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SYNTHESIS, CHARACTERIZATION AND PERSISTENT DNA BINDING, ANTI-MICROBIAL AND CYTOTOXICITY STUDIES OF NOVEL COPPER (II) COMPLEXES CONTAINING L-TRYPTOPHAN AND HETEROCYCLIC BASE

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Keywords:

1, 10-phenanthroline, copper (II) complex, cytotoxicity, DNA binding, antimicrobial activity

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ABSTRACT: The copper (II) complexes, [Cu(phen)(tryp)I] (1), [Cu(phen)(tryp)Cl], (2) [Cu(phen)(tryp)SCN] (where phen = 1,10-phenanthroline and tryp = tryptophan) has been synthesized and characterized by Infra-red Spectra, UV–Visible Spectra, EPR Spectra and elemental analysis methods. Binding interactions mode of the complexes with calf thymus DNA (CT-DNA) were investigated by UV–Vis absorption titration, fluorescence emission, cyclic voltammetry, viscometric technique. The complexes were subjected to *in-vitro* cytotoxicity studies against the human liver cancer cell, as revealed by the MTT assay method. The complex was shown to be a partial intercalation mode of binding into DNA. The results show that complex 1 exhibited potent cytotoxic effects against human cell line (HepG2) when compared to complex 2 and complex 3. The complex was screened for its *in-vitro* antibacterial activity against one Gram-positive (*staphylococcus aureus)* and two Gram-negative (*klebsiella pneumonia, Escherichia coli)* bacterial strains and for *in-vitro* antifungal activity against (*Aspergillus niger, Rhizopus species, Penicillium species).* The results suggest that all the complexes exhibit good antibacterial activity. The antifungal activity data showed that complex 1 and 3 will be inactive, whereas complex 2 show good antifungal agents towards *Aspergillus niger* fungal species.

INTRODUCTION: Deoxyribonucleic acid, DNA, is a molecule of great biological significance since it contains all the genetic information for cellular function [1]. The central role of DNA is replication, transcription, and regulation of genes has prompted the search for artificial nucleases, catalysts able to cleave the DNA molecule [2]. Studies of the interaction between transition metal complexes and DNA have been pursued in recent years.

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Transition metals that have been extensively utilized in medicinal chemistry include platinum, ruthenium, titanium, rhodium, copper, palladium, gold, and iron [3].

DNA particularly offers a wide variety of potential metal binding. Such Sites include the electron-rich DNA bases or phosphate groups that are available for direct covalent coordination to the metal, and they occur: (i) between two base pairs (intercalation) [4], (ii) in the minor groove, (iii) in the major groove, [5] and (iv) on the outside of the helix [6]. Metal complexes are known to bind to DNA via both covalent and non-covalent interactions. In covalent binding, the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N 7 [7].

Intercalative binding, as most commonly studied, is the noncovalent stacking interaction [8] resulting from the insertion of a planar heterocyclic aromatic ring between the base pairs of the DNA double helix [9]. Metallointercalators having planar N-donor heterocyclic bases are used as photochemical and chemical reagents in nucleic acids chemistry [10].

Cisplatin [cis-diamminodichloroplatinum (II)] is one of the foremost and widely used metal-based anticancer drugs for cancer therapy [11], by coordination with DNA; this interaction interferes with mitosis, causing the cancer cell to undergo apoptosis [12] but it possesses inherent limitations such as serious side effects, general toxicity, and acquired drug résistance therapeutic agents [11]. Consequently, copper is suitable as an alternative element, providing benefits in both the design and various applications of metal complexes [12].

Transition metal ions, especially the copper ions, have strong interactions with DNA, which result in conformational changes of the DNA structure [13]. Copper is an essential bio element that plays a key role in biological processes, and its complexes are preferred molecules for cancer inhibition [14]. Copper complexes containing phen ligands are useful probes for DNA duplexes.

The five-coordinated Cu (II) complexes with an N, N donor heterocyclic base ligand better prospect in DNA binding [15].

In our previous work, we synthesized and characterized DNA binding and Cleavage of some Cobalt(III) and Copper(II) Complexes [16-25]. The present aim of the work is to design ternary copper(II) complexes of bio-essential Amino acid (L-tryptophan) with phenanthroline is synthesized, characterized by DNA binding, anti-microbial activity, and anti-cancer activity. The complex [Cu(phen) (tryp) I] (1), [Cu(phen) (tryp) Cl] (2), [Cu (phen) (tryp) SCN] (3) were synthesized and characterised. The DNA binding abilities of Copper (II) complexes were examined by absorption spectroscopy, fluorescence spectroscopy, cyclic voltammetry, and viscosity measurements. The result should be of value in further understanding the binding modes of the complexes to DNA. The cytotoxic activity of complexes 1, 2, and 3 against human liver cancer cell Hep-G2 and antimicrobial activities of three copper (II) complexes against certain human pathogenic microorganisms were also reported.

EXPERIMENTAL: Common reagents such as methanol, $Cu(NO_3)_2.3H_2O$, and L-tryptophan 1,10-phenanthroline monohydrate are all analytical grade and used as received and disodium salt of calf thymus DNA (CT-DNA), and ethidium bromide were purchased from Sigma Aldrich.

Materials and Instrumentation: The infrared spectra were recorded on a Perkin Elmer spectrometer on KBr pellets (4000–400 cm^{-1}), and elemental analysis was performed on a Perkin Elmer 240 C analytical instrument. The EPR measurement was carried out at room temperature using a Bruker EMX EPR spectrometer. The UV–vis and fluorescence spectra of the complex were recorded on the Shimadzu UV-2450 spectrophotometer and Jobin Young Fluorolog 3 spectrophotometer, respectively. The cyclic voltammograms were obtained on a CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen-free conditions using a three-electrode cell with a DMF solution of TBAP (0.1M) as the supporting electrolyte. A Pt wire, glassy carbon, and the Ag/AgCl electrode (saturated KCl solution) were used as a counter, working and a reference electrode, respectively.

Synthesis of [Cu(Phen)(tryp)I] (1): The aqueous solution of L- tryptophan (2 mmol) in methanol-water (1:1), an aqueous solution of $Cu(NO_3)_2.3H_2O$ (2 mmol) was added with vigorous stirring. After 30 minutes later, the (10 ml) methanolic solution of 1,10- phenanthroline was added slowly. A few minutes later, 2 mmol of potassium iodide was added, and then the solution was stirred for about 6 h at ambient temperature. The resulting green solution was filtered and kept for slow evaporation. The green colour precipitate was obtained. The precipitate washed 3-4 times with double distilled water to remove the impurities after that it was dried at room temperature. Yield: 60%. Analytical calculation for $C_{24}H_{22}CuIN_4O_2$ (%): C, 48.10; H, 3.31; N, 11.67. Found (%): C, 48.14; H, 3.31; N, 11.88 IR (KBr, cm-[1]): 3463 br, 3244w, 1612s, 1110m, 1520m, 2925w, 850s, 722vs (br, broad; *vs*., very strong; s, strong; m, medium; w, weak) mp. 220 ºC.

