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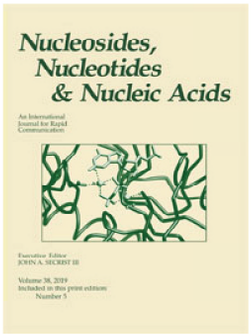

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DNA binding, photocleavage, antimicrobial and cytotoxic properties of Ru(II) polypyridyl complexes containing BOPIP ligand, (BOPIP = {2-(4-(benzyloxy) phenyl)-1H-imidazo [4,5-f] [1,2]phenanthroline})

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DNA binding, photocleavage, antimicrobial and cytotoxic properties of Ru(II) polypyridyl complexes containing BOPIP ligand, (BOPIP = {2-(4-(benzyloxy) phenyl)-1H-imidazo [4,5-f] [1,2]phenanthroline})

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ABSTRACT

A novel ligand BOPIP (BOPIP = {2-(4-(benzyloxy)phenyl)-1H-imidazo[4,5-f][1,10]phenanthroline}) and its mononuclear Ru(II) polypyridyl complexes $[Ru(phen)_2\ BOPIP]^{2+}$**(1)** (phen = 1,10-Phenanthrolene), $[Ru(bpy)_2\ BOPIP]^{2+}$**(2)** (bpy = 2,2′ bipyridyl), $[Ru(dmb)_2\ BOPIP]^{2+}$**(3)** (dmb = 4, 4′ -dimethyl 2, 2′ -bipyridine), $[Ru(Hdpa)_2\ BOPIP]^{2+}$**(4)** (Hdpa = 2,2′dipyridylamine) have been synthesized successfully and characterized by elemental analysis, UV-vis, IR, ^{1}H, ^{13}C-NMR, and ESI-MS Spectroscopy. The interaction of these complexes with CT-DNA was studied using absorption, emission techniques, viscosity measurements and molecular docking studies. The docking study also supports the binding ability of complexes obtained through the absorption and emission techniques. These studies reveal that the Four Ru(II) polypyridyl complexes bind to DNA predominantly by intercalation. The Antimicrobial activity and cytotoxicity of these complexes are also reported.

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KEYWORDS
Antimicrobial activity; DNA binding; MTT Assay; Molecular Docking; Photocleavage; Viscosity

1. Introduction

Cancer is mostly considered as a group of dreadful diseases, characterized by uncontrolled cell growth. Cancer, still proven to be one of the unruliest diseases to which humans are subjected, and as yet no practical and completely effective drugs or methods to control are available. Hence, identification of new effective, selective, and less cytotoxic anticancer agents is still one of the most pressing health issues.[1–4] DNA, the carrier of genetic information, has been identified as the primary target for a variety of anticancer drugs because of their ability to interfere DNA transcription

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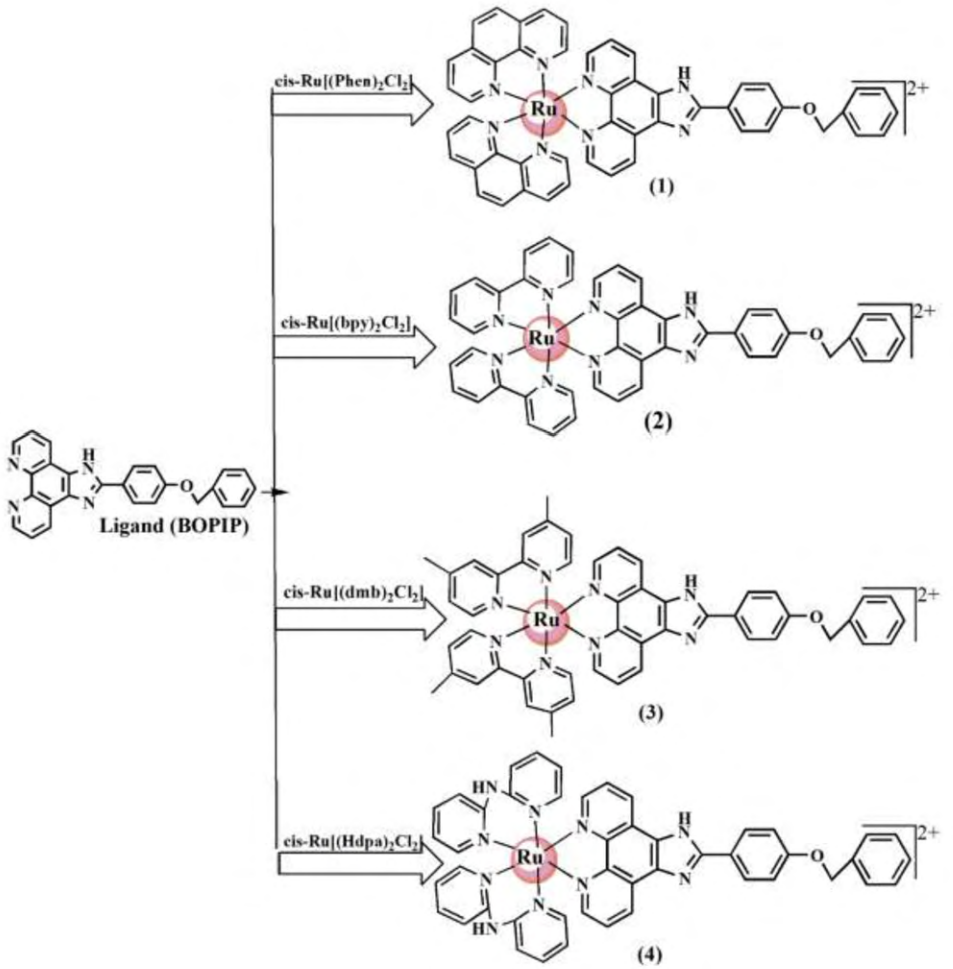
and replication, which are major steps of cell growth and division.[5] Thus, knowing and understanding drug-DNA interactions is important to comprehend the mode of action of any anticancer drug targeting DNA. DNA offers a number of sites for different covalent and noncovalent interactions with the drugs.

The field of anticancer metallodrugs is dominated by platinum-based compounds and the so-called "DNA paradigm", which presumes that the mechanism of action of metallodrugs relies on direct DNA damage.[6] The quest for alternative drugs to the well-known cisplatin and its derivatives, which are still used in more than 50% of the treatment regimes for patients suffering from cancer, is highly desirable.[7,8] The development of more efficient anticancer drugs with improved selectivity and diminished toxic side effects is currently an area of intense research. With the objective of developing compounds with a new mode of action in comparison to the established anticancer drugs cisplatin, carboplatin, and oxaliplatin for treatment of a broader range of tumors and with fewer side effects, many metal complexes were investigated in recent years for their tumour inhibiting properties.[9] New metal-based anticancer drugs may be able to widen the spectrum of treatable cancers, reduce toxic side effects, and overcome platinum resistance.

Ruthenium is the most attractive metal owing to its chemical and air stability, structural diversity, low toxicity and ability to mimic iron binding in biological system, which finally supported them as highly potent anticancer agents rather than platinum-based drugs.[10–12] Due to unique photophysical properties, ruthenium complexes have been widely applied in DNA probing, cellular imaging, protein monitoring, and anticancer activity.[13–20] Presently, ruthenium complex NKP-1339 (trans-[tetrachloridobis (1H–indazole) ruthenate(III)]) has successfully entered into the clinical trials.[21,22]

Changes in the structure of main ligand could be used to attain diverse DNA binding ability of ruthenium(II) complexes. Therefore, extensive studies on different structured ligands are necessary to further elucidate the DNA binding ability and its mechanism of Ru(II) complexes and discover some new potential anticancer reagents. In this article, we report the synthesis, characterization, DNA binding, light switching, photocleavage, cytotoxicity, and antimicrobial activity studies of the ligand 2-(4-(benzyloxy) phenyl)-1H-imidazo[4,5-f][1, 10]phenanthroline (BOPIP) and four of its ruthenium(II) complexes. $[Ru(phen)_2(BOPIP)]^{2+}$ **(1)**, $[Ru(bpy)_2(BOPIP)]^{2+}$ **(2)**, $[Ru(dmb)_2(BOPIP)]^{2+}$ **(3)**, $[Ru(Hdpa)_2(BOPIP)]^{2+}$ **(4)** (Scheme 1) The absorption & emission studies, viscosity measurements, and photocleavage studies show that the four complexes predominantly interact with DNA by an intercalative mode. The cytotoxicity of these compounds evaluated by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay.



Scheme 1. Schematic synthetic route for the preparation of complexes 1, 2, 3 and 4, Where 1 = $[Ru(Phen)_2BOPIP]^{2+}$, 2 = $[Ru(bpy)_2\ BOPIP]^{2+}$, 3 = $[Ru(dmb)_2\ BOPIP]^{2+}$, 4 = $[Ru(Hdpa)_2\ BOPIP]^{2+}$.

The cytotoxicity studies show that these compounds exhibit efficient activity against HeLa (human cervical cancer cell line) cell lines in a dose-dependent manner. The antimicrobial activity experiments show that these compounds exhibit decent antimicrobial activity.

2. Materials and methods

2.1. Materials

All reagents and solvents of analytical grade were commercial products and were used as received unless otherwise stated. 1,10-Phenanthroline-5,6-dione,[23] cis-$[Ru(phen)_2Cl_2]$. $2H_2O$, cis-$[Ru(bpy)_2Cl_2].2H_2O$, cis-$[Ru(dmb)_2Cl_2].2H_2O$,[24] and cis-$[Ru(Hdpa)_2Cl_2].2H_2O$[25] were synthesized according to literature

procedures. 4-(benzyloxy) benzaldehyde, $RuCl_3.3H_2O$, and MTT were procured from Sigma-Aldrich. 1,10-Phenanthroline monohydrate, 2,2′-bipyridine (bpy), 4,4′-dimethyl-2,2′-bipyridine (dmb), and 2,2′-dipyridyl amine (Hdpa) were acquired from Merck. Calf thymus DNA (CT-DNA) was bought from Aldrich, Supercoiled pBR322 plasmid DNA (stored at −20 °C) was obtained from Fermentas Life Sciences and was used as received. Agarose was purchased from Genei. Ultrapure Milli-Q water (18.2 mX) was used in all experiments and for preparing various buffers double-distilled water was used. The HeLa human cervical carcinoma cell line was obtained from NCCS, Pune, and was maintained in RPMI 1640 standard (Sigma Aldrich) supplemented with 10% (v/v) fetal bovine serum, 2 m.mol L-glutamine, 4.5 g L-1 glucose, 19 nonessential amino acids, and 19 antibiotics consisting of penicillin/streptomycin, gentamicin, amphotericin B, and nystatin (basal growth medium). Binding of the complexes with CT-DNA was studied in tris(hydroxymethyl)aminomethane (Tris)–HCl buffer (5 m.mol Tris–HCl, 50 m.mol NaCl, pH 7.2). A solution of CT-DNA in Tris–HCl buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.8:1 to 1.9:1, indicating the DNA was sufficiently free of protein.[26] The concentration of DNA per nucleotide was determined spectrophotometrically using a molar absorptivity of 6,600 M^{-1} cm^{-1} (260 nm).[27] Concentrated stock solutions of CT-DNA were prepared in buffer and were determined by the UV absorbance at 260 nm after 1:100 dilutions. Stock solutions were stored at 4 °C and used after not more than 4 days. Concentrated stock solutions of metal complexes were prepared by dissolving calculated amounts of metal complexes in DMSO and diluted suitably with the corresponding buffer to the concentrations required for all the experiments.

2.2. Physical measurements

The UV-Vis spectra was recorded on Shimadzu UV-2600 spectrophotometer. Cary Eclipse instrument serial number (MY12400004) Spectro fluorometer was used to record the luminescence spectral data for determining the binding constant values. IR spectra were recorded on a PerkinElmer 1605 Fourier transform IR spectrometer by means of KBr disks. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 400-MHz spectrometer with dimethyl-d6 sulfoxide (DMSO-d6) as the solvent and tetramethylsilane as the internal standard at room temperature. Elemental microanalysis (C, H, and N) was conducted by using PerkinElmer 240 elemental analyser. Electrospray ionization mass spectrometry (ESI–MS) mass spectra were recorded with a Quattro LC triple quadrupole mass spectrometer fortified with the MassLynx software program (Micromass, Manchester, UK).

2.3. Synthesis and characterization of ligand and complexes

The 1,10-phenanthroline-5,6-dione (Phendione),[23] cis-[Ru(phen)$_2$Cl$_2$], cis-[Ru(bpy)$_2$Cl$_2$], cis-[Ru(dmb)$_2$Cl$_2$], and cis-[Ru(Hdpa)$_2$Cl$_2$][24,25] were synthesized according to reported literature methods. Schematic diagram of Ru(II) complexes were shown in Scheme 1.

2.4. Synthesis of ligand [BOPIP]

The ligand was synthesized according to the procedure in the literature.[28] A mixture of phendione (0.53 g, 2.50 m.mol), 4-(benzyloxy) benzaldehyde (0.743 g, 3.50 m.mol), ammonium acetate (3.88 g, 50.0 m.mol) is liquified in glacial acetic acid (25 ml) and the ensuing solution was refluxed for 5h. A clear wine-red colour solution attained. The above solution was cooled to room temperature and transferred into distilled water, drop wise addition of Conc. NH_3 form a yellow precipitate, which was collected, washed with H_2O and dried. The crude product recrystallized with $C_5H_5N.H_2O$ and dried (Yield: 81.04%). Anal. Data for $C_{26}H_{18}N_4O$: Calcd(%): C, 77.59; H, 4.51; N, 13.9; found(%): C, 77.64; H, 4.45; N, 13.76. ES-MS(m/z) Calc: 402; found: 403 $[M+H]^+$. ^{1}H–NMR (DMSO-d$_6$, 400 MHz): δ 8.93(d,2H), 8.26(d, 2H), 7.88(m, 5H), 7.44(t, 2H),7.27(d, 2H), 7.1(d,2H), 5.22(s,2H). ^{13}C[^{1}H] NMR (400 MHz, DMSO-d$_6$, ppm): δ153.8, 153.1, 140.4, 137, 128.2, 122.6, 115.4, 114.8, 69.8. IR (KBr, cm^{-1}): 3641 (ν, N-H), 1118 (ν, C-N), 1240 (ν, C-O-C).

2.5. Synthesis of complexes

2.5.1. [Ru(phen)$_2$(BOPIP)](ClO$_4$)$_2$.2H$_2$O(1)

Cis-[Ru(Phen)$_2$Cl$_2$].2H$_2$O (0.284 g, 0.5 m.mol), BOPIP (0.201 g, 0.5 m.mol) dissolved in ethanol (25 ml) plus water (15ml) mixture and refluxed for 8h at 120 °C under N_2 atmosphere. When the light purple colour solution was obtained, it was cooled to room temperature and an equal volume of saturated aqueous $NaClO_4$ solution was added under vigorous stirring. The yellow precipitate was collected and washed with small amounts of water, ethanol and diethyl ether, then dried under vacuum (yield: 78%). Anal. data for $RuC_{50}H_{34}N_8O$: calcd (%): C, 69.51; H, 3.97; N, 12.97; found: C, 69.62; H, 3.88; N, 12.81. ES-MS(m/z) cal: 864; found: 866 $[M+H]^{+2}$. ^{1}H–NMR (DMSO-d$_6$, 400 MHz): δ 9.06(d,6H), 8.79(d, 6H), 8.21(d, 4H), 8.09(d, 2H),7.79(m, 6H), 7.2(d,2H), 5.25(s,2H). ^{13}C[^{1}H] NMR (400 MHz, DMSO-d$_6$, ppm): δ 160.5, 153.2, 147.7, 137.2, 132.2, 130.9, 128.9, 128.2, 126.8, 122.5, 116.0, 115.7, 69.9. IR (KBr, cm^{-1}): 3475 (ν, N-H), 1116 (ν, C-N), 1143 (ν, C-O-C), and 626 (ν, Ru-N).

2.5.2. $[Ru(bpy)_2(BOPIP)](ClO_4)_2.2H_2O(2)$

This complex was synthesized by adopting the same procedure as described above for Complex 1. taking a mixture of cis-$[Ru(bpy)_2Cl_2].2H_2O$ (0.260 g, 0.5 m.mol), BOPIP (0.201 g, 0.5 m.mol) (yield: 78%). Anal. data for $RuC_{46}H_{34}N_8O$: calcd(%): C, 67.72; H, 4.20; N, 13.73; found(%): C, 67.82; H, 4.23; N, 13.63. ES-MS(m/z) calc: 816; found: 817 $[M+H]^{+1}$. ^{1}H–NMR (DMSO-d_6, 400 MHz): δ 9.10(d,2H), 8.9(d, 4H), 8.84(d, 2H), 8.11(t, 4H),8.28(t, 4H), 7.86(d, 2H), 7.61(d,4H), 7.44(t, 2H), 7.34(m, 5H), 7.22(d,2H), 5.25(s,2H). ^{13}C[^{1}H] NMR (400 MHz, DMSO-d_6, ppm): δ 160.6, 157.2, 153.2, 151.8, 138.4, 137.1, 128.9, 128.3, 124.9, 122.5, 116.0, 115.7, 69.9. IR (KBr, cm^{-1}): 3444 (ν, N-H), 1078 (ν, C-N), 1143 (ν, C-O-C), and 626 (ν, Ru-N).

2.5.3. $[Ru(dmb)_2(BOPIP)](ClO_4)_2.2H_2O(3)$

This complex was synthesized as described above by taking a mixture of cis-$[Ru(dmb)_2Cl_2].2H_2O$ (0.288 g, 0.5 m.mol), BOPIP (0.201 g, 0.5 m.mol) (yield: 72.71%). Anal. data for $RuC_{50}H_{42}NO_9$: calc. C, 53.82; H, 4.04; Cl, 6.76; N, 12.02; O, 13.73; Ru, 9.64; found: C, 54.01; H, 4.32; Cl, 6.60; N, 11.94; O, 13.82; Ru, 9.50. ES-MS(m/z) calc: 1048; found: 1050 $[M+H]^{+2}$. ^{1}H–NMR (DMSO-d_6, 400 MHz): δ 8.75(d, 6H), 8.28(d, 2H), 8.07(s, 4H), 7.66(d, 4H), 7.44(t, 2H),7.17(m, 5H), 5.24(s,2H), 2.46(s, 12H). ^{13}C[^{1}H] NMR (400 MHz, DMSO-d_6, ppm): δ 160.5, 156.7, 150.0, 137.1, 132.2, 128.9, 128.2, 127.2, 122.6, 115.7, 69.9, 51.0. IR (KBr, cm^{-1}): 3444 (ν, N-H), 1133 (ν, C-N), 1141 (ν, C-O-C), and 624 (ν, Ru-N).

2.5.4. $[Ru(Hdpa)_2(BOPIP)](ClO_4)_2.2H_2O(4)$

This complex was synthesized as described above by taking a mixture of cis-$[Ru(Hdpa)_2Cl_2].2H_2O$ (0.19 g, 0.5 m.mol), BOPIP (0.201 g, 0.5 m.mol) (yield: 52.71%). Anal. data for $RuC_{47}H_{42}Cl_2N_9O_9$: calc. C, 53.82; H, 4.04; Cl, 6.76; N, 12.02; O, 13.73; Ru, 9.64; found: C, 54.01; H, 4.32; Cl, 6.60; N, 11.94; O, 13.82; Ru, 9.50. ES-MS(m/z) calc: 1048; found: 1050 $[M+H]^{+2}$. ^{1}H–NMR (DMSO-d_6, 400 MHz): δ 8.96(d, 4H), 8.25(d, 2H), 8.05(d, 2H), 7.74(t, 6H), 7.43(d, 2H), 6.97(m, 5H), 6.9(d, 6H), 5.24(s,2H), 4.2(s, 2H). ^{13}C[^{1}H] NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 153.8, 153.1, 137.1, 128.9, 128.2, 122.6, 115.9, 69.9. IR (KBr, cm^{-1}): 3444 (ν, N-H), 1133 (ν, C-N), 1141 (ν, C-O-C), and 624 (ν, Ru-N).

2.6. DNA-binding and photocleavage experiments

2.6.1. UV-Visible absorption spectral studies

The DNA-binding studies were conducted at room temperature. Concentrated stock solutions of metal complexes were prepared by

dissolving calculated amounts of metal complexes in DMSO and diluted accordingly with the corresponding buffer to the concentrations required for all the experiments. The absorption titrations were performed in Tris–HCl buffer. The absorption titrations of the complex in buffer were performed using a fixed complex concentration (20μl), to which increments of the DNA stock solution was added. Ru–DNA solutions were incubated for 5 min before the absorption spectra were recorded. The intrinsic binding constants K_b of these complexes with regard to DNA were calculated by using the following equation.[29]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA, ε_a, ε_b and ε_f correspond to the apparent absorption extinction coefficient (A_{obsd}/[complex]), the extinction coefficient for the complex in the fully bound form and the extinction coefficient for the free complex respectively. The graph was plotted between [DNA]/(ε_a-ε_f) versus [DNA] gave the intrinsic binding constant K_b. The K_b value obtained from the ratio of slope to the intercept.

2.6.2. Florescence (Luminescence) spectral studies

The luminescence titrations were performed similarly to the absorption titrations using Tris-HCl buffer. To the fixed metal concentration (10 μl), various concentrations (10–200 μl) of DNA were added. The binding constant was calculated using Scatchard equation.[30]

$$C_b = C_t\left[(F - F_0)/(F_{max} - F_0)\right] \quad (2)$$

where C_t is the total complex concentration, F is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is when the complex is maximum bound to DNA. From the Scatchard plot of r/C_f versus r, where r is the C_b/[DNA] and C_f is the concentration of the free complex, the negative slope gives the intrinsic binding constant K_b of the complexes based on the relation

$$r/C_f = K_b(1 - nr) \quad (3)$$

Quenching studies with $[Fe(CN)_6]^{4-}$ were extended under this luminescence experiment for further understanding the binding ability of these complexes with DNA. We also observed an interesting thing that these complexes are exhibiting the light switch on/off effect by taking the same concentrations of Co^{2+} and Na_2EDTA solutions in ideal concentrations of complex in fluorescence titrations.

2.6.3. Viscosity studies

Ostwald viscometer was used for the viscosity studies, Ostwald viscometer was immersed in thermo stated water bath maintained a constant temperature ($30 \pm 0.1\,^{\circ}C$) by using BPE buffer (6 m.mol Na_2HPO_4, 2 m.mol NaH_2PO_4, 1 m.mol Na_2EDTA, pH = 7.0). The used CT-DNA samples approximately 200 base pairs in average length were prepared by sonication to minimize the complexes arising from DNA flexibility.[31] Using the digital stopwatch, the flow time was recorded and each sample was repeated thrice. The recorded data were presented as $(\eta/\eta_0)^{1/3}$ versus concentration of [Ru(II)]/[DNA], where η is the viscosity of DNA in the presence of the complex, and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for the flow time of the buffer alone (t_0).

2.6.4. Photocleavage experiment

For the gel electrophoresis experiments pH 8.0 buffer of 40 m.mol Tris base, 20 m.mol acetic acid, and 1 m.mol EDTA was used. A buffer of 10 m.mol Tris–HCl and 1 m.mol Na_2EDTA was used for dilution of pBR322 DNA. Supercoiled pBR322 DNA (0.1 µg/µL) was treated with ruthenium(II) complexes with concentrations of 20, 40, 80 µl, and the mixtures were irradiated at room temperature with a UV lamp (365 nm, 10 W) for 60 min. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanole, and 30% glycerol (2 µL) was added. The samples were then analysed by 0.8% agarose gel electrophoresis at 50 V for 2 h. The gel was stained with 2 µL (from 1 mg/100 µL) ethidium bromide and photographed under UV light.[32] The gels were viewed with a gel documentation system and photographed using a CCD camera (Alpha Innotech).

[**CAUTION:** *Ethidium bromide is a mutagen and potential carcinogen. Gloves should be worn and care should be taken when handling. UV light is damaging to eyes and exposed skin. Protective eyewear and apron should be worn at all times.*]

The photocleavage experiments were also performed with singlet oxygen ($^1O_2$) inhibitor Histidine and Hydroxyl free radical ($^{\cdot}OH$) inhibitor Mannitol to establish the reactive species responsible for the photoactivated cleavage of the plasmid.

2.7. Antimicrobial studies

Antimicrobial studies were performed using standard disk diffusion method.[33] The antibacterial activity of the complexes was studied against *Escherichia coli* and *Staphylococcus aureus*. Each of the ruthenium(II) complex was dissolved in DMSO at different concentrations of 10, 20, and

40 µg. Paper disks of Whatman filter paper no. 1 were sterilized in an autoclave. The paper disks saturated with 10 µL of the ruthenium(II) complex were placed aseptically in Petri dishes containing LB agar medium inoculated separately with *E. coli* and *S. aureus*. The Petri dishes were incubated at 37 °C, and the inhibition zones were recorded after 24 h of incubation. The experiments were repeated twice and the average value was taken. The results were also compared with the results for the standard antibacterial drug Ampicillin.

2.8. Molecular docking studies

Accelry's Discovery Studio (version 2.1) was used to design lead molecules, estimate the docking interactions of a complex of drug and protein binding, and number of bonds formed by ligand with the target. The molecular docking of ruthenium complexes 1, 2, 3 and 4 was performed using LibDock.[34] LibDock is a high-throughput algorithm for docking ligands into an active binding site on the receptor, which is also a site-features docking algorithm. Accelry's CHARMm force field was used throughout the simulation before running LibDock. The crystal structure of human DNA topoisomerase 1 (TOP1) receptor was downloaded from RCSB PDB (PDB ID-1T8I), after downloading the PDB format of the protein, all the water molecules of the protein were removed by using Discovery Studio and stabilizing the charges, filling the missing residues, and generating the side chains, according to the parameters available. The receptor should be in a biologically active and stable state. After the receptor is constructed, the active site within the receptor should be recognized. The receptor may have many active sites but the one of the interest should be selected. Ruthenium complexes were sketched using the tools Chemsketch and used to dock into the target binding site. Ruthenium complex conformations aligned to receptor interaction sites and the best poses were reported at the end of docking simulations. The scoring functions have been used to estimate binding affinity to screen out active and inactive compounds during the process of virtual screening.[35]

2.9. Cytotoxicity assay in vitro (MTT Assay)

Standard MTT assay was conducted as described in the literature.[36] Cells were placed in 96-well microassay culture plates (8×10^3 per well) in 200 µL and were grown overnight at 37 °C in a 5% CO_2 incubator. Complexes 1–4, in the concentration range 1–100 µM, dissolved in DMSO (Sigma-Aldrich), were added to the wells. Control wells were prepared by addition of culture medium (200 µL). Wells containing culture medium

without cells were used as a negative control and cisplatin was used as a positive control. DMSO was used as the vehicle control. A stock solution of cisplatin (10 m.mol in DMSO) was freshly prepared for every experiment. After 48 h, 20 μL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added to each well and the plates were wrapped in aluminium foil and incubated for 4 h at 37 °C. The purple formazan product was dissolved by addition of 100 μL of 100% DMSO to each well. The absorbance was monitored at 620 nm using a 96-well plate reader. The stock solutions of the metal complexes were prepared in DMSO, and in all experiments, the percentage of DMSO was maintained in the range of 0.1–2%. DMSO by itself was found to be nontoxic to the cells until a concentration of 2%. Data were collected for three replicates each to obtain the mean values. The IC_{50} values were determined by plotting the percentage viability versus concentration on a logarithmic graph and reading the concentration at which 50% of cells remained viable relative to the control.

3. Results and discussion

3.1. Electronic absorption titrations

Electronic absorption spectroscopy is the common means to study the interaction between metal complexes and DNA.[37] For metallointercalators, DNA binding is associated with hypochromism and a redshift in the metal to ligand charge transfer (MLCT) and ligand bands.[38] This is primarily due to the intercalation, involving strong stacking interactions between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in a UV–visible band is consistent with the strength of the interaction.[39] Thus, to provide evidence for the possibility of binding of each complex to CT-DNA, spectroscopic titrations of solutions of each of the complexes with several concentrations of CT-DNA were examined. A characteristic spectral curve of the complex at different DNA concentrations is shown in Figure 1. As the DNA concentration is increased, the MLCT bands of 1 at 453 nm, 2 at 462 nm, 3 at 467 nm, and 4 at 468 nm exhibit hypochromism of about 14.46, 13.74, 11.64, and 15.01%, respectively, and bathochromism of about 2–5 nm. To further elucidate the binding strength of the complexes with regard to DNA, the intrinsic binding constant K_b was determined in each case by monitoring the changes in their absorbance in the MLCT band with increasing concentration of CT-DNA. The K_b values of 1, 2, 3, and 4 are 7.1×10^4 M^{-1}, 3.4×10^4 M^{-1}, 2.5×10^4 M^{-1}, and 8.3×10^4 M^{-1}, respectively. The values are smaller than that of those DNA metallointercalators, such as $[Ru(bpy)_2(PPIP)]^{2+}$ $K_b = (4.3\ (\pm 0.40) \times 10^4\ M^{-1})$, $[Ru(phen)_2(PPIP)]^{2+}$ $K_b = (1.13\ (\pm 0.30) \times 10^5\ M^{-1})$ and

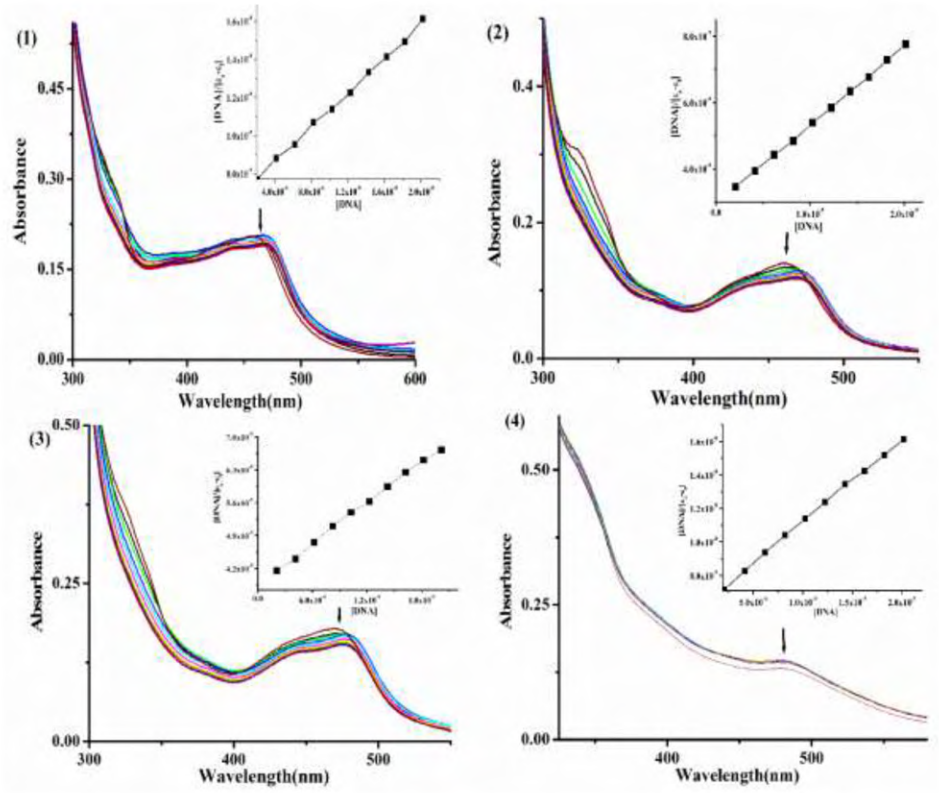


Figure 1. Absorption spectra of complexes 1–4 in absence and presence of CT-DNA in Tris-HCl buffer. Arrow shows hypochromism and bathochromism upon the increase of DNA concentration. Inserted plot,[DNA]/(ε_a-ε_f) versus [DNA] for the titration of DNA with Ru(II) complexes, which gives intrinsic binding constant (K_b).

$[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyrido-[3,2-a:2′,3′-c]phenazine, $K_b > 10^6$ M^{-1}), but bigger than that of the parent complex $[Ru(phen)_3]^{2+}$ $K_b = (5.5 \times 10^3\ M^{-1})$.[38,40,41] Since the intercalator is common in all the four complexes, the different DNA binding properties of the four complexes are due to their diverse ancillary ligands. Going from bpy to phen, the planar area and hydrophobicity increases, which may lead to a greater binding affinity for DNA. The four additional methyl groups in complex 3 relative to complex 2 employ some steric hindrance, thus averting the complex from intercalating as effectively, and so instigating a decrease in the binding constant. The flexible nonplanar hdpa ligands approach more closely and coordinate to ruthenium(II) more strongly than the rigid phen ligands[42] and the NH group in Hdpa may employ some added interactions such as hydrogen bonding with functional groups present on the edge of the DNA.[43] This would contribute significantly to the greater binding constant in contrast to the other three complexes. The K_b values of all the complexes studied are in the order $4 > 1 > 2 > 3$.

3.2. Luminescence titrations

To further understand the exact nature of the complex binding to DNA, luminescence titration experiments were performed at a fixed metal complex concentration (5 μM) in Tris buffer (pH 7.2) at ambient temperature. The change of emission intensity is related to the extent to which the complex enters into the hydrophobic environment inside the DNA. Figure 2 shows the fluorescence excitation and emission spectra for the free and bound complexes 1–4 in the presence of different amounts of CT-DNA. Excitation wavelengths of 453, 462, 467, and 468 nm were used for fluorescence measurements of complexes 1, 2, 3, and 4, respectively and emission wavelength found to be 602, 610, 618, and 627 nm. When the CT-DNA was added to the solution of the complexes 1-4, the fluorescence intensity was found to increase. The fluorescence intensities of complexes 1, 2, 3, and 4 increased by 3.26, 3.18, 3.11, and 3.83 times, respectively, compared with the intensities in the absence of CT-DNA. The emission enhancement of the complexes 1-4 in the presence of CT-DNA is much smaller than that observed for complexes $[Ru(phen)_2(PPIP)]^{2+}$, $[Ru(bpy)_2(PPIP)]^{2+}$ and $[Ru(dmb)_2(PPIP)]^{2+}$.[41] This implies that $[Ru(phen)_2(PPIP)]^{2+}$, $[Ru(bpy)_2(PPIP)]^{2+}$ and $[Ru(dmb)_2(PPIP)]^{2+}$ may interact with CT-DNA more strongly and when the complex intercalates between the DNA base pairs, the mobility of the complex is restricted at the binding site and the hydrophobic

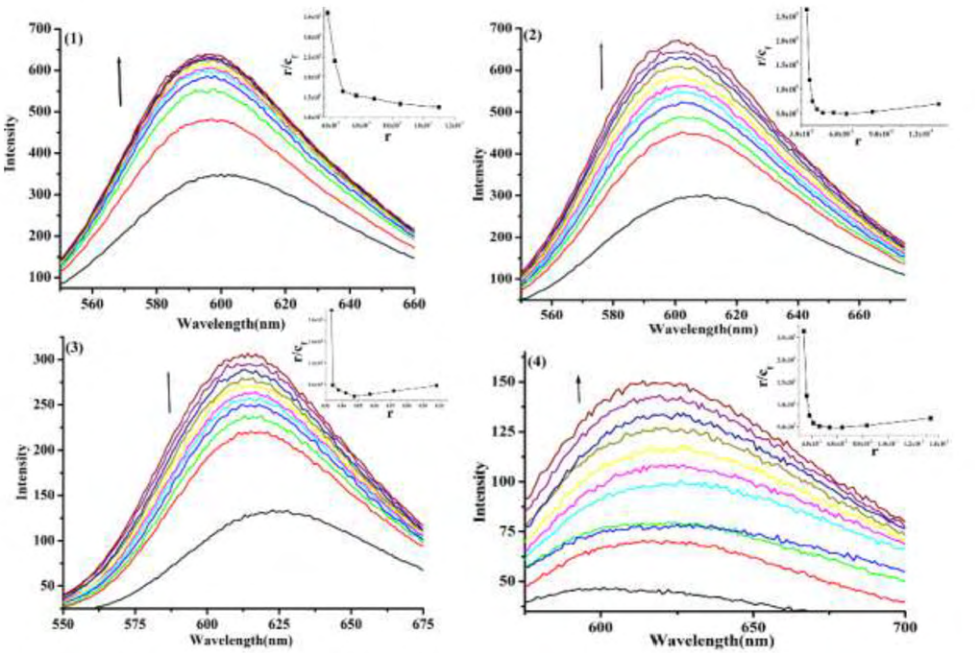


Figure 2. Emission spectra of complexes 1–4 in Tris-HCl buffer upon addition of CT-DNA. The arrow shows the intensity change upon the increase of DNA concentration. Inset: Scatchard plot of above complex, which gives binding constant (Kb).

environment inside the DNA helix reduces the accessibility of solvent water molecules to the complex, leading to a decrease of the vibrational modes of relaxation. The intrinsic binding constant from the fluorescence data was obtained from a modified Scatchard equation[30] through a plot of r/Cf versus r, where r is the binding ratio Cb/[DNA] and C_f is the free ligand concentration. Scatchard plots for the complexes were constructed from luminescence spectra, and the binding constants (K_b) were 7.29×10^4 M^{-1}, 3.61×10^4 M^{-1}, 2.57×10^4 M^{-1}, and 9.8×10^4 M^{-1} for 1, 2, 3, and 4, respectively. The binding constants calculated are in comparable with the absorption spectra.

3.2.1. Quenching studies

Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as a quencher may provide further information about complexes binding to DNA, but cannot be used to determine the mode of binding. In quenching experiments, to maintain the ionic strength so that the quenching curves remain nonlinear, KCl was added along with $K_4[Fe(CN)_6]$ such that the final and total concentration was constant at 4×10^{-3} M.[44] The Stern–Volmer quenching constant (Ksv) can be determined using the Stern-Volmer equation,[45]

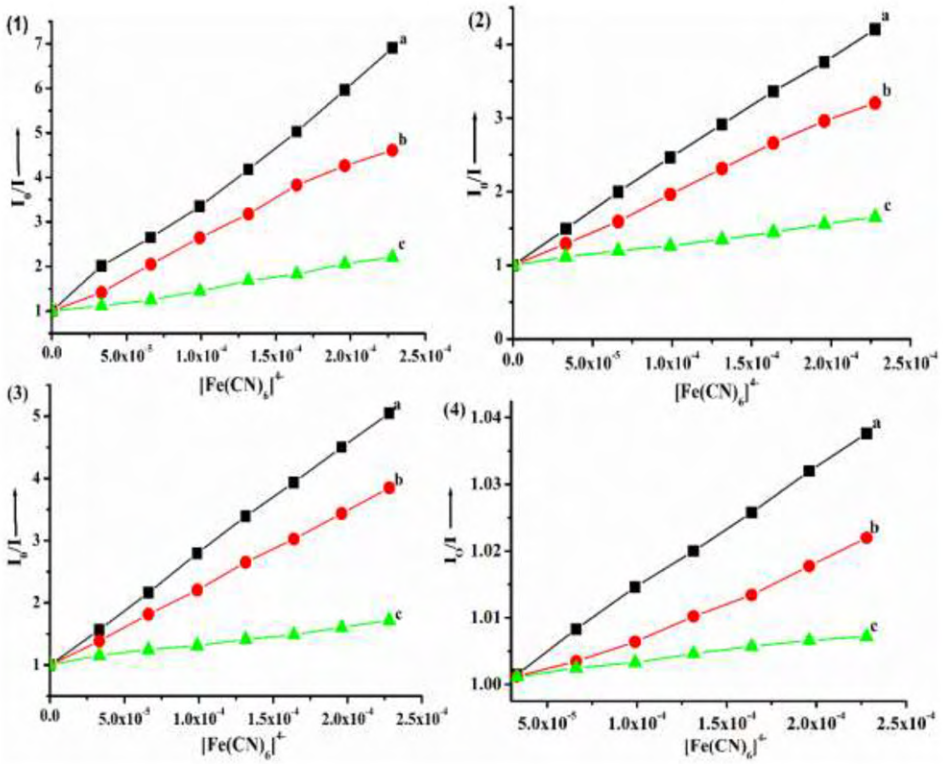


Figure 3. Quenching studies of complexes 1–4 in Tris-HCl with $[Fe(CN)6]^{4-}$ in the absence of DNA (a), presence of DNA 1:20 (b) and 1:100 (c).

Table 1. DNA binding and Ksv data for Ruthenium(II) complexes.

Complex	Absorption λ_{max} (nm) (MLCT)	Hypo chromism (%)	Absorbance binding constant (K_b)	Emission binding constant	Ksv values		
					Only Complex	Complex + DNA 1:50	Complex + DNA 1:100
$[Ru(Phen)_2BOPIP]^{+2}$ **(1)**	453	14.46	7.1×10^4	7.29×10^4	25279	16585	5542
$[Ru(bpy)_2\ BOPIP]^{+2}$ **(2)**	462	13.74	3.4×10^4	3.61×10^4	17881	12063	3053
$[Ru(dmb)_2\ BOPIP]^{+2}$ **(3)**	467	11.64	2.5×10^4	2.57×10^4	14026	9997	2804
$[Ru(Hdpa)_2BOPIP]^{+2}$ **(4)**	468	15.01	8.3×10^4	9.8×10^4	28541	17441	5847

phen: 1,10-phenanthroline, bpy: 2,2′-bipyridine, dmb: 4,4′-dimethyl-2,2′-bipyridine, bpip: 2-(4-(benzyloxy)phenyl)-1H-imidazo [4,5-f][1,10]phenanthroline, hdpa: 2,2′,-dipyridylamine, MLCT: metal-to-ligand charge transfer.

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

where I_0 and I are the intensities of the fluorophore in the absence and presence of the quencher, respectively, [Q] is the concentration of the quencher, and Ksv is the linear Stern–Volmer quenching constant. In general, positively charged free complex ions may be readily quenched by $[Fe(CN)_6]^{4-}$, whereas the complex bound to DNA can be protected from the quencher as the negative charge of $[Fe(CN)_6]^{4-}$ will be repelled by the negatively charged phosphate backbone of DNA, resulting in less quenching of the bound complex compared with the free complex. Figure 3. shows the Stern–Volmer plots for the free complexes in solution and the complexes in the presence of increasing amounts of DNA. The Ksv values for all four complexes are given in Table 1. From the quenching studies it is clear that the DNA binding affinity of complexes follows the order $4 > 1 > 2 > 3$, which is consistent with other results.[38,40,46]

3.2.2. On–off–On light switching behaviour

As shown in Figure 4 the emission spectral profile of DNA bound complex **1** elucidates the switching of emission on and off when Co^{2+} and EDTA are added, respectively. The experiments were conducted using a method similar to that developed by our research group earlier.[38,46] When the complex binds to DNA (switch on), the emission intensity is high, but when we add Co^{2+} (0.03 m.mol), the emission of DNA-bound complex **1** is quenched by Co^{2+}, thus turning the light switch off,[47,48] owing to the formation of the Co^{2+}–complex **1** heterometallic complex. When EDTA (0.03 m.mol) was added to the buffer system containing Co^{2+}–complex **1**, the emission intensity recovered again (light switch on), based on the strong coordination of Co^{2+} to EDTA (EDTA– Co^{2+}) and the complex becomes free. A similar observation was made for other three complexes. The change in luminescence of the DNA-bound complex in the presence of Co^{2+} and EDTA reveals its use in the modulation of drug therapy.

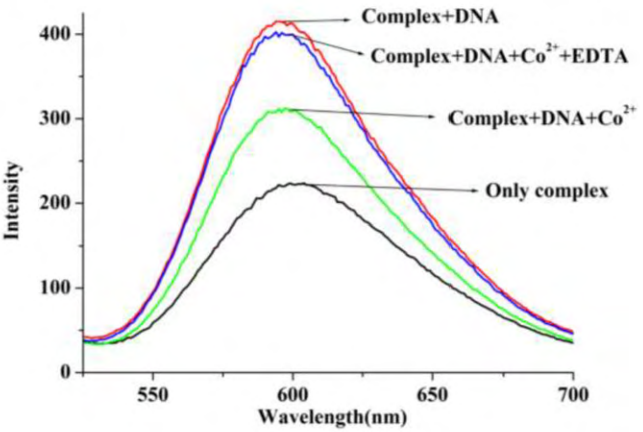


Figure 4. DNA light switch on and off experimentally showing the luminescence changes upon addition of Co^{2+}, EDTA and DNA to complex 1.

3.3. Viscosity studies

The DNA binding modes of complexes were further investigated by viscosity measurement. The viscosity measurements of DNA is regarded as the least uncertain and the critical test of a DNA binding model in solution in the absence of crystallographic data and provides strong evidence for intercalative DNA binding mode.[31,49] A classical intercalation model results in lengthening the DNA helix as base pairs are detached to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial non-classical intercalation of ligand could bend (or kink) the DNA helix and reduce its effective length.[50] For example, under suitable conditions, intercalation of dye like EtBr roots a significant increase in the overall DNA length. The effects of the complexes on the viscosity of rod-like DNA comparing with EtBr are shown in Figure 5. Though the intercalating ligand is same in all complexes, there is a small difference in the viscosity, this is due to the difference in the ancillary ligands. These further suggest that four Ru(II) complexes show an intercalative binding mode to CT-DNA, which parallel the absorption titration results. The increased degree of viscosity also supports the order of binding of the complexes to DNA as determined by other methods which follow the order EB $>4>1>2>3$ (Figure 5).

3.4. Photocleavage of pBR322 DNA

The cleavage reactions on plasmid DNA induced by ruthenium(II) complexes were performed and monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, comparatively fast migration is observed for the intact supercoiled form (form I). If scission

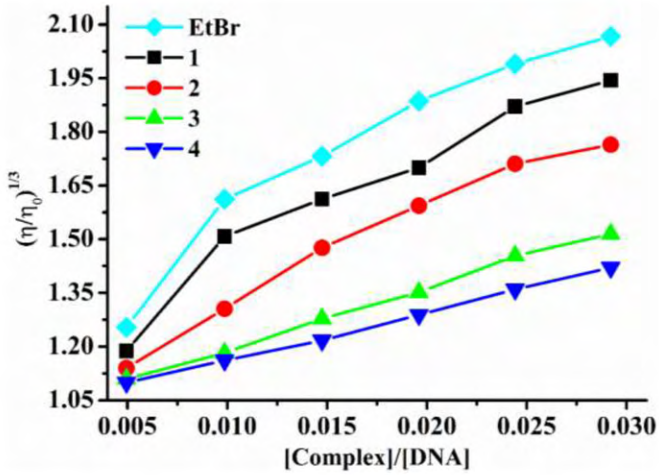


Figure 5. Viscosity studies of four complexes in BPE buffer with increasing amounts of complexes 1-4 and Ethidium bromide (EtBr) on the relative viscosity of calf thymus DNA at room temperature, a = EtBr, 1 = $[Ru(Hdpa)_2BOPIP]^{2+}$, 2 = $[Ru(Phen)_2BOPIP]^{2+}$, 3 = $[Ru(bpy)_2\ BOPIP]^{2+}$, 4 = $[Ru(dmb)_2\ BOPIP]^{2+}$.

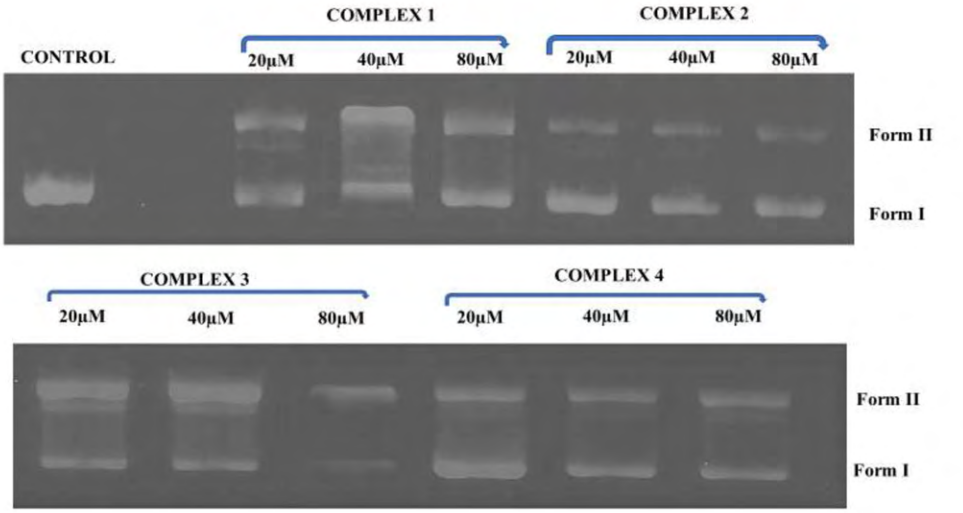


Figure 6. Photoactivated cleavage of pBR322 DNA in the absence (control) and presence of different concentrations (20, 40 and 80 μM) 0f ruthenium complexes (1–4) after irradiation under UV light for 30 minutes.

occurs on one strand (nicking), the supercoiled form will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) that migrates between form I and form II will be generated.[32] Figure 6 shows gel electrophoresis separation of pBR322DNA after incubation with different concentrations of ruthenium(II) complexes and irradiation at 365 nm for 60 min. No DNA cleavage was observed for the control, in which the metal complex was absent. When the concentration of the ruthenium(II) complexes was increased, the amount of form I gradually

decreased, whereas the amount of form II increased. Under comparable experimental conditions, all complexes showed photocleavage activity. The pBR322 DNA photocleavage results for these complexes are consistent with the results obtained for other ruthenium(II) polypyridyl complexes.[51,52] To establish the reactive species responsible for the photoactivated cleavage of the plasmid, we further investigated the influence of potentially inhibitive agents. Histidine, a naturally occurring amino acid, has been widely used as a scavenger of singlet molecular oxygen ($^1O_2$) especially during biological photooxidation processes.[53] As reported $^1O_2$ reacts with histidine to form a transannular peroxide in its imidazole ring and thus loses its ability to react with other species. Histidine is also one of the most reactive biomolecules with regard to $^1O_2$ and exists in the muscle of animal tissues. In the presence of histidine (10 m.mol) (Figure 9), cleavage was absent (form II is not observed) or very much reduced compared what was observed for the complexes with DNA (absence of histidine). This indicates that $^1O_2$ plays an important role in the photocleavage mechanism. A photocleavage experiment was also conducted in the presence of mannitol, an OH radical inhibitor (Figure 7). In the presence of mannitol, form II is formed; hence, there is no change in the cleavage pattern, which indicates that the OH, radical is not responsible for cleavage and only $^1O_2$ is responsible for photocleavage of pBR322 in presence of the ruthenium(II) complexes.

3.5. Antimicrobial activity

Complexes 1–4 were screened in vitro for their microbial activity against *E. coli* and *S. aureus* at 1 mg mL-1 concentration by the standard disk

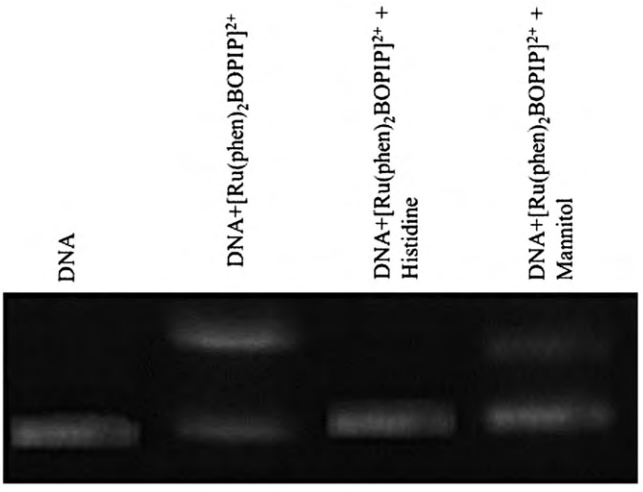


Figure 7. Photoactivated cleavage of pBR322 DNA in the presence of $[Ru(Phen)_2BOPIP]^{2+}$ complex after irradiation at 365 nm for 30 min in the presence of histidine and mannitol.

