Preparation, Characterization of Copper Complex and its Biological Activity

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ABSTRACT

Thiosemicarbazones and their metal complexes are very promising compounds which present awide range of pharmacological applications as antimicrobial, antiparasital, and antiviral agents In many circumstances the pharmacological activity of thiosemicarbazones increases upon coordination to metal ions. A new novel copper complex of thiosemicarbazide ligand has been prepared and characterized by different physical and chemical methods such as ^{1}H NMR Spectroscopy, IR spectra, elemental analysis, molar conductance, electronic spectra and magnetic susceptibilities measurements. Copper complex behaves as a neutral tridentate in nature , coordination takes place via carbonyl oxygen , N(2)H and C=S respectively .Also the study of the effect of gamma irradiation on ligand andCu(I) complex using different measurments devices. On the other side, the antibacterial activity of ligand and Cu(I) complex against gram positive (*Streptococcus pyogenes*) and gram negative(*Escherichia coli*) bacteria was investigated. The result revealed that Cu(I) complex after gamma irradiation showed a higher antibacterial activity against gram positive (*Streptococcus pyogenes*) and gram negative(*Escherichia coli*) bacteria than the ligand

Keywords: Copper complex, IR, Thermal analysis, Antibacterial, Gamma *irradiation*

INTRODUCTION

Thiosemicarbazones are Schiff based ligands which have gained importance over the decades as potential drug candidates. When coordinated to metals, they have proved as good anticancer, antimicrobial, antioxidant and antiprotozoal agents. Transition-metal based complexes hold several advantages over other metal complexes because of their better acceptability and low toxicity in biological systems **(Tahmeena et al 2015).**

Thiosemicarbazone derivatives are of special importance because of their versatile biological and pharmacological activities. Thiosemicarbazone derivatives have found application in drug development for the treatment of central nervous system disorders, of bacterial infection, as well as analgesic and antiallergic agent. Thiosemicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. Moreover, thiosemicarbazones have found their way into almost every branch of chemistry; commercially they are used as dyes,

photographic films, plastic and in textile industry **(Tada et al 2011**).

Our aim in this research work to prepare, characterized of copper(I) complex of thiosemicarbazide ligand by using different spectral, physical measurements before and after gamma irradiation and also antibacterial activity of ligand and Cu(I)complex before and after gamma irradiation were investigated against two pathogenic bacteria *Streptococcus pyogenes* as Gram-positive bacteria and *Escherichia coli* as Gram-negative bacteria to assess their antibacterial activity .

MATERIALS AND METHODES

Material

All reactions were carried out under normal atmospheric conditions. All the chemicals used in this study were of AnalaR grade and procured from Sigma, Aldrich and Fluka. Metal salts (E.Merck).

Synthesis of Ligand

The ligand of p-toludine-phenylisothio cyanate(H_2L) was prepared by mixing (0.01 mol) of desired hydrazide with(0.01 mol) of phenyl isothiocyanate in 15 ml of absolute ethanol . The reaction mixture was refluxed for 6 hrs. The reaction mixture was recrystalized several times from ethanol

Synthesis of Copper(I) Complex

Copper(I) complex of the ligand p-toludine-phenyl isothiocyanate was prepared by adding stoichiometric amount of the CuI in EtOH to a hot solution of (H_2L) in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at for certain about 6hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10} .

Measurements

Elemental analyses (C, H and N) were performed by Microanalytical unit of the Cairo Univerisity, Egypt.Metal and chlorides were estimated using standard methods

(Elboraey and El-Gammal 2015). IR absorption spectra were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometere. ^{1}H NMR spectra were recorded in d6-DMSO using 300 MHz Varian NMR spectrometer. The electronic spectra were carried out as solution (10^{-3}M) in DMF using a Perkin- Elmer Lambada 4B spectrophotometer. The molar conductivity measurements were made in DMF solution (10^{-3}M) using a Tacussel conductometer type CD6N. Magentic susceptibilities were measured at 27C^o using a modified Gouy method with Johnson Mattey balance. Energetic γ-irradiation exposure was undertaken using a γ- C^{o}60 unit at atomic energy establishment AEE at El-Naser City Egypt at an accumalated dose of 6 Mega rad in air.

Antibacterial tests

The in vitro antibacterial activity studies were carried out as described by **(Elboraey *et al*, 2016)** with some modification, the inhibitory effect of both the synthesized ligand and its complex was tested on the pathogenic Gram-positive organism *Streptococcus pyogenes* and the Gram–negative bacterium *Escherichia coli*. Biological effect of the ligand and its complex was carried out before and after exposure to gamma irradiation. Brain Heart Infusion (BHI) was used to grow *Streptococcus pyogenes* cells and Nutrient broth medium was used to grow *E.*

coli cells. Compounds under investigation were dissolved in DMSO which has no inhibition activity on both microbes. Two different concentrations (1 µg/ml, and 5 µg/ml) were prepared. Bacterial strains were prepared by activating them on the proper broth media with shaking. The bacteria were then cultured for 24 h at 37 C^o in an incubator. One ml of the standard bacterial culture was used as inoculation in a broth medium.