Synthesis of [Cu (Phen)(tryp)Cl] (2): The aqueous solution of L- tryptophan (2 mmol) in methanol- water (1:1), an aqueous solution of Cu $(NO_3)_2.3H_2O$ (2 mmol) was added with vigorous stirring. After 30 min later, the (10 ml) methanolic solution of 1,10- phenanthroline was added slowly. A few minutes later, 2 mmol of potassium chloride was added, and then the solution was stirred for about 6 h at ambient temperature. The resulting faint green solution was filtered and kept for slow evaporation. The faint green precipitate washed 3-4times with double distilled water to remove the impurities after that it was dried at room temperature. Yield: 74%. Analytical calculation for $C_{24}H_{22}CuClN_4O_2$ (%): C, 57.19; H, 3.92; N, 11.58. Found (%): C, 57.26; H, 3.97; N, 9.71; IR (KBr, cm^1): 3416br, 1388vs, 722s, 1110m, 1740w, 1634s, 854m, 1516w (br, broad; *vs*., very strong; s, strong; m, medium; w, weak) mp. 232 ºC.

Synthesis of [Cu(Phen)(tryp)SCN] (3): The aqueous solution of L- tryptophan (2 mmol) in methanol-water (1:1), an aqueous solution of Cu $(NO_3)_2.3H_2O$ (2 mmol) was added with vigorous stirring. After 30 min later, the (10 ml) methanolic solution of 1,10- phenanthroline was added slowly. A few minutes later 2 mmol of potassium thiocyanaide was added, and then the solution was stirred for about 6 h at ambient temperature. The resulting dark green solution was filtered and kept for slow evaporation. The precipitate washed 3-4times with double distilled water to remove the impurities after that it was dried at room temperature. Yield: 69%. Analytical calculation for $C_{25}H_{22}CuN_5O_2$ S (%): C, 57.03; H, 3.7 5; N, 9.71. Found (%): C, 57.07; H, 3.79; N, 9.76 IR (KBr, cm^{-1}) 3390br, 3068w, 2092vs, 1626s, 1352m, 847s, 583m, 499m, 1385s (br, broad; *vs*., very strong; s, strong; m, medium; w, weak) mp. 178-180 ºC.

Spectroscopic Studies on DNA Interaction:

Electronic Absorption Spectra: The DNA binding experiments were performed at room temperature. The DNA concentration per nucleotide was determined by electronic absorption Spectroscopy using 1 cm path length cuvettes. DNA solutions in 5 mM Tris–HCl/50 m NaCl buffer H 7.1 gave the ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.9, indicating that the DNA was sufficiently free of protein [26]. The DNA concentration was determined by measuring the UV absorption at 260 nm, taking the molar absorption coefficient (ε_{260}) of CT-DNA as 6600 M^{-1} cm^{-1}. Absorption titration experiments of copper (II) complex in Tris -HCl buffer was performed by using a fixed complex concentration to which increments of the DNA stock solutions were added. Copper (II) complex-DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded. Titration curves were constructed from the fractional change in the absorption intensity as a function of DNA concentration. The intrinsic binding constant (K_b) can be obtained by the following equation: [27]

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

Where [DNA] is the DNA concentration in M (nucleotide), ε_a is the absorption coefficient observed at a given DNA concentration, ε_f is the absorption coefficient of a complex in the absence of DNA, ε_b is the absorption coefficient of a complex when fully bound to DNA, and K_b is the intrinsic binding constant in M^{-1}. Each set of data was fitted to the above equation, and The plot of [DNA] / ($\varepsilon_a - \varepsilon_f$) *versus* [DNA] gave a slope and the *y*-intercept which is equal to $1/(\varepsilon_b - \varepsilon_f)$and $1/K_b(\varepsilon_b - \varepsilon_f)$, respectively. The intrinsic binding constant K_b was obtained from the ratio of the slope to the intercept.

Fluorescence Spectra: Fluorescence spectra were recorded with excitation at 300 nm and emission at 500 nm. The experiments were carried out by titrating complex (5 mM Tris–HCl/50 mM NaCl buffer) into the solution of DNA (1×10^{-4} M) and EtBr (8×10^{-5} M). Stern-Volmer quenching constant was calculated using the following expression [28].

$$I_0/I = 1 + K_{sv}r \ldots(2)$$

Where I_0 and I are the fluorescence intensities in the absence and presence of complex, respectively, K_{sv} is the linear Stern-Volmer quenching constant dependent on the ratio of the bound concentration of EtBr to the concentration of DNA, and r is the total concentration of complex to that of DNA. In the plot of I_0/I *versus* [Complex]/[DNA], *K*sv is given by the ratio of slop to intercept.

Viscosity: Viscosity measurements were carried out using an Ostwald-Viscometer maintained at a constant temperature of 28.0 ± 0.1 °C in a

thermostatic bath. Flow time was measured with a digital stopwatch. Each sample was measured thrice, and an average flow time was calculated. CT–DNA samples approximately 200 base pairs in average length were prepared by soliciting in order to minimize the complexities arising from DNA flexibility. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio where η is the viscosity of DNA in the presence of complex η_0 is the viscosity of DNA alone. The relative viscosity was calculated according to the relation = $(t–t_0)/t_0$, where t_0 is the flow time for the buffer, and t is the observed flow time for DNA in the presence and absence of the complex [29].

Cyclic Voltammetry Studies: Electrochemical techniques are complementary to other biophysical techniques that are applied to study the interaction between redox-active molecules and biomolecules. Double distilled water was used to prepare the buffer solutions. Cyclic Voltammetry (CV) was performed on a three-electrode system consisting of glassy carbon (GC) electrode. Before each experiment, solutions were deaerated by purging dry N_2 for 15 min, and nitrogen was kept over the solution during the experiments [30].

Antimicrobial Assay: Antimicrobialanalysis was followed using a standard agar well diffusion method to study the antimicrobial activity of compounds [31, 33]. Each bacterial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^5 colony-forming units (CFU) per mL. They had been flood-inoculated onto the surface of Media (Mueller Hinton Agar for Bacteria and Sabouraud's Dextrose agar for fungi) and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 µL (5µg compound in 500 µL DMSO) of the sample solution were poured into the wells. The plates had been incubated for 18 h at 37 °C for bacteria. Similarly, fungal plates have been incubated at room temperature for 48 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms and the solvent. DMSO was used as solvent control. Ciprofloxacin was used as a reference for anti-bacterial agents. Amphotericin B turned into used as a reference for anti-fungal agent. The tests had been executed in triplicates.

