \text{ has a solution } (V, W) = (2, 1), \text{ so } x^2 - 41y^2 = -1 \text{ is soluble.}$$

III. METHOD OF ANALYSIS

3.1: Choice 1: $t = 1$

The Pell equation is

$$x^2 = 41y^2 - 5 \tag{1}$$

Let (x_0, y_0) be the initial solution of (1) given by

$$x_0 = 6; y_0 = 1$$

To find the other solutions of (1), consider the more general Pell equation

$$x^2 = 41y^2 + 1 \tag{2}$$

whose initial solution is $(2049, 320)$ and the general solution $(\tilde{x}_n, \tilde{y}_n)$ is given by theorem 2.1 as

$$\tilde{x}_n = \frac{1}{2}f_n$$

$$\tilde{y}_n = \frac{1}{2\sqrt{41}}g_n$$

where

$$f_n = (2049 + 320\sqrt{41})^{n+1} + (2049 - 320\sqrt{41})^{n+1}$$

$$g_n = (2049 + 320\sqrt{41})^{n+1} - (2049 - 320\sqrt{41})^{n+1}, n = 0, 1, 2 \dots$$

Applying Brahma Gupta lemma between (x_0, y_0) and $(\tilde{x}_n, \tilde{y}_n)$ the sequence of non-zero distinct integer solutions to (1) are obtained as

$$x_{n+1} = x_0\tilde{x}_n + dy_0\tilde{y}_n, y_{n+1} = x_0\tilde{y}_n + y_0\tilde{x}_n \tag{3}$$

$$x_{n+1} = \frac{1}{2}[6f_n + \sqrt{41}g_n]$$

$$y_{n+1} = \frac{1}{2\sqrt{41}}[\sqrt{41}f_n + 6g_n] \tag{4}$$

The recurrence relation satisfied by the solution of (1) are given by

$$x_{n+2} - 4098x_{n+1} + x_n = 0$$

$$y_{n+2} - 4098y_{n+1} + y_n = 0 \tag{5}$$

3.2 Choice 2: $t = 3$

The Pell equation is

$$x^2 = 41y^2 - 125 \tag{6}$$

Let (x_0, y_0) be the initial solution of (6) given by

$$x_0 = 30; y_0 = 5$$

Applying Brahma Gupta lemma between (x_0, y_0) and $(\tilde{x}_n, \tilde{y}_n)$ the sequence of non-zero distinct integer solutions to (6) are obtained by equation (3) as

$$x_{n+1} = \frac{1}{2}[30f_n + 5\sqrt{41}g_n]$$

$$y_{n+1} = \frac{1}{2\sqrt{41}}[5\sqrt{41}f_n + 30g_n] \tag{7}$$

The recurrence relation satisfied by the solution of (6) are given by

$$\begin{aligned}x_{n+2} - 4098x_{n+1} + x_n &= 0 \\y_{n+2} - 4098y_{n+1} + y_n &= 0\end{aligned}\quad (8)$$

3.3 Choice 3: $t = 5$

The Pell equation is

$$x^2 = 41y^2 - 3125 \quad (9)$$

Let (x_0, y_0) be the initial solution of (9) given by

$$x_0 = 14; y_0 = 9$$

Applying Brahma Gupta lemma between (x_0, y_0) and $(\widetilde{x}_n, \widetilde{y}_n)$ the sequence of non-zero distinct integer solutions to (9) are obtained by equation (3) as

$$\begin{aligned}x_{n+1} &= \frac{1}{2}[14f_n + 9\sqrt{41}g_n] \\y_{n+1} &= \frac{1}{2\sqrt{41}}[9\sqrt{41}f_n + 14g_n]\end{aligned}\quad (10)$$

The recurrence relation satisfied by the solution of (9) are given by

$$\begin{aligned}x_{n+2} - 4098x_{n+1} + x_n &= 0 \\y_{n+2} - 4098y_{n+1} + y_n &= 0\end{aligned}\quad (11)$$

3.4 Choices 4: $t = 2k, k \in \mathbb{N}$

The Pell equation is

$$x^2 = 41y^2 - 5^{2k}, k \in \mathbb{N} \quad (12)$$

Let (x_0, y_0) be the initial solution of (12) given by

$$x_0 = 32(5)^k; y_0 = 5(5)^k$$

Applying Brahma Gupta lemma between (x_0, y_0) and $(\widetilde{x}_n, \widetilde{y}_n)$ the sequence of non-zero distinct integer solutions to (12) are obtained by equation (3) as

$$\begin{aligned}x_{n+1} &= \frac{5^k}{2}[32f_n + 5\sqrt{41}g_n] \\y_{n+1} &= \frac{5^k}{2\sqrt{41}}[5\sqrt{41}f_n + 32g_n]\end{aligned}\quad (13)$$

The recurrence relation satisfied by the solution of (12) are given by

$$\begin{aligned}x_{n+2} - 4098x_{n+1} + x_n &= 0 \\y_{n+2} - 4098y_{n+1} + y_n &= 0\end{aligned}\quad (14)$$

3.5 Choices 5: $t = 2k + 5, k \in \mathbb{N}$

The Pell equation is

$$x^2 = 41y^2 - 5^{2k+5}, k \in \mathbb{N} \quad (15)$$

Let (x_0, y_0) be the initial solution of (15) given by

$$x_0 = 70(5)^{k-1}; y_0 = 45(5)^{k-1}$$

Applying Brahma Gupta lemma between (x_0, y_0) and $(\widetilde{x}_n, \widetilde{y}_n)$ the sequence of non-zero distinct integer solutions to (15) are obtained by equation (3) as

$$\begin{aligned}x_{n+1} &= \frac{5^{k-1}}{2}[70f_n + 45\sqrt{41}g_n] \\y_{n+1} &= \frac{5^{k-1}}{2\sqrt{41}}[45\sqrt{41}f_n + 70g_n]\end{aligned}\quad (16)$$

The recurrence relation satisfied by the solution of (15) are given by

$$\begin{aligned}x_{n+2} - 4098x_{n+1} + x_n &= 0 \\y_{n+2} - 4098y_{n+1} + y_n &= 0\end{aligned}\quad (17)$$

IV. CONCLUSION

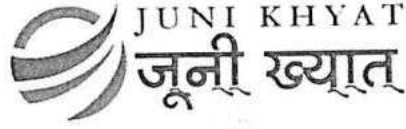
As seen above with the research put forth, solving a negative Pell's equation involving the Sophie Germain primes has provided a more intrinsic and dynamic interpretation for finding solutions to equations satisfying the similar nature.

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ABSTRACT:

The hyperbola represented by the binary quadratic equation $3x^2 - 8y^2 = -20$ is analyzed for finding its non-zero distinct integer solutions. A few interesting relations among its solutions are presented. Also, knowing an integral solution of the given hyperbola, integer solutions for other choices of hyperbolas and parabolas are presented. The formulation of second order Ramanujan Numbers with base numbers as real integers and Gaussian integers is illustrated and also the sequence of Diophantine 3-tuples are exhibited.

Keywords: Pell like equation, Binary quadratic, Hyperbola, Parabola, 2nd order Ramanujan numbers, sequence of Diophantine 3-tuples.

INTRODUCTION:

The binary quadratic Diophantine equations of the form $ax^2 - by^2 = N, (a, b, N \neq 0)$ are rich in variety and have been analyzed by many mathematicians for their respective integer solutions for particular values of a, b and N . In this context, one may refer [1-11].

This communication concerns with the problem of obtaining non-zero distinct integer solutions to the binary quadratic equation given by $3x^2 - 8y^2 = -20$ representing hyperbola. A few interesting relations among its solutions are presented. Knowing an integral solution of the given hyperbola, integer solutions for other choices of hyperbolas and parabolas are presented. The formulation of second order Ramanujan Numbers with base numbers as real integers and Gaussian integers is illustrated and also the sequence of Diophantine 3-tuples are presented.

Method of analysis:

The hyperbola represented by the non-homogeneous quadratic equation under consideration is

$$3x^2 - 8y^2 = -20 \quad (1)$$

Introduction of the linear transformations

$$x = X + 8T, y = X + 3T \quad (2)$$

in (1) leads to

$$X^2 = 24T^2 + 4 \quad (3)$$

The smallest positive integer solution for (3) is $T_0=2, X_0=10$

To find the other solutions to (3), consider the corresponding pell equation given by

$$X^2 = 24T^2 + 4 \quad (4)$$

whose general solution $(\overline{T}_n, \overline{X}_n)$ is

$$\overline{X}_n = \frac{1}{2} f_n$$

$$\overline{T}_n = \frac{1}{2\sqrt{24}} g_n$$

Where

$$f_n = (5 + 1\sqrt{24})^{n+1} + (5 - 1\sqrt{24})^{n+1}$$

$$g_n = (5 + 1\sqrt{24})^{n+1} - (5 - 1\sqrt{24})^{n+1}$$

- Employing the lemma of Brahmagupta between the solutions (T_0, X_0) & $(\overline{T}_n, \overline{X}_n)$, the general solution (T_{n+1}, X_{n+1}) to (3) is given by

$$\begin{aligned} T_{n+1} &= T_0 \overline{X}_n + X_0 \overline{T}_n \\ &= f_n + 5 * \frac{1}{\sqrt{24}} g_n \\ X_{n+1} &= X_0 \overline{X}_n + DT_0 \overline{T}_n \\ &= 5f_n + 24 * \frac{1}{\sqrt{24}} g_n \end{aligned}$$

where $n=-1, 0, 1, \dots$

In view of (2), the general solution (x_{n+1}, y_{n+1}) to (1) is given by

$$\begin{aligned} x_{n+1} &= X_{n+1} + 8T_{n+1} \\ &= 13f_n + \frac{64}{\sqrt{24}} g_n \\ y_{n+1} &= X_{n+1} + 3T_{n+1} \\ &= 8f_n + \frac{39}{\sqrt{24}} g_n \end{aligned}$$

A few numerical solutions to (1) are presented in table below:

Table: Numerical solutions

n	x_{n+1}	y_{n+1}
-1	26	16
0	258	158
1	2554	1564
2	25282	15482
3	250266	153256
4	2477378	1517078
5	24523514	15017524

Observations:

- The values of x_n and y_n are even
- $x_{n-1} \equiv 0 \pmod{2}$, $y_{2n-2} \equiv 0 \pmod{4}$ where $n=1, 2, 3, \dots$
- A few interesting relations among the solutions are given below:
 - $x_{n+3} = 10x_{n+2} - x_{n+1}$
 - $y_{n+3} = 10y_{n+2} - y_{n+1}$
 - $8y_{n+1} = x_{n+2} - 5x_{n+1}$
 - $8y_{n+2} = 5x_{n+2} - x_{n+1}$
 - $8y_{n+3} = 49x_{n+2} - 5x_{n+1}$
 - $8y_{n+1} = 5x_{n+3} - 49x_{n+2}$
 - $8y_{n+3} = 5x_{n+3} - x_{n+2}$
 - $8y_{n+2} = x_{n+3} - 5x_{n+2}$
 - $3x_{n+1} = y_{n+2} - 5y_{n+1}$
 - $3x_{n+2} = y_{n+3} - 5y_{n+2}$
 - $3x_{n+3} = 5y_{n+3} - y_{n+2}$
 - $3x_{n+1} = 5y_{n+3} - 49y_{n+2}$
 - $3x_{n+2} = 5y_{n+2} - y_{n+1}$
 - $3x_{n+3} = 49y_{n+3} - 5y_{n+1}$
 - $6x_{n+2} = y_{n+3} - y_{n+1}$
 - $80y_{n+1} = x_{n+3} - 49x_{n+1}$
 - $80y_{n+3} = 49x_{n+3} - x_{n+1}$
 - $30x_{n+3} = 49y_{n+3} - y_{n+1}$
 - $30x_{n+1} = y_{n+3} - 49y_{n+1}$
 - $16y_{n+2} = x_{n+3} - x_{n+1}$
- Expressions representing square integers:

- $\frac{1}{40}[64x_{2n+3} - 632x_{2n+2} + 80]$
- $\frac{1}{5}[79x_{2n+4} - 782x_{2n+4} + 10]$
- $\frac{1}{245}[6256y_{2n+2} - 39x_{2n+4} + 490]$
- $\frac{1}{15}[387y_{2n+2} - 39y_{2n+3} + 30]$
- $\frac{1}{15}[3831y_{2n+3} - 387y_{2n+4} + 30]$
- $\frac{1}{25}[632y_{2n+2} - 39x_{2n+3} + 50]$
- $\frac{1}{5}[632y_{2n+3} - 387x_{2n+3} + 10]$
- $\frac{1}{25}[632y_{2n+4} - 3831x_{2n+3} + 50]$
- $\frac{1}{25}[6256y_{2n+3} - 387x_{2n+4} + 50]$
- $\frac{1}{5}[6256y_{2n+4} - 3831x_{2n+4} + 10]$
- $\frac{1}{150}[3831y_{2n+2} - 39y_{2n+4} + 300]$
- $\frac{1}{400}[64x_{2n+4} - 6256x_{2n+2} + 800]$
- $\frac{1}{5}[64y_{2n+2} - 39x_{2n+2} + 10]$
- $\frac{1}{25}[64y_{2n+3} - 387x_{2n+2} + 50]$
- $\frac{1}{245}[64y_{2n+4} - 3831x_{2n+2} + 490]$

➤ Expressions representing cubical integers:

- $\frac{1}{40}[64x_{3n+4} - 632x_{3n+3} + 192x_{n+2} - 1896x_{n+1}]$
- $\frac{1}{5}[79x_{3n+5} - 782x_{3n+4} + 237x_{n+3} - 2346x_{n+2}]$
- $\frac{1}{245}[6256y_{3n+3} - 39x_{3n+5} + 18768y_{n+1} - 117x_{n+3}]$
- $\frac{1}{15}[387y_{3n+3} - 39y_{3n+4} + 1161y_{n+1} - 117y_{n+2}]$
- $\frac{1}{15}[3831y_{3n+4} - 387y_{3n+5} + 11493y_{n+2} - 1161y_{n+3}]$
- $\frac{1}{25}[632y_{3n+3} - 39x_{3n+4} + 1896y_{n+1} - 117x_{n+2}]$
- $\frac{1}{5}[632y_{3n+4} - 387x_{3n+4} + 1896y_{n+2} - 1161x_{n+2}]$
- $\frac{1}{25}[632y_{2n+5} - 3831x_{3n+4} + 1896y_{n+3} - 11493x_{n+2}]$
- $\frac{1}{25}[6256y_{3n+4} - 387x_{3n+5} + 18768y_{n+2} - 1161x_{n+3}]$
- $\frac{1}{5}[6256y_{3n+5} - 3831x_{3n+5} + 18768y_{n+3} - 11493x_{n+3}]$
- $\frac{1}{150}[3831y_{3n+3} - 39y_{3n+5} + 11493y_{n+1} - 117y_{n+3}]$
- $\frac{1}{400}[64x_{3n+5} - 6256x_{3n+3} + 192x_{n+3} - 1876x_{n+1}]$
- $\frac{1}{5}[64y_{3n+3} - 39x_{3n+3} + 192y_{n+1} - 117x_{n+1}]$
- $\frac{1}{25}[64y_{3n+4} - 387x_{3n+3} + 192y_{n+2} - 1161x_{n+1}]$
- $\frac{1}{245}[64y_{3n+5} - 3831x_{3n+3} + 192y_{n+3} - 11493x_{n+1}]$

➤ Expressions representing biquadratic integers:

- $\frac{1}{40}[64x_{4n+5} - 632x_{4n+4}] + 4f_n^2 - 2$
- $\frac{1}{40}[64x_{4n+5} - 632x_{4n+4} + 256x_{2n+3} - 2528x_{2n+2} + 240]$

- $\frac{1}{5}[79x_{4n+6} - 782x_{4n+5}] + 4f_n^2 - 2$
- $\frac{1}{5}[79x_{4n+6} - 782x_{4n+5} + 316x_{2n+4} - 3128x_{2n+3} + 30]$
- $\frac{1}{245}[6256y_{4n+4} - 39x_{4n+6}] + 4f_n^2$
- $\frac{1}{245}[6256y_{4n+4} - 39x_{4n+6} + 25024y_{2n+2} - 156x_{2n+4} + 1470]$
- $\frac{1}{15}[387y_{4n+4} - 39y_{4n+5}] + 4f_n^2 - 2$
- $\frac{1}{15}[387y_{4n+4} - 39y_{4n+5} + 1548y_{2n+2} - 156y_{2n+3} + 90]$
- $\frac{1}{25}[632y_{4n+4} - 39x_{4n+5}] + 4f_n^2 - 2$
- $\frac{1}{25}[632y_{4n+4} - 39x_{4n+5} + 2528y_{2n+2} - 156x_{2n+3} + 150]$
- $\frac{1}{5}[632y_{4n+5} - 387x_{4n+5}] + 4f_n^2 - 2$
- $\frac{1}{5}[632y_{4n+5} - 387x_{4n+5} + 4528y_{2n+3} - 1548x_{2n+3} + 30]$
- $\frac{1}{25}[632y_{4n+6} - 3831x_{4n+3}] + 4f_n^2 - 2$
- $\frac{1}{25}[632y_{4n+6} - 3831x_{4n+3} + 2528y_{2n+4} - 15324x_{2n+3} + 150]$
- $\frac{1}{25}[6256y_{4n+5} - 387x_{4n+6}] + 4f_n^2 - 2$
- $\frac{1}{25}[6256y_{4n+5} - 387x_{4n+6} + 25024y_{2n+3} - 1548x_{2n+4} + 150]$
- $\frac{1}{5}[6256y_{4n+6} - 3831x_{4n+6}] + 4f_n^2 - 2$
- $\frac{1}{5}[6256y_{4n+6} - 3831x_{4n+6} + 25024y_{2n+4} - 15324x_{2n+4} + 30]$
- $\frac{1}{150}[3831y_{4n+4} - 39y_{4n+6}] + 4f_n^2 - 2$
- $\frac{1}{150}[3831y_{4n+4} - 39y_{4n+6} + 15324y_{2n+2} - 156y_{2n+4} + 900]$
- $\frac{1}{400}[64x_{4n+6} - 6256x_{4n+4}] + 4f_n^2 - 2$
- $\frac{1}{400}[64x_{4n+6} - 6256x_{4n+4} + 256x_{2n+4} - 25024x_{2n+2} + 2400]$
- $\frac{1}{5}[64y_{4n+4} - 39x_{4n+4}] + 4f_n^2 - 2$
- $\frac{1}{5}[64y_{4n+4} - 39x_{4n+4} + 256y_{2n+2} - 156x_{2n+2} + 30]$
- $\frac{1}{25}[64y_{4n+5} - 387x_{4n+4}] + 4f_n^2 - 2$
- $\frac{1}{25}[64y_{4n+5} - 387x_{4n+4} + 256y_{2n+3} - 1548x_{2n+2} + 150]$
- $\frac{1}{245}[64y_{4n+6} - 3831x_{4n+4}] + 4f_n^2 - 2$
- $\frac{1}{245}[64y_{4n+6} - 3831x_{4n+4} + 256y_{2n+4} - 15324x_{2n+2} + 1470]$

➤ Employing linear combinations among the solutions, one obtains solutions to other choices of hyperbolas

Choice1: Let $X = 64x_{n+2} - 632x_{n+1}, Y = 129x_{n+1} - 13x_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$8[800 + 3Y^2] = X^2$$

Choice2: Let $X = 79x_{n+3} - 782x_{n+2}, Y = 1277x_{n+2} - 129x_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$80[X^2 - 100] = 3Y^2$$

Choice3: Let $X = 6256y_{n+1} - 39x_{n+3}, Y = 8x_{n+3} - 1277y_{n+1}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[6Y^2 + 60025]$$

Choice4: Let $X = 387y_{n+1} - 39y_{n+2}, Y = 8y_{n+2} - 79y_{n+1}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[225 + 6Y^2]$$

Choice5: Let $X = 3831y_{n+2} - 387y_{n+3}, Y = 79y_{n+3} - 782y_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[225 + 6Y^2]$$

Choice6: Let $X = 632y_{n+1} - 39x_{n+2}, Y = 8x_{n+2} - 129y_{n+1}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[625 + 6Y^2]$$

Choice7: Let $X = 632y_{n+2} - 387x_{n+2}, Y = 79x_{n+2} - 129y_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[25 + 6Y^2]$$

Choice8: Let $X = 632y_{n+3} - 3831x_{n+2}, Y = 782x_{n+2} - 129y_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[625 + 6Y^2]$$

Choice9: Let $X = 6256y_{n+3} - 3831x_{n+3}, Y = 782x_{n+3} - 1277y_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 24[3750 + Y^2]$$

Choice10: Let $X = 64x_{n+3} - 6256x_{n+1}, Y = 1277x_{n+1} - 13x_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[160000 + 6Y^2]$$

Choice11: Let $X = 64y_{n+1} - 39x_{n+1}, Y = 8x_{n+1} - 13y_{n+1}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[25 + 6Y^2]$$

Choice12: Let $X = 6256y_{n+2} - 387x_{n+3}, Y = 79x_{n+3} - 1277y_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[625 + 6Y^2]$$

Choice13: Let $X = 6256y_{n+3} - 3831x_{n+3}, Y = 782x_{n+3} - 1277y_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[25 + 6Y^2]$$

Choice14: Let $X = 64y_{n+3} - 3831x_{n+1}, Y = 782x_{n+1} - 13y_{n+3}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[60025 + 6Y^2]$$

Choice15: Let $X = 64y_{n+2} - 387x_{n+1}, Y = 79x_{n+1} - 13y_{n+2}$

Note that (X, Y) satisfies the hyperbola

$$X^2 = 4[625 + 6Y^2]$$

➤ Employing linear combinations among the solutions, one obtains solutions to other choices of parabolas

Choice1: Let $X = 64x_{2n+3} - 632x_{2n+2} + 80, Y = 129x_{n+1} - 13x_{n+2}$

Note that (X, Y) satisfies the parabola

$$5[X - 160] = 3Y^2$$

Choice2: Let $X = 79x_{2n+4} - 782x_{2n+3} + 10, Y = 127x_{n+2} - 129x_{n+3}$

Note that (X, Y) satisfies the parabola

$$3Y^2 = 40[X^2 - 20]$$

Choice3: Let $X = 625y_{2n+2} - 39x_{2n+4} + 490, Y = 8x_{n+3} - 1277y_{n+1}$

Note that (X, Y) satisfies the parabola

$$245X = 4[6Y^2 + 60025]$$

Choice4: Let $X = 387y_{2n+2} - 39y_{2n+3} + 30, Y = 8y_{n+2} - 79y_{n+1}$

Note that (X, Y) satisfies the parabola

$$15X = 4[225 + 6Y^2]$$

Choice5: Let $X = 3831y_{2n+3} - 387y_{2n+4} + 30, Y = 79y_{n+3} - 782y_{n+2}$

Note that (X, Y) satisfies the parabola

$$5[X - 60] = 8Y^2$$

Choice6: Let $X = 632y_{2n+2} - 39x_{2n+3} + 50, Y = 8x_{n+2} - 129y_{n+1}$

Note that (X, Y) satisfies the parabola

$$25[X - 100] = 24Y^2$$

Choice7: Let $X = 632y_{2n+3} - 387x_{2n+3} + 10, Y = 79x_{n+2} - 129y_{n+2}$

Note that (X, Y) satisfies the parabola

$$5[X - 20] = 24Y^2$$

Choice8: Let $X = 632y_{2n+4} - 3831x_{2n+3} + 50, Y = 782x_{n+2} - 129y_{n+3}$

Note that (X, Y) satisfies the parabola

$$25[X - 100] = 24Y^2$$

Choice9: Let $X = 6256y_{2n+3} - 387x_{2n+4}, Y = 79x_{n+3} - 1277y_{n+2}$

Note that (X, Y) satisfies the parabola

$$25[X - 100] = 24Y^2$$

Choice10: Let $X = 6256y_{2n+4} - 3831x_{2n+4}, Y = 782x_{n+3} - 1277y_{n+3}$

Note that (X, Y) satisfies the parabola

$$25[X - 4] = 24Y^2$$

Choice11: Let $X = 3831y_{2n+2} - 39y_{2n+4} + 300, Y = 8y_{n+3} - 782y_{n+1}$

Note that (X, Y) satisfies the parabola

$$75[X - 600] = 12Y^2$$

Choice12: Let $X = 64x_{2n+4} - 6256x_{2n+2} + 800, Y = 1277x_{n+1} - 13x_{n+3}$

Note that (X, Y) satisfies the parabola

$$50[X - 1600] = 3Y^2$$

Choice13: Let $X = 64y_{2n+2} - 39x_{2n+2} + 10, Y = 8x_{n+1} - 13y_{n+1}$

Note that (X, Y) satisfies the parabola

$$5[X - 20] = 24Y^2$$

Choice14: Let $X = 64y_{2n+3} - 387x_{2n+2} + 50, Y = 79x_{n+1} - 13y_{n+2}$

Note that (X, Y) satisfies the parabola

$$25[X - 100] = 24Y^2$$

Choice15: Let $X = 64y_{2n+4} - 3831x_{2n+2} + 490, Y = 782x_{n+1} - 13y_{n+3}$

Note that (X, Y) satisfies the parabola

$$245[X - 980] = 24Y^2$$

- Considering suitable values of x_n & y_n , one generates 2nd order Ramanujan numbers with base integers as real integers

For illustration, consider

$$\square_2 = 1564 = 1 \times 1564 = 2 \times 782 = 4 \times 391 = 17 \times 92 = 34 \times 46 \quad (*)$$

$$\text{Now, } 1 \times 1564 = 2 \times 782$$

$$\rightarrow (1564 + 1)^2 + (782 - 2)^2 = (1564 - 1)^2 + (782 + 2)^2$$

$$\rightarrow 1565^2 + 780^2 = 1563^2 + 784^2 = 30576255$$

$$1 \times 1564 = 4 \times 391$$

$$\rightarrow (1564 + 1)^2 + (391 - 4)^2 = (1564 - 1)^2 + (391 + 4)^2 = 2598994$$

$$1 \times 1564 = 17 \times 92$$

$$\rightarrow (1564 + 1)^2 + (92 - 17)^2 = (1564 - 1)^2 + (92 + 17)^2 = 2454850$$

$$1 \times 1564 = 23 \times 68$$

$$\rightarrow (1564 + 1)^2 + (68 - 23)^2 = (1564 - 1)^2 + (68 + 23)^2 = 2451250$$

$$1 \times 1564 = 34 \times 46$$

$$\rightarrow (1564 + 1)^2 + (46 - 34)^2 = (1564 - 1)^2 + (46 + 34)^2 = 2449369$$

$$2 \times 782 = 4 \times 391$$

$$\rightarrow (782 + 2)^2 + (391 - 4)^2 = (782 - 2)^2 + (391 + 4)^2 = 764425$$

$$2 \times 782 = 17 \times 92$$

$$\rightarrow (782 + 2)^2 + (92 - 17)^2 = (782 - 2)^2 + (92 + 17)^2 = 620281$$

$$2 \times 782 = 23 \times 68$$

$$\rightarrow (782 + 2)^2 + (68 - 23)^2 = (782 - 2)^2 + (68 + 23)^2 = 616681$$

$$2 \times 782 = 34 \times 46$$

$$\rightarrow (782 + 2)^2 + (46 - 34)^2 = (782 - 2)^2 + (46 + 34)^2 = 614800$$

$$4 \times 391 = 17 \times 92$$

$$\rightarrow (391 + 4)^2 + (92 - 17)^2 = (391 - 4)^2 + (92 + 17)^2 = 161650$$

$$4 \times 391 = 23 \times 68$$

$$\rightarrow (391 + 4)^2 + (68 - 23)^2 = (391 - 4)^2 + (68 + 23)^2 = 158050$$

$$4 \times 391 = 34 \times 46$$

$$\rightarrow (391 + 4)^2 + (46 - 34)^2 = (391 - 4)^2 + (46 + 34)^2 = 156169$$

$$17 \times 92 = 23 \times 68$$

$$\rightarrow (92 + 17)^2 + (68 - 23)^2 = (92 - 17)^2 + (68 + 23)^2 = 13906$$

$$17 \times 92 = 34 \times 46$$

$$\rightarrow (92 + 17)^2 + (46 - 34)^2 = (92 - 17)^2 + (46 + 34)^2 = 12025$$

$$23 \times 68 = 34 \times 46$$

$$\rightarrow (68 + 23)^2 + (46 - 34)^2 = (68 - 23)^2 + (46 + 34)^2 = 6425$$

Note:

$$2 \times 782 = 34 \times 46$$

$$\rightarrow 392^2 - 390^2 = 40^2 - 6^2$$

$$\rightarrow 392^2 + 6^2 = 40^2 + 390^2 = 153700$$

Thus 3057635, 2598994, 2454850, 2451250, 2449369, 764425, 620281, 616681, 614800, 161650, 158050, 156169, 13906, 12025, 6425 represent 2nd order Ramanujan numbers

➤ Considering suitable values of x_n & y_n , one generates 2nd order Ramanujan numbers with base integers as Gaussian integers

For illustration, consider again y_2 represented by (*),

$$\text{Now, } 1 \times 1564 = 2 \times 782 \rightarrow (1 + i1564)^2 + (782 - i2)^2 = -1834575$$

$$\text{Also, } 1 \times 1564 = 2 \times 782 \rightarrow (1564 + i1)^2 + (2 - i782)^2 = 1834575$$

Note that -1834575 & 1834575 represent 2nd order Ramanujan numbers with base integers as Gaussian integers.

In a similar manner, other 2nd order Ramanujan numbers are obtained

Therefore the triple $(26, 2k+76, 4k+188)$ represents diophantine 3-tuple with property $D(k^2 + 34k - 127)$. The repetition of the above process leads to sequences of diophantine 3-tuples whose general form $(1, c_{s-1}, c_s)$ is given by

$$(26, 26s^2 + (2k - 18)s - 2k + 8, 26s^2 + 2ks + 34s + 16), s=1, 2, 3, \dots$$

A few numerical illustrations are given in Table below:

Table: Numerical illustrations

k	$(26, c_0, c_1)$	$(26, c_1, c_2)$	$(26, c_2, c_3)$	$D(k^2 + 34k - 127)$
0	(26, 16, 76)	(26, 76, 188)	(26, 188, 352)	D(-127)
1	(26, 16, 78)	(26, 78, 192)	(26, 192, 358)	D(-92)
2	(26, 16, 80)	(26, 80, 196)	(26, 196, 364)	D(-191)

It is noted that the triple $(c_{s-1}, c_s + 26, c_{s+1}), s=1, 2, 3, \dots$ forms an arithmetic progression.

In a similar way one may generate sequences of diophantine 3-tuples with suitable property through the other solutions to (1).

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Formation of sequence of Diophantine 3-tuples:

Consider the solution to (1) given by

$$x_0 = 26, y_0 = 16$$

It is observed that

$$x_0 y_0 + k^2 + 34k - 127 = (k + 17)^2, \text{ a perfect square}$$

The pair (x_0, y_0) represents diophantine 2-tuple with property $D(k^2 + 34k - 127)$.

If c is the 3rd tuple, then it satisfies the system of double equations.

$$26c + k^2 + 34k - 127 = p^2 \tag{1^*}$$

$$16c + k^2 + 34k - 127 = q^2 \tag{2^*}$$

Eliminating c between (1*) and (2*), we have

$$-10(k^2 + 34k - 127) = 16p^2 - 26q^2 \tag{3^*}$$

Taking,

$$p = X + 26T, q = X + 16T \tag{4^*}$$

In(3*) and simplifying, we get

$$X^2 = k^2 + 34k - 127 + 416T^2$$

which is satisfied by,

$$T = 1, X = k+17$$

In view of (4*) and (1*), it is seen that

$$c = 2k + 76$$

Note that $(26, 16, 2k+76)$ represents diophantine 3-tuple with property $D(k^2 + 34k - 127)$

The process of obtaining sequences of diophantine 3-tuples with property $D(k^2 + 34k - 127)$ is illustrated below:

Let M be a 3×3 square matrix given by

$$M = \begin{pmatrix} 1 & 0 & 2 \\ 0 & 0 & -1 \\ 0 & 1 & 2 \end{pmatrix}$$

Now, $(26, 16, 2k+76)M = (26, 2k+76, 4k+188)$

Note that

$$26 \cdot (2k+76) + k^2 + 34k - 127 = \text{perfect square}$$

$$26 \cdot (4k+188) + k^2 + 34k - 127 = \text{perfect square}$$

$$(2k+76) \cdot (4k+188) + k^2 + 34k - 127 = \text{perfect square}$$

A STUDY ON INFLUENCE ON AEROBIC DANCING TRAINING ON MOTOR FITNESS PARAMETERS AND PLAYING ABILITY AMONG SOCCER PLAYERS

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ABSTRACT

In this competitive world, many people find it hard to dedicate time for physical activities like exercises, although one of their first priorities is to stay in perfect shape. Here comes the easy method of maintaining a perfect figure - dance aerobics. As the name suggests, dance aerobics is an exercise that combines the rhythmic steps of aerobics with graceful dance movements. It can be broadly divided into four types - high-impact exercises, low-impact exercises, step aerobics and water aerobics. High impact exercises involve intense jumping actions that are synchronized with the rhythmic beats of the music being played. The present study was ascertainment of motor fitness parameters where flexibility was assessed by sit and reach test and the unit of measurement was in cm, agility was assessed by 4X10 shuttle run test the unit of measurement was in minutes and dribbling ability was assessed by Warner's soccer test and the unit of measurement was in seconds. The parameters were measured at baseline and after 12 weeks of aerobic dance were examined.

KEYWORDS: Aerobic Dancing, Motor fitness, Playing ability and so on.

INTRODUCTION

Physical Education and Sports have been part and part of human life. People around the world recognize its importance for better and healthy life. Dance is an independent art form. It can exist without music accompaniment, costume or scenery. Traditionally, however a major objective in dance education has been to teach children to relate movement effectively, to accompanying sounds and to music. Too often, this objective has been implemented for encouraging children to listen to a piece of music, a song or a poem and then to develop movement sequences that fit the accompaniment.

Training has been a part of human language since ancient times. It devotes the process of preparation for some tasks. The preparation of a sportsman represents a multisided process of purposeful utility of the total complex of factor which helps in the development of the sportsman and ensure a necessary level of his / her sports performance ability.

Sports training aims at achieving high performance in sports competitions. It is a process which is spread over a long period of time and competition cum performance-oriented endeavor as well. Dance is an art, and as the expressive entity of creative movement is vital to the development of the total individual. All of the arts provide ways in which man can bring shape and order to his fragmented and repeatedly changing world. But dance provides a primary medium for expression involving the total self, not just a part, like the voice or totally separated from the physical self-live painting or sculpture. Dance and the movement that produce it, is "same" and as such, is the most intimate of expressive media.

As far as India is concerned it was profound tradition of the Indian physical culture and has been closely associated with religious practices. The activities like Yogasanas. Suriyanamaskars, Pranayama and games like Kabaddi, Kho-Kho and Atyapatya have been in vogue. Physical activities were performed

on the banks of the rivers and tanks, in the forest and caves, in the sacrificial grounds. The Great Indian Poet Kalidasa in his epic emphasized that "Physique is the base indeed for accomplishment of duty". Yoga have been part and partial of Indians. So, the physical education programme was concentrated to develop the physical health among the people.

Sports training is a scientifically based and pedagogical process of sports perfection which through systematic effect on psycho-physical performance ability and performance readiness aims at leading the sportsmen to high and highest performance (Harre-1981). The systematic and regular use of physical exercises however does not guarantee maximum improvement in performance. But, the effect of these exercises is increased by multitude of factors such as sports implements, verbal instructions, means of recovery, means of assessment of capacity, nutrition and psychological means and so on. Training is much like constructing a multi-store building. One needs materials for the building such as aerobic, anaerobic running, comprehensive conditioning, flexibility, etc. Several kinds of materials like training intensities and modalities should be utilized in an ongoing process to complete the goal of finished buildings or competitively fit athlete. Depending on the progress in the construction plan, the relative mix of all these materials will vary. As a training season develops, compressive conditioning work for strength of endurance will gradually form a transition into an emphasis on power with a substitution of intensity of volume in determining the total load.

IMPORTANCE OF AEROBIC DANCE

Aerobic dancing is a series of callisthenic exercise movements accompanied by music. The use of music is a technique of motivation that has been increased in recent years. Aerobic dance is essential to a healthy cardio-vascular system. Briefly, aerobic dance is an activity that can be sustained for an extended period of time without building an oxygen debt in the muscles. It is a type of dance that overloads the heart and lungs and causes them to work harder than they do when a person is at rest. Aerobic literally means "With air". Aerobic dance is the type of activity in which the amount of oxygen taken in is equal to the amount of oxygen required.

In this competitive world, many people find it hard to dedicate time for physical activities like exercises, although one of their first priorities is to stay in perfect shape. Here comes the easy method of maintaining a perfect figure - dance aerobics. As the name suggests, dance aerobics is an exercise that combines the rhythmic steps of aerobics with graceful dance movements. It can be broadly divided into four types - high-impact exercises, low-impact exercises, step aerobics and water aerobics. High impact exercises involve intense jumping actions that are synchronized with the rhythmic beats of the music being played.

REVIEW OF LITERATURE

Liiv H, et., al., (2014) conducted a study on international level dance sport dancer's aerobic capacity during incremental test and competition simulation in relation to the gender, dance style and international ranking. A total of 30 couples (12 Standard, 7 Latin American and 11 Ten Dance; aged 22.8 ± 6.6 years male and 22.0 ± 6.4 years female) performed an incremental treadmill test and competition simulation. In this study for the first time, we carried out longer than one round competition simulation and compared three different dance sports styles (Standard, Latin American and Ten Dance). The results showed that dancers of these three dance styles had similar aerobic capacity values. The average maximal oxygen consumption (VO_{2max}) values were 59.6 ± 5.1 and 51.2 ± 6.2 ml \cdot min⁻¹ \cdot kg⁻¹ for male and female dancers, respectively. Competition simulation showed that Latin American Dance discipline is physiologically more intensive compared to Standard and Ten Dance styles especially for the female dancers. It appeared that male and female Standard dancers tended to perform at lower intensity than anaerobic threshold (AT) during competition simulation (male $97.3 \pm 2.9\%$; female $97.9 \pm 3.6\%$), while Latin (male $101.4 \pm 2.9\%$; female $106.7 \pm$

5.9%) and Ten Dance (male $100.7 \pm 6.4\%$; female $99.2 \pm 5.6\%$) competition intensity was higher compared to AT level of athletes. The highest heart rate during competition simulation was always found during the last dances (Paso Double, Jive or Quickstep) and in the last round of each dance style. No significant relationship between VO₂max values and international rankings was registered¹.

Maruf F A, et., al., (2013) investigated the effects of aerobic dance combined with anti-hypertensive drugs on BP and number of anti-hypertensives drugs in individuals with hypertension. This open label randomized-controlled trial involved new-diagnosed male and female individuals with mild-to-moderate essential hypertension. After at least four weeks of treatment, they were randomly assigned to drug therapy (Normoretic: Hydrochlorothiazide + amiloride hydrochloride, and Amlodipine) (control: n=33) and aerobic dance combined with drug therapy (exercise: n=30) groups. Intervention in each group lasted 12 weeks. BP was measured at baseline and during and postintervention. Number of antihypertensive drugs was recorded postintervention. The BP control rates were similar between the exercises (56.7%) and control (35.5%) groups ($p=0.075$). Similarly, between groups difference in the number of drugs was not significant ($p=0.511$). This preliminary report demonstrates the tendency of aerobic dance to enhance BP control in individuals on two antihypertensive drugs without BP control².

Smol E and Fredyk A, (2012) investigated whether 6-week low intensity aerobic training programme used as a supplement to regular dance practice might improve both the aerobic capacity and psychomotor performance in female ballet dancers. To assess their maximal oxygen uptake (VO₂max) and anaerobic threshold (AT), the dancers performed a standard graded bicycle ergometer exercise test until volitional exhaustion prior to and after the supplementary training. At both these occasions, the psychomotor performance (assessed as multiple-choice reaction times) and number of correct responses to audio-visual stimuli was assessed at rest and immediately after cessation of maximal intensity exercise. The results of this study indicate that addition of low-intensity aerobic training to regular dance practice increases aerobic capacity of ballerinas with no loss of speed and accuracy of their psychomotor reaction³.

PROBLEM FOR THE STUDY

Soccer players are strong in all aspects of the physique. They need to be strong in the lower and upper body to compete at the highest level. Both tall and small players can compete. The key aspects for a soccer player are strength, stamina, speed, skills and, agility. Especially in modern day soccer, the physical performance of players is an important factor, which often decides the most crucial parts of a game. So, coaches and scientists are justifiably interested in knowing what physical challenges their players are facing during a game. the most scientists and scholars suggest poor flexibility of muscles leads to possibility of injured. So, players must undergo some exercises. The present study is an attempt

¹ Liiv, H., Jurimae, T., Maestu, J., Purge, P., Hannus, A., and Jurimae J., "Physiological Characteristics of Elite Dancers of Different Dance Styles", *European Journal of Sport Science*, 14(1): (2014): pp 429-36.

² Maruf, F.A., Akinpelu, A.O., and Salako, B.L. (2013). "Effects of Aerobic Exercise and Drug Therapy on Blood Pressure and Antihypertensive Drugs: a Randomized Controlled Trial", *African Health Science*, 13(1), (Mar- 2013): 11-9.

³ Smol, E., and Fredyk, A., "Supplementary Low-Intensity Aerobic Training Improves Aerobic Capacity and does not Affect Psychomotor Performance in Professional Female Ballet Dancers", *Journal of Human Kinetics*. 31, (Mar-2012): 79-87.

to know the aerobic dancing training methods helps to increase flexibility and stamina of soccer players.

OBJECTIVES OF THE STUDY

The following objectives were framed for the present study

1. To experiment aerobic dancing and motor fitness variables increase the performance of Soccer players
2. To find out the difference factors affecting aerobic dance and motor fitness of soccer players
3. To offer suitable suggestions to selected methods to adapt for improve effectiveness and efficiency training of soccer players

SIGNIFICANCE OF THE STUDY

The study was significant in the following ways.

1. The study might help the policy makers in educational department to implement the aerobic dancing training exercises and motivational policy which benefits the sports persons.
2. The study might help coaches and physical education teachers to understand physical, and physiological variables among UG level students
3. The study might help the coaches and physical education teachers to adopt different aerobic dancing techniques and motor fitness methods to enhance physical, and physiological variables among UG level students
4. This finding of this might act as guide to the coaches, experts to select the players who will be more suited or competent towards the particular game.

METHODS

Experimental Methods

In order to address the hypothesis presented herein, we selected 30 inter-collegiate male Soccer players from affiliated colleges of Bharathidasan University, Tiruchirappalli. The players were randomly assigned in to two equal groups namely, aerobic dance training group (ADTG) (n=15) and Control group (CG) (n=15). A pilot study was conducted to assess the initial capacity of the subjects in order to fix the load. The respective training was given to the experimental group the 3 days per weeks (alternate days) for the training period of twelve weeks. The control group was not given any sort of training except their routine.

Design

The ascertainment of motor fitness parameters where flexibility was assessed by sit and reach test and the unit of measurement was in cm, agility was assessed by 4X10 shuttle run test the unit of measurement was in minutes and dribbling ability was assessed by Warner's soccer test and the unit of measurement was in seconds. The parameters were measured at baseline and after 12 weeks of aerobic dance were examined.

Training Programme

The training programme was conducted for 60 minutes for session in a day, 3 days in a week for a period of 12 weeks duration. These 60 minutes included 15 minutes warm up, aerobic dance training for 30 minutes and 15 minutes warm down. Every three weeks of training 5% of intensity of load was increased from 50% to 80% of work load. The volume of aerobic dance training is prescribed based on the number of sets and repetitions. The equivalent in aerobic dance training is the length of the time each action in total 3 day per weeks (Monday, Wednesday and Friday). The intensity of training was pointed, so that fatigue would not be a factor during post testing.

Aerobic dance training schedule for impact per session

Training weeks	On spot marching	Sets & Repetition	Intensity
I & II	Step touch	2 X 5	50%
	Power walk	2 X 5	
	V step	2 X 5	
	Walk and kick	2 X 5	
III & IV	Step touch	2 X 6	55%
	Power walk	2 X 6	
	V step	2 X 6	
	Walk and kick	2 X 6	
V & VI	Step touch	2 X 7	60%
	Power walk	2 X 7	
	V step	2 X 7	
	Walk and kick	2 X 7	
VII & VIII	Step touch	2 X 7	65%
	Power walk	2 X 7	
	V step	2 X 7	
	Walk and kick	2 X 7	
IX & X	Step touch	2 X 8	70%
	Power walk	2 X 8	
	V step	2 X 8	
	Walk and kick	2 X 8	
XI & XII	Step touch	2 X 8	80%
	Power walk	2 X 8	
	V step	2 X 8	
	Walk and kick	2 X 8	

STATISTICAL ANALYSIS

The collected data before and after training period of 12 weeks on the above said variables due to the effect of aerobic dance was statistically analyzed with 't' test to find out the significant improvement between pre and post-test. In all cases the criterion for statistical significance was set at 0.05 level of confidence. ($P < 0.05$) based on this table the significant can be determined and resulted whether the hypothesis is accepted or rejected.

Significance of Mean Gains & Losses between Pre and Posttest Scores on Selected Variables of Aerobic Exercises Group

S.No	Variables	Pre-test mean	Post-test mean	Mean difference	Std. Deviation	DM	Statistical inference
1.	Muscular endurance	32.45	43.80	11.35	8.03	0.38	$0.007 < 0.05$ significant
2.	Grip strength	46.30	52.50	6.20	4.38	0.09	$0.203 > 0.05$ Not Significant

3.	Leg strength	35.70	41.20	5.50	3.89	0.40	0.397>0.05 Not significant
4.	Flexibility	42.90	47.30	4.40	3.11	0.82	0.031<0.05 Significant
5.	Cardio respiratory endurance	12.67	11.10	1.57	1.11	0.74	0.012<0.05 Significant
6.	Body composition	24.35	22.14	2.21	1.56	0.45	0.044<0.05 Significant
7.	Resting heart rate	70.85	67.90	2.95	2.09	0.34	0.003<0.05 Significant
8.	Peak expiratory flow rate	460.60	489.80	29.20	20.65	0.43	0.001<0.05 Significant
9.	Breath holding time	32.83	38.78	5.95	4.21	0.24	0.094>0.05 Significant
10	Cognitive Anxiety	29.40	21.80	7.60	5.37	0.60	0.437>0.05 Significant
11	Somatic Anxiety	28.00	21.00	7.00	4.95	0.53	0.186>0.05 Not Significant
12	Self Confidence	20.45	26.90	6.45	4.56	0.48	0.037<0.05 significant

The above shows the obtained 't' ratios for pre and post-test mean difference in the selected variable of muscular endurance (11.35), grip strength (6.20), leg strength (5.50), Flexibility (4.40), cardio respiratory endurance (1.57), body composition (2.21), resting heart rate (2.95), peak expiratory flow rate (29.20), breath holding time (5.95), cognitive anxiety (7.60), somatic anxiety (7.00) and self-confidence (6.45).

DISCUSSION AND FINDINGS

The present study experimented the effect of aerobic dance training on motor fitness parameters and dribbling ability of football players. The result of the study indicated that the aerobic dance training improved the motor fitness parameters such as agility and flexibility and dribbling ability. The findings of the present study had similarity with the findings of the investigations referred in this study. However, there was a significantly changes of subjects in the present study the agility, flexibility and dribbling ability was significantly improved of subject in the group may be due to the in aerobic dance. This revealed that participation in aerobic dance brings changes in the physiological and motor fitness components. Aerobic dance increases the distance covered in standing long jump which shows the improvement in explosive power of leg, improves in timings in case of agility which shows the capability in changing direction rapidly.

SUGGESTIONS OF THE STUDY:

In the present study, it is proved that selected motor ability and aerobic dancing variables are improved by performance of soccer players. Hence it is recommended to the coaches, trainers and physical educators to adopt these findings to improve motor ability and aerobic variables.

2. Aerobic dancing may be strongly recommended for the improvement of physical fitness of children.
3. Aerobic dancing may be included in fitness programmes planned for children.
4. The present study was mainly focused on soccer players. In future, related study can be conducted on players of different games.

CONCLUSION

Training is the main component and the basic form of preparing the athlete for higher level of performance. It is systematically planned preparation with the help of the exercise, which realized the main factors of influencing athlete's progress. The content of training includes all the basic type of preparation of the sportsman, physical, technical, and psychological. Through systematic training the athlete fitness level and his acquisition of vital knowledge and skill are improved.

