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DNA Binding, DNA Cleavage, BSA Binding, Antioxidant, Antibacterial Activity and Cytotoxicity Studies of Cu(II), Co(II) Complexes

Bhaskar DV[1], Ushaiah B[2], Shivaleela D[1], Jyothi P[1], Gyana Kumari C[1*]

[1]*Department of Chemistry, Osmania University, Hyderabad 500007, India*

[2]*Department of Chemistry, Nizam College, Osmania University, Hyderabad 500001, India*

ABSTRACT

A new air stable Copper(II) complexes [Cu(L) (C_2O_4) (H_2O)] (A), [Cu(L) (A.A) (H_2O)] (B), Cobalt(II) complexes [Co(L) (C_2O_4) (H_2O)] (C) and [Co(L) (A.A) (H_2O)] (D), L=2,6-bis(benzimidazole-2yl)pyridine (BZIMPY) has been synthesized from the condensation of the aqueous solution of copper, cobalt chloride salts with a hot alcoholic solution of the ligand L. The isolated complex has been deliberately characterized by elemental analysis, metal content determination, molar conductance, fast atom bombardment mass spectra, magnetic measurements, IR and electronic spectral studies. Elemental analyses and electronic spectral data suggest that the synthesized complexes have octahedral geometry. DNA binding ability was explored by different techniques which suggest that the complex bind to CT-DNA through intercalative mode and also studied the BSA binding studies. The DNA Cleavage ability of the complexes was evaluated with super coiled pBR322 DNA using gel electrophoresis. The antioxidant activity of the complexes was evaluated in vitro against DPPH radical using spectrophotometer. Furthermore, the complexes were screened for potential application, the antimicrobial activity against their effect on the growth of various strains of bacteria. Compounds were found to be significantly cytotoxic against human cancer cell lines HeLa.

Keywords: DNA binding; BSA binding; DNA cleavage; Antioxidant; Antimicrobial; Cytotoxicity

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INTRODUCTION

There has been significant development in transition metal ions coordinating ability with different benzimidazole ligands is the main scaffold in the preparation of different metal based valuable drugs. Many studies, reveals that anticancer drugs have enhanced anti-cancer activity, when administrated as their metal chelates. Benzimidazole and their metal complexes have variable physico-chemical properties, significant structural diversity, and versatile biological properties [1-3] associated such as anti-viral [4], anti-bacterial [5-7], anti-malarial, antifungal, and antitumor [8] activities. Benzimidazoles are considered as a potent pharmacological agent and the activity of the compounds highly dependent upon the nature of the hetero aromatic ring, as well as the form of the

thiosemicarbazone moiety [9,10]. The biological activity of thiosemicorbazones are assigned to their chelating ability [11] with transition metal ions bonding through three, nitrogen atoms. Benzimidazoles containing three N tridentate systems and their metal complexes stimulated widespread interest in biological activities.

To our knowledge, there is comparatively little biological research associated with metal complexes of Benzimidazole. So we find interest, in taking research in this area, that Benzimidazole and its copper (II), cobalt (II) complexes containing tridentate three N donar ligand as effective inhibitor of cell proliferation and immense anti bacterial activities. Besides that, copper, cobalt ions are the essential biologically active metalions present in various metalloenzymes. The literature survey also supports important role of Cu(II), Co(II) metal complexes with various Benzimidazolesi nteraction with DNA has been reported. DNA is basic pharmacological target to many cancer compounds, metal complexes and DNA interacts closely with their potential biological activities [12-14]. Metal based drugs used in chemotherapy for cancer treatment started in 1978 are a great innovation in bioinorganic chemistry. In the investigation of anticancer drugs DNA is considered as main target. Metal complexes aromatic moiety interacts with DNA by intercalation through major groove or minor groove binding [15] and stacking interaction. This interaction of metal complexes with DNA restrict the cell division in cancer cells. Here we reported DNA binding and cleavage activities. All the complexes have been characterized by elemental analysis, conductance, magnetic susceptibility, measurements, UV-Vis, IR, ^{1}H-NMR, and ESR spectral studies.