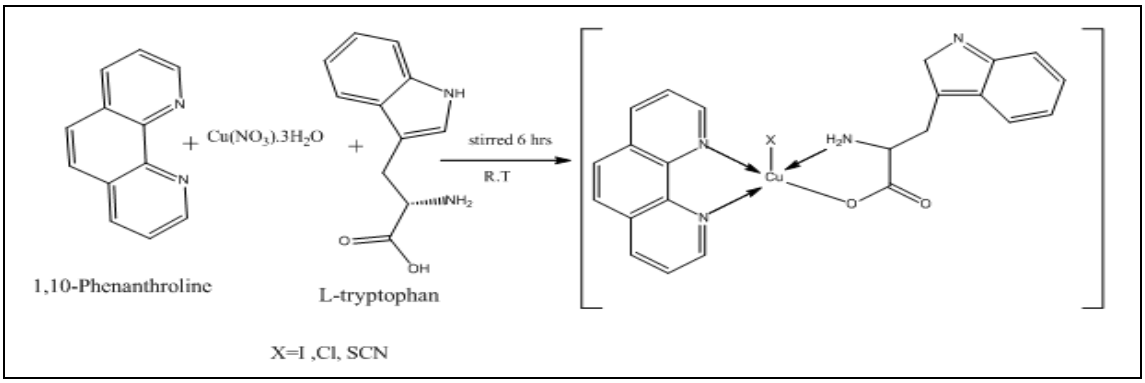
Cell Culture and MTT Assay: The Liver cancer cell line **(HEPG2)** was plated separately using 96 well plates with the concentration of 1×10^4cells/ well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in a CO2 incubator at 37 °C with 5% CO_2. The cells were washed with 200 µL of 1X PBS, and then the cells were treated with various test concentrations of compounds in serum-free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37 °C for 4 h using a CO_2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of DMSO and thoroughly mixed. The colour intensity was evaluated at 570 nm. The formazan dye turns to purple-blue color. Spectrophotometrical observance of the purple, blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graphpad prism5 software. The growth inhibitory rate of treated cells was calculated by (OD control – OD test) / OD control × 100%. Detailed experimental procedures are similar to those reported previously [34].

RESULTS AND DISCUSSION:

Synthesis and Characterization: The synthesis of the complex 1,2, and 3 presented in the scheme. The complexes were highly soluble in CH_3OH, C_2H_5OH, H_2O-Me-OH mixture, and slightly soluble in H_2O. The copper (II) complexes synthesized in the present study were characterized by UV–Vis, EPR, and IR spectroscopy. The purity of the complexes was determined by elemental analyses, which were in good agreement with the calculated values.

The IR Spectra for the complex 1, 2 and 3. In the IR spectra, the ν (NH) indole peak of tryptophan did not show any appreciable shift upon complexation, indicating the non-involvement of indole NH in metal coordination. The asymmetric and symmetric stretching vibrations of The ν_{as} (COO^{-}) and ν_s (COO^{-}) stretching vibrations for complex 1, 2, and 3 were observed at 1612, 1388 cm^{-1} (1), 1634, 1388 cm^{-1} (2), 1626, 1385 cm^{-1} (3).

The large difference between the two frequencies is greater than 200 cm^{-1} indicates that the carboxylate groups are coordinated to the metal ion in a monodentate fashion [35].



SCHEME 1: SYNTHESIS OF COMPLEX 1, 2 AND 3

The peaks corresponding to the ring stretching frequencies (ν(C=C) and ν(C=N)) at 1503, 1419 cm^{-1} of free phenanthroline were shifted to higher frequencies upon complexation indicating the coordinates of the heterocyclic nitrogen atoms to the metal ion. Copper-halide stretches are generally located in the Far-IR region; however, the presence of the strong metal-ligand vibrations in this region obscure the copper-halide stretches. The Iodide (1), chloride (2) analogues of display almost identical absorptions, with only minor shifts. For thiocyanide (3) it has been reported that ν(CN) and ν(CS) modes in M-NCS compounds fall in the range ~2040- 2080 and 780 - 860 cm^{-1}, whilst the corresponding modes in M-SCN occur in the ranges 2080 - 2120 and 680-720 cm^{-1}, respectively [36]. The characteristic stretching vibration for complex 3 observed very sharp stretching frequency appears at 2092 cm^{-1} corresponds metal sulphur bonding indicates the formation of Cu-SCN.

EPR Spectra: The EPR spectrum of the complex was recorded at a frequency 9.434 GHZ at room temperature on solid samples. It is shown in **Fig. 1a, b, c**. At room temperature, complexes 1, 2, and 3 exhibit well defined single isotropic lines at a g value of 2.046.