Sports training aims to prepare a sportsman for better performance through physical exercise. It is based on the scientific principles of aiming at education and performance enhancement. The improvement of physical fitness includes improvement of general health and organic functions as well as increasing the strength and stability of the muscle-skeletal system. Development of motor skill is also the objective of sports training. Sports activities consist of motor movement and action and their success depends largely on how correctly they are performed. Techniques of training and improvement of tactical efficiency play a vital role in the training process.

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EFFECT OF YOGASANAS ON ARM EXPLOSIVE POWER AMONG MALE ARTISTIC GYMNASTS

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ABSTRACT

Arm explosive power is one of the important motor ability. "The immediate capacity of an individual to perform in many varied stunts or athletic events is referred to as general motor ability." The purpose of this study was to find out the effect of yogasanas on arm explosive power. To achieve the purpose, 30 high school gymnasts were randomly selected from Santa Maria Higher Secondary School, Tiruchirappalli, Tamilnadu. The students were divided into two groups of 15 in each group. One group was utilized as the control group and the other as experimental group. Experimental group was given yoga training for twelve weeks. To find out Arm explosive power, softball throw for distance was administered. The data was analyzed statistically by computing mean, standard deviation and 't' test. The hypothesis was tested at 0.05 level of confidence. Significant improvement was found in arm explosive power of the experimental group due to yoga training.

Keywords: Yoga, Motor ability, Arm explosive power

INTRODUCTION

Physical education and sports help people in the development of all-round personality by improving the motor ability, and motor ability is the pre requisite for higher performance. Fitness can be achieved through participating in most enjoyable physical activity according to one's needs and ability. Yoga is one such most enjoyable activity everyone can participate. Yoga the art and science of maintaining physical and mental wellbeing that has its origin in India, is among the most ancient yet vibrant living traditions that is getting increasingly popular today. A potent stress buster, yoga is an instrument of self-evolvment and enlightenment, through physical and mental well-being. It enhances the quality of life by improving motor ability.

"The immediate capacity of an individual to perform in many varied stunts or athletic events is referred to as general motor ability."

Think for a moment, of the numerous and varied factors such as balance, flexibility, power and co-ordination, each contributing interdependently to the perfection of the total movement. Almost like the independent notes of a musical master piece, these specific factors combine to produce a symphony of movement.

Deepla (2008) studied on developing motor abilities of high school students through yoga. The subjects were given 12 weeks of Yoga training. After the training he found significant improvement in selected motor fitness. Srivastva (2009) investigated on 60 male subjects to find out the effect of yogic asanas on Motor ability and he found that the motor ability had developed through yoga training.

Yoga may be as effective as or better than exercise at improving a variety of health-related outcome measures (Ross and Thomas; 2010) Shoba (2011) studied the effect of yogasanas on motor, physiological and psychological variables and she found significant improvement in all the motor variables, physiological and psychological variables selected for the study after six weeks of yoga training.

Motor ability has been viewed as a combination of factors that are basic to all movements. Arm explosive power is one of the important motor ability. Hence the study was undertaken to find out whether yoga improves arm explosive power.

PURPOSE OF THE STUDY:

Purpose of the study was to find out the effect of yoga in improving arm explosive power.

METHODOLOGY:

To achieve the purpose 30 high school students were randomly selected from Santa Maria Higher Secondary School, Tiruchirappalli, Tamilnadu. The students were divided into two groups of fifteen in each group. One group was utilized as the control group and the other as experimental group. Experimental group was given yoga training for twelve weeks. To find out arm explosive power, softball throw for distance was administered. The data was analyzed statistically by computing mean, standard deviation and ‘t’ test. The hypothesis was tested at 0.05 level of confidence.

HYPOTHESIS:

It was hypothesized that there would be a significant difference in the Arm Explosive Power among experimental group by practicing yoga.

RESULTS AND FINDINGS:

Table-1

Table showing significance of difference between pre test and post test scores of subjects in Arm Explosive Power among experimental and control groups (N=30).

Group	Test	Mean	Standard Deviation	‘t’ value	Level of Significance
Experimental (Yoga)Group	Pre	26.466	6.207	2.107	Significant at 0.05 level
	Post	29.933	6.533		
Control (Non-Yoga) Group	Pre	23.133	4.240	0.509	Not Significant
	Post	23.700	4.387		

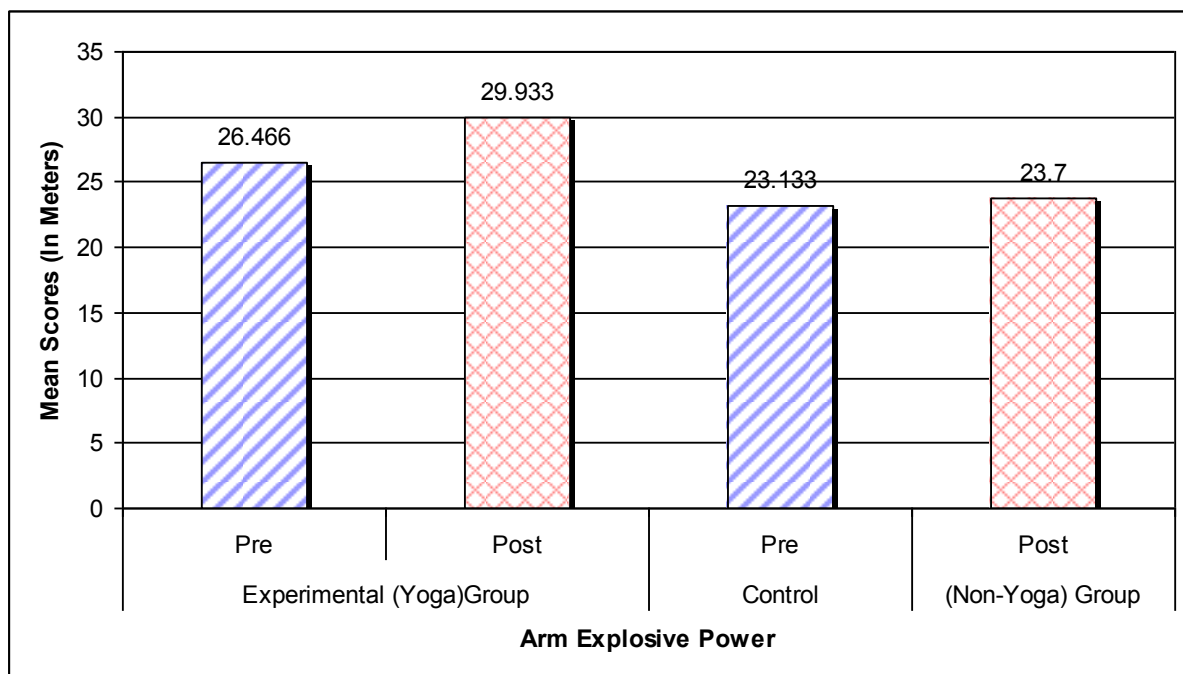


Fig.1.

The Bar graph showing comparison mean scores of Pre test and Post test scores of Arm Explosive Power (Softball Throw Test) among Control and Experimental groups.

It is observed from the above table and figure that, in experimental group the Arm Explosive Power mean scores of pre test is 26.466, which has increased to 29.933 in post test, whereas among control group the pre and post test mean scores of Arm Explosive Power are 23.700 and 23.133 respectively. It is also evident from the above table that the obtained 't' value 0.509 is less than Table value 2.05 even at 0.05 level of significance on Arm Explosive Power in control group and 2.107 is greater than Table value 2.05 at 0.05 level of significance on Arm Explosive Power in experimental group.

CONCLUSION:

On the basis of the result it was concluded that after 12 weeks of yoga training there was a significant improvement in arm explosive power among the experimental group.

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USE OF ADVANCED TECHNOLOGIES AND ALGORITHMS IN CRICKET: A SURVEY

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ABSTRACT

Cricket is the world's second most popular game that incorporates several latest technological advancements. It is an international game spanning from the 16th century to the present times. The game spread during the colonization period under British patronage. From then on till now, the development of cricket has made use of many technologies and algorithms in managing the game. Overtime, some got rejected while some are still continued for being effective. ICC is always very cautious with the provision of technologies in cricket, which is an integral part of cricket. Technology gets incorporated into the many changes in cricket like the evolution in batting, bowling, cricket gear, equipment, playing surfaces, etc, over the years. Cloud monitoring in cricket science, for instance, is a drastic evolvement in the entire range of development in cricket. Compared to the old form of cricket (from the 1800s), modern cricket (from the 2000s) proposes many advances in the bat size, boundaries size, players, etc. Overtime, all these get integrated with technology for developing the overall performance and growth of the game. As the demand for cricket players increases, simultaneously, commitment, performance level, and risk for injury also get increased automatically. A key element in incorporating the change factors in the game and in improving the performance rate by assisting players and keeping the audience active is the use of 'technology'. The emergence of technology in cricket may reflect both a negative and a positive image and effect in the game, depending on its management. To assist and to synchronize the game and the audience along with technology is all based on the advantages of technology. Some of the major interventions of technology in cricket are explored and discussed in this paper with special focus on case specific incidences which is different from most of the existing types of research being implemented in the field.

Keywords: Hot Spot Technique, No Ball detection, Card System, Location Support System

INTRODUCTION

Cricket is a universal game which millions of people are attracted to [1]. It is a bat and ball game in which every delivery is counted to be a win or a loss. In cricket counting, the overs deliveries are the crucial moments which determines the winner and losers. The work of an umpire in cricket is a challenging task of counting and estimating the overs, wickets, or boundaries. Even a little miscalculation or difference may lead to a disaster of the game. In some cases, the deliveries are disapproved by umpires and declared as no-ball by mistake. The consequence of a miscalculation of 'no- ball' in a cricket game leads to an extra delivery and run [2]. To avoid these kinds of mistakes, some technologies are adopted and implemented. In this case, if the umpire has any doubt if it is a no-ball or not, a third umpire review is requested and a television replay is formulated for better confirmation. This is one aspect in which advanced technology helps the game.

In any game, the 'decision making' based on human perception may not be accurate all the time. Moreover, it is not always possible to produce accurate judgment because of the limitations of man and man-made errors [3]. These types of errors create mass confusion and debates among the audience, cricket-fans and even the players. To overcome the no-ball issue, a computer vision-based approach is projected. In Figure 1 shows a snippet of a cricket game match with a batsman on strike posture, and another player on non-striking posture on a pitch in the ground [6]. Here, the bowler is seen delivering the ball from the lawn. The wicket-keeper and a slip-fielder are positioned there to record the scoring. This job is done along with more than 9 fielders. Also, an umpire is standing behind the non-striking wicket to calculate the ball from the player [7].



FIGURE 1. A GLIMPSE OF COMMON JUDGEMENT AND POSITIONS

LITERATURE REVIEW

In cricket, many forms of technologies have been featured in the last few decades. To name a few: Broadcasting Video, which is constructed to detect events; Semantic Analysis, which is optimized for capturing video (Rahish Tandon et al.) [8]; Hawk-Eye (HE), which is a prominent advanced technology introduced in detecting the ball trajectory. HE also helps to analyze bowling-related changes like bowler's variations and LBW helps in accurate decision making [9]. In the previous system, two major technologies such as this vision-based approach, pixel-based distance analysis (DA) [4] among the pop crease and pixel field with accurate results is optimized. As an advancement, the video feed details are provided with an extra infra-structure with broadcasting camera results. This method emphasizes a betterment in performance with least cost operation is stimulated along with sensors[5].

Hot spot and Snicko-meter are utilized by the users. This technology gives a sense of the ball touching the bat.

Based on the Spatio-temporal boundary analysis, the boundary shots are calculated. This method triggers the optical flow-analysis and color contraction features are enabled [10]. The motion sector classification is stimulated and these sectors notice every single move of the batsman and the shot details.

There are some sensor-based techniques prescribed for detecting no-ball and some other factors in cricket (Wazir Zada Khan et al.) [12]. The sensors manage to figure out the pitch and the bowler's stand shoes [13]. But apart from that, the system doesn't seem to be feasible enough to modify all the shoes of bowlers in the detection of no-ball.

In contrary to various researches, Kailas. G Dangi has proposed a sensor detection metrics using the bowling crease and popping crease. In this method, the sensors are fixed under each crease

[14]. As per the approach, the system tends to declare a legal ball as a 'no-ball' if the front foot is behind the popping crease, though it is not a no-ball according to the rules of cricket. Further, several dynamic trade-offs and optimizations are followed for choosing the best players in cricket as mentioned by Ahmed et.al. [15]. Many researchers deploy several techniques in [16] representing player selection with an evolutionary multi-objective optimization algorithm. An integer programming technique by Sharp et.al. [17] was formulated to overcome the maximization problem for T20 team-selection. The decision variable indicates whether a legitimate and over-skilled player is chosen or not. Farhana et.al. [18] have projected a speculative Support Vector Machine (SVM) related solution. These SVM machine learning metrics generate a ranked sequence of players to check their abilities. In preceding metrics, an estimation algorithm by C Deep Prakash [19] has been proposed. The systematic approach of this algorithm attains an estimation of ranking the players according to their skills. The system uses a Random-Forest based recursive feature estimation algorithm to follow the system levels. The Random-Forest approach utilizes the combination of a customized level state that accounts for IPL and T20 matches. Nihar Patel [20] prescribes a continuation of estimation model that projects a prediction algorithm. Here, the best player is predicted using the prediction metrics and operations [21, 22]. Lakkaraju [23] proposes a technique for convenient maintenance of the cricket statistics.

METHODOLOGY

Hot Spot Technique Implementation

In a sport, especially in cricket, new range technology such as 'Hot spot' is very crucial [24]. Hot-spot is an infra-red imaging system that is specifically designed to determine if the ball hits the player or any type of equipment. Hot-spot is previously used widely for military purposes, especially in war-prone areas for finding military equipment, for detecting jet-fighters, etc. It is a device that projects 90% accuracy from the batsman side.

Hot-spot is a scientifically proven technology that fulfils all the speculations surrounding accuracy related queries. In Hot-spot, two infra-red cameras are positioned at two alternative ends for better projection. These cameras leverage an axial focus on recording an image continuously. The axial focus resolves the amount of heat generated from two objects colliding at a point of friction. The cameras record a clear vision of the video and creditably project the particular incident with fine quality images. These images turn into a negative image stage delivering viewers the realistic outcome of the recent incidents. This stimulated negative image conversion technique is called a 'subtraction module'.

In the collusion process from the images, retrieved Hot-spot deliberates an accurate result which assists in proper decision making. The other sports games such as tennis, badminton, and snooker also use Hot-spot technology for better results [25, 26].

The main contributions and uses of the Hot-spot technique implementation are analysed below:

- Review verdict resolution: This technology can be useful in various metrics in cricket. The technique can solve rigid problems. At times when an umpire is confused or the umpire review is not satisfactory, then a Hot-spot technology review can be recommended from the players' side. One minor change from the part of technology may be a crucial step for a team to win or lose a match. This may become a turning point for a match.
- LBW decision making: As the cameras capture the live and real movements from the ground, an exact result can be correlated. By using the cameras or images, the correct

movement of the bat can be identified with the help of Hot-spot which can easily detect movements through the LBW.

- Minute edge detection: Deviating from the keeper's catch, even a minute edge or notch can be identified and corrected with the help of Hot-spot technology.

There are some minor drawbacks in implementing the Hot-spot technique world-wide.

- The first main drawback is the high-cost implementation in using the 'Hot-spot' apparatus.
- The second drawback is that the result implementation takes time, which tends to reduce the speed of the interesting game.

Though the Hot-spot technology is much effective, it is under trial basis at present. After trial implementation, if it performs better in speed and accuracy, the real-time implementation will get processed.

Score-Card System Implementation

Maintaining the tracks of records like scores, runs, etc., is a difficult task in cricket. To maintain the record and details, a Score-card system is emphasized. All the in and out match details are in the repository system. These files are stored and maintained in a database. The team details, team members, and their respective details are described in the forms. After every match, a live updating is structured accordingly, employing the projected software. With a single click, a scoreboard gives the entire details of a particular player or a particular match. The full details of the players, including some personal details are confidentially stored and maintained under this system.

Score-card implementation software is error-free and anyone from anyplace can use this software. All cricket viewers have access to download this software just by the click of a button. The main motive of the system is to use the software for additional benefits and details of cricket.

The main contributions of the Score-Card system are:

- At first, the system aims to compose a cricket score-board. On the scoreboard, regular updates related to cricket are promoted and provided to the viewers with live commentaries.
- If a user is held up with some other work or is not able to watch the match, this card system can provide match information and live updates.
- The score-card system will continuously post updated scores. They also leverage an adjacent team line-up in the match.
- After live matches get over, the system admin will store upcoming match details and guarantee that the team information is posted about the upcoming events or matches.

Being an international game, cricket is a special part of the lives of billions of people. For many who possess an inclination to closely follow cricket but face practical time and place constraints, this system will surely create an interesting segment of updating on the game. Announcing upcoming sequences helps the system management to load information at the match times, while providing people with live updates of matches without a video feed further helps in the expansion of the cricket game, in and off the field.

Cricket Team Selection Optimization

The scheme represents a retrospective approach in selecting teams in real-world applications. In this approach, the last 10 match records are collected for optimization.

Moreover, the player performance is speculated from the data collected from the completed matches. An integer programming technique in the python domain is stimulated. Further, the Markowitz Optimization-model is referred with the record collection. The optimization model analyzes the players' performance and optimizes the best accordingly. Then, the team selection

problem is analyzed through the financial portfolio and optimization model. Threat aversion is applied here to deal with severely inconsistent performances while the risk factors are controlled.

Location Support System

The paper promotes a detailed evaluation of the cricket supporting system. In cricket, the location provision is a major metric, useful to locate building, mobile, location-dependent applications, etc.

The location support system (LSS) allows applications executing on mobile and static modes to locate their physical location. This process of locating is implemented by using listeners who hear and analyze information from beacons that spread surrounding the building. Cricket lies under several goal metrics such as user security, decentralized management, supervision metrics, NW heterogeneity, and low-cost consumption. The beacons in the system provide data to devices despite their form of NW connection.

The designing of cricket details is driven by the following specific goals that can be applied with specified consideration under location support system:

- **User Security:** In any field or application, security is the main concern. In cricket, for storing details and maintaining users' privacy, security must be provided. The location support system is centralized in tracking systems for privacy maintenance. To construct a specific location and to address cricket related details, this system can prove very useful.
- **Decentralized Management:** Decentralized management produces a scalable way of managing and controlling all the executive operations of cricket. All the queries related to the building space, rooms, and seat occupations can be centralized under location support system.
- **NW Heterogeneity:** A high range of NW technologies exists in several building environments. In the auditorium, the users are provided with a wide range of access connected over 10/100 Mbps Ethernet. Through these services, clients can benefit from gaining knowledge on their location in an automatic manner. By this, the accommodations can maintain better network access to the system. In decoupling the cricket system process, other data communication mechanisms can be used.
- **Cost-effective metrics:** Cost is a major constrain that may lead to the discouragement of the game. Considering the building-wide deployment, Wi-Fi accessibilities and ticket charges can help in formulating the required cost.

By overcoming the above issues and executing the above applications, a proper and effective support system can be produced.

CONCLUSION

The paper presents an extensive survey on the technologies and techniques used in the development of the universal game, cricket. Various computer vision-based algorithms, particularly in detecting the often over-stepped 'no ball' situation in cricket game are analyzed. The popular techniques such as Hot-spot implementation, Score-card system, team selection optimization, and location support system used in cricket are discussed at length. An analysis of the discussed approaches portrays the location support system leading the way in the use of advanced technologies and algorithms in the cricketing world in terms of efficiency, accuracy and cost-effectiveness from all angles.

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Preparation of Zinc oxide Nanoparticles and its Characterization Using Scanning Electron Microscopy (SEM), Energy – Dispersive X-ray spectroscopy (EDAX) and Fourier Transform Infrared Spectroscopy (FT-IR)

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Abstract

A Shiny white powder Zinc oxide which has unique physical and chemical properties has wide range of applications used in paints, lotions on skin, food, electronics, biological and pharmaceuticals. A regular intake is needed to store it in the body. This paper holds the basic method of how to prepare the zinc oxide nanoparticles combining with the organic compound in an ancient and conventional method. The first part of the paper comprises of the structure seen before the process (structure of Zinc oxide). The next part is the structure obtained after the process gets completed (structure of Zinc oxide with the addend). The Studies are well analyzed by SEM, EDAX and FT-IR. There is a difference seen in the transmittance and in the absorbance level. The morphological study is done using SEM. Further the band gap is investigated by the results obtained.

Keywords: Zinc oxide, addend, EDAX, SEM, FT-IR.

1. Introduction

Richard Feynman in 1959 gave a talk mentioning “there is plenty of room at the bottom”. His idea was to bring about the n-number of combinations, the spaces, the structures and its major properties in the micro state, which are unseen or not discussed to the scientific world. The micro state is the real image of the macro state. The invention of Scanning Electron Microscopy (SEM) in the year 1981, gave a new face lift to the emerging photographic methods to study the surface morphology of tiny materials. Nano Science and Nano Technology has brought a revolution in the modern science and engineering. The combination of Zinc oxide nanoparticles with the organic compounds depicts the various sizes and shapes when they form. In recent years the application of Zinc oxide in various fields has influenced its performance among the family of elements putting it front. The optical, mechanical, chemical property of Zinc oxide brings forward its high chemical and mechanical stability too. Broad range of absorption is exhibited by Zinc oxide nanoparticles.

Structures are predominantly obtained in one dimensions 1D, two dimensions 2D and three dimensions 3D. Structures like needles, helices, nanorods, ribbons, belts, wires which come under one dimensional category are obtained from zinc oxide. The nanoparticles of Zinc oxide are seen in the form of Nano pellets, Nano-sheets, Nano plates, rectangular logs, spherical spheroids [1]. In terms of production of Nano Zinc oxide (nZnO) it takes the third place next to Nano Silicon dioxide (nSiO₂) and Nano Titanium dioxide (nTiO₂). The presence of Nano materials in the scientific market gives a platform to think about being an agent towards antibacterial activity. Zinc oxide is commonly added in sunscreens, coatings and paints to absorb UV light.

Products depending on bacteria, fungi, plants and yeasts always result in the production of nanoparticles excellently [2].

2. Experimental

a. Materials used

Preparation of Nano particles is focused here in simple manner. Zinc oxide powder (size in microns), water (Polar Solvent), magnetic stirrer.

b. Sample Preparation [A]

(Reduction of size of the Zinc powder from microns to Nano particle size)
Zinc powder is taken and then dried in shade for more than an hour, this is done to make the powder dry. Water is taken as the polar solvent, the dried zinc powder is mixed with water. This mixture is stirred for more than five hours. This is done to make the solute get mixed with the solvent. The obtained mixture is poured in plane pane and it is allowed to dry in shade. This mixture takes its own time to get dried up, nearly for more than ten days. Water content gets reduced due to evaporation. The need for the polar solvent is, it breaks the bulk segments in the zinc powder to smaller units. Obviously the size gets reduced. Here the tank water is used rather than distilled water. This leads to the presence of few other elements seen in the results.

c. Sample Preparation [B]

(Sample is prepared using leaf extract)

Leaves of lemon tree are taken, and then it is cleaned by washing it with water. Then the leaves are dropped into a bowl containing water. This system is exposed to heat, on boiling this the color of the solution gets changed. This clearly tells the presence of Nano particles. As we use the leaves of lemon tree it is obviously yellow in color (leaves are green but the extract obtained is yellow in color). Hence we get the solution the leaf extract. Zinc powder is now mixed with the extract and stirred well for nearly five hours. As we get the mixed up solution, this is kept for drying state. To obtain this, the mixed up solution is poured into plane pane. This system is kept in a shady region for the water to get evaporated. It takes more than ten days for evaporation. Finally the sample is obtained[4].

3. Measurements

Scanning Electron Microscopy (SEM) images of Zinc oxide powder nanoparticles and Zinc oxide with leaf extract sediment is taken. Energy dispersive X-ray spectroscopy EDAX from TSL Ametek for samples A, B is taken.
The FTIR study for the samples A,B is taken.

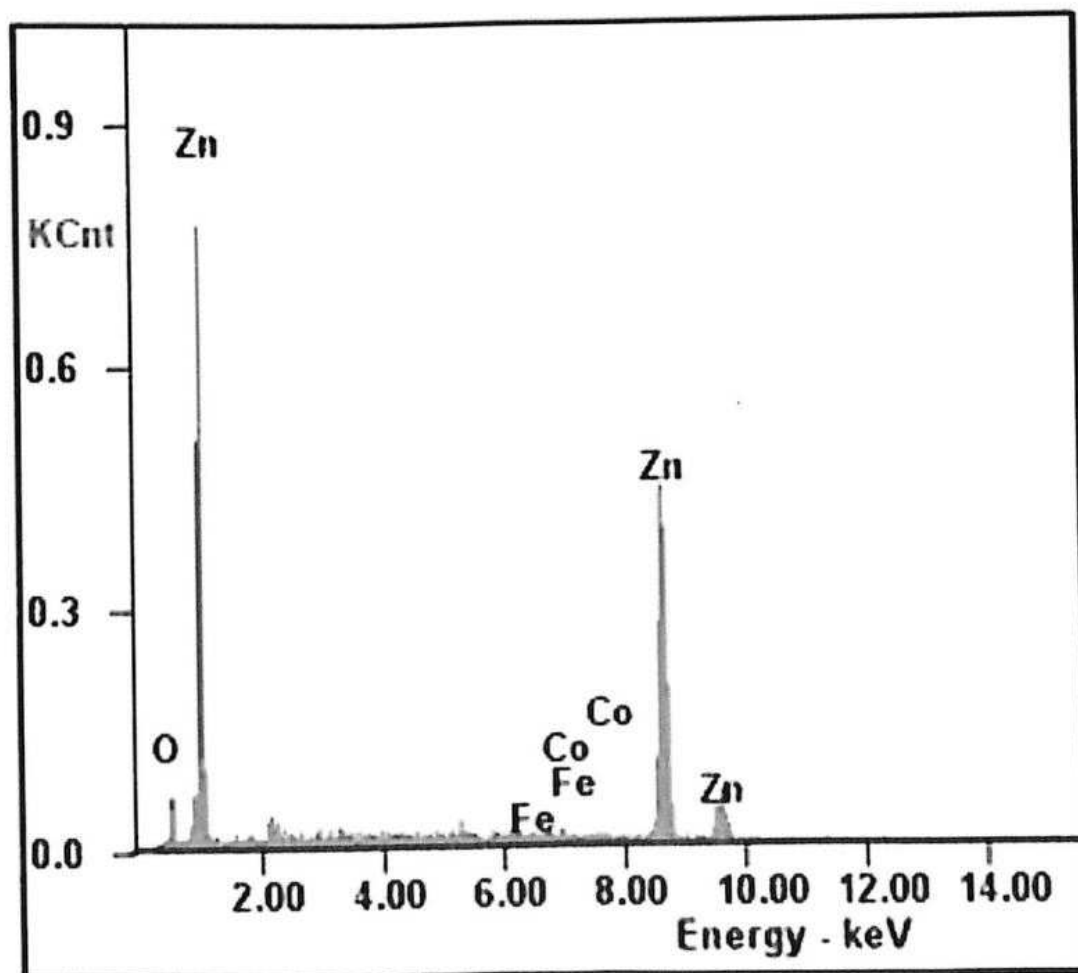
Sample A - - zinc oxide , Sample B - zinc oxide mixed with lemon extract

4. Results and Discussions

EDAX and Scanning Electron Microscopy (SEM) image of Zinc oxide powder is taken. Fig. 1 and Fig. 2 reveal the EDAX parameters of the samples A,B. The energy-dispersive X-ray spectrometer spectrum of zinc oxide nanoparticles is taken, that is the sample A. Whereas the study of EDAX tells us about the composition that is seen in the sample. The elemental composition is quite understood by having this result. Fig. 3 and Fig. 4 reveals the SEM image of the samples A,B shows a difference, there are some cracks seen on the surface of the Nano particles B, but the cracks are not seen on the surface of Nano particles sample A. This could be the reason that the biomolecules have drastically shown an impact on the surface of the sample B. The organic substance that is obtained from the leaf extract has brought these cracks on the sample B whereas we don't see it on sample A. The energy dispersive X-ray spectrometer spectrum shows the composition of elements that is seen in the samples. As tap water is used the presence of Co and Fe are seen in the figure. 1.

4.1 EDAX ANALYSIS

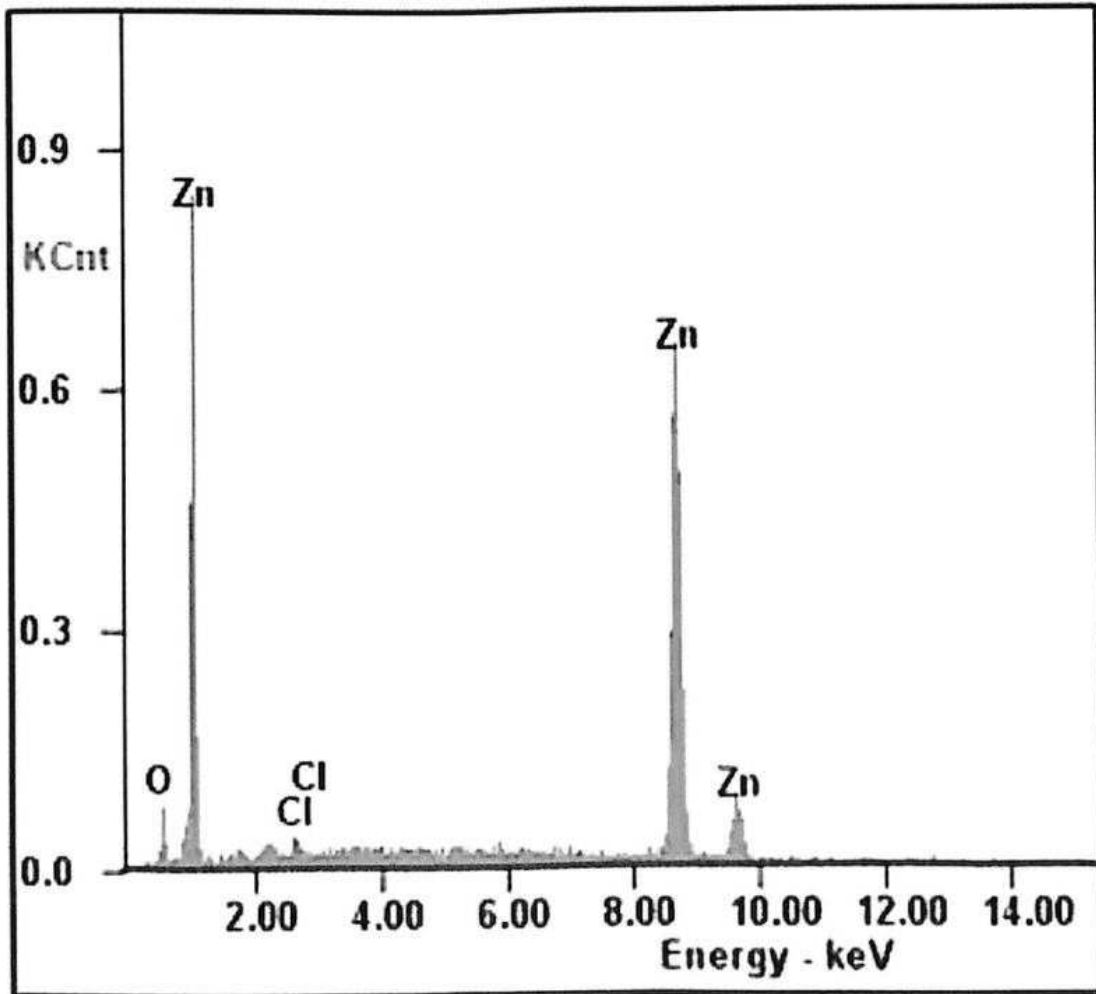
EDAX as we know it shows the composition of the elements in the sample, here the



<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>OK</i>	14.87	41.58
<i>FeK</i>	00.84	00.67
<i>CoK</i>	00.80	00.61
<i>ZnK</i>	83.49	57.14
<i>Matrix</i>	Correction	ZAF

Fig.1 - Energy-dispersive X-ray spectrometer spectrum of zinc oxide nanoparticles

elements shown in the table has the preceding letter K. This letter K implies to the K shell, whereas the electrons from the K shell gets knocked out when the sample is exposed to the incoming electrons (which is the bombarding electron). As there occurs vacancy getting formed in the K shell the neighbouring electrons from the shells L, M, N may jump into the K shell to occupy the spaces[3-4].



<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>OK</i>	10.96	33.20
<i>ClK</i>	01.24	01.70
<i>ZnK</i>	87.80	65.11
<i>Matrix</i>	Correction	ZAF

Fig.2 - Energy-dispersive X-ray spectrometer spectrum of zinc oxide mixed with lemon extract nanoparticles

The movement of the electrons from the neighbouring shells to the K shell will release the X-rays. As this process happens at the regular rate, we get the peaks seen in the graph for that element from that particular shell. At% is the atomic percentage which tells about the number of atoms in terms of percentage[5]. Whereas the Wt % is the weight percentage which tells the function of weight of the atoms. On adding, we get the total to be 100%, this gives the clear picture of the composition of the elements in the sample.

4.2 SEM ANALYSIS

The Sample A,B are clearly analyzed using SEM. The cracks caused by the organic compound

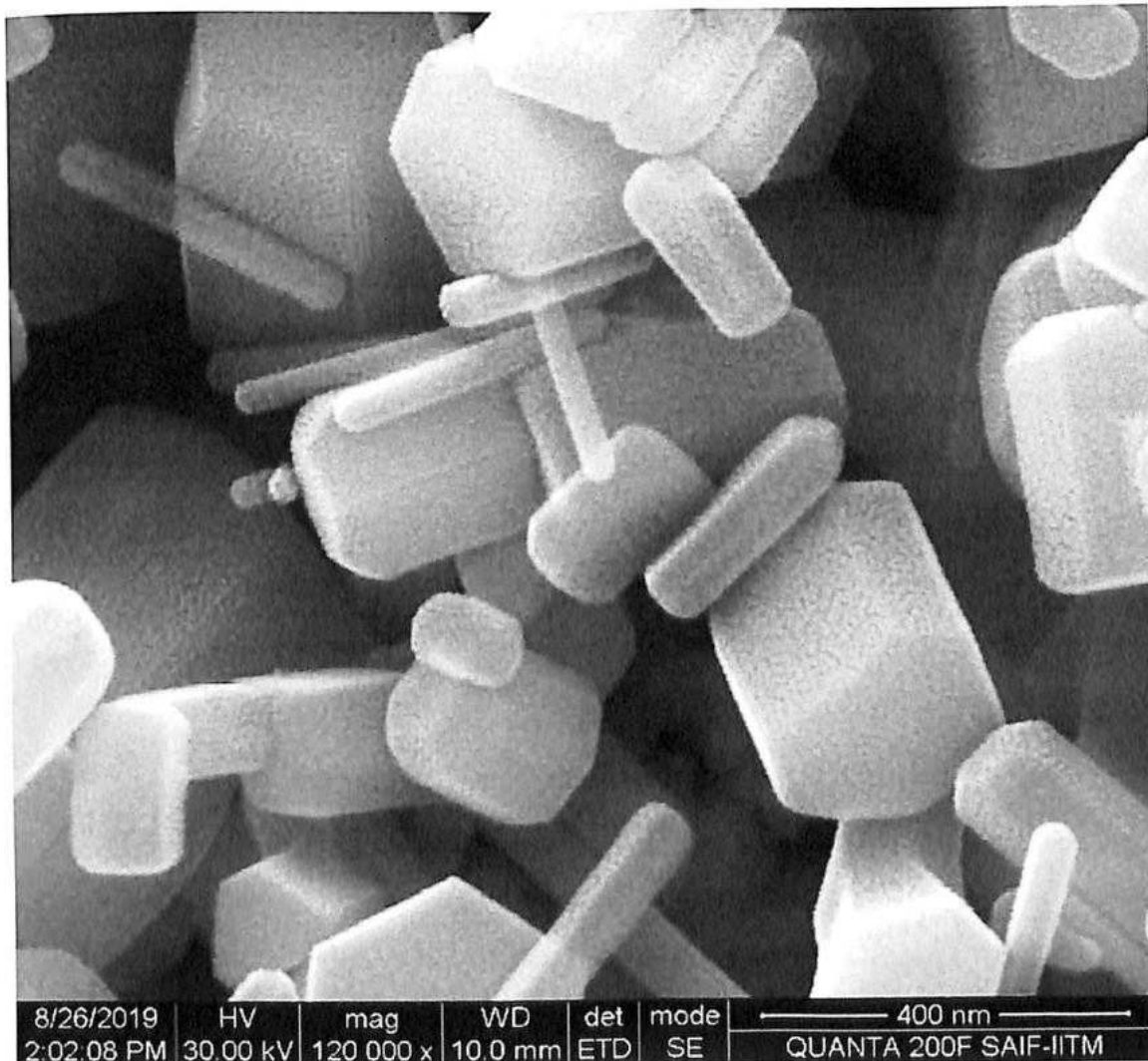


Fig. 3 - SEM- image of zinc oxide nano particles

is seen in the SEM image of the sample B. Whereas we don't find the legible cracks on sample A. The shapes shown in the picture gives us a clear idea of the structures, solid forms taking the rectangular, hexagonal, cuboid and few pellets shapes[6-7].

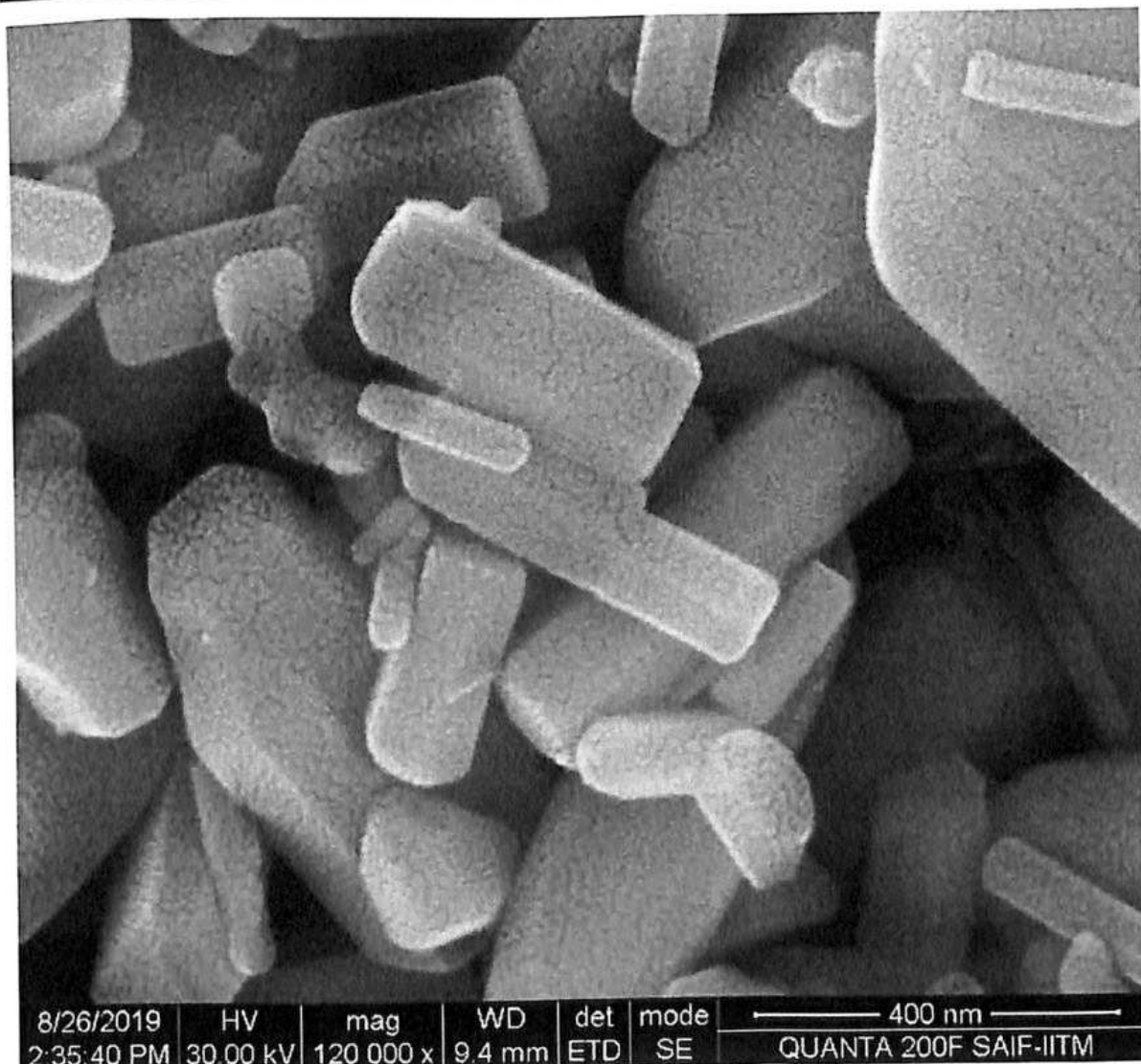


Fig.4 - SEM-image of Zinc oxide mixed with lemon extract nanoparticles

Both the samples are taken in the 400 nm magnification, this is done to have a comparative study of the samples with no deviation.

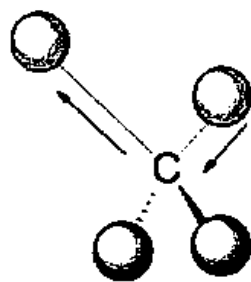
4.3 FTIR ANALYSIS

The peaks shows the occurrence of metal oxide and we know that ZnO is a metal oxide. The major peaks are to be seen below 1000 cm^{-1} to ensure that ZnO is a metal oxide. The spectrometer (Bruker) is used to find out the FT-IR analysis. We got nearly eight peaks for the sample A, this sample is an undoped or pure sample.

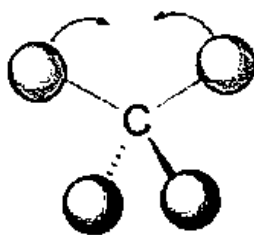
The obtained peaks fall in the range of 560.87 cm^{-1} to 3709.97 cm^{-1} . Stretching and Bending concepts are seen in the results. It is a known fact that it is easy to bend a bond rather than to stretch or on the other hand to compress[3].



symmetric stretching



asymmetric stretching



scissoring



rocking

The energy of vibrations seen in the molecular region is more over quantized than it is continuous, this put forth that a molecule can stretch or else bend at only at certain allowed frequencies. Here the molecule when exposed to electromagnetic radiation, if there occurs a matching with the vibrational modes, then it will absorb the energy from the radiation and jump to a higher vibrational energy state.

The amplitude will increase but the vibrational frequency will still remain the same. On having a note the difference in energy between the two vibrational states is fundamentally equal to the energy associated with the wavelength of the radiation that was absorbed. Figure -5 shows the undoped zinc oxide that is the Sample- A, whereas the Figure - 6 shows the organic doped material zinc oxide with lemon extract that is the Sample B.

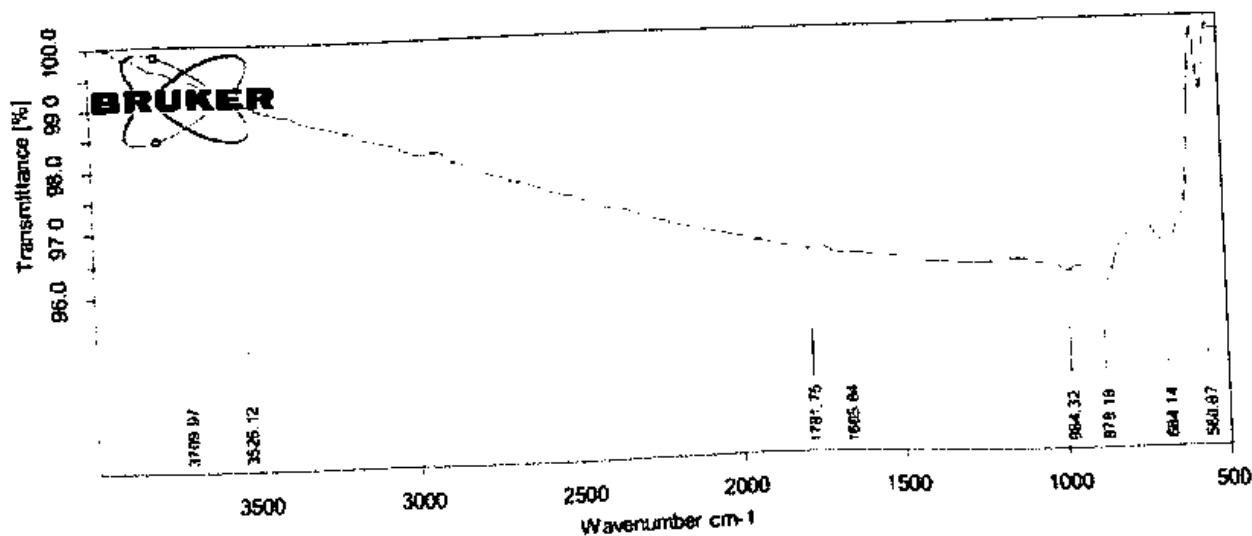


Fig. 5 - Fourier transform infrared spectroscopy spectrum of zinc oxide nanoparticles

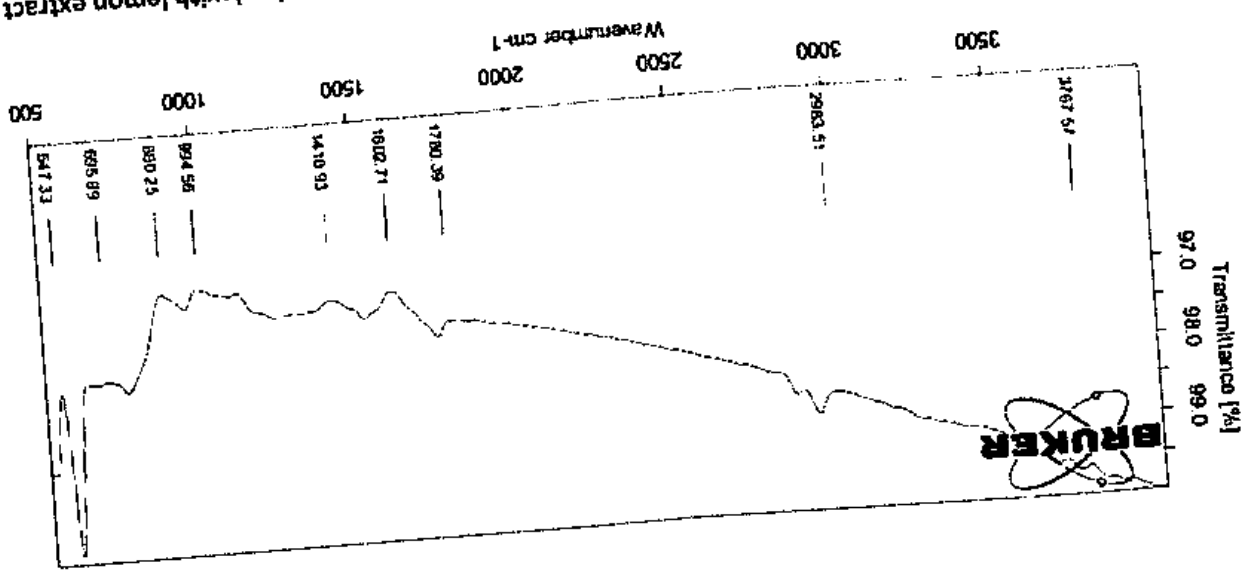
Fourier transform infrared spectroscopy spectrum of zinc oxide mixed with lemon extract nanoparticles

S.NO	ABSORPTION PEAKS OBTAINED FOR ZNO NANO PARTICLES (cm-1)	STANDARD ABSORPTION FREQUENCY RANGE (cm-1)	APPEARANCE	GROUP
1	547.33	690-515	STRONG	C-Br stretching
2	695.89	900-700	STRONG	C-H bending
3	880.25	995-985	STRONG	C=C bending
4	994.56	1440-1395	MEDIUM	O-H bending
5	1410.93	1650-1580	MEDIUM	N-H bending
6	1602.71	1800-1770	STRONG	C=O stretching
7	1780.39	3000-2840	MEDIUM	C-H stretching
8	2983.51	4000-3000	MEDIUM,SHARP	O-H stretching
9	3767.57			

Fourier transform infrared spectroscopy spectrum of zinc oxide nanoparticles

S.NO	ABSORPTION PEAKS OBTAINED FOR ZNO NANO PARTICLES (cm-1)	STANDARD ABSORPTION FREQUENCY RANGE (cm-1)	APPEARANCE	GROUP
1	560.87	600-500	STRONG	C-I stretching
2	684.14	730-665	STRONG	C=C bending
3	878.18	900-860	STRONG	C-H bending
4	984.32	1000-650	STRONG	C-H bending
5	1665.84	1675-1665	WEAK	C=C stretching
6	1781.75	1800-1770	STRONG	C=O stretching
7	3526.12	3550-3200	STRONG,BROAD	O-H stretching
8	3709.97	4000-3000	MEDIUM,SHARP	O-H stretching

Fig. 6 - Fourier transform infrared spectroscopy spectrum of zinc oxide mixed with lemon extract



5. CONCLUSION

By the simplest method the Zinc Oxide nanoparticles are prepared (this method can also be called as a slow evaporation method). The pure Zinc Oxide is sample A and the sample B is Zinc Oxide mixed with Lemon extract an organic compound. Study of EDAX with the samples have clearly shown us the composition of the elements present in the sample. Whereas the SEM image shows the minor cracks that is seen on the sample B due to the additive lemon extract. The FTIR study reveals us that peaks gets heaped out at a point 2983.1 wavenumber cm^{-1} whereas the peak sinks out at a point 1602.71 wavenumber cm^{-1} , this is highlighted in sample B on comparison with sample A. As discussed these occur due to the bonding vibration caused by the radiation which is applied on the sample.