EXPERIMENTAL SECTION

Materials and Methods

All the starting materials and solvents were procured from E. Merck, Sigma Aldrich and S.D. Fine chemicals, all Solvents used were of analytical grade and purified according to literature methods (Perrin et al. 1980). Super coiled (SC) DNA was obtained from Gene (Bangalore, India) and ethidium bromide (EB) was supplied by Sigma (USA). Tris-(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer solution was prepared using deionized distilled water using a quartz water distillation setup.

Melting point (M.P) of the complex was measured on a Galen kamp apparatus in open glass capillaries and are uncorrected. Thin layer chromatography studies were also carried out using silica gel plates from Merck. Elemental analysis was determined by micro analytical methods using an Elementar Vario EL III micro analyzer. Metal contents of the complexes were analyzed by EDTA titration method (Vogel, 1978). Molecular weight was determined by Rast method. Molar conductance of free ligand and its metal complexes was determined in DMSO (10^{-3} M) at room temperature using an Elico CM 180 conductivity meter. Magnetic susceptibility of the complex was carried out by the Gouy method using Hg [Co $(SCN)_4$] as celebrant and the diamagnetic corrections were applied in compliance with Pascal's constant (Earnshaw 1968). Electronic absorption spectra were recorded on a Schimadzu spectrophotometer (cell length, 1 cm) in the 200-1100 nm range in DMSO solution. IR spectra were recorded using KBr pellets on a FT-IR spectrophotometer, (Shimadzu 8400S) in the range 250–4000 cm^{-1}. Fast atom bombardment mass spectra of the Imine base ligand and its complexes were recorded on a JEOL SX 102/DA-600 mass spectrometer.

Ligand (BZIMPY) [2,6-Bis(benzimidazole-2yl)pyridine] (L)

Pyridine 2,6 di carboxylic acid (3.35 g, 20 mM) was mixed with 20 ml of polyphosphoric acid and o-phenylene diamine (4.70 g, 44 mM) was mixed with 20 ml poly phosphoric acid separately, these two solutions were mixed together and heated at 240 °C on temperature controlled hot plate for 4 h.The colored solution was poured into 1 litre of vigorously stirred cold water.

A bulky blue green precipitate formed was collected by filtration. It is stirred in hot *10% Na_2CO_3* solution (800 ml) for 1 h. The resulting solution is filtered and recrystallized from ethanol.

Synthesis of Metal Complexes

Synthesis of [Cu(L) (C_2O_4) (H_2O)] (A), [Cu(L) (A.A) (H_2O)] (B) (A.A=Anthranilic Acid): To the hot methanolic solution of $CuCl_2.H_2O$ (0.208 g, 0.5 mmol) added drop wise a solution of BZIMPY (L) (0.155 g,0.5 mmol) in 20 ml of methanol. The solution was refluxed for 2 h and then add oxalic acid (C_2O_4) for complex A and anthranilic acid for complex B solution with methonal stirred for 24 h at room temperature. The yellowish brown solid precipitate developed and collected by filtration, washed with DMF and dried in a desiccator (Figure 1).

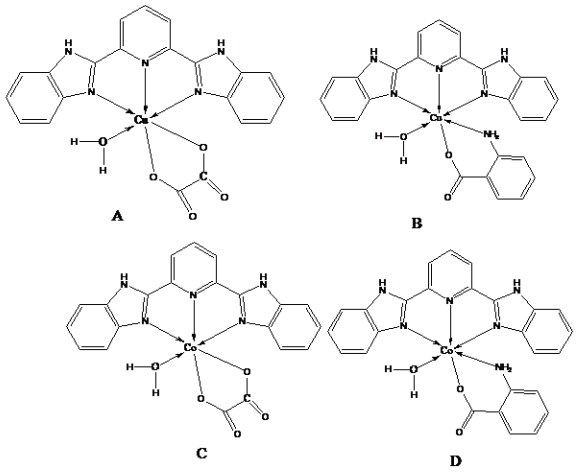

Figure 1. [Cu(L) (C_2O_4) (H_2O)] (A), [Cu(L) (A.A) (H_2O)] (B), [Co(L) (C_2O_4) (H_2O)] (C) and [Co(L) (A.A) (H_2O)] (D)

[Cu(L) (C_2O_4) (H_2O)] (A)*:* [Yield: 0.34 g, 85%] Analytical data: IR (KBr, *v* cm^{-1}): 3269, 3149 *v***(**N-H), 1567 *v* (C=N), 3420 *v* (O-H). Anal. Calcd (%): C**,** 50.20; H, 5.44; N, 17.57: Found (%) C, 50.10; H, 5.34; N, 17.57. UV-VIS (nm) 237, 320, 390; ESI-MS (m/z): 386.

