CHAPTER-V

SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT ACTIVITY AND DNA CLEAVAGE STUDY OF NOVEL COPPER (II) COMPLEX

5.1. Introduction

Design and synthesis of metal–organic hybrid complexes have drawn much attention over the years due to their fascinating architectures and topologies, including their varied potential applications as catalysts, and chemical sensors [1-4]. In modern research, functional hybrids have been inquired enormously for the remedy of several diseases like malaria, HIV, cancer, fungal and bacterial affliction etc [5]. One of the merits of employing this type of metal organic frameworks is because of the carriage of tunable inorganic-organic components. Thereby simply altering the components in these materials, the disparity in the attributes can be attuned for their prospective utilization. Amongst the d-block elements, copper is certainly the most preferred transition metal for the synthesis of novel metal-organic hybrid compounds; as it's both Cu (I) and Cu (II) ions in association with several organic joiners can shape interesting network configurations and packing motifs [6-9].

Oxygen is very essential for our living system but when it is present in excess amount in our body its turn deleterious for immune cells by the formation of reactive oxygen species (ROS). Increase amount of ROS in our body may be the reason of the development of several diseases like cancer, diabetes, obesity, rheumatoid arthritis, cognitive disorders etc as it damages normal biochemical activities in the living organism [10-11]. So to combat this foul species various antioxidants must be developed in the organism.

Therefore, herein this chapter copper (II) metal based hybrid material has been synthesized and their potential effects in alleviating oxidative disorder were studied through various biochemical methods.

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5.2. Experimental Section:

5.2.1. Synthesis of [Diaquo {bis (*p*-hydroxybenzoato-κ^1O^1)} (1-methylimidazole-κ^1N^1)} copper (II)]:

To a methanolic solution of 4-hydroxy benzoic acid (0.277g, 2mmol) (20 ml), copper nitrate (0.2417g, 1mmol) is included and the resulting blue colored mixture is stirred at room temperature for 30 minutes. To this blue coloured solution, 1-methyl imidazole (0.082g, 1 mmol) is included in a drop wise fashion. As the color of the solution changes from light blue to dark blue, a few drops of triethyl amine was mixed and after that it was refluxed for 4 hrs at 160 °C. The mother liquor was then moved into a 50 ml beaker and permitted to remain at room temperature for a couple of days. After a few days the compound was acquired as a blue crystal. Single crystals suitable for X-ray diffraction were collected by hand picking under a microscope (40X). The synthesized copper complex was soluble in most of the polar solvent except water and it did not melt up to 280 °C. Yield: 0.383 g, 78% (based on Cu). Elemental analysis calcd (%) for [Cu($C_{18}H_{20}N_2O_8$).$2H_2O$]: C 43.94, H 4.92, N 5.69, Cu 19.91; found: C 43.77, H 4.69, N 5.55, Cu 12.96.

5.3. Results and Discussion:

5.3.1. Characterization of the synthesized compound:

5.3.1.1. X-ray crystallographic Studies:

The synthesized compound crystallizes as orthorhombic system having the space group $P2_12_12_1$ with 4 formula units in the unit cell (Z = 4). Table5.1. presents the crystal data along with structure refinement variables for the synthesized Cu (II) complex.

Table 5.1. Crystal data collection and structure refinement for the complex

Crystal data	
Chemical formula	[Cu(C_{18} H_{20} N_2 O_8).2H_2O]
M_r	491.93
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	298.8 (7)
Colour, habit	Blue, Parallelepiped
Size, mm	0.50 × 0.40 × 0.20
a , b, c (Å)	7.0603 (3), 12.1658 (5), 24.2757 (8)
α, β, γ (°)	90
V (Å^3)	2085.14 (14)
Z	4
Radiation type	Mo $K\alpha$
Density (calculated), Mg/m^3	1.567
Absorption coefficient, mm^{-1}	1.106
Data collection	
Diffractometer	xcalibur, sapphire3
T_{min}, T_{max}	0.608, 0.809
θ_{max},θ_{min}	29.271, 3.453
h	-9→7
k	-16 →15
l	-25 →32
Reflections collected	6703
Unique reflections	4513
Observed reflections (>2σ(I))	4110
R_{int}	0.0363
Refinement	
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	451 /12/308
Final R indices [I>2σ(I)]	R_1 = 0.0363, wR_2 = 0.0859