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Optical, structural and electrical properties of AgSbO₃ nanotips prepared by thermal evaporation technique for thermoelectric effect applications

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ABSTRACT

The silver antimonate (AgSbO₃) nanotips prepared by thermal evaporation technique at a constant substrate temperature with molybdenum boat for coating. It was characterized by X-ray diffraction pattern and revealed a polycrystalline structure. The crystallite size was nearly 56 nm, using optical studies band gap values were measured by *Tauc* plot its value comprises between 3.0 eV and 4.2 eV and absorption coefficient is decreasing within UV range. In order to understand the Pseudo first (PFO) and second (PSO) order of kinetic of AgSbO₃ crystal are studied. Thermoelectric measurement shows a decay of thermopower effect with an increase in temperature. AFM scanned pictures show texture which looks like nanotips growing along z axis. Pseudo first order and second order models for kinetic data fitting in order to determine the rate – limiting step was analyzed for our sample.

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1. Introduction

Thermoelectric is a science and technology associated with conversion of thermoelectric converters, that is generation of electric power by Seebeck effect and refrigeration by Peltier effect [1]. The principal aim of research and development in thermoelectric materials is to fabricate semiconductors with high conversion efficiency and to assess their stability for cooling applications [2]. AgSbO₃ has received considerable attention in the last few decades, because it is used to alloy AgSbO₃ to get *n-type* material with best thermoelectric properties. The phenomenon of thermoelectricity in recent years required prominence in the technological field as a result of its application of portable refrigeration and power gener-

ation [3]. This field is dominated by the semiconducting materials, which have sufficiently large thermoelectric coefficients of practical interest. Thermoelectricity concerns with the direct generation of emf by thermal means and this involves subjecting a conductor to a temperature gradient [4]. Physically the phenomenon arises because electrons at the hot ends of the conductor can find the states of lower energy at the cold end to which they diffuse setting up an electron potential difference between the two ends. During the photocatalysis process, a material absorbs light to bring it to higher energy level and provides such energy to a reacting substance to make a chemical reaction occur [1,5]. Antimony doped silver oxide AgSbO₃ has a wide range of application including catalyst for the oxidation of phenol, conductive transparent optical thin film, electro chromic materials for printed displays, radioactive site management and heat shields [9]. Reduction of particle size is also needed for improved catalytic properties AgSbO₃ nanoparticles have been prepared by co-precipitation reaction

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method. This prepared material is used to prepare thin film by using pelletized materials [2,6]. Seebeck tested a wide range of materials including the naturally found semiconductors AgSbO_3 . It is interesting to note that if these materials had been used at that time to construct thermoelectric generator, it would have the efficiency of around 3% like that of contemporary steam engines. The Seebeck coefficient is defined as the open circuit voltage produced by a temperature between two points on a conductor where a uniform difference of 1 K exists between those points Peltier however failed to understand the implications of his findings and is not until four years later when Lenz concluded that there is a heat absorption or heat generation at the junctions and demonstrated this effect by freezing a drop of water at a AgSbO_3 junction and melting ice by reversing the current. In Thompsons effect rate of heat generated or heat absorbed in a single current carrying conductor subjected to a temperature gradient. These three relations are connected to each other by simple relationship $S = \frac{\pi}{\Delta T}$, when a thermal gradient, ΔT is applied to it will be accompanied by an electric field, ΔV is in the opposite direction this is known as Seebeck effect. The ratio $\frac{\Delta V}{\Delta T}$ is defined as Seebeck coefficient (S) and is expressed in volts per degree, $\mu\text{V}/\text{K}$. Metal thermocouples generate tens of microvolts per degree temperature difference and they are familiar temperature controlling sensors in domestic refrigerators and central heating system [3,7]. It is mentioned that PFO model might be to be valid only at the initial stage of adsorption [4,5]. The thermoelectric oxide AgSbO_3 compound is prepared by the standard solid-state reaction combined with spark plasma sintering route as cited previously [6].

2. Experimental procedure

This prepared powder was pelletized to make the thin film on ITO plate by using Thermal Evaporation Technique. Preparation method AgSbO_3 as shown in the flowchart diagram of Fig. 1. Instead of using a separate heating step to supply the energy needed to initiate reactions the component, the reaction itself supplies the energy. In co-precipitation method, the preparation of antimony doped tin oxide the following process is done. Add 25 g of $\text{AgCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.5 g of SbCl_3 and dissolved in 500 ml of deionized water. Above mixture stirred with magnetic stirrer for 45 min at 60 °C temperature. Make a solution of 20 ml of acetyl acetone and add 80 ml of methanol. Then the above solution is added slowly drop by drop wise. Take 45 min for adding the solution to the above mixture then stirring the solution 30 min, and then add ammonia to the above solution slowly drop by drop wise respective time intervals. After this precipitate formation started then keep in cool water bath for 24 h. This deionized water washed several times for removing the Cl^- ions. Then keep it at 80 °C for 2 h. This ground powder is used to deposit the thin film on ITO plate by using thermal evaporation technique. AgSbO_3 film, having 85 μm thickness, is deposited by thermal evaporation of as-synthesized NPs onto ITO substrate. Prepared with ITO substrates, having a resistivity of 8 $\Omega\text{-cm}$, by thermal evaporation, where an Ag:Sb ratio, evaporated temperature and times, which are the important processing parameters are adjusted to control the microstructure and luminescence of the film. Various AgCl_2 (99.999%, Aldrich) and SbCl_3 (99.999%, Aldrich) ratio of (90:10) are placed in a molybdenum boat is performed at the middle of the in horizontal quartz tube mounted inside muffle furnace. The substrates are placed approximately 7 cm from powder source on the downstream side of the tube. Prior to the synthesis of NPs, the quartz tube is introduced by an argon flow 90 sccm (standard cubic centimeter per minute) for 30 min to remove oxygen inside the quartz reaction tubes. Then, the temperature of the furnace is adjusted from 600 °C to 1000 °C at the rate of 10 °C min^{-1} with

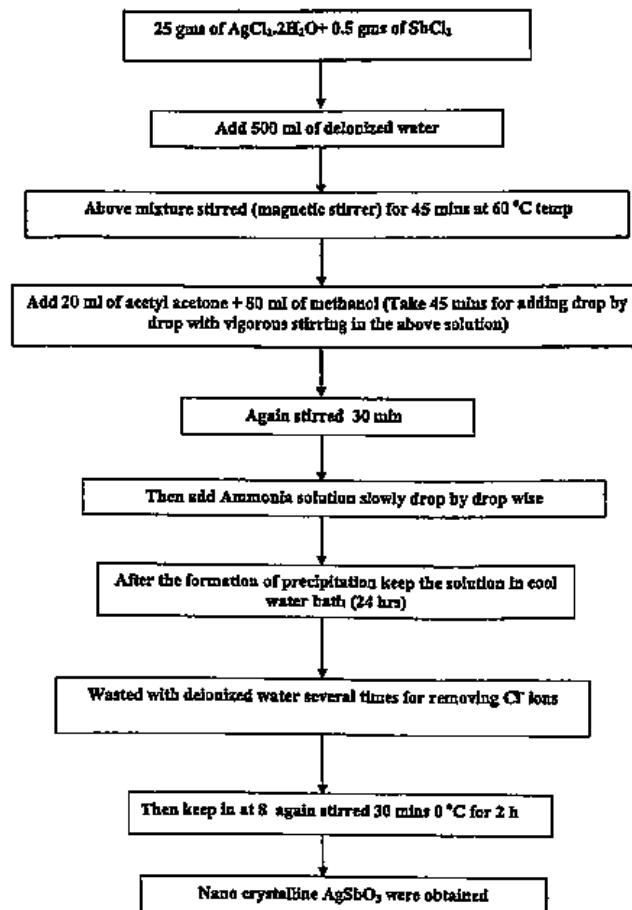


Fig. 1. Flowchart for the preparation of AgSbO_3 nanoparticles.

various evaporation times under constant argon flow of 50 sccm, after which, the system cooled to room temperature naturally, the film is removed for further studies [4]. The structural properties of AgSbO_3 thin films are investigated using PAN analytical X-ray diffractometer with $\text{CuK}\alpha$ radiation at $\lambda = 1.5406 \text{ \AA}$. The X-ray diffraction (XRD) pattern is recorded in the range of 20–80 at a scanning rate of 2° min^{-1} . Morphological studies are performed using scanning electron microscopy (SEM, Hitachi S-3400N, Japan). The atomic force microscope analyzer 2D images are obtained with tapping mode. In addition, Fig. 2 shows the schematic diagram of silver antimonite crystal structure were reported in detail.

3. Results and discussion

3.1. X-ray pattern analysis and scanning microscope observation

The crystallite size D is calculated using Scherer's formula; $D = 0.9\lambda/\beta \cos\theta$; Where ' D ' is the crystallite size, ' β ' is the broadening of diffraction line measured at half of its maximum intensity and ' λ ' is the X-ray wavelength (1.5406 Å). The calculated values are tabulated. Average crystal size for the as-prepared powder particle is 50.85 nm according to the (JCPDS file number no. 02-0697), for the annealed sample 69 nm. AgSbO_3 belongs to cubic defect pyrochlore structure with Fd_{3m} space group, the X-ray pattern analysis of sample is showed in Fig. 3. The analysis of AgSbO_3 thin film is carried out within the 20°–80°; 2 θ angle range indicating (2 2 0), (2 2 2), (4 0 0) and (4 2 2) as the main orientations. The polycrystalline aspect of sample is then revealed. The calculated values are tabulated. Average crystal size for the as-prepared pow-

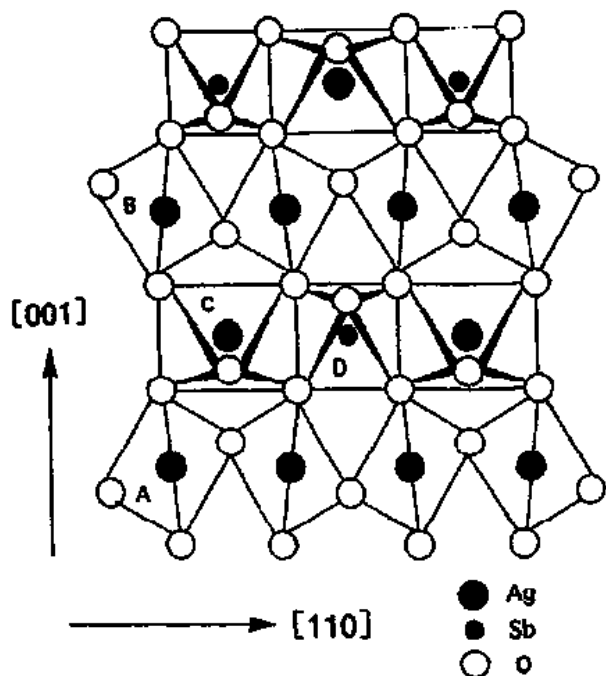


Fig. 2. Schematic diagram of silver antimonate crystal structure.

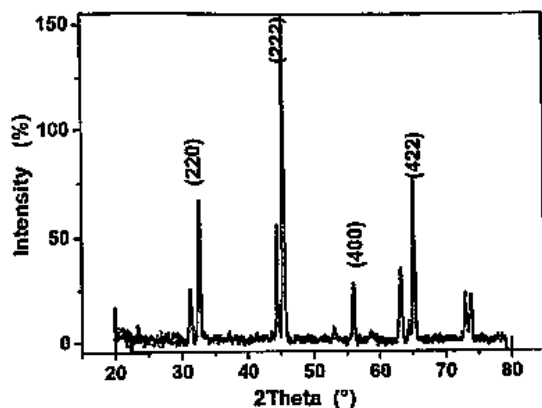


Fig. 3. X-ray diffraction pattern of AgSbO₃ nanotips.

der particle and the annealed sample are respectively 10.883 and 69 nm [5]. Grain size are calculated by Scherrer equation as

Table 1
Structural parameters of AgSbO₃ nanotips produced by thermal evaporation process.

Position (2θ)	Height [cts]	FWHM (2θ°)	d-spacing (Å)	Relative Intensity (%)
31.2584	23.79	0.4015	2.86157	15.77
32.7076	65.57	0.2676	2.73802	43.48
44.4611	54.73	0.2342	2.03771	36.29
45.6001	150.81	0.2342	1.98943	100.00
53.2854	3.93	0.8029	1.71921	2.61
56.0342	26.77	0.3011	1.64122	17.75
58.7453	3.30	0.8029	1.57178	2.19
63.1935	34.47	0.3346	1.47143	22.85
65.2050	76.78	0.3346	1.43082	50.91
72.9854	22.57	0.2676	1.29630	14.96
73.8918	18.68	0.4080	1.28157	12.38

59 nm. The Ag_xSbO₃ (x = 0.99 and 1.00) powders are crystallized in a cubic-pyrochlore structure (space group: Fd3m) as mentioned in literature [6]. The structural parameters of AgSbO₃ nanotips produced by thermal evaporation process are gathered in Table 1. The lattice parameter is 10.25 Å as listed in Table 2. As listed in Table 2, the peaks are located at 36.77 and 45.51, these values are close those, found in literature, 38° and 44°. The Fig. 4 displays the scanning electron microscope picture which taken at magnification of 12,000 with scale of a 2.5 μm and at applied voltage of 20 kV. Brightness aspect is observed showing a tangle of micro/nanostructures with no voids roughly.

3.2. UV spectrophotometer and FTIR measurements

Fig. 5 describes the variation of transmittance versus photon wavelength AgSbO₃ material within UV band. A rapid rise of transmittance from lower value to 82% when photon wavelength goes through the 280 nm–400 nm range. Based upon absorption plot (not shown here) absorption coefficient and gap energy (E_g) of AgSbO₃ materials are displayed in Fig. 6. Both E_g and absorption coefficient parameters comprise between 3.1 and 4.2 eV and 1 and 21 (×10⁻³ nm). The Ag_xSbO₃ photocatalysts with the bandgap of 2.6 eV are prepared by a conventional solid-state reaction method as mentioned previously by Kako et al., [3]. Optical bandgap determined by a Tauc plot is of 3.1 eV and a high absorption coefficient and wide bandgap are recorded in UV band around 300 nm.

FTIR spectrum analysis of silver antimonate AgSbO₃ material prepared by thermal evaporation route as shown in Fig. 7. The FTIR profile swings within 300 cm⁻¹–4000 cm⁻¹ range recording many peaks. The mains peaks are located at 653.76 cm⁻¹, 1243.88 cm⁻¹, 2360.48 cm⁻¹ respectively. The peak absorption band around 653.76 cm⁻¹ is attributed to stretching of the metal–oxygen bond in the silver antimonite, which includes the internal motion of a change in Sb–O–Sb bond length in SbO₃ structure. PL plots can be deconvoluted into three Gaussians indicating wagging absorption strong intensity at 500 cm⁻¹ and 700 cm⁻¹ respectively in bulk phase whereas the same are observed with strong and weak intensity at 840 cm⁻¹ and 900 cm⁻¹ respectively in nanophase as shown in Fig. 7. The Sb–O in plane bending is identified at 400 cm⁻¹ and 654 cm⁻¹ in bulk in nanophase. The Ag–O out of plane bending is found at 450 cm⁻¹ and 320 cm⁻¹.

3.3. The kinetic model theory and the photocatalytic analysis

The adsorption kinetics reflects the evolution of the adsorption process versus time. The latter is an important parameter, which must be taken into consideration. In addition, in the treatment of aqueous solutions, the adsorption process is intimately dependent on the other experimental parameters, such as pH, ionic strength, temperature, concentration of solute, sorbent dose, the texture of

Table 2
XRD analysis of AgSbO₃ thin film (02-0697) nanotips produced by thermal evaporation process.

Std., 2θ (degree) (From JCPDS)	Observed 2θ (degree)	Std., d (Å)	Obs., d (Å)	Miller indices (hkl)	Lattice constant Std., a (Å)	Lattice constant calculated a (Å)
36.773	32.32	3.630	3.738	220	10.25	10.36
45.514	45.66	2.960	2.9894	222		
53.137	55.64	2.560	2.619	400		
66.439	65.04	2.090	1.999	422		



Fig. 4. SEM picture of AgSbO₃ nanotips.

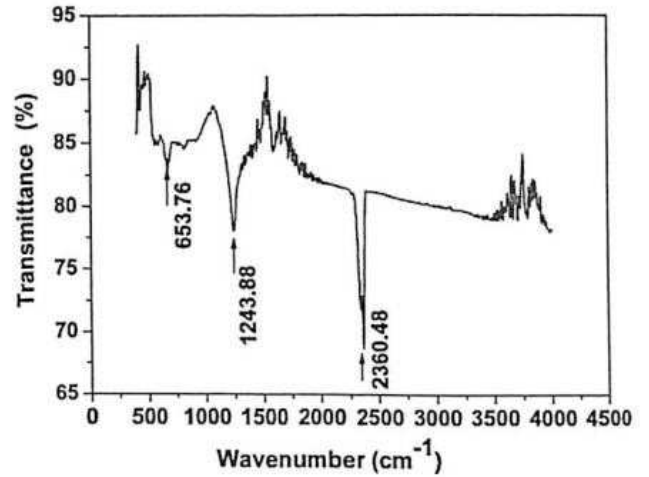


Fig. 7. FTIR Analysis variation of AgSbO₃ nanotips.

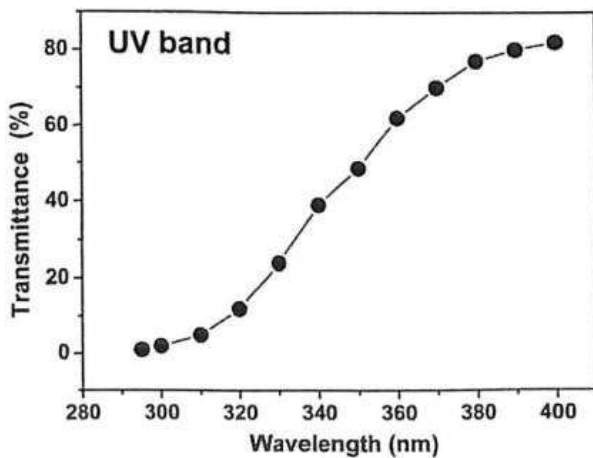


Fig. 5. Transmittance plotting versus photon wavelength (UV band) of AgSbO₃ nanotips.

adsorbent, that affect the kinetics of adsorption of an adsorbate onto any adsorbent. Therefore, the contact time where the adsorption process approaches a true equilibrium must be determined according to these parameters. The $\ln(q_e - q_t)$ versus time as showing in Fig. 8 indicating a linear lowering. By linear fit, we determine the negative slope which is found to be of 0.097. The q_t , dimensionless parameter, is the quantity taken for the photocatalytic studies with time in everyone minutes. Therefore, the PFO and PSO models have been broadly employed to describe the amount of adsorption in liquid solid interactions.

3.4. Pseudo-first order (PFO) model

Also known as Lagergren model, it describes the adsorption of solute onto adsorbent following the first order mechanism. The absorbed rate q_e of each adsorbed atom or the pseudo-first order (PFO) equation is given as [8];

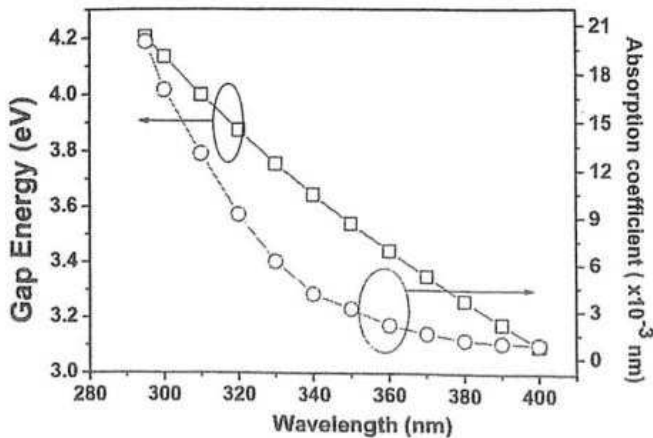


Fig. 6. Gap energy (Left) and Absorption coefficient (Right) versus photon wavelength of AgSbO₃ nanotips produced by thermal evaporation process.

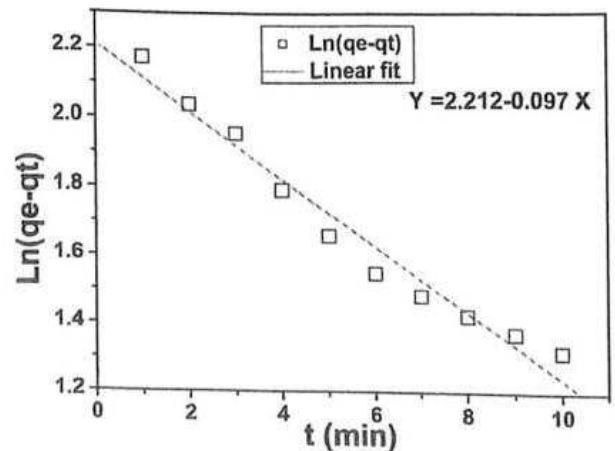


Fig. 8. Transient curve of $\ln(q_e - q_t)$ of AgSbO₃ nanotips produced by thermal evaporation process. (PFO models for the adsorption kinetics process).

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (1)$$

where K_1 (min^{-1}) is the pseudo-first order adsorption kinetic parameter or rate constant, t (min) is the contact time, q_t (mg g^{-1}) is the amount adsorbate adsorbed onto adsorbent at time t , q_e (mg g^{-1}) denotes the amount adsorbed at equilibrium or equilibrium adsorption capacity. The PFO model as shown in Fig. 8. Therefore, the amount of adsorbate adsorbed by the adsorbent at equilibrium or equilibrium adsorption capacity, q_e (mg g^{-1}), is calculated by using the following equation [8]:

$$q_e = \frac{V \cdot (C_0 - C_e)}{W} \quad (2)$$

where C_0 (mg L^{-1}) is the initial liquid-phase concentration of adsorbate, C_e (mg L^{-1}) is the equilibrium liquid-phase concentration of adsorbate, V (L) is the volume of the adsorbate solution, W (g) is the adsorbent mass. The integration of (1) with initial condition ($q_t = 0$ at $t = 0$) leads to following equation [8]:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (3)$$

The slope and intercept of plot of the $\log(q_e - q_t)$ as function of 't' provides the $K_1/2.303$ and $\log q_e$; values from which K_1 and q_e can be determined. Using the linear fit of Fig. 8, we extract the values of q_e and k_1 as 9.13 mg/g and 0.097 respectively.

3.5. Pseudo-second order (PSO) model

The pseudo-second order model assumes that the rate of adsorption of solute is proportional to the available sites on the adsorbent. And the reaction rate is dependent on the amount of solute on the surface of adsorbent. The pseudo-second order (PSO) equation is given as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (5)$$

where K_2 ($\text{g mg}^{-1} \text{ min}^{-1}$) is the pseudo-second order adsorption kinetic parameter or rate constant, t (min) is the contact time, q_t (mg g^{-1}) is the amount adsorbate adsorbed onto adsorbent at time t , q_e (mg g^{-1}) denotes the amount adsorbed at equilibrium or equilibrium adsorption capacity. Integrating (5) and noting that $q_t = 0$ at $t = 0$, the following equation is obtained in 6;

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (6)$$

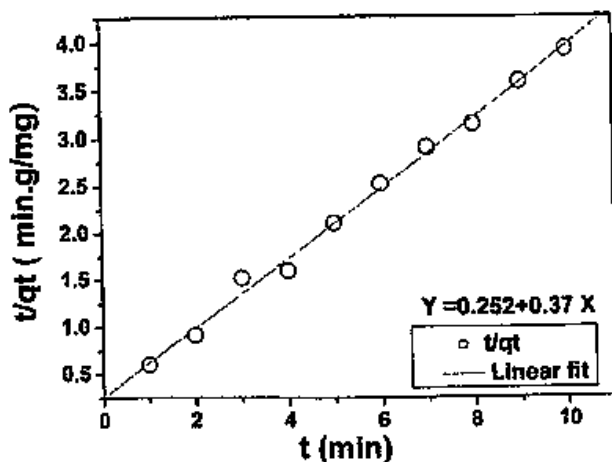


Fig. 9. Plotting of transient curve of (t/q_t) of AgSbO_3 nanotips produced by thermal evaporation process. (PSO models for the adsorption kinetics process).

The slope and intercept of t/q_t ; as a function of t plot provides the values of $1/q_e$; and $1/K_2 q_e^2$; from which q_e and K_2 can be determined. Using the plot of Fig. 9, the linear fit gives the values of K_2 and q_e which are respectively of 0.54 and 2.7 . Therefore, transient plotting shows a linear rise till 4 at 10 min. The curve slope and off set are found to be 0.369 and 0.252 respectively. The deduced parameters from Fig. 9 are a slope = 0.369 , $R = 0.994$ and the intercept = 0.252 . The variation of q_t as function of $\log t$ is fitted by linear line as shown in Fig. 10. A value of 0.032 mg/g is recorded and a slope is found to be 2.07 . The intra-particle diffusion equation is then written as;

$$q_t = k_{di} x t^{1/2} + C_1 \quad (7)$$

where k_{di} is intra-particle diffusion rate constant ($\text{mg/g min}^{1/2}$). As shown in Fig. 11, k_{di} and C_1 are respectively found to be 2.183 mg/g and -1.739 .

3.6. Coefficient of correlation

Almost given in every adsorption study, R^2 shows the degree of variability of dependent variable which is explained by all independent variables. It ranges from 0 to 1 , with values close to zero showing a perfect fit. Where q_e in mg/g , V_s volume of solution in L is, the C_0 and C_e are the initial and the equilibrium concentrations in a liquid phase (mg/l), m_{ad} is the mass of the adsorbent (g).

3.7. Thermoelectric measurements. Electronic transport property and Seebeck coefficient

The thermopower versus temperature is depicted in Fig. 12 displaying a rapid decay from $6 \mu\text{V}/^\circ\text{C}$ at 60°C to negative value at 260°C . 250 ml in 2.5 g . A quite low thermal conductivity of 0.825 W/mx K at 873 K is indicated as mentioned by sun et al., [14]. The electrical resistivity and the Seebeck coefficient 'S' are measured simultaneously from 323 to 873 K for AgSbO_3 . The temperature of semiconductor is resistivity dependent behaviour as $\rho \propto e^{C/T}$. In order to obtain thermopower a sample under test is prepared as same procedure described above. The temperature gradient is applied, and the thermoelectric voltage developed is measured apparently.

4. Electrical characterization & AFM studies

Imaginary Z'' and real Z' part of impedance is showed in cole-cole sketch as seen in Fig. 13. A semicircle, showing a radius of 80Ω , is roughly stretched. Cole-Cole plot defines approximately

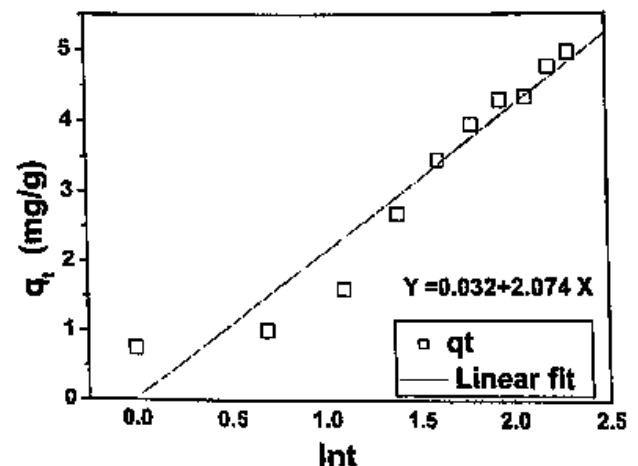


Fig. 10. Variation of q_t against $\ln t$ of AgSbO_3 nanotips.

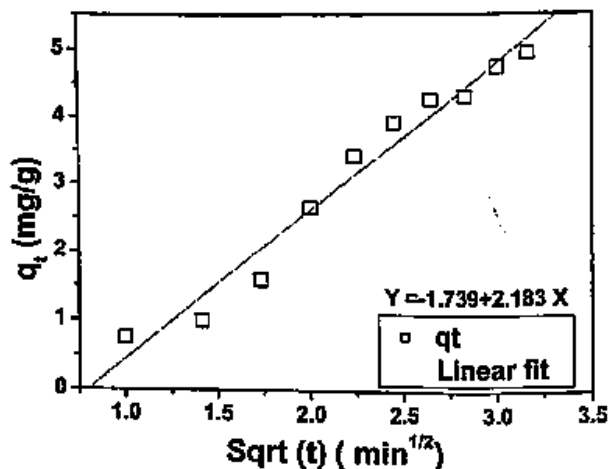


Fig. 11. The q_t parameter plots versus $\text{sqr}(t)$ of AgSbO_3 nanotips.

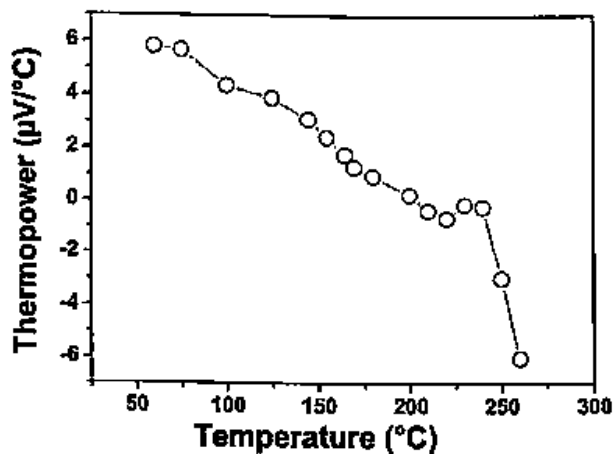


Fig. 12. Seebeck coefficient versus applied temperature of AgSbO_3 nanotips.

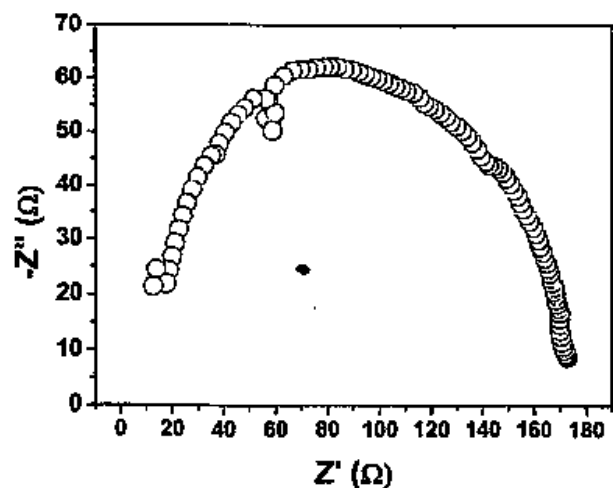


Fig. 13. Cole-Cole diagram of AgSbO_3 nanotips.

a semicircle shape which goes from 20 to 65 Ω . Negative temperature coefficient of resistance (TCR) is calculated using the following expressions;

$$\alpha_T = \frac{R_1 - R_2}{R_T(T_1 - T_2)} \tag{8}$$

$$\alpha_T = \frac{\rho_1 - \rho_2}{\rho_T(T_1 - T_2)} \tag{9}$$

where, ρ_T is the room temperature resistivity, ρ_1 and ρ_2 are the respective resistivities at temperatures T_1 and T_2 . Obtained TCR values are plotted in Fig. 14. Obtained TCR values are in the order of 10^{-3} K^{-1} . The TCR values of the film deposited at 450°C is having the maximum of -0.0922 K^{-1} . When the temperature raises from 473 K to 873 K, the temperature reduces the E, K, T and the activation energy of the electronic conductivity is nearly equal to $\rho = \rho_0 \exp(E/kT)$, where E, k, and T are the activation energy of the electronic conductivity, Boltzmann constant, and the absolute temperature, respectively. In order to understand the seebeck coefficient 'S' is usually obtained by measuring the voltage between certain temperature difference as defined as $S = dV/dT$. Negative sign of S (T) over the whole measured temperature range indicates that AgSbO_3 is an n-type semiconductor [10]. The cole-cole plot as shown in Fig. 13. A shape looks like roughly a semicircle having a diameter of 170Ω in complex Z'' , Z' plane. Hence no Dc-conductivity, which would be estimated in the occurrence of an electronic charge motion, could be established as reported by Wiggers et al., [11]. In general, the diameter of the semicircle is associated to the shunt resistance of the device and characterizes the recombination resistance of the separated carriers in the AgSbO_3 compound. This detail is in well agreements of those reported previously [12]. Atomic force microscope observation of AgSbO_3 nanotips produced by thermal evaporation process is investigated as shown in Fig. 15. The scanned 2D and 3D AFM pictures show the nanotips texture. The nanotips grow along the c-axis and the vertical cross section is displayed in the bottom of 2D AFM images [13].

5. Conclusion

The AgSbO_3 thin film is fabricated by low cost coprecipitation technique and is coated on ITO substrate by thermal evaporation process. X-rays analysis confirms the cubic crystalline structure. The transmittance increases up to 82% in UV range and an average of E_g is about 3.6 eV and absorption coefficient varies from 0.8 to $20 \times 10^{-3} \text{ nm}^{-1}$. FTIR analysis records three peaks located respectively at 653.76 cm^{-1} , 1243.88 cm^{-1} and 2360.48 cm^{-1} . PFO models for the adsorption kinetics of AgSbO_3 is displaying a decay profile. Thermopower is lowering with increase in temperature within the $50^\circ\text{C} - 300^\circ\text{C}$ range. Nyquist diagram exhibits roughly semicircle and its diameter is associated to the shunt resistance of device. TCR versus temperature exhibits a decline within the $150^\circ\text{C} - 450^\circ\text{C}$ range. Nanotips texture of AgSbO_3 is revealed by the AFM scanned 3D images.

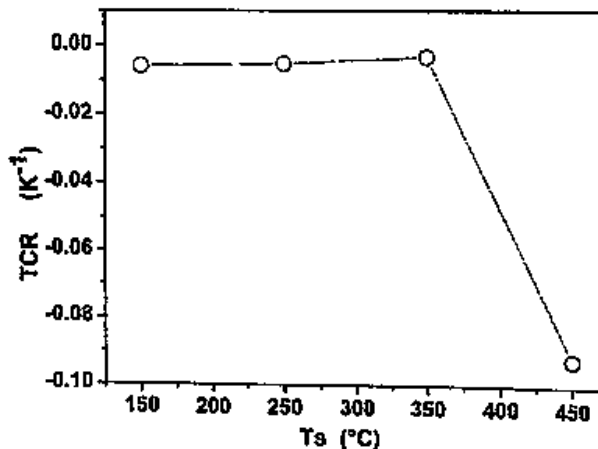


Fig. 14. TCR vs. substrate temperature of AgSbO_3 nanotips.

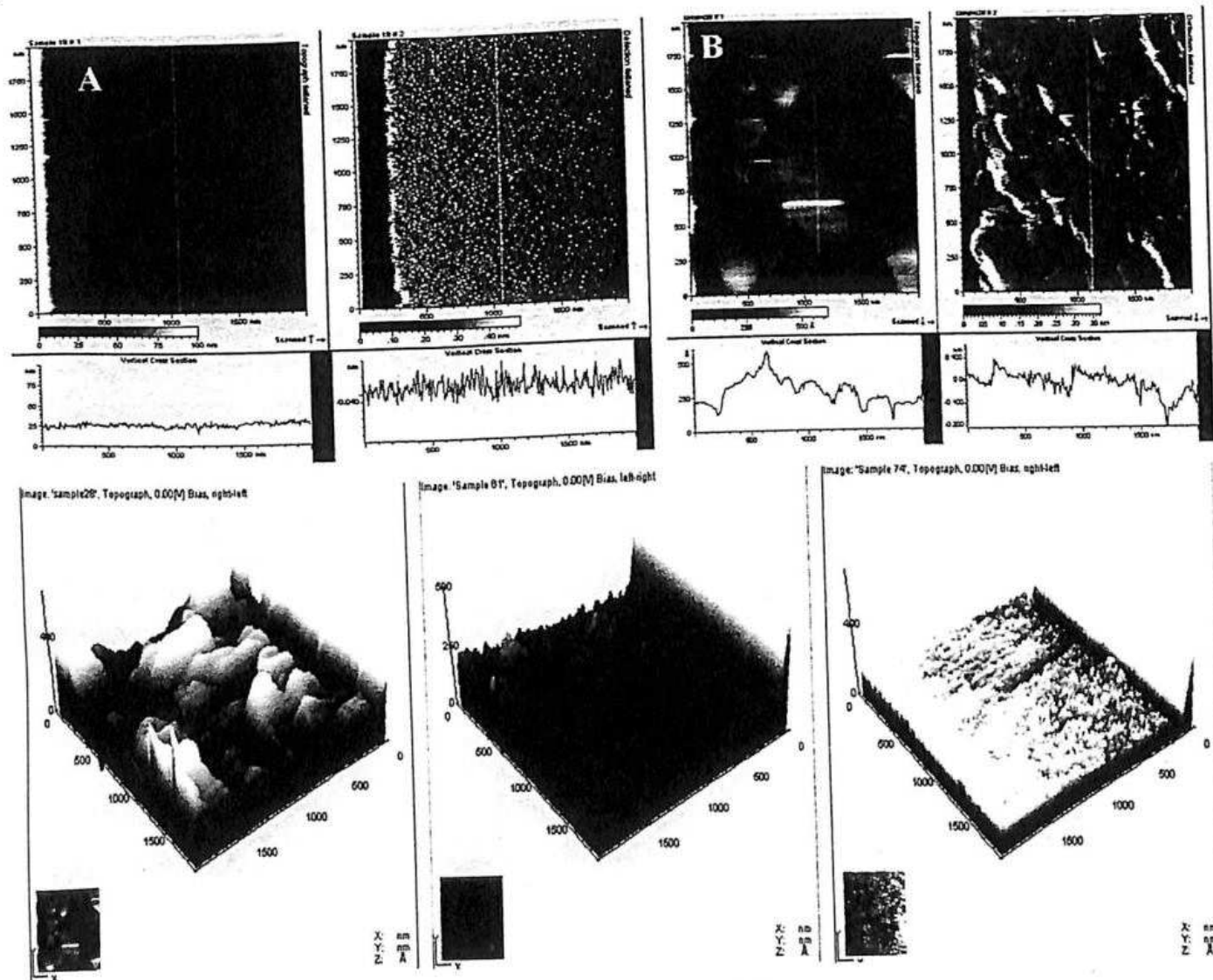


Fig. 15. 2D and 3D AFM scanned pictures of AgSbO_3 nanotips.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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From the Editor's Desk

We are happy to present to you the second issue of the second volume of DJAME Journal of Management Research. Thank you for the support and for being a part of the journey. We are aware of the challenges in maintaining quality of the papers published. I thank all my colleagues associated with editorial work for the immense help they have offered towards this publication. Our heartfelt thanks to our reviewers who help us in selecting good papers. We also sincerely thank the authors for their contributions.

In this issue we have included a mix of articles touching upon topics related to stock market, online shopping, softwares and green HRM. Discernment of the customer is a study conducted by S.Viji, which aims to find the perception of investors investing in the stock market towards the quality of service provided by the brokerage firms with reference to Coimbatore city.

The article by M.Devi, R.Chipichakkaravarthy, and S.Jaisankar attempts to identify the influencing factors in buying products online by millennial women and the decisional aid that determines the purchase decision. The work by Jeromia Muthuraj. R and Mohamed Ashik. A, tries to find a solution for identifying software which provides reliable and failure-free operations.

The entire country faced lock down when the Green HRM practices laid its way to overcome the pandemic situation. The conceptual paper by D.Saraswathi and J.J.Savithri primarily focuses on briefly understanding green HRM practices followed by organizations from the reviews made by different researchers. Also, explaining in detail the benefits of Green HRM practices to the organizations.

We request the readers to offer suggestions based on their critical evaluation and help us in improving the academic value of the journal.

Dr.J.J.Savithri

Editor

A STATISTICAL METHODOLOGY FOR IDENTIFYING RELIABLE SOFTWARE

Jeromia Muthuraj, R* and Mohamed Ashik, A**

ABSTRACT

Software plays a significant role in managing government and commercial operations. Through the support of software, anyone can manage and maintain their work easily and eliminate human errors. Nowadays there is competition among completions between the software developer's for developing failure-free and reliable software. If there are two or more softwares for the same purpose, the problem is in finding a reliable software by its performance. This work found a solution, for identifying the software which provides reliable and failure-free operations. The methodology was defendable with the statistical illustrations.

Keywords: Software reliability, ANOVA and Duncan's multiple range tests.

INTRODUCTION

The word software was first used in the late 1960's to emphasize its difference from computer hardware, hardware can be physically observed by the user. Computer software is a set of commands that tells a hardware system (computer) how to perform a task. Types of computer software can be portions into three main groups depending on their usage and application. These are system software or operating system simply referred to as the OS (Operating System), application software and programming languages. System software or operating system is the software used by the computer to translate inputs from various sources into a language which a machine can understand. Application software is Microsoft office suite which includes Word, Excel and PowerPoint. Internet Explorer, Mozilla Firefox, etc., are the applications used to access the internet.

This is a kind of computer software which is used exclusively by computer programmers. Necessarily, all the humans in this world are using the applications of the software in their day today life. Developing software is a challenging task. The challenge arises basically from the characteristics of the software itself, and because of these characteristics, the software can be said to prone towards containing faults.

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In this modern world, the applications of the software are very much essential of industry, government sector and private company. Nowadays in this technological world approximately 62% of people are using mobile phones. Mobile phones working with the support of the software. Mobile phone users also expect failure-free or reliable software. Nowadays there are many software application was developed by the software developers. There are competitions between the software developers. This paper took a problem with how to identify failure-free or reliable software if there are two or more software for the same purpose. How to identify failure-free software is the key problem. If there is more than one software for the same purpose then have to identify the reliable one for the purpose. Here it is proposed that a statistical methodology with failure information's of the software.

DATA AND ITS BACKGROUND

Justifying the methodology is very much essential in any research. The information was collected from a company which is doing commercial business. The company develops software to manage all the activities in the business, like production, stock, accounting, and employee's information. The requirements of the software were given to three different software developers to develop software with the given requirements. The software developed for the same purpose from three different software developers. The software from three different developers should be tested. The software was tested with three equal configured computer systems and the number of failures was recorded in every six hours of testing the software. The failure information is considered for the construction of statistical analysis.

METHODOLOGY

Analysis of variance

The analysis of variance (ANOVA) is used to test the significance of the difference among sample means.

Table 1: ANOVA Table for One-Way Classification

Source	Sum of Squares	Degrees of Freedom	Mean Square (Variance Estimate)	F Ratio
Between	SSB	$K - 1$	$MSB = SSB/K-1$	$F = MSB/MSW$
Within	SSW	$N - K$	$MSW = SSW/N-K$	
Total	$SST = SSB + SSW$	$N - 1$		

Where, N = Total Sample Size, K = Number of groups (treatments), SSB = Sum of squares between the groups, SSW = Sum of squares within the groups, SST = total Sum of squares, MSB = Mean sum of squares between the groups, MSW = Mean sum of squares within the groups, F = ANOVA Coefficient (F-Statistic). The one-way ANOVA is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. The main objective of analysis of variance technique is to examine if there is significant difference between the class means in view of the inherent variability within the separate classes. In one-way classification, the data are classified according to only one criterion. Specifically, it tests the null hypothesis: $H_0 = \mu_1 = \mu_2 = \dots = \mu_k$; Alternative hypothesis: $H_1 \neq \mu_1 \neq \mu_2 \neq \dots \neq \mu_k$; Where μ = group mean and k = number of groups. If one-way ANOVA yields a statistically significant result, that is the value of p is less than the significant level then accept the alternative hypothesis (H_1), which is that there is a significant difference between the groups.

Duncan's multiple range tests

Duncan's Multiple Range Test (DMRT) was initially designed by David B. Duncan as a higher-power alternative to Newman-Keuls. DMRT is more useful than the Least Significant Difference (LSD). In ANOVA, if H_0 is rejected, the results will tell there is a difference in means of the groups. However, it won't pinpoint which means are different. DMRT is a post hoc test to measure specific differences between pair of means.

RESULTS AND DISCUSSION

The data set has three variables SW1, SW2 and SW3. Each variable has 28 information's, which is the number of failures of the software in every six hours. ANOVA is a standard statistical method adopted for one-way and two-way classified data provided the underlining populations from which the data arise satisfies certain distributional assumptions, such as the parent populations are normal. Since the hypothesis is to test null (H_0) against an alternative hypothesis (H_1).

H0: There is no significant difference between the failures of the software from three different software developers.

Table 2: ANOVA table for compare means of different software

Source	Sum of Squares	Degrees of freedom	Mean Square	F - statistic	Sig. (p)
Between SW	107.167	2	53.583	12.434	.001
Within SW	349.071	81	4.310		
Total	456.238	83			

According to Table 1, the value of the ANOVA test statistic is calculated as 12.434 with significant p-value 0.001. Since the p-value obtained by lowest one, it may be decided that the data has poor evidence in favor of rejection of H0. Therefore, there is a difference between the performance levels of the software from three different software developers.

Table 3: Duncan's multiple range tests results

Software	Number of failures	Subset for alpha = 0.05	
		1	2
3	28	3.75	
1	28		5.57
2	28		6.46
Sig.		1.000	.111

Already, it is proved that there is a significant variation between the performances of the software from three different software developers. DMRT is used to verify which software is performing better based on their failure history. Table 3 gives the detail about DMRT for the software. Based on the outcome of DMRT the software is from developers three is performing enhanced than the other two companies.

CONCLUSION

The study found software from developer three may be accepted for the use of the commercial business. if there are more number of software for the use of the same purpose we have to check or test whether which is more appropriate for apply with the given circumstance and environment. This study recommends this statistical methodology is more suitable and appropriate to identify the best software.

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LINEAR MODEL FOR SHARE MARKET PREDICTION
"AN INVESTMENT IN KNOWLEDGE PAYS THE BEST INTEREST" – BENJAMIN
FANKLIN

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ABSTRACT

In this article, the future forecast for stock market price is calculated using the exponential smoothing method (linear model) also discusses changes in the stock market. There are two types of smoothing methods used: Holt and Brown. The Holt method has a lower percentage error than the Brown method. Therefore, the Holt method is the better for prediction results. Some statistical tools have been used to confirm this. The current global downfall will affect the Indian stock market in the short time period.