For growth studies, *S. pyogenes* and *E. coli* cultures were inoculated and grown aerobically on BHI broth medium and NB medium respectively. Growth was calculated turbidometrically at 650 nm using conventional Spectrophotometer, in which turbidity produced is measured by taking absorbance and compared with turbidity produced by control.

After growing bacterial cultures on media that contain the ligand, the complex and the control, absorption measurements were accomplished by spectrophotometer after 24 and 48 h of incubation to determine the number of viable cell count per milliliter of sample and were used to the calculated the inhibition Percentage.

RESULTS and DISCUSSION

The ligand (H_2L) was confirmed by elemental analysis as shown in table (1), infrared as shown in table (2) and ^1H NMR spectroscopy. The reaction of the ligand H_2L with CuI produce complex of the general formulae [Cu(H_2L) I.]. These air stable complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities were measured in DMF (10^{-3}M) solution. Table (1) shows that, the complex is non-electrolyte, indicating that coordination of the anion to the ligand. The solid complex is colored, insoluble in water, methanol and ethanol but soluble in DMF at 10^{-3}M **(Al-Shaheen and Al-Mula 2014).**

Table 1: Analytical and physical data for the ligand (H_2L, $C_{16}H_{18}N_4OS$) and Copper(I) complex

Compound	Color	Calc. (Found %)					
		C	H	N	I	Cu	Λ_M
H_2L	Buff 75	61.1 (61.3)	5.73 (5.70)	17.8 (17.9)	-	-	-
Cu(H_2L) I	Green	38.1 (38.4)	3.6 (4.2)	11.1 (11.4)	25.2 (25.2)	12.6 (12.3)	25

Where, Λ_M = molar conductivity $ohm^{-1}\ cm^2\ mol^{-1}$ in 10^{-3}M in DMF solution.

The infrared spectra of the ligand and complex

Fundamental IR spectral bands for the ligand and copper(I) complex before and after gamma-irradiation are given in Table 2 . The IR spectrum of the free ligand is characterized mainly by the strong bands at 3384 cm^{-1}, 3262 cm^{-1}, 3150 cm^{-1}, 1672 cm^{-1} and 749 cm^{-1} are attributed to the stretching frequencies of υ(N4-H), υ(N2-H), υ(N1-H) , υ (C=O) and υ(C=S) wagging vibrations, respectively**(Abou Sekkina *etal*., 2011)** . This supports the nature of the ligand H_2L as tridentate one and coordination take place via(C=O) ,(N2-H) and (C=S). The bonding mode of the ligand to metal ions has been judged by a careful

comparison of the infrared spectra of the complex with that of the free ligand. In general, the infrared spectra of the metal complex show significant changes compared to the spectrum of the free ligand. The most important diagnostic spectral bands of Cu(I) complex after and before gamma-irradiation are depicted in Table 2

Table (2): Infrared spectral bands (cm^{-1}) for ligand (H_2L) and Cu(I) complex

No.	Compound	v(N4-H)/ v(OH)	v(N2-H)	v(N1-H)	v(C=O)	v(C=S)	v(Cu-O)	v(Cu-N)
	H_2L	3384	3263	3150	1672	749	-	-
	H_2L*	3336	3185	3126	1689	750	-	-
B_1	$Cu(H_2L)I$	3431	3293	3183	1597	749	601	529
A_1	$Cu(H_2L)I$*	3434	3295	3185	1597	750	602	529

*Where * after gamma irradiation (6Mega rad)*

The IR spectra Cu(I) complex show strong bands at 3431**–**3434 cm^{-1}, 3293-3295 cm^{-1}, 3183-3185 cm^{-1} and 749-,750 cm^{-1} which attributed to the stretching frequencies of υ(N4-H), υ(N2-H), υ(N1-H) , υ (C=O) and υ(C=S)wagging vibrations, respectively. By comparing the IR spectra of Cu(I) complex before and after gamma irradiation display that the band corresponding to υ(N2-H) after gamma irradiation shift to higher frequency as compared of Cu(I) complex before gamma irradiation and high intensity of the bands of function groups after gamma irradiation.The new bands appeared at 601-602 cm^{-1} and 529cm^{-1} assigned to υ(M-O) and υ(M-N) respectively**(Meena and Jain 2014)**.

^{1}H NMR Spectra

The ^{1}H NMR spectrum of the ligand (H_2L) in DMSO –d^6 revealed a chemical shift (δ/ppm) at 9.045 ppm and at 9.733 ppm attributed to N(4)H and N(1)H. The peaks of N(1)H and N(2)H appeared as signals at 8.9 and 8.3 ppm .The singlet peak appeared at 3.331 ppm due to CH_2 group and the multiple peak at 7.4 ppm due to aromatic protons of phenyl group (El-Saied et al., 2017).