[Cu(L) (A.A) (H_2O)] (B)*:* [Yield: 0.35 g, 86%] Analytical data: IR (KBr, *v* cm^{-1}): 3266, 3149 *v***(**N-H), 1567 *v* (C=N), 3392 *v* (O-H). Anal. Calcd (%): C**,** 50.20; H, 5.44; N, 17.57: Found (%) C, 50.10; H, 5.34; N, 17.57. UV-VIS (nm) 240 320, 390; ESI-MS (m/z): 512.

Synthesis of [Co(L) (C_2O_4) (H_2O)] (C) and [Co(L) (A.A) (H_2O)] (D) (A.A=Anthranilic Acid): To the hot methanolic solution of $CoCl_2.2H_2O$ (0.208 g, 0.5 mmol) added drop wise a solution of BZIMPY (L) (0.155 g,0.5 mmol) in 20 ml of methanol. The solution was refluxed for 2 h and add oxalic acid for complex 3 and anthranilic acid for complex 4 solution with methonal then stirred for 24 h at room temperature. The yellowish brown solid precipitate developed and collected by filtration, washed with DMF and dried in a desiccator (Figure 1).

[Co(L) (C_2O_4) (H_2O)]: [Yield: 0.38 g, 85%] Analytical data: IR (KBr, *v* cm^{-1}): 3249, 3139 *v*(N-H), 1557 *v* (C=N), 3339 *v(O-H)*. Anal. Calcd (%): C, 50.120; H, 5.34; N, 17.47: Found (%) C, 50.20; H, 5.34; N, 17.87. UV-VIS (nm) 290, 340; ESI-MS (m/z): 309 (calc 309.4).

[Co(L) (A.A) (H_2O)]: [Yield: 0.34 g, 85%] Analytical data: IR (KBr, *v* cm^{-1}): 3267, 3145 *v*(N-H), 1567 *v* (C=N), 3340 *v(O-H)*. Anal. Calcd (%): C, 50.120; H, 5.34; N, 17.47: Found (%) C, 50.20; H, 5.34; N, 17.87. UV-VIS (nm) 290, 340; ESI-MS (m/z): 382 (calc 382.7).

Electronic Absorption Titration with DNA

An absorption titration experiment was carried out with a fixed amount of the Cu (II), Co (II) complex. The synthesized compound was dissolved in a solvent mixture of 1% DMSO and 99% Tris-HCl buffer (5 mM Tris-HCl; 50 mM NaCl, pH 7.1). Absorption titration experiments were performed in the absence and presence of varying concentration of CT DNA. When measuring the absorption spectra, CT-DNA was added to compound solution and to the reference solution to eliminate the absorbance of CT-DNA itself. From the absorption titration data, the binding constant (K_b) was determined using following eqn. (1).

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

Where [DNA] is the concentration of DNA, the apparent absorption coefficient, ε_a, ε_f, and ε_b correspond to $A_{obs}/[M]$, the extinction coefficient of the compounds without DNA and the extinction coefficient of the compound with DNA, respectively. In the graph of $[DNA]/[\varepsilon_a-\varepsilon_f]$ versus [DNA], K_b is given by the ratio of slope to the intercept.

Protein Binding Study

The stock solution of protein was ready by dissolving the proper amount (10 mg) solid BSA in buffer solution (5 mM Tris–HCl/50 mM NaCl at pH 7.2) then followed by stirring for 1 h and kept at 4 ^{0}C. Compounds to be tested were dissolved in DMSO (concentration is 2.0×10^{-4}M) and diluted by Tris-HCl buffer. The BSA concentration was found spectrophotometrically by known value ε_{280}=44,300 M^{-1} cm^{-1} [16]. Spectral titration by UV were done by keeping the BSA concentration constant (40 µM) and varying the concentrations of the complexes (0.00–40 µM). The change in absorbance at λ_{max}=280 nm which is characteristic band of the BSA were recorded after each titrant addition. Fluorescence emission titrations were done by keeping the BSA volume constant (20 µM) and varying the concentrations of the complexes (0.00–20 µM). Emission spectra were recorded at 290-450 nm with excitation wavelength of BSA at 295 nm and the emission at 290-450 nm.