Table 2. Antibacterial activity of ruthenium(II) complexes.

	Escherichia coli (Gram negative)			*Staphylococcus aureus* (Gram positive)		
Compound	10μg	20μg	40μg	10μg	20μg	40μg
BOPIP	3			4		
$[Ru(Phen)_2BOPIP]^{+2}$ **(1)**	6.5	10.0	12	10	12	14.0
$[Ru(bpy)_2\ BOPIP]^{+2}$ **(2)**	6.0	8.0	10	9	11	12.2
$[Ru(dmb)_2\ BOPIP]^{+2}$ **(3)**	5.5	9.2	11.5	8	11.7	13.5
$[Ru(Hdpa)_2BOPIP]^{+2}$ **(4)**	5.0	8.7	10.4	7	11.2	12.5
Ampicillin			18.0			21

Inhibition zone diameter in millimetres.

method. The results are expressed as inhibition zone diameter (in millimetres) versus the control (DMSO). The DMSO control showed negligible activity as compared with the metal complexes. The antimicrobial activity increased as the concentration of the compounds increased. The antibacterial activity data for the complexes at various concentrations (Table 2) indicate that the complexes exhibited appreciable activity against *E. coli* and *S. aureus*. The activity increased with the increase in the concentrations of the complexes. The complexes were more effective against *E. coli* than against *S. aureus* but were less effective than the standard drug ampicillin. As an increase in the lipophilic character of the complex favors its permeation through the lipid layer of the bacterial membrane, it shows more activity. These results are consistent with results from earlier studies.[54,55]

3.6. Molecular docking studies

Molecular docking studies The LibDock module from Discovery Studio was used to perform the molecular docking of ruthenium complexes 1, 2, 3 and 4 with the active site pocket residues of human DNA TOP1. Human DNA TOP1 is an essential enzyme that relaxes DNA supercoiling during replication and transcription. The topoisomerase enzymes have been researched as targets for the generation of new cancer treatments because when they are inhibited in a cell, cell death results. Therefore, inhibitors of the topoisomerase enzymes have the ability to kill all cells undergoing DNA replication, reading of the DNA for protein production, or experiencing repair of DNA damage. Subsequently, cancer cells divide much more rapidly than normal cells, the cancer cells will be slaughtered by the topoisomerase inhibitors, however, some normal cells with topoisomerase activity will also be killed. DNA TOP1 is overexpressed in tumor cells and is an important target in cancer chemotherapy. All the ruthenium complexes were docked into the active site pocket of DNA TOP1, using LibDock. According to the results obtained from LibDock simulation, all ruthenium complexes were ranked by the LibDock scores. From the results, complex 4 exhibited the highest docking scores of 137.942 kcal/mol (Figure 8). The interactions and Dock scores of the

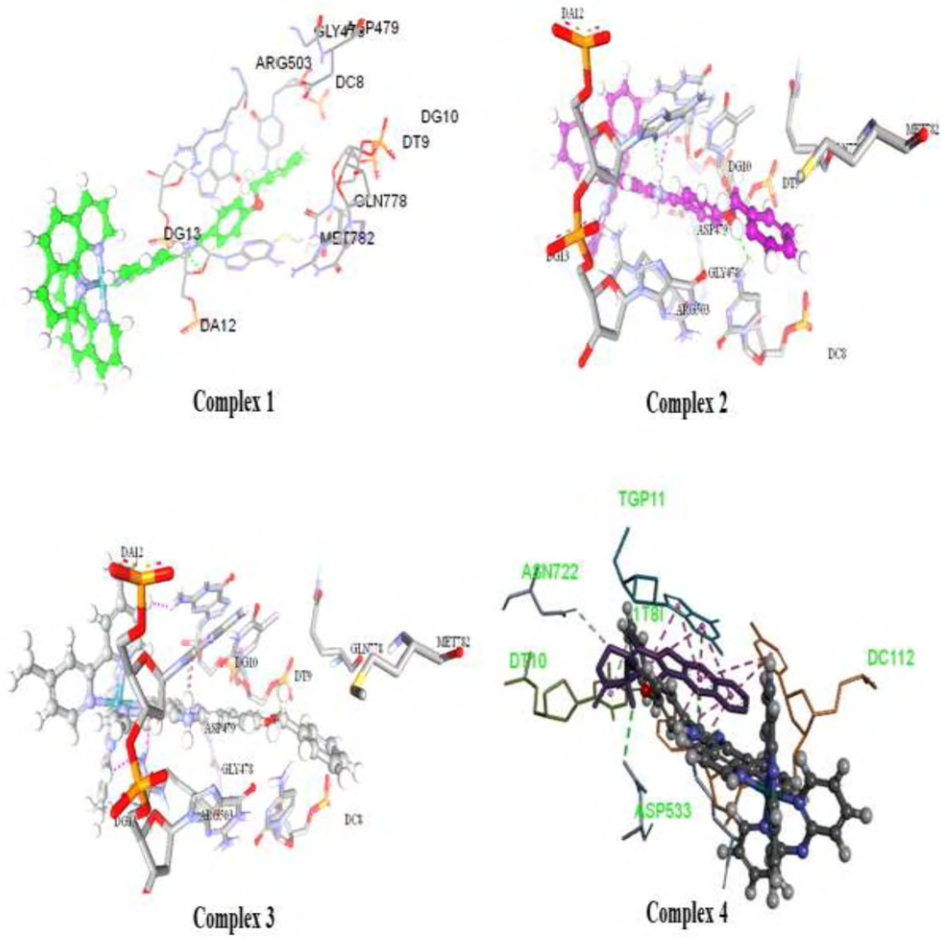


Figure 8. Molecular docking models illustrating the interaction between complexes with active site pocket residues of human DNA topoisomerase 1 (PDB ID: 1T8I) target and showing intermolecular hydrogen bonds.

Table 3. The LibDock scores and docking interactions of the ruthenium complexes (1–4) with human DNA TOP 1.

Complex	Libdock Score (K.Cal/Mole)	Interacting Residues	Interacting atoms	H-Distance
1	121.159	DC8,DT9,DG10, Gly478, Asp479, Met782, Arg503, Gln778	5:H67 - F:DA12:O4′ complex	2.2600
2	115.942	DC8, DT9, DG10, Gly478, Asp479, Met782, Arg503, Gln778	complex:H63 - F:DA12:N3 complex:H63 - F:DA12:C2 DC8:H42 - O49 complex	1.764 2.205 2.392
3	116.893	DC8, DT9, DG10, Gly478, Asp479, Met782, Arg503, Gln778	complex:H68 - F:DA12:N3 complex:H62 - F:DA12:C1′ complex:H62 - F:DA12:C2′ complex:C7 - A:ARG503:HH11	1.967 2.154 1.9070 2.0590
4	137.942	DT10, DC112, DA113, TGP11, Asn722, Asp533,	B:DT10:H3 – complex: O49	2.061

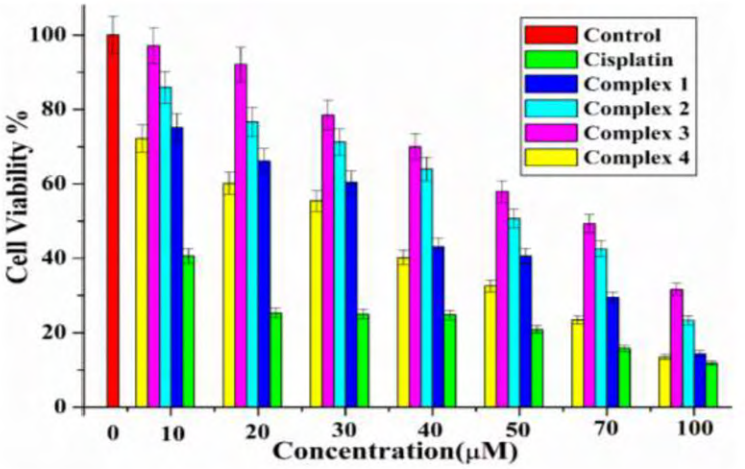


Figure 9. Cell viability of HeLa cell lines invitro treatment with complexes 1, 2, 3 and 4. Each data point is the mean standard error obtained from at least three independent experiment.

Table 4. The IC50 values for complexes 1–4 against HeLa cell lines.

S.No.	Compound	IC_{50} (μM)
1	$[Ru(Phen)_2BOPIP]^{+2}$ **(1)**	27.76
2	$[Ru(bpy)_2\ BOPIP]^{+2}$ **(2)**	31.59
3	$[Ru(dmb)_2\ BOPIP]^{+2}$ **(3)**	36.42
4	$[Ru(Hdpa)_2BOPIP]^{+2}$**(4)**	24.38
5	Cisplatin	4.81

ruthenium complexes with the active site pocket residues of human DNA TOP1 were tabulated in Table 3. The active site pocket residues of human DNA TOP1 were involved in hydrogen bonding formation with ruthenium complexes. A higher score indicates a stronger receptor–ligand-binding affinity.

3.7. In vitro cytotoxicity

The cytotoxicity activity of all four complexes and the corresponding ligand against the HeLa (human cervical cancer cell line) cell lines was evaluated by MTT assay. Cisplatin was used as a positive control and DMSO as negative control. The IC_{50} values obtained for four complexes are shown in Table 4. The tumor cells in the presence of complexes 1–4 were incubated for 48 h. The IC_{50} values for all the complexes ranged from 1 to 100 μM, suggesting that the ligand and the complexes exhibited antitumor activity against HeLa cell lines to different degrees. These compounds all exhibit relatively lower in vitro cytotoxicity against the selected HeLa cell line than cisplatin. Figure 9 showed that the cell viability decreased with increasing concentrations of complexes 1, 2, 3 and 4. Among all these, complex 4 exhibited higher in vitro cytotoxicity, with IC_{50} values of 24.38. This is may be due to the presence of an amine group (–NH–) between two pyridine moieties in Hdpa.[25]

The cytotoxicity activity of the complexes is consistent with their DNA binding abilities i.e. $4 > 1 > 2 > 3$. The obtained IC_{50} values are also comparable with the reported ruthenium (II) polypyridyl complexes.[56]

Conclusion

Four Ru(II) complexes $[Ru(phen)_2\ BOPIP]^{2+}$**(1)**, $[Ru(bpy)_2\ BOPIP]^{2+}$**(2)** $[Ru(dmb)_2\ BOPIP]^{2+}$**(3)**, $[Ru(Hdpa)_2\ BOPIP]^{2+}$**(4)** were synthesized and characterized. The absorption spectral studies, Luminescence titrations, and viscosity measurements suggest that all the four complexes bind to CT-DNA through intercalation. The intrinsic binding constants calculated through absorption studies and fluorescence spectral studies are good in agreement and complex 4 exhibits slightly higher intrinsic binding constant among four complexes. Upon irradiation, under UV light all the four complexes can cleave pBR322 DNA and proved that singlet oxygen($^1O_2$) is responsible for the cleavage of pBR322 DNA. All the four complexes exhibit the Antimicrobial activity and showed cytotoxicity against A549 (human lung tumor cell line), Du145 (human prostate cancer cell line), and HeLa (human cervical cancer cell line) cell lines. These complexes exhibit relatively lower in vitro cytotoxicity against the selected cell lines than cisplatin. Molecular docking studies support the Hydrogen bonding and Vander Wall's interactions play a major role in binding to DNA.

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References

[1] Wu, J.; Wu, S.; Shi, L.; Zhang, S.; Ren, J.; Yao, S.; Yun, D.; Huang, L.; Wang, J.; Li, W.; et al. Design, Synthesis, and Evaluation of Asymmetric EF24 Analogues as Potential Anti-cancer Agents for Lung Cancer. *Eur. J. Med. Chem.* **2017**, *125*, 1321–1331. DOI: 10.1016/j.ejmech.2016.10.027.

[2] Komor, A. C.; Barton, J. K. The Path for Metal Complexes to a DNA Target. *Chem. Commun. (Camb.)* **2013**, *49*, 3617–3630.

[3] Lin, R.; Johnson, S. G.; Connolly, P. J.; Wetter, S. K.; Binnun, E.; Hughes, T. V.; Murray, W. V.; Pandey, N. B.; Moreno-Mazza, S. J.; Adams, M.; et al. Synthesis and Evaluation of 2,7-diamino-thiazolo[4,5-*d*] Pyrimidine Analogues as Anti-tumor Epidermal Growth Factor Receptor (EGFR) tyrosine Kinase Inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2333–2337. DOI: 10.1016/j.bmcl.2009.02.067.

[4] Zheng, Y.; Zhu, L.; Fan, L.; Zhao, W.; Wang, J.; Hao, X.; Zhu, Y.; Hu, X.; Yuan, Y.; Shao, J.; Wang, W. Synthesis, SAR and Pharmacological Characterization of Novel

Anthraquinone Cation Compounds as Potential Anticancer Agents. *Eur. J. Med. Chem.* **2017**, *125*, 902–913. DOI: 10.1016/j.ejmech.2016.10.012.

[5] Syam, S.; Abdelwahab, S. I.; Al-Mamary, M. A.; Mohan, S. Synthesis of Chalcones with Anticancer Activities. *Molecules* **2012**, *17*, 6179–6195.

[6] Hartley, J. A.; Hochhauser, D. Small Molecule Drugs - Optimizing DNA Damaging Agent-Based Therapeutics. *Curr. Opin. Pharmacol.* **2012**, *12*, 398–402.

[7] Lippert, B. *Cisplatin Chemistry and Biochemistry of a Leading Anticancer Drug Wiley*; Weinheim: Germany, **1999**.

[8] Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. Antitumour Metal Compounds: More than Theme and Variations. *Dalton Trans* **2008**, *2*, 183–194. DOI: 10.1039/B712656P.

[9] Dyson, P. J.; Sava, G. Metal-Based Antitumour Drugs in the Post Genomic Era. *Dalton Trans.* **2006**, *16*, 1929–1933. DOI: 10.1039/b601840h.

[10] Mendoza-Ferri, M. G.; Hartinger, C. G.; Mendoza, M. A.; Groessl, M.; Egger, A. E.; Eichinger, R. E.; Mangrum, J. B.; Farrell, N. P.; Maruszak, M.; Bednarski, P. J.; et al. Transfering the Concept of Multinuclearity to Ruthenium Complexes for Improvement of Anticancer Activity. *J. Med. Chem.* **2009**, *52*, 916–925. DOI: 10.1021/jm8013234.

[11] Hartinger, C. G.; Metzler-Nolte, N.; Dyson, P. J. Challenges and Opportunities in the Development of Organometallic Anticancer Drugs. *Organometallics* **2012**, *31*, 5677–5685. DOI: 10.1021/om300373t.

[12] Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic Anticancer Compounds. *J. Med. Chem.* **2011**, *54*, 3–25.

[13] Gill, M. R.; Garcia-Lara, J.; Foster, S. J.; Smythe, C.; Battaglia, G.; Thomas, J. A. A Ruthenium(II) polypyridyl Complex for Direct Imaging of DNA Structure in Living Cells. *Nat. Chem.* **2009**, *1*, 662–667.

[14] Vyas, N. A.; Ramteke, S. N.; Kumbhar, A. S.; Kulkarni, P. P.; Jani, V.; Sonawane, U. B.; Joshi, R. R.; Joshi, B.; Erxleben, A. Ruthenium(II) polypyridyl Complexes with Hydrophobic Ancillary Ligand as Aβ Aggregation Inhibitors. *Eur. J. Med. Chem.* **2016**, *121*, 793–802. DOI: 10.1016/j.ejmech.2016.06.038.

[15] Puckett, C. A.; Ernst, R. J.; Barton, J. K. Exploring the Cellular Accumulation of Metal Complexes. *Dalton Trans.* **2010**, *39*, 1159–1170.

[16] Cook, N. P.; Torres, V.; Jain, D.; Martí, A. A. Sensing Amyloid-β Aggregation Using Luminescent Dipyridophenazine Ruthenium(II) Complexes. *J. Am. Chem. Soc.* **2011**, *133*, 11121–11123. DOI: 10.1021/ja204656r.

[17] Chen, Y.; Qin, M. Y.; Wu, J. H.; Wang, L.; Chao, H.; Ji, L. N.; Xu, A. L. Synthesis, Characterization, and Anticancer Activity of Ruthenium(II)-β-Carboline complex*Eur. Eur. J. Med. Chem.* **2013**, *70*, 120–129.

[18] Liu, P.; Jia, J.; Zhao, Y.; Wang, K. Z. Recent Advances on Dark and Light-Activated Cytotoxity of Imidazole-Containing Ruthenium Complexes. *Mini Rev. Med. Chem.* **2016**, *16*, 272–289.

[19] Poynton, F. E.; Bright, S. A.; Blasco, S.; Williams, D. C.; Kelly, J. M.; Gunnlaugsson, T. The Development of Ruthenium(ii) Polypyridyl Complexes and Conjugates for in vitro Cellular and in vivo Applications. *Chem. Soc. Rev.* **2017**, *46*, 7706.

[20] Vuradi, R. K.; Dandu, K.; Kumar, Y. P.; Vinoda Rani, M.; Rajender Reddy, M.; Chintakuntla, N.; Ravi, C.; Thakur, S. S.; Rao, C. M.; Satyanarayana, S. Studies on the DNA Binding and Anticancer Activity of Ru(II) polypyridyl Complexes by Using a (2-(4-(Diethoxymethyl)-^{1}H-imidazo[4,5-*f*][1,10] phenanthroline)) Intercalative Ligand. *New J. Chem.* **2018**, *42*, 846. DOI: 10.1039/C7NJ03819D.

[21] Muhammad, N.; Guo, Z. Metal-based Anticancer Chemotherapeutic Agents. *Curr. Opin. Chem. Biol.* **2014**, *19*, 144–153. DOI: 10.1016/j.cbpa.2014.02.003.

[22] Sava, G.; Bergamo, A.; Bonetti, A.; Leone, R.; Muggia, F.M.; Howell, S.B. (Eds.). *Synthesis and in Vitro (Anticancer) Evaluation of* η *6 -Arene Ruthenium Complexes Bearing Stannyl Ligands*; Humana Press: Totowa, **2009**,57–66.

[23] Yamada, M.; Nakamura, Y.; Hasegawa, T.; Itoh, A.; Kuroda, S.; Shimao, I. Synthesis and Properties of Diamino-Substituted Dipyrido [3,2-*a*: 2′,3′-*c*]Phenazine. *Bull. Chem. Soc. Japan* **1992**, *65*, 2007–2009.

[24] Sullivan, B. P.; Sullivan, D. J.; Salmon, B. P.; Mayer, T. J. Mixed Phosphine 2,2'-Bipyridine Complexes of Ruthenium. *Inorg. Chem.* **1978**, *17*, 3334–3341. DOI: 10.1021/ic50190a006.

[25] Rajendran, V.; Murali, M.; Suresh, E.; Sinha, S.; Somasundaram, K.; Palaniandavar, M. Mixed Ligand Ruthenium (II) Complexes of bis(pyrid-2-yl)-/bis(benzimidazol-2-yl)-Dithioether and Diimines: Study of Non-covalent DNA Binding and Cytotoxicity. *Dalton Trans.* **2008**, *7*(1),148–163. DOI: 10.1039/B710578A.

[26] Marmur, J. A Procedure for the Isolation of Deoxyribonucleic Acid from Micro-Organisms. *J. Mol. Bio.* **1961**, *3*, 208–218. DOI: 10.1016/S0022-2836(61)80047-8.

[27] Reichmann, M. E.; Rice, S. A.; Thomas, C. A.; Doty, P. A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid. *J. Am. Chem. Soc.* **1954**, *76*, 3047–3053. DOI: 10.1021/ja01640a067.

[28] Steck, E. A.; Day, A. R. Reactions of Phenanthraquinone and Retenequinone with Aldehydes and Ammonium Acetate in Acetic Acid Solution. *J. Am. Chem. Soc.* **1943**, *65*, 452–456.

[29] Wolfe, A.; Shimer, G. H.; Meehan, T. Polycyclic Aromatic Hydrocarbons Physically Intercalate into Duplex Regions of Denatured DNA. *Biochemistry* **1987**, *26*, 6392–6396. DOI: 10.1021/bi00394a013.

[30] Mc Ghee, J. D.; Von Hippel, P. H. Theoretical Aspects of DNA-protein Interactions: co-operative and Non-co-operative Binding of Large Ligands to a One-dimensional Homogeneous Lattice. *J. Mol. Biol.* **1974**, *86*, 469–489. DOI: 10.1016/0022-2836(74)90031-X.

[31] Chaires, J. B.; Dattagupta, N.; Crothers, D. M. Selfassociation of Daunomycin. *Biochemistry* **1982**, *21*, 3927–3932. DOI: 10.1021/bi00260a004.

[32] Barton, J. K.; Raphael, A. L. Photoactivated Stereospecific Cleavage of Double-helical DNA by Cobalt(III) Complexes. *J. Am. Chem. Soc.* **1984**, *106*, 2466–2468. DOI: 10.1021/ja00320a058.

[33] Drew, W. L.; Barry, A. L.; Toole, R. O.; Sherris, J. C. Reliability of the Kirby-Bauer Disc Diffusion Method for Detecting Methicillin-Resistant Strains of *Staphylococcus aureus*. *Appl. Microbiol.* **1972**, *24*, 240–247.

[34] Diller, D. J.; Merz , K. M. Jr. High Throughput Docking for Library Design and Library Prioritization. *Proteins* **2001**, *43*, 113–124.

[35] Jones, G.; Willet, P.; Glen, R. C. Molecular Recognition of Receptor Sites Using a Genetic Algorithm with a Description of Desolvation. *J. Mol. Biol.* **1995**, *245*, 43–53. DOI: 10.1016/S0022-2836(95)80037-9.

[36] Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 45–55. DOI: 10.1016/0022-1759(83)90303-4.

[37] Barton, J. K.; Danishefsky, A.; Goldberg, J. Tris(phenanthroline)ruthenium(II): Stereoselectivity in Binding to DNA. *J. Am. Chem. Soc.* **1984**, *106*, 2172–2176. DOI: 10.1021/ja00319a043.

[38] Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. Mixed-ligand Complexes of Ruthenium(II): Factors Governing Binding to DNA. *J. Am. Chem. Soc.* **1989**, *111*, 3051–3058. DOI: 10.1021/ja00190a046.

[39] Moucheron, C.; Mesmaeker, A. K. D.; Choua, S. Photophysics of $Ru(phen)_2(PHEHAT)^{2+}$: A Novel "Light Switch" for DNA and Photo-oxidant for Mononucleotides. *Inorg. Chem.* **1997**, *36*, 584–592. DOI: 10.1021/ic9609315.

[40] Deepika, N.; Praveen Kumar, Y.; Shobha Devi, Ch.; Venkat Reddy, P.; Srishailam, A.; Satyanarayana, S. Synthesis, characterization, and DNA binding, photocleavage, cytotoxicity, cellular uptake, apoptosis, and on-off light switching studies of Ru(II) mixed-ligand complexes containing 7-fluorodipyrido[3,2-a:2′,3′c]phenazine. *J Biol Inorg Chem*, **2013**, 18, 751–766.

[41] Tan, L.-F.; Chao, H.; Li, H.; Liu, Y.-J.; Sun, B.; Wei, W.; Ji, L.-N. Synthesis, Characterization, DNA-Binding and Photocleavage Studies of $[Ru(bpy)2(PPIP)]^{2+}$ and $[Ru(phen)2(PPIP)]^{2+}$. *J. Inorg. Biochem.* **2005**, *99*, 513–528. DOI: 10.1016/j.jinorgbio.2004.10.028.

[42] Ravi Kumar, V.; Nagababu, P.; Srinivas, G.; Reddy, M. R.; Rani, M. V.; Ravi, M.; Satyanarayana, S. Investigation of DNA/BSA Binding of Three Ru(II) complexes by Various Spectroscopic Methods, molecular Docking and Their Antimicrobial Activity. *J. Coord. Chem.* **2017**, *70*, 3790–3809. DOI: 10.1080/00958972.2017.1407410.

[43] Tu, L. C.; Chen, C. S.; Hsiao, I. C.; Chern, J. W.; Lin, C. H.; Shen, Y. C.; Yeh, S. F. Synthesis of 1-Substituted Carbazolyl-1,2,3,4-tetrahydro- and Carbazolyl-3,4-dihydro-β-carboline Analogs as Potential Antitumor Agents. *Chem. Biol.* **2005**, *12*, 1317–1324.

[44] Ang, W. H.; Dyson, P. J. Classical and Non-Classical Ruthenium-Based Anticancer Drugs: Towards Targeted Chemotherapy. *Eur. J. Inorg. Chem.* **2006**, 4003–4018. DOI: 10.1002/ejic.200600723.

[45] Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. Tris(phenanthroline)ruthenium(II) enantiomer Interactions with DNA: mode and Specificity of Binding. *Biochemistry* **1993**, *32*, 2573–2584. DOI: 10.1021/bi00061a015.

[46] Lakowicz, J. R.; Weber, G. Quenching of Fluorescence by Oxygen. Probe for Structural Fluctuations in Macromolecules. *Biochemistry* **1973**, *12*, 4161–4170. DOI: 10.1021/bi00745a020.

[47] Shobhadevi, C.; Satyanarayana, S. Review: Synthesis, characterization, and DNA-binding Properties of Ru(II) molecular "light Switch" Complexes. *J. Coord. Chem.* **2012**, *65*, 474–486. DOI: 10.1080/00958972.2011.649736.

[48] Liu, X. W.; Shen, Y. M.; Lu, J. L.; Chen, Y. D.; Li, L.; Zhang, D. S. Synthesis, DNA-binding and Photocleavage of "light Switch" complexes $[Ru(bpy)_2(pyip)]^{2+}$and $[Ru(phen)_2(pyip)]^{2+}$. *Spectrochim. Acta. Part A*. **2010**, *77*, 522–527.

[49] Chen, M.; Li, H.; Li, Q.; Xu, Z. Luminescence Properties of [Ru(bpy)(2) MDHIP](2+) modulated by the introduction of DNA, copper(II) ion and EDTA. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2010**, *75*, 1566–1570.

[50] Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. Neither DELTA.- nor.LAMBDA.-Tris(phenanthroline)Ruthenium(II) Binds to DNA by Classical Intercalation. *Biochemistry* **1992**, *31*, 9319–9324. DOI: 10.1021/bi00154a001.

[51] Satyanarayana, S.; Suh, D.; Fokt, I.; Przewloka, T.; Priebe, W.; Chaires, J. B. Parsing the Free Energy of Anthracycline Antibiotic Binding to DNA. *Biochemistry* **1996**, *35*, 2047–2053.

[52] Liu, Y. J.; Zeng, C. H.; Huang, H. L.; He, L. X.; Wu, F. H. Synthesis, DNA-binding, photocleavage, cytotoxicity and Antioxidant Activity of Ruthenium (II) polypyridyl Complexes. *Eur. J. Med. Chem.* **2010**, *45*, 564–571.

[53] Huang, H. L.; Li, Z. Z.; Liang, Z. H.; Liu, Y. J. Cell Cycle Arrest, Cytotoxicity, Apoptosis, DNA-Binding, Photocleavage, and Antioxidant Activity of Octahedral Ruthenium(II) Complexes. *Eur. J. Inorg. Chem*. **2011**, *2011*, 5538–5547. DOI: 10.1002/ejic.201100848.

[54] Tan, L. F.; Liang, X. L.; Liu, X. H. Synthesis, double Stranded DNA-binding and Photocleavage Studies of a Functionalized Ruthenium(II) complex with 7,7′-methylenedioxyphenyldipyrido[3,2-a:2′,3′-c]-Phenazine. *J. Inorg. Biochem*. **2009**, *103*, 441–447.

[55] Islam, M. S.; Hossain, M. B.; Reza, M. Y. Synthesis, Characterization and Antibacterial Activity of Mixed Ligand Complexes of Pd(II) Ions with Oxalic Acid and Heterocyclic Amines. *J. Med. Sci*. **2003**, *3*, 289–293.

[56] Morad, F. M.; Ajaily, M. M. E. L.; Gweirif, S. B. Preparation, Physical Characterization and Antibacterial Activity of Ni (II) Schiff Base Complex. *J. Sci. Appl*. **2007**, *1*, 72–78.

[57] Venkat Reddy, P.; Nagamani, C.; Reddy M, R.; Srishailam, A.; Nagasuryaprasad, K.; Deepika, N.; Yashvanth, V. V. N.; Prakasham, R. S.; Satyanrayana, S. S.; Satyanarayana, S. Synthesis and Evaluation of Invitro DNA/Protein Binding Affinity, Antimicrobial, Antioxidant and Antitumor Activity of Mononuclear Ru (II) Moxed Polypyridyl Complexes. *J. Fluoresc*. **2016**, *26*, 225–240. DOI: 10.1007/s10895-015-1705-z.

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Comparative Studies on DNA Binding, Photocleavage, and Photophysical Properties of Ru(II) Complexes Containing TIP {TIP = 2-(Thiophen-2-yl)-1*H*-imidazo[4,5-*f*][1,10]-phenanthroline} Ligand[1]

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Abstract—A thiophene contained imidazo phenanthroline ligand TIP {TIP = 2-(thiophen-2-yl)-1*H*-imidazo-[4,5-*f*][1,10]phenanthroline} and its mononuclear Ru(II) polypyridyl complexes $[Ru(phen)_2TIP]^{2+}$ (**1**) (phen = 1,10-phenanthrolene) and $[Ru(bpy)_2TIP]^{2+}$ (**2**) (bpy = 2,2'-bipyridyl), are synthesized and characterized by UV-Vis, IR, ^1H and ^{13}C NMR, and ESI-MS spectra. Interaction of these complexes with CT-DNA is studied using UV-Vis and florescence spectra, viscosity measurements and molecular docking studies. The latter supports binding ability of complexes with stability constants deduced from UV-Vis and florescence spectra. The studies reveal that both Ru(II) polypyridyl complexes bind to DNA predominantly by intercalation, and the binding constant of complex **1** is greater than that of complex **2**. Complexes **1** and **2** cleave the pBR 322 DNA under UV light.

Keywords: DNA binding, viscosity, binding constant, pBR322, antimicrobial activity

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INTRODUCTION

Cisplatin or *cis*-diamminedichloro platinum(II) is the most commonly known metal-based anticancer drug most effective against lung, head, ovarian, neck, and esophageal cancers [1]. Although cisplatin and its derivatives are efficient against the vast majority of cancers, they also induce non-cancer cells toxicity [2, 3]. In the design of new anticancer drugs [4, 5], ruthenium complexes have been tested against a number of cancer cell lines [6], and are regarded as promising candidates for alternative drugs to cisplatin and its derivatives.

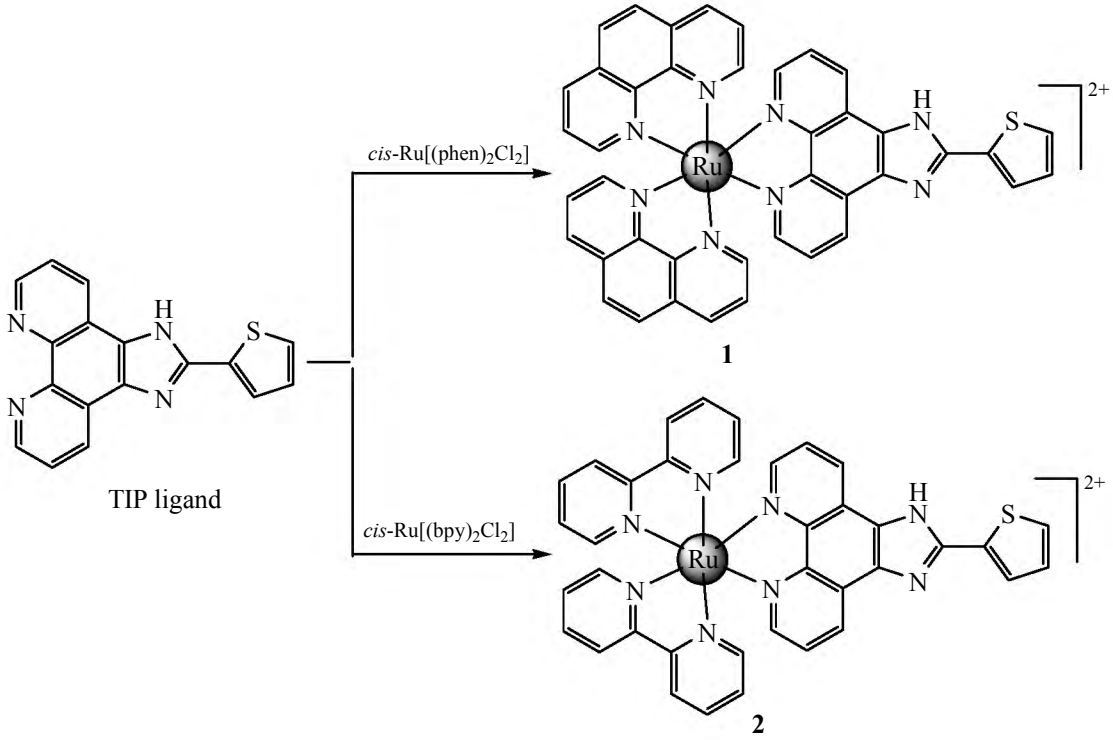
The ligand exchange kinetics of metal complexes in aqueous solutions influences upon their anticancer activity. It is quite similar for platinum and ruthenium complexes [7]. Relatively low toxicity and ability to mimic iron in binding to biomolecules made ruthenium complexes an attractive alternative to platinum-based drugs [8]. Luminescence intensity and cytotoxicity of Ru complexes are strongly ligand-dependent. This fact initiates research in fine-tuning of these properties by changing the commonly used polypyridyl ligands [9]. In this context, we synthesized two different ruthenium complexes with a common auxiliary ligand {TIP = 2-(thiophen-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline} but different chelating ligands (phen = 1,10-phenanthroline, bpy = 2,2'-bipyridine) and studied inter-actions of the complexes with CT-DNA.

EXPERIMENTAL

$RuCl_3 \cdot 3H_2O$, 2-thiophene carboxaldehyde, 1,10-phenanthroline, and 2,2'-bipyridine were purchased

[1] The text was submitted by the authors in English.

Scheme 1. Preparation of complexes **1** and **2** {[Ru(phen)$_2$TIP]$^{+2}$ (**1**) and [Ru(bpy)$_2$TIP]$^{+2}$(**2**)}.



from Sigma Aldrich, the other chemicals and solvents were obtained from local existing sources. All solvents were purified by standard procedures. The spectroscopic titration was carried out in tris-buffer (5 mM tris-HCl, 50 mM NaCl, pH = 7.2) at room temperature. Solutions of DNA in tris-buffer gave a ratio of absorbance at 260 and 280 nm of 1.8–1.9, indicating that the DNA was sufficiently free of protein [10]. Concentration of CT-DNA was determined spectrophotoetrically using the molar absorption 6600 M^{-1} cm^{-1} at 260 nm [11].

Luminescence spectral data were accumulated on a Cary Eclipse spectrofluorometer. UV-Vis spectra were recorded on an Elico-Spectrophotometer BL 198. NMR spectra were measured on an Avance-III 400 MHz spectrometer using DMSO-d_6 as a solvent. IR spectra were recorded on a Perkin-Elmer FT-IR-1605 spectrophotometer. An Ostwald Viscometer was used for viscosity measurements. Micro analysis was carried out on a Perkin-Elmer 240 elemental analyser.

1,10-Phenanthroline-5,6-dione (phendione) [12], *cis*-[Ru(phen)$_2$Cl$_2$], *cis*-[Ru(bpy)$_2$Cl$_2$] were synthesized according to the developed earlier methods [13]. Synthetic approach to Ru(II) polypyridyl complexes is presented in Scheme 1.

The TIP ligand was synthesized according to the developed earlier methods [14]. IR spectrum, ν, cm^{-1}: 3066.8 (N–H), 1076.2 (C–N), 709.8 (C–S). ^{1}H NMR spectrum, δ, ppm: 7.98 d (2H), 7.87 d (2H), 7.36 t (2H), 7.24 m (3H). ^{13}C NMR spectrum, δ, ppm: 153.60, 143.4, 132.6, 127.5, 123.4, 119.6.

Synthesis of complexes [Ru(phen)$_2$(TIP)](ClO$_4$)$_2$·2H$_2$O (1). A mixture of *cis*-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.284 g, 0.5 mM) with TIP (0.151 g, 0.5 mM) and ethanol (15 mL) was refluxed for 8 h at 120°C under the atmosphere of nitrogen. When the mixture gained light purple colour, it was cooled down to room temperature and a small amount of saturated aqueous $NaClO_4$ solution was added under vigorous stirring. The yellowish red solid was collected and washed with small amount of water, ethanol and diethyl ether, then dried under vacuum. Yield 64.6%. IR spectrum, ν, cm^{-1}: 3417.8 (N–H), 1148.2 (C–N), 719.45 (C–S), 628.87 (Ru–N). ^{1}H NMR spectrum, δ, ppm: 8.83 d (6H), 8.13 d (6H), 7.89 t (6H), 7.51 d (4H), 6.8 m (3H). ^{13}C NMR spectrum, δ, ppm: 137.8, 128.4, 126.5, 124.7, 121.8, 119.3. Found, %: C 64.69;H 3.48; N 14.79. $RuC_{41}H_{26}N_8S$. Calculated, %: C 64.47; H 3.43; N 14.67.

[Ru(bpy)$_2$(TIP)](ClO$_4$)$_2$·2H$_2$O (2). The complex was synthesized according to the method described

above for the complex **1**. IR spectrum, ν, cm^{-1}: 3447.8 (N–H), 1108.2 (C–N), 714.45 (C–S), 627.43 (Ru–N). ^{1}H NMR spectrum, δ, ppm: 8.88 d (6H), 8.34 d (4H), 8.2 t (4H), 7.6 t (4H), 7.2 t (4H), 7.03 m (3H). ^{13}C NMR spectrum, δ, ppm: 157.4, 150.3, 138.4, 136.4, 127.7, 125.6, 121.8. Found, %: C 62.11;H 3.69; N 15.69. $RuC_{37}H_{26}N_8S$. Calculated, %: C 62.08; H 3.66; N 15.65.

DNA binding. DNA binding was studied at room temperature using tris buffer (5 mM Tris-HCl, 50 mM NaCl, pH = 7.1). Absorption studies of these complexes were recorded by keeping the complex concentration constant and varying DNA concentration. Before recording each spectrum the complex-DNA solution was stored for 5 min. For each reading, we observed the absorbance change at MLCT with increasing concentration of DNA, and calculated the intrinsic binding constant (K_b) [15].

In the emission titrations the complex concentration was kept constant and upon addition of incremental DNA, spectra were recorded in the range of 400–700 nm. The binding constant (K_b) was calculated using the equation:

$$C_b = C_t[(F - F_0)/(F_{max} - F_0)],$$

where C_t is the total complex concentration, F is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is the maximum complex bound to DNA. From the scatchard equation [16], a graph r/C_f vs. r was drawn and binding constant was calculated (r is the C_b/[DNA] and C_f is the concentration of free complex.) The emission quenching experiment was carried out using $[Fe(CN)_6]^{4-}$ as a quencher in the presence or absence of DNA. The emission study (light switch on/off effect) was carried out with the equal concentrations of Co^{2+} and Na_2EDTA solutions with constant concentration of a complex.

Viscosity. Viscosity studies were carried out using an Ostwald viscometer at constant temperature (30±0.1°C) by immersing it in a thermo stated water bath using BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH = 7.0). The CT-DNA samples, ca 200 base pairs of average length, were prepared by sonication to minimize the complexes arising from DNA flexibility [17]. The measurements were carried out in triplicates for all samples. The data were presented as a graph $(\eta/\eta_0)^{1/3}$ vs concentration of [Ru(II)]/[DNA], where η is viscosity of DNA in the presence of the complex, and η_0 is viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for the flow time of the buffer alone (t_0) [18].

Photocleavage.[2] For gel electrophoresis experiments pH 8.0 buffer composed of 40 mM Tris base, 20 mM acetic acid and 1 mM EDTA was used. A buffer of 10 mM Tris–HCl and 1 mM Na_2EDTA was used for dilution of pBR322 DNA. Supercoiled pBR322 DNA (0.1 μg/μL) was treated with Ru(II) complexes **1, 2** with concentrations of 20, 40, 80 μL. The mixtures were irradiated at room temperature with UV light (365 nm, 10 W) for 60 min. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanole and 30% glycerol (2 μL) was added. The samples were then analysed by 0.8% agarose gel electrophoresis at 50 V for 2 h. The gel was stained with 2 μL (of 1 mg/100 μL) ethidium bromide and photographed under UV light. The gels were viewed with a gel documentation system and photographed using a CCD camera (Alpha Innotech).

Molecular docking studies. Accelry's Discovery Studio (version 2.1) was used to design lead molecules, estimate docking interactions of a complex of drug and protein binding, and number of bonds formed by ligand with the target. The molecular docking of complexes **1, 2** was performed using LibDock [19]. Accelry's CHARMm force field was used throughout the simulation before running LibDock. The crystal structure of human DNA topoisomerase **1** (TOP1) receptor was downloaded from RCSB PDB (PDB ID-1T8I), after downloading the PDB format of the protein, all water molecules of the protein were removed by using Discovery Studio and stabilizing the charges, filling the missing residues, and generating the side chains according to the parameters available. The receptor should be in a biologically active and stable state. After the receptor is constructed, the active site within the receptor should be recognized. The receptor may have many active sites but the one of interest should be selected. Ruthenium complexes were sketched using the tools Chemsketch and used to dock into the target binding site. Ruthenium complexes conformations aligned to receptor interaction sites and the best poses were reported at the end of docking simulations. The scoring functions have been used to estimate binding affinity

[2] CAUTION! Ethidium bromide is a mutagen and potential carcinogen. Gloves should be worn and care should be taken when handling. UV light is damaging to eyes and exposed skin. Protective eyewear and apron should be worn at all times.

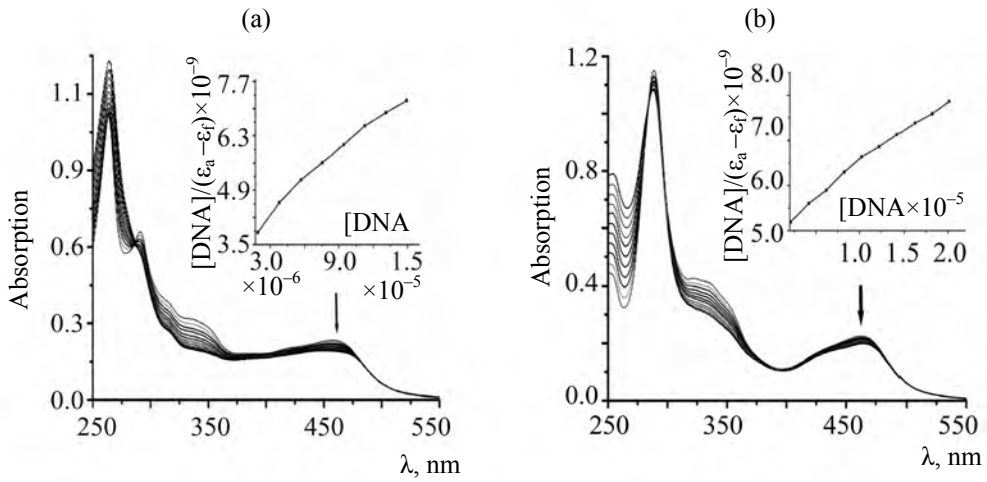


Fig. 1. Absorption spectra of complexes (a) **1** and (b) **2** in absence and presence of CT-DNA in Tris HCl buffer. Arrows show hypochromism and bathochromism upon increase of DNA concentration. Inserted plots, $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs. [DNA] for the titration of DNA with complexes.

to screen out active and inactive compounds during the process of virtual screening [20].

RESULTS AND DISCUSSION

In IR spectra the ligand band of (N–H) (3066.8 cm^{-1}) shifted to 3417.8 cm^{-1} upon complex formation. Weak bands at 628 cm^{-1} assigned to Ru–N of the complexes indicated all six Ru–N bonds that were of the same bond length, and indicated the perfect octahedral structure of the complexes. These were not observed for TIP ligand. ^{1}H NMR signals of TIP ligand shifted from 7.24–7.98 to 7.51–8.83 ppm in the spectra of complexes, confirming the complexes formation.

In UV-Vis spectra the Ru(II) complexes **1** and **2** demonstrated MLCT a band at ca 420 or 445 nm respectively, which was not recorded in the spectrum of TIP ligand.

DNA binding. *UV-Vis spectra.* UV-Vis spectra of Ru(II) complexes were significantly affected by addition of DNA (Fig.1). The bands below 400 nm pertained to π–π* transitions attributed to DNA (below 300 nm) and ligands (300-400 nm), and above 400 nm belonged to Metal-to-Ligand Charge Transfer (MLCT). Intensity of UV-Vis absorption bands of complexes **1** and **2** at 460 nm lowered on addition of CT-DNA (Fig. 1). The intrinsic binding constants (K_b)

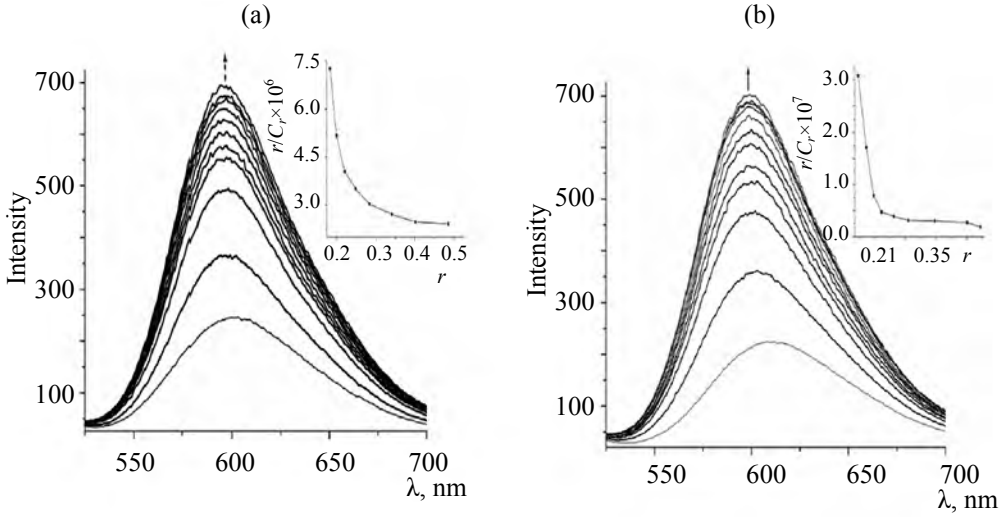


Fig. 2. Emission spectra of complexes (a) **1** and (b) **2** in Tris-HCl buffer upon addition of CT-DNA. The arrow shows the intensity change upon the increase of DNA concentration. Inset: Scatchard plot for the complexes.

Table 1. DNA binding and K_{sv} data for Ru(II) complexes **1** and **2**

Complex	Absorption λ_{max}, nm (MLCT)	Hypo chromism, %	Absorbance binding constant (K_b), M^{-1}	Emission binding constant	K_{sv} values		
					complex	complex + DNA	
						1 : 50	1 : 100
$[Ru(phen)_2TIP]^{2+}$ (**1**)	460	10.46	3.36×10^4	3.81×10^4	21423	15641	4836
$[Ru(bpy)_2TIP]^{2+}$ (**2**)	468	9.74	2.61×10^4	2.94×10^4	16541	11584	3021

Table 2. LibDock scores and docking interactions of complexes **1** and **2** with human DNA TOP 1

Complex	LibDock score, kcal/mol	Interacting residues	Interacting atoms	H-Distance
1	104.147	DC8,DT9, DG10, Gly478, Asp479, Met782, Arg503, Gln778	DG13:N···HN13:Complex	2.125
2	99.159	DC8, DT9, DG10, Gly478, Asp479, Met782, Arg503, Gln778	Complex H54–D:DT9:O4'	2.319

of the complexes calculated at 460 nm were found to be in the order of 10^4, but bigger than that of the parent complex $[Ru(phen)_3]^{2+}$ ($K_b = 5.5\times10^3$ M^{-1}), suggesting that there was a strong stacking interaction between TIP ligand of the complexes and the base pairs of the DNA. Variance in the binding ability of the complexes **1** ($K_b = 3.36\times10^4$ M^{-1}) and **2** ($K_b = 2.61\times10^4$ M^{-1}) could be explained in terms of planarity and hydrophobicity. The TIP ligand was common for both complexes, but switch from the ancillary ligand 1,10-phenanthroline (phen) to 2,2'-bipyridyl (bpy) decreased the planarity and hydrophobicity, which led to a higher binding affinity of complex **1**.

Luminescence. In the absence of DNA, the complexes **1, 2** emitted luminescence in Tris buffer. Upon addition of CT-DNA the emission intensities of complexes $[Ru(phen)_2TIP]^{2+}$ (**1**), $[Ru(bpy)_2TIP]^{2+}$ (**2**) increased (Fig. 2). The gradual increase in the luminescence of complexes was observed upon increasing concentration of DNA, which indicated that complexes could interact strongly with DNA and were protected by DNA efficiently due to hydrophobic environment inside the DNA helix, which reduced the accessibility of solvent water molecules to the duplex, and the complexes mobility was constrained at the binding site. This led to decrease in the vibrational

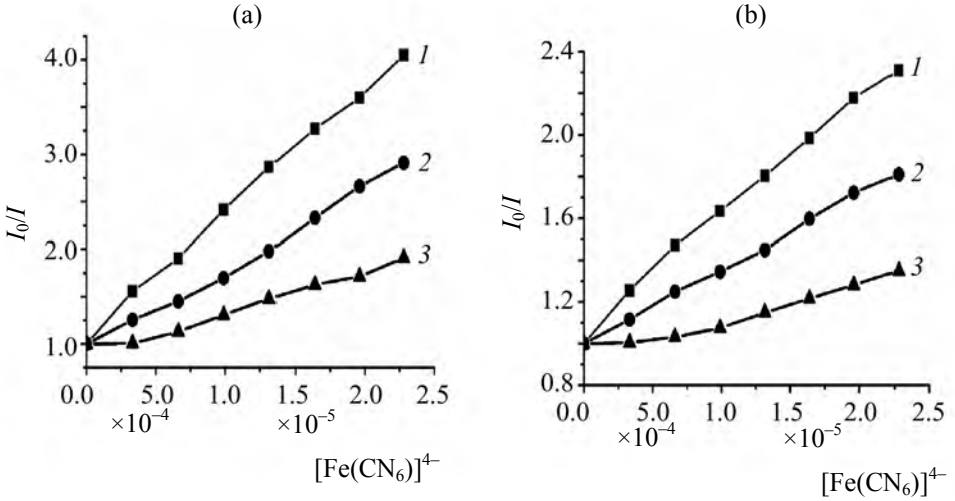


Fig. 3. Quenching of complexes (a) **1** and (b) **2** in Tris- HCl with $[Fe(CN)_6]^{4-}$ in the absence of DNA (*1*), presence of DNA 1 : 20 (*2*), and 1 : 100 (*3*).