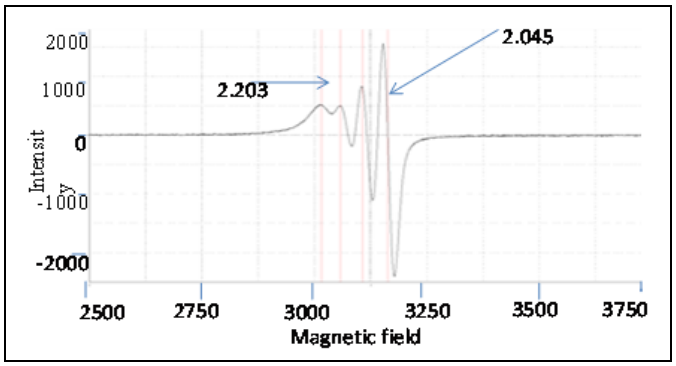


FIG. 1A: EPR SPECTRUM OF COMPLEX 1

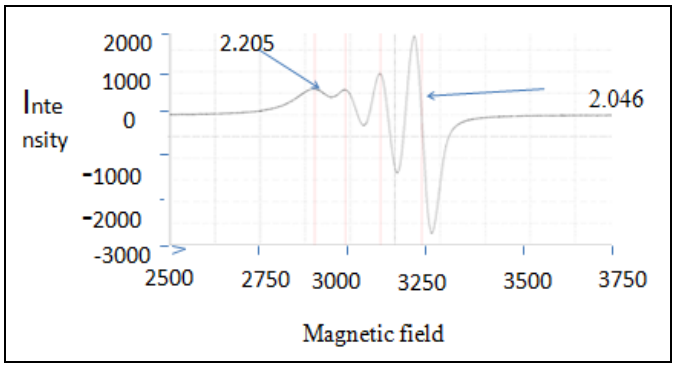


FIG. 1B: EPR SPECTRUM OF COMPLEX 2

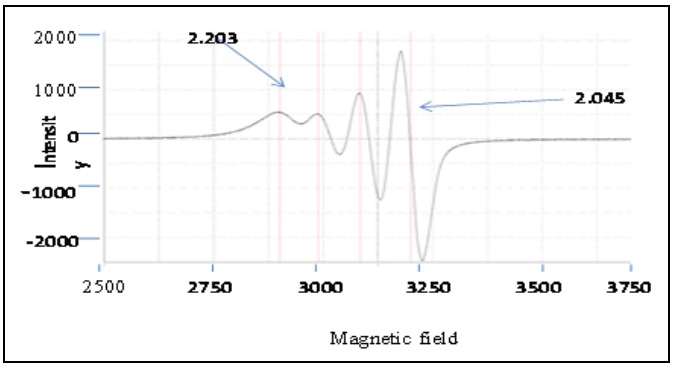


FIG. 1C: EPR SPECTRUM OF COMPLEX 3

Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. The axially elongated g∥ value of complex 1, 2 and 3 is 2.203, 2.205, 2.203 respectively g∥ values are greater than g⊥ value 2.045. The Cu (II) complex at the room temperature exhibit signal with g values is the characteristic of axial symmetry [37]. The trend, g ∥> g⊥>Ge (2.0027) showed that the unpaired electrons was localized in the dx^2-y^2 orbital of copper (II) [38].

DNA Binding:

Electronic Absorption Spectroscopic Studies: The interactions of metal complexes with DNA have been the subject of interests for the development of effective chemotherapeutic agents. The binding modes to DNA would give insights into the understanding of the biochemical mechanism of action of the complexes [39]. The electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with the metal complex. The absorption spectra of copper (II) complexes, in the absence and presence of CT-DNA, were shown in **Fig. 2**. In the UV region, the complex-3 had a two strong absorption peak at 268 nm and 293 nm, ($\varepsilon = 1.23 \times 10^4$ M^{-1} cm^{-1} & $\varepsilon=0.5 \times 10^4$ M^{-1} cm^{-1}) for complex-1 the absorption peak at 223 nm and 270 nm, ($\varepsilon=2.3 \times 10^4$ M^{-1} cm^{-1} & $\varepsilon=1.4 \times 10^4$ M^{-1} cm^{-1}) for complex-2 presented two bands 220 nm and 272 nm ($\varepsilon=1.0 \times 10^4$ M^{-1} cm^{-1} & $\varepsilon=0.7 \times 10^4$ M^{-1} cm^{-1}) which can be attributed to the π-π* transition of the coordinated phenanthroline derivatives ligand. The absorption intensity of the complexes-2 and 3 are decreased (hypochromism), and complex-1 is increased (hyperchromism), evidently after the addition of DNA, which indicated the interactions between DNA and the complexes. A similar hyperchromism has been observed for the sort bands of certain porphyrins when interacted with DNA as not yet been clearly explained [40, 41]. The hyperchromic effect may also be due to the groove binding interaction between positively charged complexes and the negatively charged phosphate backbone at the periphery of the double helix CT-DNA[42]. A plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] will give aslope $1/(\varepsilon_b-\varepsilon_f)$ and an intercept $1/K_b$ $(\varepsilon_b-\varepsilon_f)$. The K_b is the ratio of the slope and the intercept. The intrinsic binding constant (K_b) for the association of the complexes with CT- DNA (inset of **Fig. 2**) Using the absorption at 270 nm (1) and 272 nm (2) 268 nm (3) was determined as 1.34×10^{-3} (1), 4.22×10^{-3} (2) and 3.5×10^{-3} (3).

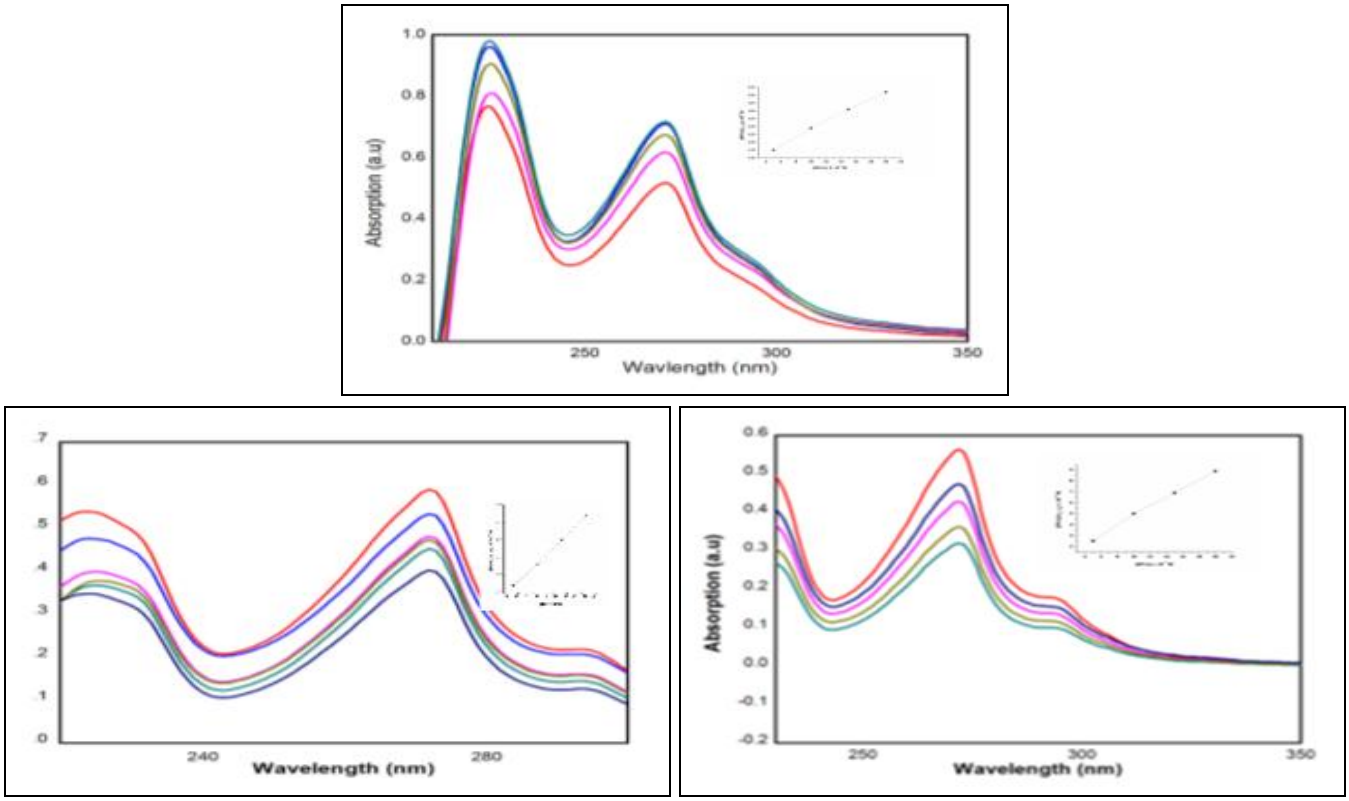


FIG. 2: ABSORPTION SPECTRAL TRACES ON ADDITION OF CT -DNA TO COMPLEXES 1, 2 AND 3 INSET PLOT OF $[DNA]/(\varepsilon_a-\varepsilon_f)$ v_s [DNA] FOR ABSORPTION TITRATION OF CT- DNA WITH COMPLEXES