Key words: Stock Market, Holt Model, Brown Model

INTRODUCTION

The stock market is an important sector that can always be hectic. One of the forces that determine a country's economy is the stock market. Everyone knows that it is challenging to know the future of stock market prices because every policy decision the government makes will affect the stock market. The Indian stock market is one of the most important in global trade, and the national stock market has always had a special respect for India's two most important stock markets. The National Stock Market is the first fully computerized stock market in India. In this paper, we take the daily closing stock price data of the five types of stock market sectors and find out their trend using an exponential smoothing approach. Here the results are obtained separately for the two types of exponential smoothing techniques (Holt and Brown). Gardner (1985) applied double exponential smoothing method and discuss with seasonal data. Holt (2004) provided a systematic development of the predicting expressions for weighted moving averages. It is discussed by seasonal and trend patterns of time series. Pal et al (2007) used Box-Jenkins and Holt Models for forecasting the Milk production. Binti et al (2016) forecast stock market price for two companies that related with gas and oil sector using smoothing techniques. Mohamed Ashik and Senthamarai Kannan (2018) the Nifty 50 closing stock market prices were computed and predicted using time series modelling methods, like exponential smoothing and autoregressive integrated moving average. In this article, we take the daily closing stock price (Rs.) data of the Auto Mobile, Bank, Financial Service, Metal, Oil & Gas sectors and apply the two types of exponential method. Using the Holt and Brown exponential methods, it has been found that any exponential method is more suited to stock market price forecasting than the other one. The data is taken from the Nifty50 website, which is approved by the Government of India. The data period is from 01.01.2019 to 31.12.2019 with 245 observations.

METHODOLOGY

Exponential Smoothing method is one of the types of time series analysis. This helps to achieve accurate results by smoothing the fluctuations in the data. Although there are many types, only the Simple, Holts and Brown methods have been applied to this article. Simple exponential smoothing is an easiest and predicting method for time series analysis. It can only be used to find one step ahead forecasts. In this case, the trend of the data and the seasonal change is not predicted. Therefore, this paper describes the methodology and prediction of the applicability of the data using only Holt and

Brown exponential smoothing methods. There is no doubt that these two methods of data are accurately predictive of future trends and seasonal changes. Holt (1957) extended simple exponential smoothing to allow the forecasting of data with a trend. The Holt forecasting model of data consists of both an exponentially smoothed level component (L_t denoted as α) and a trend component (T_t denoted as β). The level is a smoothing technique used to reduce irregularities (random fluctuations) in time series data, thus providing a clear view of the true underlying behaviour of the series. The trend represents the underlying growth in a time series. The trend component is used in the calculation of the exponentially smoothed value. The equations for the above two methods are given below.

$$L_t = Y_t + (1 - \alpha)(L_{t-1} + T_{t-1})$$

$$T_t = \beta(L_t - L_{t-1}) + (1 - \beta)T_{t-1}$$

The above equations require two smoothing constants, α and β each of which is between 0 and 1. As before control the smoothness of L_t a choice near 0 places more emphasis on past values of the time series while a value of near 1 gives more weight to current values of the series, and deemphasizes the past. [Level smoothing factor: $0 < \alpha < 1$, Trend smoothing factor: $0 < \beta < 1$]. The Brown method is not much different from the Holt method. Both are similar to a double-pistol gun, and Brown has both Trend and Level smoothing factors. The Brownian equations are inherently in SPSS software and can be obtained directly. For the one step ahead,

$$F_{t+1} = L_t + T_t$$

The forecast for two steps ahead,

$$F_{t+2} = L_t + 2T_t$$

Similarly, the k^{th} step ahead forecast,

$$F_{t+k} = L_t + KT_k$$

RESULTS AND DISCUSSION

In Figure 1 you can see whether there are outliers between the data. For this, Box-Plot statistical graphics tool is used. It is confirmed in Figure 1 that there is no outlier for all five dataset.

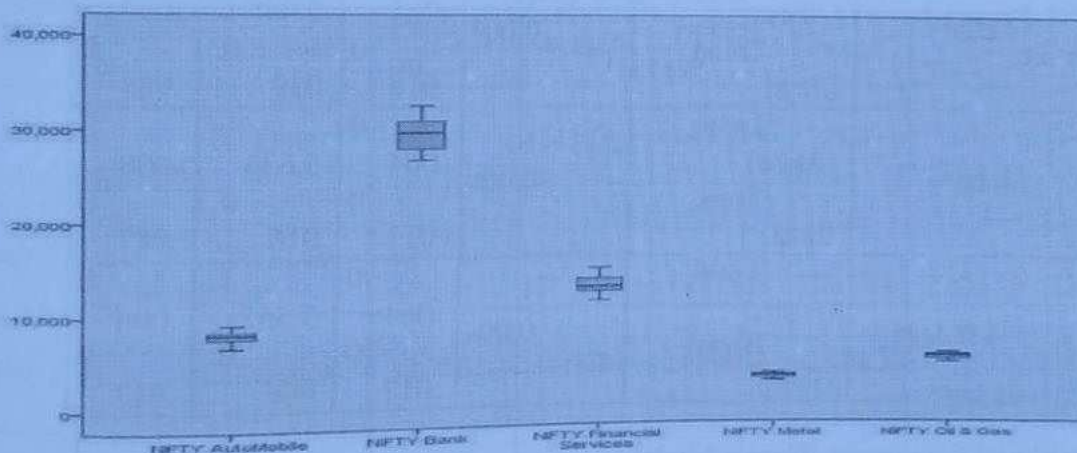


Figure 1: Box-Plot of Stock Price Dataset

Table 1: Model Statistics (Holt & Brown)

Sector	Holt's		Brown	
	R	M	R	M
Auto	0	1.	0	1.
Mobile	.957	.093	.947	.214
Bank	0	0.	0	0.

	.953	884	.941	967
Financial	0	0.	0	0.
Service	.975	793	.968	885
Metal	0	1.	0	1.
Oil & Gas	.970	301	.964	421
	0	0.	0	1.
	.959	955	.950	060

In Table 1, the R^2 and the Mean Absolute Percentage Error values are shown using exponential smoothing method for the five sectors. The smoothing factor values (level & trend), estimate, t-value and significance values are shown in table 2 & 3. The model statistics obtained by the Holt method are better than the one (Brown). Holt method MAPE value is lower than Brown method. So, Holt is more appropriate for stock price dataset.

Table 2: Model Parameters (Holt Model)

Sector	Component	Estimate	SE	T	Significance	
Auto Mobile	Level (Alpha)	1	0.00	.064	5.713	.000
	Trend (Beta)	1	0.00	.009	0.097	.923
Bank	Level (Alpha)	1	0.00	.064	5.510	.000
	Trend (Beta)	1	0.00	.012	0.068	.946
Financial Service	Level (Alpha)	1	0.00	.065	5.443	.000
	Trend (Beta)	1	0.00	.018	0.039	.969
Metal	Level (Alpha)	1	0.00	.064	5.633	.000
	Trend (Beta)	1	0.00	.012	0.016	.987
Oil & Gas	Level (Alpha)	1	0.00	.064	5.552	.000
	Trend (Beta)	1	0.00	.012	0.066	.947

Table 3: Model Parameters (Brown Model)

Sector	Component	Estimate	SE	T	Significance	
Auto Mobile	Level & Trend (Alpha)	61	0.5	.029	9.586	.000
Bank	Level & Trend (Alpha)	31	0.5	.028	8.792	.000
Financial Service	Level & Trend (Alpha)	60	0.5	.029	9.468	.000
Metal	Level & Trend (Alpha)	78	0.4	.027	7.506	.000

Oil & Gas	Level & Trend (Alpha)	0.5	0	1	0
		47	.029	9.169	.000

Figure 2 represents standard error between the predicted and actual data. It is also clear that forecast data is keeping increase with the future. The standard error difference for the actual data and large, it is possible to determine whether smoothing method is optimized for stock market price data. In terms of stock market data, the Holt Method has the potential to be an accurate forecast. Therefore, decisions made using the Holt method can help investors access less risk and reach a decisive decision about the future. The forecast graph of two exponential smoothing methods for actual dataset is shown in Figure 3.

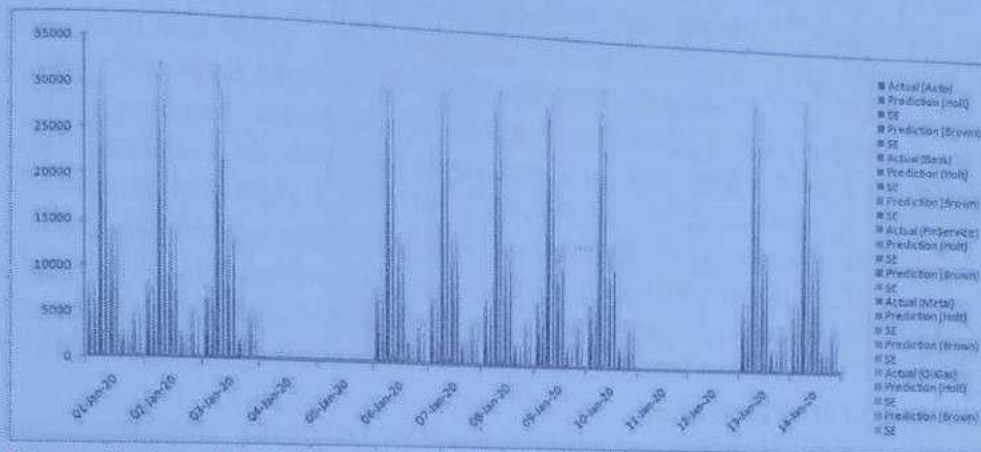


Figure 2: Standard Error of Actual & Prediction Dataset

Table 4: Actual and Prediction of Holt Model

Date	Auto		Bank		Financial Service		Metal		Oil & Gas	
	Actual	Prediction	Actual	Prediction	Actual	Prediction	Actual	Prediction	Actual	Prediction
01-Jan-20	8210.1	8244.22	32102.9	32176.6	14574.2	14568.36	2796.05	2798.93	5173.3	5175.98
02-Jan-20	8267.45	8240.14	32443.85	32191.56	14697.5	14579.3	2869.9	2796.56	5224.22	5177.77
03-Jan-20	8168.15	8236.05	32069.25	32206.51	14560.7	14590.25	2848.35	2794.19	5217.22	5179.56
06-Jan-20	7978.75	8231.97	31237.15	32221.46	14202.5	14601.19	2765.75	2791.82	5099.56	5181.35
07-Jan-20	8002.5	8227.89	31399.4	32236.42	14314.75	14612.14	2785.9	2789.44	5119.1	5183.14
08-Jan-20	7942.75	8223.81	31373.65	32251.37	14306.65	14623.09	2767.25	2787.07	5082.28	5184.93
09-Jan-20	8155.25	8219.72	32092.4	32266.32	14590.1	14634.03	2806.8	2784.7	5165.84	5186.72

10-Jan-20	8225.5	8215.64	32097.4	32281.27	14609.65	14644.98	2840.75	2782.33	5167.16	5188.5
13-Jan-20	8235.85	8211.56	32177.65	32296.23	14635.05	14655.93	2874.1	2779.96	5192.69	5190.29
14-Jan-20	8289.3	8207.47	32071.65	32311.18	14667.2	14666.87	2900.65	2777.59	5196.52	5192.08

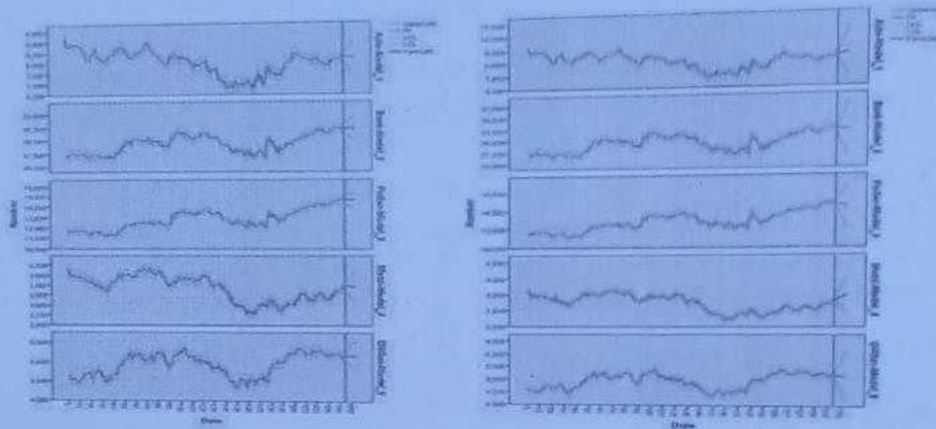


Figure 3: Forecast Graph of Holt & Brown

CONCLUSION

In this article, we take the daily stock market price data for the two types of exponential smoothing method and analyze the model validity results using appropriate methods. In the above results, the Holt Model is more likely to achieve the better result. The results of the article vary depending on the ruling government's decision policy. The current global downturn will affect the Indian stock market in the short term.

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Indian Stock Price Forecast Using Tpm Method

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Abstract:- Stock market trading plays an important role for economic growth in every developing country. India is one of the largest and electronic stock market traders in the world. Reliance Group is one of the largest commercial investors in India. In this work we find the Reliance Communication Ltd (RCOM) weekly stock trade price forecast using Transition Probability Matrix (TPM) method. From the results, it is more likely that the Small Decrease (SD) trend will be come the future weeks because it has a higher order probability chance.

Keywords:- Stock Price, Markov Chain, Higher Order TPM Matrix.

I. INTRODUCTION

The stock market sector plays an important role in a fast moving economy. The stock market in our country is the oldest and busiest. I have taken this topic to see how the future price of stock price data is set using TPM method. In a book by Taylor and Karlin (1998), A stochastic process is a family of random variables X_t , where t is a parameter running over a suitable index set T (where convenient, write $X(t)$ instead of X_t). The Markov chain is the main method underlying the stochastic process. Generally, the time series model takes into account all of the past events (data) and makes predictions. The Markov chain helps to obtain the results of future data using only present and previous present data. The uniqueness of this method is that it detects future data trends without using past data. This method was introduced in 1907 by A. A. Markov. This method is known by the following mathematical formula.

$$\Pr \{X_{n+1} = j | X_n = i, X_{n-1} = i_{n-1}, \dots, X_1 = i, X_0 = i_0\} \dots (1)$$

The important thing about Markov chain is that it converts data into matrices and then evaluates it. Due to the use of stock market data in this paper, this passage is

$$\begin{matrix} LD \\ SD \\ NC \\ SI \\ LI \end{matrix} \begin{bmatrix} 0.2 & 0.5 & 0.2 & 0.1 & 0 \\ 0.063 & 0.429 & 0.190 & 0.302 & 0.016 \\ 0 & 0.346 & 0.154 & 0.423 & 0.077 \\ 0.085 & 0.362 & 0.149 & 0.298 & 0.106 \\ 0 & 0.6 & 0 & 0.2 & 0.2 \end{bmatrix} P_{RCOM} = \begin{bmatrix} 0.200 & 0.500 & 0.200 & 0.100 & 0 \\ 0.063 & 0.459 & 0.160 & 0.302 & 0.016 \\ 0 & 0.356 & 0.154 & 0.443 & 0.077 \\ 0.085 & 0.352 & 0.149 & 0.278 & 0.106 \\ 0 & 0.600 & 0 & 0.200 & 0.200 \end{bmatrix}$$

presented in the research article of analysts who used the Markov chain method for similar stock market data. Onwukwe and Samson (2014) applied a Markov model for long-term behaviour of the closing prices of the Nigerian Bank. Huang *et al* (2017) integrated two types of Markov Chain (regular and absorbing) are used in HTC (Taiwan) stock. Singh *et al* (2017) evaluated a Markov approach in opening stock price change prediction of Nifty50. Ashik *et al* (2019) applied many statistical tools of indian stock market daily price and forecast that data. Reliance Communication Limited's week closing stock prices data are used in this paper. The duration of these data is two years (ie from June 2016 to May 2018). A total of 157 observations and these data were downloaded from the Bombay Stock Exchange website.

II. ANALYSIS AND DISCUSSION

Reliance Communications has used the MC method to calculate the stock's forecast for the week close price. First, we need to find the markovian difference for the data. The results are obtained using the formula $d_t = Y_t - Y_{t-1}$ to find the Markovian difference. MC difference is to subtract the previous week's price from the current week's price. Where Y_t is the stock price of the current week and Y_{t-1} is the stock price of the previous week. The total number of available data is currently less than the original data. So now the total number of data is 156.

Construct of the TPM Matrix:

Markov difference data is divided into 5 states and given the range. They are LD ($d_t < -5$), SD ($-5 \leq d_t < 0$), NC ($d_t = 0$), SI ($0 < d_t \leq 5$) and SI ($d_t > 5$). [LD – Large Decrease, SD – Small Decrease, SI – Small Increase, LI – Large Increase and NC – No Change.] The values of the 5 states in this data are 11, 64, 09, 48 and 24 respectively. Then, the data is converted to matrix format and the corresponding TPM matrix is found. All the results for this are shown in the matrix format below.

Higher-Order Transition Matrices for RCOM:

The higher-order TPM matrix can be used to find the equilibrium for data. An equilibrium (or Stable or stationary or invariant) distribution is a specific entity which is

unchanged by the effect of some matrix or operator, it need not be unique. Thus stationary distributions are related to eigenvectors for which the eigenvalue is unity. Higher order TPM occurred by equilibrium state of that data.

$$P_{RCOM}^{(2)} = \begin{bmatrix} 0.800 & 0.419 & 0.180 & 0.285 & 0.034 \\ 0.065 & 0.400 & 0.168 & 0.309 & 0.056 \\ 0.057 & 0.401 & 0.152 & 0.311 & 0.077 \\ 0.065 & 0.420 & 0.153 & 0.290 & 0.070 \\ 0.054 & 0.449 & 0.143 & 0.280 & 0.070 \end{bmatrix}$$

$$P_{RCOM}^{(3)} = \begin{bmatrix} 0.064 & 0.410 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.410 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.411 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.410 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.410 & 0.160 & 0.300 & 0.063 \end{bmatrix}$$

$$P_{RCOM}^{(4)} = \begin{bmatrix} 0.064 & 0.409 & 0.161 & 0.301 & 0.062 \\ 0.064 & 0.410 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.411 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.411 & 0.160 & 0.300 & 0.063 \\ 0.064 & 0.410 & 0.160 & 0.301 & 0.063 \end{bmatrix}$$

$$P_{RCOM}^{(6)} = \begin{bmatrix} 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \end{bmatrix}$$

$$P_{RCOM}^{(7)} \dots = \begin{bmatrix} 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \\ 0.065 & 0.430 & 0.140 & 0.300 & 0.065 \end{bmatrix}$$

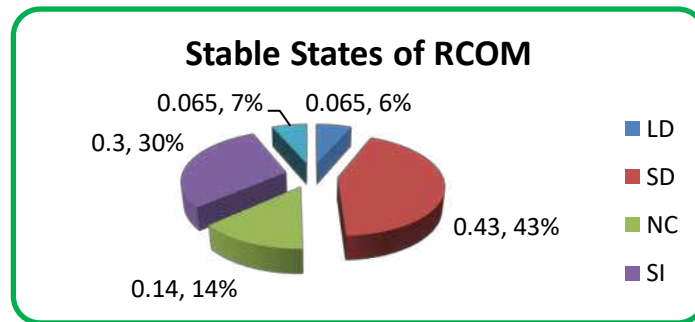
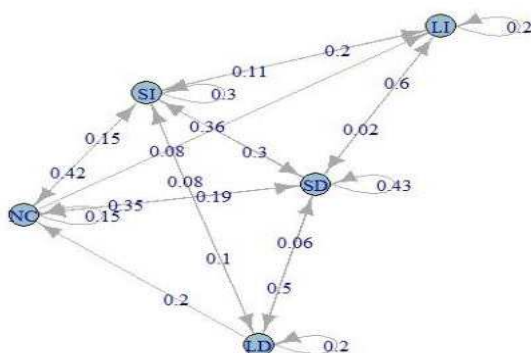


Figure 1: Pie-Chart of Equilibrium States of RCOM

The probability values for the invariant in Figure 1 are illustrated by the pie chart. The value of SD probability is that it can be seen in large part. The probability values for states can be found by higher-order TPM. Its values are 0.065, 0.430, 0.140, 0.300 and 0.065, respectively. The transition graph for the RCOM weekly stock price data is depicted in Figure 2.

Figure 2: Transition graph of Markov Chain for RCOM

RCOM weekly close price - TPM (Five States Diagram)



Forecasting: In this study, the five different states of closing price are considered which are large decrease, small decrease, no change, a small increase and large increase. An Initial State Vector (ISV) is needed to predict the future of data using the Markov Chain method. The initial state vector gives the probability of the five different states. If the state vector is denoted by $\pi^{(0)} = (\pi_1, \pi_2, \pi_3, \pi_4, \pi_5)$ then $\pi_1, \pi_2, \pi_3, \pi_4$ and π_5 gives the probability of large and small increase (up), large and small decrease (down) and remain no change: $\pi_1 = 13/156 = 0.083$; $\pi_2 = 69/156 = 0.442$; $\pi_3 = 22/156 = 0.141$; $\pi_4 = 41/156 = 0.263$ and $\pi_5 = 11/156 = 0.071$. Thus the initial state vector of RCOM stock price is

$$\pi^{(0)} = [0.083 \quad 0.442 \quad 0.141 \quad 0.263 \quad 0.071]$$

Then, to predict the probability of future data, IVS should be multiplied in the TPM. The state probabilities of the closing stock price of RCOM (157th week):

$$\pi^{(1)} = \pi^{(0)} * P_{RCOM} = [0.083 \quad 0.442 \quad 0.141 \quad 0.263 \quad 0.071] * \begin{bmatrix} 0.200 & 0.500 & 0.200 & 0.100 & 0 \\ 0.063 & 0.459 & 0.160 & 0.302 & 0.016 \\ 0 & 0.356 & 0.154 & 0.443 & 0.077 \\ 0.085 & 0.352 & 0.149 & 0.278 & 0.106 \\ 0 & 0.600 & 0 & 0.200 & 0.200 \end{bmatrix}$$

$$\pi^{(1)} = [0.076 \quad 0.440 \quad 0.142 \quad 0.268 \quad 0.074]$$

Similarly, the state probabilities of the closing stock price of RCOM (158th week) is given below:

$$\pi^{(2)} = \pi^{(1)} * P_{RCOM} = [0.076 \quad 0.440 \quad 0.142 \quad 0.268 \quad 0.074] * \begin{bmatrix} 0.200 & 0.500 & 0.200 & 0.100 & 0 \\ 0.063 & 0.459 & 0.160 & 0.302 & 0.016 \\ 0 & 0.356 & 0.154 & 0.443 & 0.077 \\ 0.085 & 0.352 & 0.149 & 0.278 & 0.106 \\ 0 & 0.600 & 0 & 0.200 & 0.200 \end{bmatrix}$$

$$\pi^{(2)} = [0.075 \quad 0.442 \quad 0.140 \quad 0.270 \quad 0.073]$$

The state probabilities of the closing stock price of RCOM (159th & 160th weeks):

$$\pi^{(3)} \pi^{(4)} = [0.075 \quad 0.443 \quad 0.140 \quad 0.270 \quad 0.072]$$

Table 1: Comparison of Actual and Forecast

Date	Actual			Prediction		Decision
	Price	Difference	State	Higher (%)	State	
30-12-2018	35.00					
06-01-2019	32.40	-2.60	SD	44.2	SD	Correct
13-01-2019	30.25	-2.15	SD	44.0	SD	Correct
20-01-2019	29.10	-1.15	SD	44.2	SD	Correct
27-01-2019	27.90	-1.20	SD	44.3	SD	Correct

III. CONCLUSION

The evaluated Markovian difference and construct the TPM of RCOM data. The higher-order TPM and ISV were calculated for the data. These data appropriate fit into the Markov Chain method because our prediction data and the original data are equal. In table, there is negligible difference between the two (original and prediction) data. Its results may be affected by certain policies taken by the government.

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பதிற்றுப்பத்து காட்டும் சேரர் வரலாறு

முனைவர் சு. சீலா

உதவிப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக் கல்லூரி (தன்னாட்சி),
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ஆய்வுச் சுருக்கம்

முவேந்தர்களில் சேர மன்னர்களின் சிறப்பு, சேரநாட்டின் எல்லை, சேரக்குடிப் பெயர், உதியன் குடும்பத்தைச் சார்ந்த சேரர்கள், பொறையன் குடும்பத்தைச் சார்ந்த சேரர்கள் ஆகியோர்களின் வெற்றிச் சிறப்புகள் குறித்து ஆராயப்பட்டுள்ளன.

முன்னுரை

பரத கண்டத்தின் தென்கோடியில் உள்ள நிலப்பகுதி, தொன்மைக் காலமாகவே சேர, சோழ, பாண்டியர் என்ற முவேந்தர்க்கும் உரியதாக இருந்து வந்தது. தமிழகத்தை ஆட்சி புரிந்த அம்முவேந்தர்களில் சேரவேந்தரே நன்கு மதிக்கப் பெற்றவர். அம்மதிப்புக் காரணம் அவர் நாட்டின் விரிவும், வளமும், வீரமும் கொடையும் ஆகும். இத்தகு சிறப்பு வாய்ந்த சேர மன்னர்களைப் பற்றி இக்கட்டுரையில் ஆராயப்படுகிறது.

சேர நாட்டின் எல்லைகள்

ஆரியர்கள் இந்தியாவிற்கு வருவதற்கு முன்பு தமிழ்நாடு,

“வடாஅது பனிபடு நெடுவரை வடக்கும்”
தெனாஅது உருகெழு குமரியின் தெற்கும்
குணாஅது கரைபொரு தொடுகடல் குணக்கும்
குடாஅது தொன்றுமுதிர் பெளவத்தின் குடக்கும்”

“தென்குமரி வடபெருங்கடல் குணகுடகடலா
எல்லை”

வடக்கே இமயமலையையும், தெற்கே குமரியையும் கிழக்கே வங்காள விரிகுடாக் கடலையும், மேற்கே அரபிக்கடலையும் எல்லையாகக் கொண்டிருந்தது.

சேரர் குடி

செந்தமிழ் வேந்தர் முவருள் சேரர் குடியினுள் முதலில் ஆண்டவர் யார் என்பதற்கான சான்றுகள் கிடைக்கவில்லை. “சோழர் கதிரோன் மரபினராகவும்” பாண்டியன் திங்கள் மரபினராகவும் (சிலம்பு. 11:23) நூல்களில் காணப்படுவதுபோல் சேரவேந்தர் இன்ன மரபினர் என்பதை நூல்களில் காணமுடியவில்லை. பிற்காலத்தோர் சேரவேந்தரைக் “கனலோன் குலத்தவராக” வழங்கினர் என்பதற்குரிய தமிழ்ச் சான்றுகள் கிடைக்கவில்லை. ஆயினும், வானவர் என்ற பெயரால் பண்டை நூல்களும் நிகண்டுகளும், சிலப்பதிகாரமும், பதிற்றுப்பத்தும் குறிக்கின்றன. இவர் குடி முற்காலத்தே தெய்வத் தொடர்பு பெற்றதென்றும் கூறுவர். “வானவர் என்ற பெயர் சீனர்க்கு இன்றும் வழங்கி வருதலால் சேரர் முதலில் சீன நாட்டிலிருந்து வந்தவராகக் கனகசபைப் பிள்ளை கருதினார்.”

பதிற்றுப்பத்துக் காட்டும் சேரமன்னர்கள்

சங்க இலக்கியத்தில் பல சேர மன்னர்கள் பற்றிய குறிப்புகள் வருகின்றன என்றாலும் பதிற்றுப்பத்தினுள் பாடப் பெற்றவர்கள் பத்துப்பேர்கள் ஆவார். இந்தப் பத்துச் சேர மன்னர்களைப் பற்றிப் பாடப் பெற்ற தொகுதியே பதிற்றுப்பத்து என்ற நூலாகும். இதில் முதல் பத்தும் இறுதிப்பத்தும் கிடைக்கவில்லை. எஞ்சிய எட்டுப் பத்துக்களைப் பாடிய சேர மன்னர்களைப் பற்றிப் பதிற்றுப்பத்து எடுத்துக் கூறுகிறது. அதன் அடிப்படையில் இவ்வியல் ஆராயப்படுகிறது.

உதியன் குடும்பத்தைச் சார்ந்தவர்கள்

(உதியஞ் சேரலாதன் கி.பி. 25-65)

உதியஞ்சேரலாதன் வெளியன் வேண்மான்
என்னும் குறுநில மன்னனுடைய மகள்

நல்லினியை மணந்து கொண்டான். இவனுக்கு இரண்டு மகன்கள். மூத்த மகன் இரண்டாம் பத்தின் தலைவனாகிய இமயவரம்பன் நெடுஞ்சேரலாதன்; இளைய மகன் மூன்றாம் பத்தின் தலைவனாகிய பல்யானைச் செல்கெழு குட்டுவன் ஆவான். இவ்வுதியஞ் சேரலாதன் முதியர்ப் பேணிய உதியஞ்சேரல் என்றும், நாடுகண் அகற்றிய உதியஞ்சேரல் என்றும் மாமூலனாரால் குறிப்பிடப்படுகிறான்.

“தலைச்சங்கப் புலவராகிய முரஞ்சியூர் முடிநாகராயரால் பாடப்பெற்ற பாரத காலத்துப் பெருஞ்சோற்று உதியஞ்சேரலாதனுக்கும், கடைச்சங்கப் புலவராகிய மாமூலனாரால் பாடப்படுகின்ற இம்முதியர்ப் பேணிய உதியஞ்சேரலுக்கும் இடைப்பட்ட கால வேற்றுமையை நோக்கும்போது இவ்விருவரும் வெவ்வேறு காலத்தவர் என்பது தெளிவாகிறது”
இமயவரம்பன் நெடுஞ்சேரலாதன் (கி.பி. 72-130)

இரண்டாம் பத்தின் பாட்டுடைத் தலைவன் இமயவரம்பன் நெடுஞ்சேரலாதன் ஆவான். இவனுடைய தந்தை உதியஞ்சேரல், தாய் நல்லினி என்பவள் ஆவாள். இவனுக்கு இரண்டு மனைவிகள் இருந்தனர். இதில் முதல் மனைவி பதுமன் என்பவனின் மகள்; இரண்டாவது மனைவி சோழன் மகன் மணக்கிள்ளி. முதல் மனைவிக்குப் பிறந்தவர்கள் களங்காய்க்கண்ணி நார்முடிச் சேரலாதனும் ஆடுகோட்பாட்டுக் சேரலாதனும் இரண்டாவது மனைவிக்குப் பிறந்தவர்கள் செங்குட்டுவனும், சிலப்பதிகாரம் செய்த இளங்கோவும் ஆவார். இவ்வுறவு முறைகள் பதிற்றுப்பத்தின் பதிகங்களாலும் சிலப் பதிகார வஞ்சிக் காண்டத்தாலும் அறியப்படுகிறது.

சிறப்புப் பெயர்கள்

வடநாட்டின் மீது படையெடுத்துச் சென்று இமயத்தில் விற்பொறித்ததால், இமயவரம்பன் என்னும் சிறப்புப் பெயர் பெற்றான். இதனை,

“அமைவரல் அருவி இமையம் விற்பொறித்து”
(பதி. பதி. 2)

எனவரும் பதிற்றுப்பத்தின் பதிகம் மூலம் அறியலாம்.

இவன் குடக்கோ நெடுஞ்சேரலாதன் (பதி.பதி.6) குடவர் கோமான் நெடுஞ்சேரலாதன் (பதி.பதி.5) சேரமான் குடக்கோ நெடுஞ்சேரலாதன் (புறம். 62.63, 368) சேரலாதன் (பதி. 11,15,18, பதி.பதி.5, அகம் 127,134) நெடுஞ்சேரலாதன் என்றெல்லாம் அழைக்கப்பட்டு வருகிறான்.

இவன் இமயத்தை எல்லையாகக் கொண்டமையால் இமயவரம்பன் என்றும், வானத்தை எல்லையாகக் கொண்டமையால் வானவரம்பன் என்றும் அழைக்கப்படுகிறான்.

இமய வெற்றி

நெடுஞ்சேரலாதனின் முதல் வெற்றி இவனது வடநாட்டுப் படையெடுப்பாகும். இதனை,

“போரிசை மரபின் ஆரியர் வணக்கி”
(பதி.பதி.2)

எனவரும் பாடலில் வடபுலத்து ஆரிய மன்னரை வென்று வணங்கியதையும், இமயத்தில் விற்பொறித்ததையும் பதிகம் குறிப்பிடுகிறது.

இவ் வெற்றிக் குறித்துச் சங்க இலக்கியங்களில் குமட்டுர்க் கண்ணாரும், பரணரும், மாமூலனாரும் மாறோக்கத்து நப்பசலையாகும் பாடியுள்ள பாடல்களில் பேசப்படுகின்றன.

“ஓங்கிய

வரையளந் தறியாப் பொன்படு நெடுங்கோட்டு
இமயம் ஓட்டிய ஏமவிற்பொறி
மாண்வினை நெடுந்தேர் வானவன்”

எனவும் இவனது இமய வெற்றி கூறப்பட்டுள்ளன. பல்யானைச் செல்கெழுகுட்டுவன் (கி.பி.100-125)

இமயவரம்பனின் தம்பி பல்யானைச் செல்கெழுகுட்டுவன். இவன் சேரன் செங்குட்டுவனின் சிறிய தந்தையும் ஆவான்.

பல்யானைச் செல்கெழுகுட்டுவனை பாலைக் கௌதமனார் பத்துப்பாடல்களில் சிறப்பாகப் பாடியுள்ளார்.

உம்பற்காட்டு ஆளுகை

“உம்பற்காடென்பது இக்காலத்து ஆனைமலைப் பகுதியாகும்”⁹ அது முன்னமே சேரராட்சியிற் சேர்ந்ததே அன்றி பிறராட்சியில் இருந்ததன்று என முனைவர் வ. குருநாதன் எழுதிய சங்ககால அரச வரலாறு என்ற நூலில் குறிப்பிட்டுள்ளார்.

உம்பற் காடாகிய ஆனைமலைத் தொடரிலேயே பழநி மலைக்கு மேற்கே பதினான்கு கிலோமீட்டர் தொலைவில் இக்காலத்து ஐவர் மலையென வழங்கும் பண்டை அயிரை மலையில் அமர்ந்த கொற்றவையாகிய தன் குல தெய்வத்தை முறைப்படி வழிபட்டான் எனக் காண்கிறோம்.

கொங்க வெற்றி

இவனது வெற்றிச் செய்தியாக இவன் அகப்பா என்பதோர் கோட்டையை அழித்த செய்தியை அறிகிறோம்.

“கடியிளைக் குண்டுகிடங்கின் நெடுமதில் நிரைப்பதனத்து அண்ணலம் பெருங்கோட்டு அகப்பா எறிந்த பொன்புனை உழிளை வெல்போர்க்குட்டுவ”¹⁰

என்று பதிற்றுப்பத்திலே பாலைக் கௌதமனார் இவ்வரசன் வெற்றி குறித்து விளித்துப் பாடுகின்றார். மேலும் சிலப்பதிகாரத்தில்,

“மிகப் பெருந்தானை யோடு இருஞ்செருவோட்டி அகப்பா எறிந்த அருந்திறல்”

களங்காய்க்கண்ணி நார்முடிச்சேரல் (கி.பி. 125-150)

நான்காம்பத்தின் பாட்டுடைத் தலைவன் களங்காய்க்கண்ணி நார்முடிச் சேரலாதன் ஆவான். இவனுடைய தந்தை இமயவரம்பன் நெடுஞ்சேரலாதன். இவன் தம்பி ஆடுகோட்டிச் சேரலாதன் ஆவார்.

சிறப்புப் பெயர்

களங்காயால் செய்த கண்ணியும், நாரால் செய்த முடியும் அணிந்து அரசப் பொறுப் பேற்றமையால் இவன் களங்காய்க்கண்ணி நார்முடிச்சேரல் என அழைக்கப்பட்டான். இதனை,

இக்களங்காய்க்கண்ணி நார்முடிச்சேரல் பூழி நாட்டைப் படையெடுத்துத் தழுவினான் என்பதும், அவன் கடம்பின் பெருவாயில் நன்னனது காவல் மரத்தை வெட்டிய செய்திகள் பின்வருமாறு கூறப்படுகிறது. இதனை,

“..... குடாஅது

இரும்பொன் வாகைப் பெருந்துறைச் செருவில் பொம்பூண் நன்னன் பொருதுகளத் தொழிய வலம்படு கொற்றம் தந்த வாய்வான் களங்காய்க் கண்ணி நார்முடிச்சேரல்”¹¹

எனவரும் கல்லாடனாரின் அகப்பாடல் மூலம் அறியலாம்.

கடல்பிறக்கோட்டிய செங்குட்டுவன் (கி.பி. 130-185)

இமயவரம்பன் நெடுஞ்சேரலாதனுக்கும் அவன் மனைவி மணங்கிள்ளி. மகள் நற்சோணைக்கும் பிறந்த மூத்த மகன் கடல்பிறக்கோட்டிய செங்குட்டுவன் ஆவான். இவனுடைய தம்பி இளங்கோவடிகள். இவன் பதிற்றுப்பத்தில் ஐந்தாம் பத்தின் பாட்டுடைத் தலைவன் ஆவான். கடல்பிறக்கோட்டிய வெற்றியின் அடியாக இவன் கடல்பிறக்கோட்டிய செங்குட்டுவன் என்று குறிக்கப் பெறுகின்றான். கடல்பிறக்கோட்டிய வேலெறிந்த வெற்றி

செங்குட்டுவன் வெற்றிகளுள் முதல் வெற்றி கடல்பிறக்கோட்டிய வெற்றியாகும். இவனது இவ்வெற்றி பல பாடல்களில் (பதி. 41, 42, 45, 46, 48, அகம்.212) பரணரால் பாராட்டிக் கூறப்படுகிறது.

மோசூர் வெற்றி

செங்குட்டுவனின் போர் வெற்றிகளுள் இரண்டாவது கூறப்படுவது மோசூர்

வெற்றியாகும். இவன் மோசூர் மன்னனுடைய முரசத்தைக் கைப்பற்றிக் கொண்டு அவனையும் கொன்று, அவனது காவல் மரத்தையும் வெட்டிக் கொணர்ந்தான் எனக் கூறப்படுகிறது. இப்போரினைக் குறித்தப் பதிற்றுப்பத்தில்,

“நுண்கொடி உழிஞை வெல்போர் அறுகை
சேணன் ஆயினும் கோளென மொழிந்து
புலம்பெயர்ந்து ஒளித்த களையாப் பூசற்கு
அரண்கள் தாவுறிஇ அணங்கு நிகழ்ந்தன்ன
மோசூர் மன்னன் முரசங் கொண்டு
நெடுமொழி பணிந்தவன் வேம்பு முதல தடிந்து
முரசு செய முரச்சிக் களிறுபலபூட்டி
ஒழுகை உய்த்தோய்”¹² (பதி. 44)

எனப் பரணர் கூறுவதை விளக்கமுறக் காணலாம்.

“பழையன் காக்கும் குழையயில் நெடுங்கோட்டு
வேம்புமுதல் தடிந்த ஏந்துவாள் வனத்துப்
போந்தைக் கண்ணிப் பொறைய”¹³

என்று இளங்கோவடிகள் இம்மோசூர் போரினைக் கூறுகின்றார்.

ஆடுகோட்பாட்டுச் சேரலாதன்

இவன் ஆறாம்பத்தின் பாட்டுடைத் தலைவன் ஆவான். இமையவரம்பன் நெடுஞ்சேரலாதனுக்கு வேளாவிக் கோமானாகிய பதுமனின் மகள் வயிற்றில் பிறந்த இளையமகனே ஆடுகோட்பாட்டுச் சேரலாதன். இவ்வரசன் களங்காய்க்கண்ணி நார்முடிச்சேரலாதன். தமிழ் ஆவான்.

சிறப்புப் பெயர்

“இச்சேரலாதனுடைய நாட்டிலிருந்து ஆட்டு நிரையைத் தண்ட காரணியத்துப் பகைவர் கவர்ந்து போனதை மீட்டுக் கொண்டு வந்தான் இச்சேரலாதன். அதனால் ஆடுகோட்பாட்டுச் சேரலாதன் என்ற சிறப்புப் பெயர் பெற்றான்.”¹⁴ என வ. குருநாதன் சங்ககால அரச வரலாறு எனும் நூலில் குறிப்பிட்டுள்ளார்.

வெற்றிச்சிறப்பு

இவனது வெற்றிச் சிறப்புகளை பின்வருமாறு காணலாம். பதிற்றுப்பத்தில்

“மடம்பெரு மையின் உடன்று மேல்வந்த
வேந்துமெய்ம்மறந்த வாழ்ச்சி
வீந்துகு போர்க்களத்து ஆடும்கோவே”
(பதி.56)

என்ற பாடலின் மூலம் போர்க்களத்தில் பகைவரை வென்று அவ்வெற்றிக் களிப்பிலே ஒள்வாள் அமலை ஆடுவான் என பாடப்பெறுகிறார்.

பொறையன் குடும்பத்தைச் சார்ந்தவர்கள் அந்துவஞ்சேரல் இரும்பொறை (கி.பி. 37-75)

உதியன் குடும்பமும் பொறையன், குடும்பமும் தாயர்கள். ‘உதியன்’, ‘இமயவரம்பன்’, ‘வானவரம்பன்’, குட்டுவன் என்பவை உதியன் பரம்பரையைக் குறிக்கும் பட்டப்பெயர்கள் ஆகும்.

அந்துவன், கடுங்கோ, மாந்தரன், பொறையன், இரும்பொறை என்பவை பொறையன் குடும்பத்தைக் குறிக்கும் பட்டப்பெயர்கள் ஆகும். ஆதன், கோதை, சேரல், சேரமான் முதலியவை இரண்டு குடும்பங்களையும் குறிக்கும் பொதுப் பெயர்களாகும்.

சேரமான் உதியஞ்சேரலுக்கு ஒத்த காலத்தவனாகவும், உடன்பிறப்பு முறைமையானாகவும் உணர வருபவர் அந்துவஞ்சேரல் இரும்பொறை.

“மடியா வுள்ளமொடு மாற்றோர்ப் பிணித்த
நெடுநுண் கேள்வி அந்துவதற்கு ஒருதந்தை
சன்ற மகள் பொறையன் பெருந்தேவி சன்ற
மகன்

செல்வக் கடுங்கோ வாழியாதன்” (பதி.பதி.7)

எனவரும் பதிற்றுப்பத்தின் ஏழாம் பதிகம் கூறுவதால் இவருடைய மகன் செல்வக்கடுங்கோ வாழியாதன் என்பது தெரிகிறது. இவ்வாறு செல்வக்கடுங்கோவுக்குத் தந்தையாகிய இவ்வந்துவஞ்சேரலைப் பதிற்றுப்பத்திலே காணப்படாத ஒரு பத்திற்குரிய தலைவன் என்றும், அப்பத்து உண்மையில் ஏழாம்பத்தாய் இருந்திருத்தல் வேண்டும் என்றும் முனைவர் வ. குருநாதன் சங்க கால அரச வரலாறு எனும் நூலில் கூறுகின்றார்.

செல்வக்கடுங்கோ வாழியாதன் (கி.பி. 75-100)

அந்துவஞ்சேரல் இரும்பொறைக்கும் ஒரு தந்தை என்பான் மகளுக்கும் பிறந்த மகன் செல்வக்கடுங்கோ வாழியாதன். இச்சேரன் மனைவி வேண்மாள். இவள் வேளாவிக்கோவின் இரண்டாவது மகள் ஆவாள். இச்செய்தி ஏழாம்பத்தின் பாட்டுடைத்தலைவன் ஆகப் பாடப்பட்டுள்ளது.

சிறந்த போராற்றல் மிக்கவன்

“வையங் காவலர் வழிமொழிந் தொழுகப்
போகம் வேண்டிப் பொதுச் சொற் பொறாஅது

ஒடுங்கா உள்ளத்து ஒம்பா ஈகைக்
கடந்தடு தானைச் சேரலாதன்”

எனவரும் பாடலடிகளில் இவரது போர்ச்சிறப்பும் மற மேம்பாடும் கூறப்படுகிறது.

தகடூர் எறிந்த பெருஞ்சேரல் இரும்பொறை
(கி.பி. 135-152)

இவன் எட்டாம் பத்தின் பாட்டுடைத் தலைவன் தகடூர் எறிந்த பெருஞ்சேரல் இரும்பொறை. இவரின் தந்தை செல்வக்கடுங்கோ வாழியாதன் ஆவார். இவர் வேளாவிக்கோமானாகிய பதுமனின் மகள் வயிற்றில் பிறந்த மகனே பெருஞ்சேரல் இரும்பொறை. இச்சேரன் அதியமான் நெடுமானஞ்சியை வென்று, தகடூரை எறித்தழித்தமையால், தகடூர் எறிந்த பெருஞ்சேரல் இரும்பொறை என அழைக்கப்படுகிறார்.

வெற்றிச்சிறப்பு

இவனது வெற்றிச்சிறப்பினை அரிசில்கிழார் பின்வருமாறு பேசுவார்.

“வெல்போர் ஆடவர் மறம்புரிந்து காக்கும்
வில்பயில் இறும்பின் தகடூர் நூறிப்

யாடுபரந்தன்ன மாவின்

ஆபரந்தன்ன யானையோன்” (பதி. 78)

எனவரும் இப்பாடலடிகளில் கொங்குநாட்டுத் தகடூரையும், அந்நாட்டு சிறப்பு கால்நடைகளாகிய ஆடுகளையும் மாடுகளையும் போன்று பல குதிரைகளும் யானைகளும் நிறைந்த படையோடு சென்று அழித்தார் என இவரது வெற்றிச் சிறப்பு கூறப்பட்டுள்ளன.

இளஞ்சேரல் இரும்பொறை (கி.பி: 152-168)

ஒன்பதாம் பத்தின் பாட்டுடைத் தலைவன் இளஞ்சேரல் இரும்பொறை இவன் குட்டுவர் இரும்பொறைக்கும், அந்துவஞ்சேரல் என்னை என்பவருக்கும் மகனாக பிறந்தவர். இவரைப் பாடிய புலவர் பெருங்குன்றூர் கிழார் ஒருவரே ஆவார்.

பதிற்றுப்பத்தில் ஒன்பதாம் பத்திலும் புறநானூற்றில் (210, 211) ஆகிய பாடல்களில் சிறப்பாகக் குறிப்பிடுகிறான். பதிற்றுப்பத்தில் குடக்கோ இளஞ்சேரல் இரும்பொறை எனக் குறிக்கப்பெறுகின்ற இவரே புறப்பாடலில் சேரமான் குடக்கோச்சேரல் இரும்பொறை எனவும் அழைக்கப்படுகிறார்.

செயற்கருஞ் செயல்கள்

இவ்வரசன் மந்திர மரபுகளை அறிந்து தெய்வத்தை வழிபட்டார் என்றும் தன் பாட்டனான மையூர் கிழானை, வேள்வியாளனை விட அறநெறி அறிந்தவனாகச் செய்தவன் எனவும் பதிற்றுப்பத்தின் பதிகம் கூறுகின்றது. இதனை,

“அருந்திறல் மரபின் பெருஞ்சதுக் கமர்ந்த
வெந்திறற் பூதரைத் தந்திவண் நிற்றி
ஆய்ந்த மரபிற் சாந்தி வேட்டு” (பதி.பதி.9)

எனவரும் பதிற்றுப்பத்தின் பதிக அடிகளால் அறியலாம். சிலப்பதிகார வஞ்சிக்காண்டத்திலும் இவனது செய்தி பேசப்படுகின்றது.

முடிவுரை

இவ்வாறு இக்கட்டுரையில் பதிற்றுப்பத்துக் காட்டும் சேரமன்னர்களின் வரலாறு, வெற்றிச் சிறப்பு ஆகியவைப்பற்றி கூறப்பட்டுள்ளது.

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TAMIL

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Dr. M. S. Batcha

பதிற்றுப்பத்தில் புறப்பொருள் ஆட்சி

முனைவர் சு. சீலா

உதவிப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக் கல்லூரி (தன்னாட்சி)

(பாரதிதாசன் பல்கலைக்கழகத்துடன் இணைவு பெற்றது), திருச்சிராப்பள்ளி, தமிழ்நாடு, இந்தியா.

ஆய்வுச்சுருக்கம்

புறம் என்ற சொல்லின் விளக்கம், புறம் பற்றிய உரையாசிரியர்களின் கருத்து, புறத்திணை, புறப்பொருளில் வீரம், புறத்திணை வகைகள், துறைகள், போருக்கான காரணங்கள், போருக்குரிய மரபுகள், போர் பற்றிய செய்திகள் ஆகியவை இக்கட்டுரையில் ஆய்வுச்சுருக்கமாகக் கூறப்பட்டுள்ளன.