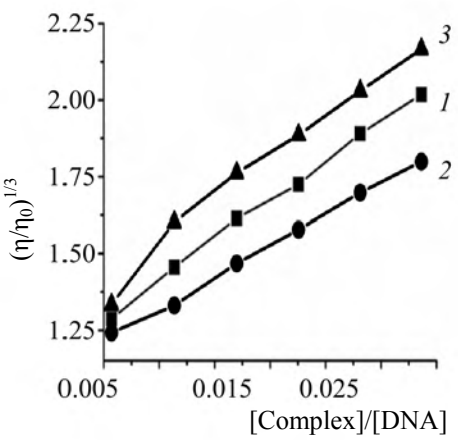


Fig. 4. Relative viscosity of DNA under the action of complexes (*1*) **1,** (*2*) **2**, and (*3*) EtBr.

modes of relaxation. Scatchard plots for complexes were constructed from luminescence spectra, and binding constants (K_b) were in the order of 10^4 (Table 1). The small divergences between the two sets of binding constants were due to different spectroscopy techniques and dissimilar calculations methods. However, the binding constants were comparable and followed the order **1** > **2**.

Quenching studies. In the emission quenching experiment, $[Fe(CN)_6]^{4-}$ was used as a quencher in the presence or absence of DNA (Fig. 3). In the absence of DNA, Ru(II) complexes were efficiently quenched, resulting in the linear Stern-Volmer plot. But in the presence of an excess of DNA quenching was low, due to highly negatively charged $[Fe(CN)_6]^{4-}$ species that could be repelled by the negative charge of DNA phosphate backbone which could impede the quenching of bound complexes. The Stern–Volmer quenching constant K_{sv} was deduced from the corresponding equation [9] (Table 1).

Viscosity. Difference in the relative viscosity of DNA on addition of complexes **1, 2** is presented in Fig. 4. As the concentration of complexes **1** and **2** increased the relative viscosity also increased, which suggested the intercalative binding mode of complexes to DNA according to the order of **1** > **2**. This was consistent with the analysis based on binding constants.

Photocleavage activity. Concentrations of Ru(II) complexes (20, 40, and 80 μM) were altered and monitored by agarose gel-electrophoresis. No DNA cleavage was observed for control in which complex was absent. The complexes **1** and **2** could effectively cleave pBR322 DNA upon irradiation by UV light for 30 min. The different cleaving efficiency was consistent with the DNA-binding affinity of the complexes.

Molecular docking studies. According to the data accumulated from LibDock simulation, the complexes were ranked by the LibDock Scores. Complex **1** exhibited the highest docking scores of 104.14 kcal/mol (Fig. 5). The interactions and Dock scores of the complexes with the active site pocket residues of human DNA TOP1 are tabulated in Table 2. The active site pocket residues of human DNA TOP1 were involved in hydrogen bonding formation with the complexes. A higher score indicated a stronger receptor–ligand-binding affinity.

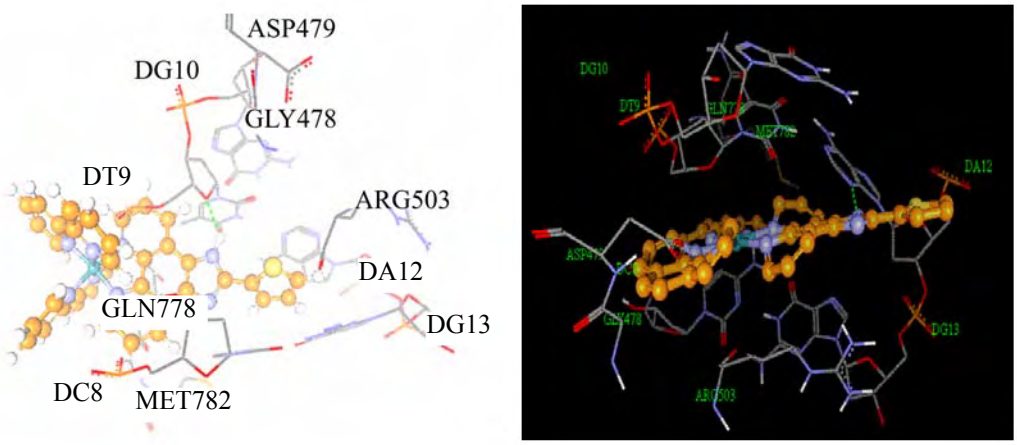


Fig. 5. Molecular docking models of the interaction between complex **1** and the active site pocket residues of human DNA topoisomerase **1** (PDB ID: 1T8I) target.

CONCLUSIONS

Two new Ru(II) polypyridyl complexes are synthesized and characterized by various spectral methods. Both complexes bind to DNA with marginally different binding constants. According to the study, the complexes can induce photo cleavage of DNA. According to viscosity measurements, the complexes can bind to DNA via intercalation. Complex **1** binds DNA more strongly and can be more efficient cytotoxic agent for cancer cells.

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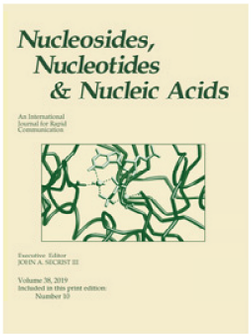
CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Cepeda, V., Fuertes, M.A., Castilla, J., Alonso, C., Quevedo, C., and Pérez, J.M., *Anti-Cancer Agents Med. Chem*., 2007, vol. 7, p. 3. doi 10.2174/187152007779314044
2. Zheng, Y.-R., Suntharalingam, K., Johnstone, T.C., Yoo, H., Lin, W., Brooks, J.G., and Lippard, S.J., *J. Am. Chem. Soc*., 2014, vol. 136, p. 8790. doi 10.1021/ja5038269
3. Fong T.T.-H., Lok, C.-N., Chung, C. Y.-S., Fung, Y.-M.E., Chow, P.-K., Wan, P.-K., and Che, C.-M., *Angew. Chem., Int. Ed*., 2016, vol. 55, p. 11935. doi 10.1002/anie.201602814
4. Abid, M., Shamsi, F., and Azam, A., *Mini-Rev. Med. Chem*., 2016, vol. 16, p. 772. doi 10.2174/1389557515666151001142012
5. Zhu, Z., Wang, X., Li, T., Aime, S., Sadler, P.J., and Guo, Z., *Angew. Chem., Int. Ed.,* 2014, vol. 53, p. 13225. doi 10.1002/anie.201407406
6. Furrer, J., and Süss-Fink, G., *Coord. Chem. Rev.*, 2016, vol. 309, p. 36 doi 10.1016/j.ccr.2015.10.007
7. Ihara, T., Ishii, T., Araki, N., Wilson, A.W., and Jyo, A., *J. Am. Chem. Soc.,* 2009, vol. 131, p. 3826. doi 10.1021/ja809702n
8. Liu, P., Liu, J., Zhang, Y.-Q., Wu, B.-Y., and Wang, K.-Z., *J. Photochem. Photobiol. B: Biol.*, 2015, p. 89. doi 10.1016/j.jphotobiol.2015.01.004
9. Svensson, F.R., Matson, M., and Lincoln, M.L.P, *Biophys. Chem.,* 2010, vol. 149, p. 102, doi 10.1016/j.bpc.2010.04.006
10. Marmur, J., *J. Mol. Biol*., 1961, vol. 3, p. 208. doi 10.1016/S0022-2836(61)80047-8
11. Reichmann, ME., Rice, SA., Thomas, C.A., and Doty, P., *J. Am. Chem. Soc.,* 1954, vol. 76, p. 3047. doi 10.1021/ja01640a067
12. Yamada, M., Tanaka, Y., Yoshimoto, Y., Kuroda, S., and Shimo, I., *Bull. Chem. Soc. Jpn*., 1992, vol. 65, p. 1006. doi 10.1246/bcsj.65.1006
13. Sullivan, B.P. and Salmon, D.J., Meyer, T., *Inorg. Chem.,* 1978, vol. 17, p. 3334. doi 10.1021/ic50190a006
14. Steck, E.A. and Day, A.R., *J. Am. Chem. Soc.,* 1943, vol. 65, p. 452. doi 10.1021/ja01243a043.
15. Wolfe, A., Shimer, G.H., and Mechan, T., *Biochemistry,* 1987, vol. 26, p. 6392, doi 10.1021/bi00394a013
16. Mc Ghee, J.D. and Von Hippel, P.H., *J. Mol. Biol.,* 1974, vol. 86, p. 469. doi 10.1016/0022-2836(74)90031-X
17. Chaires, J.B. and Dattagupta, N., Crothers, D.M., *Biochemistry,* 1982, vol. 21, p. 3927. doi 10.1021/bi00260a004
18. Satyanarayana, S., Dabrowiak, J.C., and Chaires, J.B., *Biochemistry,* 1993, vol. 32, p. 2573. doi 10.1021/bi000619015
19. Diller, D.J. and Merz Jr, K.M., *Proteins*, 2001, vol. 43, p. 113. doi 10.1002/1097-0134(20010501)43.
20. Jones, G., Willet, P., and Glen, R.C., *J. Mol. Biol.,* 1995, vol. 245, p. 43. doi 10.1016/S0022-2836(95)80037-9



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Synthesis, spectral studies, DNA binding, photocleavage, antimicrobial and anticancer activities of isoindol Ru(II) polypyridyl complexes

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Synthesis, spectral studies, DNA binding, photocleavage, antimicrobial and anticancer activities of isoindol Ru(II) polypyridyl complexes

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ABSTRACT

Three new Ru(II) polypyridyl complexes $[Ru(phen)_2CIIP]^{2+}$ (**1**) {CIIP = 2-(5-Chloro-3a H-Isoindol-3-yl)-1H-Imidazo[4,5-f][1, 10]phenantholine} (phen = 1, 10 phenanthroline), $[Ru(bpy)_2CIIP]^{2+}$ (**2**) (bpy = 2, 2′ bipyridine) and $[Ru(dmb)_2CIIP]^{2+}$ (**3**) (dmb = 4, 4′-dimethyl 2, 2′ bipyridine) were synthesized and characterized by different spectral methods. The DNA-binding behavior of these complexes was investigated by absorption, emission spectroscopic titration and viscosity measurements, indicating that these three complexes bind to CT-DNA in an intercalative mode, but binding affinities of these complexes were different. The DNA-binding constants K_b of complexes **1**, **2** and **3** were calculated in the order of 10^6. All three complexes cleave pBR322 DNA in photoactivated cleavage studies and exhibit good antimicrobial activity. Anticancer activity of these Ru(II) complexes was evaluated in MCF7 cells. Cytotoxicity by MTT assay showed growth inhibition in a dose dependent manner. Cell cycle analysis by flow cytometry data showed an increase in Sub G1 population. Annexin V FITC/PI staining confirms that these complexes cause cell death by the induction of apoptosis.

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DNA binding; fluorescence; viscosity; photocleavage; antimicrobial and apoptosis

1. Introduction

Extensive studies in the last few decades have focused on the interaction between DNA and transition metal complexes. Metal complexes can bind to DNA via non-covalent interactions such as groove, electrostatic and intercalative binding. Furthermore, a large number of useful applications of the metal complexes require that the complexes can bind to DNA via an

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intercalation mode which could induce cellular degradation.[1] Thus understanding the types of interactions of complexes with DNA is essential to design effective structure probe for DNA and provides insights for action mechanism of DNA targeted drugs. A number of Ru(II) complexes have been developed as potential cellular imaging and therapeutic agents.[2–9] The advantages of using such Ru(II) polypyridyl complexes as cellular targeting agents lies in the fact that the structural nature of the polypyridyl units will dictate the overall function of the metal complex, which includes their solubility, lipophilicity, charge, and importantly their photophysical properties.

In the search for metal complexes active against tumors and less toxic than cisplatin, ruthenium compounds[10–17] emerge as the most promising with biological features including mechanism of action, toxicity and bio distribution that are very different from those of classical platinum compounds.[18–26]

In this report, three new ruthenium(II) polypyridyl complexes (Scheme 1) were synthesized and characterized by elemental analysis, ESI-MS, ^{1}H NMR and ^{13}C NMR. The DNA-binding behaviors were studied by electronic absorption titration, photo activated cleavage and viscosity measurements, Antimicrobial and anticancer activities of these complexes were studied on the MCF7 (human breast adenocarcinoma) cell line.

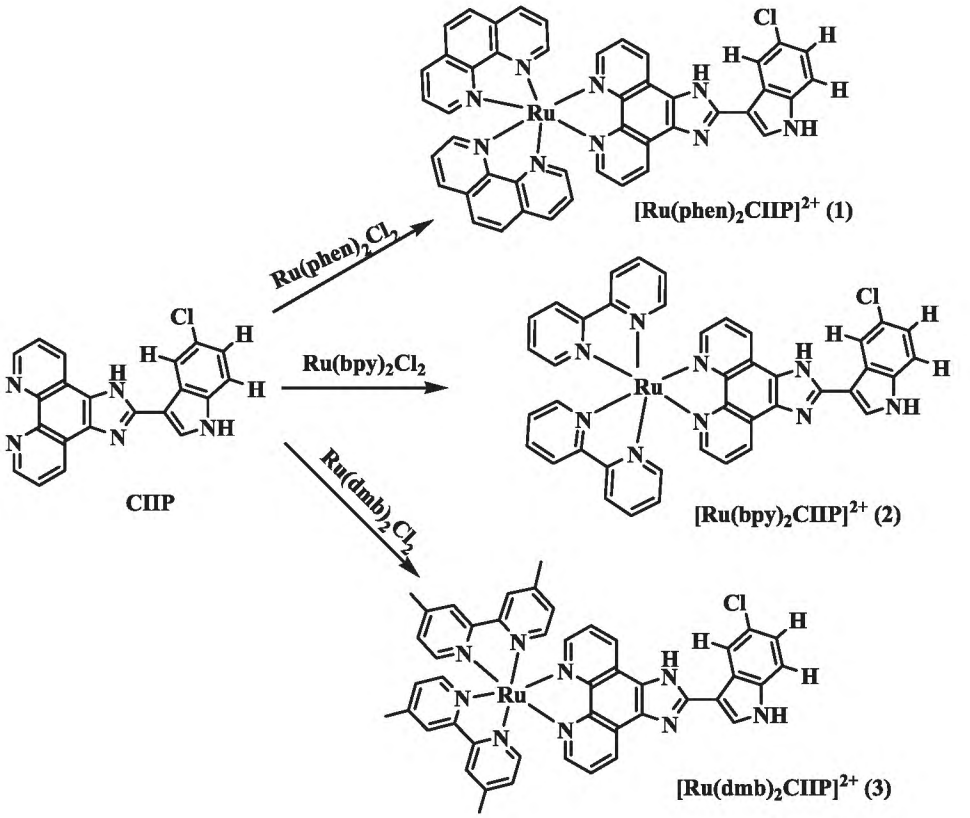
2. Materials and methods

2.1. Materials

5-Chloro indole-3 carboxaldehyde, $RuCl_3{\cdot}3H_2O$, 1, 10-Phenanthroline, 2,2′ bipyridine, and 4, 4′ dimethyl 2, 2′ bipyridine were purchased from Sigma Aldrich India. All the solvents were purified before use as per standard procedures.[27] Calf Thymus DNA (CT-DNA) was purchased from Sigma Aldrich, pBR322 super coiled plasmid DNA was obtained from Fermentas life sciences (stored at –20 °C). The Spectroscopic titration was carried out in the buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.2) at room temperature. Solutions of DNA in Tris–HCl buffer (pH = 7.2), 50 mM NaCl gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9, indicating that the DNA was sufficiently free of protein.[28] Double distilled water was used for preparing various buffers. Ampicillin for antimicrobial studies was purchased from local pharmaceuticals.

2.2. Physical measurements

For DNA absorption studies, UV-visible spectra with an Elico BL 198 and fluorescence measurements with an Elico SL 174 spectrofluorimeter were



Scheme 1. Structures of three Ru(II) Polypyridyl complexes; $[Ru(phen)_2CIIP]^{2+}$ (**1**), $[Ru(bpy)_2CIIP]^{2+}$ (**2**) and $[Ru(dmb)_2CIIP]^{2+}$ (**3**).

performed. FT-IR spectra were recorded with KBr disks on a Perkin-Elmer FT-IR-1605 spectrometer. A Bruker 400 MHz spectrometer was used for ^{1}H NMR and ^{13}C NMR spectra with DMSO-d_6 as solvent at room temperature and tetramethylsilane (TMS) as the internal standard. Microanalyses (C, H and N) were carried out with a Perkin-Elmer 240 elemental analyzer.

2.3. Synthesis and characterization

Compounds 1, 10-phenanthroline-5, 6-dione,[29] cis-$[Ru(phen)_2Cl_2]\cdot 2H_2O$, cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ and cis-$[Ru(dmb)_2Cl_2]\cdot 2H_2O$ were synthesized according to methods given in literature.[30] The synthetic scheme for the Ru(II) polypyridyl complexes are shown in Scheme 1.

2.3.1. Preparation of CIIP ligand

CIIP {2-(5-Chloro-3a *H*-isoindol-3-yl)-1*H*-imidazo[4,5-f][1, 10] phenanthroline}, was prepared by combining 1, 10-phenanthroline-5, 6-dione (1.06 g, 2.5 mM), 5-chloro indole-3 carboxaldehyde (1.2572 g, 3.5mM), ammonium

acetate (7.76 g, 60 mM) and glacial acetic acid (30 mL) and heating at reflux for 4 h as per Steck and Day,[31] cooling to room temperature, and diluting with water. Dropwise addition of ammonia gave a yellow precipitate which was collected, washed with water, dried, and purified by recrystallization from pyridine-H_2O (9:1, v/v); Yield: 0.51 g (73%), Analytical data: Elemental Analysis for $C_{21}H_{12}ClN_5$: Calc. (%): C:68.20; H: 3.27; N: 18.94; Found: C:68.18; H: 3.20; N: 18.91; ESI-MS (m/z): 370 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ: 9.04 (H–C = N), 7.30–8.71, (H–C = C) (aromatic protons), ^{13}C[^1H]-NMR (DMSO-d_6, 100 MHz): 153 (–C = N), 138–122 (–C*=C) (aromatic carbons), 118 (–C*=C–Cl).

2.3.2. Synthesis of [Ru(phen)$_2$(CIIP)](ClO$_4$)$_2$·2H$_2$O (1)

This complex was synthesized by dissolving of Cis-[Ru(phen)$_2$Cl$_2$]·2H$_2$O (0.143 g, 0.25 mM) and CIIP (0.093 g, 0.25 mM) in a mixture of ethanol (25 mL) and water (15 mL), and refluxing 70 °C for 8 h under N_2-atmosphere. At room temperature, the solution was titrated with saturated aqueous solution of $NaClO_4$ to give a brick red ppt. Then it was washed with CH_3CN-toluene (3:1) and vacuum dried. Yield: 0.39 g (67%). Elemental Analysis for $C_{45}H_{32}Cl_3N_9O_{10}Ru$, Calc. C: 50.69; H: 3.03; N: 11.82, Found: C: 50.49; H: 3.01; N: 11.76. ESI-MS (m/z): 415.56 for {[Ru(phen)$_2$(CIIP)]$^{2+}$ - $(ClO_4)_2$·2H$_2$O}. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ: 9.09 (H–C = N), 7.29–8.67, (H–C = C) (aromatic protons). ^{13}C[^1H]-NMR (DMSO-d_6, 100 MHz): 153 (–C = N), 138–122 (–C*=C) (aromatic carbons), 118 (–C*=C–Cl).

2.3.3. Synthesis of [Ru(bpy)$_2$(CIIP)](ClO$_4$)$_2$·2H$_2$O (2)

Using the complex (**1**) procedure, this complex (**2**) was synthesized with cis-[Ru(bpy)$_2$Cl$_2$]·2H$_2$O (0.13 g, 0.5 mM) in place of cis-[Ru(phen)$_2$Cl$_2$]·2H$_2$O Yield:0.36 g (66%). Elemental Analysis for $C_{41}H_{32}Cl_3N_9O_{10}Ru$, Calc. C: 48.36; H: 3.17; N: 12.38. Found: C: 48.29; H: 3.11; N: 12.31). ESI-MS (m/z): 399.14 for {[Ru(bpy)$_2$(CIIP)]$^{2+}$ $(ClO_4)_2$.2H$_2$O}. ^1H-NMR (DMSO-d_6, 400 MHz): δ: 9.17 (H–C = N), 7.31–9.02 (H–C = C) (aromatic protons), ^{13}C[^1H]-NMR (DMSO-d_6, 100 M*Hz*): 153 (–C = N), 138–122 (–C*=C) (aromatic carbons), 118 (–C*=C–Cl).

2.3.4. Synthesis of [Ru(dmb)$_2$(CIIP)](ClO$_4$)$_2$·2H$_2$O (3)

Using the complex (**1**) procedure, this complex (**3**) was synthesized with cis-[Ru(dmb)$_2$Cl$_2$]·2H$_2$O (0.144 g, 0.5 mM) in place of cis-[Ru(phen)$_2$Cl$_2$]·2H$_2$O. Yield: 0.41 g (67%). Elemental Analysis for $C_{45}H_{40}Cl_3N_9O_{10}Ru$, Calc. C: 50.31; H: 3.75; N: 11.73. Found: C: 50.28; H: 3.73; N: 11.70. ESI-MS (m/z): 419.6 for {[Ru(dmb)$_2$(CIIP)]$^{2+}$$(ClO_4)_2$.2H$_2$O}. ^1H NMR (DMSO-d_6,

400 MHz): δ: 9.16 (H–C = N), 8.85-7.18 (H–C = C) (aromatic protons), 2.08 ($-CH_3$), $^{13}C[^1H]$-NMR (DMSO-d_6, 100 MHz): 153 (–C = N), 138–122 (–C*=C) (aromatic carbons), 118 (–C*=C–Cl), 24($-CH_3$).

2.4. DNA binding studies

2.4.1. Absorption spectroscopic studies

The absorption spectroscopic titration using a fixed complex concentration (20 μM) was performed in tris-(hydroxylmethyl) amino methane hydrochloride and NaCl buffer, to this the DNA stock solution was gradually added up to the saturation point. Before the spectra were recorded the above mixture was allowed to equilibrate for 5 min. By monitoring the changes of absorption at the MLCT band with increasing concentration of DNA the intrinsic binding constant (K_b) was calculated by using the following equation.[32]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA, ε_a, ε_f and ε_b correspond to the apparent absorption coefficient A_{obs}/[complex], the extinction coefficient for the free complex and the extinction coefficient for the complex in the fully bound form, respectively. In plots of [DNA]/(ε_a–ε_f) versus [DNA], K_b is given by the ratio of slope to the intercept. It is further extended with EtBr to confirm the binding mode of complexes to DNA. A study with EtBr was performed to examine the binding potential of our complexes with CT-DNA.

2.4.2. Emission spectroscopic studies

The emission spectroscopic spectrum was recorded in tris-(hydroxylmethyl) amino methane hydrochloride and NaCl buffer a with fixed complex concentration. Before measurements, the excitation wavelength was fixed and the emission range was adjusted. The excitation intensities were monitored by varying the concentration of DNA with a fixed concentration of metal complex (20 μM). The fraction of the ligand bound was calculated from the relation $C_b = C_t\ [(F–F_0)/F_{max}–F_0)]$, where C_t is the total complex concentration, F is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA and F_{max} is when the complex is fully bound to DNA. The binding constant (K_b) was obtained from a modified Scatchard equation.[33] From a Scatchard plot of r/C_f vs r, where r is the C_b/[DNA] and C_f is the concentration of free complex and light switch 'on–off' studies were extended under this luminescence experiment.

2.5. Viscosity experiment

Viscosity experiments were carried out on Ostwald viscometer, placed in a thermostated water-bath maintained at $30 \pm 0.1\,^{\circ}C$. CT-DNA samples approximately 200 base pairs average length were prepared by sonication in order to minimize the complexes arising from DNA flexibility.[34] Flow time was measured with a digital stop-watch, and each sample was measured at least three times, using an average flow time in the calculation. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio of [Ru(II)]/[DNA],[35] where η is viscosity of DNA in the presence of the complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solutions ($t > 100\,s$) corrected for the flow time of the buffer alone (t_0).[36]

2.6. Photo activated cleavage studies of Ru(II) complexes with pBR322 DNA

Super coiled pBR322 DNA was used for the gel electrophoresis experiments, super coiled DNA was treated with Ru(II) complexes in buffer B and the solutions were then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 1 h at 80 V on a 1.0% agarose gel in Tris-acetic acid-EDTA buffer, pH = 8.2. The gel was stained with 1 μgmL^{-1} ethidium bromide and photographed with GeNei gel documentation chamber.

2.7. Antimicrobial activity

Antimicrobial activity of the complexes were screened against viz. *Eschericha coli* (*E. coli*) and *Bacillus streptococcaceae* (B.S) with positive (Ampicillin) and negative (DMSO) controls respectively. The concentrations of each complex were 1 mg/mL and 0.5 mg/mL prepared in DMSO solution and tested against spore germination of each fungus. On culture plates 10 µL of each Ru(II) complex was taken on a disc of sterilized Whatman filter paper no.1 (5 mm size). The fungal culture plates were incubated at $25 \pm 0.2\,^{\circ}C$ for 24 h. The diameters of the inhibition zones (in mm) were measured and tabulated.

2.8. Cell culture

MCF-7 cells (human breast adenocarcinoma cell line) were grown in DMEM medium with 10% fetal bovine serum (FBS), 25 mg/mL streptomycin, 25 U/mL penicillin (all from Invitrogen Corporation, Grand Island, NY). The cells were incubated at $37\,^{\circ}C$ in a humidified atmosphere containing 5% CO_2.

2.8.1. Cytotoxicity by MTT assay

The cell proliferation was determined using the 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Sigma, USA) and performed as per standard protocol. MCF7 cells were seeded 5000/well in 96 well plates and after overnight incubation; they were treated with complexes **1**, **2** and **3** from 200 µM to 3 µM for 48 h. After the stipulated time of incubation, MTT reagent was added (5 mg/mL) and incubated in the dark for 4 h at 37 °C and then media was removed and DMSO (200 µL) was added to each well for dissolving the formazan crystals. The optical density was measured at 570 nm using a microtitre plate reader. Then IC_{50} values of all three complexes were calculated. Each experiment was repeated three times.

2.8.2. Morphologic observation by phase contrast microscope

MCF7 cells were seeded and incubated overnight and then cells were treated with complexes **1**, **2** and **3** to determine then respective IC_{50} values after 48 h. Cell morphology was examined and photographed using a phase contrast microscope.

2.8.3. Cell cycle analysis

To determine the cell cycle dynamics, MCF7 cells were treated with complexes **1**, **2** and **3** for 48 h. After the incubation period, cells were collected by centrifugation and washed with PBS then fixed in 70% ethanol for 60 min at 4 °C and then cells were centrifuged to discard the alcohol and washed with PBS twice. Cells were resuspended in 500 µL of PBS containing 100 µg/mL of RNase (Invitrogen, USA) and incubated for 30 minutes and then 20 µg/mL of propidium iodide stain (Invitrogen, USA) was added and incubated further for 10 min in the dark at 37 °C before analysis. Cells with medium were used as a control. Cell cycle distribution of the cells was determined by analyzing 10,000 gated cells using a FACScan flow cytometer and Cell Quest software (FACS Calibur; Becton-Dickinson, San Jose, CA). All experiments were performed in triplicates.

2.8.4. Apoptotic analysis with annexin V FITC PI dual staining

MCF7 (1×10^6 cells) were seeded and incubated for overnight and then cells were treated with their respective IC_{50} values for 48 h and cells without treatment were kept as controls. Camptothecin (Sigma, USA) was used as early apoptotic inducer. After incubation periods, cells were pelleted down washed with PBS and Annexin V FITC/PI kit was purchased from Sigma and experiment performed according to the kit protocol.

3. Result and discussion

3.1. Synthesis and characterization

The three synthesized complexes are shown in Scheme 1. From FTIR spectra, ν_{N-H}, $\nu_{C=C}$, $\nu_{C=N}$ and ν_{M-N} of complexes were 3404, 1454, 1585 and 628 cm^{-1}, respectively. ν_{M-N} for all three complexes indicates that all six Ru→N bonds have the same bond order with an octahedral structure. ^1H NMR peaks of CIIP were slightly shifted towards to down field after complex formation, demonstrating complex formation.

3.2. DNA binding studies

3.2.1. Absorption spectroscopic studies

For metal complex and DNA interaction studies electronic absorption spectroscopy is the most useful tool.[37] This interaction is associated with hypochromism and a red shift in the metal ligand charge transfer (MLCT) and ligand bands.[38] This may be due to the intercalation of the aromatic chromophore between DNA base pairs. The degree of the hypochromism in a UV-visible band is constant with the strength of the interaction.[39,40] Thus, to provide evidence for the possibility of binding of each complex to CT-DNA, spectroscopic titrations of solution of each of the complexes with various concentration of CT-DNA were performed. Figure 1 shows the representative spectral profile of the three complexes at different DNA concentrations. The intrinsic binding constants (K_b) of the complexes were in the order of 10^6 as shown in Table 1. The binding constant was stronger for $[Ru(Phen)_2(MIPC)]^{2+}$,[41] because of the presence of electron withdrawing substituent (–Cl in CIIP) on the intercalative ligand, which increases the DNA binding affinity. Complex **3** shows the less binding strength to double helical DNA than the remaining two complexes (**1**&**2**). This reduction is caused by the presence of methyl groups at the 4 and 4′ positions of dmb (ancillary ligand), which causes steric hindrance and the complex moiety becomes electron rich, which causes a decrease in the binding affinity between the CT-DNA base pairs. The values are comparable to those of $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ $(3.56 \times 10^5 M^{-1})$ and $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ $(2.92 \times 10^5\ M^{-1})$[42] and $[Ru(phen)_2(dppca)]^{2+}$ $(3.4 \times 10^5\ M^{-1})$ and $[Ru(bpy)_2(dppca)]^{2+}$ $(2.12 \times 10^5\ M^{-1})$.[43]

3.2.2. Luminescence studies

Luminescence titration experiments were performed to further understand the exact nature of the complex binding to DNA at a fixed metal complex concentration in Tris buffer (pH = 7.2). The change of emission intensity is related to the extent to which the complex enters into the hydrophobic

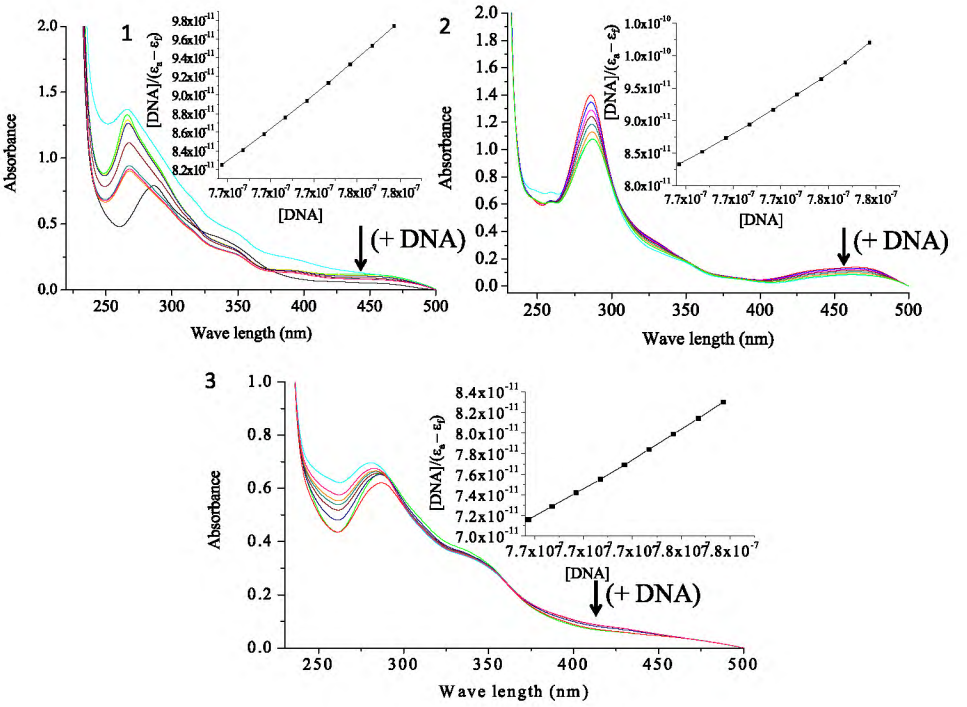


Figure 1. Absorption spectrum of three complexes $[Ru(phen)_2(CIIP)]^{2+}$(**1**), $[Ru(bpy)_2(CIIP)]^{2+}$(**2**) and $[Ru(dmb)_2(CIIP)]^{2+}$(**3**) complexes, in Tris–HCl buffer upon addition of CT-DNA. Arrow shows hypsochromic shift upon increase of DNA concentration. Inserted plot, $[DNA]/(\varepsilon_b-\varepsilon_f)$ versus [DNA] for the titration of DNA with Ru(II) complex, which gives intrinsic binding constant (K_b).

Table 1. Absorption, and Emission binding constants of ruthenium (II) complexes with CT-DNA.

Complex	K_b for absorption studies (M^{-1})	K_b for emission studies (M^{-1})
$[Ru(phen)_2CIIP]^{2+}$(1)	1.90 (±0.07)×10^6	2.32 (±0.07)×10^6
$[Ru(bpy)_2CIIP]^{2+}$ (2)	1.77 (±0.1)×10^6	2.15 (±0.04)×10^6
$[Ru(dmb)_2CIIP]^{2+}$(3)	1.26 (±0.1)×10^6	2.10 (±0.06)×10^6

environment inside the DNA as shown in Figure 2. The relative intensities of the excitation increased as DNA was added to the solution of the complex. When the complex intercalates between the DNA base pairs, the mobility of the complex is restricted at the binding site and the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the complex, leading to a decrease of the vibrational modes of relaxation. From the fluorescence data, an intrinsic binding constant was calculated by using the Scatchard equation[33] through a plot of r versus r/C_f, where r is the binding ratio $C_b/[DNA]$ and C_f is the free ligand concentration. Scatchard plots for the complexes were constructed from luminescence spectra, and the binding constants (K_b) were shown in Table 1 for **1**–**3**, respectively. The binding constants calculated are consistent with the absorption spectra.

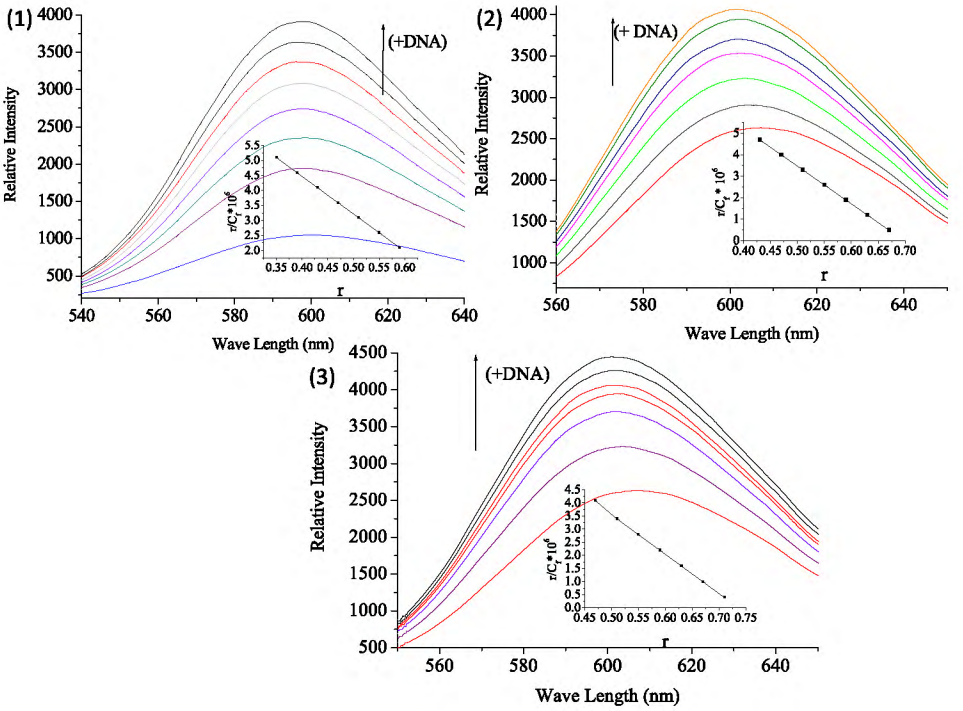


Figure 2. Luminescence spectrum of complexes $[Ru(phen)_2CIIP]^{2+}$(**1**), $[Ru(bpy)_2CIIP]^{2+}$(**2**) and $[Ru(dmb)_2CIIP]^{2+}$(**3**) in Tris–HCl buffer upon addition of CT-DNA. Arrow shows the intensity change upon the increase of DNA concentration. Inset: Scatchard plot of above complex, which gives binding constant (K_b).

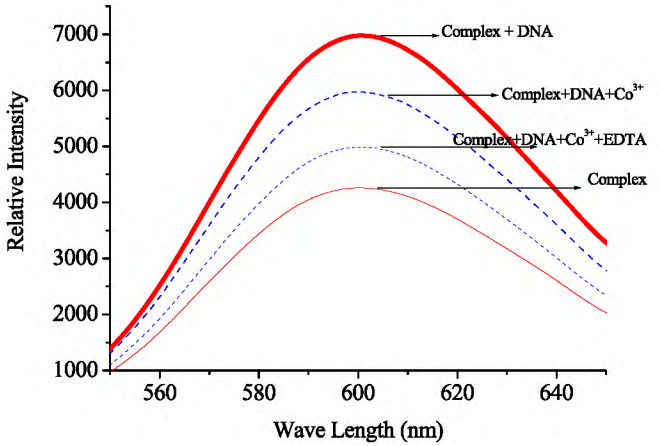


Figure 3. Luminescence modulation routes of $[Ru(phen)_2CIIP]^{2+}$ in the absence and presence of DNA by Co^{2+} and EDTA respectively.

A Further investigation was performed on the photoluminescence of DNA - $[Ru(phen)_2CIIP]^{2+}$ in the presence and absence of Co^{2+}. From Figure 3, it can be seen that when the complexes bound to DNA the emission intensity increased (switch on). By adding Co(II) ions to this resultant

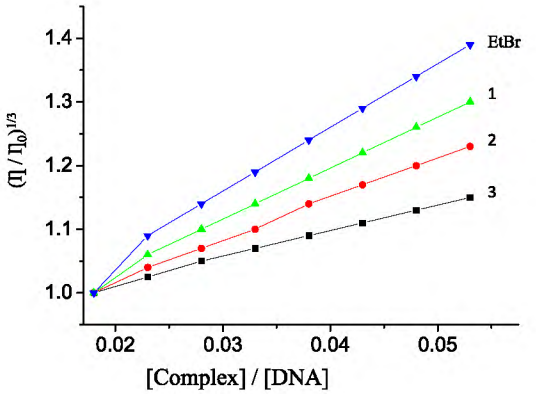


Figure 4. Effect of increasing amounts of EtBr, complexes $[Ru(phen)_2CIIP]^{2+}$(**1**), $[Ru(bpy)_2CIIP]^{2+}$(**2**) and $[Ru(dmb)_2CIIP]^{2+}$(**3**) on the relative viscosity of CT-DNA at 25(±0.1)^{0}C.

solution the emission intensity was quenched, turning the light switch off.[44,45] The addition of Co^{2+} to DNA - $[Ru(phen)_2CIIP]^{2+}$, results in the loss of intensity due to formation of a Co^{2+}- $[Ru(phen)_2CIIP]^{2+}$ heterometallic complex. However, the emission can be recovered in the presence of EDTA, thus turning the light switch on. This is because Co^{2+} was removed by EDTA, and the Co^{2+}- $[Ru(phen)_2CIIP]^{2+}$heterometallic complex cannot be formed. The present results should be of value in further developing a luminescence DNA probe.

3.3. Viscosity measurements

The consistent method for the assignment of the mode of binding of complexes to DNA is viscosity measurements. It is well-known that a classical intercalation of a ligand into DNA is known to cause a significant increase in the viscosity of a DNA solution due to an increase in the separation of the base pairs at the intercalative site and, hence, an increase in the overall DNA molecular length.[36] Figure 4 has shown that by increasing the amounts of complexes **1**, **2** and **3**, the relative viscosity of CT DNA solution increase steadily along with classical intercalator ethidium bromide. These results suggest that the three Ru(II) complexes intercalate between the DNA base pairs in the order of **1 > 2>3,** which is consistent with our interpretation based on binding constants.

3.4. Photo activated photo cleavage studies

The photo cleavage reactions of plasmid pBR322 DNA were induced by ruthenium (II) complexes and monitored by agarose gel electrophoresis. When electrophoresis is applied to circular plasmid DNA, the fastest migration of closed circular conformation (Form I) was observed. If one

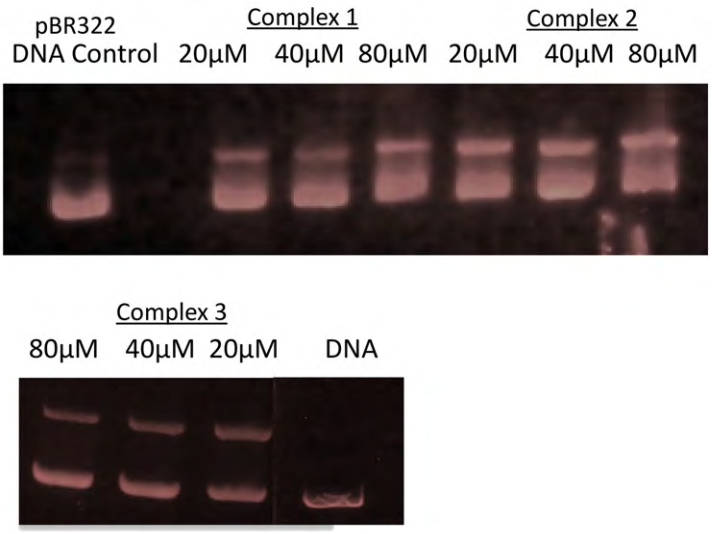


Figure 5. Photo activated cleavage studies of $[Ru(phen)_2CIIP]^{2+}$(1), $[Ru(bpy)_2CIIP]^{2+}$(2), $[Ru(dmb)_2CIIP]^{2+}$(3) with the concentration range of 20 to 80 μM against pBR322 DNA.

Table 2. Antimicrobial activity of complexes (**1**–**3**) with their minimum inhibition concentration (MIC) in mm.

Complex	Bacterial inhibition zone (mm)			
	Conc. (1000 μM)		Conc. (500 μM)	
	B.S	*E. coli*	B.S	*E. coli*
$[Ru(phen)_2CIIP]^{2+}$(1)	10.3	9.5	6.2	5.9
$[Ru(bpy)_2CIIP]^{2+}$(2)	9.3	8.9	5.9	5.7
$[Ru(dmb)_2CIIP]^{2+}$(3)	8.6	8.6	5.6	5.7
Ampicilin	14.7	13.2	8.8	8.7

strand is cleaved, the supercoil will relax to produce a slower moving nicked conformation (Form II). When both strands are cleaved, a linear conformation (Form III) is generated that migrates in between. As shown in Figure 5, no obvious cleavage was observed for the control in which metal complexes were absent (DNA alone), or incubation of the plasmid with the Ru(II) complexes in the dark. With increasing concentration of complexes, From I decreases and Form II increases gradually. At the concentration range of 20–80 μM, the three complexes can completely cleave the plasmid DNA in the sequence of concentration changes.

3.5. Antimicrobial studies

These three complexes have shown significant antibacterial activities with *Escherichia coli* (*E. coli*) and *Bacillus streptococcus* (B.S). From Table 2, inhibition zone data indicate that three complexes showed considerable

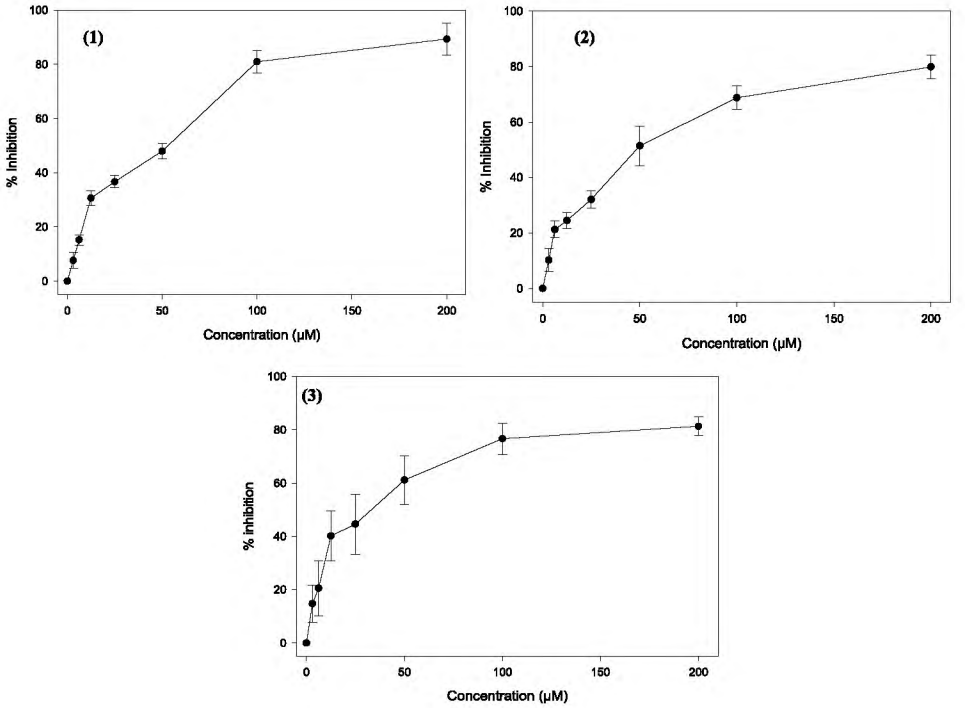


Figure 6. Cytotoxicity evaluation of complexes **1**-**3** on MCF 7 cell line by MTT assay. IC_{50} value was calculated. Data represented here are from three independent experiments.

activity against *E. coli* and *B.S* at 1 mg/mL and 0.5 mg/mL concentrations. The DMSO control showed a negligible activity and Ampicillin shows greater activity as compared with the three metal complexes. $[Ru(phen)_2CIIP]^{2+}$ showed slightly higher activity than other two complexes **2** and **3**. The antimicrobial activity is increased as the concentration of the complexes increased.

3.6. MTT assay

All three complexes showed significant inhibition on MCF 7 cells in comparison to control. IC_{50} values were calculated using an MTT assay. The IC_{50} value for complex **1** was 52.7 μM whereas for complex **2** and **3** it was 48.7 and 33.5 μM as shown in Figure 6.

3.6.1. Morphological observations

The effect of three complexes on morphology of MCF 7 cells was observed by phase contrast microscope. After treatment of cells with complexes **1**–**3** for 48 h, it was observed that cell growth was inhibited in a significant manner when compared to control cells as shown in the Figure 7.

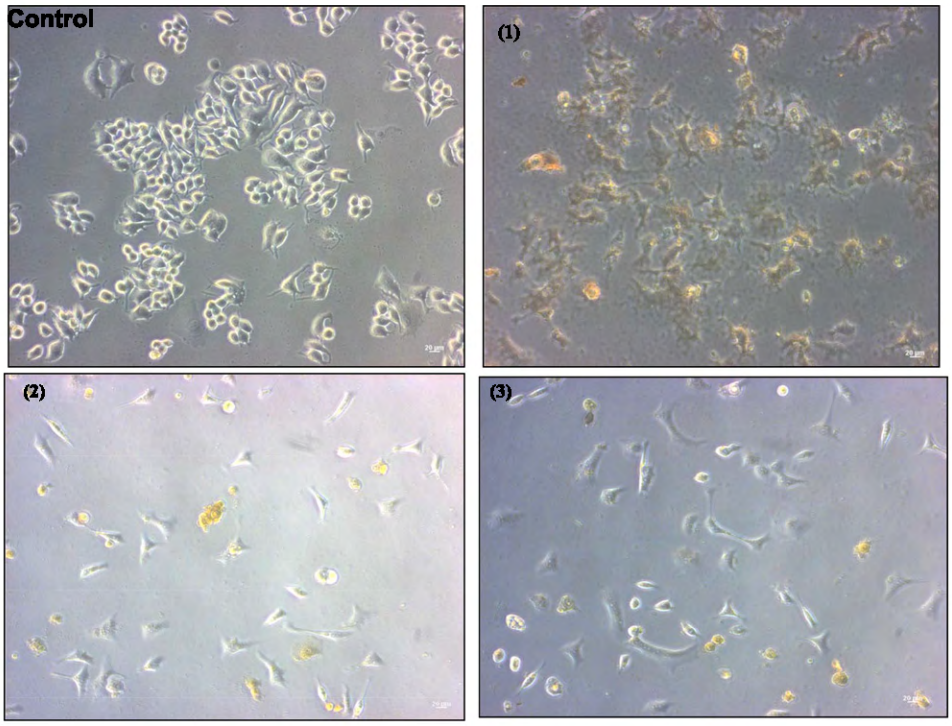


Figure 7. Morphological examination of MCF7 cells by Phase Contrast Microscope.

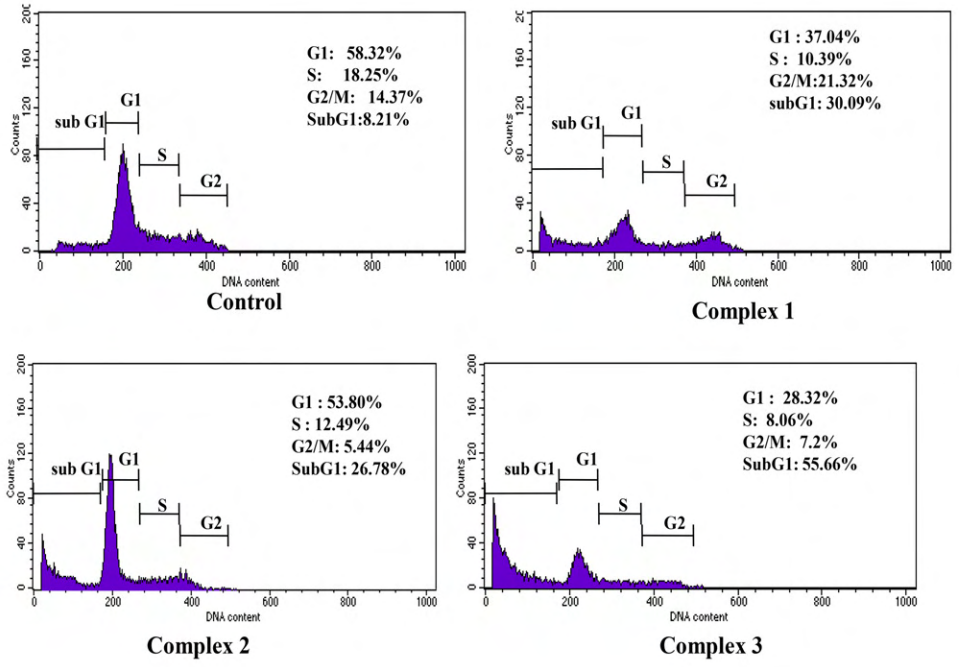


Figure 8. Cell cycle analysis by PI staining followed by Flow cytometry.