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X-ray analysis report revealed that the asymmetric unit contains one Cu (II) ion, one 1- methyl imidazole, two benzoic acid moiety and two coordinated H_2O molecules. In addition two guest H_2O molecules are also present in the asymmetric unit (Fig.5.1.).

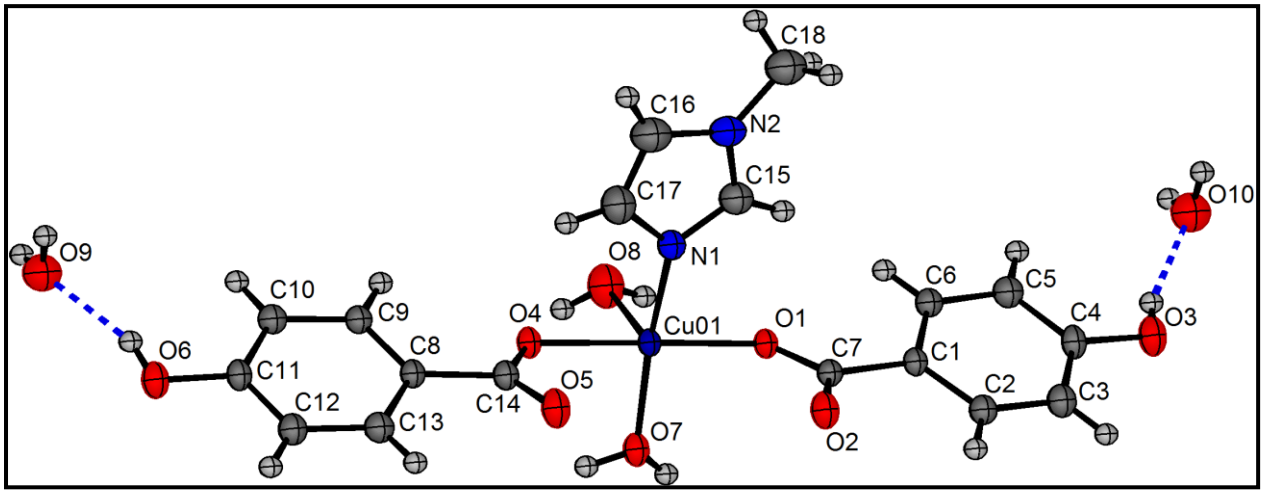

Fig.5.1. Coordination geometry of Cu(II) ion in the complex.

The synthesized complex molecule adopts a square pyramidal geometry having the molecular formula $[Cu(C_{18}H_{20}N_2O_8).2H_2O]$. In Fig.5.1. three dimensional layout of the complex molecule has been displayed. Fig.5.2. disclosed the supramolecular framework of the complex formed via both intramolecular and intermolecular H-bonding between coordinated H_2O molecules and the guest H_2O molecules.