DNA Cleavage Activity

DNA Cleavage experiment of SC pBR 322 DNA has been carried out by gel electrophoresis. The Cu(II), Co(II) complexes were dissolved in DMSO (15 µM) and treated with pBR-322 plasmid DNA in Tris-HCl buffer (100 mM, p^H 7.2) and NaCl (0.05 M). The test samples were incubated for a period of 2 h at 37 ^{0}C and then loaded into an agarose gel chamber wells along with reference plasmid DNA and then electrophoresed at 100 eV constant voltage up to 45 min. Consequently, the gel was stained by ethidium bromide solution for 15 mints and then photographed under UV light (gel doc BIO RAD).

Antimicrobial Activity Procedure

Preparation of bacterial pathogens: The overnight cultures (100 µL) of each bacterium were loaded into a 20 mL of sterile nutrient broth and incubated for a period of about 8 hr to stabilize the culture. A loopful of the standard cultures were used for the antibacterial assay.

Antibacterial Assay

Muller Hilton agar was prepared and sterilized. A 20 mL of media was poured in petri dishes and allowed for solidification. The bacterial lawn culture was made using a sterile cotton swab and labeled. The wells were made in the media with the help of whatman paper slips with centers at least 6 mm. Recommended concentration 10, 20 µL of the test sample 1 mg/mL in water was introduced in the respective wells. The plates were incubated immediately at 37 ^{0}C for 12 hours. Activity was located by calculating the diameter of the region showing complete inhibition (mm). A zone of inhibition was compared with the drug [17].

Cytotoxicity

The cytotoxic effect of complexes was carried out using a standard MTT assay [18]. The samples to be tested were dissolved in DMSO in the concentration range of 1-100µM. Cells were seeded in a 96-well plate and kept in 5% CO_2 attachment and grown for 48 h. Then the cells were treated with various concentrations of the complex dissolved in DMSO and incubated for 24 h. After completion of incubation the culture medium was removed and 15µL of the MTT dye solution (5 mg/ml in phosphate-buffered saline) (PBS) was added to each well and incubated for 4 h. After 4 h incubation in the dark, MTT was discarded and DMSO (100 (L/well) was added to solubilize the purple product. The absorbance at 620 nm in each well was measured with a Elisa reader (Thermo Scientific Multi Scan EX) by keeping medium without complex as a control. The IC_{50} values were calculated from the plotted absorbance data of the dose response curves.

RESULTS AND DISCUSSION

DNA Binding Studies

Electronic absorption titration with DNA: The interaction of the complexes with DNA was investigated using absorption spectra [19]. The absorption spectra of Cu (II), Co (II) complexes, in the absence and presence of CT-DNA (at a constant concentration of the complex) is given in Figure 2 as the concentration of DNA was increased the MLCT transition bands of complexes, exhibited hypochromism. These spectral characteristics may suggest that there are some interactions between the complex and CT DNA. In order to compare quantitatively, the binding strength of the complexes, the intrinsic binding constant K_b of complexes with DNA was determined from the decay of the absorbance monitored for complexes (Figure 2). The intrinsic binding constant K_b of the complexes with CT-DNA were determined from the Eq (1) are $2.1 \times 10^4 M^{-1}$, $2.4 \times 10^4 M^{-1}$ for Cu (II) complexes A, B and $1.2 \times 10^4 M^{-1}$, $1.4 \times 10^4 M^{-1}$ for Co (II) complexes C, D.