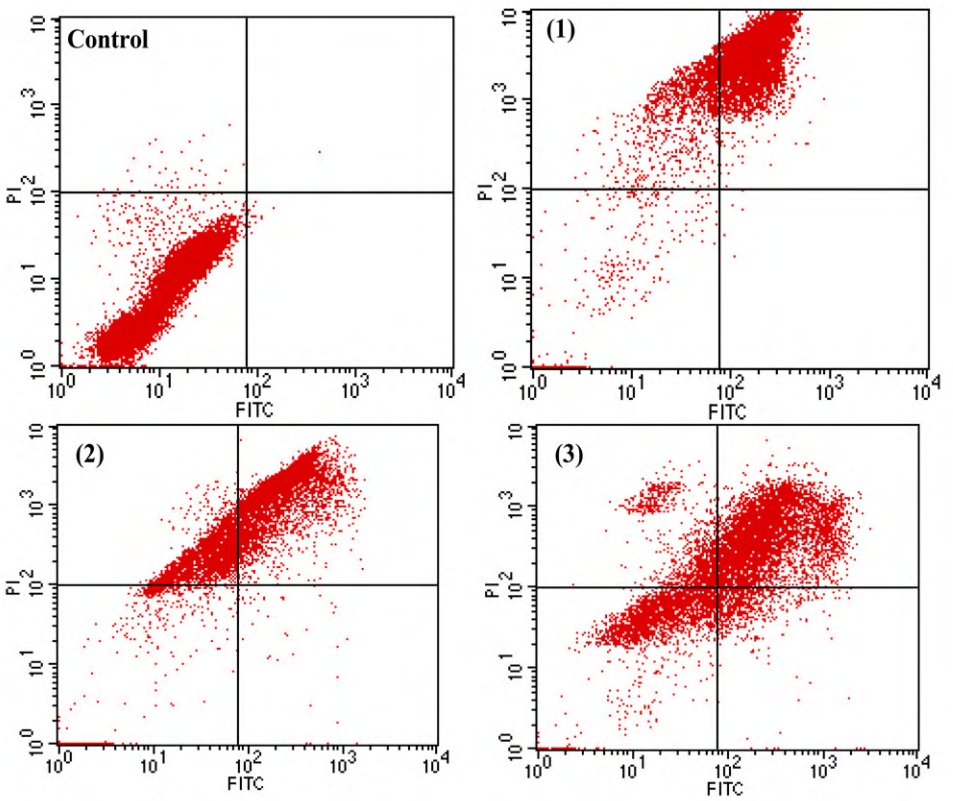


Figure 9. Determination of Apoptosis by Annexin V FITC/PI staining followed by Flow Cytometry.

3.6.2. Cell cycle dynamics

Cell cycle analysis following treatment with complexes **1**–**3** in MCF 7 cells showed significant increase in the subG1 population (hypodiploid DNA content and cell death) of cells as shown in Figure 8. These results suggested that all three complexes were effective in causing cell death.

3.6.3. Induce apoptosis

To investigate the possible mechanism causing cell death, cellular apoptosis was assessed by Annexin V/PI staining followed by flow cytometry. Results had shown that apoptotic percentages of MCF-7 cells were increased on treatment with complexes **1**, **2** and **3** as shown in Figure 9. These results suggested that treatment with these complexes led to translocation of phosphotidylserine from inner to outer leaflet of the plasma membrane, which is an indicator of early apoptosis.

4. Conclusion

Three new ruthenium(II) polypyridyl complexes $[Ru(phen)_2(CIIP)]^{2+}$(**1**), $[Ru(bpy)_2(CIIP)]^{2+}$ (**2**) and $[Ru(dmb)_2(CIIP)]^{2+}$(**3**) were synthesized and

characterized. The absorption, emission and viscosity studies revealed that all the three Ru(II) complexes bind to DNA through an intercalative mode, and the binding affinity of three complexes are in the order **1** > **2**>**3**, due to the planarity and steric hindrance of the ancillary ligands. In a gel electrophoresis experiment, all three complexes can cleave effectively pBR322 DNA in different forms. Antimicrobial activity indicated that complex **1** was more active compared to other complexes against all tested microorganisms. All the synthesized ruthenium polypyridyl complexes showed cytotoxicity against MCF7 cell line and these complexes caused cell death by induction of apoptosis. These results may be useful in understanding the interactions of complexes with DNA and also useful in the development of new metal based anticancer agents.

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References

[1] Wang, B. D.; Yang, Z. Y.; Crewdson, P.; Wang, D. Q. Synthesis, Crystal Structure and DNA-Binding Studies of the Ln(III) Complex with 6-Hydroxychromone-3-Carbaldehyde Benzoyl Hydrazone. *J. Inorg. Biochem.* **2007**, *101*, 1492–1504. DOI: 10.1016/j.jinorgbio.2007.04.007.

[2] Niyazi, H.; Hall, J. P.; O'Sullivan, K.; Winter, G.; Sorensen, T.; Kelly, J. M.; Cardin, C. J. Crystal Structures of Lambda-$[Ru(Phen)_2dppz]^{2+}$ with Oligonucleotides Containing TA/TA and at/at Steps Show Two Intercalation Modes. *Nature Chem.* **2012**, *4*, 621–628. DOI: 10.1038/nchem.1397.

[3] Nonat, A. M.; Quinn, S. J.; Gunnlaugsson, T. Mixed f-d Coordination Complexes as Dual Visible- and near-Infrared-Emitting Probes for Targeting DNA. *Inorg. Chem.* **2009**, *48*, 4646–4648. DOI: 10.1021/ic900422z.

[4] Ryan, G. J.; Quinn, S.; Gunnlaugsson, T. Highly Effective DNA Photocleavage by Novel "Rigid" Ru(Bpy)3-4-Nitro- and -4-Amino-1,8-Naphthalimide Conjugates. *Inorg. Chem.* **2008**, *47*, 401–403. DOI: 10.1021/ic700967y.

[5] Zeglis, B. M.; Pierre, V. C.; Barton, J. K. Metallo-Intercalators and Metallo-Insertors. *Chem. Commun.* **2007**, *44*, 4565–4579. DOI: 10.1039/b710949k.

[6] Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. Photoreactions of Ruthenium(II) and Osmium(II) Complexes with Deoxyribonucleic Acid (DNA). *J. Photochem. Photobiol. B, Biol.* **1997**, *40*, 91–106.

[7] Erkkila, K. E.; Odom, D. T.; Barton, J. K. Recognition and Reaction of Metallointercalators with DNA. *Chem. Rev.* **1999**, *99*, 2777−2795.

[8] Song, H.; Kaiser, J. T.; Barton, J. K. Crystal Structure of Δ-$[Ru(bpy)_2dppz]^{2+}$ bound to mismatched DNA reveals side-by-side metalloinsertion and intercalation. *Nat. Chem.* **2012**, *4*, 615–620. DOI: 10.1038/nchem.1375.

[9] Gill, M. R.; Thomas, J. A. Ruthenium(II) Polypyridyl Complexes and DNA-from Structural Probes to Cellular Imaging and Therapeutics. *Chem. Soc. Rev.* **2012**, *41*, 3179–3192. DOI: 10.1039/c2cs15299a.

[10] Bergamo, A.; Gaiddon, C.; Schellens, J.; Beijnen, J. H.; Sava, G. Approaching Tumour Therapy beyond Platinum Drugs: Status of the Art and Perspectives of Ruthenium Drug Candidates. *J. Inorg. Biochem.* **2012**, *106*, 90–99. DOI: 10.1016/j.jinorgbio.2011.09.030.

[11] Pillozzi, S.; Gasparoli, L.; Stefanini, M.; Ristori, M.; D'Amico, M.; Alessio, E.; Scaletti, F.; Becchetti, A.; Arcangeli, A.; Messori, L. NAMI-A Is Highly Cytotoxic toward Leukemia Cell Lines: Evidence of Inhibition of KCa3.1 Channels. *Dalton Trans.* **2014**, *43*, 12150–12155. DOI: 10.1039/C4DT01356E.

[12] Trondl, R.; Heffeter, P.; Kowol, C. R.; Jakupec, M. A.; Berger, W.; Keppler, B. K. NKP-1339, the First Ruthenium-Based Anticancer Drug on the Edge to Clinical Application. *Chem. Sci.* **2014**, *5*, 2925–2932. DOI: 10.1039/C3SC53243G.

[13] Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K. KP1019, a New Redox-Active Anticancer Agent–Preclinical Development and Results of a Clinical Phase I Study in Tumor Patients. *Chem. Biol.* **2008**, *5*, 2140–2155. DOI: 10.1002/cbdv.200890195.

[14] Oszajca, M.; Kuliś, E.; Stochel, G.; Brindell, M. Interaction of the NAMI-A Complex with Nitric Oxide under Physiological Conditions. *New J. Chem.* **2014**, *38*, 3386–3394. DOI: 10.1039/C3NJ01631E.

[15] He, X.; Jin, L.; Tan, L. DNA-Binding, Topoisomerases I and II Inhibition and in Vitro Cytotoxicity of Ruthenium(II) Polypyridyl Complexes: $[Ru(Dppz)_2L]^{2+}$ (L = Dppz-11-CO_2Me and Dppz). *Spectrochim. Acta Part A* **2015**, *135*, 101–109. DOI: 10.1016/j.saa.2014.06.147.

[16] Selvamurugan, S.; Ramachandran, R.; Viswanathamurthi, P. Ruthenium(II) Carbonyl Complexes Containing S-Methylisothiosemicarbazone Based Tetradentate Ligand: Synthesis, Characterization and Biological Applications. *Biometals*. **2013**, *26*, 741–753. DOI: 10.1007/s10534-013-9649-8.

[17] Miserachs, H. G.; Cipriani, M.; Grau, J.; Vilaseca, M.; Lorenzo, J.; Medeiros, A.; Comini, M. A.; Gambino, D.; Otero, L.; Moreno, V. Antitumor and Antiparasitic Activity of Novel Ruthenium Compounds with Polycyclic Aromatic Ligands. *J. Inorg. Biochem.* **2015**, *150*, 38–47. DOI: 10.1016/j.jinorgbio.2015.06.007.

[18] Mjos, K. D.; Orvig, C. Metallodrugs in Medicinal Inorganic Chemistry. *Chem. Rev.* **2014**, *114*, 4540–4563. DOI: 10.1021/cr400460s.

[19] Bratsos, L.; Jedner, S.; Gianferrara, T.; Alessio, E. Ruthenium Anticancer Compounds: challenges and Expectations. *Chimia.* **2007**, *61*, 692–697. DOI: 10.2533/chimia.2007.692.

[20] Jiang, G. B.; Yao, J. H.; Wang, J.; Li, W.; Han, B. J.; Lin, G. J.; Xie, Y. Y.; Huang, H. L.; Liu, Y. J. The Induction of Apoptosis in BEL-7402 Cells through the ROS-Mediated Mitochondrial Pathway by a Ruthenium(II) Polypyridyl Complex. *New J. Chem.* **2014**, *38*, 2554–2563. DOI: 10.1039/C3NJ01467C.

[21] Yu, Q.; Liu, Y.; Xu, L.; Zheng, C.; Le, F.; Qin, X.; Liu, Y.; Liu, J. Ruthenium(II) Polypyridyl Complexes: Cellular Uptake, Cell Image and Apoptosis of HeLa Cancer

Cells Induced by Double Targets. *Eur. J. Med. Chem.* **2014**, *82*, 82–95. DOI: 10.1016/j.ejmech.2014.05.040.

[22] Pierroz, V.; Joshi, T.; Leonidova, A.; Mari, C.; Schur, J.; Ott, I.; Spiccia, L.; Ferrari, S.; Gasser, G. Molecular and Cellular Characterization of the Biological Effects of Ruthenium(II) Complexes Incorporating 2-Pyridyl-2-Pyrimidine-4-Carboxylic Acid. *J. Am. Chem. Soc.* **2012**, *134*, 20376–20387. DOI: 10.1021/ja307288s.

[23] Kostova, I. Ruthenium Complexes as Anticancer Agents. *Curr. Med. Chem.* **2006**, *13*, 1085–1107.

[24] Huang, H. L.; Li, Z. Z.; Liang, Z. H.; Liu, Y. J. Cell Cycle Arrest, Cytotoxicity, Apoptosis, DNA-Binding, Photocleavage, and Antioxidant Activity of Octahedral Ruthenium(II) Complexes. *Eur. J. Inorg. Chem.* **2011**, *36*, 5538–5547. DOI: 10.1002/ejic.201100848.

[25] Vuradi, R. K.; Avudoddi, S.; Putta, V. R.; Kotha, L. R.; Yata, P. K.; Sirasani, S. Synthesis, Characterization and Luminescence Sensitivity with Variance in pH, DNA and BSA Binding Studies of Ru(II) Polypyridyl Complexes. *J. Fluoresc.* **2017**, *27*, 939–952. DOI: 10.1007/s10895-017-2029-y.

[26] Ravi Kumar, V.; Kamakshi, D.; Praveen Kumar, Y.; Vinoda Rani, M.; Rajender Reddy, M.; Nagamani, C.; Ravi, C.; Suman Thakur, S.; Mohan Rao, C.; Satyanarayana, S. Studies on the DNA-Binding and Anticancer Activity of Ru(II) Polypyridyl Complexes by Using (2-(4-(Diethoxymethyl)-1*H* Imidazo[4,5-f] [1,10] Phenanthroline)) Intercalative Ligand. *New J. Chem.* **2018**, *42*, 846–859. DOI: 10.1039/C7NJ03819D.

[27] Perrin, D. D.; Annarego, W. L. F.; Perrin, D. R. *Drying of Solvents and Laboratory Chemicals*, 2nd edn. Pergamon Press: New York; **1980**.

[28] Marmur, J. A Procedure for the Isolation of Deoxyribonucleic Acid from Micro-Organisms. *J. Mol. Biol.* **1961**, *3*, 208–218. DOI: 10.1016/S0022-2836(61)80047-8.

[29] Yamada, M.; Tanaka, Y.; Yoshimoto, Y.; Kuroda, S.; Shimo, I. Synthesis and Properties of Diamino-Substituted Dipyrido [3,2-a:2′,3′-c] Phenazine. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1006–1011. DOI: 10.1002/chin.199231204.

[30] Sullivan, B. P.; Salmon, D. J.; Meyer, T. Mixed Phosphine 2,2′- Bipyridine Complexes of Ruthenium. *Inorg. Chem.* **1978**, *17*, 3334–3341. DOI: 10.1021/ic50190a006.

[31] Steck, E. A.; Day, A. R. Reactions of Phenanthraquinone and Retenequinone with Aldehydes and Ammonium Acetate in Acetic Acid Solution. *J. Am. Chem. Soc.* **1943**, *65*, 452–456. DOI: 10.1021/ja01243a043.

[32] Wolfe, A.; Shimer, G. H.; Meehan, T. Polycyclic Aromatic Hydrocarbons Physically Intercalate into Duplex Regions of Denatured DNA. *Biochemistry.* **1987**, *26*, 6392–6396. DOI: 10.1021/bi00394a013.

[33] Mc Ghee, J. D.; Von Hippel, P. H. Theoretical Aspects of DNA Protein Interactions: co-Operative and Non-co-Operative Binding of Large Ligands to a One-Dimensional Homogeneous Lattice. *J. Mol. Biol.* **1974**, *86*, 469–489. DOI: 10.1016/0022-2836(74)90031-X.

[34] Chaires, J. B.; Dattagupta, N.; Crothers, D. M. Selfassociation of Daunomycin. *Biochemistry.* **1982**, *21*, 3927–3932. DOI: 10.1021/bi00260a004.

[35] Cohen, G.; Eisenberg, H. Viscosity and Sedimentation Study of Sonicated DNA–Proflavine Complexes. *Biopolymers.* **1969**, *8*, 45–55. DOI: 10.1002/bip.1969.360080105.

[36] Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. Neither Delta- nor Lambda-Tris(Phenanthroline)Ruthenium(II) Binds to DNA by Classical Intercalation. *Biochemistry.* **1992**, *31*, 9319–9324. DOI: 10.1021/bi00154a001.

[37] Bhat, I.; Tabassum, S. Synthesis of New Piperazine Derived Cu(II)/Zn(II) Metal Complexes, Their DNA Binding Studies, Electrochemistry and anti-Microbial Activity: validation for Specific Recognition of Zn(II) Complex to DNA Helix by Interaction with Thymine Base. *Spectrochim. Acta Part A* **2009**, *72*, 1026–1033. DOI: 10.1016/j.saa.2008.12.037.

[38] Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. Mixed-Ligand Complexes of Ruthenium(II): Factors Governing Binding to DNA. *J. Am. Chem. Soc.* **1989**, *111*, 3051–3058. DOI: 10.1021/ja00190a046.

[39] Moucheron, C.; Mesmaeker, A.; Choua, S. Photophysics of $Ru(Phen)_2(PHEHAT)^{2+}$: a Novel "Light Switch" for DNA and Photo-Oxidant for Mononucleotides. *Inorg. Chem.* **1997**, *36*, 584–592. DOI: 10.1021/ic9609315.

[40] Pyle, A. M.; Barton, J. K. Probing Nucleic Acids with Transition Metal Complexes. In: Lippard, S. J., Ed. *Bioinorganic Chemistry*; Wiley: New York, **1990**; pp. 413–475.

[41] Srishailam, A.; Yata Praveen, K.; Nazar, M.; Venkat Reddy, P.; Deepika, N.; Veerababu, N.; Satyanarayana, S. Synthesis, DNA-Binding, Cytotoxicity, Photo Cleavage, Antimicrobial and Docking Studies of Ru(II) Polypyridyl Complexes. *J. Fluoresc.* **2013**, *23*, 897–908. DOI: 10.1007/s10895-013-1209-7.

[42] Reddy, K. L.; Reddy, Y.; Satyanarayana, S. DNA-Binding and Photocleavage Properties of Ru(II) Polypyridyl Complexes with DNA and Their Toxicity Studies on Eukaryotic Microorganisms. *Nucleosides Nucleotides Nucleic Acids*. **2009**, *28*, 953–968. DOI: 10.1080/15257770903307060.

[43] Yata, P. K.; Shilpa, M.; Nagababu, P.; Reddy, M. R.; Kotha, L. R.; Gabra, N.; Satyanarayana, S. Study of DNA Light Switch Ru(II) Complexes: synthesis, Characterization, Photocleavage and Antimicrobial Activity. *J. Fluoresc.* **2012**, *22*, 835–847. DOI: 10.1007/s10895-011-1018-9.

[44] Liu, X. W.; Shen, Y. M.; Lu, J. L.; Chen, Y. D.; Li, L.; Zhang, D. S. Synthesis, DNA-Binding and Photocleavage of "Light Switch" Complexes $[Ru(Bpy)_2(Pyip)]^{2+}$ and $[Ru(Phen)_2(Pyip)]^{2+}$. Spectrochemica. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2010**, *77*, 522–527. DOI: 10.1016/j.saa.2010.06.030.

[45] Chen, M.; Li, H.; Li, Q.; Xu, Z. Luminescence Properties of $[Ru(Bpy)_2MDHIP]^{2+}$ Modulated by the Introduction of DNA, Copper(II) Ion and EDTA, Spectrochemica Acta Part a Mol. *Biomol. Spectrosc.* **2010**, *75*, 1566–1570.

SCATTERED COMPUTING WITH REAL TIME AND DISTRIBUTED DEVICES

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ABSTRACT

In this paper the challenges in computer systems research posed by the emerging field of scatered computing. It first examines the relationship of this new field to its predecessors: distributed systems and mobile computing. It then identifies four new research thrusts: effective use of smart spaces, invisibility, localized scalability, and masking uneven conditioning. Next, it sketches a couple of hypothetical scatered computing scenarios, and uses them to identify key capabilities missing from today's systems. The article closes with a discussion. The goal of this article is to help us understand the challenges in computer systems research posed by scatered computing.

Keywords: scatered computing, Nano technology, peer, interfaces.

1 REAL TIME COMPUTING SYSTEM HISTORY

Scatered computing aims to revolutionize the current paradigm of human computer interaction. Computers have been used in various aspects of human life, but in most cases human beings have to adapt their behavior to each system. Scatered computing is a computing environment computing systems weave themselves in the fabric of everyday life and become invisible. Invisibility is the most important aspect of Scatered computing. The user is exposed to a few sets of services available to him/her and is oblivious to the complex system implementing those services. This takes the human-computer interaction into a whole different dimension, where the user is surrounded by a complete smart environment with devices/sensors communicating with each other and aggregating their functionalities to provide a set of consolidated services. The terms Scatered computing and Pervasive computing are used interchangeably , but they are conceptually different. Scatered computing uses the advances in Mobile computing and Pervasive computing to present a global computing environment. Mobile computing is about elevating computing services and making them available on mobile devices using the wireless infrastructure. The focus here is to reduce the size of the computing devices so that they can be carried anywhere or by providing access to computing capacity through high-speed networks. But Mobile computing has some limitations. The computing model does not change considerably as we move since the computing devices cannot acquire the context information and adjust accordingly. Pervasive computing, on the other hand, is about acquiring context from the environment and dynamically building computing models dependent on context. Pervasive computing is invisible to human

users and yet provides useful computing services. Scatered computing aims to provide Pervasive computing environments to a human user as s/he moves from one location to another. A Scatered computing environment can be built in two ways. The traditional approach is to achieve it by using Mobile computing and Pervasive computing together, in which the mobile devices remember the information about past environments they operated in and activate when we reenter into a known environment or proactively build up services as we walk into new environments. This allows context information to be stored on the Web and then shared across Pervasive computing environments via the Web to provide context-aware services. Scatered computing began in the Electronics and Imaging Laboratory of the Xerox Palo Alto Research Center (PARC)

Scatered computing is the third wave of computing technologies to emerge since computers first appeared:

• **First Wave** - Mainframe computing era: one computer shared by many people, via workstations.
• **Second Wave** - Personal computing era: one computer used by one person, requiring a conscious interaction. Users largely bound to desktop.
•**Third Wave** – Scatered (initially called scatered) computing era: one person, many computers. Millions of computers embedded in the environment, allowing technology to recede into the background.

1.1SCOPE IN DISTRIBUTED SYSTEMS

The field of distributed systems arose at the intersection of personal computers and local area networks. The research that followed from the mid-1970s through the early 1990s created a conceptual framework and algorithmic base that

has proven to be of enduring value in all work involving two or more computers connected by a network Whether mobile or static, wired or wireless, sparse or scatered. This body of knowledge spans many areas that are foundational to scatered like…
• **Remote communication**, including protocol layering, remote procedure call, the use of timeouts, and the use of end to- end arguments in placement of functionality
• **High availability**, including optimistic and pessimistic replica control mirrored execution, and optimistic recovery
• **Security**, including encryption-based mutual authentication

1.2 MOBILE COMPUTING

Four key constraints of mobility forced the development of specialized techniques. The results achieved so far can be grouped into the following broad areas:
• **Mobile networking**, including Mobile IP ad hoc protocols and techniques for improving TCP performance in wireless networks.
• **Mobile information access**, including disconnected operation bandwidth-adaptive file access and selective control of data consistency.
• **Support for adaptive applications**, including transcoding by proxies and adaptive resource management.

2 SCATERED COMPUTING

Technology is moving from personal computers (PCs) to handheld, intelligent, and everyday devices with imbedded technology and connectivity

Scatered computing is a rapidly developing area of Information and communications Technology (ICT). The term refers to the increasing integration of ICT into people lives and environments, made possible by the growing variability of microprocessors with inbuilt communications facilities. Scatered computing has many potential applications, from health and home care to environmental monitoring and intelligent transport systems. This briefing provides an overview of scatered computing and discusses the growing debate over privacy, safety and environmental implications.

Scatered computing has been in development for almost 15 but still remains some way from becoming a fully operational reality. Some core technologies have already emerged, although the development of battery technologies and user interfaces pose particular challenges. It may be another 5-10 years before complete PCS become widely available. This depends on market forces, industry, public perceptions and the effects of any policy/regulatory frameworks. There have been calls for more widespread debate on the implications of scatered computing while it is still at an early stage of development

2.1 EFFECTIVE USE OF SMART SPACES

A space may be an enclosed area such as a meeting room or corridor, or a well-defined open area such as a courtyard or quadrangle. By embedding computing infrastructure in building infrastructure, a smart space brings together two worlds that have been disjoint until now .The fusion of these worlds enables sensing and control of one world by the other.

2.2 INVISIBILITY

The second thrust is *invisibility*. The ideal expressed by Weiser is complete disappearance of scatered computing technology from a user's consciousness. In practice, a reasonable approximation to this ideal is *minimal user distraction*. If a scatered computing environment continuously meets user expectations and rarely presents him with surprises, it allows him to interact almost at a subconscious level.

2.3 LOCALIZED SCALABILITY

The third research thrust is *localized scalability*. As smart spaces grow in sophistication, the intensity of interactions between a user's personal computing space and his/her surroundings increases. Scalability, in the broadest sense, is thus a critical problem in scatered computing. Previous work on scalability has typically ignored physical distance. A Web server or file server should handle as many clients as possible, regardless of whether they are located next door or across the country. The situation is very different in scatered computing. Here, the density of interactions has to fall off as one moves away; otherwise, both the user and his computing system will be overwhelmed by distant interactions that are of little relevance.

2.4 CONNECTIVITY

Scatered computing systems will rely on the interlinking of independent electronic devices into broader networks. This can be achieved via both wired (such as Broadband (ADSL) or Ethernet) and wireless networking technologies (such as Wi-Fi or Bluetooth), with the devices themselves being capable of assessing the most effective form of connectivity in any given scenario. The effective development of scatered computing systems depends on their degree of interoperability, as well as on the convergence of standards for wired and wireless technologies.

2.5 DEVICES

PCS devices are likely to assume many different forms and sizes, from handheld units (similar to mobile phones) to near-invisible devices set into 'everyday' objects (like furniture and clothing). These will all be able to communicate with each other and act 'intelligently'. Such devices can be separated into three categories:

• **Sensors:** input devices that detect environmental changes, user behaviors, human commands etc.

• **Processors:** electronic systems that interpret and analyses input-data;

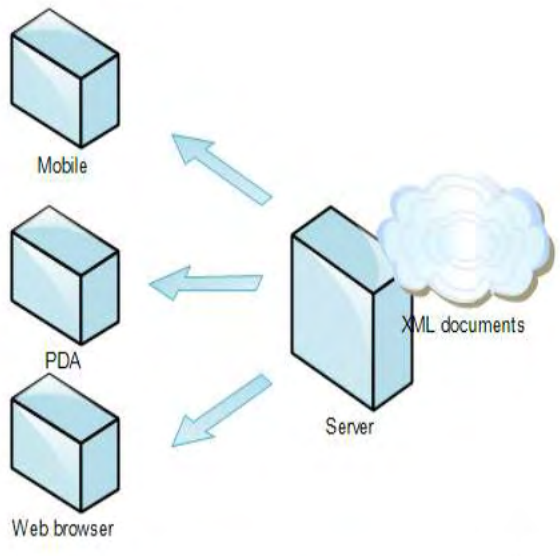
• **Actuators:** output devices that respond to processed information by altering the environment via electronic or mechanical means. For example, air temperature control is often done with actuators. However the term can also refer to devices which deliver information, rather than altering the environment physically. There are many visions for the future development of PCS devices. Several research groups are endeavoring to produce networks of devices that could be small as a grain of sand. The idea is that each one would function independently, with its own power supply, and could also communicate wirelessly with the others. These could be distributed throughout the environment to form dense, but almost invisible, pervasive computing networks, thus eliminating the need for overt devices.2

At the other extreme, augmented reality would involve overlaying the real world with digital information. This approach emphasizes the use of mobile technologies, geographical positioning systems and internet-linked databases to distribute information via personal digital companions. Such devices could come in many forms:

Children might have them integrated into school bags, whereas adults might use devices more closely resembling personal digital assistants (PDAs). Ultimately a spectrum of devices may become available. These will range from miniaturized (potentially embedded in surrounding objects) to a variety of mobile (including handheld and wearable) devices. While these could exist as standalone systems, it is likely that many will be interlinked to form more comprehensive systems

2.6 USER INTERFACES

User interfaces represent the point of contact between ICT and human users. For example with a personal computer, the mouse and keyboard are used to *input* information, while the monitor usually provides the *output*. With future user interfaces the input might be visual information – for example recognizing a person's face, or responding to gestures. Three very different forms of human-computer interaction are postulated: **active**, **passive** and **coercive.**



3 HUMAN-COMPUTER INTERACTIONS (HCIS)

Active: Users could have overt control over scatered computing technologies and devices in the environment. This could be achieved through language-based interfaces, allowing users to issue direct spoken or written commands

Passive: Scatered computing could disappear into the background. People would no longer know they were interacting with computers. The technology would sense and respond to human activity, behavior and demands intuitively and intelligently (for example, lighting altering in reaction to users' location, mood and activity).

Coercive: Scatered computing could control, overtly or covertly, lives and environments (for example if a device did not have an off-switch or a manual over-ride). Decisions made by developers (such as programming a system in accordance with health and safety regulations), development errors, unintended device interactions and malicious interference could all lead to loss of user control, and could possibly have negative implications for users.

4 ADVANTAGE

We increasingly rely on the electronic creation, storage, and transmittal of personal, financial, and other confidential information, and demand the highest security for all these transactions and require complete access to time-sensitive data, regardless of physical location. We expect devices -- personal digital assistants, mobile phones, office PCs and home entertainment systems -- to access that information and work together in one seamless, integrated system. Scatered computing gives us the tools to manage information quickly, efficiently, and effortlessly.

It aims to enable people to accomplish an increasing number of personal and professional transactions using a new class of intelligent and portable appliances or "smart devices" embedded with microprocessors that allow users to plug into intelligent networks and gain direct, simple, and secure access to both relevant information and services.. It gives people convenient access to relevant information stored on powerful networks, allowing them to easily take action anywhere, anytime.

Scatered computing simplifies life by combining open standards-based applications with everyday activities. It removes the complexity of new technologies, enables us to be more efficient in our work and leaves us more leisure time and thus scatered computing is fast becoming a part of everyday life.

5 APPLICATIONS

Scatered computing could have a range of applications, many of which may not yet have been identified. Applications in healthcare, homecare, transport and environmental monitoring are among the most frequently cited, as discussed below. Research is taking place in industry and academia, often collaboratively, handsome government activities are underway.

5.1 DOMICILIARY CARE

Improved methods for monitoring health and wellbeing could allow people to live longer in their own homes. Sensors embedded in items of clothing, for example, might allow constant monitoring of heart rates, body-mass index, blood pressure and other physiological variables. Further sensors embedded throughout the home could detect movement and fluctuations within the ambient environment (such as temperature change) to alert care-workers to any irregularities

5.2 ENVIRONMENTAL MONITORING

Scatered computing provides improved methods to monitor the environment. It will allow for continuous real-time data collection and analysis via remote, wireless devices. However, this poses significant challenges for PCS developers. Devices may be required to withstand harsh environmental conditions (such as heat, cold and humidity).

5.3 INTELLIGENT TRANSPORT SYSTEMS

Scatered computing technologies are being employed in the development of intelligent transport systems to try to alleviate these costs. Such systems seek to bring together information and telecommunications technologies in a environment. Computers are already incorporated into modern cars via integrated mobile phone systems, parking sensors and complex engine management systems. Intelligent transport systems take this process further by introducing 'intelligent' elements into vehicles. Vehicles could become capable of receiving and exchanging information 'on the move' via wireless technologies and be able to communicate with devices integrated into transport infrastructure, alerting drivers to traffic congestion, accident hotspots, and road closures. Alternative routes could be relayed to in-car computers, speeding up journey times and reducing road congestion.

5.4 HEALTH CARE

Scatered computing offers opportunities for future healthcare provision in the UK, both for treating and managing disease, and for patient administration. For instance, remote sensors and monitoring technology might allow the continuous capture and analysis of patients' physiological data. Medical staff could be immediately alerted to any detected irregularities. Data collection on this scale could also provide for more accurate pattern/trend analysis of long-term conditions such as heart disease, diabetes and epilepsy. Wearable sensors may offer greater patient mobility and freedom within hospitals and save both time and money by reducing the need for repeated and intrusive testing. Hospital administration could also be transformed. Patients might be tagged with wristbands containing digital photographs and medical notes. These wristbands would allow patients to be traced more effectively through hospital administration systems, reducing the risk of misidentification and treatment errors.

6 ISSUES

There are engineering problems to be solved before many of the envisaged applications of PCS can become a reality.

6.1 ENGINEERING ISSUES

The complexity of PCS systems means that their communications, software and hardware are likely to suffer from faults. These might be accidental, or the result of deliberate attempts to damage the system.3 If systems are interconnected it will be harder to establish who is responsible if something goes wrong.

6.2 PRIVACY, SECURITY AND SAFETY

Scatered computing systems may have implications for privacy, security and safety, as a result of their ability to: • gather sensitive data

6.2.1 PRIVACY

PCS could be embedded in places considered private, such as the home. The advent of scatered computing may mean that data can be collected without a person's knowledge or consent. Some argue that this could violate existing data protection law4. Data mining involves processing large quantities of data to spot patterns and trends. In terms of consumer data, this can lead to more effective targeted marketing.

6.2.2 SAFETY AND SECURITY

Scatered computing also gives rise to debate over safety. Integrated transport systems could involve road vehicles having actuating devices that intervene in the driving process, possibly responding to hazards more quickly than humans. For example the new Mercedes S-Class features an active braking system that can detect rapidly slowing vehicles in front, activating the brakes without driver intervention. While this may help avoid accidents, there are also potential risks.

6.2.3 Technological measures

It is argued that privacy, safety and security can be better protected if appropriate procedures and protocols are integrated into PCS at the design level rather than implemented retrospectively. Three measures are frequently cited as vital in establishing robust security measures:

• The volume of transmitted data should be kept to a minimum;

• Data that require transmission should be encrypted and sent anonymously (without reference to the owner);

• Security should be treated as an ongoing and integral element of PCS. These principles are accepted by many centers of PCS research and development. However, consumer groups such as the NCC say that developers need to give more consideration to privacy issues. The NNC argues that in the case of RFID,6 privacy issues were considered only late in development and have still not been fully addressed.

7 EMERGING SCATERED (SCATERED) TECHNOLOGIES

7.1 Peer-to-Peer (P2P) networking

Napster popularized the application of P2P (peer-to-peer networking) products and now the same technology has begun to sing a business melody. The basic idea behind it being the sharing of files and programs and communicating directly with people over the Internet, without having to rely on a centralized server.

7.2 WIRELESS TECHNOLOGY

Wireless Internet connection helps access the Net through cellular phones, Personal Digital Assistants (PDAs) and Wireless laptops and this technology proposes enormous business opportunities. The sales force can avail real-time access to inventory records; price lists, order and customer account status and can book a sale almost instantaneously. Constant communication with wireless gadgets (that cost many degrees lesser than a laptop) can ensure that there is a constant feedback loop thus ensuring a new way of reaching customers.

7.3 THE TAPESTRY OF DISTRIBUTED COMPUTING

Distributed computing is the processing power of thousands of PCs aggregated to create a super computer. A centralized server subsidizes a large computing task in to smaller bits. It then assigns those bits to thousands of desktop computers, each of which does a small task and returns the results to the server.

7.4 VOICE COMPUTING

Voice recognition software will soon allow users to switch on their computers by just talking to them. Even documents can be edited through voice commands. We'll finally be reaching out to the frontier where man will be able to talk to all his machines and command them to do as he wishes. In effect, we are talking about an e-web or the embedded web where the Internet's role as content provider and shopping assistant morphs into that of companion and advisor.

7.5 E-WEB

Embedded devices in cars, refrigerators, shop floors, hospital rooms extend the Internet's role beyond content providers and shopping assistants to companion and advisors. The Next-Gen web will be more interactive with a swarm of specialized devices like sensors, and other appliances, all with Internet access and the ability to communicate. Scatered computing illustrates a world that we are moving towards, quite rapidly. The promise of 'convenience' implies that islands of technologies will soon converge and simplify life even further. We will finally be witness to the long promised shift to convergence. Computing will no longer be a monopolizing activity that shackles us to our desktops.

8 CONCLUSION

Scatered computing refers to embedding computers and communication in our environment. Scatered computing provides an attractive vision for the future of computing. The idea behind the scatered computing is to make the computing power disappear in the environment, but will always be there whenever need or in other words it means availability and invisibility. Scatered computing will be a fertile source of challenging research problems in computer systems for many years to come. Solving these problems will require us to broaden our discourse on some topics, and revisit long-standing design assumptions in others. We will also have to address research challenges in areas outside computer systems. These areas include human-computer interaction (especially multimodal interactions and human-centric hardware designs), software agents (with specific relevance to high-level proactive behavior), and expert systems and artificial intelligence (particularly in the areas of decision making and planning). Capabilities from these areas will need to be integrated with the kinds of computer systems capabilities discussed in this article.

REFERENCES

[1] Inutile, S.S., K. Larson, J.S. Beaudin, J.Nawyn, E. Munguia Tapia, and P. Kaushik, A living Laboratory for the design and evaluation of scatered computing interfaces, in Extended Abstracts of the 2005 Conference on Human Factors in Computing Systems. 2005, ACM Press: New York, NY.

[2] "Scatered Computing: Vision and Challenges" by Prof. M. Satyanarayanan, Carnegie Mellon University, *IEEE Personal Communications*, 2001

[3] Dept. of Electronics & Communication Engg. GEC Thrissur, Embedded/Real Time Systems: Concepts, Design and Programming â€œ The Ultimate Reference by Dr. K.V.K.K Prasad Electronics Maker May 2004 Vol.5

[4] IEEE Scatered Computing Magazinewww.computer.org/scatered/

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HISTOPATHOLOGICAL STUDIES IN C*HANA PUNCTATUS* AND *MACROBRACHIUM ROSENBERGII* EXPOSED TO SONATA

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ABSTRACT

The present study is aimed to assess the histological damage caused to the fish C*hana punctatus* and prawn *Macrobrachium rosenbergii* were exposed to lethal concentration (15.39ppm &12.09ppm) to Sonata(Fungicide). Light microscopic studies exhibited severe histopathological changes in the Gill, Liver and Brain. The histopathological changes in the gill of fish include: epithilial lifting, degenerated secondary lamella, curling of secondary filaments and degenration of epithelial cells and gill of prawn include Detached cuticle, Degeneration of epithelium in secondary gill lamelle, Necrosis., Infiltrations of haemocytes, Edema reupture of epithelial cells., Hypertrophy, Hyperpalsia., Disarrangement of secondary gill lamellae, Disruption of pillar cells and Enlargement of scondary gill lamellae. The histopathological changes in the Liver of fish include: blood cells among haptocytes, appearance of blood streaks among hepatocytes, formation of vacuoles and degeenrated hepato pancreatic tissue and Liver of prawn include haemorrage, vacuoles, necrosis and blood vesseles. The changes in the Brain of fish include: Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area and brain of prawn include Pyknotic nuclei, Pyknotic nuclei with dense eosinophilic cytoplasamm, Vacuolated spaces, Prolifiration of gilala cells and Gliosis and nodule formation.

KEYWORDS: Sonata, Hypertrophy, Hyperpalsia, epithilial lifting, Pyknotic nuclei, Gliosis.

INTRODUCTION

In order to meet the growing population needs and demands, use of agrochemicals is inevitable for enhanced food production. Pesticides are highly effective substances used in control of pest and vectors of human disease. The increasing use of pesticides has caused

concerns about their effects on human health and the environment. Inspite of potential applications in agriculture, horticlture and other allied fields, they also exert some disadvantages, they include toxicity to animals, plants and human beings. Persistence of some of these chemicals in the environment and their subsequent entry into aquatic systems causes a great havoc. Pesticides and fungicides exert their toxic action on arthropods, mussels, fishes, frogs, turtles, water birds and other wild life too. Excessive use leads to bioaccumulation in farm workers, fruits, vegetables, nuts and food crops, consumers, and it also causes biomagnification at various trophic levels of the food chain. Although Indian average consumption of pesticide is far lower than many other developed economies, the problem of pesticide residue is very high in India.[1] Fungicides also threatens non target aquatic and terrestrial organisms through drift either by consumption or by ground water contamination. They enter water from agriculture and run off. The pollution of normal waters with synthetic chemicals has caused serious problems to the aquatic biota.[2,3,4,5] Fish and prawn are useful bioindicators and integrators of contaminants. They accumulate in gills, liver, kidney, and fat and induces metabolic changes associated with these organs.

MATERIALS AND METHODS

Animal collection

The fish and prawn specimens samples of the two varities namely *Channa punctatus* and *Macrobrachium rosenbergii* were collected from the freshwater lake located in waddepelly cheru, Warangal district. Fish measuring 14-15cms in length and weighing 250-300gms and prawn measuring 14-18cms in length and weighing 25-30gms specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies.

The fish and prawn were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2oC. During the period of acclimatization, the fish and prawn were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms[6] were followed. After Acclimatization, Fishes and prawns were divided into groups and treated with concentrations of 10 and 20 ppm biofungicide sonata at time points 48, 72 and 96 hrs. to decide the lethal toxicity (LC50). The LC50 values were calculated the using probits analysis based on finney's (1952) table.

Tissue collection

Gill, liver, and brain tissues were isolated from normal (not exposed to the toxicant) and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouins solution for 48 hr, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut of 4-6µ (microns) thickness; stained with Hematoxylin-Eosin (dissolved in 70% alcohol)[7] and were mounted in Canada balsam. Histopathological lesions were examined and photographed with the help of Intel Pentium QX3 computer attached microscope under 400X lens.

RESULTS

Gills

No histopathological changes were observed in the gill of the control fish and prawn. The structural details of the gill of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 1A&B. The most common changes in 15.39ppm & 12.09ppm concentrations of Sonata Fungicide were epithilial lifting, degenerated secondary lamella, curling of secondary filaments and degenration of epithelial cells and in prawn include Detached cuticle, Degeneration of epithelium in secondary gill lamelle, Necrosis., Infiltrations of haemocytes, Edema reupture of cpithelial cells., Hypertrophy, Hyperpalsia., Disarrangement of secondary gill lamellae, Disruption of pillar cells and Enlargement of scondary gill lamellae. The histological changes noticed in the pesticide exposed and control fishes and prawns are shown in fig.1 A(A1) & FIG.1B(A1).

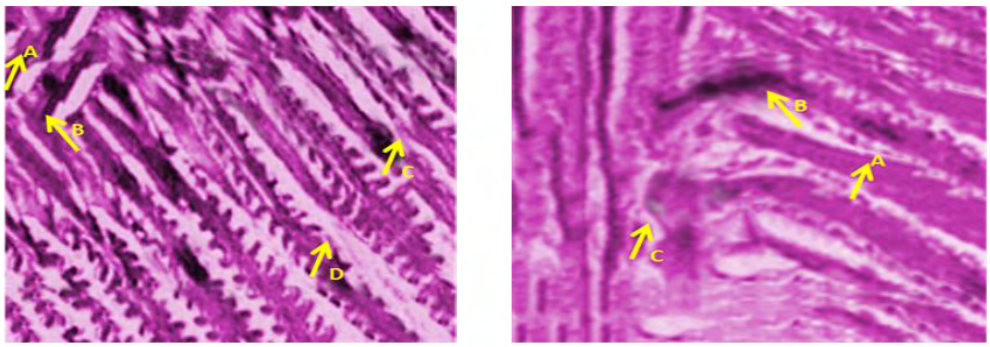
Liver

No histopathological changes were observed in the liver of the control fish and prawn. The structural details of the liver of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 2A&B. In the liver tissues of fish and prawn exposed to sonata concentrations of 15.39 ppm and 12.09 ppm, blood cells among haptocytes, appearance of blood streaks among hepatocytes, formation of vacuoles and degeenrated hepato pancreatic tissue and Liver of prawn include haemorrage, vacuoles, necrosis and blood vesseles were seen (Figs. 2B-. The histological changes noticed in the pesticide exposed and control fishes and prawns are shown in Fig.2A(A1) & Fig.2B(A1).

Brain

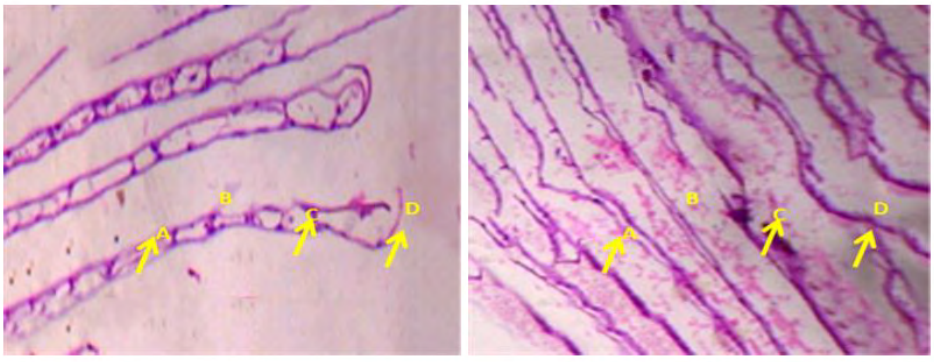
No histopathological changes were observed in the brain of the control fish and prawn. The structural details of the brain of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 3A&B. The most common changes in15.39ppm &12.09ppm concentrations of Sonata Fungicide were Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area and in prawn include Pyknotic nuclei, Pyknotic nuclei with dense eosinophilic cytoplasamm, Vacuolated spaces, Prolifiration of gilala cells and Gliosis and nodule formation. The histological changes noticed in the pesticide exposed and control fishesand prawns are shown in fig.3 A(A1) & FIG.3B(A1).

Gill



A1 **B1**

Fig 1A: Histopathology Studies of *Chana Punctatus* in Gill samples treated at 96 hrs (A&B); A1: Control: B1 Treated: In Control, A.Central Axis. B. Erythrocyte. C. Primary Gill Lamella. D. Secondary gill lamella: In Treated A. Efithilial Lifting. B. Curling of secondary gill filaments C. Degenretaed secondary lamella.

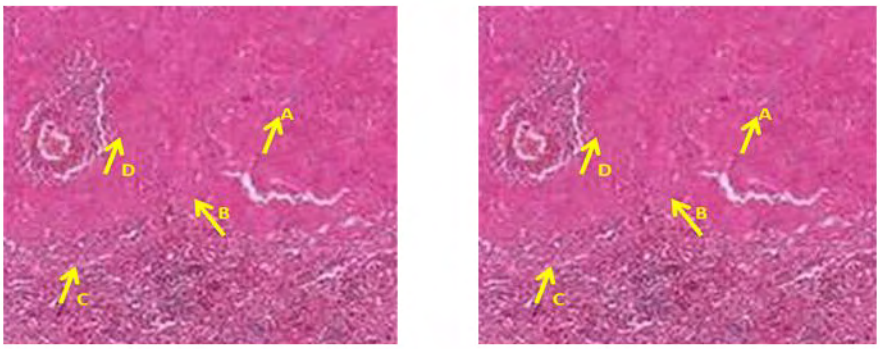


A1 **B1**

Figure1B: Histopathology Studies of *Macrobrachium* in Gill samples treated at 96 hrs (A1 &B1); A1 - Control: B1- treated; In control: A. Pillar cells. B. Incites. C. Detached cuticle. D. Degeneration of epithelium in secondary gill lamelle. In treated: A. Necrosis.

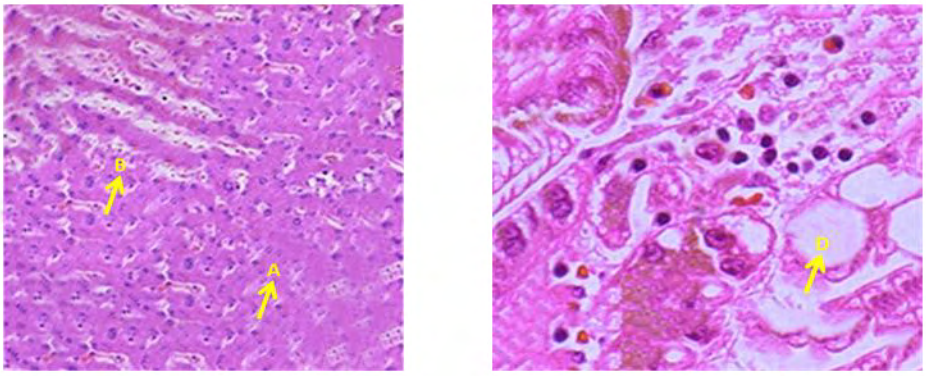
B. Infiltrations of haemocytes. C. Disarrangement of secondary gill lamellae. D. Disruption of pillar cells.

Liver



A1 **B1**

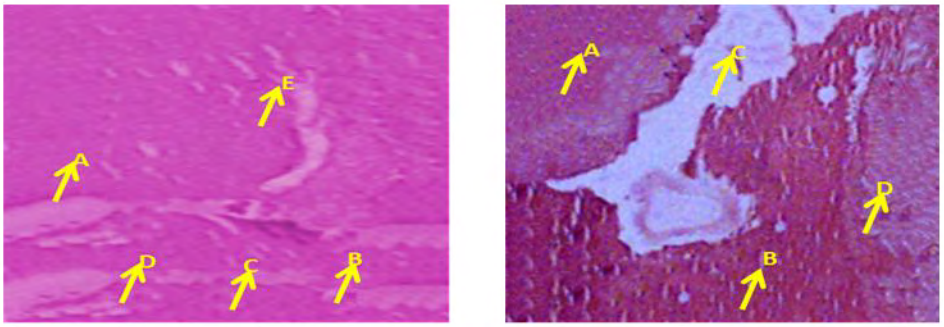
Figure 2 A: Histopathology Studies of *Channa Punctatus* in Liver samples treated at 96 hrs (A1 &B1). A1 Control: B1Treated. In Control, A.Hepatic cell. B. Nucleus. C. Lipid and glycogen granules: In Treated A. Degenerated hepato pancreatic tissue. B. Blood cells among hepatocytes C. Apperaence of blood streaks among hepatocytes. D. Formation of vacuoles.



A1 **B1**

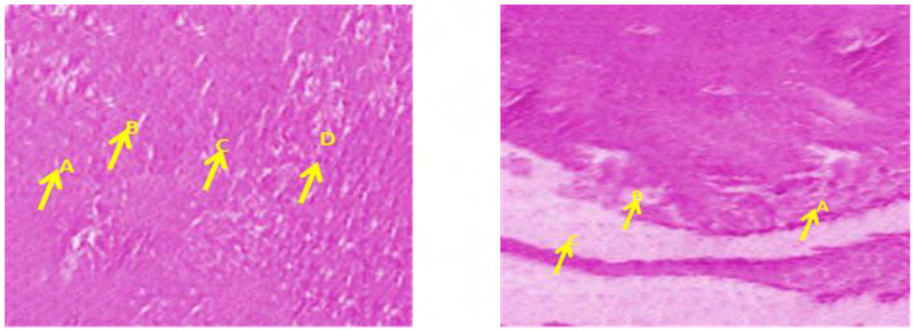
Figure 2 B: Histopathology Studies of *Macrobrachium* in Liver samples treated at 96 hrs (A1 &B1); A1 -Control: B1- Treated: A. Hepatic cells. B. Hepatic pancreas. C. Haemorrage. D. Vacuoles.

Brain



A1 **B1**

Figure 3 A: Histopathology Studies of *Channa Punctatus* in Brain samples treated at 96 hrs (A1 & B1); A1 Control: B1Treated: In Control, A Dorsal olfactory area. B. Ventral olfactory area. C. Septal area. D. Tractus olfactoriuos medialis. E Tractus olfactorius lateralis: In Treated A. Degenerated dorsal olfactory area B. Degenerated Ventral olfactory area C. Blood streeks. D. Degenerated septal area.