முன்னுரை

சங்க இலக்கியம் தொகை, பாட்டு என்ற இருவகையில் தொகுக்கப்பட்டுள்ளது. அகத்திணை, புறத்திணை உள்ளீடுகள் பாடுபொருளாக அமைந்துள்ள சிறப்பு சங்க இலக்கியத்திற்கு உண்டு. இதில் பதிற்றுப்பத்து நூலில் காணப்படும், புறப்பொருள் ஆட்சிகளில் திணை, துறை வகுக்கப்பட்டு, போருக்கான காரணங்கள், மரபுகள், போர்முறைகள் வெற்றிச்சிறப்பு ஆகியவை இக்கட்டுரையில் ஆராயப்படுகிறது.

புறம் என்ற சொல்லின் விளக்கம்

“சங்க இலக்கியத்தில் புறம் என்ற சொல்லிற்குப் பொருளாக வெளியிடம், அந்நியம் பக்கம், முதுகு, அலர்மொழி காலம் ஆகிய வெவ்வேறான பொருளைக் குறிக்கும் வகையில் இடம் பெற்றுள்ளது.”¹

என தமிழ் அகராதி விளக்கம் தருகிறது.

புறம் பற்றிய உரையாசிரியர்களின் கருத்துக்கள்

“புறப்பொருள் என்பதற்கு மறஞ்செய்தலும், அறஞ்செய்தலும் அவற்றான் ஆய பயன் பிறர்க்கு புலனாதல்”²

என்ற பொருளில் இளம்புரணர் கூறுகின்றார். இதேபோல நச்சினார்க்கினியர் அகத்திற்குரிய

விளக்கத்திணைக் கூறி இதனை ஒழிந்தன புறம் எனக் கூறுகின்றார். இவ்வாறு இளம்புரணர் அகப்பொருளை இன்ன பாக்களிலேயே பாடுதல் வேண்டுமென வரையறுத்தவர்கள் புறப்பொருளுக்கு அவ்வாறு ஒரு வரையறையை விதிக்கவில்லை எனக் கூறுகின்றார்.

புறத்திணை நோக்கம்

புறப்பொருள் மேலீடாக அமைந்த இத்தன்மையானது அகத்தில் புறமும், புறத்தில் அகமும் தேவையாகிறது. ஒருவரின் அகவாழ்க்கை செம்மையாக அமைந்தால்தான் அவனுடைய புறவாழ்க்கையும் போற்றுவதாக அமையும் இதனை,

“புகழ்புரிந்த இல்லிலோர்க்கு இல்லை
இகழ்வார்முள்
ஏறுபோல் பீடு நடை”³

என்ற திருக்குறளில் மூலம் அறியலாம்.

வீட்டிலும் நாட்டிலும் நிலவும் அமைதியே தன் கவலை இன்மைக்குக் காரணம் என்பார் பிசிராந்தையார்.

“யாண்டு பலவாக நரையில் ஆகுதல்

.....
சான்றோர் பலர்யான் வாழும்உனரே”⁴

மேற்கண்ட இப்பாடலில் வீடும் நாடும் சிறப்புற்று இருப்பதால் நானும் நரையற்றேன் என்கின்றார்.

புறப்பொருளின் வீரம்

தொடக்க கால மனிதன் ஒரு குழுவில் உணவு தேடுவதிலும் தன்னைக் காத்துக் கொள்வதிலும் தமக்கு வேண்டிய பொருளை தம்மிடம் இல்லாத நிலையில் மற்றொரு குழுவில் ஆளுமை செலுத்துவதில் போரின் பண்பையே பெற்றிருந்தான்.

“ஒருவனை ஒருவன் அடுதலும் தொலைதலும்
புதுவதன்று இவ்வுலகத் தியற்கை”

இப்பாடல் வரியில் கற்காலத்திலேயே ஆளுமை
புடுத்துவதும், அடிமை கொள்வதும் தமிழ் மரபில்
வீரம் பற்றிய செய்தியாக வந்துள்ளது.

புறத்திணைப் பற்றிய தொல்காப்பியர் கருத்து
புறத்திணைப் பற்றிய கருத்தினைத்
தொல்காப்பியர்,

“மக்கள் நுதலிய அகன்ஐந் திணையும்
கட்டி ஒருவர் பெயர் கொள்ப் பெறாஅர்”

“புறத்திணை மருங்கின் பொருந்தின் அல்லது
அகத்திணை மருங்கின் அளவுதல் இலவே”

“புறத்திணை என்பது சமுதாயத்தில் ஓர்
உறுப்பாக அமைந்த தனிமனிதனின் சமூகந்
குறித்த நினைவுகளையும், உணர்வுகளையும்,
செயல்களையும் எடுத்துக் காட்டுவதாக
அமைகிறது.”⁴ இவ் விளக்கங்களால்
அகத்திணைக்கு அமைந்த விளக்கத்தைப் போல
அகப்பொருளில் பாடுதற்கமைந்த விதிமுறைகளைப்
போல, புறத்திணைக்கும் புறப்பொருளுக்கும்
அமையவில்லை என டாக்டர் கு.வெ.
பாலசுப்பிரமணியன், சங்க இலக்கியத்தில்
புறப்பொருள் எனும் நூலில் விளக்கம் தந்துள்ளார்.

புறத்திணை வகைகள்

ஏழு அகத்திணைகளுக்குப் புறமாக ஏழு
புறத்திணைகள் அமையும் என்பது தொல்காப்பியர்
கருத்தாகும் என்பதை,

“கைக்கிளை முதலாப் பெருந்திணைப்
இறுவாய்
முற்படக் கிளந்த எழுதிணை என்ப”⁵

“அகத்திணை இலக்கணம் அரில்தப
உணர்ந்தோர்
புறத்திணை இலக்கணம் திறப்படக் கிளப்பின்
வெட்சி தானே குறிஞ்சியது புறனே
உட்குவரத் தோன்றும் ஈரேழ் துரைத்தே”⁶

என ஏழாக இருதிணைகளை வகுத்து ஒவ்வொரு
அகத்திணைக்குப் புறமாக ஏழு புறத்திணைகள்

சுறுப்படுகின்றன. தொல்காப்பியர் ஏழாகப் பகுத்த
நிலையை புறப்பொருள் வெண்பாமாலை ஆசிரியர்
ஐயனாரிதனார் பன்னிரண்டாகப் பிரித்துள்ளார்.
அவை வெட்சி, கரந்தை, வஞ்சி, காஞ்சி, உழிஞை,
நொச்சி, தும்பை, வாகை, பாடாண், கைக்கிளை,
பெருந்திணை என வகைப்படுத்தலாம்.
தொல்காப்பியத்தில் திணைகள் வெட்சி, வஞ்சி,
காஞ்சி, உழிஞை, வாகை, பாடாண் காஞ்சி எனப்
பிரிக்கப்படுகிறது.

திணை

பதிறறுப்பத்தில் பாடாண் திணை மட்டுமே
இடம்பெற்றுள்ளது. கிடைத்த எட்டு மன்னர்களின்
பாடல்களுக்கும் பாடாண் திணையை வகுத்து,
செந்துறைப் பாடாண் பாட்டு 36 பாடல்களிலும்,
வஞ்சித்துறைப் பாடாண் பாட்டு 10 பாடல்களிலும்,
விறலியாற்றுப்படை காட்சி வாழ்த்து ஆகிய 6
பாடல்களிலும், இயன்மொழி வாழ்த்து 5
பாடல்களிலும், வாகைத்துறை பாடாண்பாட்டு 3
பாடல்களிலும், பரிசில் துறைப் பாடாண்பாட்டு,
தும்மை அரவம் ஆகிய 2 பாடல்களிலும், நாடு
வாழ்த்து, பெருஞ்சோற்றுநிலை, களவழி, குரவை
நிலை, ஒள்வாள் அமலை, பாணாற்றுப்படை,
உழிஞை அரவம் முல்லை ஆகிய துறைகள் ஒரு
பாடலிலும் துறைகளாக அமைந்துள்ளன.

பாடாண் திணை

தொல்காப்பியர் வகுத்துள்ள புறத்திணை
ஏழினுள் இறுதிக் திணையாக வைத்து
எண்ணப்படுவது பாடாண் திணையாகும். பாடாண்
என்பது பாடுதல் வினையாகிய தொழிலையோ,
அவரால் புகழ்ந்து பாடப்படும் ஆண்மகனையோ
குறிப்பது அன்று. புலவர் பாடும் புகழினை
விரும்பிய மன்னன் தம்முடைய அறிவு, ஆற்றல்,
ஈகை முதலிய பண்புகளுடன் ஆளும் தன்மையைக்
குறித்து வழங்குவது பாடாண் என்ற சொல்லாகும்.
தொல்காப்பியர்,

“பாடாண் பகுதி கைக்கிளைப் புறனே”

என வகுத்து இதில் கடவுள் வாழ்த்து, வாழ்த்தியல்
மங்கலம், செவியறிவுறுத்தல், வசை என எட்டாகப்
பாகுபடுத்தியுள்ளார்.

தொல்காப்பியத்திற்கு பின் எழுந்த புறப்பொருள் வெண்பா மாலையில் திணைகளுக்குப் படலம் என பெயரிட்டு அழைத்துள்ளார். பாடாண் திணையைப் பாடாண் படலம் என்றே கட்டியுள்ளார். “பாடாண் திணையைத் தனியாகவும், கைக்கிளைப் படலத்தை தனியாகவும் இலக்கணம் ஆக்கியுள்ளார். இதில் பாடாண் திணை பாடப்படும் ஆண்மகனது ஒழுகலாறு”⁹ என துரை. பட்டாபிராமன் என்பவர் புறத்திணை இலக்கியம்-பதிற்றுப்பத்து எனும் தலைப்பில் இந்தியப் பல்கலைக்கழகத் தமிழாசிரியர் மன்ற ஆய்வுக்கோவையில் விளக்கியுள்ளார்.

போருக்கான காரணங்கள்

போருக்குரிய காரணங்களாகப் பல உரைக்கப்படுகின்றன. இயல்பான மறப்பண்பு, மண்ணாசை, இடஞ்சிறிதென்னும் ஊக்கமேலீடு அரசர்க்கு அரசராகத் திகழும் உட்கோள், அரசரிமைத் தடை விளைதல், அரசரிமையார்க்கெனும் சிக்கல், மானக்குறை உண்டாக மற்றையோர் நடத்தல், மகள் மறுத்தல் ஆகிய காரணங்களால் போர் தோன்றக் கூடும் என்பார்.¹⁰ என அறிஞர் வித்தியானந்தன் அவர்கள் கூறுகிறார். பழைய இந்து சமூகத்தில் போர்கள் நடைபெற்றமைக்குரிய உளவியற் காரணங்களாக, 1. சாதிச் சமூகப் பிரிவுகளில் போரைத்தொழிலாகப் பெற்றவரின் மனநிலை, 2. போர்த்தொழிலின்றி வாழ இயலாத மனநிலை, 3. வீரச்செயல்களில் நாட்டம், 4. உடல்திணவு, 5. தற்காப்பு எண்ணம், 6. வெகுளியும், பொறமையும் 7. பிறர்மீது ஆளுகை செலுத்தும் நோக்கம் ஆகியவற்றை ஆய்வறிஞர் குறிக்கின்றார். டாக்டர் கு.வெ. பாலசுப்பிரமணியன் சங்க இலக்கியத்தில் புறப்பொருள் நூலில் குறிப்பிடுகிறார்.

வீரம் நிறைந்த சமூகத்திலும் தலைவன் ஒட்டிலா வலிமையும் ஆற்றலும் உடையவனாகக் கூறப்படுகிறான். இதனை,

“களம்புக லோம்புமின் றெவ்வீர் போரெதிர்ந் தெம்முரு முளனொரு பொருநன் வைகல் எண்டேர் செய்யுந் தச்சன்” (புறம் - 87)

எனத் தலைவனின் போராற்றல் உரைக்கப்படுகிறது. உடல் வலிமையும், போராற்றலும், வீர உணர்வுமே தலைமைக்கும், போற்றுதலுக்கும், புகழுக்கும் உரியனவாகும். இத்தகைய வீர உணர்வே எல் லாவற்றுக்கும் உரிய தகுதியாக கருதப்பட்டிருக்கிறது.

போருக்குரிய மரபுகள்

சங்ககாலத் தமிழகப் போர்முறைகளில் சில வியத்தகு மரபுகள் காணப்படுகின்றன. வேந்தர்கள் அடையப் பூச்சுடுவதோடு எப்பொழுதெல்லாம் போர் நிகழ்கின்றதோ அப்பொழுதெல்லாம் அதற்கேற்ற பூவையும் சூடுதல், படைவீரர்கள் அடையாளப் பூச்சுடுதல் ஆகியன தொல்காப்பியராலே குறிக்கப்பெறுகின்றன. தமிழர் வாழ்வில் அகத்திலும் புறத்திலும் மலர்கள் குறியீடுகளாகவும், மங்கலப் பொருளாகவும் மகிழ்ச்சிக்குரியனவாகவும் விளங்குகின்றன. கண்ணியும் கோதையும் தொடையலும் பிணையலும் தாரும் மாலையும் ஆரமும் என்றின்ன பல்வகையால் மலர்களைச் சேரகட்டிப் புறத்திணை நிகழ்ச்சிகளில் ஆடவரும், அகத்திணை நிகழ்ச்சிகளில் மகளிரும் புனைந்து கொள்ளும் இயற்கை வாழ்வு நெறியைச் சங்க இலக்கியங்கள் காட்டுகின்றன. இதில் பதிற்றுப்பத்திலும் மலர் சூடும்நிலை கூறப்படுகிறது.

புறத்திணைப் போர்நிகழ்ச்சி ஒவ்வொன்றிலும் ஒரு குறிப்பிட்ட மலர் சூடும் வழக்கம் பிறமொழி இலக்கியங்களில் எங்கும் இல்லை என குறிப்பது கருதத்தக்கதாகும். வெட்சி, வஞ்சி, உழிஞை, தும்பை, வாகை, காஞ்சி என்னும் ஆறுவகை மலர்கள் புறத்திணையில் இடம்பெறுவன. பண்டைக் கிரேக்க வீரர் வெற்றிக்குப்பின் தலைக்கவசத்தின் மேல் இறகு சூடிக்கொள்ளும் வழக்கம், தமிழர் மரபில் வாகை மலரைச் சூடும் வழக்கத்தை ஒத்துள்ளது. எனினும் போர்ச்செயல் ஒவ்வொன்றுக்கும் ஒரு குறிப்பிட்ட மலர் சூடும் வழக்கம் வேறு எங்கும் இருந்ததில்லை.

போருக்குப் புறப்படுவோன், ஊர்க்கயத்தில் நீராடி, வெள்ளாடை உடுத்திச் செல்லும் மரபை,

“மூதூர் வாயிற் பனிக்கய மண்ணி
மன்ற வேம்பி னொண்குழை மலைந்து”¹²

என்ற பகுதிகள் எடுத்தியம்புவதைக் காணலாம்.

போருக்குப் புறப்படுமுன் விரிச்சிக் கேட்டலும், புள் முதலியவற்றால் நிமித்தம் காணலும், உன்ன மரத்தின் நிலை நோக்கலும், முழுத்தம் பார்த்தலும் ஆகிய நம்பிக்கைச் செயல்கள் உண்டு என்பதை,

“காலனும் காலம் பார்க்கும் பாராது
வேவீண்டு தானை விழுமியோர் தொலைய
வேண்டிடத் தடுஉம் வெல்போர் வேந்தே”
என்ற புறநானூற்றுப் பாடலிலும், பதிற்றுப்பத்தில்

“புன்கா லுன்னத்துப் பகைவ னொங்கோ”¹³
(பதி. 61)

என்ற பாடல் அடிகளாலும் அறியலாம்.

போருக்குரிய வேணிற் காலத்தில் பொருதலைத் தொடங்குவோர் கார்காலம் வந்தவுடன் போரை நிறுத்திவிட்டு ஊர் திரும்புவர். வாடைப் பாசறையில் தங்கியிருந்தலும் உண்டு. கார் காலத்தில் காதல் உணர்வு காரணமாகப் படை வீரர்கள் ஊர் திரும்புவார்கள். “கார் காலத்தில் படைகளின் இயக்கம் செம்மையாக இராது என்பதால் போரை நிறுத்தி ஊர் நண்ணுவர் என்பது பொருத்தமான காரணமாகும்.”¹⁴

போர் பற்றிய செய்திகள் (அ) போர் முறைகள்

அரசர்கள் தங்களின் பாதுகாப்பிற்கும் நாட்டு மக்களின் பாதுகாப்பிற்காகவும் பல்வேறு படைப்பிரிவுகளை வைத்திருந்தனர். அவற்றில் குறிப்பிடத்தக்க வகை, காலாப்படை, குதிரைப்படை, யானைப்படை, தேர்ப்படை ஆகிய படைகளாகும். இதனை,

“தானை யானை குதிரை என்ற
நோனார் உட்கும் மூவகை நிலையும்”

எனத் தொல்காப்பியம் மூவகைப் படையினை எடுத்துக் கூறுகிறது. அதனை அடிப்படையாக வைத்து பதிற்றுப்பத்தில் மூவகைப் பாடல்கள் கூறப்படுகின்றன. புறநானூற்றில்,

“கடுஞ்சினத்த கொல்களிறுங்
கதழ்பரியகலிமாவும்
நெடுங்கொடிய நிமிர்ந்தேரு நெஞ்சுடைய
புகன்மறவரும்என
நான்குடன் மாண்ட யரசின் கொற்றம்”

என நாற்படை பற்றியும் கூறப்படுகிறது. நாற்படைக்கும் உரியவராக சேரர், சோழர், பாண்டியர் ஆகியோர் குறிக்கப் பெறுகின்றனர். கொடைப்பண்பு மிக்க குறுநில மன்னர்களிடம் யானை, குதிரை தேர் ஆகியன படைப்பொருளாக மட்டுமன்றிக் கொடைப் பொருளாகவும் விளங்குகின்றன.

பதிற்றுப்பத்தில்,

“விழவு வீற்றிருந்த வியலுள் ஆங்கண்
கோடியர் முழுவின் முன்னர் ஆடல்
வல்லா னல்லன்; வாழ்க அவன் கண்ணி
வலம்படு முரசம் துவைப்ப வாளுயர்த்து
இலங்கும் பூணன் பொலங்கொடி உழிஞையன்
மடம்பெரு மையின் உடன்றுமேல்வந்த
வேந்துமெய்ய் மறந்த வாழ்ச்சி
வீந்துகு போர்க்களத்து ஆடுங்கோவே”¹⁵
(பதி.56)

போர்க்களத்தில் வெற்றிக் கூத்தாடுவதற்குச் சேரலாதன் வல்லவனே அல்லாமல் கூத்தர் முன்பாகக் களிக்கூத்து ஆடிக் களித்தலில் வல்லவன் அல்லன் என்பதையும் விளக்குகிறது.

வெற்றிச் சிறப்பு

இமயவரம்பன் நெடுஞ்சேரலாதனின் வெற்றிச் சிறப்பினைக் குமட்டுர்க்கண்ணனார் ஏழு பாடல்களில் பாடியுள்ளார். இதனை,

“சிவந்த காந்தன்முதல் சிதைமுதிற்
புலவவில் உழவிற புல்லாள் வழங்கும்
புல்லிலை வைப்பிற புலஞ்சிதை யரம்பின்
அறியாமையான் மறந்ததும் பெதிர்ந்தநின்
பகைவர் நாடும் கண்டுவந்தி சினே”
(பதி.15)

இப்பாடலில் பகைவரது புலன்களை அழிக்கின்ற போர் மறப்பண்பு கூறப்படுகிறது.

பல்யானைச் செல்கெழுகுட்டுவனின் வெற்றிச் சிறப்பினை பாலைக் கௌதமனார் ஆறு பாடல்களில் பாடியுள்ளார். அதனை,

“காடுறு கடுநெறியாக மன்னிய
முருகுடன்று கறுத்த கலியழிமுதூர்
உரும்பில் கூற்றத் தண்ணீரின்
திருந்து தொழில் வயவர் சீறிய நாடே”
(பதி. 26)

இப்பாடல் முருகப் பெருமான் வெகுண்டு செல்வக்களிப்பை இழந்து தவித்த படை மறவர்கள் வெகுண்டு பொருதழிந்த நாடுகளின் அழிவைக் கூறுவதாக அமைகிறது.

களங்காய்க்கண்ணி நார் முடிச் சேரலின்
மறமாண்பினை நான்கு பாடல்களில் கூறியுள்ளார்.
இதனை.

“புரைசால் மைந்தநீ ஓம்பன் மாறே
உரைசான் றனவாய் பெருமநின் வென்றி
இடுங்களிற் றியானை இலங்கவான்
மருப்பொடு” (பதி. 35)

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“கைவில் இளையர் கடவுள் பழிச்ச
மறப்புலிக் குழு உக்குரல் செத்து வயக்களிறு
.....
மாயிருஞ் சென்னி அணிபெற மில்லைச்சிச்”
(பதி. 41)

இப்பாடலின்மூலம் வெற்றிச்சிறப்பு கூறப்படுகிறது.
முடிவுரை

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விளக்கப்பட்டுள்ளது.

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முதன்மைப் பதிப்பாசிரியர்
முனைவர் சி. சித்ரா



தமிழ்த்துறை
பாரதியார் பல்கலைக்கழகம்
கோயம்புத்தூர் - 641 046.

**நாட்டுப்புற
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வாழ்த்துரை

'பயிற்றிப் பல கல்வி தந்து இந்தப் பாரை உயர்த்திட வேண்டும்' என்ற பாரதியின் கனவுகளை நனவாக்கிவரும் பாரதியார் பல்கலைக்கழகம் உயர்கல்விக்காகப் பல ஆக்கப்பூர்வமான செயல்களைச் செய்து உலகின் தலைசிறந்த பல்கலைக்கழக வரிசையை நோக்கி வீறுநடை போட்டுக் கொண்டிருக்கிறது. மகாகவி பாரதியின் நினைவு நூற்றாண்டான இந்த ஆண்டில் பாரதியார் பல்கலைக்கழகம் தனது மணிமுடியில் பல்வேறு விலைமதிப்பற்ற சாதனை இரத்தினங்களைச் சூடிக் கொண்டிருக்கிறது. அத்தகு இரத்தினங்களுள் தமிழ்த்துறையின் இரத்தினமும் ஒன்று என்று கூறுவதில் பெருமகிழ்ச்சியடைகிறேன்.

'உறுதி கொண்ட நெஞ்சினாய் வா வா வா' என்பான் பாரதி. அந்த மகாகவியின் வாக்கினை முழுமையாக உள்வாங்கிக் கொண்டவர்கள் தமிழ்த்துறையினர். அதனால்தான் பொது முடக்கக் காலகட்டத்திலும் இலக்கியம் இலக்கணம் சார்ந்த நான்கு பன்னாட்டு மாநாடுகளை இணையவழி நடத்தி வியக்க வைத்தார்கள்.

தற்போது நாட்டுப்புறவியலுக்காக ஒரு பன்னாட்டு மாநாட்டைச் செம்மையறத் திட்டமிட்டு நடத்துகின்றனர். சர்வதேச அளவிலும் இந்திய அளவிலும் முத்திரை பதித்த நாட்டுப்புறவியல் அறிஞர் பெருமக்களை நேரடியாக அழைத்து நடத்தவிருக்கும் பன்னாட்டு நாட்டுப்புறவியல் மாநாடு உலக நாட்டுப்புறவியல் துறைக்கும் பாரதியார் பல்கலைக்கழகத்திற்கும் தமிழ்த்துறைக்கும் பெருமை சேர்க்கும் வகையில் திட்டமிடப்பட்டுள்ளது.

திருவிழா நடைபெறுவதற்குச் சில நாட்களுக்கு முன்பாகவே களை கட்டிவிடும் கிராமத்தைப் போன்று மாநாடு நடைபெறுதற்கு

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7. நல்லூர் கலம்பகம், புலவர் வை.க.சிற்றம்பலம் (1994)
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9. கட்டுரையாளர் பெயர்: 'கலாபூஷணம்' எஸ்.சிவானந்தராஜா, முகவரி: செட்டிகுறிச்சி, பண்டத்தரிப்பு, யாழ்ப்பாணம், ஸ்ரீலங்கா, தொலைபேசி: 0094 767567140/0094 217901445



நாட்டுப்புற மக்களின் வாழ்வியல் முறைகள்

முனைவர் இரா. சுந்தரவேல்

முகவுரை

நாட்டுப்புற இலக்கியங்கள் எழுத்தறிவில்லாத உழைக்கும் மக்களின் கூட்டுப் படைப்புகளாகும். இவை சில நேரங்களில் தனி மனிதர்களால் படைக்கப்பட்டாலும்கூட, வாய்மொழியாகப் பரவும்போது பலருடைய எண்ணங்களையும், கருத்துகளையும் பிரதிபலித்துக் காட்டுகின்றன. இவ்விலக்கியங்கள் மக்களின் அன்றாட வாழ்வில் பங்குப்பெறும் உயர் இலக்கியங்களாகத் திகழ்வதோடு அவர்களின் வாழ்க்கை முறையையும், உணர்வையும், இலட்சியத்தையும் ஒளிவு மறைவின்றி வெளிப்படுத்தவும் செய்கின்றன. இவ்வாறு அமைந்த நாட்டுப்புற இலக்கியத்தில் மக்களின் வாழ்க்கையைப் பற்றி இக்கட்டுரையில் காண்போம்.

நாட்டுப்புற இலக்கியம்

மனித சமுதாயத்தின் ஆரம்ப காலத்திலிருந்து மக்களின் உணர்ச்சியை உள்ளது உள்ளவாறு கூறியும், அவர்களது பழக்கவழக்கங்களை எளிய நடையில் தெளிவாக வெளிப்படுத்தியும் காட்டியது நாட்டுப்புற இலக்கியமாகும். இவை மக்களது வாழ்க்கையில் பிறந்து, வளர்ந்து, மக்களின் வாழ்க்கைக்கு பயன்படுவதால் இதனை மக்கள் இலக்கியம் எனலாம். நாட்டுப்புற இலக்கியங்கள் நாட்டுப்புற மக்களின் உயிர் இலக்கியங்களாகத் திகழ்வதோடு மக்களின் வாழ்க்கை

உதவிப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி - 01.

முறைகளையும் காலத்தினால் ஏற்பட்ட மாற்றங்களையும் சுட்டுவனவாக அமைந்துள்ளன.

நாட்டுப்புற இலக்கியத்தின் தோற்றம்

மக்களின் வாழ்வோடு பொருந்திய இலக்கியமே காலமெனும் கடுவெள்ளத்திற்கு எதிராக நீச்சலிட்டு நெடுங்காலம் வாழ்கின்றது. அத்தகைய இலக்கியங்கள் நம்மிடையே பெருகி விரிந்து கிடக்கின்றன. இவை உழவனால் பயிரிடப்படாமல் தானே முளைத்து, வளர்ந்து, மலர்ந்து மணம் வீசும் காட்டுமலர்களைப் போன்றவை. நாட்டுப்புறப்பாடல்கள் இயற்கையோடு இயைந்து வாழ்ந்த மக்களின் உணர்ச்சி வெளிப்பாடாகும். மொழியறியாத காட்டுமிராண்டிகளாக மக்கள் வாழ்ந்த காலத்தில்கூட மனிதன் தன் உணர்ச்சிகளைப் பல்வேறு ஒலிகளின் மூலம்தான் வெளிப்படுத்தினான். மொழியறியாத குழந்தைகூட இசையைக் கேட்டு மயங்குகிறது. நாகம்கூட மகுடியின் இசை கேட்டு ஆடுகிறது. எனவே மக்கள் இசையின் தன்மையை உணர்ந்த நாளிலிருந்து நாட்டுப்புற இலக்கியம் தோன்ற ஆரம்பித்துவிட்டது எனக் கூறலாம்.

வளர்ச்சி

தொல்காப்பியர் காலத்திற்கு முன்பிருந்தே வழங்கிவரும் இவ்விலக்கியத்தைப் படைத்தவர் மக்களையாடிலும் இதனை வாழ்வித்தவர், வளர்த்தவர் தமிழ்நாட்டில் மிகச் சிறந்த குடிகளாகப் போற்றப்பட்ட துடியர், பாணர், பறையர், கடம்பர், பள்ளர் என்பவரே ஆவார்.

கலைநயமும், இசைநயமும் உடைய இவர்கள் ஊர்விட்டு ஊர், நாடுவிட்டு நாடு நடந்து சென்று அரசர்களையும், மற்றவர்களையும் கண்டு பாடியும், ஆடியும் மகிழ்வித்து வந்தனர். இவர்கள் செவிவழியாக நாட்டுப்புற இலக்கியத்தைப் போற்றி வந்தனரேயன்றி ஏட்டில் எழுதி வைக்கவில்லை.

மேலை நாடுகளிலும் இத்தகைய குடியினர் நாடோடிகளாகத் திரிந்து நாட்டுப்புற இலக்கியத்தை வளர்த்திருக்கின்றனர். பல

அறிஞர்கள் அவர்கள் பாடிய பாடல்களை விரிவாக ஆய்ந்து எழுதியுள்ளனர். இந்தப் பாடல்கள் முதலில் வீரத்தைக் குறிக்கும் பரணிப் பாடல்களாகவே உண்டாகியிருக்க வேண்டும் என்பது அவர்களின் நம்பிக்கை ஆகும்.

'பழங்காலத்தில் பைந்தமிழ் இசை வளர்த்த பாணர்கள் ஏர்க்களம் பாடுதல், போர்க்களம் பாடுதல் முதலான வழக்கங்களைக் கொண்டிருந்தனர். அவ்வாறு ஏர்க்களம் பாடியும், போர்க்களம் பாடியும் வருணித்த பகுதிகள் தமிழ்நாட்டின் முலை முடுக்குகளினெல்லாம் வெகுவாகப் பரவி அவையே மக்களிடம் பரிணமித்திருக்க வேண்டும். அப்பாடல்களைக் கேட்டு வழிவழியாகப் பாடி வந்தமையால் காலம் செல்லச் செல்ல ஆதி வடிவம் சிதைந்து அது நாடோடி பாடலாக மாறியிருக்க வேண்டும்.'

நாட்டுப்புற இலக்கியத்தின் கூறுகள்

தமிழகத்தில் மேலை நாட்டு ஆய்வாளர் வரையறுத்த அளவுக்கு நாட்டுப்புற இலக்கியத்தைத் தெளிவாக எவரும் வரையறுக்கவில்லை என்றாலும் தொல்காப்பியர் காலம் முதல் இன்றுவரை அது வளர்ச்சிபெற்று வரும் தன்மை நோக்கின் ஆங்கில ஆய்வாளர் காட்டும் எல்லையிலும் சிறந்த வகையில் எல்லை கடவா நெறியில் மக்கள் உள்ளத்தைப் பிணிக் கும் வகையில் சிறந்துள்ளதை அறியலாம்.

சங்கப் புலவர்கள் ஐவகை நிலத்தும் வாழ்ந்த மக்களின் படைப்புகளைப் பாடினர். குறிஞ்சிப்பண், முல்லைப்பண் ஆகியவற்றிற்குப் பாணன், பாடினி ஆகியோர் பண் அமைத்தனர். சிலப்பதிகாரத்தில் கானல்வரி, வேட்டுவவரி, ஆய்ச்சியர் குரவை, குன்றக்குரவை எனப்பட இடங்களில் நாட்டுப்புற இலக்கியக் கூறுகள் இடம் பெறுகின்றன. நாயன் மார்களாலும், ஆழ்வார்களாலும் இது பெரிதும் போற்றப்பட்டுள்ளது. பரணி, கலம்பகம், பிள்ளைத்தமிழ், பள்ளு, குறவஞ்சி ஆகிய சிற்றிலக்கியங்கள் நாட்டுப்புற இலக்கிய வகையைச் சார்ந்தவையே. பாரதிதர்சன், பாரதியார் ஆகிய பிற்காலக்

கவிஞரும் நாட்டுப்பாடல்களில் நெஞ்சம் பறிகொடுத்து அதன்வழித் தம் இலக்கியத்தில் பாடல்களை எளிய முறையில் அமைத்தனர்.

நாட்டுப்புற இலக்கியத்தில் மக்களின் தொழில்கள்

உலக இயக்கத்தின் உயிர்நாடி தொழிலாகும். உழைப்பால் எதையும் உருவாக்க முடியும். வேலை செய்யும்போது களைப்பும், 'கடினமும் தோன்றியிருக்க நாட்டுப்புறத்து மக்கள் பாடுகிறார்கள். ஆடிப்பாடி வேலை செய்தால் அலுப்பிருக்காது' என்ற கூற்றிற்கேற்ப வேலை செய்கின்றனர்.

உழவர்களின் நிலை

ஒரு நாற்றாண்டுக்கு முன் நமது நாட்டில் முக்கியத் தொழில்கள் விவசாயமும், நெசவுமாகவே இருந்து வந்தன. பிற தொழில்கள் எல்லாம் இவற்றைச் சார்ந்தே இருந்தன. கொல்லன், தச்சன் முதலியவர்கள் உழவுக்குத் தேவையான பொருட்களை உற்பத்தி செய்யும் வேறு தொழில்கள் இல்லை. வண்ணான், நாவிதன், கொத்தன் போன்றோர்கள் தனி மனிதனது சக வாழ்விற்கு உதவி புரிந்தவர்கள். இத்தகைய உற்பத்தி முறையில் மனித உழைப்பே முக்கியமான உற்பத்தி சக்தியாக இருந்தது. இச்சக்தி கிராம சமுதாயத்தினுள்ளே கட்டுப்படுத்தப்பட்டிருந்தது. பெரும்பாலும் கோவில்கள் இவ்வுழைப்பின்மீது ஆதிக்கம் புரிந்தன. அதற்கடுத்தாற்போல் நிலவுடைமையாளர்களும் உயர் குலத்தினரும் ஆதிக்கம் புரிந்தார்கள். கோயில் நிலங்களையோ அல்லது நில உடைமையாளர்களின் நிலங்களையோ குத்தகைக்குப் பயிரிட்டு வந்தார்கள் எனலாம்.

உழவுக் காளை

உழவதற்குப் பயன்பட்ட மாடுகள் மனிதனுக்கு நிலையாகத் தங்கும் வாழ்க்கையை ஏற்படுத்திக் கொடுத்தது. அவனுக்குச் செல்வமும் பெருகிற்று. எனவே 'மாடு' என்ற சொல்லுக்கே 'செல்வம்' என்ற பொருள் வந்தது.

மாட்டைக் கவர்ந்து சென்று செல்வத்தைப் பெருக்கிக் கொள்ளலாம் என்று அதனைப் பழக்கத் தெரிந்தவர்கள்

பழங்காலத்தில், எண்ணினர். பழக்கத் தெரிந்தவர்களே மாட்டைப் பாதுகாக்கப் போராடினர். இதனை 'வெட்சி' என்று புறப்பொருள் இலக்கண நூல்கள் கூறுகின்றன.

சிறசில பண்டைய குழுவின் காளையை, நந்தியென்றும், பகவான் என்றும் வணங்கினர். சமண முனிவர் ரிஷபதேவர் என்று முதல் தீர்த்தங்கரருக்குப் பெயரிட்டு காளையை அவரது அடையாளமாக்கினர். பிற்கால சைவம் அதனை சிவனுக்கு வாகனமாக்கியது. மனிதன் காட்டும் காளையைப் பழக்கித் தனது வேலைக்குப் பயன்படுத்தியதையே புராணக்கதை கூறுகிறது.

தற்காலத்தில் உழவுக்குப் பயன்படும் காளையை உழவர்கள் போற்றுகிறார்கள். முதல் உழவுக்குக் காளையை அழைத்துச் செல்லும்போது அதனை அலங்கரித்துப் பிள்ளையார் பூசைப்போட்டு உழவு தொடங்குகிறார்கள்.

நடுகை

நடுகை வேலை பள்ளர், பறையர் சாதிப் பெண்கள் மட்டுமே செய்யும் வேலையாகும். பருவ வேலைகளிலேயே நாற்றுப்பிடுங்கி நடுவது தான் மிகவும் நுட்பமான வேலையாகும். முதுகு குனிந்து நெடுநேரம் வேலை செய்ய வேண்டும். சரியான இடைவெளிவிட்டு நாற்றை நட்டுச் செல்வதற்குப் பயிற்சியும் அனுபவமும் வேண்டும். நடுகைப் பாடல்கள் காதலைப் பொருளாகக் கொண்டனவும், அனுதாபம், இரக்கம், கொடுமை, பரிதாபம் இவைகளைப் பொருளாகக் கொண்டனவும் உள்ளன.

'நடுகைப் பாடல்கள் தற்பொழுது நிரம்பக் கிடைப்பதில்லை. ஆனால் சுமார் 600 வருடங்களுக்கு முன்னால் நடுகைப் பாட்டையும் அதற்கு முன் பள்ளர் ஆடும் ஆட்டத்தைக் கண்டும், கேட்டும் ரங்கநாதர் கோயில் அரையரொருவர் அவற்றைக் கற்றுக் கொள்வதற்காகப் பறைச்சேரியிலேயே சென்று தங்கிவிட்டாரென்று ஸ்ரீரங்கம் கோயில் வரலாற்றைக் கூறும் கோயிலொழுது எனும் நூல் குறிப்பிடுகிறது. சுமார் 300 ஆண்டுகளுக்கு முன் தோன்றிய அழகர் கோயில் பள்ளியில் நடுகைப் பாடல்கள் பல காணப்படுகின்றன.'²

விவசாய வேலைகளில் மிகவும் கடினமானது நாற்று நடுவதுதான். இவ்வேலையைச் செய்வது பள்ளர், பறையர் குலப்பெண்களே. வரப்பைச் சுற்றி ஆண்கள் நின்று கொண்டு நாற்றுக் கட்டுகளைச் சுமந்து வயல் வெளியில் நிற்கும் பெண்களை நோக்கி வீசி எறிவர். பெண்கள் குனிந்து முதுகு நிமிராமல் விரைவாக நாற்று முடிகளை எடுத்து நடுவார்கள். தற்காலத்தில் நடுகை நடும் பெண்கள் மனத்திற்குள்ளேயே ஏதோ ஒரு பாட்டை முணுமுணுத்துக் கொள்கிறார்கள். வரப்பிலுட்கார்ந்து கொண்டு ஆண்களும் பாடுவார்கள். பாடல்களைக் கேட்டுக் கொண்டு வேலையும் விரைவாக நடக்கும் என்பர்.

களை எடுத்தல்

நடுகையைப் போலவே களை எடுத்தலும் பெண்களின் வேலையாகும். பயிரைப் போலவே தோன்றும் களைகளைக் கூர்ந்து நோக்கி பிடுங்கியெடுக்க வேண்டும் சற்று அயர்ந்தால் களைக்குப் பதில் பயிர் கையோடு வந்துவிடும். களை எடுத்தலும் நடுகையைப் போலவே சலிப்புத் தரும் வேலை. சலிப்புத் தன்மை தோன்றாமலிருக்க வயல் வரப்பிலுள்ள ஆண்களும் வயலில் களை எடுக்கும் பெண்களும் சேர்ந்துப் பாடுவர்.

'வாய்க்கால் வரப்பு சாமி
வயக்காட்டுப் பொன்னுசாமி
களை எடுக்கும் பெண்களுக்கு
காவலுக்கு வந்த சாமி'

அறுவடைப் பாடல்கள்

கதிர் முற்றியபின் ஆண்களும் பெண்களுமாகக்கூடி அறுப்பர். அவ்வாறு அறுக்கையில் பெண்கள் அரிவாளின் பெருமையைப் பாடுவர். ஆடவரைவிடப் பெண்டிரே மேலானவர் என்னும் காதற் பாடல்களும் காணப்படுகின்றன.

கதிர் சுமத்தல்

அறுத்த கதிரைக் கட்டாகக் கட்டித் தலையில் சுமந்து கொண்டு ஆண்களும் பெண்களுமாய் வரிசையாய் வருவர்.

தலையில் இருக்கும் கட்டானது கொண்டையை ஆட்டிக்கொண்டு சல்சல் என மேலும் கீழும் குதிப்பதுபோல் தோன்றும். அத்துடன் உடுக்கை அடித்துக் கொண்டு வழிநடைப் பாடல் ஒன்றையும் பாடிவிட்டால் அத்தொழில் அலுப்பேது, களைப்பேது என்பர்.

நாட்டுப்புற கிலக்கியத்தில் பண்பாடு

பண்பாடு மக்கள் வாழ்க்கையில் முக்கிய பங்கு வகிக்கிறது. ஒரு மனிதன் பண்பாட்டுடன் வாழ்ந்தால் தான் வீடும், சமுதாயமும், நாடும் சிறந்தோங்கி வளர முடியும். மக்களின் வாழ்க்கை முறை, பழக்கவழக்கங்கள், நம்பிக்கைகள் இவற்றைப் பற்றி அறிந்து கொள்ள உதவுவதே பண்பாடாகும்.

பண்பாடு

ஒரு நாட்டின் பண்பாட்டை அளக்கும் அளவுகோல் அதன் மக்கள் தொகையோ, நகரமுகோ செல்வமோ அன்று சிறந்த பண்புகளால் நிறைந்து வாழும் மக்களே ஆவார். படைப்பினங்களிலே மனித இனம் உயர்ந்த நிலை பெற்றதாகும்.

'மாவும் மாக்களும் ஐயறிவினவே
மக்கள் தாமே ஆறறி வுயிரே'

என்று தொல்காப்பியர் நாற்பா வகுப்பதற்கு அடிப்படையாய் அமைந்தது மக்களிடம் காணப்படும் பண்பேயாகும்.

பண்பாட்டைக் குறிப்பன

'பெரும்பாலும் சிறந்த வாழ்க்கைக்கு அடிப்படையாக அமைகின்ற உள்ளப் பாங்கின் வெளிப்பாட்டையே பண்பாடு என்கிறோம். அந்த வெளிப்பாடு சுவையுணர்வாகவும், நடையுடை பாவனைகளாகவும் தோன்றும். அப்பண்பாடில்லாதவனைக் காட்டுமிராண்டி என்கிறோம். வாழ்வின் பற்பல போக்குகள் அமைந்த பல்வேறு நிலைகளையும் இந்தப் பண்பாடென்பது குறிக்கும். உடலைப் பற்றிய நன்னிலை மனத்தைப் பற்றிய தூய்மை நிலை, பேச்சின் இனிமை இவையெல்லாம் பண்பாட்டில் அடங்கும்' என்று தெ.பொ.மீனாட்சி சுந்தரம்பிள்ளை குறிப்பிடுகிறார்.

நாட்டுப்புறப் பாடல்களும் பண்பாடுகளும்

'கற்றாரைக் காமுறச் செய்யும் பாடல்கள் நிறைந்து நாகரிகம், பண்பாடு, பழக்கவழக்கங்கள், நம்பிக்கைகள் ஆகியவற்றின் கொள்கலமாய் அமைந்தது நாட்டுப்புற இலக்கியம் என்று ச.வே.சுப்பிரமணியன் குறிப்பிடுகிறார்.'⁷⁷ நாட்டுப்புறப் பாடல்கள் கவிஞர்களின் கைத்திறனின்றி உணர்ச்சிகள் வார்த்தைகளாக ஒலி நயத்துடன் இயற்கையாக அமைந்தவை. செய்யுளுக்கூரிய எதுகை, மோனை, உவமை, கற்பனை முதலிய பண்புகள் காணப்பட்டாலும் இயற்கையால் அமைந்தவையே அன்றி வலிய நுழைந்தவை அல்ல. நாட்டுப்புறப் பாடல்கள் தொன்மைக்கால மக்களின் வாழ்க்கை முறையை, எண்ணங்களின் வெளிப்பாட்டை, சிந்தனைத் திறனை, உழைப்பின் உயர்வை, பொழுது போக்கின் தன்மையை இக்கால மக்கள் அறிந்து கொள்ள உதவும் அரிய சாதனமாக விளங்குகின்றன.

பழக்கம்

'பழக்கம் என்பது கற்கும் செயலாகும். இது தனி மனிதனிடம் இயல்பாக வந்தமைக்கு இன்றியமையாதக் கூறாகக் கருதப்படுகிறது. நனவுடன் தொடங்கிய செயல் நாளடைவில் நனவின்றியே நிகழக்கூடியதாக ஆகிவிடும் செயலையே பழக்கம் என்பர்.' பழக்கம் எனும் சொல்லாட்சி பழகுதல் அல்லது பயிற்சியாதல் எனவும் கூறலாம். தனி மனிதனின் செயல்கள் தொடர்ந்து அவனால் செய்யப்பட்டு வரும்போது நாளடைவில் இது பழக்கமாகிவிடுகிறது. பழக்கமானதும் ஒரு செயலின் வெளிப்பாடு முன்பிருந்ததைவிட முறையாக பண்பட்டு வெளிவருவதை காணலாம். பழக்கம் என்பது இளமைக் காலத்தும், முதுமை காலத்தும் மனித இயல்போடு இணைந்து வருவது, 'இளமையில் கல்' எனும் வர்க்கு இளமைக் காலத்திலேயே நல்ல பழக்கங்கள் உருவாக வேண்டும் எனும் அடிப்படையில் எழுந்ததாகும்.

வழக்கம்

'வழக்கம் எனும் சொல் இலக்கண இலக்கியங்களில் கூட்டுக் குழுவினரின் செயல் களைக் குறிப்பதற்குப்

பயன்படுத்தப்பட்டுள்ளது'. தனி மனிதனிடமிருந்து பழக்கத்தைச் சமூகம் ஏற்றுக் கொண்டு அதனைத் தொடர்ந்து செயல்படுத்தும் நிலையில் வழக்கமாகிறது. தனிமனிதன் ஒருவன் பழகிப்போன முறையில் திரும்பத் திரும்பச் செய்துவரும் ஒரு குறிப்பிட்ட செயலைப் பல மனிதர் ஒன்றாகச் சேர்ந்து செய்கின்றபொழுது அது வழக்கம் எனப் போற்றப்படுகிறது என்று குறிப்பிடுவர். ஒரு பழக்கத்தைப் பல தலைமுறையினர் இடைவெளியின்றித் தொடர்ந்து செய்து வரும்போது வழக்கமாறி நிலைபெறு அடைகிறது. வழக்கம் என்பது சமுதாயத்திற்குரியதாக அமைந்துவிடுகிறது.

நாட்டுப்புற இலக்கியத்தில் மக்களின் வெளிப்பாடு

மனித இனம் தோன்றிய நாளிலிருந்தே இறைவழிபாடு இருந்து வந்துள்ளது. மனிதனின் நாகரிக வளர்ச்சிக்கேற்ப பல மாற்றங்கள் ஏற்பட்டுள்ளன. வாழ்வாங்கு வாழும் மக்கள் வாழ்வில் கோயில்கள் சிறந்த இடத்தைப் பெறுகின்றன. நோயற்ற வாழ்வையும் குறைவற்ற செல்வத்தையும் தருபவன் இறைவன் என்னும் நம்பிக்கை தொன்று தொட்டு மக்களிடம் உள்ளமையால் இறைவனை வழிபடுகின்றனர். இறைவனிடத்துத் தமக்குள்ள பக்தியையும், அன்பினையும் புலப்படுத்தும் பாங்கிலும் இறைவனின் உயர்வைச் சிறப்பிக்கும் தன்மையிலும் மக்கள் விழாக்களை எடுக்கின்றனர்.

வழிபாட்டின் தோற்றம்

இயற்கையின் ஆற்றலைக் கண்டு அஞ்சிய மனிதன் அதன் சீற்றத்திற்கு ஆளாகாமல் இருக்கலாம் என்று நம்பினான். அதனடிப்படையில் இயற்கை வழிபாடுகளும், விழாக்களும் தோற்றம் பெற்றன. பலிகள் தருவதன் மூலம் இயற்கையினை வயப்படுத்திவிடலாம் என்ற அடிப்படையில் விழாக்களும், கொண்டாட்டங்களும் தோன்றின. காலப்போக்கில் இயற்கைக்கு உருவம் கொடுத்து வழிபடத் தொடங்கினர். பழங்கால மனிதன் இடி, மின்னல், மழை போன்றவற்றைக் கண்டு அஞ்சினான். நோய் முதலியவற்றிற்கு ஆவிகளே காரணமென எண்ணினான். அவ்வாறானவை வழிபட ஆரம்பித்தான்.

'கோயிலில்லா ஊரில் குடியிருக்க வேண்டாம்' என்ற பழமொழிக்கேற்ப நல்லது நடந்தால் தெய்வ அருள் என்றும், தீயவை நடந்தால் தெய்வத்தின் கோபம் என்றும் இன்றும் மக்கள் நம்புகின்றனர். இவ்வாறு மக்களிடையே வழிபாடு தோற்றம் பெற்றது.