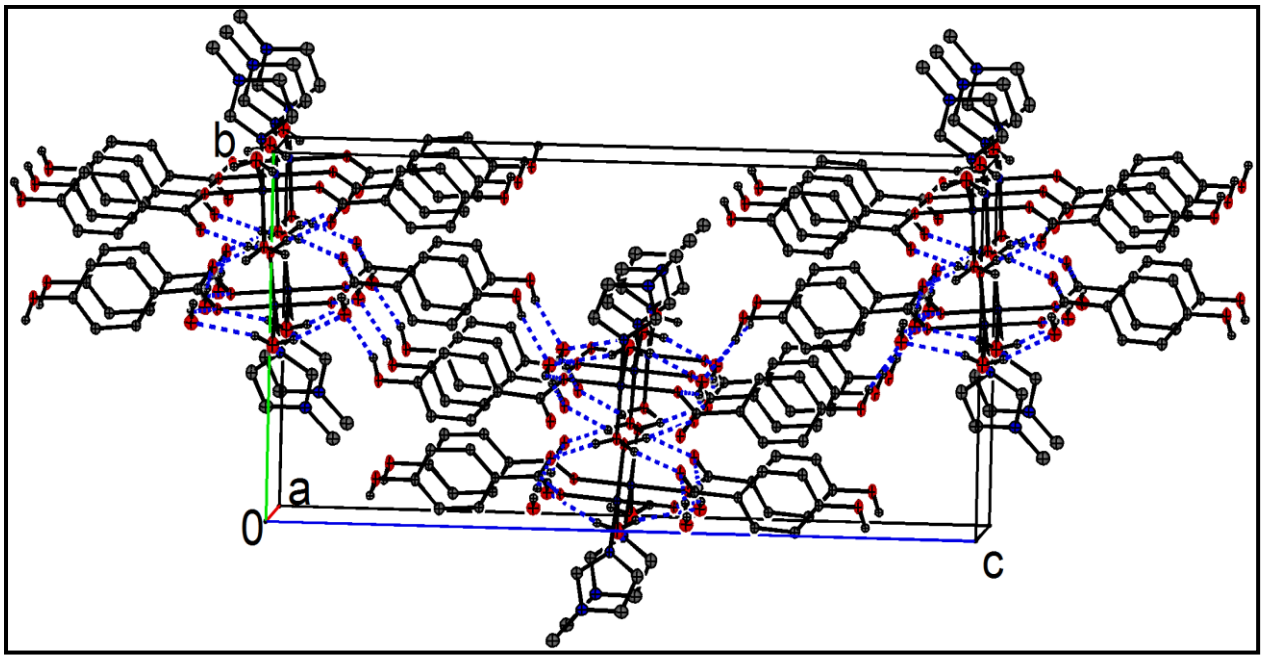

Fig.5.2. Crystal packing and hydrogen bonding within the framework.

5.3.1.2. FTIR Spectroscopic study:

Infra-red spectra always offer productive details regarding metal ligand bonding. The FTIR spectrum of the synthesized complex has been depicted in Fig.5.3. and it showed two characteristic bands emerging at 1595 cm^{-1} and 1389 cm^{-1} for asymmetric (υ_{as}) and symmetric (υ_s) stretching of (COO-) group respectively [12-13]. The large separation value of $\Delta\upsilon = \upsilon_{as} - \upsilon_s = 206$ cm^{-1} implies un-symmetrical monodentate behavior of the -COO group. A broad band was noticed in the region 3222-3459 cm^{-1} which is related to the presence of coordinated H_2O molecules in the structure of the complex meanwhile the presence of Cu-N bond was confirmed by the appearance of peak at 632 cm^{-1}. The data of IR spectra supports the structure obtained from single crystal XRD.