A1 **B1**

Figure 3 B: Histopathology Studies of *Macrobrachium.rosenbergii* in Brain samples treated at 96 hrs (A1 & B1); A1 Control: B1Treated: In Control, A Dorsal olfactory area. B. Ventral olfactory area. C. Septal area. D. Tractus olfactoriuos medialis. In Treated, A.Pyknotic nuclei. B. Pyknotic nuclei with dense eosinophilic cytoplasam. C. Vacuolated spaces and nodule formation.

DISCUSSION

The gills, which participate in many significant functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants.[7,8,9] Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the

external environment and the blood and thus serve as a barrier to the entrance of contaminants.[10,7,11,12]

Liver, the first organ to encounters any foreign molecule through portal circulation is subjected to more damage.[13] Liver is an important organ of detoxification which breaks down toxic substances and metabolites of administered substances. This breakdown is carried out by endoplasmic reticulum of hepatocytes. Due to these reasons the hepatic cells are damaged severely.[13] reported that in fish *Tilapia mosanmbica* exposed to the toxicant resulted in vacuolation and necrosis in liver.[14] reported that *Channa punctatus* under Malathion toxicity showed the degenerative changes in liver.[15] reported that in teleost fish Nemachelius denesoni (Day) exposure to phosphamidon caused highly vacuolated and claudy swelling and even the connective tissue was damaged in liver.[16] reported significant alterations in the hepatic cell count and the nucleocyton plasmic index in the liver of zebra fish *Brachydanio rerio* (cyprinid) exposed to 0.9 mg/l concentration of Malathion.

Like gills and liver in sonata treated *Chana punctatus* fish, Pathological changes were observed in brain samples also. Changes include Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area. Similar changes were observed by[17] reporting swelling of the axon, atrophy, necrosis and pycnosis in the fish *Ctenopharyngodon idellus* under fenvalerate toxicity, and[18] on *Cirrhinus mrigala* exposed to the sublethal and lethal concentrations of technical grade as well as 20% EC of Chlorpyrifos for 8 days and the severity of damage is more in lethal exposures than in sublethal exposures. Quinalphos technical grade caused more degenerative changes in brain than in 25% EC exposures (Plate VI.3, Fig. B, C, D and E).[19] reported that hexachlorocyclohexane was neurotoxic and induced vacuolation of brain parenchyma and moderate swelling of pyramidal cells of the cerebrum and opined that vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. Loss of Nissle substances and glial cell reaction, with evidence of glial nodule formation in places, were proof of the the neurotoxic nature of the chemical.

In the present investigation, gill, liver and brain tissues shows changes in their structures were observed during acute and sublethal sonata exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues. The present study also demonstrated that sonata has a high oxidative-stress-inducing potential in *Chana*

punctatus and *Macrobrachium rosenbergi*i and gill is the most sensitive organ in both acute as well as sub lethal concentration.

CONCLUSION

All the histopathological observation indicated that exposure to lethal concentrations of sonata caused destructive effect in the gill, liver and brain tissues of *C. Punctatus and M.rosenbergii*. Gil, liver and brain histopathological alterations, such as those observed in this study and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish and prawn. As a conclusion, the findings of the present histological investigations demonstrated a direct correlation between pesticide exposure and histopathological disorders observed in several tissues.

REFERENCES

1. Abhilash, P. C. and Nandita Singh.: Indian scenario J. Hazardous Materials, 2009; 165(1-3): 1-12.
2. Arillo, A., Margiocco, C., Melodia, F. and Mensi, P.: Chemosphere, 1982; 11(1): 47-58.
3. Reber, H.H. and Borkott H.: Natural Resources and development, 1983; 17: 29- 39.
4. Ganesan, R.M., Jebakumar, S.K.D. and Jayaraman, J.: Pro. Ind. Acad. Sci., (Anim. Sci.), 1989; 98: 51-55.
5. Anitha Kumari, S. and N.S.R Kumar.: Environ. Biol, 1997; 18(1): 11-16.
6. APHA: American Public Health Association, 16 edn (1995).
7. Humason, G.L.: Animal tissue techniques 111 (edition) (1972).
8. POLEKSIC, V. and MITROVIC-TUTUNDZIC, V.: Cambridge: Cambridge Univ. Press, 1994; 339-352.
9. MAZON, AF., MONTEIRO, EAS., PINHEIRO, GHD. and FERNANDES, MN.: Brazilian Journal of Biology, 2002; 62(4A): 621-631.
10. FERNANDES, MN. and MAZON, F.:Enfield: Science Publishers, 2003; 203-231.
11. MALLAT, J.: Canandiam Journal Fish of Aquatic Science, 1985; 42: 630-648.
12. Hinton, D. E. & D. J. Laurén.: In: McCarthy, J.F. & L.R. Shugart (Eds.). Boca Raton, Lewis Publishers, 1990; 51-65.
13. Jayantha Rao, K.: Ph.D., Thesis, S.V. University, Tirupati, A.P., India, 1982.
14. Dubale, M.S and Shah, P.: Indian Journal of Experimental Biology, 1979; 17(7): 693-697.
15. Rashatwar and Ilysas. Toxicol. Lett, 1984; 16: 249-252.
16. ANSARI, B.A. & K. KUMAR.: Sci. Total Environ, 1988; 76(I): 63-68.

17. Yacobu: Ecotoxicol. Environ. Monit, 1999; 12: 9-15.

18. Tilak, K.S., K. Veeraiah and D. Koteswara Rao. Pollut. Res, 2005b; 24: 101-111.

19. Das BK, Mukherjee SC.: Veterinarski Arch, 2000; 70(4): 169-180.

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ANTIOXIDANT STUDIES ON SONATA (FUNGICIDE) INDUCED STRESS IN CHANA PUNCTATUS AND MACROBRACHIUM ROSENBERGII

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ABSTRACT

Acute toxicity tests (48hr, 72hr, 96 hr LC50& Lethal concentration) of Sonata were conducted with two species (fish and prawn) viz. *Chana punctatus* and *Macrobrachium rosenbergii*. At the end of each trail, the animals were dissected and their organs viz. Liver, Gill and Brain were isolated for determination of antioxidant activity. Sonata was significantly more toxic in prawn than fish with 96hr LC 50 values of 12.094ppm and 15.399ppm respectively. Activity of SOD, Catalase, GSH and LPO increased with increasing sonata concentrations in the test mediums. The fish and prawn were kept in control conditions(without sonata stress) showed maximum activity of catalase, SOD,GSH and LPO. Among fish and prawn organs Liver appeared as a target organ that accumulates significantly higher contents of sonata followed by that of gill and brain.

KEYWORDS: Acute toxicity tests *Chana punctatus* and *Macrobrachium rosenbergii*.

INTRODUCTION

Pesticides cover a wide range of compounds used in pest control; including insecticides, fungicides, herbicides, rodenticides and molluscicides.[1,2] Pesticides and fungicides exert their toxic action on arthropods, mussels, fishes, frogs, turtles, water birds and other wild life too. Protection of wild life and water quality is possible when pesticide used selectively, and in combination with other pest control measures in a safe manner. The pollution of surface waters and contamination of aquatic life can be avoided. Excessive use leads to bioaccumulation in farm workers, fruits, vegetables, nuts and food crops, consumers, and it also causes biomagnification at various trophic levels of the food chain. Application of these compounds is most effective and accepted mean for protection of crops.[3] They accumulate

in liver, kidney, salivary glands and fat. Fish are useful bioindicators and integrators of contaminants. Pesticides generally affect the biological active molecules like carbohydrates, proteins, lipids and enzymes. Depletion of oxygen content occurs in medium when pesticides, chemicals, sewage and other effluents contaminating organic matter are discharged into water bodies.

One of the important manifestations of toxic action of chemicals is overstimulation or depression of respiratory activity. Respiration also plays an important role in study in aquatic toxicology. Aquatic orgnisms like prawns, fish, bivalves, crabs respire through gills and it causes reduction in oxygen consumption and leads to physiological imbalance in the organism.[4]

The extensive advantages of the utilization of pesticides are incompletely balanced by generous condition cost. An ecologically pervasive problem is widespread environmental contamination by pesticides, including the chemical compounds residues in aquatic life.[5] The contamination of water sources by pesticides may affect non target aquatic organisms including fish.[6,7,8]

Fish-toxin relationship has impressive significance in the investigation of fish populace and vitality designs in fish stocks of a particular area.[9] The field of fishes and pesticide contamination plainly shows that impacts of individual pesticides on various physiological and biochemical parts of fishes have been broadly examined by an expansive number of specialists.[10,11,12] Through the examinations involving the impact of individual pesticides on fishes have produced valuable information, the problem of pesticidal pollution becomes magnified when the runoff waters from cultivable land drain cause a wide spectrum of different pesticides accumulate into a particular fresh water body such as ponds, lakes or rivers.

Since the physiological changes that occur when organisms are exposed to sublethal levels of pressure could include rate of feeding as well as respiration and excretion, the net outcome could be a change in energy available for growth and reproduction. Since, pollutants uptake from water the most important route is gill the primary target and may be one of the first organs to exhibit symptoms of sublethal toxicity. Besides, there are biochemical parts like starches, for example, proteins and lipids that can likewise go about as vitality sources.[13]

MATERIALS AND METHODS

Animal collection: The fish and prawn specimens samples of the two varities namely *Channa punctatus* and *Macrobrachium rosenbergii* were collected from the freshwater lake located in waddepelly cheru, Warangal district. Specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies. Fish measuring 14-15cms in length and weighing 250-300gms and prawn measuring 14-18 cms in length and weighing 25-30gms specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies.

The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2^{o}C. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms,[14] were followed.

Fungicide selected for the study: Procurement of technical grade SONATA fungicides technical grade 00.0% purity was supplied by Hyderabad chemical supplies limited Hyderabad.

Sonata acute toxicity assays: The lethal concentrations ensure death even before noticing the behavioural abnormalities.[15] reported that sublethal exposures to longer periods may be dangerous to the organisms. Even when the animal is exposed to low concentrations continuously, many behavioural abnormalities and physiological alterations would be observed. In the present study 96 hr LC50 value was selected as sublethal concentration to study the behavioural alterations and physioloical alterations (As per the recommendations of committee on toxicity studies.[16,14]

Enzyme assays: The fish and prawn used in the acute (48-hr,72-hr,96-hr LC50) and lethal test trails were weighed and removed from the media. All the fish were dissected and organs viz. liver, brain and gills were removed. These organs were kept at -80°C for the further enzyme assays and biochemical analyses.

Preparation of extract: To remove RBCs the dissected organs of each fish and prawn were washed with phosphate buffer (pH 6.5), Organs were weighed and homogenate was prepared in phosphate buffer (0.2M, pH 6.5) with a ratio of 1: 4, respectively. These tissues

homogenates were then centrifuged at 10,000 rpm for 15 minutes at 4◦C. The clear supernatant was preserved and used for further enzyme analysis.

***1. Superoxide dismutase assay*:** The activity of superoxidedismutase was determined by measuring its ability to inhibit the O2- dependent reaction or to inhibit the photo-reductionof nitro-blue tetrazolium (NBT) by superoxide.[16]

***2. Catalase assay*:** The crude enzyme was subjected to enzyme assay and the activities of catalase were measured by following the method of Beer and Sizer (1952).[17] Catalase activity was concluded by measuring its ability to decrease the H2O2 concentration per minute at 240 nm.

3. Glutathione Reductase activity: The activity of Glutathione reductase was determined by measuring its ability to maintains adequate levels of reduced cellular GSH by Carlberg and Mannervik.[17]

4. Lipid peroxidation activity: The activity of Lipid peroxidation was determined by measuring of MDA has been used as an indicator of lipid peroxidation by Utley *et al.*[18]

Statistical analysis: The significance of sample mean between control and Sonata treated fish and prawn was tested using Student's "*t*" test.

RESULTS

The effect of sublethal concentration (15.399 ppm & 12.094 ppm) of sonata on metabolic enzyme activities in gill, liver and brain of *C. punctatus* and *Macrobrachium rosenbergii* is represented in Table 1 and 2. The antioxidant enzyme activities viz., SOD, CAT, GSH and LPO in fish *Chana punctatus* and in prawn *Macrobrachium rosenbergii* exposed to sublethal concentration of Sonata for 48hrs, 72hrs and 96hrs showed significant alterations when compared to control groups. A significant ($p < 0.05$) decrease in SOD activity was observed in gill, liver and brain of *Chana punctatus* and *Macrobrachium rosenbergii* throughout the study period when compared to the control group. The catalase activity in gill, liver and brain of sonata treated fish and prawn showed a significant ($p < 0.05$) decrease throughout the study period. The GSH activity in gill, liver and brain of sonata treated fish and prawn showed a significant ($p < 0.05$) decrease throughout the study period. The LPO activity in gill and brain showed a significant increase ($p < 0.05$) throughout the study period.

Table 1: SOD, Catalase, GSH, MDA activity in the Gill, liver and Brains of C. punctatus (n=5), exposed to the sublethal concentration of Sonata for 48,72,96hrs. The values given are mean ± S.E.M. *= p<0.05.

	Chana punctatus									
		Brain			Liver			Gill		
	Incubation	Control	Experiment		Control	Experiment		Control	Experiment	
			10 PPM	20PPM		10 PPM	20PPM		10 PPM	20PPM
SOD (IU/Mg/protein)	**48hrs**	581±1.78	483±1.42	352±1.43	332±0.62	261±0.90	122±1.21	63±1.56	50±1.31	44±0.79
	72hrs	662±0.71	544±0.81	486±1.22	447±1.75	397±1.30	269±0.83	70±0.78	58±0.52	39±1.73
	96hrs	735±0.96	653±1.01	535±0.99	523±0.05	423±0.09	323±0.99	82±1.00	50±0.45	39±0.56
Catalase (µmol/mg/protein)	**48hrs**	52±0.90	45±1.32	32±1.34	42±1.09	38±0.57	33±1.19	23±1.55	11±0.65	7±1.19
	72hrs	63±1.15	49±1.19	38±1.42	55±0.75	49±0.62	33±0.83	41±1.39	32±0.71	20±1.48
	96hrs	81±0.85	86±0.99	94±0.96	68±0.96	81±1.05	91±0.65	66±0.89	69±0.84	84±0.54
GSH (mU/mg/protein)	**48hrs**	702±0.95	582±1.39	422±1.10	441±1.23	389±0.79	312±1.14	115±1.29	87±0.87	62±1.45
	72hrs	783±0.82	676±1.42	579±1.23	512±1.35	443±0.90	333±1.36	154±1.48	139±1.21	129±1.69
	96hrs	815±0.58	915±1.56	996±0.98	598±0.98	625±1.91	721±0.58	172±0.68	175±0.95	197±0.87
MDA (nmol/mg/protein)	**48hrs**	6.68±0.94	7.36±1.16	8.29±1.22	4.66±0.84	5.88±1.61	5.99±0.88	0.54±0.89	0.83±1.10	1.7±0.78
	72hrs	8.03±0.99	9.25±1.05	12.1±1.28	6.11±1.09	7.63±1.52	8.72±0.92	1.39±0.92	1.91±1.33	2.9±1.13
	96hrs	12.58±0.58	13.49±0.96	18.7±1.45	11.78±1.58	9.58±1.68	11.05±1.02	1.76±0.68	2.54±0.84	5.1±0.85

Table 2: SOD, Catalase, GSH, MDA activity in the Gill, liver and Brains of M.rosenbergii (n=5), exposed to the sublethal concentration of Sonata for 48,72,96hrs. The values given are mean ± S.E.M. *= p<0.05.

	Macrobrachium rosenbergii									
		Brain			Liver			Gill		
	Incubation	Control	Experiment		Control	Experiment		Control	Experiment	
			10 PPM	20PPM		10 PPM	20PPM		10 PPM	20PPM
SOD (IU/Mg/protein)	48hrs	21.4±0.21	14.6±0.65	12.4±0.54	18±0.45	13±0.48	8±1.01	23.2±0.42	11.5±0.21	5.6±0.11
	72hrs	26±0.18	15±0.84	9.7±0.98	12±0.58	8±0.78	4±1.24	26.9±0.68	16.8±0.56	7.8±0.48
	96hrs	31±0.47	25±0.65	19.2±0.67	25±0.96	20±0.62	14±0.95	28.5±0.54	21.5±0.64	16.9±0.52
Catalase (µmol/mg/protein)	48hrs	24.8±0.21	21.5±0.84	17.4±0.47	22.4±0.58	12.4±0.34	7.9±0.48	29.5±0.62	17.5±0.15	9.5±0.48
	72hrs	29.5±0.19	19.9±0.65	13.8±0.58	27.9±0.69	17..7±0.68	14.5±0.78	33.4±0.84	26.8±0.39	13.5±0.68
	96hrs	37.6±0.35	25.6±0.48	11.1±0.24	32.4±0.47	23.9±0.75	12.1±0.96	38.7±0.56	24.5±0.96	11.8±0.56
GSH (mU/mg/protein)	48hrs	1.65±0.15	1.47±0.58	1.34±0.47	1.54±0.64	1.37±0.58	1.24±0.47	3.25±0.13	2.68±0.68	1.85±0.74
	72hrs	1.99±0.28	1.89±0.91	1.57±0.82	1.97±0.38	1.89±0.91	1.57±0.82	6.89±0.35	2.65±0.89	4.47±0.89
	96hrs	2.32±0.48	1.92±0.99	1.38±0.96	2.35±0.79	1.92±0.99	1.28±0.96	9.9±0.59	9.6±0.97	7.5±0.95
MDA (nmol/mg/protein)	48hrs	0.89±0.18	1.14±0.28	1.28±0.24	0.46±0.34	0.89±0.87	1.01±0.98	0.86±0.21	1.02±0.35	1.24±0.85
	72hrs	1.25±0.34	1.59±0.67	1.86±0.54	0.98±0.56	1.48±0.95	1.68±1.05	1.12±0.56	1.35±0.47	1.58±0.99
	96hrs	2.65±0.84	2.84±0.59	2.99±0.68	1.23±0.24	1.96±0.65	2.05±1.24	1.24±0.69	1.48±0.69	1.96±1.02

DISCUSSION

The toxicity of many contaminants in aquatic organisms is mediated through oxidative damage when reactive oxygen species (ROS) are formed. Under normal conditions, ROS are removed from the cell by the action of antioxidant defence systems. If the production of ROS is in excess, the balance between the formation and removal of ROS will be destroyed and it will produce the oxidative stress.[19] Lipid peroxidation (LPO) has been reported as a major contributor to the loss of the cell function under oxidative stress conditions and it is usually indicated by TBARS in fish.[20] To minimise the potential toxic effects of ROS, fish have evolved an enzymatic antioxidant defence system composed of SOD, CAT, GPx, GR and other molecules to inhibit the formation of oxygen radicals.[19] SOD is a primary oxygen radical scavenger of tissues converting the superoxide anion radical to H2O and H2O2.[21] CAT and GPx act cooperatively as scavengers of hydrogen peroxide (both enzymes) and other hydroperoxides (GPx).[22] GR plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathways.[23]

In the present investigation tissue specific responses in the activities of antioxidant enzymes such as SOD, catalase, GSH and LPO were observed during acute and sublethal sonata exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues and also the varied concentration of Sonata in these tissues as reported by Monteiro[24] The present study also demonstrated that sonata has a high oxidative-stress-inducing potential in *Chana punctatus* and *Macrobrachium rosenbergi*i and gill is the most sensitive organ in both acute as well as sub lethal concentration.

CONCLUSION

The results of the present investigation indicate that acute and sublethal exposure of sonata induces significant changes in the enzymatic profiles in *C. Punctatus and Macrobrachium rosenbergii*. The presence of such level of sonata in the natural environment is dangerous to the ecosystem and will definitely affect the survival of fish. Gills, due to their large surface area and permeability, are the primary sites for absorption. The experimental data obtained with *C. Punctatus and Macrobrachium rosenbergii* can be considered as a useful reference for comparisons with biomarker responses of organisms living in polluted environments. These parameters can be used as biomarkers in assessing the pesticide toxicity in aquatic ecosystem.

REFERENCES

1. Anwar, W. A: Environ Health Perspect, 1997; 105(4): 801-6.
2. Heath RGM, Claassen M (eds). WRC Report No. 428/1/99. (1999).
3. Bolognesi C.: Mut Res., 2003; 543: 251-272.
4. Mulley, H.E. and Beck, J.N.: Bull. Environ. Contam. Toxicol, 2012; 44: 768-804.
5. B.A. Freedman, J.D.: Biol. Chem, 1981; 256: 10986-1099.
6. Hernández F, Morell I, Beltrán J, López FJ., 1993; 37: 303-312.
7. Rastogi, A. and Kulshrestha, S.K.: Bull. Environ. Contam. Toxicol, 1990; 45: 742-747.
8. Parvathi, M.L.S., Reddy, C.S. and Chetty, N.: Pollution Research, 2000; 19: 345-351.
9. Peña-Llopis S, Ferrando MD, Peña JB.: Aquatic Toxicol, 2003; 65: 337–360.
10. Rameshthangam P. and Ramasamy, P.: Aquaculture, 2006; 254: 32–39.
11. Mishra, R. and Shukla, S.P.: Ecotoxicology and Environmental Safety, 1998; 56: 425-433.
12. Torreblanca, A., Diaz-Mayans, J., Del Ramo, J., and Nunez, A.: Comp. Biochem. Physiol, 1987; 86C: 219–224.
13. APHA : American Public Health Association.: 16 edn, (1995).
14. Anderson JM, Peterson MR.: Science, 1969; 164-440.
15. Beachamp and Fridovich Superoxidedismutase activity, 1971.
16. Beer and Sizer Catalase activity, 1952.
17. Carlberg and Mannervik Glutathione reductase activity, 1985.
18. Utley.et.al Lipid peroxidation activity, 1967.
19. Li Z.H., Zlabek V., Grabic R., Li P., Machova J., Velisek J., Randak T.:Aquatic Toxicology, 2010; 98: 297-303.
20. Oakes K.D., Van der Kraak G.J.: Aquatic Toxicology, 2003; 63: 447-463.
21. Nordberg J., Arner E.S.J.: Free Radical Research Biology and Medicine, 2001; 31: 1287–1312.
22. Gate L., Paul J., Ba G.N., Tew K.D., Tapiero H.: Biomedicine and Pharmacotherapy, 1999; 53: 169–180.
23. Cazenave, J., Wunderlin, D.A., Bistoni, M.A., Am´e, M.V., Krause, E., Pflugmacher, S., Wiegand, C,: Aquat. Tox-icol, 2005; 75: 178–190.
24. D.A. Monteiro, F.T. Rantin, A.L. Kalinin.:Comp. Biochem. Physiol. C, 2009; 149: 40-49.

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ACETYLCHOLINESTERASE GENE EXPRESSION STUDIES ON SONATA (FUNGICIDE) INDUCED STRESS IN *CHANNA PUNCTATUS* AND *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

Acute toxicity tests (48hr, 72hr, 96hr LC50& Lethal concentration) of Sonata were conducted with two species (fish and prawn) viz. *Channa punctatus* and *Macrobrachium rosenbergii*. Acetylcholinesterase (AChE) is a key enzyme in the nervous system. It terminates nerve impulses by catalysing the hydrolysis of neurotransmitter acetylcholine. After serving as a neurotransmitter, acetylcholine is hydrolyzed by acetylcholinesterase (AChE). Inhibition of AChE is considered to be a specific biomarker for exposure to fungicides. In the present study, Acetylcholinesterase AChE genes were used in *Channa punctatus* and *Macrobrachium rosenbergii* brain were analyzed using real-time Polymerase chain reaction PCR to determine alterations in gene expression levels after Sonata fungicide treatment. The AChE gene was isolated from fish and prawn brain by RT-PCR methods using degenerate primers.RNA isolated from two samples of Channa punctatus and Macrobrachium rosenbergii and they were subjected to agarose gel electrophoresis.. cDNA (Complementary DNA) will be synthesized from RNA and complementary DNA will be subjected to RT-PCR. The amplified fragment of 800 nucleotides generated by PCR was cloned, and sequence analysis showed 80% nucleotide identity with AChE of the Electrophorus electricus. Specific primers of the fish and prawn AChE gene were then synthesized and used in the examination of AChE gene expression in brain tissue of fish and prawn exposed to sub lethal concentrations of sonata (36.05, 18.19 and 15.39ppm) and (18.40, 15.39 and 12.09ppm) for 48 hr, 72hr and 96 hr. RT-PCR was used to compare with the amplified GAPDH gene. Acetylcholinesterase gene expression got down regulated in *Channa punctatus and* up regulated in *Macrobrachium rosenbergii* after exposing of fungicides in comparison to the control group.

KEYWORDS: ***Acetylcholinesterase, Fungicide, Sonata, Channa punctatus, Macrobrachium rosenbergii, Real-time PCR.***

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INTRODUCTION

Agriculture is the primary source of food for millions of people living in this world. More importantly, it is a major supplier of raw material and manpower for manufacturing and services sectors. Sustainable agronomic development is at the heart of implementing the economic development policies. In India, policy makers continue to consider agricultural development as the most important objective of planning and policy. Pesticides play an important role in keeping the plant healthy. They protect the plant from many outrageous diseases. However, both animals and human beings exposed to pesticides suffer from health problems. Currently, in the Asian continent, India is the largest producer of pesticides. With respect to using pesticides, it ranks twelfth in the world. Compared to many other countries, India's average consumption of pesticides is low. However, the problem of pesticide residue is high.[1] With increase of pest attacks, the use of chemical pesticides is also increased. When pesticides are used, the crop loss amounts to 20-30% of total crop. When the pesticides are not used, the loss amounts to 50-80% of the total crop. Pesticides have become popular because they are convenient to use, inexpensive and provide quick control of pests. The use of pesticides has increased significantly since 1950. Every year, about 2.5 million tons of industrial pesticides are produced and used across the world (USEPA, 2005). The pollution of water sources by pesticides can affect even the non-target aquatic organisms like the fish .[2-3] In the investigation of fish populace and vitality designs in fish stocks of a particular area, the fish-toxin relationship plays an important role.[4] A review of writing in the field of fishes and pesticide contamination plainly shows that impacts of individual pesticides on various physiological and biochemical parts of fishes have been broadly examined by an expansive number of specialists.[5-7] When the literature in the field of fishes and pesticide contamination is examined, it is revealed that impacts of individual pesticides on various physiological and biochemical parts of fishes are thoroughly analyzed by a number of specialists. Because the physiological changes that happen when organisms are subjected to sublethal levels of pressure could comprise of rate of feeding, and respiration and excretion, which ultimately effects the final outcome could be a difference in energy available for growth and reproduction. Acetyl cholinesterase is an enzyme that through its hydrolytic activity degrades the neurotransmitter acetylcholine into its components choline and acetate. This enzyme is found in the neuromuscular junctions and cholinergic nervous system. It is mainly involved in the termination of synaptic transmission and has a high catalytic activity. In the present study, AchE gene expression was carried out on *Channa punctatus and Macrobrachium rosenbergii*.

MATERIALS AND METHODS

Animal collection

The fish and prawn samples of the two verities namely *Channa punctatus* and *Macrobrachium rosenbergii* were collected from the freshwater lake located in Waddepally, Warangal district. Fish measuring 14-15 cms in length and weighing 250-300 gms and prawn measuring 14-18 cms in length and weighing 25-30 gms specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies. The specimens of fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2^{o}C. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms[8] were followed.

Fungicide selected for the study

Procurement of technical grade SONATA fungicides was supplied by Hyderabad chemical supplies limited, Hyderabad.

Experimental design

The fish and prawn used in the acute (48-hr, 72-hr,96-hr LC50) and lethal test trails were weighed and removed from the media. Tissue processing after the desired time period, control and treated animals were sacrificed by cervical dislocation. All the fish and prawn were dissected and organs viz. liver, brain and gills were removed. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. These organs were kept at -80°C for the further enzyme assays and biochemical analyses and histopathological observations. In this study, Brain tissue is taken for gene expression studies.

Total RNA isolation

The total RNA was isolated by using Trizol method according to Chomczynski and Mackey K. (1995). RNA will be isolated from brain of *Channa punctatus* and *Macrobrachium rosenbergii* and were subjected to agarose gel electrophoresis. The pictures were taken using a gel doc (Bio-Rad).

Reverse transcription-polymerase chain reaction (RT-PCR)

Complementary DNA(c DNA) was synthesized from RNA by using one step takara kit method. Primers of AChE gene were designed in conserved region of fishes and other organisms from GENBANK using CODEHOP program. Primer

For the PCR reaction, 2 µl of cDNA from each synthesis was added to 10 µl of 'Syber PCR master mix' containing 5X PCR buffer, 10 mMdNTP, 25 mM $MgCl_2$and 5 U of Taq DNA polymerase (Fermentas, USA). Twenty µM of each pair of the primers was added, and the final volume was adjusted to 14 µl with nuclease free water. The mixtures were denatured at 94^{o}C for 3 min. Thirty five cycles of PCR were carried out with denaturation at 94 ^{o}C for 45 sec, annealing at 57^{o}C for 30 sec, and extension at 72^{o}C for 1 min, followed by a final extension period of 5 min. PCR products were analyzed by electrophoresis on 1% agarose gels stained with GelStar Nucleic Acid Gel Stain (Cambrex Bio Science Rockland, Inc.).

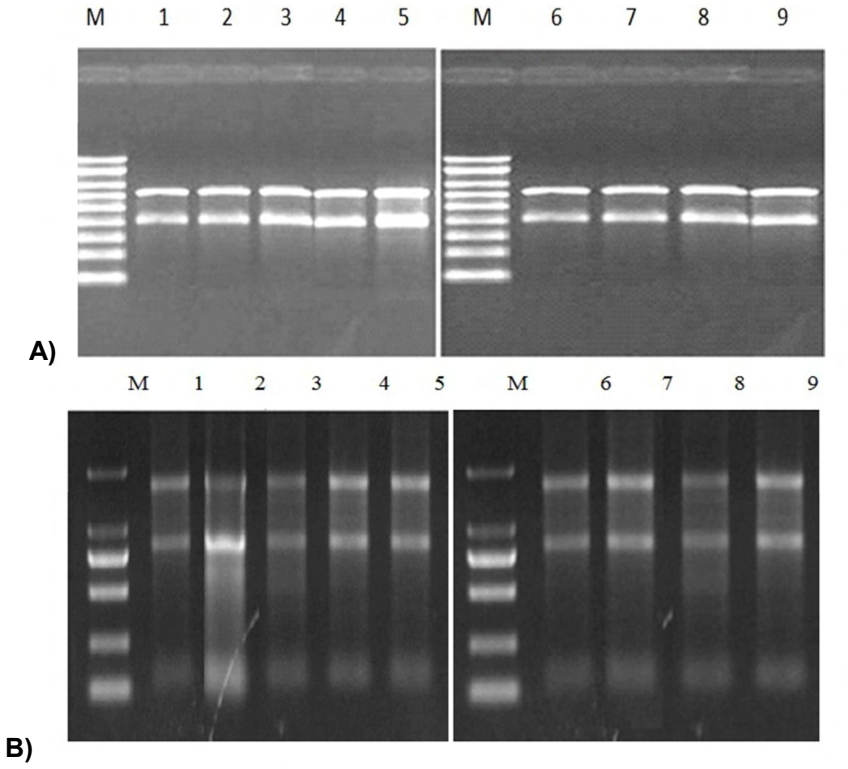

Figure 1

A) Agarose gel images of RNA isolation of brain Samples of Channa punctatus: M-Marker, 1-Control 48 hours, 2- dose I48 hours, 3- dose II 48 hours, 4- Control 72 hours , 5- dose I 72 hours, 6- dose II 72 hours, 7- Control 96 hours, 8- dose I 96 hours , 9- dose II 96 hours.

B) Macrobrachium rosenbergii-M-Marker, 1-Control 48 hours , 2- dose I 48 hours, 3- dose II 48 hours, 4- Control 72 hours , 5- dose I 72 hours, 6- dose II 72 hours, 7- Control 96 hours, 8- dose I 96 hours, 9- dose II 96 hours.

RESULTS AND DISCUSSION

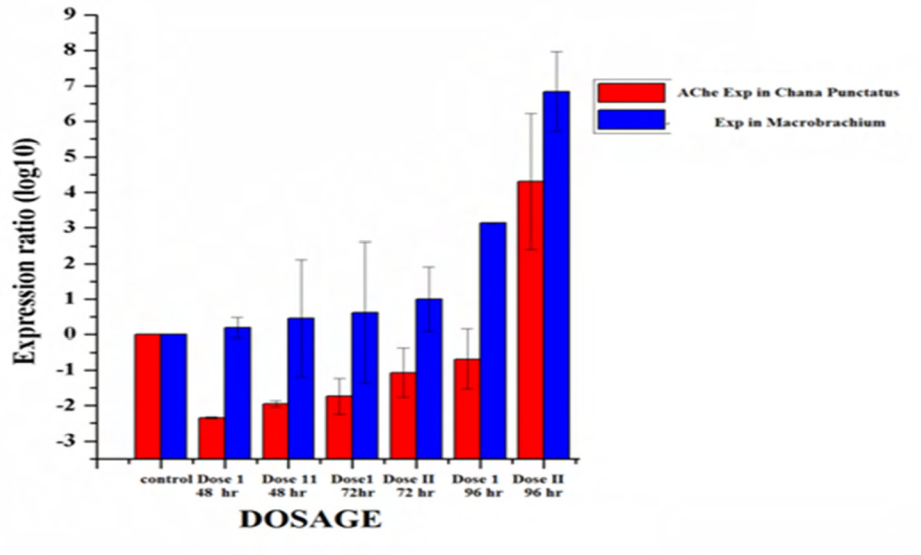

Figure 2

Comparative Graph of AchE gene Expression in Channa punctatus and Macrobrachium rosenbergii after treatment from 48 hr to 96 hr.

Comparative Graph reveals AchE gene expression in *Channa punctatus* and *Macrobrachium rosenbergii* after treatment from 48 hr to 96 hr. In 48 hr treatment expression fold of *Channa punctatus*is down regulated as compared to control but *Macrobrachium rosenbergii* has little high expression fold which represent gene up regulated as compared to control in Dose 1 and Dose II. In 72 hr treatment, expression fold of *Channa punctatus* again down regulated as compared to control whereas *Macrobrachium rosenbergii* is up regulated in both set of dosage i.e., Dose 1 and Dose II. In 96 hr treatment, Dose I again shows down regulation for *Channa punctatus* and *Macrobrachium rosenbergii* again is found undergoing up regulation. In Dose II *Channa*

punctatus shows little up regulation with treatment whereas *Macrobrachium rosenbergii* shows very high expression which suggest good up regulation of gene with treatment. In this study, expression fold for *Macrobrachium rosenbergii* is up regulated with increase in time for both set of dosage but *Channa punctatus* up regulates in 96 hr treatment in dose II. as shown in figure 2. Acetylcholinesterase is an enzyme whose hydrolytic activity degrades the neurotransmitter acetylcholine into its components choline and acetate. This enzyme is found in the neuromuscular junctions and cholinergic nervous system. It is mainly involved in the termination of synaptic transmission and has a high catalytic activity. It hydrolyses 25000 acetylcholine molecules per second.[9] Theactivity of this enzyme has been shown to reduce upon methylmercury exposure in Medekafish.[10] In the present study Acetyl Cholinesterase AChE genes were used in *Channa punctatus* and *Macrobrachium rosenbergii* brain by using real-time PCR to determine alterations in gene expression levels after Sonata fungicide treatment. The outcome of RT-PCR analysis revealed that Acetyl Cholinesterase gene expression got down regulated in *Channa punctatus and* up regulated in *Macrobrachium rosenbergii* after getting exposed to fungicides induction. Acetyl Cholinesterase genes AChE gene expression is an important part of the cholinergic neuronal systems for the protection of CNS-central nervous system homeostasis.[11] Up-regulation may be ascribed to the feedback response of transcription to depressed cholinergic neurotransmission, leading to elevated levels of brain acetylcholine following sonata exposure. An autologous feedback response could regulate transcriptional elevation from the AChE gene through insecticide complexes acting on signalling intracellular pathways.[12] They are found in the brain of the mouse that diisopropyl fluorophosphate (DEF) (an OP insecticide) and pyridostigmine (a CB insecticide) increased levels of AChE mRNA over controls within 30 minutes; using sagittal hippocampal brain slices of mice.Even moderate changes in neuronal excitability may direct to overt modulations in brain gene expression.[12] Therefore, the up-regulation and down regulation of these two genes in *Channa punctatus* and *Macrobrachium rosenbergii* are responsible for changing fatty acid biosynthesis after Sonata fungicide treatment. This alteration was first reported in relation to Sonata fungicide treatment toxicity in fishes and prawns. The levels of acetylcholine are continuously regulated by the hydrolytic enzyme acetylcholinesterase (AChE), which rapidly degrades acetylcholine in the periphery and the brain. AChE is expressed in cholinergic neurons and neuromuscular junctions as well as tissues that are not innervated by cholinergic neurons.[13-14] In order to verify whether or not the AChE gene could be modulated when fish and prawn were exposed to the fungicides, the gene expression of AChE was investigated. The results showed that Sonata can alter the gene expression of AChE in fish and prawn of brain.

CONCLUSION

The early biological changes caused by xenobiotics may not be manifested as the pathological findings, but rather as inductions in the expression of the genes. In the case of the AChE gene, we observed such inductions in the fish and prawn following the exposure to sonata, indicating a potential biomarker for the early detection of fungicide contamination. Results from this study indicated that gene expression technique is quite sensitive, rapid and reliable than AChE enzyme activities and should be applied as a screening tool for detection of the fungicides contamination in fish and prawn.

AUTHORS CONTRIBUTION STATEMENT

S. Swetha carried out the experiment and wrote the manuscript with the support of Dr. E.Narayana who also supervised the experiment.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Abhilash PC, Singh N. Pesticide use and application: An Indian scenario. J Hazard Mater. 2009;165(1–3):1–12. DOI: 10.1016/j.jhazmat.2008.10.061
2. Hernández DA, Lombardo RJ, Ferrari L, Tortorelli MC. Toxicity of ethyl-parathion and carbaryl on early development of sea urchin. Bull Environ Contam Toxicol. 1990;45(5):734–41. DOI: 10.1007/bf01700994
3. Rastogi A, Kulshrestha SK. Effect of sublethal doses of three pesticides on the ovary of a carp minnowRasbora daniconius. Bull Environ Contam Toxicol. 1990;45(5):742–7. DOI: 10.1007/bf01700995
4. Parvathi ML, Reddy DC CA. In Vivo Recovery and Long Term Effect of Phosalone on Total Lipid and Trigl ycerides in Freshwater Fish, Tilapia Mossambica (Peters). Pollut Res. 2000;19(3):345–51.
5. Ferrando MD, Alarcon V, Fernandez-Casalderrey A, Gamon M, Andreu-Moliner E. Persistence of some pesticides in the aquatic environment. Bull Environ Contam Toxicol. 1992;48(5). DOI: 10.1007/bf00195997
6. Ramaswamy M, Thangavel P, Dhanalakshmi S, Govindaraj P, Karuppiah D. Comparative Study on the Synergistic and Individual Effects of Dimecron and Cuman L on Oxygen Uptake and Haematological Parameters of a Freshwater Edible Fish, Sarotherodon mossambicus (Peters). Bull Environ Contam Toxicol. 1996;56(5):796–802. DOI: 10.1007/s001289900116
7. Pulla R MK. Acute toxicity of LABS containing detergent 'Shudh'to juvenile (fingerling) stage of Indian major carp. Labeo rohita. 1998;34(7).
8. Eaton AD, Clesceri LS, Greenberg AE FM. Standard methods for the examination of water and wastewater. Am public Heal Assoc. 2005;21:1600.

9. Prosser CL BF. Comparative animal physiology. Am J Med Sci. 1962;243(6):818.
10. Lee C-C, Peters PJ. Neurotoxicity and Behavioral Effects of Thiram in Rats. Environ Health Perspect. 1976;17:35. DOI: 10.2307/3428607
11. Damodaran T V, Jones KH, Patel AG, Abou-Donia MB. Sarin (nerve agent GB)-induced differential expression of mRNA coding for the acetylcholinesterase gene in the rat central nervous system. Biochem Pharmacol. 2003;65(12):2041–7. DOI: 10.1016/s0006-2952(03)00160-6
12. Kaufer D, Friedman A, Seidman S, Soreq H. Anticholinesterases induce multigenic transcriptional feedback response suppressing cholinergic neurotransmission. Chem Biol Interact. 1999;119–120:349–60. DOI: 10.1016/s0009-2797(99)00046-0
13. Somnuek C, Cheevaporn V, Saengkul C BF. Variability in acetylcholinesterase upon exposure to chlorpyrifos and carbaryl in hybrid catfish. Sci Asia. 2007;33(301–5). Available from: http://scienceasia.org/2007.33.n3/v33_301_305.pdf
14. Zinkl JG, Shea PJ, Nakamoto RJ, Callman J. Brain cholinesterase activity of rainbow trout poisoned by carbaryl. Bull Environ Contam Toxicol. 1987;38(1):29–35. DOI: 10.1007/bf01606553

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DECLINING FEMALE LABOR FORCE PARTICIPATION IN THE EMERGING YOUNGEST NATION – A STUDY OF RURAL WOMEN IN THE STATE OF TELANGANA

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ABSTRACT

India, by 2020 is set to become the world's youngest nation with its population's average age at 29 years. The demographic dividend that our country gains cannot deliver positive results unless and until the human resources are harnessed effectively and efficiently. The present study endeavors to explore the reasons for declining female labor force participation in the rural areas of Telangana state. Random sampling technique was used to collect data from 200 respondents. The survey reveals the fact that education by itself do not facilitate women empowerment in rural areas. There is a dire necessity to focus on providing alternative employment sources to agriculture to the qualified women.

Keywords: Harnessed, human resources, empowerment, facilitate, employment.

INTRODUCTION

India is the biggest democracy in the world with a population of 1.2 billion. India is set to experience a dynamic transformation as the population burden of the past turns into a demographic dividend. In three years, India will become the world's youngest country. By the year 2020, India's average age will come to rest at 29 years. The findings of the 'State of the Urban Youth, India 2012: Employment, Livelihoods, Skills,' a report published by IRIS Knowledge Foundation in collaboration with UN-HABITAT trace that the population in the age-group of 15-34 increased from 353 million in 2001 to 430 million in 2011. Current predictions suggest a steady increase in the youth population to 464 million by 2021 and finally a decline to 458 million by 2026. By 2020, India is set to become the world's youngest country with 64 per cent of its population in the working age group. With the West, Japan and even China aging, this demographic potential offers India and its growing economy an unprecedented edge that economists believe could add a significant 2 per cent to the Gross Domestic Product (GDP) growth rate[1].

The demographic dividend that our country gains cannot produce positive results unless and until the human resources are harnessed effectively and efficiently. As stated by Aristotle "good citizens make a good state" it is a dire necessity to chisel the emerging young citizens and equip them with proper education, knowledge and skills to arrive at a good state. Demographic

dividend if goes hand in hand with poverty, illiteracy, gender bias, social and economic inequality, terrorism and social unrest will ultimately lead to peril.

ORIGIN OF RESEARCH PROBLEM

A survey conducted by Young Lives India - the Indian Chapter of Young Lives, a study of childhood poverty in 2016, funded by the University of Oxford, UK – in undivided Andhra Pradesh since 2002 revealed that 'half of the women stay home or were married by age 22 and do not prefer to work'[2].

The logical link that education should lead to jobs is broken in India. In rural India, 67% of girls who are graduates do not work. In towns and cities, 68.3% of women who graduate don't have paid jobs, says a 2015 report by the United Nations Development Programme (UNDP), Women's Voices, Employment and Entrepreneurship in India[3].

These data lend weight to other studies that show Indian women are at a significant and possibly widening disadvantage. Gap between men and women has widened on political empowerment, healthy life expectancy and basic literacy, resulting in India slipping 21 places to 108 in 2017 from 87 in 2016 on the Global Gender Gap Index of the World Economic Forum, FactChecker reported on November 3, 2017[4].

The present study is to identify the reasons for the women confining themselves to homes though they are adequately qualified to take up employment. This study also identified the measures to be taken to bring women into the active workforce thereby providing them economic justice and economic development for the nation.

CONSTITUTIONAL PROVISIONS

The Indian constitution has provided many provisions to facilitate women's right to economic justice and proper working conditions for women. The Directive Principles enshrined in Part IV of the constitution is an instrument of instructions to both union and state governments in ushering a classless and socialistic society.

a) **Article 23(a)** - the state to secure for men and women equally the right to an adequate means of livelihood.
b) **Article 39(b)** - the state to secure equal pay for equal work for both Indian men and women.
c) **Article 39(e)** - the state is required to ensure that the health and strength of women workers are not abused and that they are not forced be economic necessity to enter avocations unsuited to their strength.
d) **Article 42** – the state shall make provision for securing just and humane condition of work and maternity relief.

INTER-DISCIPLINARY RELEVANCE

The present study carries interdisciplinary relevance at large. To one degree or another, solutions to social, political, intellectual, and economic problems do not lie in a single focus. The

present study provides a background for approaching issues holistically through an understanding of the complexity and interconnectedness of gender bias as a stigma in economic development. This study draws the fiber from Political Science, Economics and Sociology to weave a fabric to derive the reasons for gender inequality and the reasons for the educated women not joining into the workforce.

SIGNIFICANCE OF THE STUDY

Women empowerment, gender equality and contribution of women to the economic development of the country carry a lot of significance when India is about to become the youngest nation in the world. The demographic advantage that the country is going to get shall be full-fledged only when there is inclusive development catering to the needs of all the sections of population.

But, unfortunately even after seven decades of independence, the governments and civil society were complacent towards women empowerment. Social and cultural norms too, at large stood as barriers for women to explore new opportunities. The recent statistics throw light on the fact that nearly 50 per cent of women in both Telangana and Andhra Pradesh states are confining to homes after the age of 22 in spite being educated. India has one of the lowest female participation rates in the world, ranking 120th among the 131 countries for which data are available. Even among countries with similar income levels, India is at the bottom, together with Yemen, Pakistan and Egypt. Worse still, the rate has been declining since 2005.

This is a matter of concern as women's paid employment is known to increase their ability to influence decision-making within the household, and empower them more broadly in society as a whole.

In this context this study tries to elucidate the reasons for low female labor participation in the state of Telangana and thereby attempts to derive at measures to address this lacuna.

LIMITATIONS OF THE STUDY

The present study is confined to understanding the reasons for low women labor participation in rural areas of Telangana state. This leaves a larger gaps like urban scenario, political participation of women, and cultural factors to be studied in future research.

REVIEW OF LITERATURE

It is imperative to have a thorough review of the previous studies on this topic to understand what the other research scholars have already explored through their research studies, books and articles. Prominent among them are:

1. Policy Research Working Paper titled "Precarious Drop - Reassessing Patterns of Female Labor Force Participation in India" written by Luis A. Andres, Basab Dasgupta, George Joseph, Vinoj Abraham and Maria Correia, Publsihed by World Bank Group - South Asia Region Social Development Unit, April 2017. This research paper provides

a description of nearly two decades of patterns and trends in female labor force participation in India from 1993–94 to 2011–12.

2. Research Paper on ”Empowerment of Women Representatives in Panchayati Raj Institution in Gulbarga District in Karnataka” authored by Dr.K.N.Doddamani and published by Quest Journals Journal of Research in Agriculture and Animal Science Volume 2 ~ Issue 3 (2014) pp:09-14. This paper tries to explore women empowerment by providing political rights at grass root level.
3. ‘Half a Billion Rising: The Emergence of the Indian Women” book written by Anirudha Dutta and published by Rupa Publications Private Limited. This book analyzes the change drivers and the repercussions of present-day gender revolution. It also surveys how society at large and men in particular are reacting to the rise of women power.

OBJECTIVES

1. To estimate the extent of the recent decline in female labor force participation in the target area.
2. To assess the reasons for women not joining into work force.
3. To examines and assesses the contribution of various demographic and socioeconomic factors in explaining the female labor force participation decision and the recent drop.
4. To analyze weather education is the sole factor enough to help women to attain economic liberty by gaining employment.
5. To identify solutions to bring more women to embark on the journey for achieving economic liberty by attaining employment opportunities.

RESEARCH METHODOLOGY

In the completion of the study empirical and descriptive methods are adopted, specifically the methodology adopted is **Exploratory**, to inquire the extent of women participation as labor force in target area; **Descriptive**, to making careful observations and detailed documentation of reasons for women confining to homes and; **Explanatory**, in the sense, to analyze the observed phenomenon.

The conclusions derived in this study are based on primary data through questionnaires and secondary data through books, journals, magazines, news papers and internet. The selection of respondents was through random sampling. This survey aimed at eliciting information on the respondent’s marital status, educational qualifications, skills acquired and their participation into labor force. The research design adopted for the study is the preparation of questionnaires to collect the opinion of 200 respondents from different sections of women in rural areas of Telangana, with special focus on women belonging to Scheduled Tribes. Survey was conducted in villages like Janakinagar, Sithal thanda Gudibanda, Chilukuru, Narayanapuram, Dondapadu, Balajinagar, Ramalaxmipuram of Suryapet district of Telangana state.

FINDINGS & DISCUSSIONS

Jawaharlal Nehru once remarked, **"I have long been convinced that a nation's progress is intimately connected with the status of its women"**. In the wake of waves of economic liberalization, the condition of India – when thought of in terms of economic and human development- has improved dramatically.

Yet, while the status of women has arguably improved in both the public and private spheres, their ability to access opportunities in this newly liberalized economy remains precarious.

India's Female Labor Force Participation (FLFP) rate has remained visibly low; the ILO (2013) ranks India's FLFP rate as 121 out of 131 countries, one of the lowest in the world. In 2013, India had the lowest FLFP rate in South Asia, with the exception of Pakistan. Globally, only parts of the Arab world held a lower FLFP rates than India in the same year[5].

Moreover, the FLFP rate dropped from 49.0 percent to 37.8 percent in rural areas between 2004-05 and 2009-10 (NSSO, 2011), despite an impressive annual GDP growth rate of around 8.6 percent, and an annual population growth rate of 1.74 percent. The same pattern continued into the most recent round of the National Sample Survey (NSSO) in 2011-12. Among the Asian economies, only China experienced a marginally higher drop in FLFP rate from 1990 to 2013. However, in comparison to India, China's FLFP rate remained considerably higher at 64 percent. Pakistan, which had lower FLFP than India in 2013, experienced a sharp rise in women's participation in the labor force during 1990-2013. Further, for the first time in recent history, estimates suggest that between 2004-05 and 2009-10, not only was there a decline in India's FLFP rate, but also a shrinking of the total female labor force.

If the number of women who quit jobs in India between 2004-05 and 2011-12 (the last year for which census data is available), was a city, it would, at 19.6 million, be the third-most populated in the world, after Shanghai and Beijing.

Only 27% Indian women are currently in the labor force. Among G-20 countries, only Saudi Arabia is worse, IndiaSpend reported on April 9, 2016. Within South Asia in 2013, India had the lowest rate of female employment after Pakistan. In over two decades preceding 2013, female labor force participation in India fell from 34.8% to 27%, according to an April 2017 World Bank report[6].

India's female labor force participation rate, at 24%, was below the world average of 39% in 2016, according to World Bank data. India was ranked 172 among 185 nations for which data were available.

Not only were fewer women training themselves for the labor market, far more women were married by age 22 in 2016 than men, according to research by Young Lives India – the India chapter of Young Lives, a study of childhood poverty funded by the University of Oxford, UK – in undivided Andhra Pradesh since 2002.