Fluorescence Spectral Studies: In order to further investigate the interaction mode between the copper complexes and CT-DNA, the fluorescence titration experiments are performed. The fluorescence titration experiments, especially the EB fluorescence displacement experiment, have been widely used to characterize the interaction of complexes with DNA by following the changes in fluorescence intensity of the complexes [43]. The intrinsic fluorescence intensities of DNA and that of EB in Tris–HCl buffer are low, while the fluorescence intensity of EB will be enhanced on the addition of DNA as its intercalation into the DNA. Therefore, EB can be used to probe the interaction of complexes with DNA. If the complexes can intercalate into DNA, the binding sites of DNA available for EB will be decreased, and hence the fluorescence intensity of EB will be quenched. So the Competitive binding studies using DNA with bound ethidium bromide (EtBr) were carried out for the complexes 1, 2, and 3. The results in **Fig. 3** showed that the fluorescence intensity of CT-DNA-EB decreased remarkably with the addition of copper (II) complexes, which indicated that the complexes could bind to DNA and replace EB from the CT-DNA-EB system [44]. The above data was analyzed by means of the Stern–Volmer equation [28].

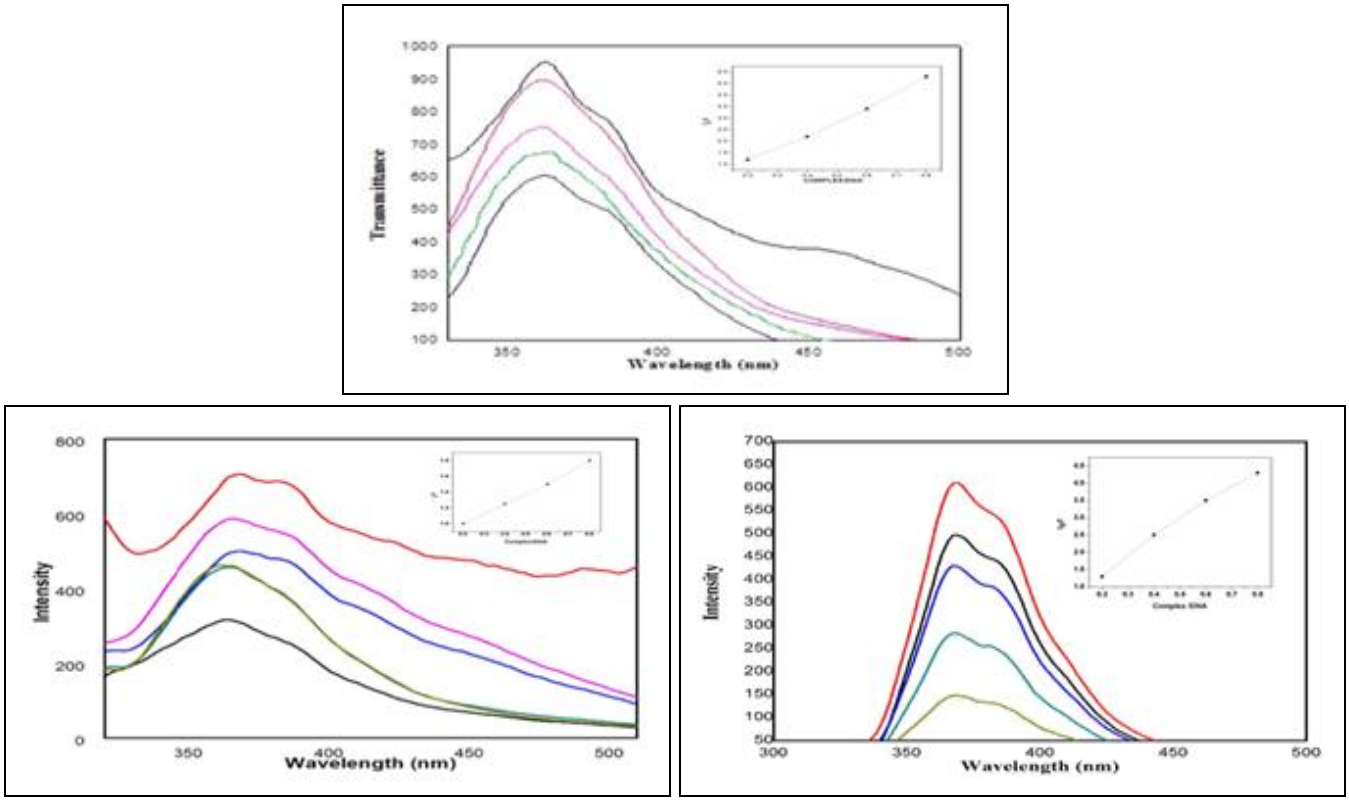


FIG. 3: FLUORESCENCE EMISSION SPECTRA OF COMPLEXES 1, 2 & 3 (EXCITED AT 300, 290 & 330 nm). THE INTENSITY OF THE COMPLEX WAS INCREASED WHEN THE ADDITION OF CT- DNA, EB SYSTEM (4×10^{-5}M EB, 4×10^{-5} CT –DNA

Cyclic Voltammetric Studies: The application of cyclic voltammetry to study the interaction between complex and DNA provides a useful complement to the previously utilized methods of investigation such as UV–Vis and viscosity experiments [45]. The typical cyclic voltammogram of a 0.01mM solution of copper (II) complex without and with DNA at glassy carbon (GC) electrode in DMF were carried out **Fig. 4**. In the forward scan, a single cathodic peak was observed, which corresponds to the reduction of the complex. In the reverse scan, no anodic peak was observed, which indicates that the process is irreversible. When CT-DNA is added to a solution of complex, the cathodic peak was observed, which indicates a marked decrease in the peak current intensity and shift of peak potential [46, 47]. The cyclic voltammetric behavior was not affected by the addition of a very large excess of DNA, indicating that the decrease of the peak current of the complex after the addition of DNA due to the binding of copper (II) complex to the DNA [48].