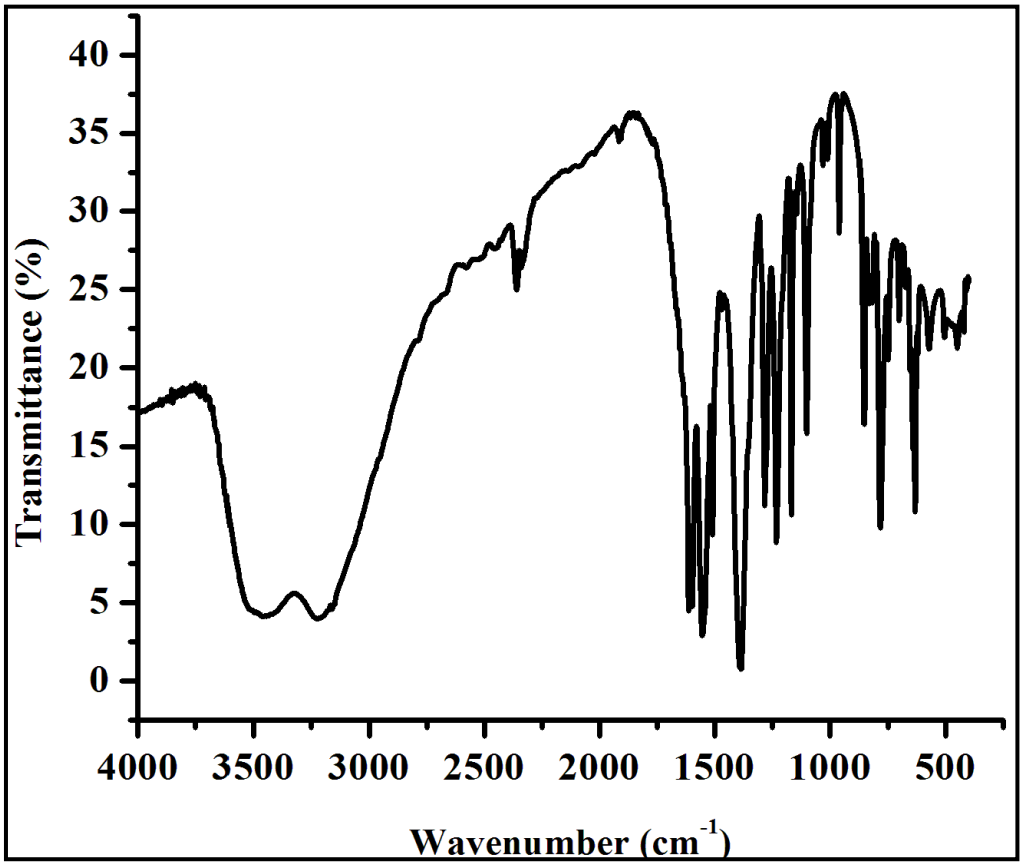

Fig.5.3. FTIR spectrum of the complex**.**

5.3.1.3. Magnetic moment and EPR analysis:

The magnetic moment, μ_{eff} of the complex was found to be 1.92 B.M and it is in well acceptance of the presence of an unpaired electron in synthesized Cu (II) compound [14]. The μ_{eff} value is greatly propped by the X-band EPR spectrum. The recorded EPR spectrum is characteristic of square pyramidal geometry. A perusal of the *g*-values [$g_e < g_\perp < g_\parallel$ (2.002 < 2.15 < 2.215)] suggests that $d_{x^2-y^2}$ orbital contains the unpaired electron having the electronic configuration $(e_g)^4(a_{1g})^2(b_{2g})^2(b_1)^1$.

5.3.2. Molecular Docking Analysis:

The molecular Docking investigation revealed a high binding affinity of the compounds with the membrane receptor proteins. Among them Kappa opioid receptor and mu opioid receptors showed highest interaction of -8.2kcal/mol and -8.4kcal/mol (Fig.5.4) both molecular view and secondary protein structure). Such a range of binding affinity predicts spontaneous reaction when present in biological system. The opioid receptors like kappa, delta and mu are all effective in Central Nervous system, especially mu opioid receptor are against to morphine [15]. Thus, the torment assuaging action of the compound can be anticipated.