முன்னோர் வழிபாடு, ஆவியுலக வழிபாடு, அச்ச உணர்வு வழிபாடு, இயற்கை வழிபாடு, குலதெய்வ வழிபாடு, ஊர்த்தெய்வ வழிபாடு, பெருந்தெய்வ வழிபாடு, சிறுதெய்வ வழிபாடு, மாரியம்மனுக்குக் கரக வழிபாடு, குலக்குறி வழிபாடு, குறியும் வழிபாடும் இவ்வாறு பல வழிபாட்டு வகைகள் உள்ளன.

நீறைவுரை

இவ்வாய்வின் வழியாக, நாட்டுப்புற இலக்கியம் மக்களின் உள்ளத்து உணர்ச்சிகளை உள்ளது உள்ளவாறு வெளிப்படுத்தும் பாங்கில் அமைந்துள்ளது. உழைக்கும் தொழிலாளி கலப்பையைப் பயன்படுத்தி முன்னேறினான். இவ்வாறு முன்னேறிய தொழிலாளி வயலில் வேலை செய்யும்போது தம் சோர்வு தெரியாமல் இருக்கப் பாடல்களைப் பாடினான். அப்பொழுது நாட்டுப்புறப்பாட்டு அவனுக்குத் துணை புரிந்தது. நாட்டுப்புறப் பாடல்கள் அம்மக்களின் பழக்கவழக்க மரபுகளை அறிந்து கொள்ளப் பயன்பட்டன. மக்களின் அச்சத்தைப் போக்குவதற்குரிய வழிபாடு இன்றியமையாததாக இருந்துள்ளது என்பதைப் பாடல்கள் வழியாக அறியமுடிகிறது.

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2. வானமாமலை.நா, தமிழர் நாட்டுப்பாடல், ப.456
3. மேலது நூல், ப.461
4. தொல்காப்பியர், தொல்காப்பியம், மரபியல், நூ.32
5. சுப்பிரமணியன்.ச.வே, தமிழ் நாட்டுப்புற இயல் ஆய்வு, ப.7

நாட்டுப்புறக் கதைப்பாடல்களில் பழக்க வழக்கங்கள்

முனைவர் மு. சுபாஷினி

முகவுரை

பண்டைக் காலச் சமுதாயம் வாழ்ந்த முறைமைகளை இலக்கியங்கள் தெளிவுபடுத்துகின்றன. நாட்டுப்புற மக்களின் வாழ்க்கை முறைகளில் பழக்கவழக்கங்களை எவ்விதம் பின்பற்றி வந்தனர் என்பதை நாட்டுப்புறக் கதைப்பாடல்கள் வாயிலாக ஆராயும் விதத்தில் இக்கட்டுரை அமைந்துள்ளது. நாட்டுப்புறச் சமுதாயம் என்பதற்கு, 'ஒருபடித்தான மக்கள்தொகை மற்றும் பண்பாடு உடைய தனிப்படுத்தப்பட்ட ஒரு சிறிய சமுதாயம் - அடிப்படையானதொழில் பாகுபாடு, முறைப்படுத்தப்படாத, தெளிவற்ற மதிப்பீடு முறை, சாதாரண உத்திமுறை, நிலையான வரன்முறை மரபு சார்ந்த தலைமைப் பதவி, உறுதியான இரத்த மற்றும் குலமரபுச் சின்னம் சார்ந்த உறவுமுறை இணைப்பு, பெருகும் போற்றுதலுக்குரிய மரபு, மதம் மற்றும் மந்திரத்துக்கு அதிக முக்கியத்துவம் அளித்தல் ஆகியவை இச்சமுதாயத்தில் காணப்படும்' என்று நா.இராபர்ட் சத்திய சோசப் அவர்கள் கலைச்சொல் விளக்க அகராதியில் விளக்கம் தருகிறார்.

நாட்டுப்புறக் கதைப்பாடல்களில் பழக்க வழக்கங்கள்

மனித நாகரிகத்தில் முன்பிருந்தே சில பழக்க வழக்கங்களை மக்கள் மேற்கொண்டிருந்தனர். தொழில்கள் மாறியதால் சமுதாய மாற்றமும் நிகழ்ந்தது. சமுதாய மாற்றத்தால் பழக்க வழக்கங்களும் காலத்திற்கேற்ப மாறி

உதவிப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி - 01

Occurrence of Brown Vine Snake in Pachamalai Hills, India



Roadkill of Brown Vine Snake found in Pachamalai, Tamil Nadu, India.

Tropical Asian vine snakes *Ahaetulla* spp. are widely distributed in the Asian mainland, peninsular India, parts of the Sundaic region and their surrounding islands (Mallik et al. 2020). They further reported that there are 17 lineages in *Ahaetulla* within its distribution range. Pachamalai Hills are located (11.303° N & 78.654° E) in

north-east of Tamil Nadu, India covering an area of 14,122 km² at an altitude ranging from 500–1,000 m.

On 25 January 2021, a dead snake was found on the road (11.303° N & 78.654° E around 680m) leading to Pachamalai Hills. It was assumed to have been killed by vehicular traffic and was identified as

Brown Vine Snake *Ahaetulla sahyadrensis* based on the morphological characters given by Mallik et al. (2020).

As the Western Ghats population of *A. pulverulenta sensu lato* is diagnosed to be distinct at species rank from the Sri Lankan population, Mallik et al (2020) recently provided a new replacement



name *Ahaetulla sahyadrensis*. Thus, *A. pulverulenta* is considered endemic to Sri Lanka. The length of the snake was 138 cm. The dorsal side of the body was light brown in colour with dark brownish anteriorly converging bars from nape to midbody. The head had dark brownish rhomboid markings with a deep eye-stripe from the nostril to nape. Venkatraman et al. (1997) reported this species in Siruvani foot hills of Western Ghats, Tamil Nadu. However, it was not included in the list published by Daniels (1994) for the Eastern Ghats of Tamil Nadu. Hence, it is a new addition to Pachamalai Hills.

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Ministry of Environment,
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Deep Sea

Faunal Diversity in India



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ZOOLOGICAL SURVEY OF INDIA

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PELAGIC AVIFAUNA



V. Gokula^{*1} and C. Venkataraman²

Pelagic birds spend greater part of their life on open Ocean and return to land for a shorter period only to breed. As they are found only far out to sea, understanding their status and ecology is often challenging. Hence, unlike other group of birds, pelagic birds are poorly studied in India. India, with a long coastline of nearly 7500 km, shares its maritime boundary with seven countries. India harbours around 72 pelagic species of birds belonging to five orders and nine families. Of the 72 species, 15 breed in Indian Territories while the rest are mere migrants/vagrants to India. Birds belonging to phaethontiformes and suliformes are truly pelagic in habitat and often sighted in poor numbers. Habitat loss, climate change, and pollution are the immediate threats to pelagic birds.

Keywords: oceanic, pelagic, marine, avifauna

INTRODUCTION

Pelagic birds spend a significant portion of its life on open Ocean and return to land for a shorter period only to breed. They have salt glands to extract excess salt accumulated due to the intake of oceanic prey and marine water and thus without accumulating toxic levels of salt in their bloodstreams. These two unique characters differentiate the pelagic birds from the rest of the birds. However, as there are disagreements about what makes a bird species truly pelagic, even birds that do not share the above mentioned characters are also treated as pelagic birds. Pelagic birds belong to the orders namely Phaethontiformes (tropic birds), gaviiformes (loons/divers), procellariiformes (storm petrels, petrels, shearwaters), suliformes (frigatebirds, gannets and boobies), and charadriiformes (gulls, terns, skimmers, skuas). However, there are disagreements among scientists in the inclusion of all the species of birds belonging to above mentioned orders as pelagic birds. Birds belonging to other orders due to their strong association with coastal habitats are considered near-pelagic despite the fact that they are not seabirds in the formal sense (eg. Cormorants and pelicans).

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Besides, wings of true pelagic birds are best suited to fly effortlessly for long periods without rest for thousands of miles offshore. They do occasionally rest or float on the surface of the water for a brief period. Being oceanic in habit, they feed on marine organism such as fish, squid, and crustaceans by plunging/skimming. Besides, they are opportunistic/refined kleptoparasites. As they are found only far out to sea, understanding their status and ecology is often challenging. Pelagic touring is the only way to study the pelagic birds. As it is very expensive and time consuming, opportunistic birding by professional/non professional on a cruise vacation is often included to understand the status of pelagic birds. Hence, unlike other group of birds, pelagic birds are poorly studied in India.


India has around 7500 km coastline. It shares its coastal boundary with seven countries namely Indonesia, Pakistan, Thailand, Sri Lanka, Maldives, Bangladesh, and Myanmar. It has a 12 nautical-miles territorial sea and 200 nautical miles exclusive economic zone covering a territory of nearly 314,400 square kilometers. Successful delineation of maritime boundary is very much important for scientific explorations. India has maritime boundary demarcated with Pakistan in 1968, with Maldives in 1976, with Sri Lanka in 1976, with Indonesia in 1977, with Thailand in 1978, with Myanmar in 1986, and with Bangladesh in 2014. However, Sir Creek issue with Pakistan and fisherman issue of Sri Lanka are the two major maritime disputes in which India is involved at present. India's offshore islands are Andaman and Nicobar Islands, situated in the Bay of Bengal, and Lakshadweep, situated in the Arabian Sea. Andaman has 204 Islands while Nicobar has 19 Islands. Lakshadweep comprises 36 islands. Birds that exploit the above mentioned Indian marine territory are only dealt in the present study.

India has diverse habitat types (tidal flats, rocky shore, coastal dunes, mudflats, mangroves, wetlands, marshes, seaweed and seagrass bed, deltaic plains, lagoons, estuaries, and coral reefs) for pelagic birds to forage and breed. However, unlike forest birds and wetland birds, no systematic study on species level or community level on pelagic birds has been undertaken in India, leading to a paucity of data. Hence, the available records were based on wind-blown individuals sighted/collected from onshore and a few offshore expeditions (Hume, 1876; Abdulali, 1970; Madsen, 1990; Mohan, 1989; Lainer, 2003; Pande *et al.*, 2007, Mathew and Ambedkar, 1964; Daniels, 1992). Praveen *et al.* (2011) initiated the first exclusive pelagic bird survey by conducting three offshore expeditions from Kerala. Subsequently, Praveen (2014) conducted another survey off the west and east coast. Recently, Baidya *et al.* (2017) conducted a survey off the Goa.

PELAGIC BIRDS OF INDIA

In general, India harbours around 72 species of pelagic birds (Table 1) belonging to five orders (phaethontiformes, gaviiformes, procellariiformes, suliformes, and charadriiformes) and nine families (Phaethontidae, Gaviidae, Oceanitidae, Hydrobatidae, Procellariidae, Fregatidae, Sulidae, Laridae, and Stercorariidae).

Three species belonging to phaethontiformes namely Red-billed Tropicbird *Phaethon aethereus* Red-tailed Tropicbird *Phaethon rubricauda* and White-tailed Tropicbird



Phaethon lepturus are recorded in India. All the three are truly pelagic in habitat. Among the three species, the white-tailed tropicbird was recorded from Rameshwaram, Tamil Nadu by Balachandran (1992), from River Barrak by Hume (1877) and Rasmussen and Anderton (2005), at Kovalam, Kerala by (Sashikumar *et al.*, 2011) and from northern Kerala by Palot (2011). Redbilled tropic bird was recently sighted at Kozhikode by Palot (2003). Harrison (1983) reported that among the three species, only red-tailed tropic bird breeds in Nicobar Islands.


Two species belonging to gaviiformes namely Red-throated Loon *Gaviastellata* Arctic Loon *Gavia arctica* are recorded in India (Ali and Ripley 1983). Both are treated as vagrant to India. However, Red-throated Diver, an holarctic species, breeding largely north of 50° N and far into the high arctic, and wintering mainly along the north coasts of the Atlantic and Pacific Oceans as well as in several major lakes and seas such as the Great Lakes and the Black, Caspian and Mediterranean Seas (del Hoyo *et al.*, 1992), was not reported from India. Available two records are from Nepal (Ticehurst 1927; Tebb *et al.*, 2004). Unusual weather phenomenon was reported possible reason for these records in Nepal. Two reports (Jones, 1922; Steijn and Vries, 2009) confirmed the presence of Black-throated Diver/Arctic Loon *Gavia arctica* (Linnaeus, 1758) in Indian boundary.

Three species belonging to the order Procellariiformes and the family Oceanitidae namely Wilson's Storm-petrel *Oceanites oceanicus*, White-faced Storm-petrel *Pelagodroma marina*, and Black-bellied Storm-petrel *Fregetta tropica* are recorded in India. Wilson's Storm-petrel breeds in the sub-Antarctic region in the Southern Ocean and is considered a non-breeding visitor off the western coast of India, Lakshadweep (Rasmussen and Anderton 2005) and Indo-Sri Lankan water (Phillips, 1955; Sashikumar *et al.*, 2011; Robertson, 1994). Harrison (1983) reported that White-faced Storm-petrel and Black-bellied Storm-petrel are rare visitors or vagrants to southwest coastal waters of India. Among the storm petrels, Wilson's storm-petrel is reported the commonest (Chandran *et al.*, 2011; Praveen *et al.*, 2011; Karuthedathu *et al.*, 2013)

One species belonging to the order procellariiformes and the family Hydrobatidae namely Swinhoe's Storm-petrel *Hydrobates monorhis* are recorded in India. Rasmussen and Anderton (2005) reported Swinhoe's Storm-petrels as unconfirmed non-breeding visitor to India, however, sashikumar *et al.* (2011) and Vivek *et al.* (2011) confirmed its range in Indian coast while Chandran *et al.* (2011) reported it as common.

Ten species belonging to the order procellariiformes and the family Procellariidae namely Cape Petrel *Daption capense* Barau's Petrel *Pterodroma barau* Wedge-tailed Shearwater *Ardenna pacifica* Short-tailed Shearwater *Ardenna tenuirostris* Flesh-footed Shearwater *Ardenna carneipes* Streaked Shearwater *Calonectris leucomelas* Cory's Shearwater *Calonectris borealis* Tropical Shearwater *Puffinus bailloni* Persian Shearwater *Puffinus persicus* Jouanin's Petrel *Bulweria fallax* are recorded in India.

Flesh-footed Shearwater is the most common seabird in west coast (Chandran *et al.*, 2011, Karuthedathu *et al.*, 2013, Palot, 2008). Praveen *et al.* (2011) reported Wedge-tailed Shearwater from the coast of Kannur on May 28, 2011. Karuthedathu *et al.* (2013) reported



Streaked Shearwater from Indian Coast. Sashikumar *et al.* (2011) reported Persian Shearwater from Indian coast. The Jouanin's Petrel was recorded by Karuthedathu *et al.* (2012), Sashikumar *et al.* (2011), Robertson (1995), and Rahmani (2012). No recent records are available for the other species.

Three species belonging to the order Suliformes and the family Fregatidae namely Lesser Frigatebird *Fregata ariel* Great Frigatebird *Fregata minor* Christmas Frigatebird *Fregata andrewsi* are recorded in India. Karuthedathu *et al.* (2015) recorded Lesser Frigatebird and Christmas Frigatebird from east and west coast of India.

Three species belonging to the order Suliformes and the family Sulidae namely Red-footed Booby *Sula sula* Brown Booby *Sula leucogaster* Masked Booby *Sula dactylatra* are recorded in India. Karuthedathu *et al.* (2012) and Sashikumar *et al.* (2011) reported Masked Booby as one of the most frequent windblown species. Jamalabad (2013) recorded Brown Booby while Karmakar (2011) reported Red-footed Booby.

37 species belonging to the order Charadriiformes and the family Laridae namely Brown Noddy *Anous stolidus* Lesser Noddy *Anous tenuirostris* Black Noddy *Anous minutus* Common White Tern *Gygis alba* Indian Skimmer *Rynchops albicollis* Little Gull *Hydrocoloeus minutus* Sabine's Gull *Xema sabini* Black-legged Kittiwake *Rissa tridactyla* Slender-billed Gull *Larus genei* Brown-headed Gull *Larus brunnicephalus* Black-headed Gull *Larus ridibundus* Franklin's Gull *Larus pipixcan* Pallas's Gull *Larus ichthyaetus* Sooty Gull *Larus hemprichii* Mew Gull *Larus canus* Lesser Black-backed Gull *Larus fuscus* Caspian Gull *Larus cachinnans* Arctic Herring Gull *Larus smithsonianus* Sooty Tern *Onychoprion fuscatus* Bridled Tern *Onychoprion anaethetus* Little Tern *Sternula albifrons* Saunders's Tern *Sternula saundersi* Common Gull-billed Tern *Gelochelidon nilotica* Caspian Tern *Hydroprogne caspia* Whiskered Tern *Chlidonias hybrid* White-winged Tern *Chlidonias leucopterus* Black Tern *Chlidonias niger* River Tern *Sterna aurantia* Roseate Tern *Sterna dougallii* Black-naped Tern *Sterna sumatrana* Common Tern *Sterna hirundo* White-cheeked Tern *Sterna repressa* Arctic Tern *Sterna paradisaea* Black-bellied Tern *Sterna acuticauda* Lesser Crested Tern *Thalasseus bengalensis* Sandwich Tern *Thalasseus sandvicensis* and Greater Crested Tern *Thalasseus bergii* are recorded in India.

Praveen (2013) recorded Bridled Terns, Sooty Terns (wind-blown species to the coasts- Karuthedathu *et al.*, 2012), Brown Noddy, Great Crested Tern, Lesser Crested Tern, Sandwich Tern, Common Terns, Little tern, Whiskered tern and Gull-billed Terns, Brown-headed Gull, Black-headed Gull, Pallas's Gulls and Lesser Black-backed Gulls from Kerala.

Five species belonging to the order Charadriiformes and the family Stercorariidae namely Long-tailed Jaeger *Stercorarius longicaudus* Arctic Jaeger *Stercorarius parasiticus* Pomarine Jaeger *Stercorarius pomarinus* South Polar Skua *Catharacta maccormicki* Brown Skua *Catharacta Antarctica* are recorded in India. Praveen (2013) recorded Parasitic Jaeger *S. parasiticus* as the most common Jaeger species, with most sightings during September and April along with Pomarine Jaeger *S. pomarinus* from the Karnataka and Kerala coasts. Praveen *et al.*, 2013 and Praveen (2013) confirmed the presence of South Polar Skua *S. maccormicki* from the Thiruvananthapuram coast.

Of the 72 species of pelagic birds, 15 species of birds breed in Indian Territories while other species of birds are non-breeder to India. Birds belonging to phaethontiformes and suliformes are truly pelagic in habitat. In general, all these truly pelagic birds are sighted in poor numbers.

Table 1. Pelagic birds of India

Sl. No.	Order	Family	Common Name	Scientific Name
1	Phaethontiformes	Phaethontidae	Red-billed Tropicbird	<i>Phaethon aethereus</i>
2	Phaethontiformes	Phaethontidae	Red-tailed Tropicbird	<i>Phaethon rubricauda</i>
3	Phaethontiformes	Phaethontidae	White-tailed Tropicbird	<i>Phaethon lepturus</i>
4	Gaviiformes	Gaviidae	Red-throated Diver	<i>Gavia stellata</i>
5	Gaviiformes	Gaviidae	Black-throated Diver	<i>Gavia arctica</i>
6	Procellariiformes	Oceanitidae	Wilson's Storm-petrel	<i>Oceanites oceanicus</i>
7	Procellariiformes	Oceanitidae	White-faced Storm-petrel	<i>Pelagodroma marina</i>
8	Procellariiformes	Oceanitidae	Black-bellied Storm-petrel	<i>Fregetta tropica</i>
9	Procellariiformes	Hydrobatidae	Band-rumped Storm-petrel	<i>Hydrobates castro</i>
10	Procellariiformes	Hydrobatidae	Swinhoe's Storm-petrel	<i>Hydrobates monorhis</i>
11	Procellariiformes	Procellariidae	Cape Petrel	<i>Daption capense</i>
12	Procellariiformes	Procellariidae	Barau's Petrel	<i>Pterodroma barau</i>
13	Procellariiformes	Procellariidae	Wedge-tailed Shearwater	<i>Ardenna pacifica</i>
14	Procellariiformes	Procellariidae	Short-tailed Shearwater	<i>Ardenna tenuirostris</i>
15	Procellariiformes	Procellariidae	Sooty Shearwater	<i>Ardenna grisea</i>
16	Procellariiformes	Procellariidae	Flesh-footed Shearwater	<i>Ardenna carneipes</i>
17	Procellariiformes	Procellariidae	Streaked Shearwater	<i>Calonectris leucomelas</i>
18	Procellariiformes	Procellariidae	Cory's Shearwater	<i>Calonectris borealis</i>
19	Procellariiformes	Procellariidae	Tropical Shearwater	<i>Puffinus bailloni</i>
20	Procellariiformes	Procellariidae	Persian Shearwater	<i>Puffinus persicus</i>

Sl. No.	Order	Family	Common Name	Scientific Name
21	Procellariiformes	Procellariidae	Bulwer's Petrel	<i>Bulweria bulwerii</i>
22	Procellariiformes	Procellariidae	Jouanin's Petrel	<i>Bulweria fallax</i>
23	Suliformes	Fregatidae	Lesser Frigatebird	<i>Fregata ariel</i>
24	Suliformes	Fregatidae	Great Frigatebird	<i>Fregata minor</i>
25	Suliformes	Fregatidae	Christmas Island Frigatebird	<i>Fregata andrewsi</i>
26	Suliformes	Sulidae	Abbott's Booby	<i>Papasula abbotti</i>
27	Suliformes	Sulidae	Red-footed Booby	<i>Sula sula</i>
28	Suliformes	Sulidae	Brown Booby	<i>Sula leucogaster</i>
29	Suliformes	Sulidae	Masked Booby	<i>Sula dactylatra</i>
30	Charadriiformes	Stercorariidae	Long-tailed Skua	<i>Stercorarius longicaudus</i>
31	Charadriiformes	Stercorariidae	Arctic Skua	<i>Stercorarius parasiticus</i>
32	Charadriiformes	Stercorariidae	Pomarine Skua	<i>Stercorarius pomarinus</i>
33	Charadriiformes	Stercorariidae	South Polar Skua	<i>Stercorarius maccormicki</i>
34	Charadriiformes	Stercorariidae	Brown Skua	<i>Stercorarius antarcticus</i>
35	Charadriiformes	Laridae	Brown Noddy	<i>Anous stolidus</i>
36	Charadriiformes	Laridae	Lesser Noddy	<i>Anous tenuirostris</i>
37	Charadriiformes	Laridae	Black Noddy	<i>Anous minutus</i>
38	Charadriiformes	Laridae	White Tern	<i>Gygis alba</i>
39	Charadriiformes	Laridae	Indian Skimmer	<i>Rynchops albicollis</i>
40	Charadriiformes	Laridae	Black-legged Kittiwake	<i>Rissa tridactyla</i>
41	Charadriiformes	Laridae	Sabine's Gull	<i>Xemasabini</i>
42	Charadriiformes	Laridae	Slender-billed Gull	<i>Chroicocephalus genei</i>
43	Charadriiformes	Laridae	Brown-headed Gull	<i>Chroicocephalus brunnicephalus</i>
44	Charadriiformes	Laridae	Black-headed Gull	<i>Chroicocephalus ridibundus</i>
45	Charadriiformes	Laridae	Little Gull	<i>Hydrocoloeus minutus</i>
46	Charadriiformes	Laridae	Franklin's Gull	<i>Leucophaeus pipixcan</i>
47	Charadriiformes	Laridae	White-eyed Gull	<i>Ichthyaetus leucophthalmus</i>
48	Charadriiformes	Laridae	Sooty Gull	<i>Ichthyaetus hemprichii</i>
49	Charadriiformes	Laridae	Pallas's Gull	<i>Ichthyaetus ichthyaetus</i>
50	Charadriiformes	Laridae	Mew Gull	<i>Larus canus</i>
51	Charadriiformes	Laridae	Lesser Black-backed Gull	<i>Larus fuscus</i>
52	Charadriiformes	Laridae	Caspian Gull	<i>Larus cachinnans</i>

Sl. No.	Order	Family	Common Name	Scientific Name
53	Charadriiformes	Laridae	Mongolian Gull	<i>Larus smithsonianus mongolicus</i>
54	Charadriiformes	Laridae	Sooty Tern	<i>Onychoprion fuscatus</i>
55	Charadriiformes	Laridae	Bridled Tern	<i>Onychoprion anaethetus</i>
56	Charadriiformes	Laridae	Little Tern	<i>Sternula albifrons</i>
57	Charadriiformes	Laridae	Saunders's Tern	<i>Sternula saundersi</i>
58	Charadriiformes	Laridae	Gull-billed Tern	<i>Gelochelidon nilotica</i>
59	Charadriiformes	Laridae	Caspian Tern	<i>Hydroprogne caspia</i>
60	Charadriiformes	Laridae	Whiskered Tern	<i>Chlidonias hybrida</i>
61	Charadriiformes	Laridae	White-winged Tern	<i>Chlidonias leucopterus</i>
62	Charadriiformes	Laridae	Black Tern	<i>Chlidonias niger</i>
63	Charadriiformes	Laridae	River Tern	<i>Sterna aurantia</i>
64	Charadriiformes	Laridae	Roseate Tern	<i>Sterna dougallii</i>
65	Charadriiformes	Laridae	Black-naped Tern	<i>Sterna sumatrana</i>
66	Charadriiformes	Laridae	Common Tern	<i>Sterna hirundo</i>
67	Charadriiformes	Laridae	White-cheeked Tern	<i>Sterna repressa</i>
68	Charadriiformes	Laridae	Arctic Tern	<i>Sterna paradisaea</i>
69	Charadriiformes	Laridae	Black-bellied Tern	<i>Sterna acuticauda</i>
70	Charadriiformes	Laridae	Lesser Crested Tern	<i>Thalasseus bengalensis</i>
71	Charadriiformes	Laridae	Sandwich Tern	<i>Thalasseus sandvicensis</i>
72	Charadriiformes	Laridae	Greater Crested Tern	<i>Thalasseus bergii</i>

Table 2. Status of Pelagic birds of India

Sl. No.	English and scientific name	Status	Habitat	Breeding status	Records
1	Red-billed Tropicbird <i>Phaethon aethereus</i>	Rare	Pelagic	Non-breeder	West coast
2	Red-tailed Tropicbird <i>Phaethon rubricauda</i>	Rare	Pelagic	Breeder (in Nicobar)	Nicobar
3	White-tailed Tropicbird <i>Phaethon lepturus</i>	Rare	Pelagic	Non-breeder	East coast
4	Red-throated Diver <i>Gavia stellata</i>	-	-	-	No recent record
5	Black-throated Diver <i>Gavia arctica</i>	Rare	Less partially pelagic	Non-breeder	Hariyana and Assam
6	Wilson's Storm-petrel <i>Oceanites oceanicus</i>	Rare	Pelagic	Non-breeder	Laccadives, West coast, Indo-Sri lankan coast

Sl. No.	English and scientific name	Status	Habitat	Breeding status	Records
7	White-faced Storm-petrel <i>Pelagodroma marina</i>	Rare	Pelagic	Non-breeder	West coast
8	Black-bellied Storm-petrel <i>Fregetta tropica</i>	Rare	Pelagic	Non-breeder	West coast
9	Band-rumped Storm-petrel <i>Hydrobates castro</i>	-	-	-	No recent record
10	Swinhoe's Storm-petrel <i>Hydrobates monorhis</i>	Rare	Pelagic	Non-breeder	East coast
11	Cape Petrel <i>Daption capense</i>	Rare	Pelagic	Non-breeder	Indo-Sri Lankan Coast
12	Barau's Petrel <i>Pterodroma barauii</i>	Rare	Pelagic	Non-breeder	Indo-Sri Lankan Coast, Laccadives
13	Wedge-tailed Shearwater <i>Ardenna pacifica</i>	Rare	Pelagic	Non-breeder	Laccadives, West coast
14	Short-tailed Shearwater <i>Ardenna tenuirostris</i>	-	-	-	No recent record
15	Sooty Shearwater <i>Ardenna grisea</i>	-	-	-	No recent record
16	Flesh-footed Shearwater <i>Ardenna carneipes</i>	Rare	Pelagic	Non-breeder	Laccadives, East coast
17	Streaked Shearwater <i>Calonectris leucomelas</i>	Rare	Partially Pelagic	Non-breeder	Indo-Sri lankan Coast, East Coast
18	Cory's Shearwater <i>Calonectris borealis</i>	-	-	-	No recent record
19	Tropical Shearwater <i>Puffinus bailloni</i>	-	-	-	No recent record
20	Persian Shearwater <i>Puffinus persicus</i>	Rare	Pelagic	Non-breeder	East coast
21	Bulwer's Petrel <i>Bulweria bulwerii</i>	Rare	Pelagic	Non-breeder	East coast
22	Jouanin's Petrel <i>Bulweria fallax</i>	Rare	Pelagic	Non-breeder	East coast, West Coast, Laccadives
23	Lesser Frigatebird <i>Fregata ariel</i>	Rare	Pelagic	Non-breeder	East coast, West coast
24	Great Frigatebird <i>Fregata minor</i>	Rare	Pelagic	Non-breeder	East coast, West coast
25	Christmas Island Frigatebird <i>Fregata andrewsi</i>	Very rare	Pelagic	Non-breeder	East coast, West coast
26	Abbott's Booby <i>Papasula abbotti</i>	-	-	-	No recent record
27	Red-footed Booby <i>Sula sula</i>	Very rare	Pelagic	Non-breeder	West coast
28	Brown Booby <i>Sula leucogaster</i>	Very rare	Pelagic	Non-breeder	West coast
29	Masked Booby <i>Sula dactylatra</i>	Rare	Pelagic	Non-breeder	Laccadives, West Coast
30	Long-tailed Skua <i>Stercorarius longicaudus</i>	-	-	-	No recent record
31	Arctic Skua <i>Stercorarius parasiticus</i>	Rare	Partially Pelagic	Non-breeder	West Coast, East Coast

Sl. No.	English and scientific name	Status	Habitat	Breeding status	Records
32	Pomarine Skua <i>Stercorarius pomarinus</i>	Rare	Partially Pelagic	Non-breeder	West coast
33	South Polar Skua <i>Stercorarius maccormicki</i>	Rare	Partially Pelagic	Non-breeder	West coast
34	Brown Skua <i>Stercorarius antarcticus</i>	Rare	Partially Pelagic	Non-breeder	West coast, Indo-Sri lankan Coast
35	Brown Noddy <i>Anous stolidus</i>	Rare	Pelagic	Breeder (in Laccadives)	Laccadives, Andamans and Nicobar
36	Lesser Noddy <i>Anous tenuirostris</i>	-	-	-	No recent record
37	Black Noddy <i>Anous minutus</i>	Rare	Pelagic	Non-breeder	Laccadives
38	White Tern <i>Gygis alba</i>	Rare	Pelagic	Non-breeder	No recent record
39	Indian Skimmer <i>Rynchops albicollis</i>	Common	Less partially pelagic	Non-breeder	East Coast, West Coast, Inland
40	Black-legged Kittiwake <i>Rissa tridactyla</i>	-	-	-	No recent record
41	Sabine's Gull <i>Xema sabini</i>	-	-	-	No recent record
42	Slender-billed Gull <i>Chroicocephalus genei</i>	Rare	Partially Pelagic	Non-breeder	East Coast, West Coast
43	Brown-headed Gull <i>Chroicocephalus brunnicephalus</i>	Common	Partially Pelagic	Non-breeder	Laccadives, West coast
44	Black-headed Gull <i>Chroicocephalus ridibundus</i>	Common	Partially Pelagic	Non-breeder	Laccadives, West coast
45	Little Gull <i>Hydrocoloeus minutus</i>	Rare	Less partially pelagic	Non-breeder	Coastal and Inland waters
46	Franklin's Gull <i>Leucophaeus pipixcan</i>	-	-	-	No recent record
47	White-eyed Gull <i>Ichthyaetus leucophthalmus</i>	-	-	-	No recent record
48	Sooty Gull <i>Ichthyaetus hemprichii</i>	Rare	Partially Pelagic	Non-breeder	East Coast
49	Pallas's Gull <i>Ichthyaetus ichthyaetus</i>	Less common	Partially Pelagic	Non-breeder	East Coast, west coast
50	Mew Gull <i>Larus canus</i>	Rare		Non-breeder	No recent record
51	Lesser Black-backed Gull <i>Larus fuscus</i>	-	-	-	No recent record
52	Caspian Gull <i>Larus cachinnans</i>	Less common	Less partially pelagic	Non-breeder	Inlands, East Coast, West Coast
53	Mongolian Gull <i>Larus smithsonianus mongolicus</i>	-	-	-	No recent record
54	Sooty Tern <i>Onychoprion fuscatus</i>	Common	Partially Pelagic	Breeder (in Vengula Rocks, Laccadives)	west coast

Sl. No.	English and scientific name	Status	Habitat	Breeding status	Records
55	Bridled Tern <i>Onychoprion anaethetus</i>	Common	Partially Pelagic	Breeder (in Vengula Rocks)	west coast
56	Little Tern <i>Sternula albifrons</i>	Common	Less partially pelagic	Breeder	Inlands, East Coast, West Coast
57	Saunders's Tern <i>Sternula saundersi</i>	Common	Less partially pelagic	Breeder (unconfirmed)	Coastal and Inland waters
58	Gull-billed Tern <i>Gelochelidon nilotica</i>	Common	Less partially pelagic	Breeder (in Bengal, Orissa)	East coast, West coast
59	Caspian Tern <i>Hydroprogne caspia</i>	Common	Less partially pelagic	Breeder (in Gujarat)	East coast, West coast
60	Whiskered Tern <i>Chlidonias hybrida</i>	Common	Less partially pelagic	Breeder (in kashmir, Assam)	Inlands, East Coast, West Coast
61	White-winged Tern <i>Chlidonias leucopterus</i>	Common	Less partially pelagic	Non-breeder	Inlands, East Coast, West Coast
62	Black Tern <i>Chlidonias niger</i>	-	-	-	No recent record
63	River Tern <i>Sterna aurantia</i>	Common	Non-pelagic	Breeder	Almost all parts of India
64	Roseate Tern <i>Sterna dougallii</i>	Less common	Less partially pelagic	Breeder (in offshore Islands)	Andamans and Nicobar
65	Black-naped Tern <i>Sterna sumatrana</i>	-	-	-	No recent record
66	Common Tern <i>Sterna hirundo</i>	very common	Non-pelagic	Breeder (in ladakh)	Inlands, East Coast, West Coast
67	White-cheeked Tern <i>Sterna repressa</i>	Common	Less partially pelagic	Breeder (in Vengula Rocks)	Inlands, East Coast, West Coast
68	Arctic Tern <i>Sterna paradisaea</i>	very common	-	-	No recent record
69	Black-bellied Tern <i>Sterna acuticauda</i>	very common	Less partially pelagic	Breeder	Almost all parts of India
70	Lesser Crested Tern <i>Thalasseus bengalensis</i>	Common	Partially Pelagic	Breeder (in Laccadives)	Esatcosat, west coast
71	Sandwich Tern <i>Thalasseus sandvicensis</i>	Less common	Partially Pelagic	Non-breeder	east coast, west coast
72	Greater Crested Tern <i>Thalasseus bergii</i>	Common	Partially Pelagic	Breeder (in offshore Islands)	East coast, west coast

CONSERVATION AND MANAGEMENT

The pelagic birds of India, besides sea/ocean, exploits habitats like mangroves (covers about 6,749km and situated in East Coast (about 4700km²), West Coast (about 850 km²), and Andaman and Nicobar Islands about (1190 km²) with Lakshadweep atoll), coral Reefs (occurs in Gulf of Kachchh and Lakshadweep in Arabian Sea, Gulf of Mannar, Andaman and Nicobar Islands in Bay of Bengal, off-shore reef in Malvan, Maharashtra), coastal sand dunes (a poorly documented ecosystem), mudflats (covering an area of more than 38,000 km²), salt Marshes (covers 1696.3796 sq. km), and seagrass beds (along the Gulf of Mannar and Palk Bay, in the lagoons of islands from Lakshadweep and Andaman and Nicobar (Bay of Bengal)). Besides serving as a habitat for birds, mangroves play a major role in carbon sequestration. In order to protect the oceanic birds and the above mentioned habits, the Government of India has already formed a National network of 38 Marine and Coastal Protected Areas (MCPAs) that forbid extractive uses and employ sustainable management practices. Besides, there are another 100 PAs as National Parks or Wildlife Sanctuaries. However, lack of funds, lack of man power, and inadequate coordination between public and policy makers adversely affect the above system. However, potential sites like Bhusandpur and Tinimuthan, Burnt Island (Bandra) Vengurla Rocks, Mahul-Sewree Creek, Thane Creek, Point Calimere, Great Vedaranyam Swamp, Chilika Lake, Gulf of Mannar Biosphere Reserve, and Pulikat Bird Sanctuary are brought under the above scheme and well protected to conserve the oceanic birds.

Sutherland *et al.* (2012) identified 45 threats faced by the shorebirds around the world and placed under three categories: natural (volcanoes and cyclone), current anthropogenic (habitat conversion, climate change) and future issues (microplastics, global hydro security and changes in sedimentation rates). However, in India, threats can be largely linked to habitat loss. The human population increase has considerably reduced the potential habitats available for shorebirds. Loss of key habitats, particularly stopover habitats for migrants on the migratory pathway and breeding ground for migrants, has significant consequences for a number of pelagic species. As areas become less, overcrowding, competition, and increased risk of disease transmission among shorebirds will be inevitable on the existing habitats.

Disturbance from human activities has a high energetic cost to shorebirds and may compromise their capacity to build sufficient energy reserves to undertake migration (Goss-Custard *et al.*, 2006; Weston *et al.*, 2012). Hence, even a small disturbance capable of altering a habitat unusable for birds can exacerbate pelagic bird population declines, as unusable habitat is equivalent to habitat loss. Besides, climate change has the potential to affect migratory shorebirds and their habitats by reducing the extent of coastal and inland wetlands or through a poleward shift in the range of many species (Chambers *et al.*, 2005; Iwamura *et al.*, 2013).

Overharvesting of marine resources can lead to decreased productivity and changes in prey distribution and availability (MacKinnon *et al.*, 2012). Hence, controlled harvesting should be introduced for the conservation of oceanic birds. Further, accidental killing of

pelagic birds requires further investigation.

CONCLUSION

India harbours around 72 species of pelagic birds belonging to five orders and nine families. Of the 72 species, 15 breed in Indian Territories while the rest are mere migrants/vagrants to India. Birds belonging to phaethontiformes and suliformes are truly pelagic in habitat and often sighted in poor numbers. Studies on ecological requirements of the pelagic birds are the immediate need for the conservation and management of these species in India.

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VADUVUR AND SITHERI LAKES, TAMIL NADU, INDIA: CONSERVATION AND MANAGEMENT PERSPECTIVE

V. Gokula & P. Ananth Raj

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Vaduvur and Sitheri lakes, Tamil Nadu, India: conservation and management perspective

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Abstract: Wetlands are declining globally. Hence, it is reasonable to assume that most existing wetlands are impacted to some degree by human land-use that in turn caused population declines in many wetland-dependent taxa. The National Wetland Atlas has classified Tamil Nadu as a wetland-rich state as they occupy 6.92% of geographic area. However, studies on wetlands are limited in Tamil Nadu. Hence, an attempt was made to identify the threats to the Vaduvur and Sitheri lakes and their associated fauna. In total, 118 species of birds belonging to 87 genera, 48 families and 18 orders in Vaduvur Lake and 87 species of birds belonging to 71 genera, 48 families and 16 orders in Sitheri Lake were recorded. A total of 28 zooplankton species were recorded in both the lakes comprising 14 species of rotifers, six species of cladocerans, five species of copepods, two species of ostracods, and one species of protozoa. A total of 15 species of fishes were identified from the sellers who catch fishes from the Sitheri Lake. The physico-chemical parameters of water varied according to the seasonal fluctuations in rainfall pattern. In general, wetland management for waterbirds of these two lakes should focus on providing suitable nesting habitats and available food resources for dependant avifauna. Management of invertebrates, amphibians, and fishes in these two lakes is one technique that can be used to provide foraging opportunities for waterbirds. An integrated approach and increased co-operation would result in the rational use of this freshwater resource leading to improved standards of living around this lake.

Keywords: Illegal trade, poaching, threats.

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INTRODUCTION

Wetlands are declining globally. Between 1993 and 2007, the global acreage of wetlands decreased by 6% (Prigent et al. 2012). Hence, it is reasonable to assume that most existing wetlands are impacted to some degree by human land-use that in turn caused population declines in many wetland-dependent taxa. Wetlands have many successional stages and hydroperiods, represented in close proximity, thus, managing wetlands effectively requires an understanding of basic ecosystem processes besides using appropriate management techniques that depend on target species, coastal versus interior wetlands, available infrastructure, resources, and management objectives.

The National Wetland Atlas, prepared by the Ahmedabad (Gujarat) Space Application Centre (SAC), Indian Space Research Organization (ISRO), has classified Tamil Nadu as a wetland-rich state as they occupy 6.92% of the geographic area. One-hundred-and-twenty-five species of birds including both migratory and resident that depend on wetlands fully or partly and 28 other species found in the vicinity of wetlands are known from Tamil Nadu. Although Tamil Nadu State has several wetlands, studies are limited to very few: Point Calimere (Sugathan 1982), Kaliveli (Pieter 1987), Singanallur Lake (Reginald et al. 2007), Pallikaranai (Raj et al. 2010), Karaivetti (Gokula 2010), Vaduvor (Gokula & Raj 2011), and Sitheri (Gokula & Raj 2015). The majority of research work on wetland management in Tamil Nadu relates to the limnological and ornithological aspects. Nevertheless, the land-use changes and socio-economic activities leading to changes in limnological and biodiversity aspects of these wetlands have not been explored substantially. Moreover, the national water sector agenda pays little attention to wetland management resulting in over exploitation of wetland's resources. Hence, an attempt was made to identify the threats to the Vaduvur and Sitheri lakes and their associated fauna.

STUDY AREA

The Vaduvur Lake, situated between 10.698–10.706 °N & 79.309–79.322 °E, spread over c. 128ha and Sitheri Lake, situated between 10.712–10.728 °N & 79.323–79.342 °E, spread over c. 87ha, are located at a distance of 20km from Mannargudi, a town, situated between 10.636–10.677 °N & 79.432–79.450 °E in Tiruvarur District in Tamil Nadu. The Vaduvor Lake was declared a bird

sanctuary by the forest department in July 1999. The bunds help in holding the water up to an average depth of c. 2.5m. Vegetation of the lake consists of *Prosopis chilensis*, *Azadirachta indica*, *Tamarindus indica*, and *Acacia nilotica* including planting of *A. nilotica* by the forest department under the Sanctuary Management Program. The Sitheri Lake is currently being maintained by the public works department, Tamil Nadu, however, the forest department of Tamil Nadu has a plan to bring this wetland and its components under the protected areas network. The Vennaru River is the main source of water in addition to the monsoon (largely from the north-east) for both the lakes.

METHODS

Birds were counted using direct count method from selected vantage points following Bibby et al. (1992) and Sutherland (1997). Counts were made four times in a month during which birds were observed from 06.00–10.00 hr and 16.00–18.00 hr, being their most active periods of the day from September 2010 to February 2012. No count was done during extreme weather conditions. The water quality of the lakes was assessed using the standard methods described by APHA (1996). Identification of zooplankton was done by following Alfred et al. (1973) and Adoni et al. (1985). Zooplankton samples were collected from the two lakes by towing a plankton net, made up of bolten silk with a mesh size of 100µm, from surface water to 1m depth. One-hundred litre of water from the lakes was filtered through the zooplankton net and collected planktons were preserved in 5% formalin. Planktons were identified up to species level. Planktons were enumerated using SedgwickRafter chamber and species richness and diversity were calculated. Fish collected by the local people were inspected and identified up to the species level, based on which, a list of fish species for each lake was prepared. Jayaram (1999) was followed to identify the fish fauna. Several visits were made around the lakes and villages nearby for collecting information on threats to avifauna and two lakes. Formal and informal interviews were conducted with local people to prepare a list of threats to these two lakes and dependant avifauna. During the fieldwork, anthropogenic activities, viz., hunting, illegal fishing, and woodcutting (if any) were monitored and quantified (if possible) following Joshua & Johnsingh (1994). Only fishing and illegal hunting of birds were identified as threats to the wetlands. People who are directly or indirectly involved in the above said two



threats were approached for additional quantitative (if possible) and qualitative information. Fish markets were periodically visited for collecting information on illegal bird trade. Informal interviews were conducted with those traders for further details. Wherever permission was granted, quantification were made on the number of individuals and species of birds involved in the trade. The methodology was based on the principles and procedures of the Australian/New Zealand Standard for Risk Management ISO 31000:2009 (Standards Australia 2009; AZ/NZS 4360:1999) and HB 203: 2000 Environmental Risk Management – Principles and Process (Standards Australia 2009).

RESULTS AND DISCUSSION

In total, 118 species of birds belonging to 87 genera, 48 families, & 18 orders in Vaduvur Lake and 87 species of birds belonging to 71 genera, 48 families, & 16 orders in Sitheri Lake were recorded and the details are given elsewhere (Gokula & Raj 2011, 2015). The numerical differences may be attributed to the combination of any of the factors, viz., size and location of wetlands, proximity to other wetlands, water level, foraging opportunity, food availability, availability of nest-sites, inter and intra specific competition, human pressure, site fidelity of bird species, and site history. Besides regular common migrants, both the lakes harbour Near Threatened bird species, viz.: Darter *Anhinga melanogaster*, Painted Stork *Mycteria leucocephala*, and Oriental White Ibis *Threskiornis melanocephalus*. Among the bird species recorded, the most numerous were Garganey *Anas querquedula* and Northern Shoveller *Anas clypeata*, however, individuals of a majority of the species were poor in numbers. Little Grebe *Tachybaptus ruficollis*, Little Cormorant *Phalacrocorax niger*, Little Egret *Egretta garzetta*, Cattle Egret *Bubulcus ibis*, Indian Pond Heron *Ardeola grayii*, and Pheasant-tailed Jacana *Hydrophasianus chirurgus* nest in Sitheri; while, Little Grebe, Asian Openbill *Aanastomus oscitans*, Cattle Egret, Black-crowned Night Heron *Nycticorax nycticorax*, Grey Heron *Ardea cinerea*, Little Egret, Little Cormorant *Microcarbo niger*, Pheasant-tailed Jacana nest in Vaduvur Lake. In both the wetlands, birds breed largely during and after the north-east monsoon as reported by Subramanya (2005) for majority of the heronries of Tamil Nadu.

A total of 28 zooplankton species were recorded in both the lakes comprising 14 species of rotifers, six species of cladocerans, five species of copepods, two

species of ostracods, and one species of protozoa (Table 1). A greater number of species of zooplanktons was recorded during November to June followed by May to July (Table 2). The diversity of planktons was more during the monsoon rather than in the summer in both the lakes, which is in contrast to other such studies carried out in Tamil Nadu. Manikam et al. (2014, 2017) reported high diversity of planktons in summer and attributed it to favourable temperature and availability of food in the form of bacteria, phytoplankton, and suspended detritus during the season. Hence, a detailed long-term study is needed to confirm it further.

The Sitheri Lake is a very good fishery resource. A total of 15 species of fishes were identified from the sellers who catch fishes from the Sitheri Lake and the Vennaru River, a prime source for the two lakes (Table 3). Thiyagesan & Nagarajan (1995) reported the negative impacts of the over exploitation of aquaculture and fisheries resources in inland and coastal wetlands of the eastern coast of India on their bird life.

With respect to water quality the changes in water chemistry has been considered to exert influence in the distribution of many aquatic plant species (Catling et al. 1986; Shay & Shay 1986; Chee & Vitt 1989; Engelhardt & Ritchie 2001; Lentz-Cipollini & Dunson 2006). As waterbirds and wetland dependant birds depend directly or indirectly on aquatic fauna and flora which in turn depend on water chemistry, birds' distribution is expected to change with changes in water chemistry. As anticipated the physico-chemical parameters of water varied according to the seasonal fluctuations (Table 4). The limnological variables showed two distinct clusters: July to December with high rainfall and January to April with less/no rainfall (Figure 1). The rainfall (both from north-west and south-east monsoons between June to December) and lack of rainfall (between January to May) showed greater influences in the values of water quality parameters in both the lakes.