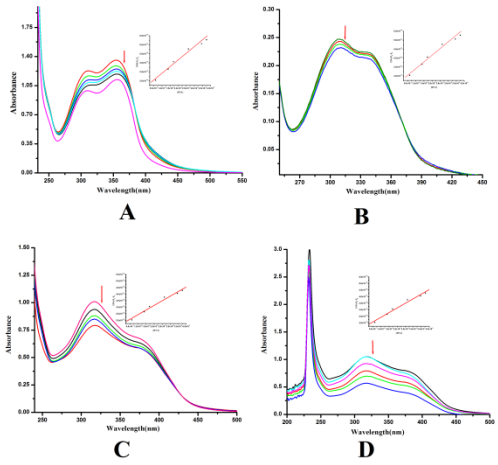

Figure 2. Absorption spectra of Cu(II), Co (II)complexes in TriseHCl buffer upon addition of CT-DNA. [M]=30 mM. The graph shows the decrease in absorbance upon the addition of CT-DNA concentration. Inset: plot of [DNA]/(εa-εf) versus [DNA] for the titration of DNA with Complexes A,B,C and D

Viscosity

With the aim of further clarifying the binding of the complexes with DNA, The experiment of the DNA viscosity, by the addition of a complex solution, the viscosity of DNA is responsive to DNA extension. Our results (Figure 3) Divulge that complexes generated an increase in viscosity of CT DNA that be a sign of an intercalative mode. We could have observed different patterns in an increase of viscosity indicates different intercalation. The linear enhancement in viscosity of DNA produced by complex indicates that the interaction of this complex involves a classic intercalation [20,21] with the participation of the phenyl molecule. This different behavior could explain the strong interaction of complexes deduced from the thermal denaturation.

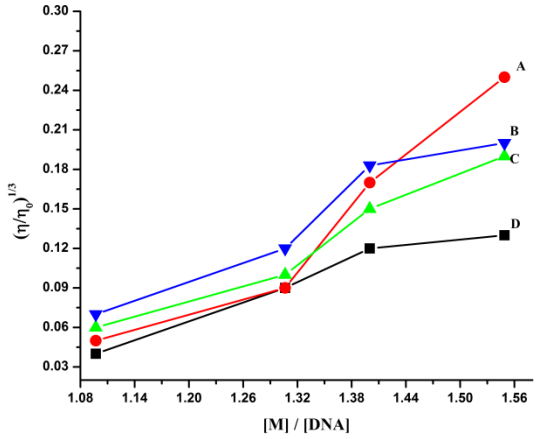

Figure 3. Effect of relative viscosity increasing amount of ethidium bromide (EBr) A, B,C and D complexes of CT-DNA at 25 (±0.1) ^{0}C. [DNA]=0.5 mM

Fluorescence Quenching Studies

The competitive experiment of complexes with EB have been explored by fluorescence emission spectroscopy, Fluorescence emission experiments were conducted in buffer (5 mM Tris–HCl, 50 mM NaCl at pH 7.1) to examine whether it is able to replace EB bound to CT DNA. The emission spectra of EB CT DNA adduct was recorded by

the titration with the metal complexes**.** In this experiment EB emits intense fluorescence by reason of good intercalation of the planar EB phenanthridine ring with DNA With the concentration of compound increased which can equal or more strongly intercalate with DNA than EB, a quenching of the DNA-induced EB fluorescence emission may appear. The emission intensity decreases with the increasing concentration of complexes. In our investigation the intensity of fluorescence decreased due to complexes will influence displacement of bound EB with DNA. The metal complexes do not show fluorescence peaks at room temperature in solution or in the presence of CT DNA, the excited peak observed at 517 nm. The addition of this complex solution to a EB DNA containing solution does not influence quenching of free EB fluorescence. Therefore, the intensity of the fluorescence emission spectra of EB DNA decreased. The volume of the metal complexes was mixed between 0 and 100 μM. After every addition of the complex solution to DNA-EB adduct, the solution was allowed to incubate at 25 ^{0}C for 5 min before recording the spectrum. The Stern–Volmer quenching constant [22] and was measured for complex using the equation as given by the eqn. (2).

$$I_0/I=1+K_q\, r \qquad (2)$$

Where I_0 represent the fluorescence intensity in the absence of the compound and I represent the fluorescence intensity in the presence of compounds. K_q is a Stern-Volmer quenching constant, *r* is the concentration of the compound. In the quenching plot (Figure 4) of I_0/I versus [complex], *K*q is given by the ratio of the slope to the intercept. The K_q value is 2.44×10^4 M^{-1}, 2.48×10^4 M^{-1} for Cu(II) complexes A, B and 2.44×10^4 M^{-1} 2.54×10^4 M^{-1} for Co(II) complexes C, D respectively, reveals that the metal complexes intercalate with DNA.