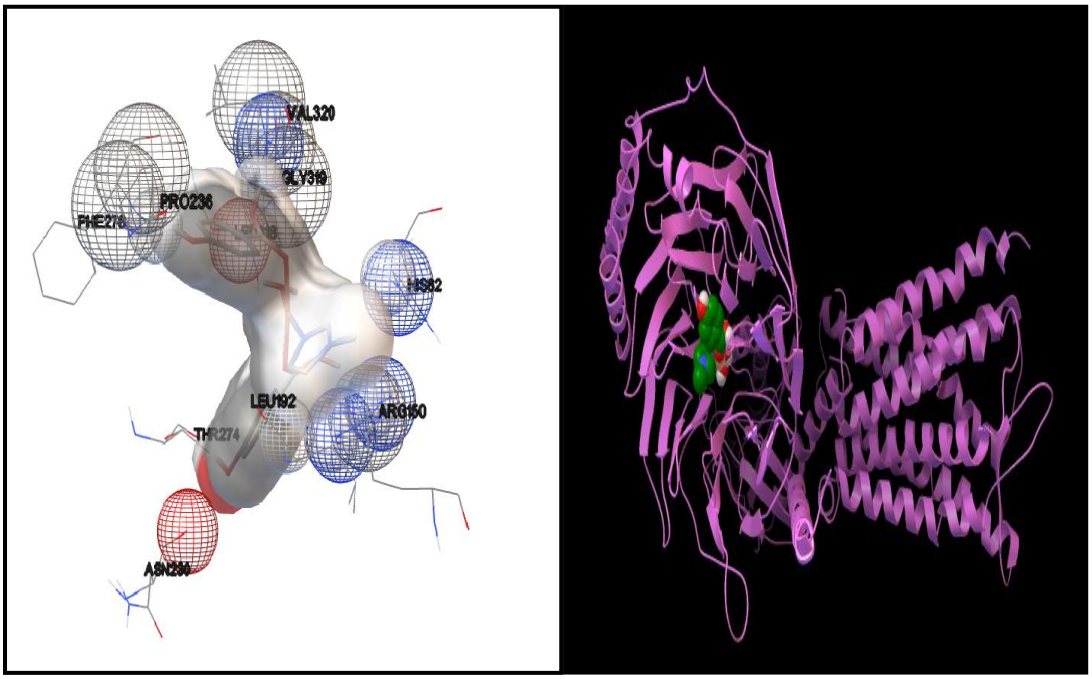

Fig.5.4. Molecular Docking interaction

5.3.3. DNA cleavage study:

The DNA cleavage reaction was investigated by gel electrophoresis technique. The DNA cleavage activity was determined by monitoring the transformation of supercoiled pBR322 DNA (Form I) to nicked circular form (Form II) and linear form (Form III) [16]. To perform cleavage reaction, solutions of pBR 322 DNA were prepared in Tris-HCl buffer and each reaction mixture contains 20μM DNA, 200μM H_2O_2 and 5-15μM synthesized complex. After incubation for 2 hours, 2 μl of loading buffer and 1% agarose gel (containing 1 μg/cm^3 EB) was added to each reaction mixture tube. The electrophoresis was performed in Tris-acetic acid-EDTA buffer for 2h at 50V. After the completion of electrophoresis, the gel was captured photographically under UV light.

The electrophoresis study clearly showed that the Cu(II) complex have acted on DNA as there was noticeable difference in bands of the complex with respect to band of the control DNA (Fig.5.5). The synthesized complex is highly efficient to convert supercoiled form of DNA (Form I) into nicked circular form (Form II). The appearance of smears in the gel photograph supporting radical cleavage. As the synthesized metal complex was found to cleave DNA, so it can be inferred that the Cu(II) complex will inhibit the growth of pathogens.