These data lend weight to other studies that show Indian women are at a significant and possibly widening disadvantage. Gap between men and women has widened on political empowerment, healthy life expectancy and basic literacy, resulting in India slipping 21 places to

108 in 2017 from 87 in 2016 on the Global Gender Gap Index of the World Economic Forum, FactChecker reported on November 3, 2017.

"India remains the fastest growing economy in the world and it will get a big boost from its approach to GST which will - reduce the cost of doing business for firms, reduce logistics costs of moving goods across states, while ensuring no loss in equity," said Junaid Ahmad, World Bank Country Director in India. "Low female labor force participation, however, remains a serious concern. Higher level of women participation in the economy can help propel India closer to double digit growth"[7].

This paper attempts to conducts an empirical examination to better understand the socioeconomic milieu and demographic dynamics of this downslide in FLFP during the current decade. This study, through the data collected, endeavors to track insights into finding the reasons for the decline in FLFP and to explore the drivers to steer the women population into the productive activity there by ushering a gender equitable and socialist society.

1. Table 1 reflect that out of 200 respondents of the target area 76% belong to ST, 20% belong to SC and 04% belong to BC.

Community wise categorisation of respondents

Sl. No	Caste	Respondents
1	SC	40
2	ST	152
3	BC	8
4	OC	0

2. Table 2 highlights the educational qualifications of the respondents. Out of the respondents 15% are Post Graduates, 32% are Graduates, 33% completed Intermediate, 17% SSC and 3% are illiterates.

Educational Qualifications of Respondents

Sl. No	Qualification	Respondents
1	Illiterates	6
2	SSC	34
3	Intermediate	66
4	Graduation	64
5	Post Graduation	30

3. Table 3 details about the Employment Oriented Courses done by the respondents. This data reflects that 9% undertook Nursing training, 16% done TTC/B.Ed and 1% underwent Lab Technician training. This survey reveals that nearly 74% of the respondents are not seriously inclined to pursue any certified employment oriented course

for practical employment. Though majority of the respondents have regular academic qualifications, they are least equipped with employability skills.

Employment Oriented Courses

Sl. No	Course	Respondents
1	Nursing	18
2	TTC/B. Ed	32
3	Lab Technician	2
4	Mushroom Culture	0
5	Librarian	0

4. Table 4 highlights about the Skill Enhancement Training undertook by the respondents. This data reflects that 32% undertook Basic Computer Course, 29% tailoring, 10% Beautician, and 29% failed to undertake any kind of skill enhancement training.

Skill Enhancement training by respondents

Sl. No	Course	Respondents
1	Basic Computers	64
2	Tailoring	58
3	Beautician	20
4	Mobile Repair	0
5	Untrained	58

5. Table 5 explores the present daily grind of the respondents. The survey show that 58% are either house wives or confined to homes, 16% are pursuing agriculture, 15% are engaged in private jobs, 1% are in Government Service, 2% are running business and 8% are continuing their caste based occupation.

Present occupation of the respondents.

Sl. No	Present Occupation	Respondents
1	House Wife	116
2	Agriculture	32
3	Private Job	30
4	Government Job	2
5	Business	4
6	Traditional Occupation	8

6. Table 6 examines the participation of women in Self-help groups. The study projects that 19% are members of DWACRA group, 4% are members of Sthrinidhi and 78% are inactive in any of the self-help groups.

Membership in Self-help groups

Sl. No	SHG	Respondents
1	DWACRA	38
2	STHRINIDHI	8
3	OTHERS	0

OBSERVATIONS

1. The logical link that education should lead to jobs do not hold ground among women in India in rural areas. India's first chief statistician and country Head for the International Growth Centre's India Central Programme Mr. Pronab Sen observed that, "More girls are being educated than boys, but do not know where they are going,"[8] .

   The survey sheds light on the fact that, though 97% of the respondents are educated (15% are Post Graduates, 32% are Graduates, 33% completed Intermediate, 17% passed SSC) only 18% of the women joined into work force.
2. Out of 97% of educated women, 58% are confining to homes without pursuing any active employment. Either they are married or are disinterested in pursuing any employment.
3. The survey reveals that the rural society is in favor of educating the girls, but stands far behind in giving equal opportunity with men to pursue employment and attain economic independence. The data extol that 71% of the women have undertaken Skill Enhancement Training (32% Basic Computer Course, 29% Tailoring, 10% Beautician). But, of these girls, only 18% have taken up employment which needs to be addressed.
4. 24% of the educated women are under employed and still pursuing traditional occupations like agriculture and caste based occupations (16% agriculture & 8% caste based occupations).
5. **F**ewer jobs in agriculture have not been replaced by alternative jobs considered suitable for women.
6. 78% of the women are not active participants of any self – help groups which are initiated to give a credit base to women at rural areas.
7. Cultural and societal prejudices still play a pivot role in deciding the status of women in the society. Social norms about appropriate behavior for women and the enforcement of these norms by parents, in-laws and husbands dictates their ability to seek employment. The 2011 Indian Human Development Survey finds that a sizeable number of women need to take permission from a family member to even go to the market or health centre, said Rohini Pande of Harvard Kennedy School[9].

SUGGESTIONS

1. Women in rural areas, because of their social and cultural milieu, family obligations and for security reasons are unable to travel to cities for pursuing employment. Ergo, it is dire necessity to provide more employment opportunities for women in the rural areas.

2. Obvious solution to bring more women into workforce is skilling. It is important to skill young women to meet what industry demands. Department of Women Development & Child Welfare, Government of Telangana has in the recent past has launched number of projects for women like – Arogya Lakshmi, Balamrutham, Early Childhood Care and Education, Supplementary Nutrition Programme, Care and Nutrition Counselling Service and Child Protection[10]. Though the above projects are playing an instrumental role in taking care of health and hygiene of women, there is an immediate need to formulate policies that provide employment opportunities to women in the rural areas.
3. Telangana is one of the few states where a separate Department is functioning for development and welfare of women and children. Simultaneously the programmes like Deen Dayal Upadhyaya Grameen Kaushalya Yojana, TG SERP (Society for Elimination of Rural Poverty) ect, need to be enhanced to cover majority of women population in the state.
4. Rajiv Gandhi Scheme For Empowerment of Adolescent Girls **(RGSEAG) Sabla** is a centrally sponsored programme initiated in 2011. As a pilot project this programme is launched in 03 districts of Telangana i.e, Adilabadh, Mahaboobnagar and Hyderabad[11]. Analyzing the pros and cons of this programme, there is a need to initiative such kind of programmes in all the districts of the state.
5. Woman-friendly workplaces need to be brought about. There's a dire necessity of infrastructure that would enable women's participation in the workplace.
6. Education on gender sensitization is of primary necessity. The societal norms that limit women to four walls need to be shattered.
7. Hostels for working women and crèches for their children.
8. The role of companies in nurturing gender diversity by providing equal number of jobs to women, and also to pay equal pay for equal work.
9. To bring a change in the attitude of women. They themselves seem inclined to choose trades that are traditionally considered women oriented: beauty and healthcare for instance. "Social norms and a lack of information often limit women's opportunities to so-called traditional jobs, closely linked to typical ideas of what women can and cannot do.

CONCLUSION

The present survey display a startling fact that nearly 58% of the women in the target area of study are confined to domestic boundaries, though 97% of them are educated and are qualified to take up positive employment. Many reasons like lack of alternative to agriculture to provide employment opportunities to women in rural areas, patriarchic social base, cultural prejudices, family burden, poor transport facilities to work place etc are playing an important role in confining the women to their homes. This gender bias needs to be addressed to bring about social and economic justice. If women participated in the economy on a par with men, India could increase GDP by up to 60%, or $2.9 trillion, by 2025, according to a 2015study by the McKinsey

Global Institute, a think tank. At present, women contribute a mere 17% to the country's GDP, well below the global average of 37%.

REFERENCES

1. www.thehindu.com >17 April/ referred on 23-12-2017.
2. https://www.younglives.org.uk > content referred on 24-12-2017.
3. 'Women's Voices: Employment and Entrepreneurship in India, Published by United Nations Development Fund (UNDP), July, 2016.
4. https://www.fckchecker.in > global-gender-gap-index, referred on 24-12-2017.
5. Policy Research Working Paper titled "Precarious Drop - Reassessing Patterns of Female Labor Force Participation in India" written by Luis A. Andres, Basab Dasgupta, George Joseph, Vinoj Abraham and Maria Correia, Published by World Bank Group -South Asia Region Social Development Unit, April 2017, Pg. 2.
6. www. Indiaspend.com > Cover Story> Why Indian Workplaces Are Losing Women: Our Nationwide Investigation Begins, August 2017.
7. www.governancenow.com > regular-story >Low female labour force in India: World Bank, May 2017.
8. http://scroll.in > article > Why are Indian women increasingly dropping out of work?, August 2017.
9. 'Getting India's Women into the Workforce: Time for a small approach, by Rohini Pande, Published in Ideas For India on 10 Mar, 2017.
10. wdcw.tg.nic.in > WDCW Department, browsed on 5/01/2018.
11. www.nmew.gov.in > index 1 > Rajiv Gandhi Scheme for Empowerment of Adolescent Girls, browsed on 5/01/2018.

GRAPHS

Table 1: Community wise categorisation of respondents.

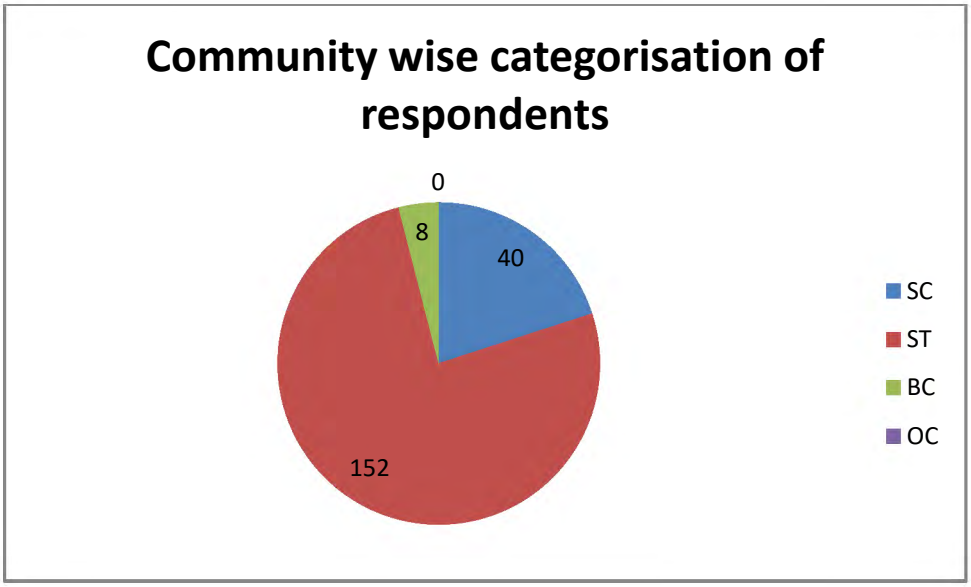


Table 2: Educational Qualifications of respondents.

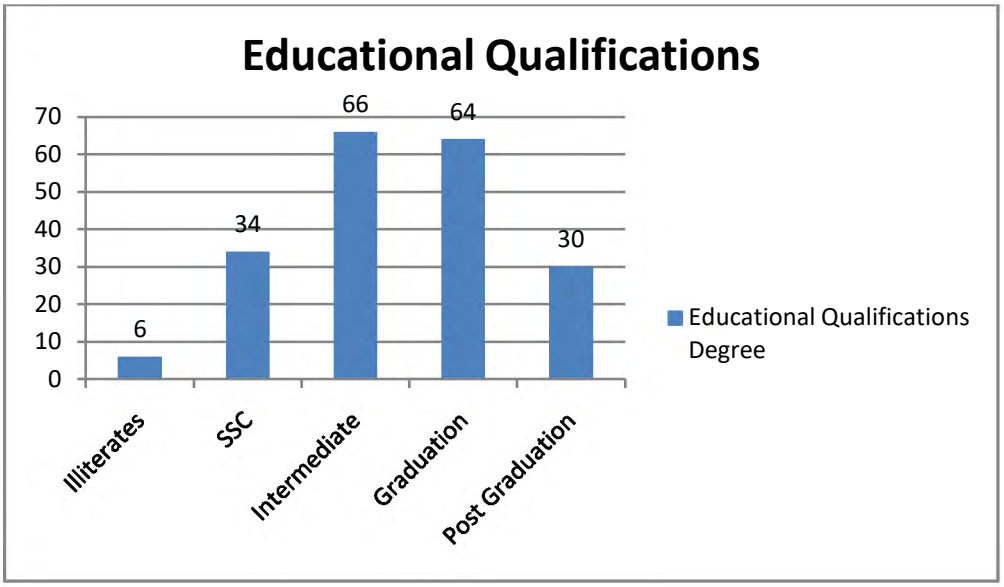


Table 3: Employment Oriented Courses undertaken by respondents,

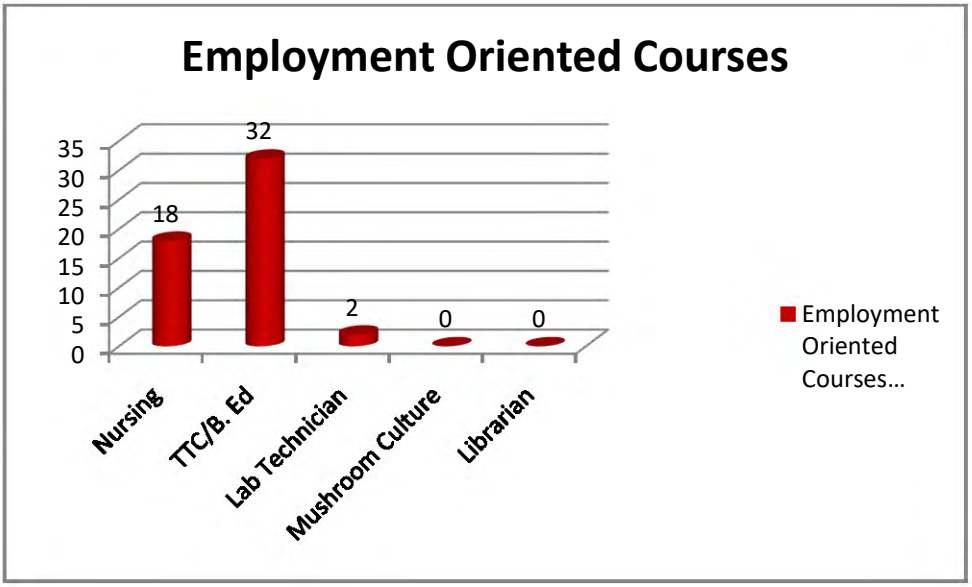


Table 4 – Skill Enhancement Training.

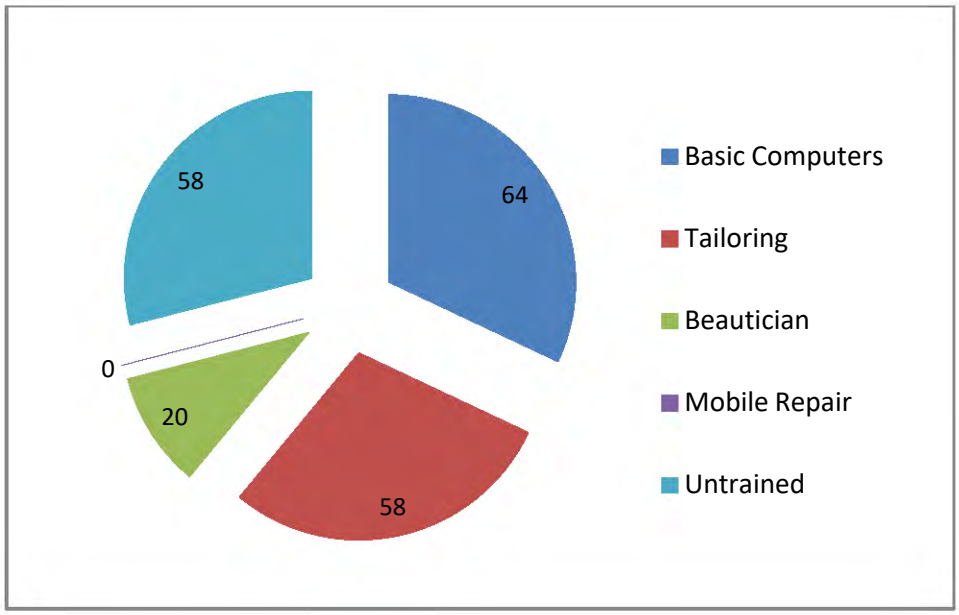


Table 5: Present Occupation of the respondents.

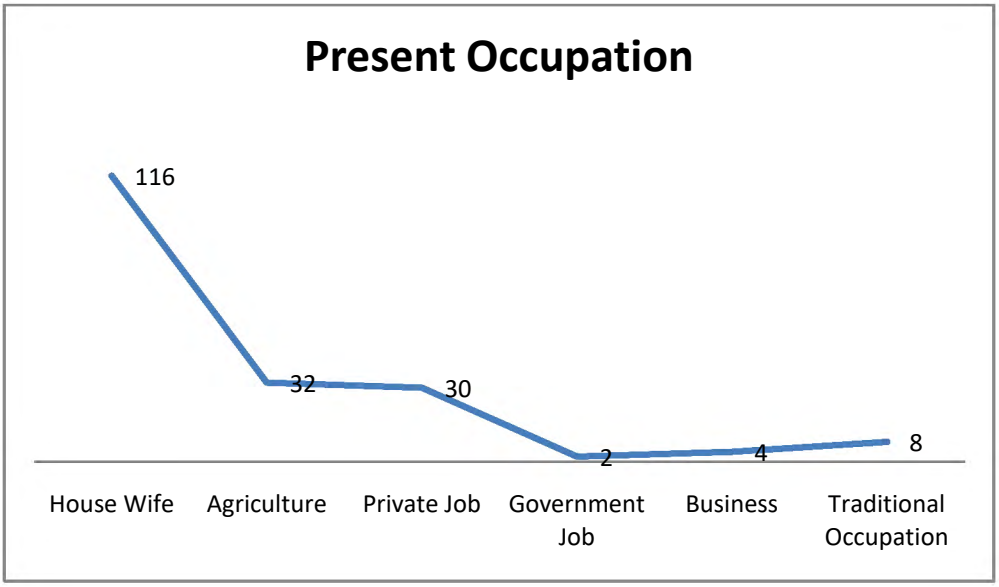
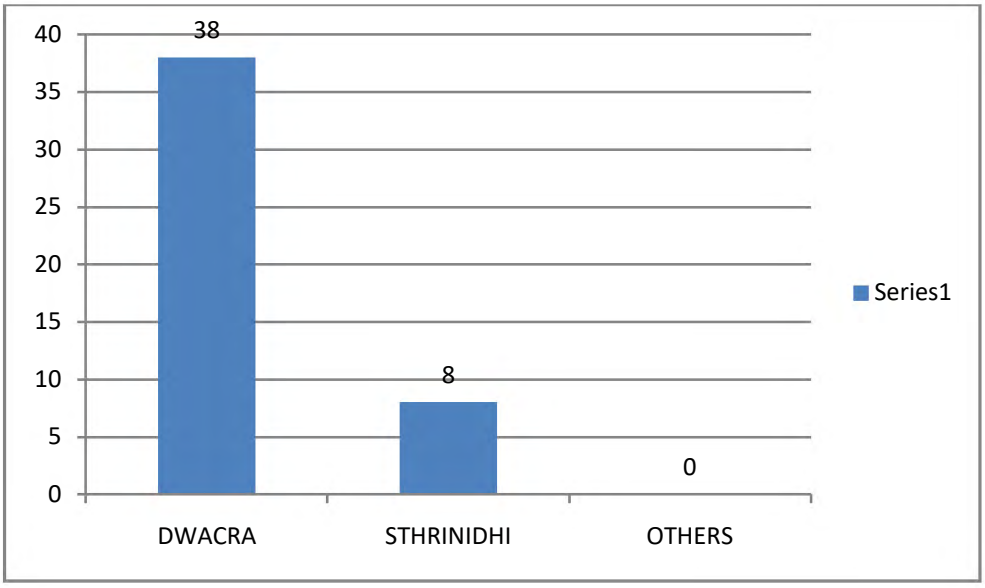


Table 6 – Participation in Self-help groups.



Hakimiat-e-Ilahia & Iqamat-e-Deen: The Core of Maududi's Political Ideology

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Abstract: This paper discusses the twin basic concepts of A.A. Maududi's vision of Islam and Politics. *Hakimiat-e-Ilahia* in plain English means Sovereignty of the God Almighty and *Iqamat-e-Deen* means establishment of Islam as a political force in, off course, Muslim majority states. This paper also examines Maududi's understanding of Islam. For him, Islam is not merely a religion but a way of life, that implies the political, social and legal aspects of human life. According to him, accepting Islam means 'giving oneself into complete subjugation to the Divine Commands.

Introduction: Abul Ala Maudūdi has been a well recognised name in the realms of Islamic theology as well as Political Science especially to those who have been concerned with Indian sub-continent, Middle East, or with just Political Islam. Many distinguished writers, such as Charles J. Adams, E.I.J. Rosenthal, Aziz Ahmed, Kalim Bahadur, S. V. Raza Nasr, have taken up his political thought but none focussed upon the core of his thought namely *Hakimiyat-e-Ilahia* and *Iqamat-e-Deen*, i.e. Divine Sovereignty and Establishment of the Religion respectively. Divine, for Maududi, is the Omnipotent and Omnipresent God Almighty to Whom he prefer to call *Allah*. In fact these two concepts are essentially one as the second is the process to realize the first. This concept is the natural corollary of his understanding of, what he called as, four basic terns of Quran, the Holy book of Islam.

Main content

Maudūdi's interpretation of what he called 'the four basic terms of the Qur'ān' led him to form the core of his theo-political theory namely, Hākimiat-e-Ilāhia, (Sovereignty of God) and Iqāmat-e-Deen, (establishment of Islam These are the two basic themes of all his theological and political, theoretical and practical discourses. Though interdependent and sometimes used interchangeably the earlier is the means to realise the later. The later is also not the ultimate aim, rather it is a means to achieve the final goal that is success in the life hereafter.

Hakimiat-e-Ilahia (Divine Sovereignty)

Hakimiat-e-Ilahia, is the central idea of Maudūdi's political thought. He calls it as the most fundamental and most revolutionary concept of the Quran.[1]According to Maududi, the Qur'an possesses its own concept of Universe that insists that Universe is the creation of Allah, all the humans, animals, heavens, earth, sun, moon, stars, in brief, each and every thing in the Universe is created by Him. Maudūdi quotes several verses from the Qur'an in this regard:[2]

> "Your Guardian-Lord is Allah, Who created the heavens and the earth in six Days, then He established Himself on the Throne (of authority): He draweth the night as a veil O'er the day, each seeking the other in rapid succession: He created the sun, the moon, and the stars, (all) governed by laws under His Command. Is it not His to create and to govern? Blessed be Allah, the Cherisher and Sustainer of the Worlds!"[3]
>
> "It is He Who created the heavens and the earth in true (proportions)."[4]
>
> "Say: "Allah is the Creator of all things: He is the One, the Supreme and Irresistible"."[5]
>
> "He rules (all) affairs from the heavens to the earth: in the end will (all affairs) go up to Him, on a Day, the space whereof will be (as) a thousand years of your reckoning."[6]
>
> From the above Quranic verses, it is evident that Allah is the Master, Ruler, Manager, and Administrator of all His creation.[7] He is Omnipresent, Omniscient, and Self-consistent. He never needs any other's help or assistance. No one has any share in His attributes or creation. If he wishes to destroy the Universe or any thing in it, or to punish any one, no one can refrain Him from doing so or rescue any body from His wrath. The Quran further says:[8]

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[1] *Islamic Law*, p. 166
[2] See also *Qur'ān*, 2:29, 4:1, 56:58-72., *Understanding the Qur'ān,* Vol. I. p. 58; vol. II. p. 5., *Tafhîm*, Vol. V. p. 257
[3] *Quran.*, 7:54., See also *Understanding the Qur'ān,* vol. III. p. 32
[4] *Ibid.*, 6:73., See also *Understanding the Qur'ān,* vol. II. p. 243
[5] *Ibid.*, 13:16., See also *Understanding the Qur'ān,* vol. IV. p. 231
[6] *Qur'ān* 32:5., See also *Tafhim,* vol. IV. p. 38
[7] See also *Qur'ān,* 20:6, 30:26, 32:5.,*Understanding the Qur'ān,* vol. V. p. 181; *Tafhîm*, vol. III. p. 750; vol. IV. p. 38
[8] See also *Qur'ān*, 2:107, 3:154, 6:57, 13:16, 16:17, 18:26, 25: 2, 28:70, 30:4,
Understanding the Qur'ān, vol. I. pp. 100 & 293; vol. II. p. 237; vol. IV. pp. 231 & 319; vol. V. p. 101. *Tafhîm*, vol. III. pp. 433, 659 & 731.

> "Say: "Have ye seen (these) 'Partners' of yours whom ye call upon besides Allah? Show me what it is they have created in the (wide) earth. Or have they a share in the heavens?" Or have We given them a Book from which they (can derive) clear (evidence)? Nay, the wrong-doers promise each other nothing but delusions. It is Allah Who sustains the heavens and the earth, lest they cease (to function): and if they should fail, there is none -not one- can sustain them thereafter: verily He is Most Forbearing, Oft-Forgiving."[9]

From the above Quranic verses it is clear that all the attributes and characteristics of Allah are exclusively concentrated in Him alone; no one else in the Universe possesses these attributes. He is Irresistible, Infallible, Self-subsisting, Acquainted with every thing, Preserver of Safety, and Protector, Eternal, Ever-awaken, Exalted in Might. All authority and power is in His hands; every thing is under His control; profit and loss is His blessings. Every one is answerable to Him but He is answerable to none. No one can deny or ignore His Command.[10] To establish the sovereignty of God Maudūdi quotes the following Quranic verses:[11]

> "Knowest thou not that to Allah belongeth the dominion of the heavens and the earth? And besides Him ye have neither patron nor helper."[12]
>
> "He is the Irresistible, (watching) from above over His worshippers; and He is the Wise, Acquainted with all things."[13]
>
> "Allah is He, than Whom there is no other god; the Sovereign, the Holy One, the Source of Peace (and Perfection), the Guardian of Faith, the Preserver of Safety, the Exalted in Might, the Irresistible, the Supreme: Glory to Allah! (High is He) above the partners they attribute to Him."[14]
>
> Divine Sovereignty, for Maududi, is the logical consequence of the Quranic concept of the Universe. The Quran mentions in unequivocal words that the Ruler and Sovereign of the Universe is the Ruler and Sovereign of the humans too. He is the Omnipotent, Omnipresent, Omniscient, Irresistible, Supreme and Exalted; His powers are Universal, Comprehensive and Inalienable to Him; He does not have any counterpart in His powers, nor even wife or children:
>
> "He to Whom belongs the dominion of the heavens and the earth: no son has He begotten, nor has He a partner in His dominion: it is He Who created all things, and ordered them in due proportions."[15]
>
> "They have no protector other than Him; nor does He share His Command with any person whatsoever."[16]
>
> The entire universe is one organic system that is being controlled by one authority.[17] He created the Men and exclusively enjoys the authority to govern them and regulate their affairs; no one else is authorise to command or adjudicate them:
>
> "Is it not His to create and to govern? Blessed be Allah, the Cherisher and Sustainer of the Worlds!"[18]

In the physical sphere of the Universe, Maudūdi writes, God's Sovereignty has been established by coercion over each and every object including the mankind. Mankind, like other objects, is subject to His Will without any choice. It makes no difference whether men acknowledge His Supremacy or not. However, in volitional sphere mankind is physically, not morally, free for acceptance or rejection of His Sovereignty. Mankind is morally bound to acknowledge His Sovereignty by its own covenant with Him on the *yaom-e-alast* i.e. the day of Covenant. The Quran claims that Almighty drew the souls of all the human beings whom He intended to create until the Day of Judgement and made them testify. All have taken the oath of allegiance that they would obey and worship Him:

> "When thy Lord drew forth from the Children of Adam from their loins, their descendants, and made them testify concerning themselves, (saying): 'Am I not your Lord (Who cherishes and sustains you)?' They said: 'Yea! We do testify!" (This), lest ye should say on the Day of Judgement: "Of this we were never mindful'."[19]

Then, the God Almighty inculcated this Covenant in their sub-consciousness and very nature, like other pieces of knowledge. Hence, regarding any other entity as their sovereign amounts to the breach of the Covenant that is inherent in their instinct.[20] This is so inherent in the human nature that the non-believers and polytheists too did not deny the existence of the God, nor that the whole mechanism of universe is functioning in accordance with His commands:

> "If thou ask them, Who created them, they will certainly say, Allah: how then are they deluded away (from the Truth)?" [21]
>
> "Say: "Who is it that sustains you (in life) from the sky and from the earth? Or who is it that has power over hearing and sight? And who is it that brings out the living from the dead and the dead from the living? And who is it that rules and regulates all affairs?" They will soon say, 'Allah'."[22]

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[9] *Qur'ãn,* 35:40-41., See also *Tafhîm,* vol. IV. pp. 239-240
[10] *Khilãfat,* pp 13-20
[11] See also *Qur'ãn*, 2:204&255, 3:25 & 83, 5:1, 6:18, 7:128, 10:65 & 107, 13:9 & 41, 18:27, 23:88, 28:23, 36:83, 48:11, 59:23, 67:1, 72:23, 85:13-16, 95:8, *Understanding the Qur'ãn,* vol. I. pp.161,196, 244 & 269; vol. II. p. 20; vol. IV. p.47, 71, 227 & 247; *Tafhîm,* vol. IV. p. 273; vol. V. p. 415; vol. VI. p.41& 120.
[12] *Qur'ãn,* 2:107., See also *Understanding the Qur'ãn,* vol. I. p. 100
[13] *Ibid.,* 6:18., See also *Understanding the Qur'ãn,* vol. II. p. 220
[14] *Ibid.,* 59:23., See also *Tafhîm,* Vol. V. p. 416
[15] *Ibid.,* 25:2., See also *Tafhîm,* vol. III. p. 433
[16] *Ibid.,* 18:26., See also *Understanding the Qur'ãn,* vol. V. p. 101
[17] *Islamic Law,* p. 170
[18] *Al Qur'ãn*, 7:54., See also *Understanding the Qur'ãn,* vol. III. p. 32
[19] *Ibid.,* 7:172., See also *Understanding the Qur'ãn,* vol. III. p. 97
[20] *Understanding the Quran*, Vol. III. pp. 97-100
[21] *Al Qur'ãn*, 43:87., See also *Tafhîm,* vol. IV. p. 553
[22] *Ibid.,* 10:31., See also *Understanding the Qur'ãn,* vol. IV. p. 31

> The Quran claims, according to Maududi, are so-politically oriented that it would be difficult to limit them to the religious arena.
>
> Allah has said: "Take not (for worship) two gods: for He is just One God: then fear Me (and Me alone)."[23]
>
> The Quran repeatedly uses the political terms like Kingship, Lord and Sovereign to explain the relationship of God with man and His other creations.[24]

He is kind enough to sent messengers and prophets to remind the Covenant and elucidate His attributes to the Mankind that they should recognise Him and acknowledge His Sovereignty intentionally, consciously and with their own will and wish, as the rejection would cause unending punishment in their eternal life.

According to Maududi, the real mission of all the prophets, right from the Adam to Prophet Muhammad, have been to call the Mankind towards acknowledging Hākimiate Ilāhia, Sovereignty of God Almighty over the entire life of man and to seek Iqāmate Deen, establishment of His deen on His earth.[25] The dispute between the prophets and the non-believers is that the prophets demanded absolute obedience to Allah and complete acknowledgement of His sovereignty in the religious, moral, social, cultural, economic, political and all other fields, but those in power refused to forego their authority and acknowledge that of Allah. As far as the concept of God over universe is concerned, in Maudūdi's opinion, it is accepted by most of the people to whom these prophets have been sent. For instance, the Pharaoh, Maudūdi writes, claimed godhood but he could not have mean that he was the Creator of the heavens and the earth. Nor any man in his good senses could claim that. The Pharaoh could not even mean that only he should be worshipped, for the Egyptians worshiped a host of deities along with the Pharaoh. His claim to godhood could only mean that he wanted to be obeyed as the King and the sovereign of the people of Egypt. And this position is similar with the claims of modern states for legal and political sovereignty.[26] These prophets have demanded their people to establish the Rule of the Allah over the earth. While explaining the 40th verse of Sura-e-Yousuf, Maudūdi writes:

> "This verse determines that the power to adjudicate and right to rule (in other words, Sovereignty) is exclusively for Allah. Here, there is no such word or reason on which basis this Sovereignty could be confined in the sense of universal sovereignty. The Sovereignty of Allah is as much Political and legal, Moral and ideological, as it is universal. And there are explicit evidences in the Quran itself that reserved all such kinds of sovereignty exclusively to Allah alone. Hence, The Quran determines that Allah is not only *Rabb* and *Ilāh* of humans but He is *Malik*, the King of the humans too: 'Say: I seek refuge of (Allah) the Lord of Mankind, the King of Mankind'. It says that Allah is the King of the dominion and no one else share in His Kingship:
>
> 'Say: "O Allah! Lord of Power (and Rule), Thou givest Power to whom Thou pleasest, and Thou strippest off Power from whom Thou pleasest.' (3:26)
>
> 'Say: Praise be to Allah, Who begets no son, and has no partner in (His) dominion.' (17:111)
>
> 'Your Guardian-Lord is Allah, Who created the heavens and the earth in six Days, then He established Himself on the Throne (of authority): He draweth the night as a veil O'er the day, each seeking the other in rapid succession: He created the sun, the moon, and the stars, (all) governed by laws under His Command. Is it not His to create and to govern? Blessed be Allah, the Cherisher and Sustainer of the Worlds!' (7:54)" [27]

Hence, acceptance of Islam, for Maududi, implies the acceptance of His sovereignty and rejection of this claim is rejection of Islam; the Quranic concepts of the Unity of God and the Divine Sovereignty are so vitally intertwined that the negation of one amounts to the negation of the other ipso facto. Hence, he equates acceptance of any other's supremacy in any aspect of life, including political with shirk, polytheism. For him, mere association in command involves association in worship. He quotes several verses from the Quran:

> "Yet there are men who take (for worship) others besides Allah, as equal (with Allah): they love them as they should love Allah, but those of Faith are overflowing in their love for Allah. If only the unrighteous could see, behold, they would see the Punishment: that to Allah belongs all power, and Allah will strongly enforce the Punishment."[28]
>
> "Allah is He, than Whom there is no other god; the Sovereign, the Holy One, the Source of Peace (and Perfection), the Guardian of Faith, the Preserver of Safety, the Exalted in Might, the Irresistible, the Supreme: Glory to Allah! (High is He) above the partners they attribute to Him."[29]
>
> "These are the limits ordained by Allah; so do not transgress them if any do transgress the limits ordained by Allah, such persons wrong (themselves as well as others)."[30]

According to Maududi, mankind is bound to follow His Laws and Commands. The believers in Him have no choice except to submit themselves to His Laws. They can neither amend these laws by themselves nor be content with laws made by others. Permitting others to legislate for them or following the laws made by others or according to their own whims will amount to shirk, polytheism:

According to Maududi, He(the God Almighty) has already caused legislation and communicated it to the Mankind through His Book, namely the Quran, and the practices of the Prophet Muhammad. These laws are ultimate and final, and un-amendable in any circumstances. However, the people of knowledge among the Muslims can deduce subsidiary laws from the Quran or the Practices of the Prophet. They can even infer a new law, if any novel situation arises, on the basis of the basic principles given in the Quran and Hadith.[31]

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[23] *Ibid.*, 6:51., See also *Understanding the Qur'ãn,* vol. II. p. 235
[24] *Islamic Law*, p 170
[25] *Ibid*, p.175-176
[26] *Ibid*, p. 166 & 172
[27] *Islami Riyasat*, pp 365-366.
[28] *Al Qur'ãn* 2:165., See also *Understanding the Qur'ãn,* vol. I. p. 133
[29] *Ibid.*, 59:23., See also *Tafhim*, vol. V. p. 515
[30] *Al Qur'ãn,* 2:229., See also *Understanding the Qur'ãn,* vol. I. p. 177
[31] *Tafhimat,* vol. III. pp. 10-16

In brief, the Sovereignty lies exclusively with Almighty and no other person or the institution enjoys any share in it, not even the state. In fact, the basic attribute of the Islamic State is that it lacks sovereignty on it its part; it is a mere vicegerent of Almighty on the earth to uphold His Commands. According to Maududi, the concept of political and legal sovereignty of Allah is the first among the basic principles of Islam and no Islamic constitution can be drafted unless the political and legal sovereignty of Allah is accredited in that. The Islamic constitution must embody that it is subservient to Allah, acknowledges Him as the Supreme Ruler and His Commands have binding effect upon it.[32]

Maudūdi not only explains the necessity of Divine Sovereignty in Islam but he also proved that the Divine Sovereignty is the only possibility if sovereignty means absolute power and unlimited authority. He touches upon the most difficult question and the most disputed issue of political science that haunted several political philosophers and they pleaded to discard it altogether, including Bodin and Austin, that whether any sovereign on earth ever enjoyed or will enjoy the absoluteness as required by many of the political thinkers? In other words, Is it possible for any human sovereign to be, after all, a sovereign? In fact, it is impossible for any human being to have such independence, absoluteness, exclusiveness, eternity, inalienability and originality as required by Bodin,[33] Hobbes,[34] Austin,[35] and other political philosophers. According to Maudūdi such sovereignty is beyond the sphere of human competence. No emperor or autocratic ruler have, in reality, ever enjoyed or have been enjoying such absolute powers. His powers are limited by various internal and external factors that are beyond his control. In democratic systems also no one can determine where the sovereignty actually lies as everyone has been controlled by others and every one's authority is being checked by others. In fact, for Maududi, there is no one among the humans or other creatures who possesses such attributes of sovereignty. There is only one such being in the Universe and that is Allah, the God Almighty.[36] He writes:

> "The root cause of all difficulties in respect to this question is a basic fallacy: the political philosophers have tried to place the cap of sovereignty on man being for whom it was never intended and whom it can never fit. Keeping in view the attributes of sovereign, no human being or human organisation can really claim title to it. And when sovereignty is forced upon human beings, it results in confusion on all hands."[37]

The second question, posed by Maududi, is 'Who possesses the right to have sovereignty?' He says that even if anyone has provided someone with such a status it would be inappropriate to follow his commands because no one has the authority to make him sovereign. One may try to justify it on the basis of popular choice but the people themselves have no such authority to handover such a power to anybody. Hence, authority of such sovereign will be void. According to Maududi, the paradox can be solved only, as done by the Quran, by having a supra-natural being, the God Almighty, as the legal sovereign.[38] He argues:

"The Quranic concept of sovereignty is simple. God is the Creator of the Universe. He is its real Sustainer and Ruler. It is His Will that prevails in the cosmos all around. As all Creation is His, His command should also be established and obeyed in man's society. He is the real sovereign and His Will should reign supreme as law."[39]

Thus, Maudūdi proves the Divine sovereignty not only on the authority of the Quran but by the very words of the western philosophers.

In brief, Islam, according to Maududi, by it's virtue, claims *Hakimia-te-Ilahia*, Divine Sovereignty, over the earth and the entire life of man. He argues that Islam is not submission to and acceptance of Lordship and Omnipresence of the God Almighty in mere physical sphere of the life, but it requires a complete and exclusive submission to and acceptance of His exclusive Authority, Omnipotent Sovereignty, and Suzerainty in all spheres of volitional life also. It implies that Islam, in Maudūdi's view, demands *Hakimiat-e-Ilahia,* i.e., the exclusion of the right of Sovereignty in a State for the God Almighty alone and it requires Iqāmate Deen, establishment of Islam as the political power.

Iqamat-e- Deen (Establishment of Islam)

Maududi, though, places great significance on the Concept of Divine Sovereignty but he is not content with a mere declaration that the constitution declares Allah as the Supreme Head of the Islamic State while as the entire system is functioning against His Will and Commands. *Hakimiat-e-Ilahia,* for him, is a mean to achieve *Iqamat-e-Deen*, the establishment of Islam as political authority. Iqamat-e-Deen, for him, does not mean mere establishment of a system of *Salat* and *Zakat*, or performing Hajj etc. In fact it is the establishment of the Will and Wish of Allah, as ordained in the Quran and *Sunnah*, in each and every field of human activity, right from private morals to foreign policy; it is enforcement of His Commands in social and political arena as it is enforced in religious sphere.

In brief, *Iqamat-e-Deen*, for Maududi, means establishment of Islam in its complete form and shape without any curtailment or compromise, through the political force. It also implies seeking political power and dominance to Islam that it becomes the dominant and decisive force on earth, as it does not descend to remain in a subservient position. Hence, Islam has no choice except to seek the reign of state for itself or for those who share its faith as it presents a complete system or way of life. If it has not sought, the others will do so and consequently, deprive it with ninety percent of its content. According to Maududi, such coercion is natural and unavoidable; it will be on the part of either Islam or any other religion. Islam possesses, for Maududi, preference in this regard since it is the embodied Will of the very Creator of this Universe.

Hence, according to Maududi, for Muslims, if they wish to lead their lives according to Islam, there is no choice except to submit their entire life to the Will of the God, conduct all their individual as well as congregational, private as well as public affairs, and resolve all their disputes according to His injunctions. Islam is a complete and comprehensive *deen*, way of life, it neither to resolve with any segregation between private and public life, nor to accept that Muslims will follow the commandments of God in their private affairs but follow the other's rules and regulations in their public life. Islam not only lays down principles

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[32] *Islami Riyasat*, p. 367
[33] Sukhbir Singh, *A Histoy of Political Thought*, vol. I. pp. 405-406
[34] *Ibid*, vol. I. pp. 443-447
[35] *Ibid*, vol II. pp. 65-69
[36] *Islami Riyasat*, pp. 314-315
[37] *Islamic Law*, p. 166
[38] *Islami Riyasat*, p. 316
[39] *Islamic Law*, p. 166

of morality, and ethics but also regulates the social, economic and political fields. It prescribes criminal procedure, fiscal and economic regulations as it enunciates the methods of prayer and pilgrimage. According to Maududi, an individual's claim of Islam is doubtful if he will not follow His commandments in all aspects, including political, of his life as *deen* involves all of them. For instance, in 24th verse of *Sura-e-Baqrah*, Maudūdi quotes, the criminal law of Islam has been called as *Din-ul-lah*, i.e., the religion of Allah. It means that *deen* does not merely mean prayers, fasting, pilgrimage; criminal law is also a part and parcel of it and it is obvious that political power is necessary to realise it. A *deen* without government in its hands, for him, is like a blue print of a building, not the building itself. Hence, if anyone have been performing religious rites according to Islam but, on the other hand, adopting the criminal procedure made by someone else by his will he is trying to follow multiple of *deens* at once.[40]

In Maudūdi's view, Islam did not descend to provide faithful subjects to the rulers. If the aim of Islam was mere submission to the existing system and rulers it would lead to inconsistency, as it means that Islam is presenting itself as the best *deen* and insisting upon its complete following but at the same time it is instructing its followers to submit themselves to the existing system and obey its commands as against to the commandments of Islam.

He argues that if it were be the nature of Islam the Arab pagans would prove themselves more secular and accommodating than the Britons. In fact, they were well aware that Islam presents a complete way of life and it demands, obviously, the authority and power to rule over them and regulate their entire lives according to the Will of the God and acceptance of Islam means submission of their free will to the Will of the God. It was the basic reason for their antipathy towards Islam. For Maududi, the real objective of Islam is to remove the lordship of man over man and to establish the Kingdom of God on Earth, that is expressed by his coined word '*Iqamat-e-Deen'*. It is, for Maududi, necessary, essential and inevitable to realise Islam. *Namāz, Rozah, Zakāt, Hajj* etc., are not the real objectives of Islam, but all are meant as preparation for a greater task namely *Jihād,* i.e., to stake one's life and everything else to achieve the *Iqamat-e-Deen. Iqamat-e-Deen* is necessary and unavoidable because, according to Maududi, the rotten political systems are the root of all the evils of the world. In fact, the bad character of government, largely, generates evils in the society. Power and wealth rests in its hands; laws are framed and enforced by it. Hence, it can either restrict or permit theses evils. According to Maududi, whatever evils there are in the present society, they are because the reins of government are in the hands of most wicked and mischievous elements of mankind. In order to effect reforms among the people and to bring them on the path of righteousness there is no remedy except to set right the mutilated shape of government. It will be impossible to stop drinking, gambling, adultery, and other such things by mere sermons, but it can be exterminated easily by an act of government. Whoever really wants, Maudūdi writes, to root out mischief and chaos from God's earth and is genuinely anxious to ameliorate the condition of God's creation, it is useless for him to work as a mere preacher. He should stand up to finish the government run on wrong principles, snatch power form wrong-doers and establish a government based on correct principles and following a proper system. After suggesting the remedy-measure, that is seeking power by the right-minded people, to eradicate the evils of the society, Maudūdi tackled with the root-cause of the government's badness. According to him, the rule of man over man is the basis of ignoble government. The reform he suggested is that there should not be lordship of man over man but that of God over man and this is what the Islam want to introduce. The striving for this is Jihad and the establishment of noble and God-fearing and God-guided government is *Iqamat-e-Deen*.

Conclusion:

The above observations show that establishment of the Divine-Rule on earth is the ultimate aim of Islam, as it is envisages by Maududi. He made the God Almighty as not only physical sovereign of the universe but the political and legal sovereign of the state also. For him Islam means complete subjugation to the Commandments of the God in personal as well as congregational life. Religion, for him, is not limited to the personal sphere but its scope includes the political realm also. However, Maududi never-ever advocated terrorism or violent methods. He seeks the revolution through the propagation and peaceful means.

[40] *Fundamentals of Islam*, p. 255

Muslim Socio-religious Movements & Ideologies In Colonial India

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Abstract: The exclusive aim of this paper is to present the theo-political ideology that is presented by various theo-reformative movements among Muslims in the Indian sub-continent during colonial rule, hence, the history and organisational details of these movements have been consciously avoided. Hence, the stress has been on the ideologies and beliefs of several famous Muslim leaders and organisations of that period such as Mahmud ul Hasan and Reshmi Rumal Tehreek, Nanotawi and Deobandi Tehreek, Titu Mir and Tariqa-e-Muhammadiya, Haji Shariatullah and Faraedhi Tehreek, Qanunji and Tehreek-e-Ahl-e-Hadith, Syed Ahmed Khan and Aligarh Tehreek, Chiragh Ali and Rationalism, Titu Mir and Tariqa-e-Muhammadiya, Haji Shariatullah and Faraedhi Tehreek, Qanunji and Tehreek-e-Ahl-e-Hadith, Syed Ahmed Khan and Aligarh Tehreek, Chiragh Ali and Rationalism, Shibili Numani and Historical Glorification, Muhammad Ali Jauhar & Khilafat Movement, Iqbal and Pan-Islamism, Maulana Ilyas and Tablighi Jamat, Inayatullah Mashriqi & Khaksar Tehreek, Obaidullah Sindhi and Hijrat Movement, Jamiyat ul Ulama & Composite Nation Theory, Khudai Khidmatgar, Muslim League & Muslim Nationalism, Maulana Maududi & Jamat-e-Islami.

Introduction : For the Indian Muslims the post-Ghadar 1857 era was of a great uncertainty in the religious as well as political spheres. They have had already lost all their hopes for re-glorification due to failure in 1857.[1] This unsuccessful attempt ruined the Muslims in almost all aspects.[2] They had been facing severe economic crisis due to loosing of political power. Further, adoption of English replacing Persian as official language made the situation more aggrieved. There was no provision of teaching English under the traditional *madarsa* system; learning English for majority of Maulvis was tantamount to heresy. Therefore, majority of the Muslims themselves from the modern education system that, in due course resulted in lack of qualified Muslim candidates for public services. This phenomenon, in addition to the government's deliberate policy of alienation, resulted in less representation in public services, comparing to the other communities.[3]

Further, in 1924, Muslims faced another debacle by dissolution of Ottoman Caliphate that was the last symbol, though nominal, of their political unity and sovereignty as well as hope and inspiration.

Main Content

Mahmud ul Hasan and Reshmi Rumal Tehreek

The Muslim intelligentsia, at the above juncture, had no clear-cut solution to these surmounting problems. Some of the Muslim leaders were in British camp while some others were under the influence of Gandhi ji. Sir Syed Ahmed Khan and his followers had been vehemently advocating the British presence in India regarding it as a God's blessing. However, Maulana Abdul Aziz, Mawlana Mahmood al Hassan and Maulana Obaidullah Sindhi was still in favour of an armed struggle against the British.[4] For many of such Mullas British India was a *dar al-harb*, land of War. They initiated the Indian Muslims to migrate to Afghanistan or any neighbourhood Muslim country.[5] In 1870, Maulana Abdul Azeez revived the jihadi tradition of Shah Ismail Shaheed and Ahmed Shah Barelvi (1786-1831). He re-organised *Mujāhideen* in the Northwestern province and started armed struggle against the British but failed due to un-matching power.[6]

Again, in the beginning of the twentieth century, Mawlana Mahmood al Hassan, then Nazim of the Darul uloom Deoband, strived hard to revive the above tradition of *Mujahideen*. He founded an underground movement, *Reshmi Rumal Tehreek*. He sketched to strike the English army through Durra e Khyber, banking upon the promises of Afghanistan, Iran, and Turkey. In 1918, he sent his most trustworthy fellow Obaidullah Sindhi to Afghanistan to negotiate with the rulers of Afghanistan and Iran as well as to reorganise the *Mujāhideen,* who were overzealous to teach a lesson to the Briton. In the same year, he arrived at Hejaz to acquire the military favours from the Turkish government. The Turkish War Minister Anwar Pasha, promised to provide military assistance in this endeavour. Meanwhile, Obaidullah Sindhi established a government-in-exile with several associates of the '*Ghadar Movement*' at Kabul. But, the entire endeavour failed due to leaking of the secret to the British government as the British succeeded in arresting

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[1]See for the causes of the War, Sir Syed, *Asbaab-e Baghaawate Hind*, & Zakaria, Rafiqh. *Rise of Muslims in Indian Politics*, Bombay, 1970, pp.3-7., Hence, Zakaria.

[2]See for details on Muslims' persecution by the British, Peter Hardy, *The Muslims of British India*, Cambridge University Press, 1972, pp. 70-79. Hence, Hardy.

[3]For details see, Zakaria, pp.7-25.

[4]For details see, Hardy, p. 84.

[5]Qadhi Mohd. Aslam Saif, *Tahreek-e Ahl-e Hadith, Tareekh Ke Aainay mein*, Al-Kitab International, New Delhi,1996, p.252; See for detail discussion on *dar ul-harb*, *Ibid*, pp. 109-115.

[6]Aziz Ahmed, *Islamic Modernism in India & Pakistan*, London, 1967, p. 20. Hence, Aziz Ahmed.