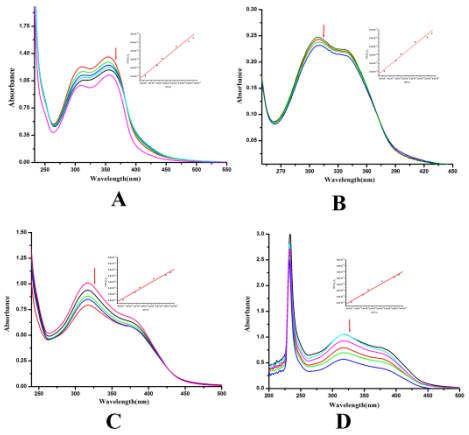

Figure 4. Emission spectra of A, B,C and D complexes in Tris HCl buffer at 25 ^{0}C upon addition of CT-DNA. The arrow shows the intensity change upon increasing complex concentration. Figure 6 Absorption spectra of BSA and BSA with compounds A, B,C and D. The arrow shows the absorbance changes with increasing complexes concentration. Inset shows the plot of 1/(A–A_0) vs. 1/[Complex]

Protein Binding Studies

Fluorescence quenching of BSA with Cu(II), Co(II) complexes were investigated. The fluorophores of amino acid residue's tryptophan, tyrosine and phenylalanine present in BSA and hence BSA emits intensely when it is excited. In fact the fluorescence of BSA is mainly contributed by tryptophan alone. The emission intensity depends on two tryptophan units such as 134 and 212 when excited at 295 nm. The protein emission intensity is depends on the

sensitivity of polar environment of tryptophan and quenching groups. Upon addition of the complex solution (0-20 µM) to BSA solution emission intensity bands are affected. Fluorescence quenching reveals to any assay which results a decrease of the fluorescence intensity from a fluorophore due to a different molecular binding sites go along with a red shift of 78 nm for the complex I and red shift of 33 nm for the complexes II-IV respectively (Figure 5). The main fact to result red shift is the active site in protein is hidden in a hydrophobic environment. The interaction of complexes with BSA fluorescence intensity is shown in Figs Fluorescence quenching is determined by the Stern–Volmer equation [23].

$$I_0/I=1+ Kq \times [Q]=1+K_{SV} \times [Q] \qquad (3)$$

where I_0 and I represent the fluorescence intensities in the absence and in the presence of quencher, respectively the K_{sv} values are calculated as 3.3×10^4 M^{-1} (complex A), 3.1×10^4 M^{-1} (complex B) 2.5×10^4 M^{-1} (complex C) 2.3×10^4 M^{-1}(Complex D)

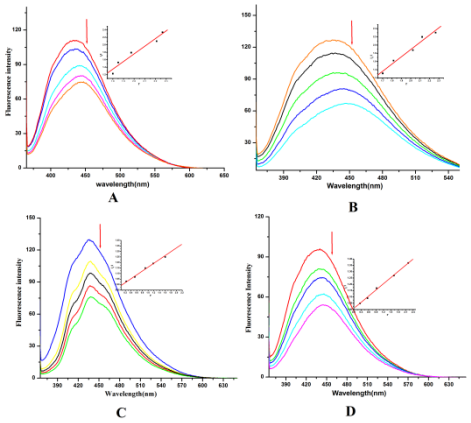

Figure 5. The emission spectrum of BSA (λ exi=295 nm; λ emi=425 nm) in the presence of increasing amounts of compounds A, B,C and D. The arrow shows the fluorescence quenching upon increasing the concentrations of the compounds. The insets show the Stern–Volmer plot and Scatchard plot of the complexes with BSA

DNA Cleavage Activity

The interaction of plasmid PBR 322 DNA with Cu (II), Co (II) complexes was carried out by using gel electrophoresis. DNA cleavage was accomplished by performing the gel electrophoresis for naturally obtain, circular form (Form I) generating to the nicked circular (Form II). When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be perceived for the super coil form (Form I), slower migration will be observed for nicked circular form (Form II). The DNA cleavage pictures are shown in Figure 6, reference control DNA (In Fig. Lane control) does not exhibit any activity [24].