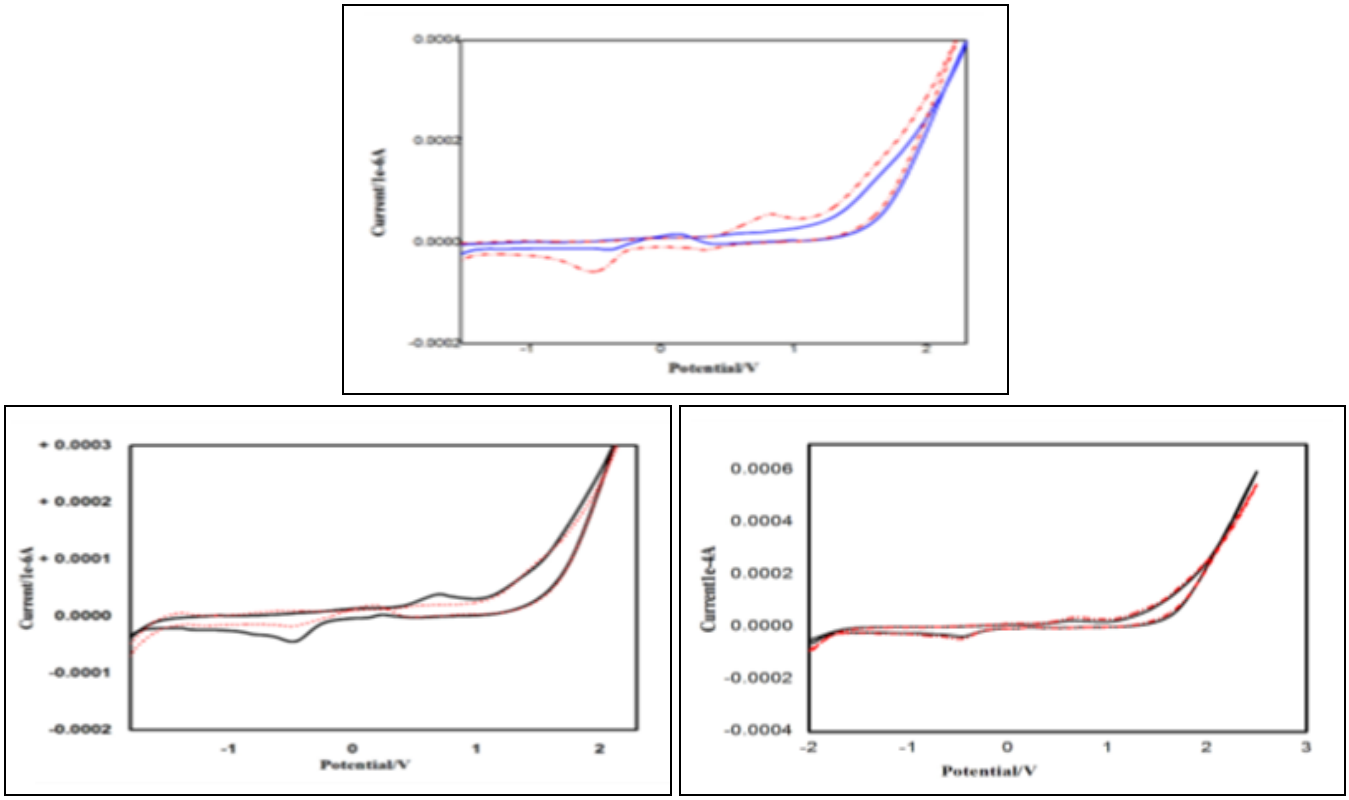


FIG. 4: CYCLICVOLTAGRAM OF COMPLEXES 1, 2 AND 3 (1 mM) iN THE ABSENCE (—) AND IN THE PRESENCE (- - -) OF CT-DNA

Viscosity Measurements: To explore further the binding mode of the present copper (II) complexes viscosity measurements on solutions of calf thymus DNA incubated with the complexes was carried out [49].