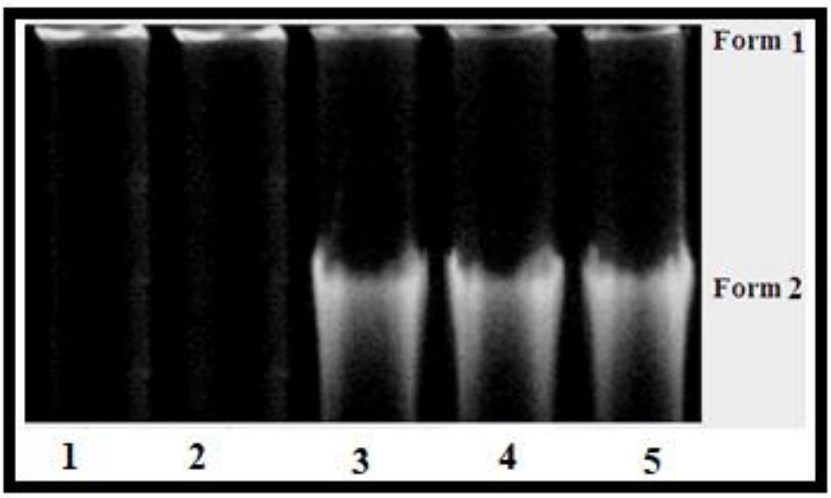

Fig. 5.5. Changes in the agarose gel electrophoretic pattern of pBR322 plasmid DNA induced by H_2O_2 Cu(II) complex. Lane (1): DNA control, Lane (2): DNA + H_2O_2, Lane (3-5): DNA+ Cu(II) complex + H_2O_2, [complex] = 5, 10, 15μM respectively

5.3.4. *In vitro* antioxidant activity

In the present study, novel Cu (II) complex shows high antioxidant activity compared to respective standard compounds (Fig.5.6.). DPPH activity has been extensively used for screening free radical scavenging activity at its low concentration. Cu (II) complex shows DPPH activity in a dose dependent manner. The highest dose 20 µg/ml concentration shows the highest percent of inhibition (81.63 ± 2.52 µg/ml) has been demonstrated in Fig.5.6. Similar results were found in case of hydroxyl radical and hydrogen peroxide radical scavenging assay. The percent of inhibition in case of hydroxyl radical (62.56 ± 4.34 µg/ml) is much higher than the standard compound Mannitol (31.31 ± 0.85 µg/ml) and hydrogen peroxide (65.81 ± 5.27 µg/ml) also much higher than standard sodium pyruvate (7.64 ± 0.68 µg/ml). Hypochlorous acid, superoxide anion, singlet oxygen, nitric oxide, peroxinitrite and lipid peroxidation shows similar results where percent of inhibition gradually increases in a dose dependent manner and shows better scavenging activities than standard compound each. The better antioxidant activity of any compound has potential and reduces the risk of oxidative damages with least side effects [17]. Therefore, in the present study in practice several antioxidant and free radica scavenging activities were carried out with our sample of interest. Now days, due to the environmental pollutions human beings are exposed to H_2O_2, which may enter in our body by normal physiological function. Hydrogen peroxide directly or indirectly converted to hydrogen and water and may produced hydroxyl radical (.OH) that can cause lipid peroxidation and DNA damage. Therefore, scavenging activity of H_2O_2 by novel Cu (II) complex proves beneficial for health. On the other hand, peroxinitrite ($OONO^-$), a reactive nitrogen species alleviating chances of carcinogenesis [18]. Therefore, inhibition of peroxinitrite by Cu (II) complex may prevent the chances of carcinoma. Cu (II) complex also prove that it has the ability to scavenge proxynitrate, hydroxyl radical, superoxide, singlet oxygen and other free radicals that cause the damaging consequence in our biological system. Thus, novel Cu (II) complex might prove to be a solution in hindrance of various diseases related to oxidative stress and can be considered as a good remedial approach.