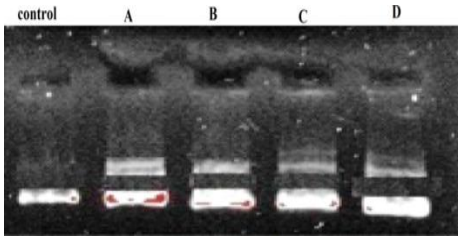

Figure 6. Photoactivated cleavage of pBR322 DNA in the presence A, B,C and D after irradiation at 365 nm. edition of complexes of 20, 30,40, and 50 µL

Antioxidant Activity

An antioxidant experiment was performed by using DPPH is a stable free radical, Scavenging DPPH free radical is the well-known mechanisms, which was working at antioxidants inhibit lipid oxidation. To scavenging free radicals, earlier reported different methods. In this experiment, the method of scavenging DPPH free radical was used to evaluate the antioxidant nature of testing compounds dissolved in DMSO solution, is shown in Figure 7. When the DPPH radical scavenging activities of the compound recorded at different concentration of compound between 100 mg L^{-1} to 0 mg L^{-1}, DPPH radical scavenging activity was increased with the concentration of the compound. Ascorbic acid and Trolox are used as reference antioxidants to compare free radical scavenging activity of the complexes. Reference compounds DPPH scavenging activity found higher than the test compound at all the concentrations and their DPPH radical scavenging activities were found to be 90.1% and 91.6%, at a concentration of 100 mg L^{-1}, respectively [25-27].

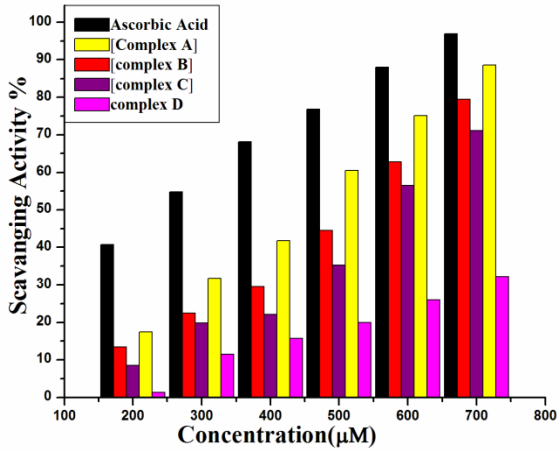

Figure 7. Radical-scavenging activity on DPPH radicals (%) of the A, B, C and D complexes

Antibacterial Activity of Metal Complexes and Commercial Drugs

Antibacterial activity [26] of metal complexes and the commercial drugs (Ampicillin) were checked against four bacterial pathogens such as, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*. Metal complexes were shown better result in the form of zone of inhibition in culture plates (Table 1).

Table 1. Anti bacterial activity

Name of bacteria pathogens	Complex I (1 mg/1 ml)	Complex II (1 mg/1 ml)	Complex III (1 mg/1 ml)	Complex IV (1 mg/1 ml)	Ampicillin (1 mg/1 ml)
Bacillus subtilis	4.82	6.83	5	2.78	13.2
Klebsiella pneumonia	3.65	4.98	2.7	1.76	9.9
Escherichia coli	3.71	5.6	2.4	2	9.6
Staphylococcus aureus	2.78	5.3	4.8	2.12	2.78

Against four pathogens, this is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept and Tweedy's chelation theory. The zone of inhibition of the 20 µL metal complex show in the culture plates is *Escherichia coli* (5.67 mm), *Klebsiella pneumonia* (4.98 mm), *Bacillus subtilis* (6.83 mm) and *Staphylococcus aureus* (5.39 mm) (Figure 8).