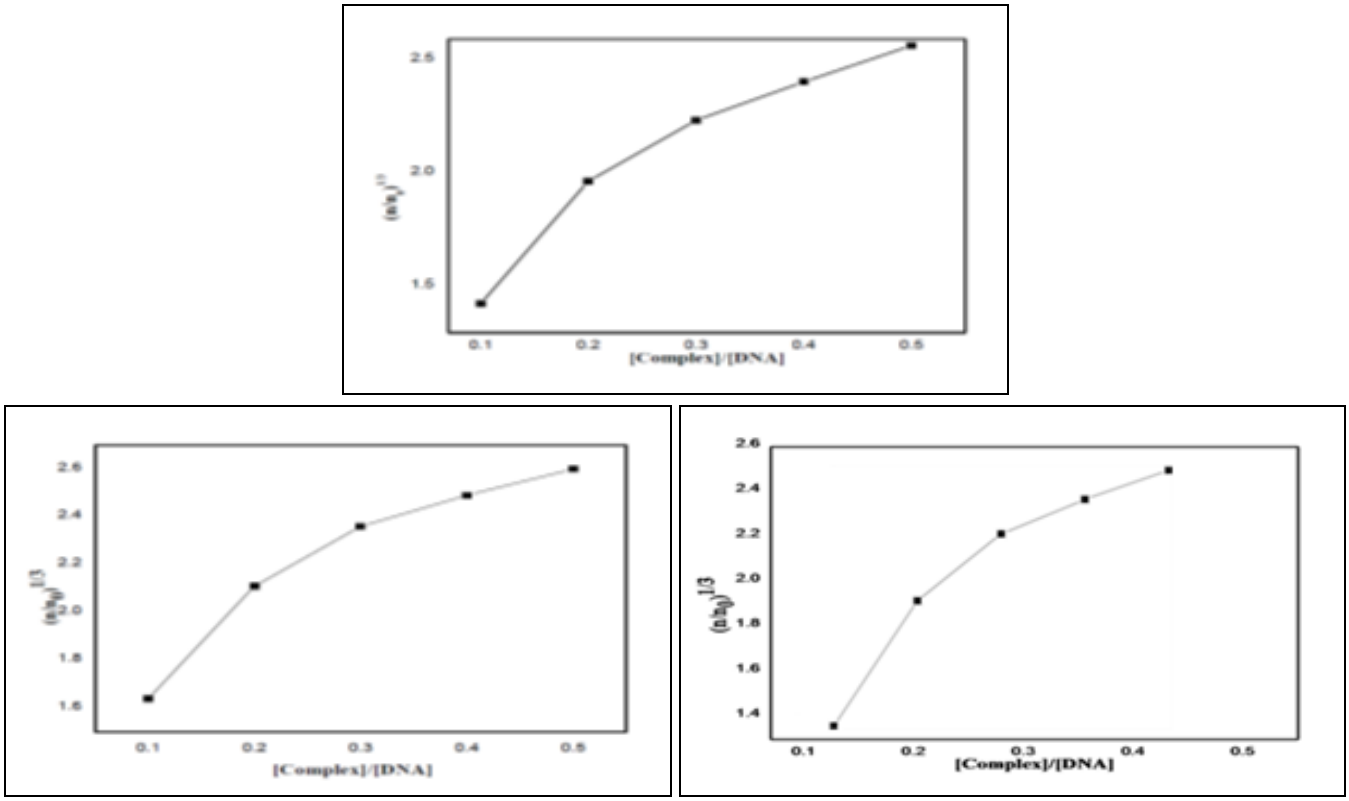


FIG. 5: EFFECT OF INCREASING AMOUNT OF COMPLEXES ON THE RELATIVE VISCOSITIES OF CT-DNA AT 25 ºC FOR COMPLEXES 1, 2 AND 3

Since, the relative specific viscosity (η/η_0), (η and η_0) are the specific viscosities of DNA in the presence and absence of the complexes, respectively) on DNA reflects the increase in contour length associated with the separation of DNA base pairs caused by intercalation, a classical intercalator such as ethidium bromide could cause a significant increase in viscosity of DNA solutions. In contrast, partial or non-classical intercalation of the ligand could bend or kink DNA resulting in a decrease in its effective length with a concomitant increase in its viscosity of complexes (1, 2, 3) [50]. The plots of relative specific viscosities versus 1/R (= [Cu] / [DNA]) are shown in **Fig. 5 (1, 2, 3)**. The complexes expect, show a slight increase in viscosity of DNA on increasing the concentration of the complexes (1, 2, 3). However, the increase in the viscosity was much less compared to that of potential intercalators like ethidium bromide in the same DNA concentration range. This observation leads us to support the above spectral studies, which suggest that the complex interactions with DNA *via* partial intercalation between DNA base pairs, which is similar to the interaction of copper (II) complex with DNA. The complexes appear to prevent the partial intercalation of the phen ring to DNA base pairs and hence increase in viscosity.

Antimicrobial Activity: The copper (II) complex was screened for its *in-vitro* antimicrobial activity against certain pathogenic bacterial and fungal species using standard agar well diffusion method [51-55]. The complexes were found to exhibit considerable antimicrobial activity against Gram-positive, Gram negative bacteria and fungi. The test solutions were prepared in dimethyl sulphoxide, and it is used as a solvent control Gram-positive (*Staphylococcus auerus*), and Gram-negative (*Klebsella pneumonia, Escherichia coli*,) bacteria were grown in nutrient agar medium and incubated at 37 ºC for 18 h followed by a frequent subculture to fresh medium and were used to test bacteria. The **Fig. 6** and **7** represents the antibacterial activities of complexes (1, 2 and 3).

TABLE 1: ANTIBACTERIAL ACTIVITY (DIAMETER OF ZONE INHIBITION, IN mm) OF COPPER (II) COMPLEXES

S. no.	Microorganisms	Control	1	2	3	Ciprofloxacin
			Zone of inhibition in mm			
1	*Staphylococcus aureus*	-	30	30	30	30
2	*Klebsiella pneumoniae*	-	8	18	10	13
3	*Escherichia coli*	-	22	22	22	10

FIG. 6: ANTIBACTERIAL ACTIVITIES (DIAMETER OF ZONE INHIBITION, IN mm) OF COPPER (II) COMPLEXES

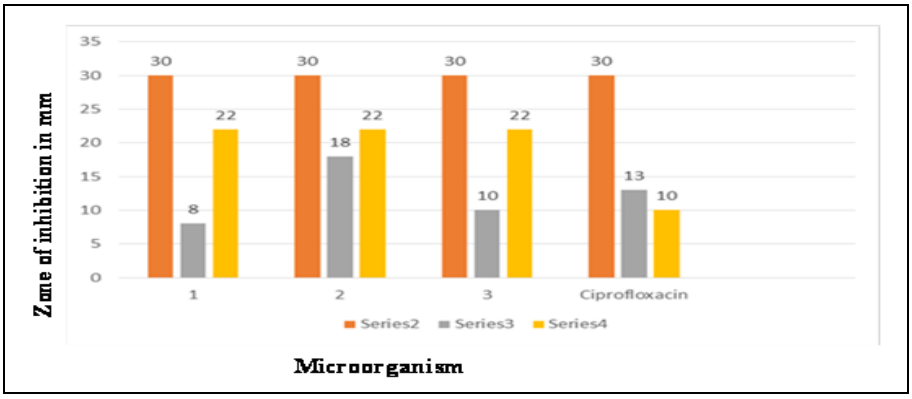


FIG. 7: BAR GRAPH INDICATES ANTIBACTERIAL ACTIVITY OF COMPLEXES 1, 2 AND 3

Similarly fungal plates were incubated at room temperature for 48 h. The fungi *Aspergillus niger, Rhizopus species Penicillium species* are grown as a seaboard dextrose agar medium were incubated for 48 h followed by periodic sub-culturing to fresh medium and were used to test fungus. The **Fig. 8** and **9** represents the antifungal activities of the complexes (1, 2 and 3). Then the petri plates were inoculated with a loop full of bacterial and fungal culture and spread throughout the petri plates uniformly; the tests were carried out in triplicates. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms and the solvent. These are summarized in the **Table 1** and **2**. The antibacterial and antifungal activity the standard used as a Ciprofloxacin and Amphotericin B, respectively. It may be concluded that copper (II) complexes inhibit the growth of bacteria to a higher extent than towards fungi

TABLE 2: ANTI-FUNGAL ACTIVITIES (DIAMETER OF ZONE INHIBITION, IN MM) OF COPPER (II) COMPLEXES

S. no.	Microorganisms	Control	1	2	3	Ciprofloxacin
			Zone of inhibition in mm			
1	*Aspergillus niger*	-	-	10	-	11
2	*Rhizopus sps*	-	-	-	-	10
3	*Penicillium sps*	-	-	-	-	14

FIG. 8: ANTI-FUNGAL ACTIVITIES (DIAMETER OF ZONE INHIBITION, IN mm) OF COPPER (II) COMPLEXES

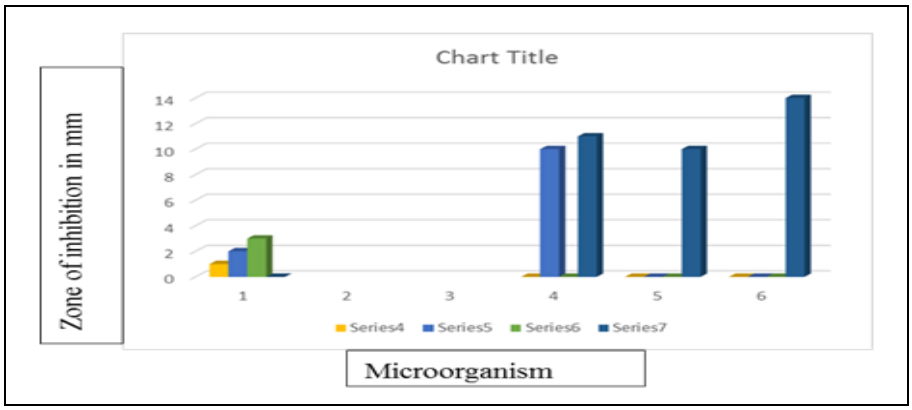


FIG. 9: BAR GRAPH INDICATES ANTIFUNGAL ACTIVITIES OF COMPLEXES 1, 2 AND 3

Cytotoxic Activity: The cytotoxicity of the complex to be used as chemotherapeutic agents was studied using MTT assay. The ability of the complex on HepG2 cells was tested with or without various concentrations (25–500µg/ml) of the complex for 24 h. Cells incubated with different concentrations using normal as control. After the incubation period, MTT assay was carried out to calculate the cell death percentage. For each concentration of the complex cells were incubated in triplicate.