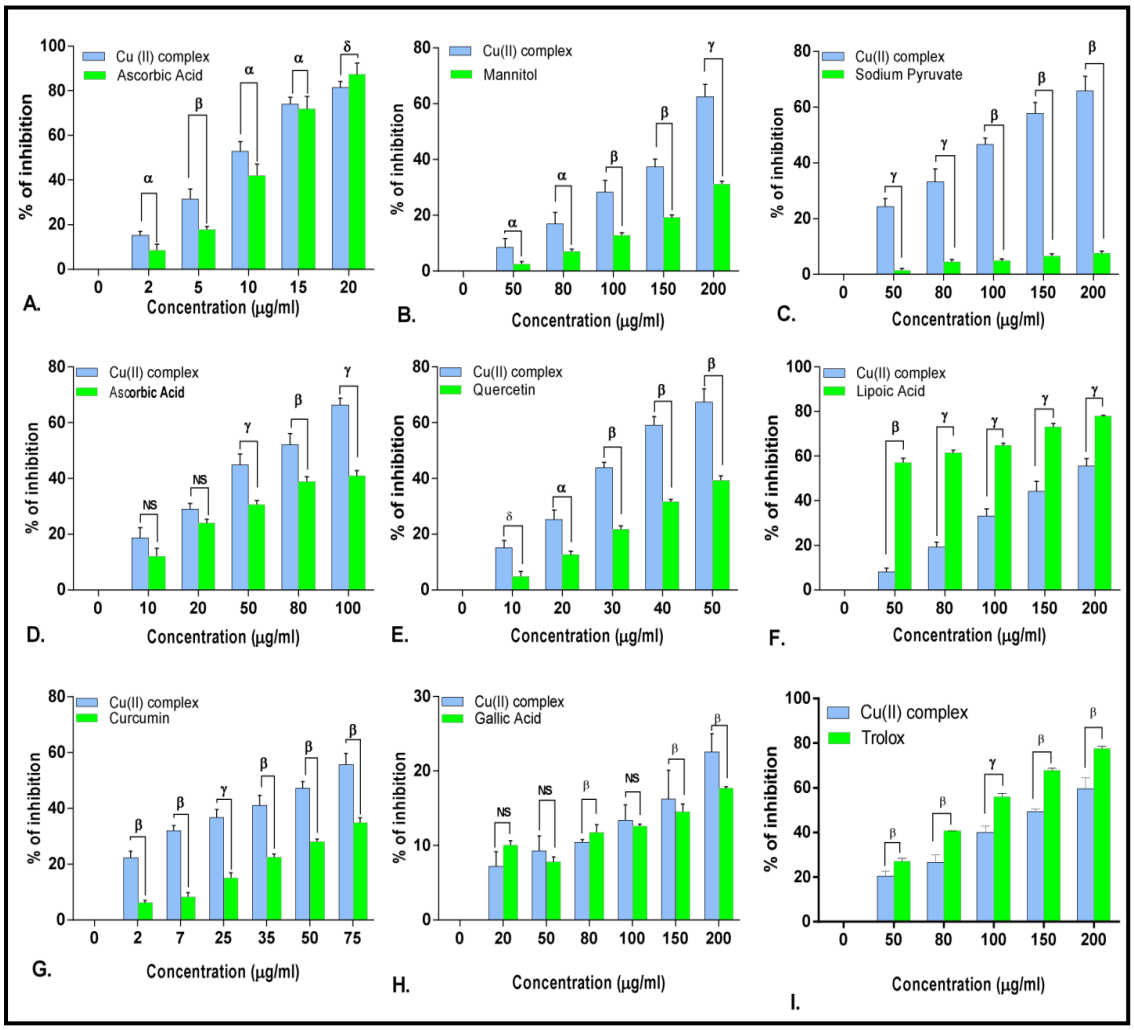

Fig.5.6. Antioxidant activity of Cu (II) complex. (A) DPPH activity; (B) Hydroxyl radical activity; (C) Hydrogen peroxide; (D) Hypochlorous acid activity; (E) Superoxide anion; (F) Singletoxygen; (G) Nitric oxide; (H) Peroxinitrite; (I) Lipid peroxidation. Data expressed as mean ±S.D (n = 6). α p< 0.05; β p< 0.01; γ p< 0.001; NS - Non significant when compared with standard.

5.4. Conclusion:

The blue colored newly synthesized metal-organic hybrid complex has Parallelopiped shaped orthorhombic crystals with space group $P2_12_12_1$. The three-dimensional structure of the complex was stabilized due to extensive inter- molecular hydrogen bonding interactions. The compound shows a great probability of being an analgesic drug although it can have a long way to go to establish it also as a standard drug. In the present study it is clear that the novel Cu (II) complex aided in the revival of oxidative stress.

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