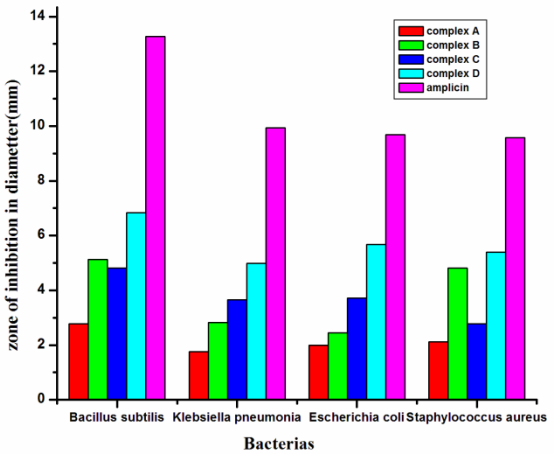

Figure 8. Antibacterial Activity of A, B, C and D metal complexes

Cytotoxic Assay *In-Vitro*

Cytotoxic studies evaluated by using MTT assay, HeLa (Human cervical cancer cell line) and MCF-7 (breast cancer cell line) tumor cell lines were treated with different concentrations (10 µM, 20 µM, and 30 µM) of prepared samples, the percentage of cell viability have shown in Figure 9. Cell death is studied by treating the HeLa cancer cells with 30 µM of samples for 24 h and then observing them for cytological changes by adopting DAPI staining.

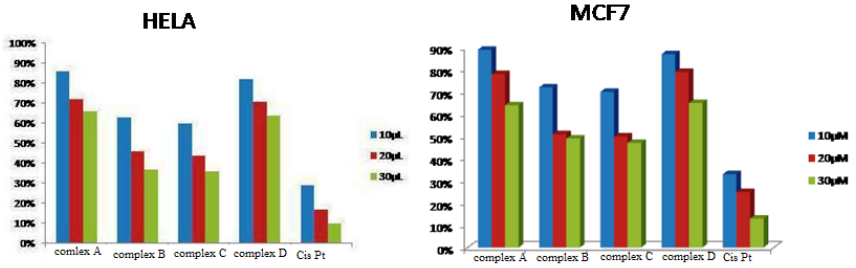

Figure 9. Confocal microscopy images of HeLa(Human cervical cancer cell line) tumor cell lines were treated with 30 µM of prepared samples. a) HeLa cells with bright field b) HeLa cells stain with DAPI and observed by confocal microscopy (excitation 488 nm, emission 600–620 nm). c) HeLa cells were treated with A, B, and D and d) cells were treated with A, B, and D complex an arrow shows blebbing of the nuclei

The representative morphological changes observed on addition of synthesized samples such as nuclear swelling, cytoplasmic blebbing and late apoptosis indication of dot-like chromatin condensation is shown in Figure 10. The increase in the number of abnormal cells to increase in concentration of compounds reveals the concentration effect of study compounds on cytological changes [18]. The results of the current investigation predict the order for apoptosis-inducing effect as; Cisplatin >Cu (II) complex > Co(II) complex. And indicate that they have relatively good antitumor activity.

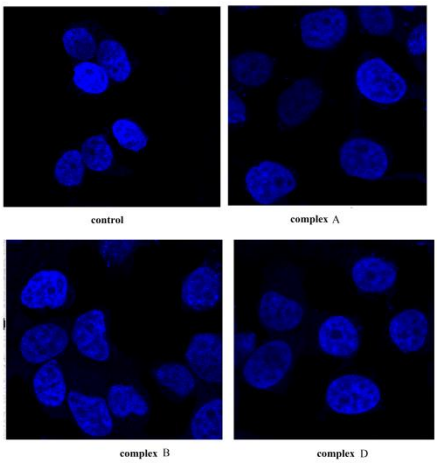

Figure 10. Plot of the percentage of cell viability at various concentrations of the compounds [Cu(L) (C_2O4) (H_2O)] (A), [Cu(L) (A.A) (H_2O)] (B), [Co(L) (C_2O_4) (H_2O)] (C) and [Co(L) (A.A) (H_2O)] (D) Cis platin, HeLa cells and MCF-7 cellines

CONCLUSION

A new Cu (II), Co(II) complexes has been synthesized and structurally characterized. Based on the elemental analysis and spectral data, the geometry of metal complexes is assigned as octahedral. The DNA binding ability of the complexes has been studied by different methodologies. The results suggest that the complex bind through intercalative mode. etalcomplexs binds BSA protein. Anti-bacterial studies of these complexes reveal that it shows better activity when compared to that of the amplicine drug. The antiproliferative activities of the metal complexes were investigated against HeLa cell lines. According to the results; both of the compounds have shown cell selective activity against HeLa cell lines. Metal complexes also exhibiting better nuclease and antioxidant activity.

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