Mahmud ul Hasan along with his followers at Mecca itself. They were sent to Malta for life imprisonment.[7]

Nanotawi and Deobandi Tehreek

The bulk of Ulama believe that the only way for Indian Muslims to be saved is to rejuvenate and revitalise their faith.[8] They were more afraid of the Western ideology than her political domination. They were very much anxious about the existence of Islam and Muslims in India. Maulana Qasim Nanotawi (1832-1880) had strongly believed that popularising religious education among the Muslims was the need of the hour lest they would perish. He resorted only to the disseminating of theological knowledge because he thought that the government has already taken up the task of providing modern and scientific knowledge.[9] Hence, he along with Maulana Haji Imdadullah Muhajir Makki and Maulana Rashid Ahmed Gangohi decided, in 1867, to establish a *Deeni Madarsa* at Deoband exclusively for Islamic education, unadulterated by Western influence. Hence, modern sciences and English could not found a place in its curriculum. Being products of the Waliullahi school[10] they were more concerned about the conservation of the *Hanafi Maslak* and Deobandi tradition.

However, one may note that the objectives of the establishment of Deoband were not that simple.[11] All the three founders were not just academic luminaries of high excellence but active participants in the War of Independence at Shāmli as well.[12] In fact, there was a profound underlying aim, namely re-gaining the theo-political glory of the past. For them, there was no difference between Shāmli and Deoband, but of weapons. At Shāmli armed struggle was tried, but here at Deoband intellectual and peaceful means were adopted.[13]

The presence of physical training of quasi-military nature, that led the people to remark sarcastically that it was rather a *madarsa-e harbia* instead of a *madarsa-e Arabia*, envisages the minds of its founders.[14] However, later it became a neglected aspect of the curriculum as the people at Deoband, with exception of a few, had forgotten the real aim of its founding father.

Titu Mir and Tariqa-e-Muhammadiya

Titu Mir (1782 - 1831), a reformist turned rebel, founded a movement, *Tariqa-e-Muhammadiya*, in Bengal. This was in fact a socio-religious reformist movement that soon acquired the character of an armed rebellion against British due to their continuous support to the oppressive landlords.

Titu preached against polytheism (*shirk*) and innovations (*bid'aat)* at first, but soon found himself embroiled in a conflict with local zamindars and English indigo growers as he fought back against their repressive methods. He petitioned before British but in vain. Then he opted to take matters into his own hands, forming a *Mujahid* militia and training them in local weapons such as the *lathi*. British authorities took serious note of these activities and sent offensives against them. Titu bravely defeated at least three of such offensives but could not sustain for a long time before the well equipped British forces and martyred on 19th November 1831 after five days of fierce battle.[15]

Haji Shariatullah and Faraedhi Tehreek

Haji Shariatullah (1781-1840) after living in Mecca for twenty years founded the Faraedhi Tehreek in Bengal to lead Bengalian Muslims to the correct path of Islam. The term *Faraedhi* is derived from '*fardh'*, i.e. obligatory duties enjoined by Allah. Haji Shariatullah, on the other hand, used the word in wider meanings to refer to all religious obligations mandated by the Qur'an and the Sunnah.

Bengali Muslims, while ignoring the real Islam, had been indulging in several un-Islamic customs, rituals, and ceremonies. Shariatullah emphasised the five pillars of Islam, urged on complete acceptance and strict adherence to pure Islam, and condemned all innovations like *Chhuttee, Puttee, Chilla, Shabgasht, Fatiha, Milad, Urs, Taziah* as polytheism. He emphasised on '*Adl*, justice, equality, and the Islamic concept of *Ukhuwah*, brotherhood. He used the terms *ustaad* and *shagird*, instead of *peer* and *mureed*, to denote his relation with his disciples.

On the political side, Haji Shariatullah declared British regime as anti-Islam and Muslims. He pronounced that it is not true to perform Juma payer in absence of a legitimate Caliph.

This movement become famous in the districts of Dhaka, Tippera, Noakhali, Bakerganj, Faridpur, Mymensingh, Chittagong, and the adjacent province of Assam. In 1831, he was forced to leave his base, Ramnagar. Due to continuance tension with Hindu Zamindars and Indigo growers this movement converted this movement into a militant-reformist organisation. He, almost a century before to Gandhiji, ordered his followers to resist illegal cess and ban on cow slaughter. To take rid off him the Hindu lords, in 1837, accused him of attempting to set up a kingdom on the lines of Titu Meer. They also brought numerous lawsuits, with the help of

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[7]Faruqi. Ziya ul Hasan, *Deoband School and the Demand for Pakistan*, 1967, pp. 59-62., Hence, Faruqi.

[8]Zakaria, p. 26.

[9]For details on introduction of modern education by the British see, Hardy, pp. 90-91 & De Bary. Wm. T., *Sources of Indian Tradition,* NY, 1958, pp. 35-37, Hence, De Bary.

[10]Most of the Muslim Movements of British India, many of whom were antagonistic to each other such as Ahnaf and Ahl-e-Hadith, surprisingly trace back their origin in Shah Waliullah's writings. His theo-political thought keeps the Islam alive in the Indian sub-continent. (Riyadh Ahmed, *Mawdoodi and Islamic state*, Lahore: P.P.H 1976, p. 15).

[11]Mawlana Mahmud al-Hasan's remarks are crucial in this regard. He questioned, when he was suggested by the administration of *Dar al-'Uloom* to keep away from politics, "Did our revered teacher (Nanotawi) lay the foundations of this *madarsah* for mere educational purposes? It was founded in my presence and, as far as I know, one of its main objects was to compensate for the losses in 1857. Those interested only in education are free to do as they like but I stand for those objects which the founder of the *Dar al-'Uloom* had in view and for whose achievement he worked hard". (Faruqi, p. 59, n. 1)

[12]*Ibid*, p. 21.

[13]*Ibid*, p. 23.

[14]*Ibid*, pp. 30-37.

[15]'Titu Mir', *Wikipedia*, Electronic edn, 2008 cited to Rabiya Khatoon, *Titumirer Bansher Kella*, 1981.

European indigo planters against the Faraedhis. He was arrested by the police several times for allegedly causing agrarian disturbances in Faridpur.

On Shariathullah's demise his son Muhsinuddin Ahmad alias Dudu Miyan presides over the movement and brought an agrarian character to the movement.[16]

Qanunji and Tehreek-e-Ahl-e-Hadith

Muhammad Siddiq Hasan Khan al Qanunji (1832-1890) founded the *Ahl-e-Hadith* movement, in the late Ninteeth century on the ideas of Shah Waliullah (d. 1763), Syed Ahmad Shaheed (d. 1831) and Qadhi Ash-Shaukani (d. 1832). Its aim was to bring religious reform by denouncing *taqlid*, i.e. the following of any particular Imam amongst the four Imams of *Ahl-e-Sunnah*, as *bid'a*, i.e. sinful innovation. They were nicknamed as Indian Wahabis as ideologically they were akin with Muhammad b. Abdul Wahab of Najad.

Sadiq Hasan married the third Begum of Bhopal, Shah Jahan (reigned 1868-1901), that made his position strong enough to combat the traditional Indian '*ulama*, who were Hanafites. He compiled more than 200 books in three languages namely Persian, Arabic, and Urdu. He established a far-reaching network to sell his books and buy others for him. This challenges the common view that the nineteenth century India was just a periphery that did not participated in the intellectual developments and the trends of Islamic centres.[17]

Syed Ahmed Khan and Aligarh Tehreek

Sir Syed Ahmed Khan (1817 - 1898), who was also a product of the traditional education, on the other side, had radical views with regard to the nature and curriculum of education among Muslims. He had a strong belief that the key for salvation of Muslim community lies in their learning of English language, modern education and adopting western culture and civilisation. He insisted upon Muslims that they must abhor all those habits and beliefs that were in contrast to the western culture, civilisation, morality, or modern sciences. He pronounced that the real Islam would not be an impediment to the proposed modernisation as the *wahi*, revelation and natural laws are identical and connote the words and works of the Almighty respectively. The words of God i.e. Qur'ãn must be in harmony with the works of God i.e. Nature. Hence, the Qur'ãn cannot contradicts the law of nature. Similarly, *wahi* and reason are identical. *Wahi* acts as an instinct in lesser forms of life while the reason as a revelation-instinct operates in scientific investigation.[18] Hence, anything that descended through *wahi* cannot contradict to facts discovered by science and should be seen in that context.[19] He considered *Jihād* as a defensive warfare and slavery as the product of historical Islam, not the real and revealed Islam.[20]

His approach was purely materialistic and he was concerned with betterment of the mundane life of Muslims. For this sake, he was ready to interpret the Qur'ãn according to his modernistic and naturalistic point of view. In order to tune the Qur'ãnic verses with the western criteria he formulated his own fifteen principles of exegesis. He divided the Ayats of Qur'ãn into two kinds, basic and iconic. The former constitutes the basis of Islam, hence, cannot be altered while various interpretations in the later might be permissible as per the needs of the times that may be different from that of Prophetic period.[21] He advocated that all Muslims have the right of an iconic or analytical interpretation of the Qur'ãn. The bases of interpretation, said Sir Syed, were to be *usūl,* the basic principles and not *furū',* the trivial principles derived therefrom. Similarly, those Ayahs of the Qur'ãn that are referring to the specific historical situations cannot be a basis for interpretation.[22] To rid with the unaccommodating *ahādith* he, like Goldziher and Schacht, challenged the authenticity of all the classical collections, including *Sahih Bukhāri* and *Sahih Muslim*.[23]

Further, for the sake of developing cordial relations between Christianity and Islam he has written two books, *Tabaiin al-Kalām* and *Risala der Ta'ām ahl al-Kitab*, in which he advocated that the Muslims should remove the social barriers with regard to the Christians.[24]

While dealing all these, he was dare enough to criticise the British policy of discrimination against the Indians. He left the *Agra Durbar* unattended when he noticed that the chairs for Indian guests were arranged on a lower level to those of Europeans.[25]

However, Sir Syed concentrated upon the popularisation of western education and social reforms among the Muslims. After a thorough analysis, he concluded that the root cause of all the backwardness and sufferings of the Muslims was their abhorrence to the English language, western education and sciences. He realised that if Muslims were not acquainted with the modern education

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[16]"Faraezi Tehreek", *Wikipedia*, Electronic edn., 2008.

[17]*Ibid*, cited to Claudia Preckel, *The Begums of Bhopal*, Rolibooks, New Delhi.

[18]Aziz Ahmed, pp. 42-43 & Ikram. *Indian Muslims & Partition of India*, ND, p.55., Hence, Ikram.

[19]Sir Syed, on this assumption, strived to reconcile the Darwinian evolutionism with the Islamic tenets of Creation. For him, Adam connotes human nature. He considered the legend of Fall of Adam and all the Prophetic miracles, including the *M'eraj*, as metaphorical, legendary, or symbolic. Angels were either the properties of created things or divine moral support against overwhelming odds and Satan signifies dark passions of man. Similarly, Djinns were either wilder men living in forests or projections of evil, diseases and other calamities. He viewed the soul as pragmatic reality and *wahy* and Gabriel as instinctive flash upon the mind of the prophet. (Aziz Ahmed, pp. 43-44 & 47-48)

[20]Sir Syed regarded the Prophet's expeditions as a defensive warfare-mechanism. *Ibid*, p. 50.

[21]Sir Syed, on this basis, pronounced that the simple interest drawn from banks or government institutions is permissible. *Ibid*, pp. 45 & 52-54., However, astonishingly he supported *purdah* system among the Muslim women. (Zakaria, p. 244)

[22]Aziz Ahmed, p. 42.

[23]*Ibid*, p. 49.

[24]Zakaria, pp. 237-238.

[25]S. M. Ikram, Modern Muslim India and the Birth of Pakistan, Delhi, 1991, p. 42., Hence, Modern Muslim India.

their condition will not be improved and they could not get an honourable position amongst the nations of the world. He, in 1864, founded the Scientific Society for introducing modern education among the Indian Muslims. He started with the establishment of a modern school at Ghazipur and encouraged the others to establish such institutions at the district level. However, he, unlike his predecessor Nanotawi, stressed upon English and modern sciences. He caused the translation of useful English books into Urdu. However, his great achievement in the field of education is the establishment of Anglo-Muhammadan Oriental College in 1874, at Aligarh on the model of Cambridge University with English as the medium of instruction. It aimed at the scientific education, broad mindedness, liberalisation of ideas, and a pragmatic approach to politics. This college in course of time developed into Aligarh Muslim University.[26]

These endeavours produced a small but talented intelligentsia that contributed much in development of political consciousness among the Muslims and created dynamics in the fossilised society. It provided the leadership for Muslims to check the growing influence of the Congress. Moreover, the neo-elite, who had little sympathy towards Islam and its tenets, later played an active role in Pakistan movement as they believed in Muslim nationalism with respect to Indian subcontinent.

On the political front, Sir Syed was convinced that the British rule over India has been beneficial and a Divine blessing to the Indians, especially to the Indian Muslims. Hence, he remained loyal towards the British throughout his life and preached the same. He condemned the efforts to present the Mutiny of 1857 as a Muslim revolt and started a magazine, *The Loyal Muhammadans of India*, to correct the false impression of British officials.[27] His loyalism can be divided in three phases. From 1859 to 1870, he tried to persuade, on the one hand, the British authorities that until the Indian Muslims were free to perform their religious obligations there was no theological reason for them to revolt against the British,[28] and, on the other, the Muslim community that the British rule over India was in their interest. In the second phase, from 1870 to 1884, his objective was to check the advent of pan-Islamism, which he considered as a dangerous political adventurism. In the third phase, from 1887 to 1898, he led the Muslims towards political separatism. He on the basis of Urdu-Hindi controversy and the communal riots that followed, it is concluded that these two nations could not be united into a composite nation.[29] He succeeded in the first and third phase of his loyalism, but on the question of pan-Islamism he failed to attract the Muslim intelligentsia as well as masses.[30]

In 1884, with regard to the self-government, he suggested a political pattern based on triangular, Hindu, Muslim, and British, participation. He was convinced that if anyone from Hindu or Muslim community rule the country, peace couldn't be maintained; therefore, the British rule is inevitable to retain the peaceful co-existence of all the communities in India. He was very critical of the Congress and always considered it as a Hindu organisation. He was afraid of any political alliance with the Hindus. In his view, it could lead only to the eventual domination and subjugation of the Muslim minority to the Hindu majority. Therefore, he opposed the Congress' stance to appease the minority, in the form of Khilāfat Movement. He reacted sharply when the Congress elected a Muslim, Badruddin Tayyabji, as its president. He condemned it as the beginning of erosion in Muslim community, which was numerically in minority, educationally backward, politically immature, and economically weak.[31] He had certain reservations regarding the association with the Congress; firstly, he was afraid of the cultural dominance of the Hindus in the Congress that association with such an organisation would cause loss of identity for the Muslims and eventually they would absorb, like the Buddhists and Jains, into the Hindu community. Secondly, that the Congress' anti-British attitude would ruin the Muslim community again as it happened after the Mutiny of 1857. Hence, he pronounced that the Muslim's alliance with the Congress would cause 'a loss to this world as well as the next'.[32] He founded two organisations one in 1888, and another in 1894 to counteract the Congress' influence on Indian Muslims.[33]

In brief, Sir Syed's Naturalism rejected or reinterpreted all those elements in Islam that contradicted the modern science or western culture and civilisation; for this sake, he strived to rationalise the minutiae of dogma and liberalise the Islamic law. Thus, in this endeavour he disowned three-fourth of Islam. His Rationalist speculation, in which he was close to the Mu'tazilites, and loyalist Occidentalism can be summed up in the following six points:

1. A rationalistic approach to Islam;
2. A readjustment of Islamic traditions and customs in accordance with the changing times, i.e. according to western criterion;
3. An active interest in the history and literature of Islam;
4. A new approach that was based on *Deen* but in tune with the Western civilisation;
5. A better understanding of the Christians and their culture and civilisation; and
6. A loyalism towards the British combined with Muslim separatism.[34]

Though, Sir Syed did not formally establish any organisation, his theo-socio-political endeavours has been generally referred as 'Aligarh Movement'.

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[26] Aziz Ahmed, pp. 34-38. see for details, Ikram, pp. 31, 37-40.

[27] Ikram, p. 30., see also, Hardy, pp.84-85.

[28] Lord Mayo, by a letter, dt. 30th May 1871, suggested W.W. Hunter to analyse that whether the Indian Muslims have a religious duty to rebel against the Briton in the present situation? (Hardy, p. 85, see also, pp. 62-70)

[29] Zakaria, p. 82.

[30] Aziz Ahmed, p. 33.

[31] *Ibid*, p. 34.

[32] Zakaria, p. 84.

[33] For more details on these association see, *ibid*, pp. 66-70 & 82-85.

[34] *Ibid*, p. 240.

Chiragh Ali and Rationalism

Chiragh 'Ali (1844-1895), a radical disciple of Syed Ahmed Khan who called himself as Mu'tazili,[35] went a step ahead of his master. He considered the Prophet Mohammad, peace be upon him, only as a reformist, whose main concern was to improve the moral standards of Arabs and conditions of Women, bring monotheism in the place of polytheism. He, in unequivocal words, repudiated the authenticity of the *Ahadith* and rejected *Ijma'* as a source of law. He expressed reservations with regard to the authenticity of the traditional sources of law.[36] He also suggested that too much sanctity should not be attached to the Prophet, his words and practices.[37] He envisaged that there are several references to nature and principles of nature. He endeavoured to identify *mutlaq*, i.e. absolute and *muqayyad* i.e. conditional verses in Qur'ãn and developed the notion that when the context and the ruling are comparable, an 'absolute' ayah should be interpreted in the light of a 'conditional' ayah. According to him, Islam does not provide any social system; however, Muslims in places where they were in majority have started to identify their social systems with the Qur'an. Islamic jurisprudence was essentially a reflection of such social experiences of ninth and tenth centuries. It may be still practicable in fossilised and static Muslim societies. But, in countries like India, Algeria and Turkey, which have been exposed to West, some of its sections become outdated and require re-writing; they, he insisted, should develop new laws. He disowned the legal disabilities against non-Muslims as having no real theological basis. He also considered jihād as a defensive mechanism irrelevant to the development of modernist Islam.[38] For him, all references of the Qur'ãn with regard to the sword was false, meant only to malign Islam as the Qur'ãn itself declared in unequivocal words that 'there is no compulsion in religion'.[39]

He pleaded for religion-politics dichotomy as the Prophet, in his opinion, never mingled religion with state.[40] With regard to the real politics of the Colonial India, Chiragh Ali, like his master Sir Syed followed a loyalist. He generally opposed the Muslim participation in the Congress as well as their subscription to the view of pan-Islamism. However, he was sympathetic with certain aspects of nationalist movement, such as inter-communal harmony and peaceful coexistence with Hindus.[41]

Shibili Numani and Historical Glorification

Shibili Numani (1857-1914) strived to establish a synthesis between the extreme orthodoxy of traditional *ulama* and extremist modernism and naturalism of western educated intelligentsia. He favoured of English education and modernisation of certain social institutions, but to the extent that it did not harm the religious foundations of Islam. He discarded the superfluous and the ridiculous things crept into the Indian Muslim while adhered to essentials of *Deen*.

He was the driving force behind the establishment of *Nadwat al-Ulama* at Lucknow as a synthesis between the orthodoxy of Deoband and modernism of Aligarh. He admired many things in the Western civilisation and did not mind to borrow the ideas and institutions from Europe or for that matter from anywhere, but only if these were absolutely essential for the regeneration of Islam. However, he was to measure the Western ideas and values by the Islamic yardstick. Hence, he disagreed with Sir Syed in many of his theological interpretations. For instance, he, in quite contrast to Sir Syed, considered reason as the handmaid of religion.[42]

With regard to the treatment of non-Muslim subjects, he suggested liberal measures. He discarded discriminatory practices against *dhimmis* in erstwhile Islamic states as the personal attitude of the rulers, not essentially Islamic.[43]

Shibili popularised the concept of Islamic historiography among Indian Muslims with a profound aim to revive the glories of Islam, at least in the hearts and minds of the new generation. He tried hard to establish a synthesis between traditional Islamic sciences with the modern Western one. He appreciated Orientalists' research towards cultural and religious resources of Islam to establish a historical as well as scientific perspective simultaneously to the Islamic studies.[44]

Shibili has taken the refuge of history with two fold aims: one, to bring the Muslim community out of dismay and gloom that became its destiny since the unsuccessful War of 1857, by reminding their past glories. secondly, to convince the *'ulama* that in the earliest period of Islam, even in the reign of orthodox caliphs, several ideas and institutions have been borrowed from different sources alien to Islam, and he succeeded to a considerable extent in both his objectives.

Muhammad Ali Jauhar & Khilafat Movement

Muhammad Ali Jauhar (1879-1930) was a Pan-Islamist in action. His loyalty towards Islam was beyond the national considerations. At the onslaught of European powers over the Ottoman Empire he represented the anguish and anxiety of Indian Muslims regarding the fate of Caliphate, which had been regarded as the symbol of secular power and unity of Muslims worldwide. During the First World War, he, in his *Comrade*, begged the Allies to win over the Turks by compensating the losses inflicted upon them, in order to keep them away from the Germans. Despite these appeals, the Britain wrested many of Turkish territories from her, which shocked the Muslim opinion in the subcontinent. Yet, the Muslims still believed that they could pressurise the British and this could bring good to their Turkish brethren. Hence, they, under the leadership of Muhammad Ali, caused, in 1920, the *Khilāfat*

[35] *Ibid*, p. 242.
[36] Aziz Ahmed, pp. 57-61.
[37] Zakaria, p. 242.
[38] Aziz Ahmed, pp. 59-62.
[39] Zakaria, p. 242.
[40] *Ibid*, p. 242.
[41] Aziz Ahmed, p. 65.
[42] Zakaria, pp. 251-253.
[43] Aziz Ahmed, pp. 81-83.
[44] *Ibid*, p. 78.

movement.

Congress, at that moment, was keen to attract Muslim support. In fact, it had been since its inception, in 1885, striving to draw the Muslims towards it to cast off the Hindu colour and to present itself as a true representative body of all the Indians. The *Khilāfat* question provided an excellent opportunity to it. Hence, it decided to take the matter as its own. The entire nation under the dual leadership of Gandhi and Muhammad Ali stood united for the Turkish cause. However, this alliance could not last long as the Muslims turned hostile towards the congress again when it took-back the non-cooperation movement on the excuse of infiltration of violence into it, while Muslim masses, including Muhammad Ali, considered it as a lame excuse and moved further away from the Congress. The *Khilāfat* movement hung up until the Turks themselves abolished the Caliphate in 1924. Muhammad Ali died during his visit to London to attend the first Round Table Conference.[45]

Obaidullah Sindhi and Hijrat Movement

Maulana Obaidullah Sindhi (d. 1944), a convert from Sikhism and a prominent disciple of Maulana Mahmud al-Hasan (1851-1920) was a real dynamic. He, besides taking part in the *Reshmi Rumal ki Tehreek,* initiated another movement called as *Hijrat* Movement. This movement regarded India as *dar al-harb* and encouraged Muslim-migration to any of the Muslim countries, preferably to Afghanistan. At Kabul, he succeeded in establishing a government-in-exile with several associates of the '*Ghadar* Movement'.

On the political side, he followed the composite nation theory of Deoband Ulama as a bargaining strategy for achieving independence. He was first to develop the idea of linguistic nationalities in India. He envisaged free India as a confederation of linguistic and cultural nationalities.

Among the leading theories, socialism attracted him most. Astonishingly, he attributed this idea to the certain writings of Shah Waliullah. In his view, Islam was basically and inherently socialistic. He has seen the Communist revolution in the USSR as close to Islam. He envisaged that the Muslims should themselves evolve a mechanism based on *deen* to establish economic justice in the society. *Jihād*, for him, was the basis of organisation of Islamic social revolution. However, this should be achieved through non-violent method. He identified victory to Islamic social revolution on social scale as fulfilment of God's blessings on earth.

The difference, in his view, between the social revolution preached by Islam and communism was that the former believes in God while the later denies him. He connotes the Qur'ãnic concept of *Jama'a* with Communist concept of revolutionary party.[46]

Iqbal and Pan-Islamism

Allama Iqbāl (1875-1938), a one-in-all personality of the Muslim intelligentsia, was in fact an embodiment of the orthodox Orientalism and modern Occidentalism. He was convinced that a reconstruction of shariah had become essential due to the revolutionary changes in modern times. The traditional interpretations were good for the times in which they were made but in the context of the modern conditions, they become obsolete. He felt the need of a great *faqih* who could interpret Islam correctly in the light of new developments. According to some sources he put his hopes on Maududi.

Iqbāl suggested certain valuable reforms as an immediate concern, for instance, the proper protection of the rights of women and their education. He supported *purdah* and polygamy; condemned wasteful expenditure in Muslims marriages and other ceremonies. He pursued the Muhammadan Educational Conference to establish a Reforms Section under its auspices.

In the field of education, Iqbāl stressed upon the industrial education. In his view, there was no hope of progress without it.[47] However, he opposed neither traditional theological education nor modern education of general nature.

Iqbāl's contribution to the legal thought of Islam was his advocacy for widening of the span and weight of *Ijmā'* and *Ijtihād*. He envisaged that the power of *Ijtihād* should be taken from the individual representatives of different schools of jurisprudence and vested in a representative assembly of Muslims. The consensus reached in such a body was *Ijmā'* for him. He approved amendments in the Muslim law. In his view, it can be adjusted in accordance with the exigencies of the present society, as he thought it a non-sacred or this-worldly element of Islamic faith. For him, the freezing of Islamic Law by the so-called *masalik* or *mazahib* is an artificial phenomenon. This can be addressed by returning to the *Ijtihād*.[48]

In his view, Islam is a moral ideal along with a specific type of state by which he means a society under a legal system and a moral ideal.[49] He was against religion-politics dichotomy since he was convinced that in Islam there is no such duality between spirit and matter, consequently, between Church and State. According to him, both were organic to each other. He rejected the idea to limit the religion in the private sphere of the individual life. He envisaged that the Prophet's experiences created a society or social order that in turn provided us basic outlines of a state with implied legal ideology. Hence, the spiritual objective of the Islam is so imbedded in its social order that one's rejection would gradually lead to the others negation. He was not content with accepting Islam in private life but rejecting the same in congregational matters and establishing separate national identities on the basis of region or race, colour or clan.

He was against the establishment of national states among Muslims and considered it as against the humanising and universalising spirit of Islam. On the contrary, he advocated al-Afghani's Pan-Islamism. In his narrative poem, *Jawed Nāma,* he presented his ideal Islamic state in the words of al-Afghani.

Nevertheless, he did not see any contradiction between pan-Islamism and demand for Pakistan as he considered the Indian case a distinct one. He rejected the idea of a composite nation of all Indians communities on the basis that establishment of a state on

[45] De Bary, pp. 216-220.

[46] Aziz Ahmed, pp. 195-201.

[47] Zakaria, pp. 259-262.

[48] Aziz Ahmed, pp.154-155.

[49] *Ibid*, p. 160.

national considerations was unimaginable to a Muslim as it gives priority to other things over Islam. He, in unequivocal words, condemned Maulana Hussein Ahmed Madani for his statement that a composite nation could be constituted in India based on homeland.[50] He pointed out that in modern terminology homeland was a political concept that contradicted with Islam. In his view, the moral consciousness, emotional and psychological homogeneity that was required to constitute the essence of nation was not present in India.[51] He suggested an autonomous province comprising of Muslims majority provinces of the subcontinent under an Indian federation to solve the communal problem. In his view, the diversity between Hindu and Muslim communities is a undeniable fact and it must be recognised, otherwise, it would harm both the communities.[52] He writes:

> "I would like to see the Punjab, North-West Frontier Province, Sind and Baluchistan amalgamated into a single State. Self government within British Empire, or without British the British Empire, the formation of a consolidated North-West Indian Muslim state appears to me to be the final destiny of the Muslims, at least of North-West India."[53]

Later this idea, in the hands of Jinnah, eventually led to the Pakistan Resolution for a sovereign Muslim State.

Maulana Ilyas and Tablighi Jamat

Maulana Muhammad Ilyas Kandhalavi (1885-1944) founded the Tablighi Jamat, in late 1920s, at Mewat as an apolitical and purely missionary movement. According to him, he inspired by a dream during the Hajj in 1926. The word '*Tabligh*' means 'to propagate'; so *Tablighi Jamat* stands for an organisation to propagate Islam.

However, its sphere of activity has been, right from its establishment, limited to the Muslim community only. Maulana Ilyas' motto was '*Aye Musalmano! Musalman Bano*', ('O Muslims! Become real Muslims'). He had the opinion that the first priority should be given to develop true Islam among Muslims. He aims to revive the Muslim society by inculcating practice of basic obligations of Islam, especially the *Namaz*. He enunciated six-point formulae to be followed in order to become true Muslims. These are:

1. Firm belief in the Kalimah
2. Concentration and Devotion in prayer
3. 'Ilm (knowledge) and Dhikr (to remind the God)
4. Respect towards Muslims
5. Ikhlas-e-Niyyat (good intent)
6. Dawat wa Tabligh (conveying of the message and propagation)

Tabligh was essentially a deobandi in its tenants but neither the Jamat nor the Darul uloom ever acknowledges this fact. Further, in many places even Ahle Hadees have associated with it. In fact, it was formed in turbulent period when there was a general feeling that the Muslims would perish their identity if they did not practically adhere to their religion.

After Malulana Ilyas' demise (d. 1944), Maulana Muhammad Yusuf Kandhalvi (1917-65) became second *Amir*, followed by Maulana Inamul Hassan (1965-95). Now, in India, the Jamat is divided into two factions namely *Shurai* and Nizamuddeni has been headed by Maulana Sa'ad sahib while as the Shurai group was under the collective leadership of number of senior Maulanas of the jamat. *Fazail-e-Amal*, composed by Maulana Zakriya has been practically the basic literature of the Tabligh.[54]

Inayatullah Mashriqi & Khaksar Tehreek

Inayatullah Mashriqi (1888-1963) was the founder of *Khaksar Tehreek*. He believed that there could not be any conflict among the basic teachings of various religions because If such a thing existed, it would mean that the Creator have been sent conflicting messages to the same creation. He examined the basic principles of different religions and concluded that all prophets' teachings were closely related to the evolution of humankind as one and united specie, in contrast to animal species. He couldnot believe there was a contradiction and conflict in the Universe, and he couldn't believe the battle between religions was true. If such a thing existed, then either religion was a lie, and the messenger was an impostor who misled and upset society, or he was misprojected by his followers. In his opinion, Religions were basically the Science of collective evolution of humankind and the purpose of prophets was to unite the humanity, not to divide them; the basic principles of all religions are one and the same.

In 1930, Mashriqi founded the Khaksar Tehreek after retiring from the British service. He was instrumental in steering the Muslims toward freedom. Mashriqi, his family, and a considerable number of Khaksars were frequently imprisoned. In 1938, he announced a fourteen point-charter in which the first is as follows:

> "We *Khaksars* are determined to establish, by destroying all sectarian feelings and religious bigotry (but keeping religion intact), an egalitarian, non-partisan and tolerant order which would ensure a fair deal to all nations and their rightful growth, and which will be based on virtue, struggle, action and supreme justice."[55]

In this critical situation, the two centres of Muslim education and thought, viz. Deoband and Aligarh, 'the two facets of the theological heritage of Waliullahi tradition',[56] presented two different and extremely antagonistic approaches.

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[50] Iqbāl, Tasadduq Hussein Tāj, ed. *Madhamīn-e-Iqbāl*. pp. 180-196.

[51] Aziz Ahmed, pp. 156-163.

[52] De Bary, pp. 211-215.

[53] *Ibid*, p. 215.

[54] "Tabligh Jamat" *Wikipedia*, Electronic edn, 2008; Alex Alexiev, 'Tablighi Jamāt: Jihad's Stealthy Legions', in *Middle East Quarterly*, Winter 2005, vol. XXII. No.1.

[55] "Mashriqui", *Wikipaedia*, Electronic edn, 2008

[56] Mawlana 'Obaid Ullah Sindhi remarked that the *Dar al-'Uloom*, Deoband and Muhammadan Anglo-Oriental College, Aligarh are two facets of Waliullahi tradition, Aziz Ahmed, p. 104.

Jamiyat ul Ulama & Composite Nation Theory

There was, on one hand, the Composite Nation Theory or United Indian Nationalism, presented by Indian National Congress and seconded by *Jami'yat-e-'Ulama-e-Hind*, founded in 1919, by the *'Ulama* of Deoband, Nadwah and Firangi Mahal under the leadership of Maulana Hussein Ahmed Madani.[57] Maulana Rashid Ahmed Gangohi, Maulana Mahmood al-Hasan and Maulana 'Obaidullah Sindhi, were under the impression that an armed struggle was inappropriate at that time, also subscribed to that theory.[58] This theory advocates that the Hindus and Muslims of the Indian subcontinent together constitute a composite nation as they share the same homeland. Hence, they should live united under single government in a single sovereign state, by a federal arrangement, with their respective religious identities.[59] It considers the homeland as the very core of the nationalism. Hussein Ahmed pronounced:

> "We, the inhabitants of India, in so far as we are Indians, have one thing in common and that is our Indianness which remains unchanged in spite of our religious and cultural differences. As the diversities in our appearances, individual qualities and personal traits and colour and stature do not affect our common humanness, similarly, our religious and cultural differences do not interfere with our common associations with our homeland. - - - - - This is what I mean by the '*Muttahidah Qawmiyat*'."[60]

Maulana Abul Kalam Azad (1888-1958), a Congress-man by conviction, provided a theological basis for this theory. He connoted the situation in India with that of Medina immediately after the *Hijrah*. According to him, in the Covenant, concluded between the Prophet and the people of Medina, Muslims as well as Jews and Pagans were described as a single community. He presented it as a precedent to constitute the composite nation of all the Indians irrespective of their religion.[61]

It was obvious that the Jamiyat had been advocating geographical considerations, instead of religious affiliation, as the basis for the constitution of a nation. Hence, it, on this basis, repudiated the very idea of Pakistan. Jamiyat's anxiety about the demand for Pakistan was three-folded;

- Firstly, it was suspicious about the British designs.[62]
- Secondly, it was anxious about the safety and security of the Muslims left in India as minorities after the division.[63]
- Thirdly, it was thinking with a missionary viewpoint that the division will surely cease, or at least impede, their objective of the propagation of Islam among the Hindus.[64]

Maulana Hussein Ahmed Madani wrote in this regard:

> "And Islam being a missionary religion, it is its duty, so far as possible, to absorb others in itself, not to reject them. This is why we should not hate our neighbouring peoples even if they hate us, if they call us unclean and impure."[65]

> "The great object of an over-all spread of Islam in the whole of India cannot be realised by appealing to passions of hatred and antagonism. It is the non-Muslims who are the field of action for the *'tabligh'* of Islam and form the raw material for this splendid activity. Today, by propagating hatred towards the Hindus, this field is being closed and this material wasted. It is contrary to the universal message of our great Prophet."[66]

> "Our object is to bridge the gulf of hatred, which is being created by the protagonists of the scheme of Pakistan. We are opposed to the idea of limiting the right of missionary activities of Islam within any particular area."[67]

Objectives of the Jamiyat involved the preservation and promulgation of shariah in the Indian sub-continent. Though, shariah in this regard was limited to a mere application of the Islamic personal law into the private lives of Muslims. It was suspicious that the Western educated leadership of the League would not allow it to enforce even this abridged version of shariah and would impose their modernist alterations and naturalist interpretations in its proposed Muslim state. They felt more secure in India than in Pakistan. Although, some of the league-leaders have given the assurance that Pakistan would be an Islamic State, but Jinnah's statements ran

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[57] It is noteworthy that a small section of the ulama-e Deoband was against the composite nation theory and collaboration with the Congress. Mawlana Ashraf 'Ali Thanawi (1863-1943) was the leader of this group. He along with Mawlana Shabbir Ahmed 'Uthmani and few other ulama-e-Deoband defected from the Jamiyat ul Ulama e Hind and established Jamiyat ul Ulama e Islam in 1946. They with the blessings of Mr. Jinnah counteracted the formers' activities. However, the bulk of the Deobandi ulama were against the demand for Pakistan. Faruqi, pp. 102-103, n. 4.

[58] Aziz Ahmed, p. 190.

[59] *Ibid*, p.104.

[60] *Ibid*, pp. 103-104.

[61] *Ibid*, p. 189.

[62] *Ibid*, pp.106-111.

[63] *Ibid*, pp. 111-114.

[64] *Ibid*, pp. 114-115.

[65] *Ibid*, p. 116.

[66] *Ibid*, p. 117.

[67] *Ibid*,

contrary to it.[68] He declared his secular policy unequivocally in some of his speeches:

> "You may belong to any religion or caste or creed- that has nothing to do with the business of the State. - - -You will find that in course of time Hindus would cease to be Hindus and Muslims would cease to be Muslims, not in the religious sense, because that is the personal faith of each individual, but in the political sense as citizens of the State."[69]

The ulama belonging to Jamaiyat were aware of the fact. Therefore, they were unwilling to support a sheer worldly scheme that, in their view, has no relevance to Islam, rather, harmful in terms of the future of Islam and Muslims in Hindu-India.

Khudai Khidmatgar

Another close ally of Congress among the Muslim organisations was *Khudai Khidmatgar* founded by Khan Abdul Ghaffar Khan, a Pakhtoon popularly known as Sarhadi Gandhi, in the Second decade of Twenteenth centurry, with a strong belief in Gandhi ji's notion of non-violence. He regarded it as the true religion of the Prophet. He addressed his fellow men:

"I am going to give you such a weapon that the police and the army would not be able to stand against it. It is the weapon of the Prophet, but you are not aware of it. That weapon is patience and righteousness. No power on earth can stand against it."

Over one-lakh members joined the organisation and became a legend for their peaceful opposition. However, they suffered a lot in the hands of the British army and police. On April 23, 1930, The British opened fire in Peshawar on the unarmed Khudai Khidmatgars gathered to protest against the arrest of their leader. This brutal incident killed 200-250 *Khudai Khidmatgars.*

Through political setup, strikes, and non-violent opposition, this organisation has achived some success and become a dominative force in the NWFP. The political wing of the movement was led by Ghaffar Khan's brother Dr. Jabbar Khan. He was Chief Minister of the province for more than two decades, roughly from the late 1920s to 1947. After the independence Jinnah dismissed his government for his inclination towards India.

Ghaffar Khan was a staunch opponent of the partition. As the leader of Pakhtuns he was more comfortable in India than Pakistan. Hence, in 1946, he was attacked by the supporters of partition at Peshawar. He had been always labelled as anti-Pakistani and kept under house arrest by all successive Pakistani governments till his death in 1985.

Muslim League & Muslim Nationalism

Muslim League, founded in 1906,[70] comprising largely of Aligarh based English-educated Muslim elite,[71] under the leadership of Muhammad Ali Jinnah, on the other hand, presented its 'Two Nation theory' which insisted that the Indian Muslims were a nation in itself, distinct from the Hindus and other communities by any definition of a nation and by all canons of International law. Jinnah argued that Indian Muslims have their own culture, civilisation, language, literature, art and history, therefore, they are a nation in their own and require a sovereign homeland for them, consisting of the Muslim majority areas of the subcontinent.

The League under the leadership of Muhammad Ali Jinnah launched a massive Pakistan Movement, in 1940, to pressurise the British to divide India into two sovereign states; one for Hindus and other for Muslims, before leaving the country. This demand reflected the sentiments of middle class Muslims who, being backward in all spheres, were frightened with their Hindu counterparts in an independent India. It was the outcome of the mixed feeling of fear and pride; fear of all sorts and the pride of being the erstwhile lords of the subcontinent.

Muslim League raised the cry of 'Islam is in danger' to attract the masses. Soon it became a popular goal, in the Muslim-dominated provinces, to be achieved at any cost.[72] Jinnah was ready to sacrifice the twenty million Muslims living as minorities in different parts of India in the interests of the Muslim majority provinces.[73]

It is in this theo-political context, at the advent of the fifth decade of the twentieth century, that Maudūdi presented his own theory of comprehensive Islam that at once rejected all the above. In his view, Islam descended to establish God Almighty's Will all over the earth, hence, acquiring just a piece of land for Muslims was out of context and un-Islamic. Muslims were distinct from Hindus but they were not a nation in western sense; they were rather *Ummah*, i.e. an ideological party; a party with a specific mission, namely propagation of Islam across the globe.

Maulana Maududi & Jamat-e-Islami

On 26th August, 1941 Maulana Maudūdi formally established the *Jamat-e-Islami* at Lahore to moblise the Muslim Ummah towards a real essence of Islam. He adopted a Qur'ān-&-Sunnah-enlightened-rationalistic approach. In his opinion Muslims are not a nation but an *Ummah*, meaning of which is close to a 'party'. He regarded nationalism as opposed to Islam. He opposed both Composite nationalism and separatism. He opposed identifying Islam with any other theory or ideology. state' on the basis of Islam.[74]

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[68] Faruqi, pp. 118-120.

[69] *Ibid*, p. 121.

[70] Zakaria, p. 110.

[71] It is noteworthy that entire western educated Muslim intelligentsia was not behind the demand for Pakistan. A secular minded group, having luminaries such as Muhammad 'Ali Jauhar, Abul Kalam Azad, Zakir Hussain, Muhammad Mujib, 'Abid Hussein in its fold, stood for the composite nationalism. (Aziz Ahmed, p. 194)

[72] *Ibid*, pp. 94-95.

[73] *Ibid*, p. 112.

[74] *Ibid*, p. 52.

Maududi's ideology begins, revolves round, and runs with *Hakimiyat-e-Ilahia* (Divine Sovereignty) and *Iqamāt-e-Deen* (establishment of Islam). He envisages a Universal State that would be beyond the geographical and racial considerations. His justification for this state has been '*al-ardhu li-llah, al-mulku li-llah,* i.e. the land belongeth to God Almighty, hence, the right to rule belongeth to God Almighty alone. In fact, the need and justification for an Islamic state, for him, follows from the nature of universal order. It is a part of a broad integrated theology that is based upon the Sovereignty of the very Creator of the Universe. In Maudūdi's view, this Sovereignty has been enforced automatically in the physical and natural sphere of life. However, in volitional life, the human beings have physical liberty to acknowledge his sovereignty or refuse to do so, but morally they are bound to acknowledge as they have concluded an agreement with Him on the *Yaom-e Alast.*[75] However, he was inclined to a peaceful way through propagation and persuasion to establish the Islamic state. Hence, after establishment of Pakistan he migrated to it and took active participation in its *realpolitik* and influenced the formation of Pakistan-constitution.

Conclusion:

The above analysis shows how confused Muslims were there after the failure in the first War of Independence. There was no unified and dynamic leadership at this juncture. Various leaders have presented their own solutions and approaches on the basis of their own understanding of the problem. Maulana Mahmud ul Hasan adopted the path of armed struggle against the new regime but in vain. Maulana Nanotawi thought it would be wise to resort to *Dars wa Tadress* in the unfavourable situation but not to forget the actual aim of revolution. Later, disciples of his school favoured composite nation theory while as Iqbal and Jinnah rejected this and presented a vertical division of India on the basis of religion. Meanwhile, intellectuals like Iqbal and Afghani, and Maulana Obaidullah Sindhi presented their own solutions in the form of Pan- Islamism and Islamic Socialism respectively. Various Ahl-e-Hadees movements and Maulana Ilyas's Tablighi Jamat found the solution in religious Puritanism and revivalism respectively. However, Maulana Maududi's approach was unique and all-embracing at once. He neither rejected any of the above approaches in toto nor adopted any of that in whole, with exception of rejection of Islamic Socialism. He presented Islam as a complete scheme or system of life that engulfs all the aspects of the life, spiritual as well material, individual as well congregational, social as well as political. He rejected the composite nation theory on the ground that the Muslims have a separate identity and a nation on their own, that is called by him as Muslim Ummah. Simultaneously, he rejected the demand for a Muslim Homeland on the ground that it would not be appropriate for a nation like Muslim Ummah to have homeland like other western nations. He also stressed upon Islamic Puritanism and reviving the Quran and Sunnah but did not gone to the extent of Ahl-e-Hadith. However, his ideas could not gain popular support among Muslims as it lacks a short term achievable goal.

[75]The Day of Covenant. Qur'ān claims that Almighty drew the souls of all the human beings whom He intended to create until the Day of Judgment and made them testify. All have taken the oath of allegiance that they would obey and worship Him.

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Mesoporous carbon supported MgO for CO_2 capture and separation of CO_2/N_2

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Abstract−Mesoporous carbon derived from pongamia pinnata fruit hulls was used as support to incorporate magnesium oxide for the study of CO_2 adsorption and separation of CO_2/N_2. All synthesized adsorbents were characterized by PXRD, N_2 adsorption-desorption isotherms, Raman and SEM with EDX techniques. Characterization results revealed the existence of magnesium oxide on mesoporous carbon. CO_2 adsorption on MgO incorporated mesoporous carbon was higher than bulk mesoporous carbon, due to the electrostatic interaction between magnesium oxide and CO_2. High CO_2 adsorption capacity 1.68 mmol/g was obtained for 10 wt% MgO incorporated mesoporous carbon at 298 K, 1 bar compared to remaining loadings, because of the high content of MgO. However, the N_2 adsorption capacity decreased with the increase of MgO content due to a decrease in surface area and no interaction of the N_2 molecule with the adsorbent. The selectivity of CO_2/N_2 was higher on 10 wt% MgO incorporated mesoporous carbon and the value was 40. The heat of CO_2 adsorption was 36 KJ/mol at low coverage of CO_2, and CO_2 adsorption capacity was constant in each adsorption cycle over the same adsorbent.

Keywords: MgO, Mesoporous Carbon, CO_2 and N_2 Adsorption, Selectivity, Heat of CO_2 Adsorption

INTRODUCTION

Carbon dioxide is one of the environmental pollutant gases causing global warming. It is produced by the consumption of fossil fuel, high growth of petrochemical, automobile industries, and power plants [1]. CO_2 concentration can be minimized by the development of an alternative energy source until commercial energy sources have to use for the production of energy. Carbon dioxide in the atmosphere can be reduced by carbon capture and separation. In power plants, a large amount of CO_2 is liberated that is absorbed by the use of liquid amine solutions. However, a large amount of energy is required for the regeneration, and volatile organic compounds are liberated which damage the pipeline system [2]. Adsorption is one of the best techniques to reduce the concentration of CO_2. In this process, energy consumption and damage to the pipeline system are less. So far, commercially available carbon materials [3], zeolites [4], clays [5] and silica materials [6] have been used for CO_2 capture and separation.

Activated carbon is one of the most abundant carbon materials [7]. However, large-scale synthesis is hindered because of the non-renewable source. Porous carbon was synthesized from renewable sources such as waste tea [8], coffee grounds [9], cotton stalk [10], peach stone and olive stone [11,12], biodiesel solid residue [13] and rice husk [14]. Pongamia pinnata fruit hulls are also a source for the porous carbon synthesis. Bio-oil is produced from pongamia pinnata seeds. During the production of bio-oil, pongamia pinnata fruit hulls are thrown without any commercial use. From the fruit hulls, we have synthesized mesoporous carbon to capture CO_2. CO_2 adsorption capacity on mesoporous carbon can be enhanced by the incorporation of basic metal oxides, amine or heteroatom which generates basic sites to capture CO_2.

Iron oxide-doped MCM-41 has shown CO_2 adsorption capacity 0.87 mmol/g at 298 K, 1 bar [15]. CeO_2 incorporated mesoporous carbon showed CO_2 adsorption 1.77 mmol/g at 303 K, 1 bar [16]. Cu_2O and NiO incorporated porous carbon showed high CO_2 adsorption capacity compared to bulk porous carbon [17,18]. S-doped microporous carbon has shown 3.7 mmol/g of CO_2 adsorption at 298 K, 1 bar [19]. N-enriched activated carbon from Procambarus Clarkii shells has shown 2.55 mmol/g of CO_2 adsorption at 298 K, 1 bar [20]. MgO modified mesoporous silica has shown 1.34 mmol/g of CO_2 at 303 K [21]. Similarly, MgO supported titanium oxide showed 0.48 mmol/g of CO_2 adsorption at 298 K [22]. In all reported adsorbents, the CO_2 adsorption capacity was higher due to electrostatic interaction between a metal oxide and CO_2. In this article, we studied CO_2 adsorption on mesoporous carbon and magnesium oxide incorporated mesoporous carbon in low pressure at 298 K. Moreover, the selectivity of CO_2/N_2, the heat of CO_2 adsorption and multiple CO_2 adsorption cycles was studied.

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EXPERIMENTAL

1. Materials

All analytical grade chemicals such as magnesium nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$), Orthophosphoric acid (H_3PO_4) were purchased from all commercial sources and used without further purification. Laboratory purified double distilled water was used for the synthesis of adsorbents. Ultra-high pure gases such as helium, carbon dioxide, nitrogen were purchased from local suppliers in India.

2. Synthesis of Adsorbents

Pongamia pinnata fruit hulls were collected from the forest region of Telangana, India. The fruit hulls were dried and crushed into a fine powder, then chemically activated using orthophosphoric acid with 1 : 1 (w/w%) followed by drying at 100 °C for 12 h, then calcined at 723 K for 4 h in a nitrogen atmosphere. The obtained product was washed with distilled water until neutral pH was obtained, then vacuum dried at 373 K for 12 h. Finally, we got mesoporous carbon [23]; it was denoted as MC. Magnesium oxide incorporated mesoporous carbon was synthesized by the impregnation method. The desired quantity of magnesium nitrate hexahydrate was dissolved in 10 mL distilled water, then 1 g of mesoporous carbon was added to it. The mixture was stirred at room temperature for 1 h, then dried at 373 K for 12 h. The dried product was calcined at 723 K for 4 h in the nitrogen atmosphere. Finally, we got magnesium oxide incorporated mesoporous carbon; it was denoted as xMgO/MC, where x represents the weight percentage of magnesium oxide (x=2, 5 and 10).

3. Characterization

Powdered X-ray diffraction patterns were recorded on Rigaku MiniFlex600 X-ray diffractometer using Ni-filtered Cu K_α radiation (λ=1.54 A°) in the scan range 2θ=10-80°. N_2 adsorption-desorption isotherms were measured using Micromeritics ASAP 2020 surface area and porosity analyzer at 77 K. Prior to adsorption study, about 0.1 g of sample was degasified at 473 K for 4 h under vacuum. The specific surface area was calculated by the BET method. Total pore volume at a relative pressure of 0.99 and micropore volume by the t-plot method was calculated from N_2 adsorption-desorption isotherms. Raman spectra were recorded using LabRAM HR800 Raman spectrometer having laser wavelength 514 nm. Morphological image with metal composition was obtained from ZEISS Sigma 300 Scanning electron microscope analyzer.

4. CO_2 and N_2 Adsorption Measurement

CO_2 and N_2 adsorption isotherms were measured on Micromeritics ASAP 2020 analyzer at low pressure 0-100 kPa at 298 K. Sample temperature was controlled by the thermostatic bath which was connected to water circulating jacket. Free space of the sample was measured using helium gas. About 0.1 g of sample was degasified similar to N_2 adsorption-desorption isotherm measurement at 77 K, then cooled to room temperature for the study of CO_2 and N_2 adsorption isotherms. The selectivity of CO_2/N_2 was calculated using the initial slope of each isotherm by Henry's Law. The heat of CO_2 adsorption was calculated by the Clausius-Clapeyron equation using adsorption isotherms measured at 283, 298 and 303 K. Multiple CO_2 adsorption cycles were also carried out, after desorbing adsorbed CO_2 at 473 K for 2 h under vacuum.