Fig. 11 clearly illustrates that there is a clear decrease in the viable cell number in the cells incubated with complex in a concentration-dependent manner.

The viability of cells incubated without any compound was considered as 100%, and the percentage of viable cells incubated with compounds are given as relative to the control. The IC_{50} values of complex 1, 2 and 3 19.19 ± 0.14, 19.84 ± 0.32, 21.50 ± 1.24 µg/ml respectively [58, 59].

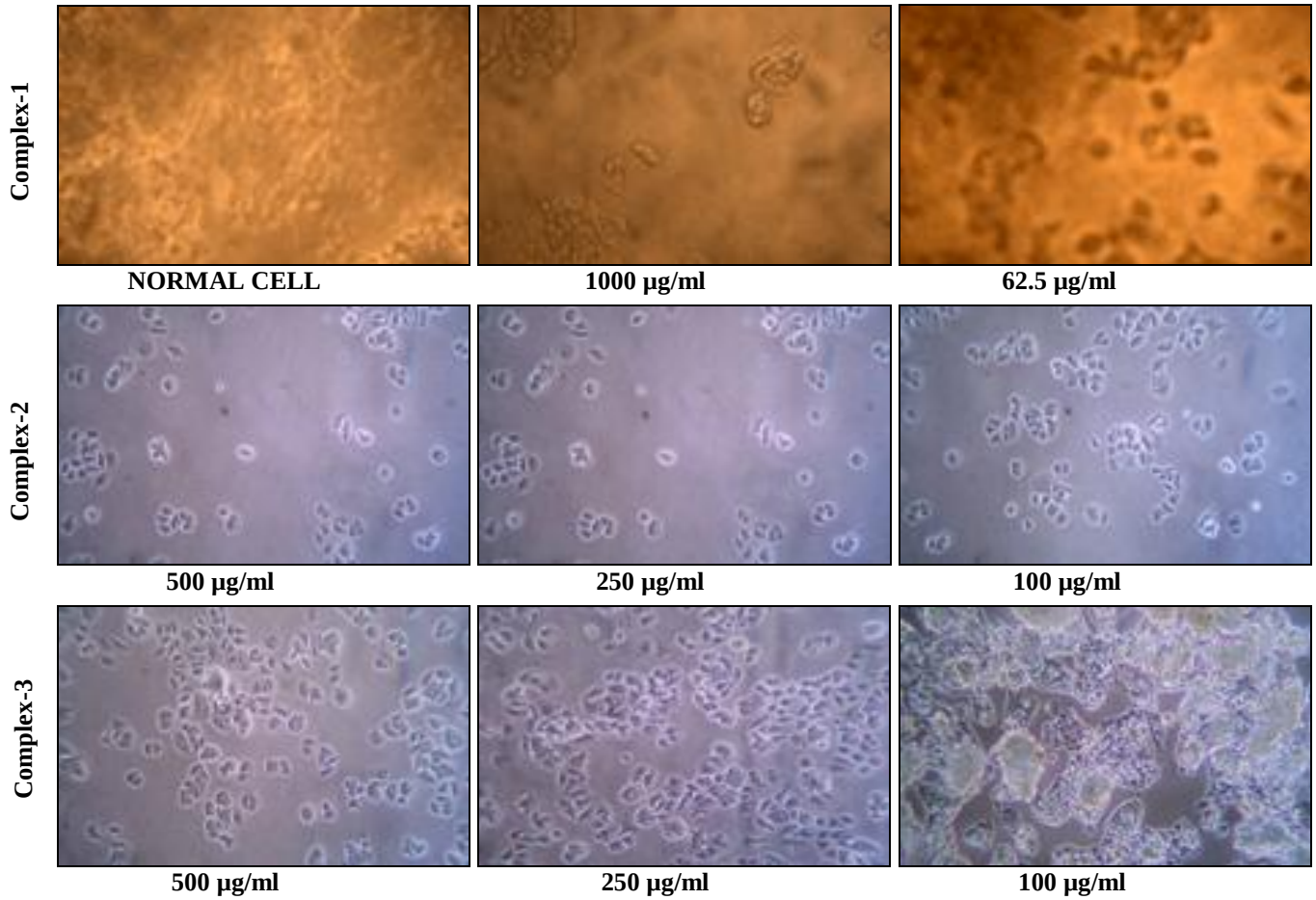


FIG. 10: ANTICANCER ACTIVITY OF COPPER (II) COMPLEXES 1, 2 AND 3

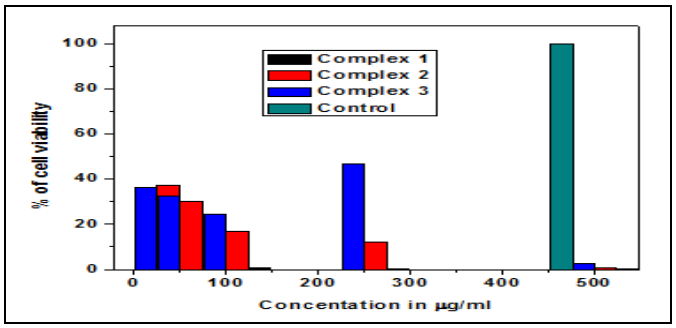


FIG. 11: CELL VIABILITY OF HepG2 CELLS AFTER TREATMENT WITH COMPLEXES 1, 2 AND 3 AT DIFFERENT CONCENTRATION at 24 h

CONCLUSION: In this study, a novel copper (II) complexes having N, O-donor has been synthesized and characterized. The planarity and extended conjugation of the phenanthroline base has a profound effect on the persistent DNA binding of the copper complex. The effectiveness of binding induces a change in the physio-chemical and spectroscopic methods confirms interactive mode of intercalation of the complex with CT-DNA. The results suggest from antimicrobial assay of all the copper (II) complexes exhibits good antibacterial activity against both Gram-positive and Gram-negative bacteria whereas complexes 1 and 3 will be inactive against fungal species. The complex-2 shows good antifungal activity towards *Aspergillus niger*. The results show that complex 1 exhibited potent cytotoxic effects against human cell line (HepG2) concludes its potential role in medicine as antiseptic and anticancer agent.

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