Three major villages are situated around these two lakes: Vaduvur Vadpathi (2,289 individuals belonging to 575 families of which 1,154 are males while 1,135 are females), Vaduvur Melpathi (3,010 individuals belonging to 817 families of which 1,478 are males while 1,532 are females), and Vaduvur Thenpathi (3,412 individuals belonging to 896 families of which 1,673 are males while 1,739 are females). The socio-economic status of the people of these villages revealed that both the lakes play a vital role in the livelihood of many people. Agriculture is the main occupation of the people of these villages and they greatly depend on the lake for irrigation and other domestic purposes. Paddy is the main crop cultivated

Table 1. Species of planktons recorded in Vaduvur and Sitheri lakes during the study period.

	Vaduvur Lake	Sitheri Lake
Group	Species	Species
Protozoa	<i>Vorticella</i> sp.	<i>Vorticella</i> sp.
Rotifera	<i>Brachionus calyciflorus</i>	<i>Brachionus calyciflorus</i>
Rotifera	<i>B. quadridentatus</i>	<i>B. quadridentatus</i>
Rotifera	<i>B. forticula</i>	<i>B. forticula</i>
Rotifera	<i>Euchlanis</i> sp.	
Rotifera	<i>Horella brehmi</i>	<i>Horella brehmi</i>
Rotifera	<i>Lepadella</i> sp.	
Rotifera	<i>Mytilina</i> sp.	<i>Mytilina</i> sp.
Rotifera	<i>Notholca</i> sp.	<i>Notholca</i> sp.
Rotifera	<i>Trichotria</i> sp.	<i>Trichotria</i> sp.
Rotifera	<i>Trichocera rattus</i>	<i>Trichocera rattus</i>
Rotifera	<i>Testudinella patina</i>	<i>Testudinella patina</i>
Rotifera	<i>Asplanchna brightwelli</i>	
Rotifera	<i>Lecane lunaris</i>	<i>Lecane lunaris</i>
Rotifera	<i>L. bulla</i>	<i>L. bulla</i>
Cladocera	<i>Alonella</i> sp.	<i>Alonella</i> sp.
Cladocera	<i>Bosmina longirostris</i>	<i>Bosmina longirostris</i>
Cladocera	<i>Daphnia carinata</i>	<i>Daphnia carinata</i>
Cladocera	<i>Diaphanosoma</i> sp.	
Cladocera	<i>Diaphanosoma</i> sp.	<i>Diaphanosoma</i> sp.
Cladocera	<i>Moina daphnia</i>	<i>Moina daphnia</i>
Copepoda	<i>Calonoid copepod</i>	<i>Calonoid copepod</i>
Copepoda	<i>Heleodiptomus viduus</i>	<i>Heleodiptomus viduus</i>
Copepoda	<i>Mesocyclops hyalinus</i>	<i>Mesocyclops hyalinus</i>
Copepoda	<i>Thermocyclops</i> sp.	<i>Thermocyclops</i> sp.
Copepoda	<i>T. crassus</i>	<i>T. crassus</i>
Ostracoda	<i>Cypris</i> sp.	<i>Cypris</i> sp.
Ostracoda	<i>Stenocypris malcolmsoni</i>	<i>Stenocypris malcolmsoni</i>

around these two wetlands and it is grown three times in a year. The first crop is known as ‘Kuruvai’ (the short-term crop) with a duration of three and a half to four months from June–July to October–November. The second crop called the ‘Thaladi’ has a duration of five to six months from October–November to February–March. The third is the ‘Samba’ (the long term) crop and has a duration of almost six months from August to January. During the cultivation periods, in particular, between the months of October and January, the agriculture fields are water-logged with aquatic invertebrates. Thus, the agriculture fields surrounding these two wetlands and in nearby villages not only act as a unique foraging ground but also provide various foraging opportunities

Table 2. Species richness and diversity of planktons recorded during various months of the study area.

	Vaduvur	Sitheri	Vaduvur	Sitheri
months	Taxa_S	Taxa_S	Shannon_H	Shannon_H
Sep-10	19	16	2.795	2.642
Oct-10	23	19	2.963	2.784
Nov-10	28	24	3.132	3.009
Dec-10	28	24	3.127	3.007
Jan-11	21	17	2.841	2.636
Feb-11	17	16	2.614	2.566
Mar-11	13	12	2.336	2.272
Apr-11	8	8	1.895	1.895
May-11	9	9	2.062	2.062
Jun-11	8	8	1.934	1.934
Jul-11	8	8	1.992	1.992
Aug-11	11	11	2.322	2.322
Sep-11	18	16	2.768	2.655
Oct-11	23	19	2.9	2.729
Nov-11	28	24	3.09	2.961
Dec-11	27	24	3.018	2.934
Jan-12	20	17	2.73	2.596
Feb-12	17	15	2.566	2.487

to the waterbirds and wetland dependant birds during their stay at in these two lakes. The water-logging of agricultural fields often attracts waterbirds, especially when they are close to other wetlands (Nagarajan & Thiyagesan 1996; Kahlert et al. 2007). Moreover, paddy fields support the highest bird diversity when they are water logged with abundant aquatic insects, worms, snails, and tadpoles (Deep 2008). Moreover, when they are flooded in winter they often provide a good feeding habitat for large numbers of birds (Chan et al. 2007). Croplands that are flooded to a shallow depth act as temporary foraging grounds for waders. Some species appear to need very large rice-fields while others prefer smaller ones and edge habitats (Burton et al. 2002). Hence, lakes with sufficient water and surrounded by agriculture fields with agricultural activities are more crucial to sustain the population of waterbirds and wetland dependent birds that traditionally inhabit any wetland. Due to unusual drought and fall in rainfall due to climate change, lack of interest in agricultural practices, and conversion of agricultural land into human habitation, agricultural activities in agriculture fields have drastically been declining not only around these lakes but also in the entire district. Recently, the Federation of Tamil Nadu Agricultural Associations reported that

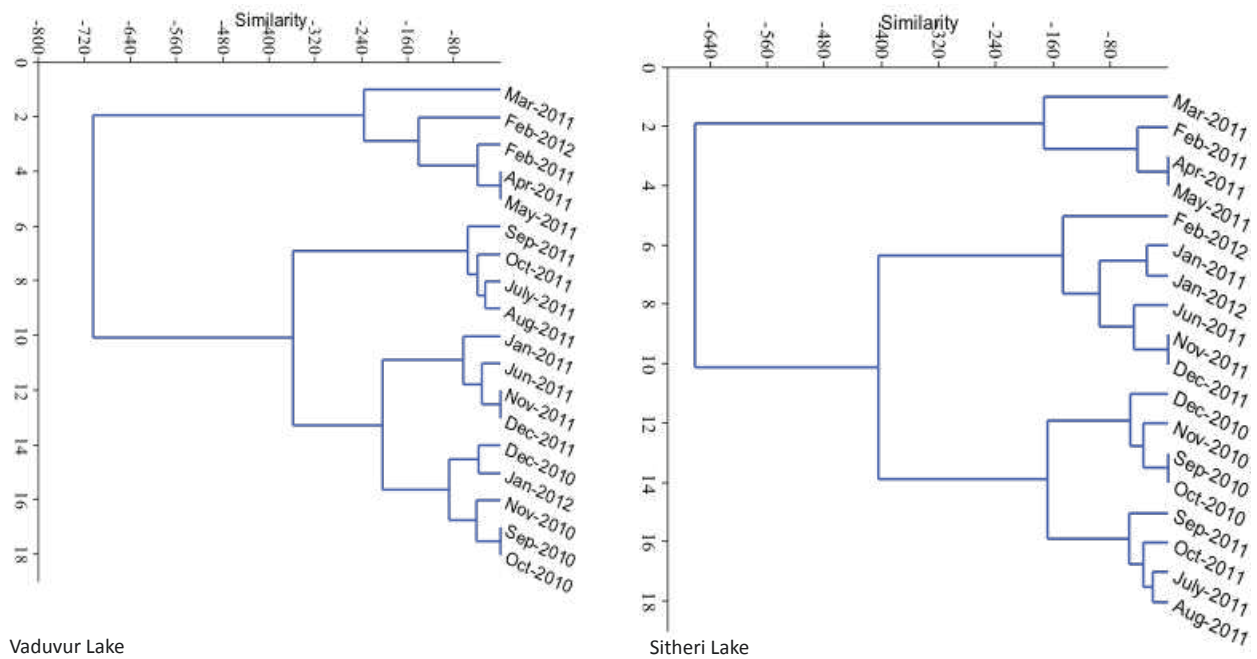


Figure 1. Dendrogram showing seasonal clusters for water quality parameters recorded in Vaduvur and Sitheri lakes.

from 2001 to 2011, nearly 8,67,582 farmers have stopped agriculture due to various reasons, including huge losses. Interestingly, there was an increase in the number of agricultural labourers during the period, as the farmers sell their land for real estate and prefer to work on daily wages. They attributed the reason for the plight of the farmers to wrong import policies of the government, unremunerative prices for farmers’ produce, industrialization and urbanization, coupled with the failure to divert the rain and other waters going to sea to the farmers’ fields, by linking rivers.

As majority of the lakes in Tamil Nadu go dry in summer, it is presumed that waterbirds move from places lacking adequate water to places with adequate water ignoring site fidelity. Although, both these lakes go 90% dry in the month of May, they still attract a significant number of waterbirds from other parts. The Spot-billed Pelican *Pelecanus philippensis* being a late arrival (largely during late November) particularly in the southern districts of Tamil Nadu performs its breeding activity until the month of April (February to April being crucial months to raise the young ones) while all other species complete their breeding activities largely by January. As majority of the lakes go dry from the month of February onwards in Tamil Nadu, the movement of pelicans from their traditional sites to new lakes with sufficient water is common (Gokula 2011). The Vaduvur Lake is one such wetland that supports pelicans during the above said crucial months. Hence, in order

to support Spot-billed Pelican, a near threatened bird, proper steps have to be taken to sustain the water level during April and May.

Various kinds of threats such as excessive fishing, poaching of birds, cattle grazing, fuel-wood collection, siltation, weed invasion, and pollution were identified particularly for the lakes during the study period. Earlier Wolstencroft et al. (1989) reported that these were the major threats in Asia in various wetlands. Thiyagesan & Nagarajan (1995) listed similar threats to the coastal wetlands of Tamil Nadu, southern India. Divakaran (2000) also noticed a majority of these threats in different islands of the Gulf of Mannar, southern India, causing great havoc for bird life there. The forest department of Tamil Nadu has protected the Vaduvur Lake under the bird sanctuary category thus the lake is comparatively free from above said illegal activities such as fishing and poaching of birds. All such activities have been prevailing in the Sitheri Lake.

Waterbird harvest is widespread, long-standing, and an important activity for local communities around the world. In many countries, the harvest takes place as a primary food source, but sport or recreational hunting is also popular; however, waterbird harvest has not been a popular activity in India since time immemorial and it may either be due to the availability of food resources in plenty or due to the culture. Sport or recreational hunting of waterbirds, however, was a part of the recreational activities of kings/maharajas

Table 3. List fish species recorded in the Sitheri Lake, Tamil Nadu, India.

	Fish	Vernacular name	National status, Global status (in parenthesis)
	Cyprinidae		
1	<i>Barilius bendelisis</i>	Vannathikendai	LRnt/N (LC)
2	<i>Puntius sophore</i>	Kullakendai, Mochakendai	LRnt/N (LC)
3	<i>labeo calbasu</i>	Karupan sel, Selukendai	LRnt/N (LC)
4	<i>Cirrhinus reba</i>	reba	VU (LC)
5	<i>Puntius conchonius</i>	Vallikendia	VU (LC)
6	<i>Rasbora daniconius</i>	Bhavanikendia	NE (LC)
	Cobitidae		
7	<i>Lepidocephalus thermalis</i>	Ayirai	NE (LC)
	Bagridae		
8	<i>Mystus cavasius</i>	Naikeluthi	LRnt/N (LC)
9	<i>Mystus vittatus</i>	Vazhppu	VU (LC)
10	<i>Mystus bleekeri</i>	Keluthi	VU (LC)
	Cichlidae		
11	<i>Eetroplus suratensis</i>	Sella kasu, Puradi, Salladai meen	NE (LC)
12	<i>Eetroplus maculatus</i>	Sethakendai, Bommi	NE (LC)
	Gobiidae		
13	<i>Awaous gutum</i>	Ulluvai, Kalulluvai	VU (LC)
	Clariidae		
14	<i>Clarias batrachus</i>	Thalmeen, Thal Kendia	VU (LC)
	Mastacembelidae		
15	<i>Mastcembelus armatus</i>	Aarraah	VU (LC)

EN —Endangered | VU—Vulnerable | LRnt—Lower Risk near threatened | NE—Not Evaluated | LC—Least concern (IUCN status). Status nationally as per CAMP assessment (Molur & Walker 1998).

and it continued until the British colonial period. Later, the Indian Wildlife (Protection) Act 1972 prevented this activity to be practiced anywhere in India. Still a nomadic community called ‘Narikurava’ in Tamil Nadu hunts birds for food as well as commercial purposes. In the interview, some admitted they supply birds dead or alive, specific or common to customers depending on their needs. Some suggested they should be given controlled hunting permits enabling them to make a sustainable living while protecting wildlife. Due to their small population size and the insignificant demand for wild birds among the public comparing others (fish, mutton, domestic chicken) in the market, however, it is generally assumed that hunting is well below the sustainable utilization, a level commonly regarded as a

cornerstone in the conservation of nature.

In the present study, frequent visits were made to fish markets of Vaduvur and Mannargudi (a town situated 12km away from the study area) to assess the wild bird trade from 2009 to 2015 (Table 5 & Image 1). In total 68 visits were made of which wild bird trade was found on 26 occasions. On all the occasions, (except three), no same person was found trading in wild birds. Two (belonging to Narikurava) persons involved in wild bird trade were sighted on three occasions. When approached for informal interviews we found many illegal wild bird traders made good their escape, while a very few stayed and engaged in conversation. People belonging to Narikurava though afraid to be photographed with birds, revealed facts like where and how they caught the birds. In total, 974 birds belonging to 21 species, 11 families and eight orders were recorded in the wild bird trade. It even included the Spot-billed Pelican, a Near Threatened bird. Among the orders, Coconiiformes dominated with seven species of birds followed by Gruiformes with five species of birds. Among the bird species, White-breasted Waterhen (89), Little Egret (87), Common Coot (76), and Water Cock (73) were sold in more numbers. Although wild bird trade was found in all the months of the year, it was more frequent during November to January. All the species of birds were largely bought for the purpose of meat. On one occasion, a crow was found sold to a customer and the enquiry with the trader revealed that it was for the purpose of black magic. He also revealed that they do supply crow on request occasionally for the above said purpose. The traders also revealed that all the birds were caught from the paddy fields surrounding the wetlands during early morning and late evening hours using indigenous traps (such as clap trap, mesh nets, and nooses). Although the forest officials frequently intercept, and arrest those involved in the hunting of wild birds in and around Vaduvur area, patrolling larger areas surrounding these two wetlands is not possible and feasible with the existing work force in the forest department. Often, arrested people are booked under the provisions of the Indian Wildlife (Protection) Act 1972. In the early 1970s and 1980s, over 150 families of different communities from Nagapattinam, Thanjavur, and Thiruvarur districts were involved in trapping migratory shorebirds and ducks that used to frequent the coastal wetlands, in several lakhs, during the migratory season (October to April). Now, many bird trappers have shifted to fishing as they were looked down upon for carrying on this illegal profession. Cattle egrets and pond herons are often bought by roadside restaurants and wine shops to serve and sell as

Table 4. Descriptive statistics of water quality parameters recorded during various months of the study area.

Water Quality Parameters	Descriptive Statistics: Vaduvur Lake						Descriptive Statistics: Sitheri Lake					
	Min	Max	Mean	SE	V	SD	Min	Max	Mean	SE	V	SD
Turbidity (NTU)	8.00	32.00	18.56	1.72	53.08	7.29	8.00	30.00	17.39	1.61	46.60	6.83
Total Dissolved Solids	270.00	595.00	394.89	20.80	7785.75	88.24	265.00	560.00	385.67	20.37	7472.12	86.44
Electrical Conductivity MicS/cm	415.00	912.00	605.56	31.88	18292.73	135.25	415.00	897.00	595.67	29.25	15404.40	124.11
pH	6.74	8.00	7.38	0.06	0.08	0.28	6.40	7.90	7.33	0.08	0.10	0.32
Alcalinity pH as CaCO ₃ (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alkalinity Total as CaCO ₃ (mg/l)	105.00	222.00	146.17	6.87	848.97	29.14	108.00	223.00	143.89	6.89	855.28	29.25
Total Hardness as CaCO ₃ (mg/l)	81.00	238.00	126.06	8.94	1437.59	37.92	78.00	237.00	123.22	8.71	1366.89	36.97
Calcium as Ca(mg/l)	24.00	61.00	33.33	2.11	79.88	8.94	24.00	56.00	31.44	1.81	58.97	7.68
Magnesium as Mg (mg/l)	4.00	20.00	10.56	0.93	15.44	3.93	5.00	19.00	10.00	0.82	12.00	3.46
Iron Total as Fe (mg/l)	0.00	2.50	0.72	0.16	0.44	0.66	0.00	2.20	0.67	0.14	0.36	0.60
Manganese as Mn (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Free Ammonia as NH ₃ (mg/l)	0.00	1.13	0.48	0.08	0.11	0.33	0.00	1.10	0.43	0.07	0.08	0.28
Nitrite as NO ₂ (mg/l)	0.00	0.45	0.14	0.04	0.03	0.16	0.00	0.45	0.13	0.04	0.02	0.15
Nitrate as NO ₃ (mg/l)	0.00	5.00	0.67	0.38	2.59	1.61	0.00	4.00	0.56	0.30	1.67	1.29
Chloride as Cl (mg/l)	48.00	127.00	91.06	5.71	585.94	24.21	43.00	120.00	88.83	5.17	480.50	21.92
Fluoride as F (mg/l)	0.00	0.40	0.19	0.02	0.01	0.08	0.00	0.40	0.14	0.02	0.01	0.08
Sulphate as SO ₄ (mg/l)	2.00	36.00	23.44	2.67	128.26	11.33	6.00	34.00	23.00	2.30	94.94	9.74
Phosphate as PO ₄ (mg/l)	0.08	1.64	0.60	0.11	0.20	0.45	0.07	1.54	0.59	0.10	0.19	0.43
Tidy's as O	0.40	1.84	0.94	0.11	0.20	0.45	0.40	1.70	0.93	0.10	0.19	0.43



Table 5. Various species of birds recorded in the illegal trade.

	Common name	Used in trade as/for			Available months	Availability rating	Frequency of occurrence	Number of visits	Total number birds found
		Aviculture/pet use	Meat	Sport					
1	Little Grebe <i>Tachybaptus ruficollis</i>	*	*		Nov–Feb	Frequent	18	26	67
2	Spot-billed Pelican <i>Pelecanus philippensis</i>		*		Nov–Jan	Rare	1	26	1
3	Little Cormorant <i>Phalacrocorax niger</i>	*	*		Nov–Jan	Less Frequent	4	26	12
4	Little Egret <i>Egretta garzetta</i>		*		Nov–Jan	Frequent	22	26	87
5	Grey Heron <i>Ardea cinerea</i>		*		Nov–Jan	Less frequent	4	26	5
6	Large Egret <i>Casmerodius albus</i>		*		Nov–Jan	Less frequent	5	26	8
7	Cattle Egret <i>Bubulcus ibis</i>		*		All the months	Very frequent	26	26	67
8	Indian Pond-Heron <i>Ardeola grayii</i>		*		All the months	Very frequent	26	26	56
9	Black-crowned Night Heron <i>Nycticorax nycticorax</i>		*		All the months	Very frequent	26	26	69
10	Asian Openbill-Stork <i>Anastomus oscitans</i>		*		Nov–Jan	Rare	2	26	2
11	White-breasted Waterhen <i>Amaurornis phoenicurus</i>		*		Nov–Jan	Frequent	16	26	89
12	Water Cock <i>Gallinula cinerea</i>		*		Nov–Jan	Frequent	14	26	73
13	Purple Moorhen <i>Porphyrio porphyrio</i>		*		Nov–Jan	Frequent	15	26	67
14	Common Moorhen <i>Gallinula chloropus</i>		*		Nov–Jan	Frequent	16	26	68
15	Common Coot <i>Fulica atra</i>		*		Nov–Jan	Frequent	18	26	76
16	Pheasant-tailed Jacana <i>Hydrophasianus chirurgus</i>		*		Nov–Jan	Frequent	14	26	56
17	Gull-billed Tern <i>Gelochelidon nilotica</i>		*		Nov–Jan	Frequent	14	26	45
18	Common Tern <i>Sterna hirundo</i>		*		Nov–Jan	Frequent	14	26	46
19	Little Brown Dove <i>Streptopelia senegalensis</i>	*	*	*	All the months	Frequent	19	26	45
20	Asian Koel <i>Eudynamis scolopacea</i>		*		All the months	Frequent	15	26	34
21	House Crow <i>Corvus splendens</i>					Based on order	1	26	1
	Total								974

chicken. Regardless of months, Cattle Egrets and Pond Herons are trapped every day for this purpose. Hence, proper awareness programmes to other communities and alternate sources of livelihood for Narikurava are essential to wean them away from their traditional but destructive profession. All these birds involved in the illegal trade play a very significant role in the agro-ecosystem as they feed on various insect species and thereby control the pest population.

Anand (1999) reported desiltation was not only useful in terms of improvement of irrigation and fisheries potential, but also to the increase of wildlife diversity and use. During the rainy season the eroded soil from their catchments, gets dumped into these lakes, which in turn reduces the water holding capacity of the lake.

Siltation, a serious problem, results in low water depth thereby facilitating the invasion of weed patches. Vallenweider (1968) reported that water bodies with less water depths would be more affected by eutrophication problems. The *Ipomoea aquatic* (weed) invasion was very extensive in these lakes. Anand (1999) observed that the *Ipomoea* invasion changed the water quality and reduced the primary production and nutrient cycle. As a result the weeds should be cleared either manually or by application of weedicide. Such a step will increase the irrigation potential of the lake and improve the condition for the wildlife, especially waterbirds.

The lake area is used by surrounding villagers for grazing their domestic livestock especially during summer. This intensive cattle grazing could result in



Image 1. Various species of waterbirds found in the local market. © V. Gokula

breaking the nutrient cycle of the lake. Further, the trampling cattle might harden the soil surface and reduce the aeration of the lake. Earlier Meganathan (2002) also expressed similar apprehensions for the freshwater lakes of Tamil Nadu. The local people must be educated in this aspect. The surrounding village people are using the lake for washing their livestock. The livestock are allowed to freely drink and bathe in this lake. This cattle washing pollutes the water and acts as a deterrent for waterbirds. Hence, cattle washing should be prohibited in the lake.

Another threat is wood collection for fuel by the local villagers from the lakes and its immediate surroundings. *Acacia*, *Zizypus*, and *Prosopis* were the plants cut for fuel wood. They are the roosting and nesting places for birds like openbill storks and night herons. Dickson et al. (1995) stated that protection of vegetation along the sides of the wetlands is important to retain water quality and accommodate wildlife including breeding birds. Hence, this vegetation, especially at the northern region of the Vaduvor Lake and the entire Sitheri Lake

must be given full attention and protection to prevent human disturbances to nesting activities through wood removal. In 2015, *Prosopis chilensis*—then roosting and nesting sites for several species of birds, were completely removed by the people, which in turn affected the avifauna

Although many of the heronries in Tamil Nadu, despite the stench emanating from the nesting activities of the birds, are zealously protected by villagers (e.g., Kanjirankulam, Udayamarthandapuram, Vettangudi, Vedanthangal, and Koonthakulam), villagers in and around the Vaduvor Lake lack such interest towards protection of birds. Usage of crackers and musical instruments by villagers are very common during festival times in Vaduvor Lake area. A prominent Kothandaramar Temple and a community temple are situated around the Vaduvor Lake. Although, festivals of Kothandaramar Temple largely come between June and August, disturbance to birds by the devotees are considerably less as birds are less during these months. Frequent family functions held at the community temple

situated at the edge of the lake, however, cause a major threat to the breeding birds particularly during the migration and breeding seasons. It has been suggested that the greatest and most depressing problem in conservation is not habitat loss or overexploitation but the human indifference to such problems (Balmford 1999). Overcoming such indifference is likely to depend on providing both the opportunities to appreciate areas and species, and education to highlight the ecological, aesthetic, cultural, spiritual, recreational, and economic importance. Education is one of the major techniques available to conservationists through which change in behaviour or compliance with new legislation can be achieved. Moreover, maintaining protected areas is easier if there is public support, which often leads to political and financial support and greater adherence to rules and regulations (Shepard & McNeely 1998). Hence, a proper public awareness program has to be initiated about the conservation of birds and lakes among the public.

As the lake is situated on one side of the Trichy-Mannargudi main road, vehicular sound is a great threat to the breeding birds. High decibel noise often disturbs the breeding activities of the birds, and frail chicks. Hence, usage of horns by vehicles should be banned from start to end of the lake at least during the peak breeding season of birds.

Pesticides, manures, and fertilizers are being increasingly used to ensure greater production of food in the nearby paddy fields around the lake. Some of these chemicals find their way into soils, water and other parts of the environment as a result of direct application or by indirect means. Hence, it is also necessary to monitor the water for possible pesticide contaminants since the lake is also the main source of water supply for agricultural consumption. In addition to awareness, volunteers should be trained to monitor the breeding population of birds and other threats to birds and wetlands.

CONCLUSION

In general, wetland management for waterbirds of these two lakes should focus on providing suitable nesting habitats and available food resources for dependant avifauna. Management of invertebrates, amphibians, and fishes in these two lakes is one technique that can be used to provide foraging opportunities for waterbirds. Most species often rely much on nearby aquaculture fields thus a straightforward 'farm crisis' may badly affect the avifauna of these two lakes. Hence,

agricultural activities around the two lakes should be encouraged. The water level and water quality of the lake should be properly maintained to cater to the needs of both irrigation and wildlife. Periodic desilting should be initiated with proper care and planning to provide a variety of depth levels. Cattle grazing and cattle washing in the lake should be totally prohibited. The weed *Ipomoea* should be removed totally. Poaching of waterbirds should be stopped by effective steps, such as better vigil and weaning of nomadic life from wildlife hunting by educating them and providing alternative livelihood. An awareness campaign must be conducted so that the local public realizes the significance of the lake in terms of their wildlife values and need to utilize them judiciously and sustainably for mutual benefit. There is an excellent potential for developing these lakes as very good tourist attractions since these lakes are situated near other famous tourist areas such as Point Calimere Wildlife Sanctuary, Karaivetti Lake, and other cultural heritage sites (such as Tharangambadi, Thanjavur, and Velankanni). Ecotourism would increase the income of the local people. Hence, an integrated approach and increased co-operation would result in the rational use of this freshwater resource leading to improved standards of living around this lake.

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Sublethal effects of phenol on histology of selected organs of freshwater fish *Mystus vittatus*

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Abstract

Acute toxicity (96 h LC₅₀) of phenol was analyzed in the cat fish *Mystus vittatus* in static bio-assay over a 96-h exposure period using probit method. The 24, 48, 72, and 96 h LC₅₀ values (with 95% confidence limits) of phenol for fingerling catfish were found out as 13.98, 13.17, 12.62, and 12.21 mg/l respectively. Investigations pertaining to the histopathological sections have shown high degree of pathological lesions observed in various parts like gill, liver intestine, and kidney of the fish species. Analysis of gill section revealed observable changes in the experimental species such as fusion, malformation at the tip of secondary lamellae, vacuolation, hyperplasia, and epithelial damage. Exposure of phenol showed cytoplasmic vacuolation, tissue damage, and loss of hepatic cell wall in the liver of experimental organism. Lesions of tissue damage at the epithelial site, inflammation, and clumping of adjacent villi made of columnar epithelium have been observed in the intestine of fish, and also the excretory part of the fish kidney revealed various changes like glomerular atrophy, damage of Bowman's capsule, vacuolization, and degeneration of renal epithelium. The current study on histological changes observed in the experimental organisms has thrown light on the current scenario which poses threat and danger to the whole aquatic ecosystem, and this study plays a vital role in assessing the aquatic pollution.

Keywords Catfish · *Mystus vittatus* · Acute toxicity · Phenol · Histology

Introduction

Nowadays, due to rapid industrialization, modern agriculture (application of synthetic fertilizers and various insecticides), and domestic sewage, many aquatic environments in India are

experiencing complicated problems of pollution (Reddy and Rawat 2013). These pollutants hold highly destructive effects on balancing the ecosystem and wide variety of aquatic organisms (Farombi et al. 2007). Phenol is an eco-toxin and ubiquitous pollutant of aquatic ecosystem. It is extensively used as a reference toxicant for many bioassays relevant to environmental health. Phenols are used as the components of dyes, polymers, drugs, and other organic substances. In India, it is the main effluent from the pulp paper industry, which is used as a bleaching agent (Singh and Chandra 2019). Bioaccumulation of phenolic compounds or chemicals which disturb endocrine glands was studied in wild fish (Yin-Zhi and et al. 2019). The sublethal effects of phenol on hematological parameters of catfish showed various abnormalities (Moraes et al. 2015).

Phenol-containing effluent is being directly or indirectly discharged at a rate of 20–100 cubic meters per metric ton of product into the river and lake, and which contributes to the toxicity to the aquatic environment (Gavrilescu 2008; Petra et al. 2015). According to Gustavo et al. (2015) and Li-Ping et al. (2018), the adverse effect of pulp paper industrial

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effluents on reproductive system of fishes has been reported in multiple ways, i.e., masculinization, lower plasma sex hormone, reduced gonad size, and reduced vitellogenin in the female, circulating sex hormone, fecundity, delayed maturity and change in secondary sex characteristics. Shirdel and Kalbassi (2016) reported that various histopathological lesions in gill and intestine tissues of the endangered Caspian brown trout (*Salmo trutta caspius*) exposed to different nonylphenol concentrations.

Li et al. (2018) reported that phenolic compounds inhibit development of fathead minnows by slowing down mitochondrial respiration and heartbeat of embryos. Butchiram et al. (2013) reported the histopathological changes in various vital tissues such as gills, liver, and kidney in the freshwater fish *Labeo rohita* exposed with sublethal concentration of phenol for a period of 8 days. Morphological, behavioral, and histopathological alterations in African catfish *Clarias gariepinus* were observed upon exposure with sublethal concentration of phenol (Ibrahim 2011). Severe gill architectural changes in *Oreochromis mossambicus* were reported owing to exposure to sublethal concentration of phenol (Remya et al. 2014).

However, very few studies have been carried out so far on histological changes induced by phenol. The experimental studies carried out by Tilak et al. (2006) in *Catla catla* and Abdel-Hameid (2007) in *Oreochromis aureus* revealed severe modulatory pathological variations in the fish exposed to phenol. Butchiram et al. (2013) noted phenol-induced pathological lesions in *Labeo rohita*. Sabry et al. (2009) also studied histological and histochemical alterations in phenol-exposed *Oreochromis aureus* juveniles.

In this study, *Mystus vittatus* was used as an experimental fish; it has both ornamental food and commercial value. This fish is a widely spread species occurring in the Ganga-Brahmaputra and Indus river basins. The objective of this current study throws light on various histopathological changes in gills, liver, intestine, and kidney of *Mystus vittatus* exposed to sublethal concentration of phenol for 10, 20, and 30 days. The aim of histopathological studies was mainly to assess the extent of internal pathological lesions caused due to excess accumulation of phenolic compounds. Pathological studies bear direct testimony to the toxic nature of phenol on the tissues.

Materials and methods

Test chemicals

The analytical grade phenol ($\geq 99.5\%$ purity, CAS number of 108-95-2) was obtained from New India Chemical Enterprises, Cochin, India, and used without processing for this current experiment.

Animal maintenance

The healthy and stable fingerlings of *Mystus vittatus* weighing 08 ± 0.7 g and measuring 7 ± 0.5 cm were procured from the nearby places of Adirampattinam, Tamil Nadu, India. The fishes collected from the respective area were safely brought to the laboratory and transferred them into a large cement tank (1000-L capacity). They were allowed to acclimatize to the current environment for about 1-month period. During this period, the fishes were fed with rice bran and groundnut oil cake which has trace amount of cadmium. They were fed only once in a day. The water was renewed daily to avoid storage and contamination of excretory materials which could be toxic to the fish leading to mortality. The feeding procedure has been stopped 24 h prior to the commencement of the experiment. Fishes observed with abnormal behavior were removed. The water used in this current study was completely free from chlorine and possesses the following physico-chemical characteristics such as temperature 28 ± 0.13 , pH 7.6 ± 0.04 , salinity 1.2 ± 0.13 ppt, D.O. 5.6 ± 0.2 mg/L, and total hardness 35 ± 0.5 mg/L. Before commencing the experiment, a suitable number of fishes were transferred into two glass tanks and they were fixed with aerator.

Preparation of stock solution and determination of 96 h LC₅₀ value of phenol

Stock solution of phenol was prepared by dissolving 1 g of phenol in an appropriate amount of water. For determination of median tolerance limits or LC₅₀, different concentrations of phenol (10, 11, 12, 13, and 14 mg/L) were prepared from the stock and added in separate glass tanks with 50-L water capacity. Triplicates were necessitated for each concentration of stock solution, into which 10 fishes of similar size and weight were introduced. The test water was renewed after 24 h of post experimental setup, and freshly prepared phenol was added to maintain its stability. Simultaneously control of 30 fishes in three different glass tanks was maintained under similar conditions. The fish mortality was recorded after 24, 48, 72, and 96 h respectively, and median lethal concentration (LC₅₀) values were calculated by the Finney method (1971). One-tenth of the LC₅₀ value for 96 h was utilized for estimating the sublethal concentration (Sprague 1973).

Sublethal studies

Test for sublethal toxicity requires 100 fingerlings selected based on the requirement and categorized into four groups (one control and three experimental setups) each glass tank holding 25 fishes. The desired concentration (1/10 of 96 h

Table 1 Percent mortality of *Mystus vittatus* exposed to different concentrations of phenol for different periods (24–96 h)

Hours of exposure	LC ₅₀	LCL	UCL	Regression equation	Calculated χ^2 value	Table χ^2 value
24	13.98251	14.46604	13.51515	$Y = -7.9605 + 11.31343X$	2.609604	11.07
48	13.17809	13.73593	12.64291	$Y = -10.28271 + 13.64708X$	13.2491	11.07
72	12.62277	12.809	12.43923	$Y = -11.8393 + 15.2924X$	7.508591	12.59
96	12.21073	12.42394	12.00118	$Y = -9.340177 + 13.19557X$	7.583908	11.07

LCL, lower concentration level; UCL, upper concentration level

LC₅₀) of the toxicant was added, and the setup is allowed to maintain constant concentration of the toxicant. The experiment was conducted for 30 days, and at an interval of every 10 days, samples were analyzed. It has been observed there was no specification of fish mortality during the aforementioned treatment period. At the end of 10th, 20th, and 30th day period, fishes were randomly selected and libation of fish has been done to study the histological patterns.

Histology

During 10th, 20th, and 30th day of experimental tenure, fishes were randomly selected and atoned and gills are removed. The gills were fixed using Bouin’s fluid, later processed (Gurr 1950), and hardened using paraffin wax (58–60C). Serial sections of 8- μ m thickness were cut, and paraffin was removed from the gill sections and

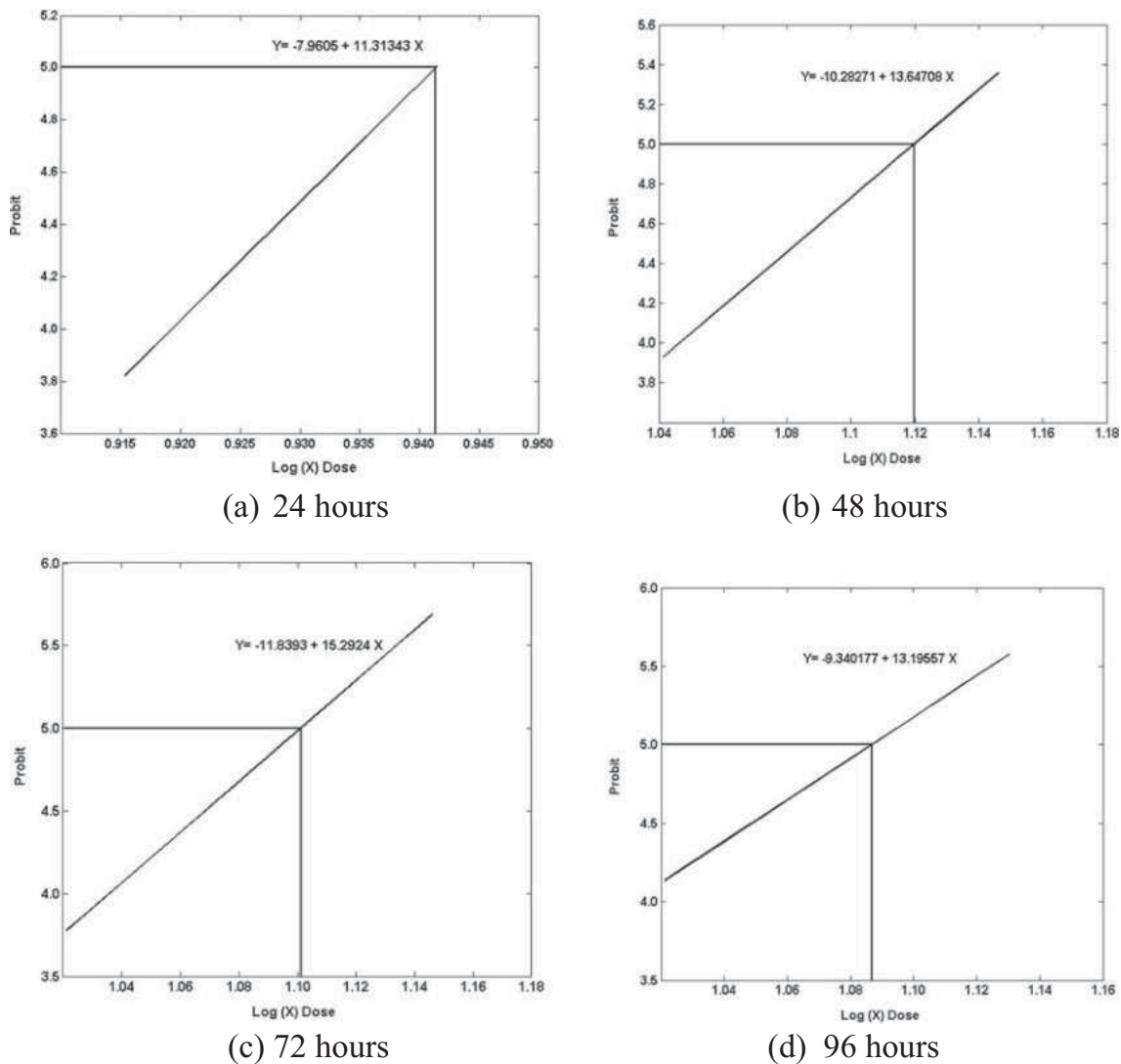


Fig. 1 Regression line (based on profit analysis) of log concentration of phenol vs percent mortality of fish for **a** 24, **b** 48, **c** 72, and **d** 96 h

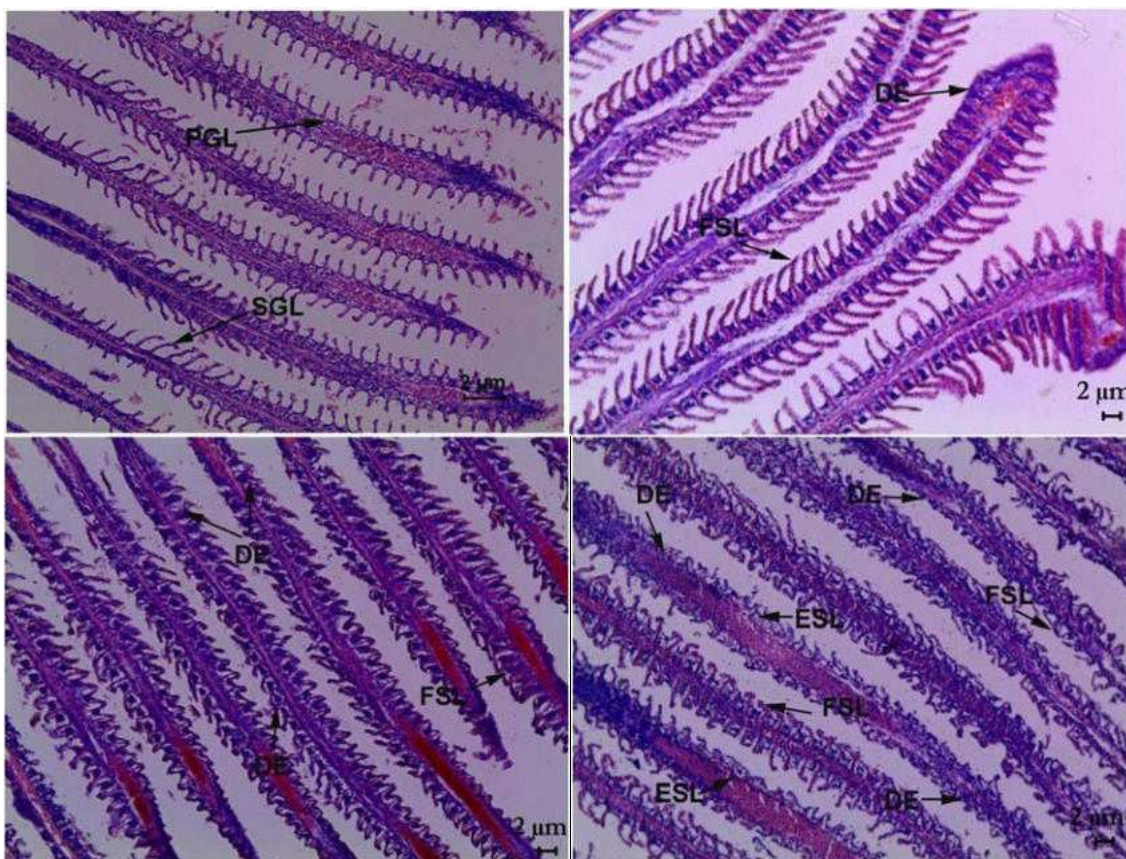


Plate 1 Histopathological lesions in the gill. (1) Control. (2) 10 days treated. (3) 20 days treated. (4) 30 days treated. PGL, primary gill lamellae; SGL, secondary gill lamellae; FSL, fusion of secondary lamellae; DE, degeneration of epithelium; ESL, erosion of secondary lamellae

stained with hematoxylin and finally counterstained with aqueous eosin.

Results

LC₅₀ value—96 h

Acute toxicity test was carried out and the results are tabulated in Table 1. The LC₅₀ value was calculated using probit analysis and result was found to be 12.21 mg/l for 96 h of exposure to phenolic compounds (Fig. 1a, b, c, d). During the course of study, it was observed that the behavioral patterns of the control fish were normal, whereas abnormal behavior such as erratic swimming movement, prolonged surface activity, spooling of high mucous secretion from body, and restlessness has been observed in those fishes which are exposed to sublethal concentration of phenolic components.

Histological observation of the control fish gill

The control fish showed laterally compressed leaf-like structures of its primary gill lamellae. Each primary gill lamella

bears thin, slender, finger-like structures called secondary gill lamellae which are attached on either side of the primary lamellae. The lamellae consist of numerous vascular cells with blood lacunae covered by thin epithelial layer (Plate 1, 1–4).

Histological alterations in phenol-treated fish gill

Several pathological lesions appeared on the gills of phenol-treated fishes. Gills were characterized by the cohesion, malformation on the tip of secondary lamellae, vacuolation, hyperplasia, and degeneration of epithelium. At the end of the test exposure periods (10, 20, 30 days), severe pathological lesions were seen in both 20 and 30 days of 10% sublethal concentration-treated fishes (Plate 1, 1–4).

Histological observation of the control fish liver

Histological observation of normal fish liver indicates the presences of hepatic cell mass and cord-like structure. The cells are hexagonal in shape and the size is larger with centrally located nucleus (Plate 2, 1–4).

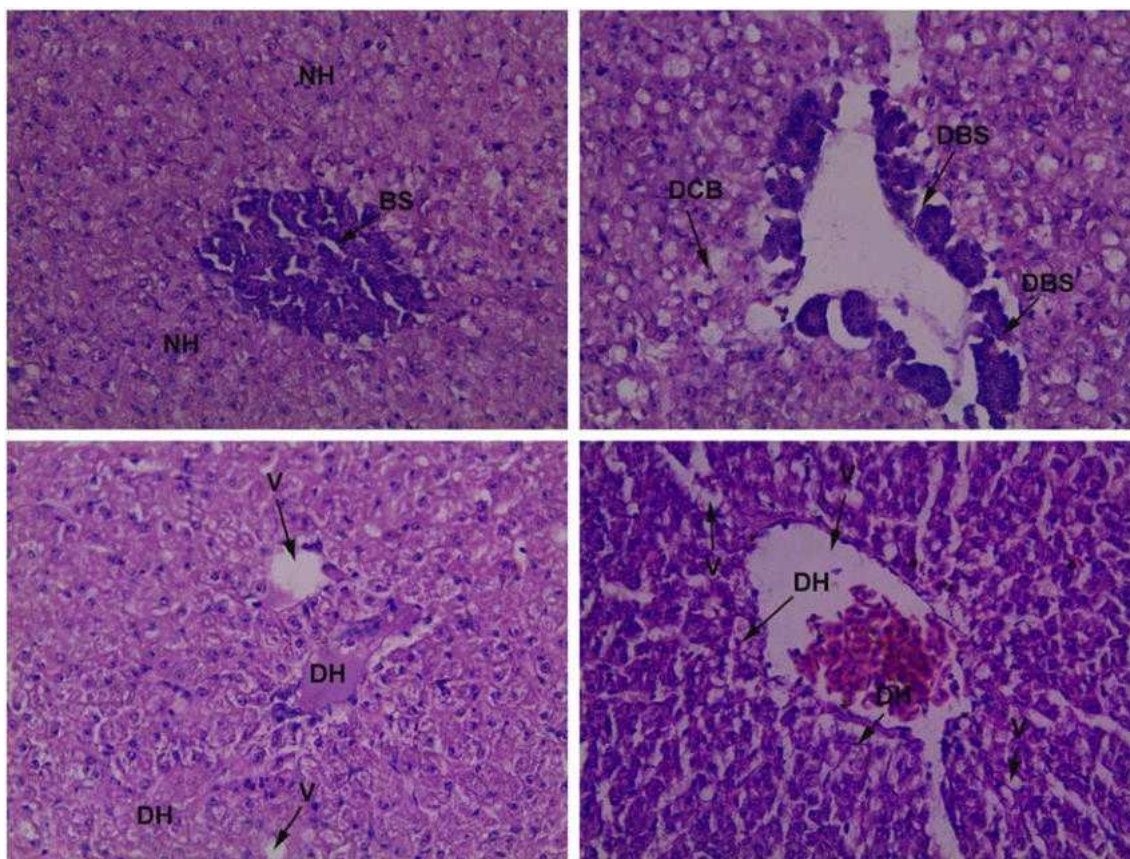


Plate 2 Histopathological lesions in the liver. (1) Control. (2) 10 days treated. (3) 20 days treated. (4) 30 days treated. NH, normal hepatocytes; BS, blood sinus; DCB, disintegration of cell boundaries; DBS, dilation of blood sinusoids; V, vacuolization; DH, degeneration of hepatocytes

Histological alterations in phenol-treated fish liver

Fish exposed to sublethal concentrations of phenol indicated several pathological changes in liver tissue. In 10, 20, and 30 days treated fishes, the liver tissue revealed many necrotic cells. In most of the hepatic cells, the integrity of the cell wall was completely lost. Intracellular vacuolation was also apparent. The liver tissue was found severely injured in 10% sublethal concentration of phenol of 30 days treated fishes compared to 10 and 20 days treated fishes. In most of the places, cytoplasmic vacuolation and necrosis were all common (Plate 2, 1–4).

Histological observation of the control fish intestine

The intestine of control fish consists of simple columnar epithelium which was thin and slender. The columnar epithelial cells arranged uniformly in the mucosa layer had a distinct double-layered tunica propria (Plate 3, 1–4).