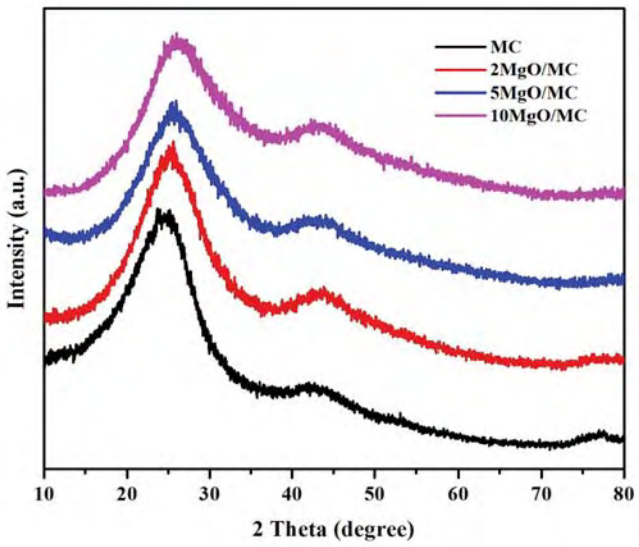

Fig. 1. XRD patterns of MC and MgO incorporated MC.

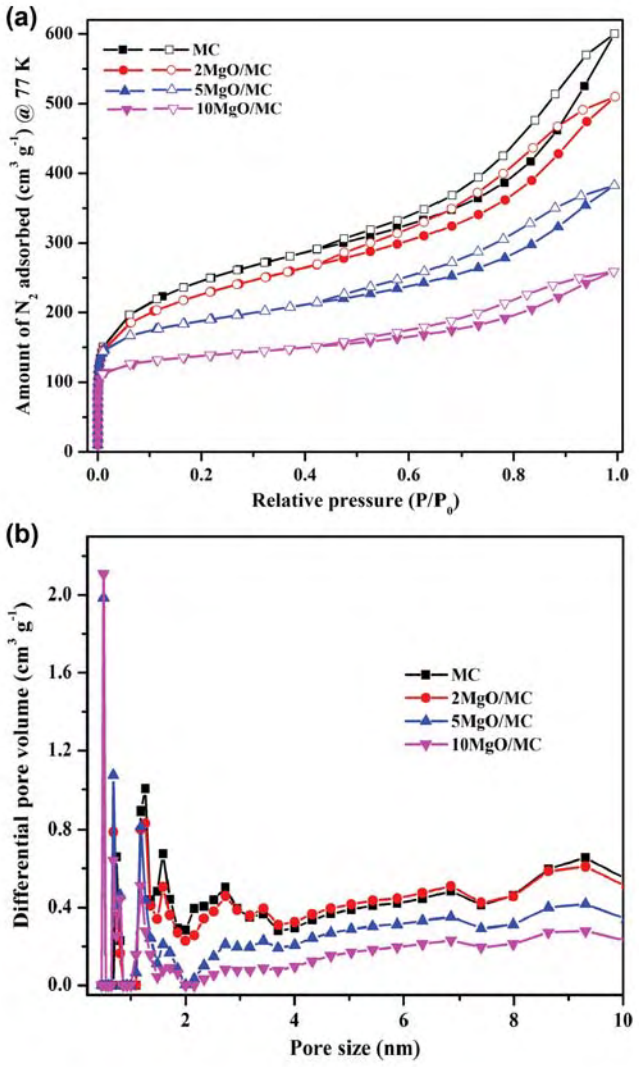

Fig. 2. (a) N_2 adsorption-desorption isotherms and (b) pore size distribution of MC and MgO incorporated MC.

RESULTS AND DISCUSSION

The X-ray diffraction pattern of mesoporous carbon and MgO incorporated mesoporous carbon samples are shown in Fig. 1. MC shows two broad diffraction peaks at 2θ=24.2° and 43.66° having planes (002) and (100), respectively, which are characteristic peaks of mesoporous carbon [24]. Moreover, MgO incorporated mesoporous carbon samples also show diffraction peaks similar to mesoporous carbon and no diffraction peaks related to MgO appeared. It indicates that MgO was well-dispersed over mesoporous carbon. However, the intensity of major diffraction peaks of MC decreased with the increase of MgO loading and shifted towards higher angle. Similar results have been reported on nickel loaded MCM-41 for hydrogen storage [25].

From N_2 adsorption-desorption isotherm, the porosity of carbon material can be found. Fig. 2 shows N_2 adsorption-desorption isotherms of MC and MgO incorporated MC at 77 K. Textural properties are presented in Table 1. MC shows a large amount of N_2 uptake below the relative pressure of 0.1 and a hysteresis loop above the relative pressure of 0.4. The isotherm pattern of MC is similar to type-I and type-IV of classification of porous materials by IUPAC [26], which represent that MC has micro and mesopores. The specific surface area, total pore volume and pore size of MC were 840 m^2/g, 0.94 cm^3/g, and 4.4 nm, respectively. Similarly, MgO incorporated MC samples show the same isotherm pattern similar to MC. But, the amount of N_2 uptake is less. As the content of MgO increased, the amount of N_2 uptake was decreased. Hence, a change in textural properties has been observed. The specific surface area was decreased to 421 m^2/g, the total pore volume 0.34 cm^3/g and pore size 3.8 nm. However, micropore surface area and micropore volume were increased, which indicates that incorporated MgO has occupied mesopores of MC.

Raman analysis is used to determine the crystallinity of carbon material. Fig. 3 shows the Raman spectra of MC and 10MgO/MC. Mesoporous carbon shows two Raman bands at 1,325 cm^{-1} and 1,580 cm^{-1} which correspond to D-band and G-band, respectively [27]. D-band represents disordered carbon and G-band represents graphitic carbon. The ratio of the intensity of bands (I_D/I_G) represents the degree of graphitization. In mesoporous carbon, the intensity of G-band is higher than D-band. It represents that mesoporous carbon has a graphitic nature. The I_D/I_G value was 0.95 for MC and 0.97 for 10MgO/MC. By incorporation of MgO, the graphitic nature of MC decreased [28]. Morphology with the elemental composition of MC and 10MgO/MC is shown in Fig. 4. MC shows irregular shaped carbon particles (Fig. 4(a)). Same morphology was replicated in 10 wt% MgO incorporated mesoporous carbon, which indicates that magnesium oxide was homogenously distributed over carbon surface (Fig. 4(b)). The amount of magnesium was calculated from EDX and the value was 4.92 wt%.

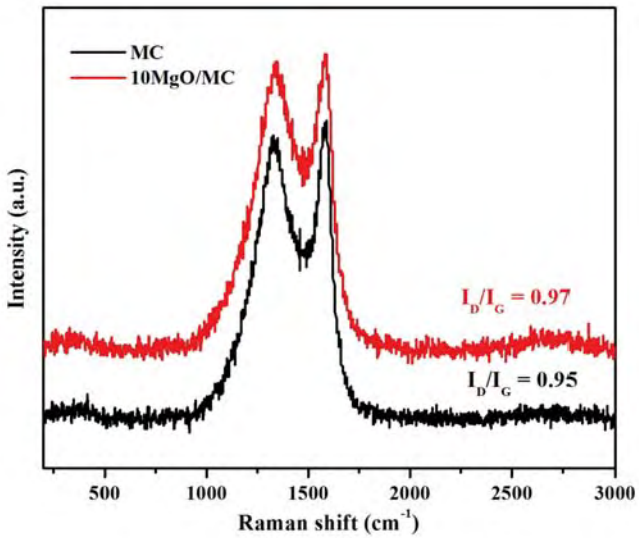

Fig. 3. Raman spectra of MC and MgO incorporated MC.

Fig. 5(a) shows single component adsorption isotherm of CO_2 on MC and MgO incorporated MC samples in low pressure at 298 K. With the increase of CO_2 pressure, the amount of CO_2 adsorption capacity was increased. CO_2 adsorption capacity was 0.9 mmol g^{-1} for MC, 1.0 mmol g^{-1} for 2MgO/MC, 1.5 mmol g^{-1} for 5MgO/MC and 1.68 mmol g^{-1} for 10MgO/MC at 298 K, 1 bar. The CO_2 adsorption capacity of MgO incorporated MC samples was higher compared to MC because of electrostatic interaction between MgO and CO_2. With the increase of MgO content, the amount of CO_2 adsorption capacity increased due to the high content of magnesium oxide. It could be confirmed by calculating the amount of

Table 1. Textural properties of MC and MgO incorporated MC

Adsorbent	S_{BET}[a] (m^2 g^{-1})	S_{micro}[b] (m^2 g^{-1})	V_{total}[c] (cm^3 g^{-1})	V_{micro}[d] (cm^3 g^{-1})	V_{meso}[e] (cm^3 g^{-1})	V_{meso}[f] (%)	Pore size[g] (nm)
MC	840	225	0.93	0.11	0.82	88	4.4
2MgO/MC	743	189	0.79	0.11	0.68	86	4.2
5MgO/MC	591	293	0.59	0.16	0.43	73	4.0
10MgO/MC	421	263	0.40	0.14	0.26	65	3.8

[a]Multipoint BET surface area
[b]Micropore surface area by t-plot
[c]Total pore volume at P/P_0=0.99
[d]Micropore volume by t-plot
[e]Mesopore volume=$V_{total}-V_{micro}$
[f]Mesopore volume (%)=V_{meso}/V_{total}
[g]Average pore size by BET method (4V/S.A)

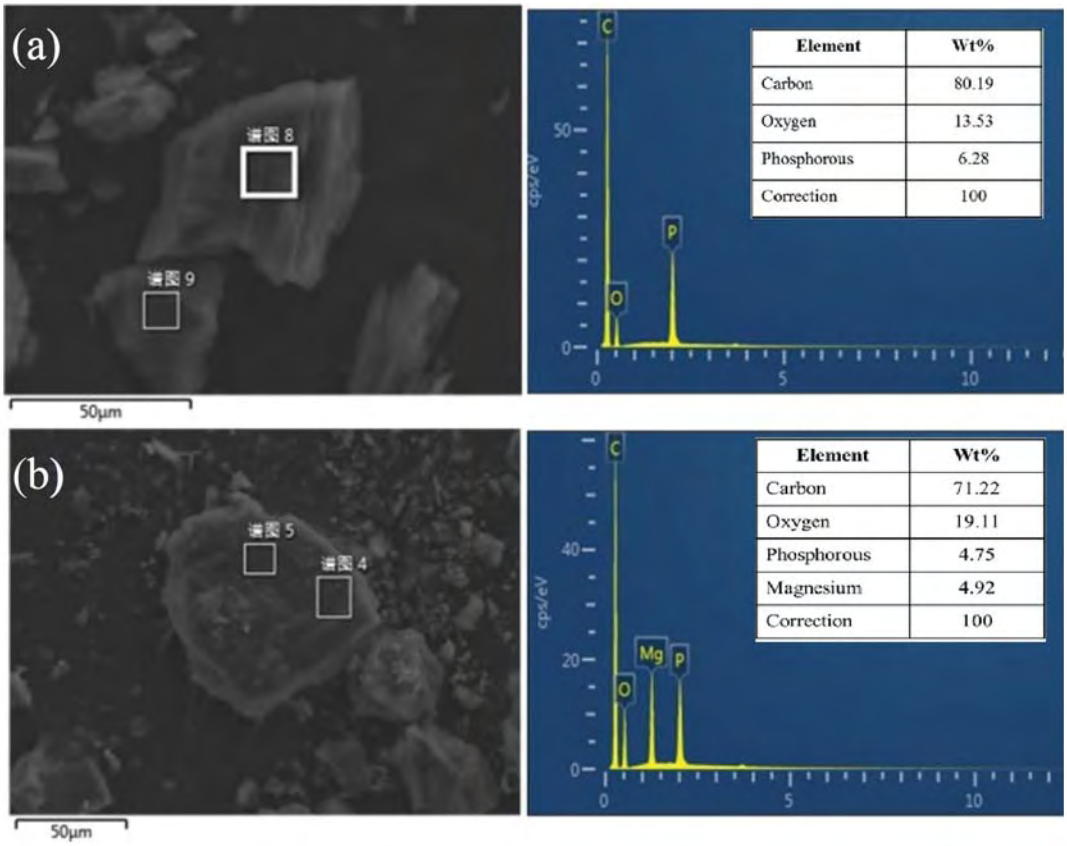

Fig. 4. SEM with EDX of (a) MC and (b) 10MgO/MC.

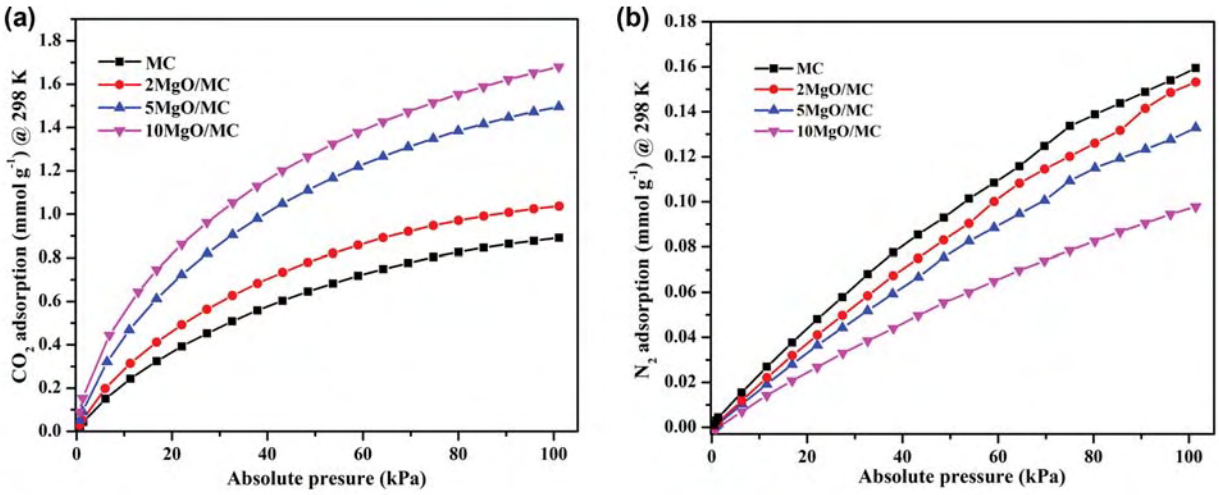

Fig. 5. (a) CO_2 and (b) N_2 adsorption isotherms of MC and MgO incorporated MC at 298 K.

CO_2 adsorption on unit surface area (Fig. 6(a)). CO_2 adsorption on the unit surface area of MgO incorporated mesoporous carbon samples was higher than porous carbon. Hence, CO_2 adsorption depends on the surface chemistry of the adsorbent instead of surface area. Fig. 5(b) shows N_2 adsorption of synthesized adsorbents under a similar condition of CO_2 adsorption. The N_2 adsorption capacity was 0.16 mmol g^{-1} for MC, 0.15 mmol g^{-1} for 2MgO/MC, 0.13 mmol g^{-1} for 5MgO/MC and 0.09 mmol g^{-1} for 10MgO/MC at 298 K, 1 bar. The decrease in N_2 adsorption capacity with the increase of MgO content was due to the decrease in surface area.

The difference in adsorption capacity of CO_2 and N_2 is helpful for studying the selectivity of CO_2 over N_2. In industrial flue gas, CO_2 is a major component gas, so it is essential to study the selectivity of CO_2/N_2. It was calculated using the initial slope of each isotherm in low pressure by Henry's law [29]. Fig. 6(b) shows the

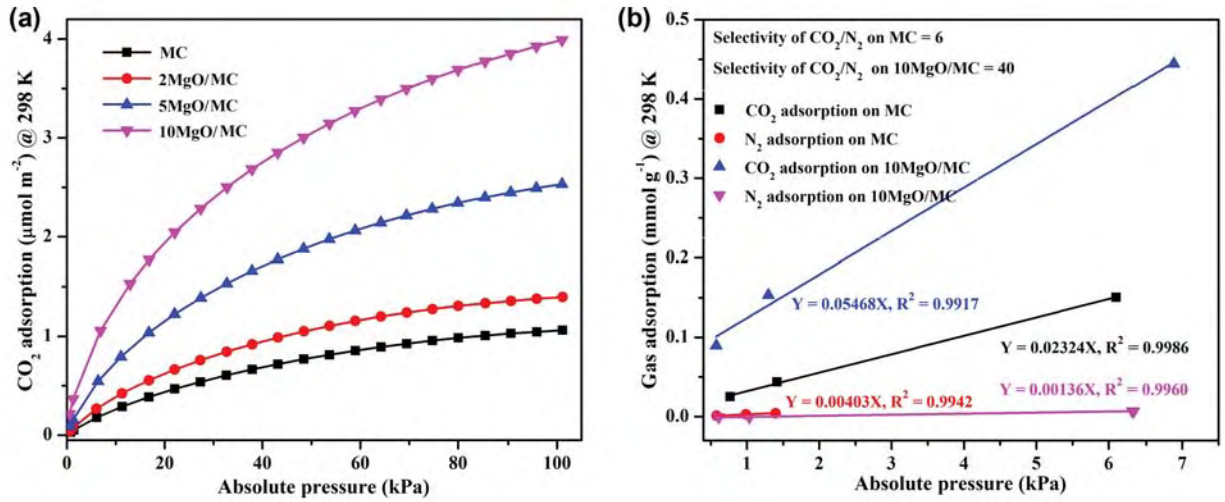

Fig. 6. (a) CO_2 adsorption on unit surface area, (b) selectivity of CO_2/N_2 on MC and 10MgO/MC.

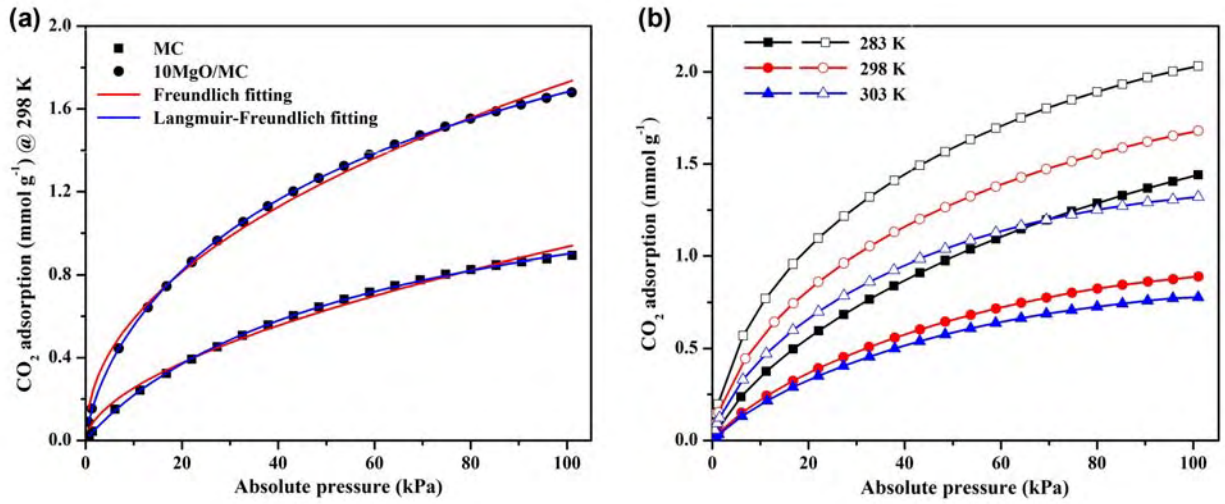

Fig. 7. (a) Fitting curves of experimental CO_2 adsorption data (b) CO_2 adsorption of MC (closed symbol) and 10MgO/MC (open symbol) at different temperatures.

Table 2. Fitting parameters of Freundlich and Langmuir-Freundlich models

Adsorbent	Freundlich model			Langmuir-Freundlich model			
	k_F (kPa^{-1})	n	R^2	Q_{max} (mmol g^{-1})	K (kPa^{-1})	n	R^2
MC	0.0688	1.7666	0.9909	1.4988	0.0195	1.0609	0.9997
10MgO/MC	0.2005	2.1378	0.9942	3.2968	0.0395	1.4077	0.9999

selectivity of CO_2/N_2 on MC and 10MgO/MC. The selectivity value was 6 for MC and 40 for 10MgO/MC. High selectivity value on 10MgO/MC was due to the high adsorption of CO_2. To describe the adsorption of CO_2 on the adsorbent, experimental CO_2 adsorption data of all synthesized adsorbents was fitted with Freundlich and Langmuir-Freundlich models [30]. These models can be expressed as follows.

$$\text{Freundlich model: } Q = k_F P^{1/n} \quad (1)$$

$$\text{Langmuir-Freundlich model: } Q = Q_{max}\frac{KP^{1/n}}{1+KP^{1/n}} \quad (2)$$

where Q is adsorption capacity at equilibrium in mmol/g, Q_{max} is maximum adsorption capacity in mmol/g, P is pressure in kPa, k_F and K are Freundlich, Langmuir-Freundlich coefficients and n is heterogeneity factor. Fitting curves of experimental CO_2 adsorption data of MC and 10MgO/MC are shown in Fig. 7(a) and fitting parameters are presented in Table 2. Langmuir-Freundlich model was well-fitted with experimental CO_2 adsorption data of both adsorbents with regression coefficient (R^2) higher than 0.999, and Q_{max} was higher on 10MgO/MC.

The interaction between adsorbent and adsorbate can be known by calculating the heat of adsorption using the Clausius-Clapeyron

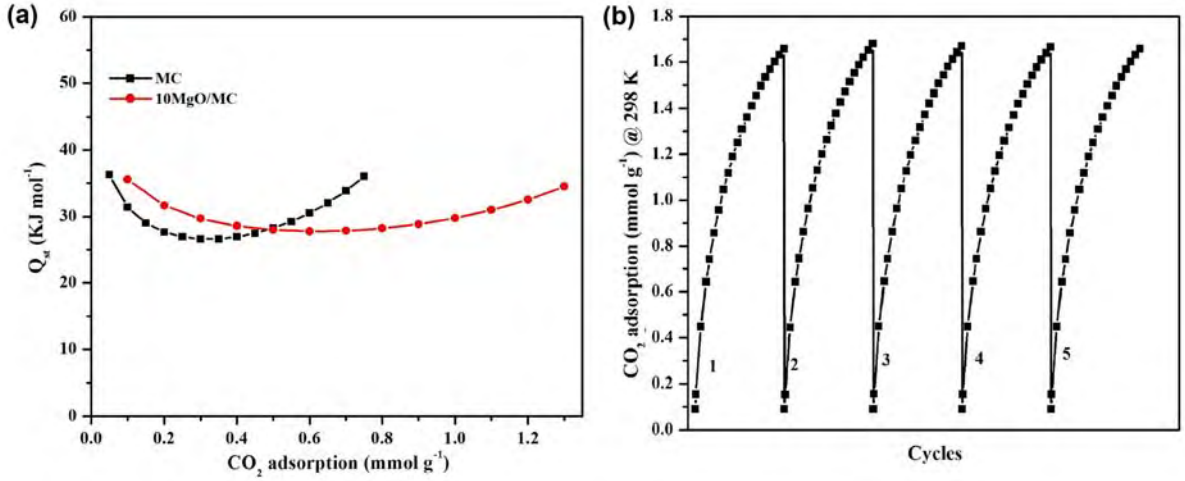

Fig. 8. (a) Heat of CO_2 adsorption on MC and 10MgO/MC, (b) CO_2 adsorption cycles of 10MgO/MC at 298 K.

Table 3. Comparison of CO_2 adsorption capacity and selectivity with reported adsorbents

Adsorbent	CO_2 adsorption at 298 K, 1 bar (mmol g^{-1})	Selectivity (CO_2/N_2)	Reference
NiO/mesoporous carbon	2.02 (303 K)	17.6	[16]
Fe_2O_3 doped MCM-41	0.87	-	[15]
Zeolite-13X	1.70	-	[32]
Karanja seed cake	1.78 (343 K)	-	[33]
MgO/Al_2O_3	1.60 (333 K)	-	[34]
N-doped microporous carbon	1.9	21	[29]
MgO/mesoporous carbon	1.68	40	Present work

equation [31]:

$$\ln P = \frac{-Q_{st}}{RT} + C$$

Here, P is pressure in kPa, T is the absolute temperature in K, R is universal gas constant (8.314 J/K·mol), C is constant and Q_{st} is the heat of adsorption. The partial pressure at different temperatures for the fixed amount of gas uptake can be obtained from the Langmuir-Freundlich model. By drawing a graph between ln P versus 1/T with straight line fitting, we can obtain the slope. Finally, Q_{st} was calculated from the slope. For the calculation of heat of CO_2 adsorption on MC and 10MgO/MC, we measured CO_2 adsorption at 283 K and 303 K also (see Fig. 7(b)). A decrease in CO_2 adsorption capacity was observed on both adsorbents with the increase of temperature. Fig. 8(a) shows the heat of CO_2 adsorption with gas adsorption capacity on MC and 10MgO/MC. The heat of CO_2 adsorption was 36.3-36.0 KJ/mole for MC and 36-34.5 KJ/mole for 10MgO/MC. At low coverage of CO_2, the heat of CO_2 adsorption for 10MgO/MC was higher than MC. It was due to the strong interaction between MgO and CO_2. The Q_{st} was decreased to 34.5 KJ/mole with the increase of CO_2 adsorption. For both adsorbents, the heat of CO_2 adsorption was increased after the minimum with an increase of CO_2 adsorption capacity. It was due to the heterogeneity of the adsorbent.

Adsorption stability of an adsorbent can be known by multiple adsorption cycles. Fig. 8(b) shows multiple CO_2 adsorption cycles of 10MgO/MC at 298 K. Before study of each adsorption cycle, the adsorbent was degasified at 473 K for 2 h under vacuum. 10MgO/MC showed constant CO_2 adsorption capacity up to five cycles. The CO_2 adsorption of 10MgO/PC was compared with some of the reported adsorbents (Table 3). The CO_2 adsorption capacity value was between the adsorption capacity of NiO supported on mesoporous carbon [16], iron oxide doped MCM-41 [15] and Zeolite-13X [32]. Hence, it is one of the adsorbents that has shown good adsorption capacity and selectivity.

CONCLUSIONS

Mesoporous carbon and MgO incorporated mesoporous carbon samples were used as an adsorbent for the study of CO_2 capture and separation. The presence of MgO on mesoporous was confirmed by all characterization techniques. 10MgO/MC showed high adsorption of CO_2 1.68 mmol/g at 298 K, 1 bar, which was higher than MC by electrostatic interaction between CO_2 and MgO. High selectivity of CO_2 over N_2 was 40 and heat of CO_2 adsorption was 36 KJ/mole at low coverage of CO_2 on 10MgO/MC. Stable CO_2 adsorption capacity was maintained in each adsorption cycle. Therefore, mesoporous carbon derived from pongamia pinnata fruit hulls can be used as an adsorbent and support to incorporate metal oxides to study CO_2 adsorption and separation.

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REFERENCES

1. S. Hosseini, I. Bayesti, E. Marahel, F. Eghbali Babadi, L. Chuah Abdullah and T. S. Y. Choong, *J. Taiwan Inst. Chem. Eng.*, **52,** 109 (2015).
2. D. Aaron and C. Tsouris, *Sep. Purif. Technol.*, **40**, 321 (2005).
3. M. K. Al Mesfer and M. Danish, *J. Environ. Chem. Eng.*, **6**, 4514 (2018).
4. R. Seabra, A. M. Ribeiro, K. Gleichmann, A. F. P. Ferreira and A. E. Rodrigues, *Micropor. Mesopor. Mater.*, **277**, 105 (2019).
5. J. Pires, M. Bestilleiro, M. Pinto and A. Gil, *Sep. Purif. Technol.*, **61**, 161 (2008).
6. C. Knöfel, J. Descarpentries, A. Benzaouia, V. Zeleňák, S. Mornet, P. L. Llewellyn and V. Hornebecq, *Micropor. Mesopor. Mater.*, **99**, 79 (2007).
7. B. B. Saha, S. Jribi, S. Koyama and I. I. El-Sharkawy, *J. Chem. Eng. Data*, **56**, 1974 (2011).
8. I. I. Gurten, M. Ozmak, E. Yagmur and Z. Aktas, *Biomass Bioenergy*, **37**, 73 (2012).
9. S. Rattanapan, J. Srikram and P. Kongsune, *Energy Procedia*, **138**, 949 (2017).
10. H. Deng, G. Li, H. Yang, J. Tang and J. Tang, *Chem. Eng. J.*, **163**, 373 (2010).
11. T. Uysal, G. Duman, Y. Onal, I. Yasa and J. Yanik, *J. Anal. Appl. Pyrolysis*, **108**, 47 (2014).
12. S. M. Yakout and G. Sharaf El-Deen, *Arabian J. Chem.*, **9**, S1155 (2016).
13. X. Zhao, W. Li, F. Kong, H. Chen, Z. Wang, S. Liu and C. Jin, *Mater. Chem. Phys.*, **219**, 461 (2018).
14. Y. Gao, L. Li, Y. Jin, Y. Wang, C. Yuan, Y. Wei, G. Chen, J. Ge and H. Lu, *Appl. Energy*, **153**, 41 (2015).
15. K. C. Chanapattharapol, S. Krachuamram and S. Youngme, *Micropor. Mesopor. Mater.*, **245**, 8 (2017).
16. M. Li, K. Huang, J. A. Schott, Z. Wu and S. Dai, *Micropor. Mesopor. Mater.*, **249**, 34 (2017).
17. B. J. Kim, K. S. Cho and S. J. Park, *J. Colloid Interface Sci.*, **342**, 575 (2010).
18. D. I. Jang and S. J. Park, *Fuel*, **102**, 439 (2012).
19. J. Shi, N. Yan, H. Cui, Y. Liu and Y. Weng, *J. Environ. Chem. Eng.*, **5**, 4605 (2017).
20. W. Cai, S. Zhang, X. Hu and M. Jaroniec, *Energy Fuels*, **32**, 9701 (2018).
21. H. Zhao, W. Yan, Z. Bian, J. Hu and H. Liu, *Solid State Sci.*, **14**, 250 (2012).
22. H. Jeon, Y. J. Min, S. H. Ahn, S.-M. Hong, J. S. Shin, J. H. Kim and K. B. Lee, *Colloids Surf., A*, **414**, 75 (2012).
23. M. A. Islam, S. Sabar, A. Benhouria, W. A. Khanday, M. Asif and B. H. Hameed, *J. Taiwan Inst. Chem. Eng.*, **74**, 96 (2017).
24. B. Chen, Z. Yang, G. Ma, D. Kong, W. Xiong, J. Wang, Y. Zhu and Y. Xia, *Micropor. Mesopor. Mater.*, **257**, 1 (2018).
25. S. J. Park and S. Y. Lee, *J. Colloid Interface Sci.*, **346**, 194 (2010).
26. S. Brunauer, P. H. Emmett and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).
27. W. Tian, Q. Gao, Y. Tan, K. Yang, L. Zhu, C. Yang and H. Zhang, *J. Mater. Chem. A*, **3**, 5656 (2015).
28. S. Cheng, L. Zhang, H. Xia and J. Peng, *Green Process. Synth.*, **6**, 487 (2017).
29. M. Saleh, J. N. Tiwari, K. C. Kemp, M. Yousuf and K. S. Kim, *Environ. Sci. Technol.*, **47**, 5467 (2013).
30. C. Goel, H. Bhunia and P. K. Bajpai, *J. Environ. Chem. Eng.*, **4**, 346 (2016).
31. J. Yan, Y. Yu, C. Ma, J. Xiao, Q. Xia, Y. Li and Z. *Li, Appl. Therm. Eng.*, **84**, 118 (2015).
32. J. McEwen, J. D. Hayman and A. Ozgur Yazaydin, *Chem. Phys.*, **412**, 72 (2013).
33. K. Upendar, T. V. Sagar, G. Raveendra, N. Lingaiah, B. V. S. K. Rao, R. B. N. Prasad and P. S. S. Prasad, *RSC Adv.*, **4**, 7142 (2014).
34. S. Zhang, W. Cai, J. Yu, C. Ji and N. Zhao, *Chem. Eng. J.*, **310**, 216 (2017).

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HISTOPATHOLOGICAL STUDIES IN C*HANA PUNCTATUS* AND *MACROBRACHIUM ROSENBERGII* EXPOSED TO SONATA

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ABSTRACT

The present study is aimed to assess the histological damage caused to the fish C*hana punctatus* and prawn *Macrobrachium rosenbergii* were exposed to lethal concentration (15.39ppm &12.09ppm) to Sonata(Fungicide). Light microscopic studies exhibited severe histopathological changes in the Gill, Liver and Brain. The histopathological changes in the gill of fish include: epithilial lifting, degenerated secondary lamella, curling of secondary filaments and degenration of epithelial cells and gill of prawn include Detached cuticle, Degeneration of epithelium in secondary gill lamelle, Necrosis., Infiltrations of haemocytes, Edema reupture of epithelial cells., Hypertrophy, Hyperpalsia., Disarrangement of secondary gill lamellae, Disruption of pillar cells and Enlargement of scondary gill lamellae. The histopathological changes in the Liver of fish include: blood cells among haptocytes, appearance of blood streaks among hepatocytes, formation of vacuoles and degeenrated hepato pancreatic tissue and Liver of prawn include haemorrage, vacuoles, necrosis and blood vesseles. The changes in the Brain of fish include: Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area and brain of prawn include Pyknotic nuclei, Pyknotic nuclei with dense eosinophilic cytoplasamm, Vacuolated spaces, Prolifiration of gilala cells and Gliosis and nodule formation.

KEYWORDS: Sonata, Hypertrophy, Hyperpalsia, epithilial lifting, Pyknotic nuclei, Gliosis.

INTRODUCTION

In order to meet the growing population needs and demands, use of agrochemicals is inevitable for enhanced food production. Pesticides are highly effective substances used in control of pest and vectors of human disease. The increasing use of pesticides has caused

concerns about their effects on human health and the environment. Inspite of potential applications in agriculture, horticlture and other allied fields, they also exert some disadvantages, they include toxicity to animals, plants and human beings. Persistence of some of these chemicals in the environment and their subsequent entry into aquatic systems causes a great havoc. Pesticides and fungicides exert their toxic action on arthropods, mussels, fishes, frogs, turtles, water birds and other wild life too. Excessive use leads to bioaccumulation in farm workers, fruits, vegetables, nuts and food crops, consumers, and it also causes biomagnification at various trophic levels of the food chain. Although Indian average consumption of pesticide is far lower than many other developed economies, the problem of pesticide residue is very high in India.[1] Fungicides also threatens non target aquatic and terrestrial organisms through drift either by consumption or by ground water contamination. They enter water from agriculture and run off. The pollution of normal waters with synthetic chemicals has caused serious problems to the aquatic biota.[2,3,4,5] Fish and prawn are useful bioindicators and integrators of contaminants. They accumulate in gills, liver, kidney, and fat and induces metabolic changes associated with these organs.

MATERIALS AND METHODS

Animal collection

The fish and prawn specimens samples of the two varities namely *Channa punctatus* and *Macrobrachium rosenbergii* were collected from the freshwater lake located in waddepelly cheru, Warangal district. Fish measuring 14-15cms in length and weighing 250-300gms and prawn measuring 14-18cms in length and weighing 25-30gms specimens were brought to the laboratory immediately and analysed for various biological and nutritive value studies.

The fish and prawn were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2oC. During the period of acclimatization, the fish and prawn were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms[6] were followed. After Acclimatization, Fishes and prawns were divided into groups and treated with concentrations of 10 and 20 ppm biofungicide sonata at time points 48, 72 and 96 hrs. to decide the lethal toxicity (LC50). The LC50 values were calculated the using probits analysis based on finney's (1952) table.

Tissue collection

Gill, liver, and brain tissues were isolated from normal (not exposed to the toxicant) and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouins solution for 48 hr, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut of 4-6µ (microns) thickness; stained with Hematoxylin-Eosin (dissolved in 70% alcohol)[7] and were mounted in Canada balsam. Histopathological lesions were examined and photographed with the help of Intel Pentium QX3 computer attached microscope under 400X lens.

RESULTS

Gills

No histopathological changes were observed in the gill of the control fish and prawn. The structural details of the gill of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 1A&B. The most common changes in 15.39ppm & 12.09ppm concentrations of Sonata Fungicide were epithilial lifting, degenerated secondary lamella, curling of secondary filaments and degenration of epithelial cells and in prawn include Detached cuticle, Degeneration of epithelium in secondary gill lamelle, Necrosis., Infiltrations of haemocytes, Edema reupture of cpithelial cells., Hypertrophy, Hyperpalsia., Disarrangement of secondary gill lamellae, Disruption of pillar cells and Enlargement of scondary gill lamellae. The histological changes noticed in the pesticide exposed and control fishes and prawns are shown in fig.1 A(A1) & FIG.1B(A1).

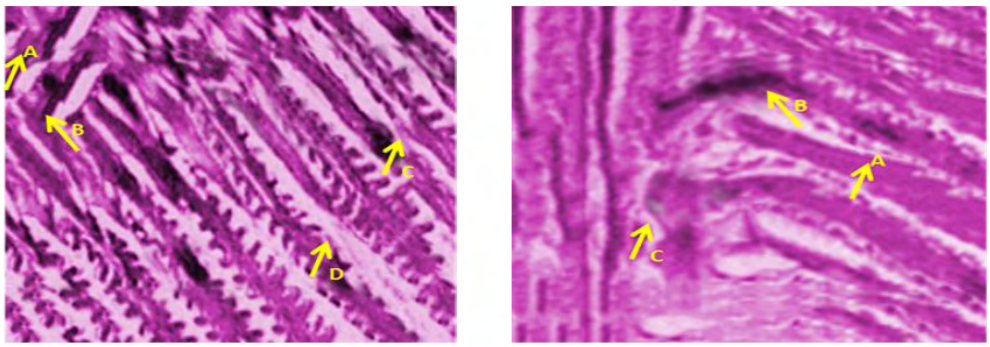
Liver

No histopathological changes were observed in the liver of the control fish and prawn. The structural details of the liver of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 2A&B. In the liver tissues of fish and prawn exposed to sonata concentrations of 15.39 ppm and 12.09 ppm, blood cells among haptocytes, appearance of blood streaks among hepatocytes, formation of vacuoles and degeenrated hepato pancreatic tissue and Liver of prawn include haemorrage, vacuoles, necrosis and blood vesseles were seen (Figs. 2B-. The histological changes noticed in the pesticide exposed and control fishes and prawns are shown in Fig.2A(A1) & Fig.2B(A1).

Brain

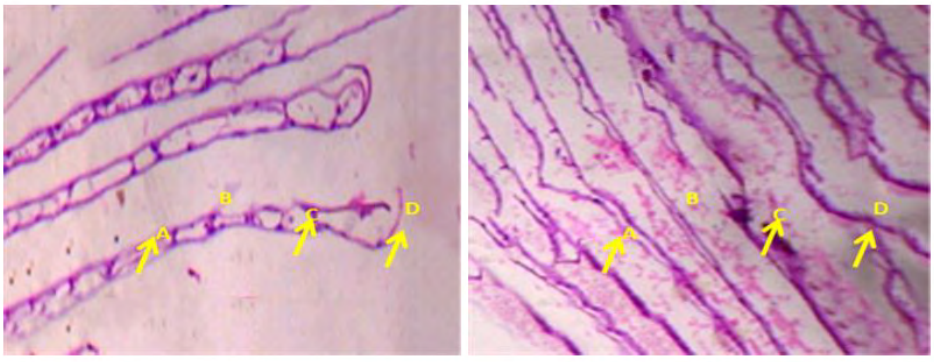
No histopathological changes were observed in the brain of the control fish and prawn. The structural details of the brain of control *C. Punctatus* and *M.rosenbergii* are shown in Fig. 3A&B. The most common changes in15.39ppm &12.09ppm concentrations of Sonata Fungicide were Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area and in prawn include Pyknotic nuclei, Pyknotic nuclei with dense eosinophilic cytoplasamm, Vacuolated spaces, Prolifiration of gilala cells and Gliosis and nodule formation. The histological changes noticed in the pesticide exposed and control fishesand prawns are shown in fig.3 A(A1) & FIG.3B(A1).

Gill



A1 **B1**

Fig 1A: Histopathology Studies of *Chana Punctatus* in Gill samples treated at 96 hrs (A&B); A1: Control: B1 Treated: In Control, A.Central Axis. B. Erythrocyte. C. Primary Gill Lamella. D. Secondary gill lamella: In Treated A. Efithilial Lifting. B. Curling of secondary gill filaments C. Degenretaed secondary lamella.

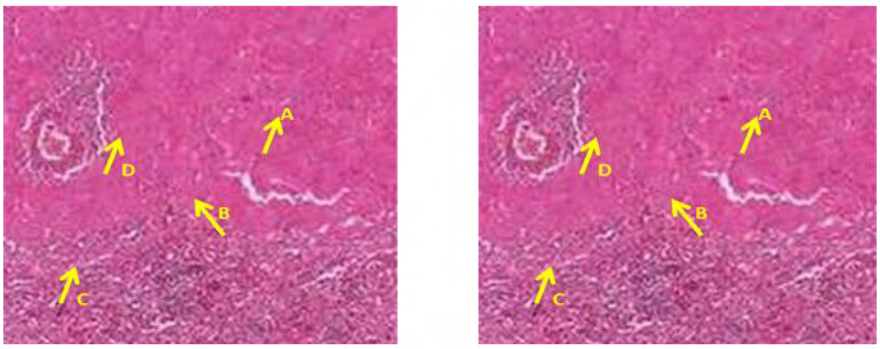


A1 **B1**

Figure1B: Histopathology Studies of *Macrobrachium* in Gill samples treated at 96 hrs (A1 &B1); A1 - Control: B1- treated; In control: A. Pillar cells. B. Incites. C. Detached cuticle. D. Degeneration of epithelium in secondary gill lamelle. In treated: A. Necrosis.

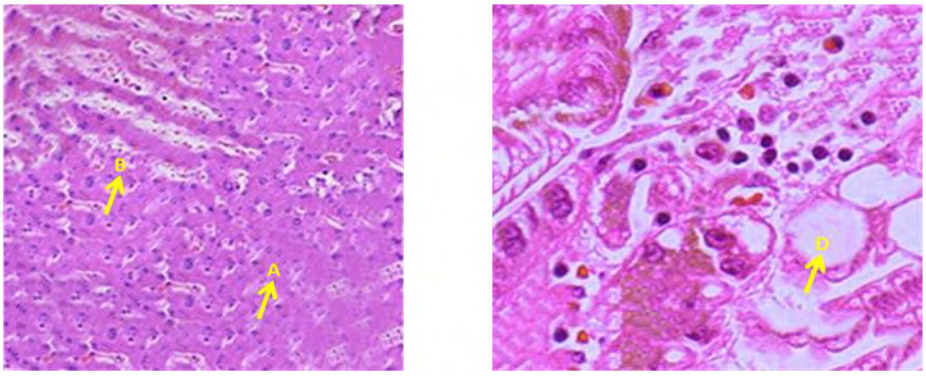
B. Infiltrations of haemocytes. C. Disarrangement of secondary gill lamellae. D. Disruption of pillar cells.

Liver



A1 **B1**

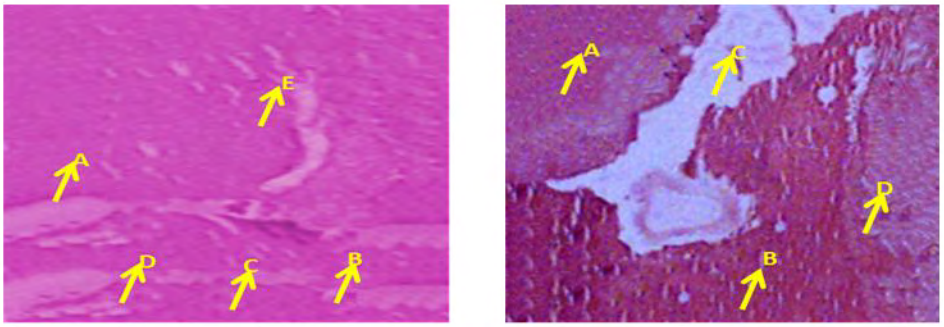
Figure 2 A: Histopathology Studies of *Channa Punctatus* in Liver samples treated at 96 hrs (A1 &B1). A1 Control: B1Treated. In Control, A.Hepatic cell. B. Nucleus. C. Lipid and glycogen granules: In Treated A. Degenerated hepato pancreatic tissue. B. Blood cells among hepatocytes C. Apperaence of blood streaks among hepatocytes. D. Formation of vacuoles.



A1 **B1**

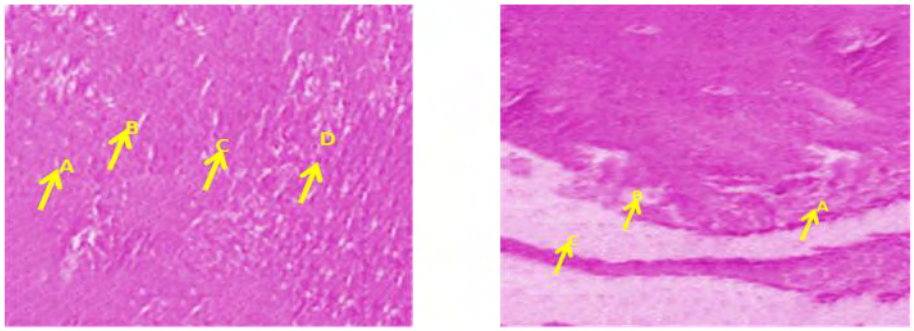
Figure 2 B: Histopathology Studies of *Macrobrachium* in Liver samples treated at 96 hrs (A1 &B1); A1 -Control: B1- Treated: A. Hepatic cells. B. Hepatic pancreas. C. Haemorrage. D. Vacuoles.

Brain



A1 **B1**

Figure 3 A: Histopathology Studies of *Channa Punctatus* in Brain samples treated at 96 hrs (A1 & B1); A1 Control: B1Treated: In Control, A Dorsal olfactory area. B. Ventral olfactory area. C. Septal area. D. Tractus olfactoriuos medialis. E Tractus olfactorius lateralis: In Treated A. Degenerated dorsal olfactory area B. Degenerated Ventral olfactory area C. Blood streeks. D. Degenerated septal area.



A1 **B1**

Figure 3 B: Histopathology Studies of *Macrobrachium.rosenbergii* in Brain samples treated at 96 hrs (A1 & B1); A1 Control: B1Treated: In Control, A Dorsal olfactory area. B. Ventral olfactory area. C. Septal area. D. Tractus olfactoriuos medialis. In Treated, A.Pyknotic nuclei. B. Pyknotic nuclei with dense eosinophilic cytoplasam. C. Vacuolated spaces and nodule formation.

DISCUSSION

The gills, which participate in many significant functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants.[7,8,9] Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the

external environment and the blood and thus serve as a barrier to the entrance of contaminants.[10,7,11,12]

Liver, the first organ to encounters any foreign molecule through portal circulation is subjected to more damage.[13] Liver is an important organ of detoxification which breaks down toxic substances and metabolites of administered substances. This breakdown is carried out by endoplasmic reticulum of hepatocytes. Due to these reasons the hepatic cells are damaged severely.[13] reported that in fish *Tilapia mosanmbica* exposed to the toxicant resulted in vacuolation and necrosis in liver.[14] reported that *Channa punctatus* under Malathion toxicity showed the degenerative changes in liver.[15] reported that in teleost fish Nemachelius denesoni (Day) exposure to phosphamidon caused highly vacuolated and claudy swelling and even the connective tissue was damaged in liver.[16] reported significant alterations in the hepatic cell count and the nucleocyton plasmic index in the liver of zebra fish *Brachydanio rerio* (cyprinid) exposed to 0.9 mg/l concentration of Malathion.

Like gills and liver in sonata treated *Chana punctatus* fish, Pathological changes were observed in brain samples also. Changes include Degenerated dorsal olfactory area, degenerated Ventral olfactory area, blood streeks and degenerated septal area. Similar changes were observed by[17] reporting swelling of the axon, atrophy, necrosis and pycnosis in the fish *Ctenopharyngodon idellus* under fenvalerate toxicity, and[18] on *Cirrhinus mrigala* exposed to the sublethal and lethal concentrations of technical grade as well as 20% EC of Chlorpyrifos for 8 days and the severity of damage is more in lethal exposures than in sublethal exposures. Quinalphos technical grade caused more degenerative changes in brain than in 25% EC exposures (Plate VI.3, Fig. B, C, D and E).[19] reported that hexachlorocyclohexane was neurotoxic and induced vacuolation of brain parenchyma and moderate swelling of pyramidal cells of the cerebrum and opined that vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. Loss of Nissle substances and glial cell reaction, with evidence of glial nodule formation in places, were proof of the the neurotoxic nature of the chemical.

In the present investigation, gill, liver and brain tissues shows changes in their structures were observed during acute and sublethal sonata exposure which may indicate the different rates of free radical generation and different antioxidant potentials of these tissues. The present study also demonstrated that sonata has a high oxidative-stress-inducing potential in *Chana*

punctatus and *Macrobrachium rosenbergi*i and gill is the most sensitive organ in both acute as well as sub lethal concentration.

CONCLUSION

All the histopathological observation indicated that exposure to lethal concentrations of sonata caused destructive effect in the gill, liver and brain tissues of *C. Punctatus and M.rosenbergii*. Gil, liver and brain histopathological alterations, such as those observed in this study and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish and prawn. As a conclusion, the findings of the present histological investigations demonstrated a direct correlation between pesticide exposure and histopathological disorders observed in several tissues.

REFERENCES

1. Abhilash, P. C. and Nandita Singh.: Indian scenario J. Hazardous Materials, 2009; 165(1-3): 1-12.
2. Arillo, A., Margiocco, C., Melodia, F. and Mensi, P.: Chemosphere, 1982; 11(1): 47-58.
3. Reber, H.H. and Borkott H.: Natural Resources and development, 1983; 17: 29- 39.
4. Ganesan, R.M., Jebakumar, S.K.D. and Jayaraman, J.: Pro. Ind. Acad. Sci., (Anim. Sci.), 1989; 98: 51-55.
5. Anitha Kumari, S. and N.S.R Kumar.: Environ. Biol, 1997; 18(1): 11-16.
6. APHA: American Public Health Association, 16 edn (1995).
7. Humason, G.L.: Animal tissue techniques 111 (edition) (1972).
8. POLEKSIC, V. and MITROVIC-TUTUNDZIC, V.: Cambridge: Cambridge Univ. Press, 1994; 339-352.
9. MAZON, AF., MONTEIRO, EAS., PINHEIRO, GHD. and FERNANDES, MN.: Brazilian Journal of Biology, 2002; 62(4A): 621-631.
10. FERNANDES, MN. and MAZON, F.:Enfield: Science Publishers, 2003; 203-231.
11. MALLAT, J.: Canandiam Journal Fish of Aquatic Science, 1985; 42: 630-648.
12. Hinton, D. E. & D. J. Laurén.: In: McCarthy, J.F. & L.R. Shugart (Eds.). Boca Raton, Lewis Publishers, 1990; 51-65.
13. Jayantha Rao, K.: Ph.D., Thesis, S.V. University, Tirupati, A.P., India, 1982.
14. Dubale, M.S and Shah, P.: Indian Journal of Experimental Biology, 1979; 17(7): 693-697.
15. Rashatwar and Ilysas. Toxicol. Lett, 1984; 16: 249-252.
16. ANSARI, B.A. & K. KUMAR.: Sci. Total Environ, 1988; 76(I): 63-68.

17. Yacobu: Ecotoxicol. Environ. Monit, 1999; 12: 9-15.

18. Tilak, K.S., K. Veeraiah and D. Koteswara Rao. Pollut. Res, 2005b; 24: 101-111.

19. Das BK, Mukherjee SC.: Veterinarski Arch, 2000; 70(4): 169-180.