Histological alterations in phenol-treated fish intestine

Histological alterations have been observed in the intestine of fish treated with phenolic compounds at an interval of 10, 20,

and 30 days respectively. Changes have been observed in the phenolic-treated fish intestine such as tissue damage in the intestinal epithelium, inflammation, and binding of adjacent villi made of cuboidal epithelium. At the end of 30 days exposure to 10% sublethal concentration, these lesions were more prominent to observe (Plate 3, 1–4).

Histological observation of the control fish kidney

In control fish, the excretory part of the kidney was made of maximum nephrons. Each nephron comprises of Malpighian corpuscle and a coiled uriniferous tubule. The corpuscle was made up of glomerulus and Bowman’s capsule (Plate 4, 1–4).

Histological alterations in phenol-treated fish kidney

The fish exposed to the sublethal concentrations of phenol showed complete atrophy of glomeruli, degeneration of Bowman’s capsule, vacuolization, and damage of renal epithelium. Thereafter, the changes were further analyzed and found maximum on 30 days exposure to 10% sublethal concentration of phenol treatment (Plate 4, 1–4).

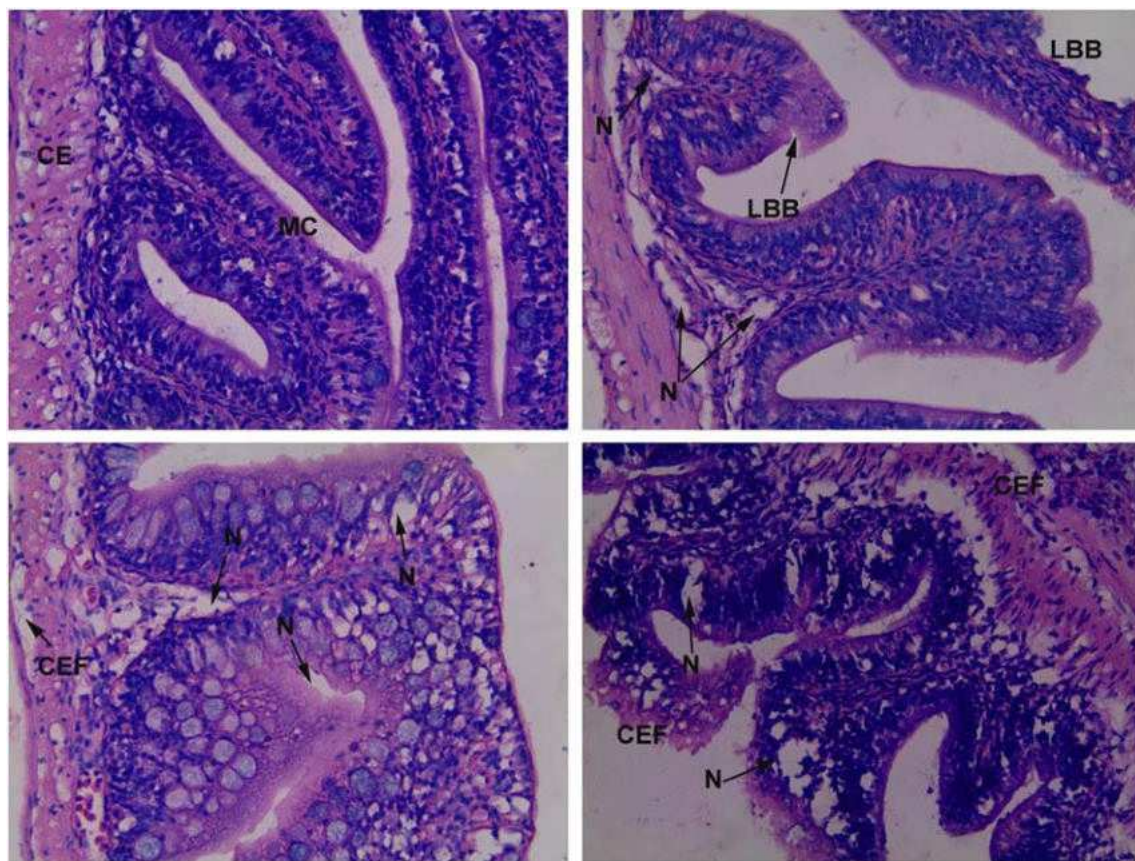


Plate 3 Histopathological lesions in the intestine. (1) Control. (2) 10 days treated. (3) 20 days treated. (4) 30 days treated. MC, mucus cells; CE, columnar epithelium; LBB, loss of brush border; N, Necrosis; CEF, columnar epithelial fusion

Discussion

A survey on LC₅₀ values of fish exposed to phenolic treatment with respect to the time interval reveals that the maximum variations occurred in the current study due to the time schedule followed and type of fish used for the purpose of study (Verma et al. 1981; Saha et al. 1999; Abdel-Hameid 2007; Verma et al. 1980; Gupta et al. 1982; Chagon and Hlahowskyj 1989; Hori et al. 2006; Sannadurgappa et al. 2007; Gad and Saad 2008; Ibrahem 2012; De Moraes et al. 2015).

In fish, gills take over majority of the functions such as respiration and osmoregulation. It remains in close contact with the external environment and sensitive water quality parameters, which are considered to be the primary target of various contaminants (Camargo and Martnez 2007; Fernandes and Mazon 2003). Pathological changes observed in the gills of fish exposed to phenol were characterized by the cohesion of secondary lamellae, damage of epithelium, hypertrophy, erosion of secondary lamellae, and malformation at the tips of the gill lamellae.

The fish exposed to sublethal concentrations of phenol revealed the loose arrangement of hepatic cells with vacuolation, dilation of blood sinusoids, cytolysis, and necrosis.

Phenol-induced histopathological changes in the liver of fishes have been reported by many authors. Butchiram et al. (2013) observed enlargement of nuclei in liver cells, atrophic cells, and enlarged sinusoids in liver of *Labeo rohita* due to phenol toxicity in addition to formation of number of vacuoles and loose arrangement of cells, similar to the observations in the present study. Sabry et al. (2009) suggested that the extent of liver damage due to phenol treatment was dose and duration dependent. In the present investigation also, with highest sublethal concentration of the phenol at 30 days of exposure, histopathological changes were more pronounced. Similar observations were also reported by Abdel-Hameid (2007) in the fish *Oreochromis aureus* exposed to phenol. Radha Krishnan and Hemalatha (2010) observed, in cadmium chloride-treated *Channa striatus*, vacuolation, blood vessel congestion, and necrosis to be occasionally present. Narayan and Singh (1991) also reported that *Heteropneustes fossilis* when exposed to Thiodon showed degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in the liver. The present observations in *Mystus vittatus* are highly comparable with the previous reports on the histopathology of fishes.

The fish exposed to the sublethal concentration of phenol showed the occurrence of inflamed lamina propria, fusion of columnar epithelium, and loss of brush border. These

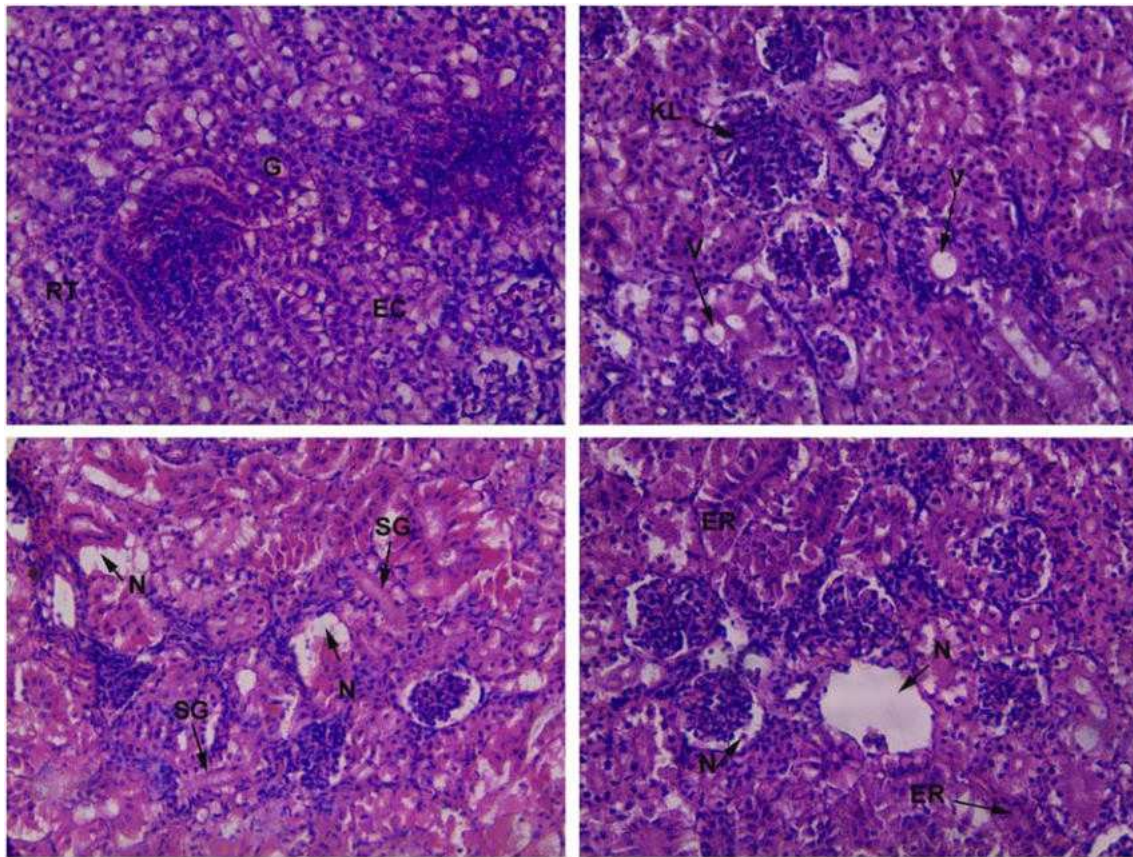


Plate 4 Histopathological lesions in the kidney. (1) Control. (2) 10 days treated. (3) 20 days treated. (4) 30 days treated. G, glomeruli; RT, renal tubules; EC, epithelial cells; KL, karyolysis; V, vacuolization; N, necrosis; SG, shrinkage of glomeruli; ER, enlargement of renal tubule

observed histological deformities in the intestine are in agreement with the previous report on the histopathology of other species of fishes.

Fish exposed to the sublethal concentration of phenol showed shrinkage of glomeruli, vacuolation, enlargement of renal tubules, necrosis, and hyperplasia. These changes were the same as those reported by Persis and Kalaiarasi (2003) who worked on pathological changes of the kidney in the fresh water catfish *Mystus vittatus* exposed to pesticide dimethoate. Rashatwar and Ilyas (1984) have reported the histopathological changes in the kidney to lead and noted swelling of renal tubules in *Nemacheilus denisonii* acutely exposed to phosphamidon. Mohapatra and Noble (1992) have observed vacuolation of epithelial cells of renal tubules, enlargement of renal tubules, and extensive desquamation in nuvan-treated mullet *Liza partisia*. Necrosis and vacuolation in the kidney were observed by Iqbal et al. (2004) in *Cyprinus carpio* exposed to nitrate. Sukumar and Karpagaganapathy (1986) observed a number of striking changes in the histological structure of the kidney of *Colisa lalia* exposed to carbofuran for a period of 30 days. Prashanth (2011) studied histopathological changes in the kidney of *Cirrinus mrigala*, exposed to cypermethrin. Remya et al. (2014) corroborated severe gill

histopathological lesions due to phenol exposure such as architectural loss, necrosis, desquamation of epithelial layer, hyperplasia, and telangiectasis in *Oreochromis mossambicus*. Similarly, Ibrahim (2011) reported severe pathological lesions in various vital internal organs like skin, gills, brain, and liver of African catfish *Clarias gariepinus* due to sublethal phenol exposure. The results of the present observations in *Mystus vittatus* exposed to sublethal concentrations of phenol were in agreement with those of the earlier workers, especially in the degeneration and shrinkage of glomeruli, vacuolation, and necrosis.

Conclusions

This study explained and concluded that the phenolic compound is moderately toxic to catfish fingerlings, *Mystus vittatus*. Histopathological studies revealed that modifications have been observed in the structural patterns of gills, hepatic, intestinal and renal cells. The results indicate that *Mystus vittatus* is a potential experimental indicator for the aquatic pollution and could be a suitable species for examining water quality parameters.

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Authors' contributions Kannayiram Muthukumaravel-conception and design

Natarajan Vasanthi-conduct experiment

Arumugam Stalin-literature search

Lubna Alam-contribution to the manuscript preparation

Bharathi Santhanabharathi-statistical Analysis and interpretation

Mohamed Saiyad Musthafa-final approval of the article

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Compliance with ethical standards

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Consent to participate Not Applicable

Consent to publish Not Applicable

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Ultrastructural alteration in Gill and Hepatopancrease of freshwater prawn *Macrobrachium rosenbergii* exposed to ^{60}Co gamma radiation

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Abstract

The present study was designed to evaluate the impact of gamma radiation (^{60}Co) on freshwater prawn *Macrobrachium rosenbergii* by using electron microscopic (SEM, TEM) studies. One set of prawns (experimental group) was irradiated (3, 30, 300, and 3000 mGy) by Theratron Phoenix TeleCobalt Unit [P-33], while other set of prawns (control group) was maintained (non-irradiated) separately. Scanning electron microscopic observations of gills and hepatopancreas showed fused and swollen lamella, abnormal gill tips, wrinkled lamellar epithelium, and necrotic epithelium surface in irradiated groups, while no such abnormalities were obvious in the control group. Transmission electron microscopic studies showed damaged nucleus, granulated mitochondria, vacuoles with crystalline granular inclusions, destructed membrane, vacuoles filled with granules, rough endoplasmic reticulum with residual bodies, shrunken mitochondria, dilated rough endoplasmic reticulum, and dilated cisternae of the Golgi body in irradiated groups. The structural abnormalities of vital organs could affect physiological functions such as respiration, osmo-ionic regulation and storage, secretion of the gills, and hepatopancreas, which in turn could adversely affect the growth and survivability of *M. rosenbergii*.

Keywords *Macrobrachium rosenbergii* · ^{60}Co · SEM · TEM

Abbreviations

^{60}Co	Cobalt-60
SEM	Scanning electron microscopic
TEM	transmission electron microscopic
ICRP	International Commission on Radiological Protection
TLD	Thermo luminescence disks
BARC	Bhabha Atomic Research Centre
GVN	G. Viswanathan
PBS	Phosphate-buffered saline
OsO_4	Osmium tetroxide
mGy	milligray

Introduction

Radiation is one of the most widespread sources of environmental stress in living environment and their exposure causes oxidative stress and metabolic changes in the living organisms (Mohamed 2011). The International Commission on Radiological Protection (ICRP) emphasized the need to protect non-human biota from the potential effects of ionizing radiation (ICRP 2007). The severity of a toxicant can be measured at the level of the molecular, cellular, tissue, organ, individual, or population (Moore 1985). The detection of responses to toxicants at the cellular or tissue level is of great value and where histopathological studies reflected the impact of toxicants on metabolic processes at the cellular level (Triebekom and Kohler 1996; Mathur and Gupta 2008).

Scanning electron microscope (SEM) helps to study the organ's morphological characteristics and abnormalities if any in treated samples. Transmission electron microscope (TEM) studies reveal the cell organelle features and their pathological conditions in treated samples. Ultrastructural alterations were studied by several authors for assessing the effects of organic chemicals and metals (Segner and Braunbeck

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1998). In the last few decades, many studies focused on the influences of exogenous factors on crustaceans. Bhavan and Geraldine (2000) reported that always a close correlation exists between different kinds of stresses and ultrastructure of tissues.

The gill is used as a model organ for assessing the environmental radiation impact on testing the species and performs multifunctions like gas exchange (McMahon and Wilkens 1983), osmo-ionic homeostasis (Mantel and Farmer 1983), and transbranchial NaCl absorption and water excretion (Kirschner 2004). A Crustacean hepatopancreas acts as a primary organ responsible for absorption and storage of ingested materials and plays a major role in accumulating, neutralizing, and eliminating harmful chemicals (Abdelmeguid et al. 2009). The present study was designed to evaluate the impact of gamma radiation (^{60}Co , 3, 30, 300, and 3000 mGy) on freshwater prawn *Macrobrachium rosenbergii* by using electron microscopic (SEM, TEM) studies.

Materials and methods

Experimental design and Gamma irradiation

Macrobrachium rosenbergii was collected from the river Cauvery (11° 29' N; 79° 50' E), Tiruchirappalli, Tamil Nadu, India, acclimatized in Environmental Research Laboratory, Jamal Mohamed College, Tiruchirappalli, and fed with boiled chopped goat liver ad libitum (Stalin et al. 2013). Prawns ($n = 10$) were irradiated (3, 30, 300, and 3000 mGy) along with Thermo Luminescence Disks (TLD, BARC, India) for dose measurement (Sadiq Bukhari et al. 2012) by using Theratron phoenix (P-33) Tele cobalt unit (^{60}Co radionuclide source) in GVN Cancer Cure Research Centre and Hospital, Tiruchirappalli (Tamil Nadu, India). Control (non-irradiated) and irradiated groups were maintained in the laboratory for the next 96 h and were sacrificed.

Scanning electron microscopic studies

The animals were anesthetized on ice for 3 min, and gills and hepatopancreas were dissected out following Poljaroena et al. (2010). Gills and hepatopancreas were fixed in 2.5% Glutaraldehyde in 0.1 M PBS, pH 7.4, at 4 °C for 24 h and were post-fixed in 1% Osmium Tetraoxide (OsO_4) in 0.1 M PBS, pH 7.4 for 1 h and dehydrated through increasing concentrations of ethanol. Tissues were coated with platinum and palladium in a Hitachi ion-sputtering apparatus (E2500). The processed tissues were examined in a Hitachi S-2500 scanning electron microscope (SEM) operating at 15 KV in the Centre for an Advanced Study in Botany, in the University of Madras, Chennai (Tamil Nadu, India) following Cheng et al. (2010).

Transmission electron microscopic studies

The gills and the hepatopancreas tissues were kept in vivo condition prior placing it into the fixative medium (fixed in 3% Glutaraldehyde and post-fixed by 1% OsO_4). Tissues were dehydrated in ascending graded alcohol and cleared by propylene oxide. Tissues were molded with epoxy resin, kept in an incubator at 60 °C for 48 h, cooled down (Manush et al. 2007), sectioned (1 μm) through ultra microtome (Leica ultracut UCT) with a glass knife, and stained by toluidine blue. The sections were transmitted in Tecnai TR spirit (Netherland) and photographed in Histopathological Unit, Christian Medical College (CMC), Vellore (Tamil Nadu, India).

Histological gradations

Semi-thin sections were cut at 1- μm thickness and stained with methylene blue. The histological changes if any were examined and ranked semiquantitatively (Mishra and Mohanty 2008; Stalin et al. 2013): none (0%), mild changes (< 10%), moderate changes (10 to 50%), severe changes (50 to 70%), and extended severe changes (> 70%) in prawn tissues. Data was represented as the mean of slides (25 slides/organ) for each prawn, i.e., 250 slides per group.

Results

Scanning electron microscopic studies

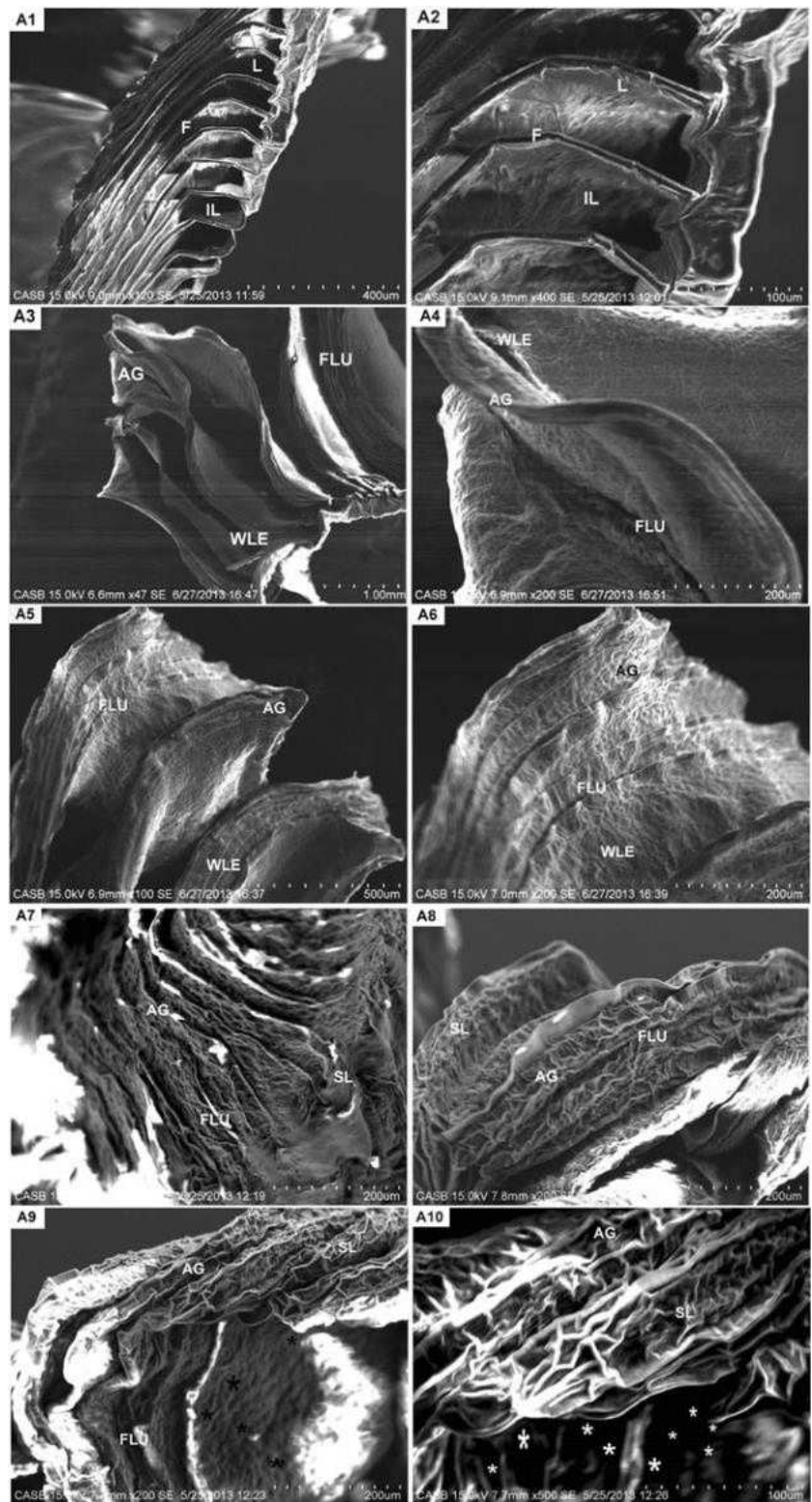
Gill

Scanning electron micrographs of gills of *M. rosenbergii* in the control prawn show the filament (F), lamella (L), interlamellar region (IL), and pillar cells (Fig. 1 (A1 and A2)). Gills of *M. rosenbergii*, treated with 3 mGy and 30 mGy Cobalt-60 gamma, showed fused lamellae (FLU), abnormal gill tip (AG), and wrinkling of the lamellar epithelium (WLE) (Fig. 1 (A3 and A4; A5 and A6)). The 300-mGy Cobalt-60 gamma-treated to *M. rosenbergii* showed swollen lamellae (SL) besides fused lamellae (FLU) and abnormal gill tip (AG) (Fig. 1 (A7 and A8)). Epithelial necrosis with and without blood emerging (asterisk shaped) besides fused lamellae (FLU), abnormal gill tip (AG), and swollen lamellae (SL) was observed in the gills of the animal treated with 3000-mGy Cobalt-60 gamma radiation (Fig. 1 (A9 and A10)).

Hepatopancreas

Scanning electron micrographs of hepatopancreas of *M. rosenbergii* in the control prawn were found with B cells, S cells, L-lipid droplets, and epithelial in normal structure

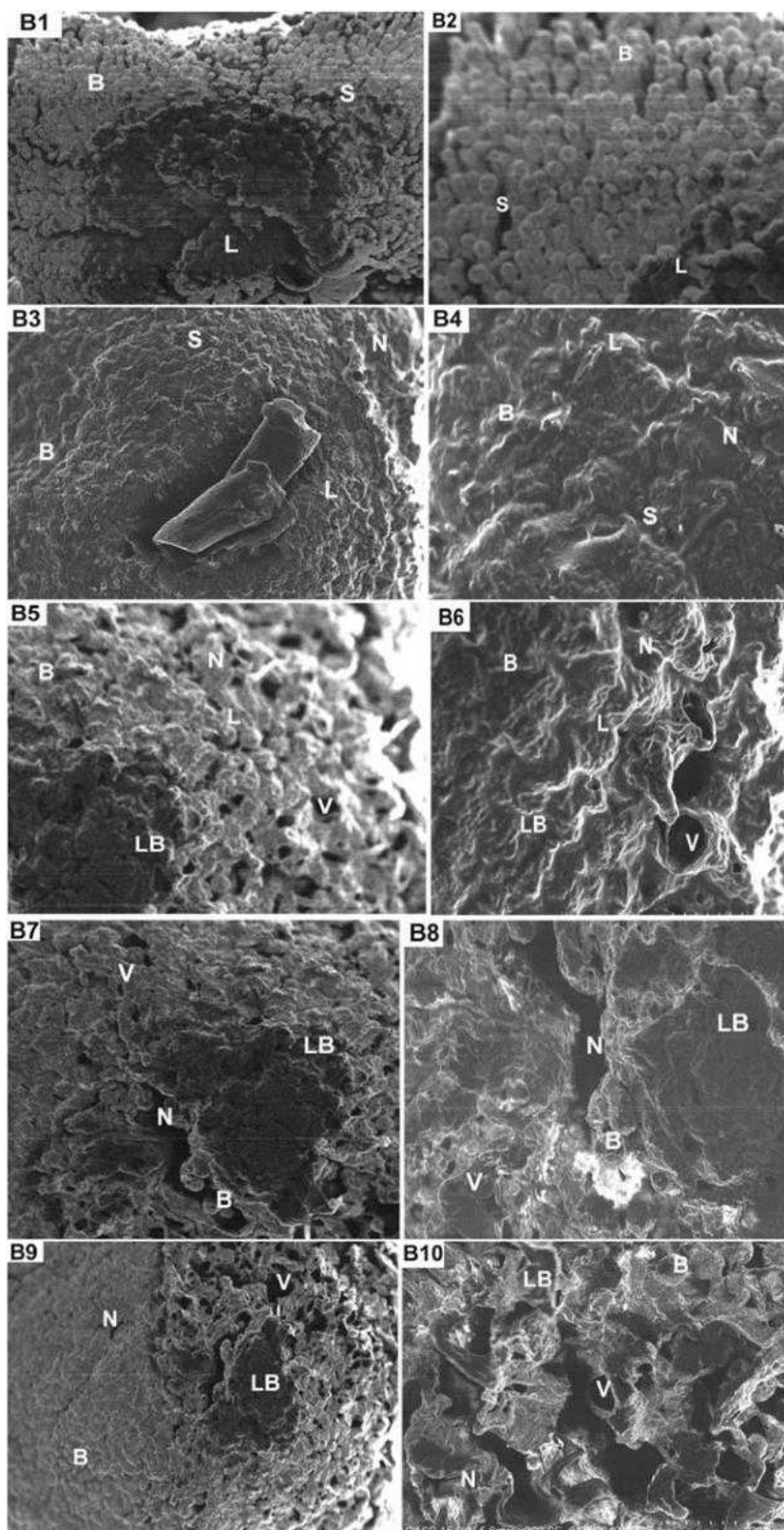
Fig. 1 Scanning electron microscopic images of control and ⁶⁰Co gamma irradiated freshwater prawn *M. rosenbergii*. (A) Gill: Control (A1–A2), 3 mGy (A3–A4), 30 mGy (A5–A6), 300 mGy (A7–A8), and 3000 mGy (A9–A10) groups. F filament, L lamella, IL interlamellar region, FLU fusion of lamellae, AG abnormal gill tip, WLE wrinkling of the lamellar epithelium, SL swollen lamellae, * epithelial necrosis



(Fig. 2 (B1 and B2)). However, the hepatopancreas of *M. rosenbergii* treated with 3-mGy Cobalt-60 gamma showed with large lipid droplets inside the B cell, extrusion of large and small lipid (L) droplets, few completely mineralized

lamellae body (LB), and irregular bulbous surface with necrotic crystalline fragments (N) (Fig. 2 (B3 and B4)). About 30-mGy Cobalt-60 gamma-irradiated prawn showed small and rare lipid droplets inside a B cell (decaying surface) (B), large

Fig. 2 Scanning electron microscopic images of control and ⁶⁰Co gamma irradiated freshwater prawn *M. rosenbergii*. (B) Hepatopancreas: Control (B1–B2), 3 mGy (B3–B4), 30 mGy (B5–B6), 300 mGy (B7–B8), and 3000 mGy (A9–A10) groups. B B cells, S S cells, L lipid droplets; N necrosis; L (circle) large lipid droplets inside the B-cell; LB extrusion of large and small lipid droplets, lamellae body; V vacuoles formation, B small and rare lipid droplets inside a B cell (decaying surface)



lipid droplets inside the B cell (L), extrusion of large and small lipid droplets, lamellae body (LB), vacuoles formation(V), and necrosis (N) (Fig. 2 (B5 and B6)). About 300-mGy

Cobalt-60 gamma-treated prawn was observed with extrusion of large and small lipid droplets, lamellae body (LB), vacuoles formation (V), and small and rare lipid droplets inside a B cell

(decaying surface) (B) (Fig. 2 (B7 and B8)). Scanning electron micrographs of *M. rosenbergii* treated with 3000-mGy Cobalt-60 gamma showed small and rare lipid (L) droplets inside a B cell disintegrated apical part of a cell and decaying surface, necrotic epithelium surface, formation of vacuoles (V), and lamellae body (LB) (Fig. 2 (B9 and B10)).

Transmission electron microscopic studies

Gill

Transmission electron microscopic observation of control prawn gill showed pillar cell (PC) and hemocoelic space (HS). The lamellar region of the gills showed an epithelial cell with cuticle (C), nucleus (N), and mitochondria (M) associated with infolded cell membranes (IC) (Fig. 3 (A1 and A2)). Cobalt-60 gamma-irradiated (3 mGy) prawn gills showed HS, the E cell with centrally placed nucleus (N), few mitochondrial swelling with degenerated cristae, and vacuolated area with degenerated mitochondria (MV). The lamellar region characterized by elevated cuticle (EC), vacuoles with crystalline granular inclusion (VC), and granular cells (Fig. 3 (A3 and A4)). About 30-mGy-irradiated prawn was observed with necrosis of mitochondria (M), damaged nucleus (N), lamellar region characterized by elevated cuticle (EC), and vacuoles with crystalline granular inclusion (VC) (Fig. 3 (A5 and A6)). About 300-mGy gamma-irradiated prawn was observed with necrosis of mitochondria (M), damaged nucleus (N), elevated cuticle (EC), vacuoles with crystalline granular inclusion (VC), formation of vacuoles (V), and granular cells (GC) (Fig. 3 (A7 and A8)). About 3000-mGy-irradiated prawn gills showed damaged nucleus (N), mitochondria with granular cell (Gc) formations, VC found at the basal regions of the gills, mitochondrial degeneration and devoid of cristae, and MV in adjacent intralamellar septal cell(s) (Fig. 3 (A9 and A10)).

Hepatopancreas

The hepatopancreas of control prawns showed E cell with centrally placed nucleus (N), few mitochondria (M), rough endoplasmic reticulum (RER), and rich in glycogen granules (G) and without microvilli (Fig. 4 (B1 and B2)). Hepatopancreas of 3 mGy irradiated group of prawns showed absorptive (R) cell with centrally placed nucleus (N), large lipid vacuoles (LV), hepatocyte vacuoles filled with granular inclusions (VG), cytoplasm with many electron dense granules (EG), shrunken mitochondria (SM), dilated rough endoplasmic reticulum (DRER), and dilated cisternae of the Golgi body (DGB) in the tubule epithelium (Fig. 4 (B3 and B4)). About 30-mGy treated prawn was observed with vacuolated cell with granular inclusions (VG),

nucleus (N), dilated cisternae of the Golgi body (DGB), dilated Golgi body complex (GC), lipid vacuoles (LV), residual bodies in rough endoplasmic reticulum (RB), shrunken mitochondria (SM), and dilated rough endoplasmic reticulum (DRER) (Fig. 4 (B5 and B6)). About 300-mGy treated prawn was observed with necrotic nucleus (N), glycogen granules (G), microvilli (MV), dilated cisternae of the Golgi body (DGB), dilated Golgi body complex (GC), lipid vacuoles (LV), residual bodies in rough endoplasmic reticulum (RB), shrunken mitochondria (SM), and dilated rough endoplasmic reticulum (DRER) (Fig. 4 (B7 and B8)). Lipid vacuoles LV, vacuolated cell with granular inclusions (VG), shrunken mitochondria (SM), dilated rough endoplasmic reticulum (DRER), dilated cisternae of the Golgi body (DGB), and residual bodies (RB) formation in RER were observed in the hepatopancreatic tubule epithelium of 3000 mGy irradiated prawns (Fig. 4 (B9 and B10)).

Histological gradations

In irradiated groups, the histological anomalies increase as dose increases. Maximum histological changes were found at 3000 mGy followed by lower level. Semiquantitative scoring of gill and hepatopancreas abnormalities observed by scanning electron microscopic studies was done (Table 1). Similar scoring was done for the gill and hepatopancreas abnormalities observed by transmission electron microscopic studies (Table 2).

Discussion

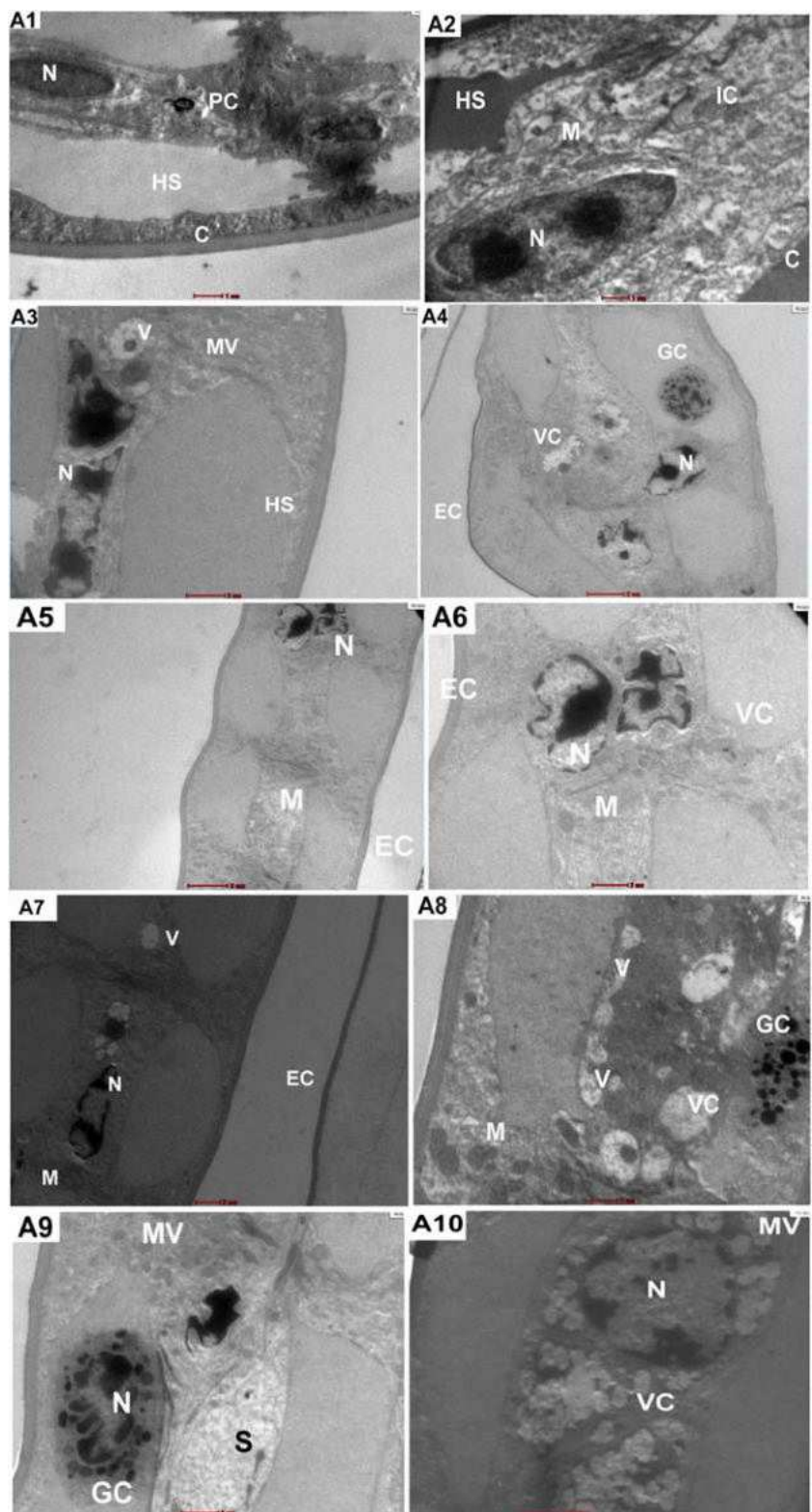
The impact of radiation on biota has been identified as priority area for future research within both scientific and regulatory communities especially in crustaceans in aquatic ecosystem (Fuller et al. 2015). In this study, the effects of ^{60}Co gamma radiation at dose level of 3, 30, 300, and 3000 mGy on the gill and hepatopancreas of freshwater crustacean *M. rosenbergii* were investigated by electron microscopic studies.

Scanning electron microscope

Gill

The SEM study showed fused to swollen lamellae (FLU-SL), abnormal gill tip (AG), and wrinkling of the lamellar epithelium (WLE) which may reduce the surface area and in turn likely to lead to decreased ionic permeability as suggested by McNamara and Lima (1997). Segner (1987) reported that gill epithelial cells in decapods crustaceans undergo structural reorganization in response to low dose radiation.

Fig. 3 Transmission electron microscopic images of control and ⁶⁰Co gamma irradiated freshwater prawn *M. rosenbergii*. (A) Gill: Control (A1–A2), 3 mGy (A3–A4), 30 mGy (A5–A6), 300 mGy (A7–A8), and 3000 mGy (A9–A10) groups. PC pillar cell, HS hemocoelic space, C epithelial cell with cuticle, N nucleus, M mitochondria, IC infolding of cell membrane, MV vacuolated mitochondria, EC elevated cuticle in lamellar region, VC vacuoles with crystalline granular inclusion

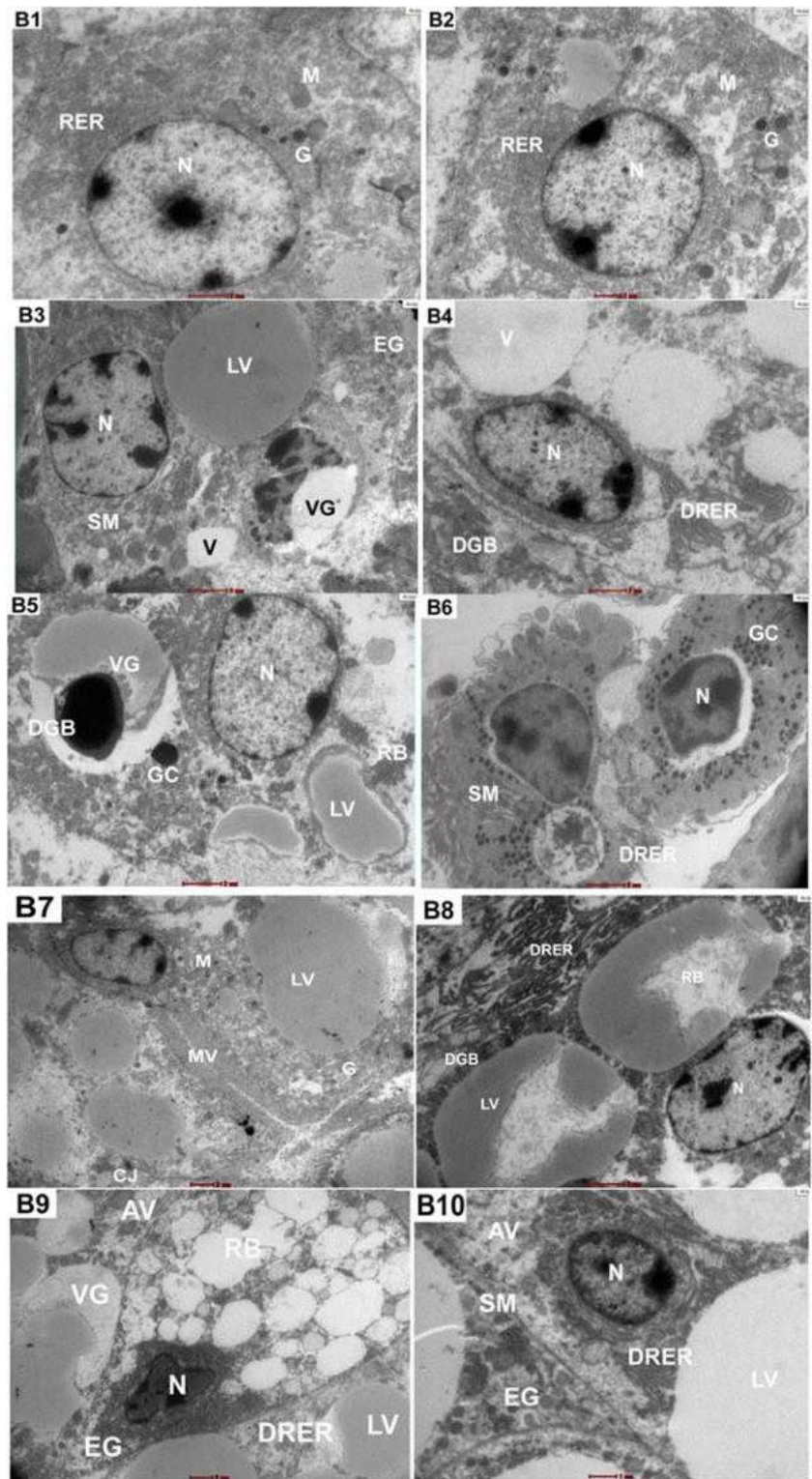


Hepatopancreas

Hepatopancreas of irradiated group of prawns showed various levels of abnormalities in various organelles. Icelly and Nott

(1980) reported several types of granulate inclusion, responsible for regulation of the concentrations of metals in the blood, in cells. Hence, gamma radiation exposure may disturb the regulations of the concentrations of metals. They may act

Fig. 4 Transmission electron microscopic images of control and ⁶⁰Co gamma irradiated freshwater prawn *M. rosenbergii*. (B) Hepatopancreas: Control (B1–B2), 3 mGy (B3–B4), 30 mGy (B5–B6), 300 mGy (B7–B8), and 3000 mGy (A9–A10) groups. E embryonic cell, N nucleus, M mitochondria, RER rough endoplasmic reticulum, G glycogen granules, R absorptive cell, LV lipid vacuoles, VG vacuolated cell with granular inclusions, EG electron dense granules, SM shrunken mitochondria, DRER dilated rough endoplasmic reticulum, DGB dilated cisternae of the Golgi body, RB residual bodies in rough endoplasmic reticulum



as a “barrier” to the diffusion of potentially harmful amounts of essential and non-essential metals into the blood. Hence, it

is likely that the gamma radiation exposure may disturb the regulations of the concentrations of metals.

Table 1 SEM grading of Gill and Hepatopancreas of control and ⁶⁰Co gamma irradiated *M. rosenbergii*

Histological parameters	Gamma irradiation dose (mGy)				
	Control	3	30	300	3000
Gill					
Fusion of lamellae	-	+	+	++	++
Abnormal gill tip	-	+	+	++	+++
Wrinkling of the lamellar epithelium	-	++	+	+	-
Swollen lamellae	-	-	+	++	++++
Epithelial necrosis	-	-	+	++	+++
Mean	-	+	++	++	+++
Hepatopancreas					
Lipid droplets in B cells	-	+	+	++	+++
Lipid body extrusion	-	+	++	+++	+++
Necrotic crystalline fragments	-	+	++	++	++
Necrotic epithelial surface	-	-	+	++	++++
Vacuoles	-	-	+	++	++++
Mean	-	+	++	++	+++

- none (0%), + mild (< 10%), ++ moderate (10 to 50%), +++ severe (50 to 70%), +++++ extended severe (> 70%)

Table 2 TEM grading of Gill and Hepatopancreas of control and ⁶⁰Co gamma irradiated *M. rosenbergii*

Histological parameters	Gamma irradiation dose (mGy)				
	Control	3	30	300	3000
Gill					
Mitochondria swelling	-	++	++	+++	+++
Elevated cuticle	-	++	+	+	-
Vacuole with granular inclusion	-	++	++	+++	++++
Granular Cell	-	++	++	+++	+++
Necrotic Nucleus	-	+	+	++	+++
Vacuolated cytoplasm	-	-	+	++	+++
Mean	-	+	++	++	+++
Hepatopancreas					
Large lipid vacuole	-	+	+	++	+++
Vacuoles with granules	-	+	++	+++	+++
Electron dense granules	-	+	++	++	+++
Shrunken mitochondria	-	+	++	+++	++++
Dilated rough endoplasmic reticulum	-	+	+	+++	++++
Dilated Golgi body	-	++	+	++	++++
Residual bodies	-	+	++	+++	+++
Mean	-	+	++	+++	++++

- none (0%), + mild (< 10%), ++ moderate (10 to 50%), +++ severe (50 to 70%), +++++ extended severe (> 70%)

Transmission electron microscopic

Gill

TEM study revealed mitochondrial swelling with degenerated cristae, vacuolated area with degenerated mitochondria, vacuoles with crystalline granular inclusion and granular cells, necrosis of mitochondria, damaged nucleus (N), lamellar region characterized by elevated cuticle, and formation of vacuoles (V) and granular cells. Manush et al. (2007), in the gills of *M. rosenbergii* exposed to 35 °C, observed severe degeneration of mitochondria with no cristae, irregular nuclear membrane and marginated chromatin. Gills of *M. olfersii* showed flattened hemilamella, narrow, hemolymph-filled space, and layers of pillar cells (Freire and McNamara 1995).

Hepatopancreas

The electron microscopic studies in controlled hepatopancreas of *M. rosenbergii* showed a normal arrangement with intact nuclear membrane, less number of nuclear pores and vacuoles, eccentric position of nucleolus, and more number of microvilli (Ramalingam and Ramarani 2007). Tissue destruction and bacterial accumulation found in the hepatopancreatic tubular lumen of *Penaeus vannamei* infected with *Vibrio anguillarum* (Esteve and Herrera 2000). The hepatocytes possessed distinct boundaries and apical membranes showed long microvilli which represented the brush border during TEM analysis (Abdelmeguid et al. 2009).

The folded basal lamina in the tubules evidenced in *P. argentinus* and *P. monodon* exposed to pesticides (Vogt 1987). TEM study on *P. serratus* exposed to 100 ng/μL petroleum compound revealed cellular disorganization and increased thickness in basal lamina, degeneration of the hepatopancreatic epithelium. No attempt has however been made to assess the impact of (mGy) of ⁶⁰Co gamma radiation at low level (Sadiq Bukhari et al. 2012). Hence, the present work is a novel attempt to study the ⁶⁰Co gamma (3, 30, 300, and 3000 mGy) radiation-induced ultrastructural studies in adult freshwater crustaceans (prawn) *Macrobrachium rosenbergii*.

Conclusion

The impact of ⁶⁰Co gamma radiation was studied on *Macrobrachium rosenbergii*. Ultrastructural damages were found in gills and hepatopancreas of *M. rosenbergii* exposed even at 3-mGy level. However, no lethality was observed in the select dose levels. Hence, it is recommended that radiation output may be kept at below 3-mGy level in order to protect the biota from radiation effect.

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Authors' contributions Stalin Arumugam contributed to the study conception, study design, study methods, data collection, analysis, and first draft preparation. Suganthi Palai and Mathivani Subramanian contributed to the improvement of study design and study methods. Gokula Varadharajan contributed to the data analysis, data presentation and improving the first draft. Stalin Arumugam, Suganthi Palani, Mathivani Subramanian, and Gokula Varadharajan commented on earlier versions of the manuscript, and read and approved the final manuscript.

Data availability Not applicable.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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