The electronic spectra of the ligand and copper(I) complex

The electronic spectra of ligand and Cu(I) complex were recorded in DMF solution (10^{-3}M). In UV spectra of ligand shows λ_{max} at 272 nm with a shoulder band. It indicates that in DMF solution the ligand exists in thiol form. While in Cu(I) ions have the d^{10} configuration and therefore their complexes should not exhibit any d-d transition. The copper complex of this ion was found to be diamagnetic and octahedral geometry **(**Haddad *et al.* 2013**).**

Thermal Studies (TGA) before and after γ-Irradiation

The thermal behavior of the ligand and $Cu(H_2L)I$, $Cu(H_2L)I$*complexes synthesized before and after γ-irradiation are shown and Figs.(1 and 2). The thermal behavior of the ligand and copper complexes before and after γ-irradiation was investigated by thermogravimetric studies in temperature range 25-800 °C. The nature of proposed chemical change with temperature and percent of metal oxide obtained shown in Figs.(1 and 2)

The ligand before and after γ –irradiation

The TG curve of the ligand before and after γ-irradiation shows that it is thermally stable till 140°C. Also the TG curve shows three decomposition steps (Fig.1) in the temperature range 140-550ºC; 125-510°C with total weight loss of Calc. 99.0% (Found 99.9%); Calc.100% (Found 100%) before and after γ-irradiation respectively. Before γ-irradiation the thermogravimetric analyses curves of the ligand reveal that gamma irradiation induced thermal stability to the material than after irradiation **(Abousekkina *et al.,* 2011).** These results agree well with the composition of the ligand determined from elemental analysis and IR spectrum.

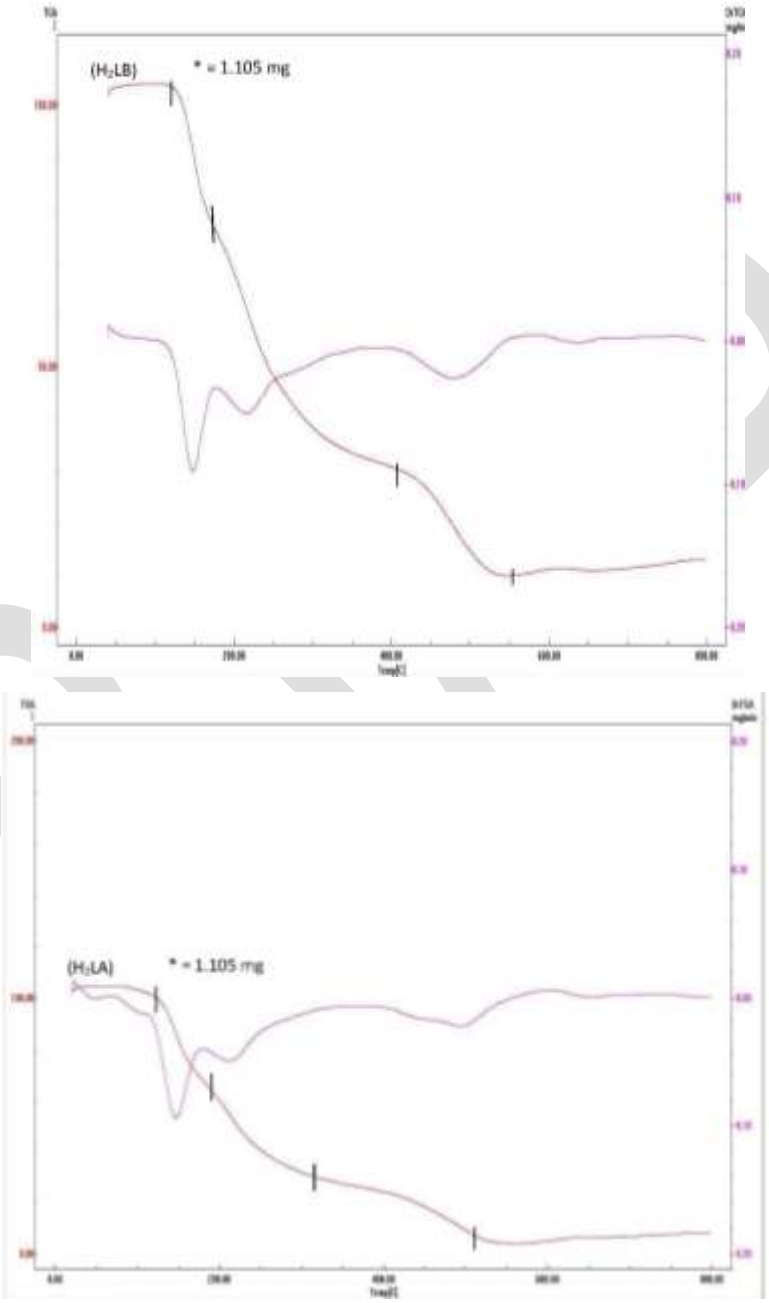

Fig .1: TGA/ DTG curves of ligand before irradiation (H_2L_B), after irradiation (H_2L_A)

Copper complexes before and after γ-irradiation

The TGA curve of copper complexes before and after γ- irradiation (B_1 and A_1) (Fig.2). The TG curve exhibit gradual decomposition at a temperature range 195–570°C, leaving CuO as final residue at 570°C. The TG curve after γ-irradiation is similar to before γ-irradiation.

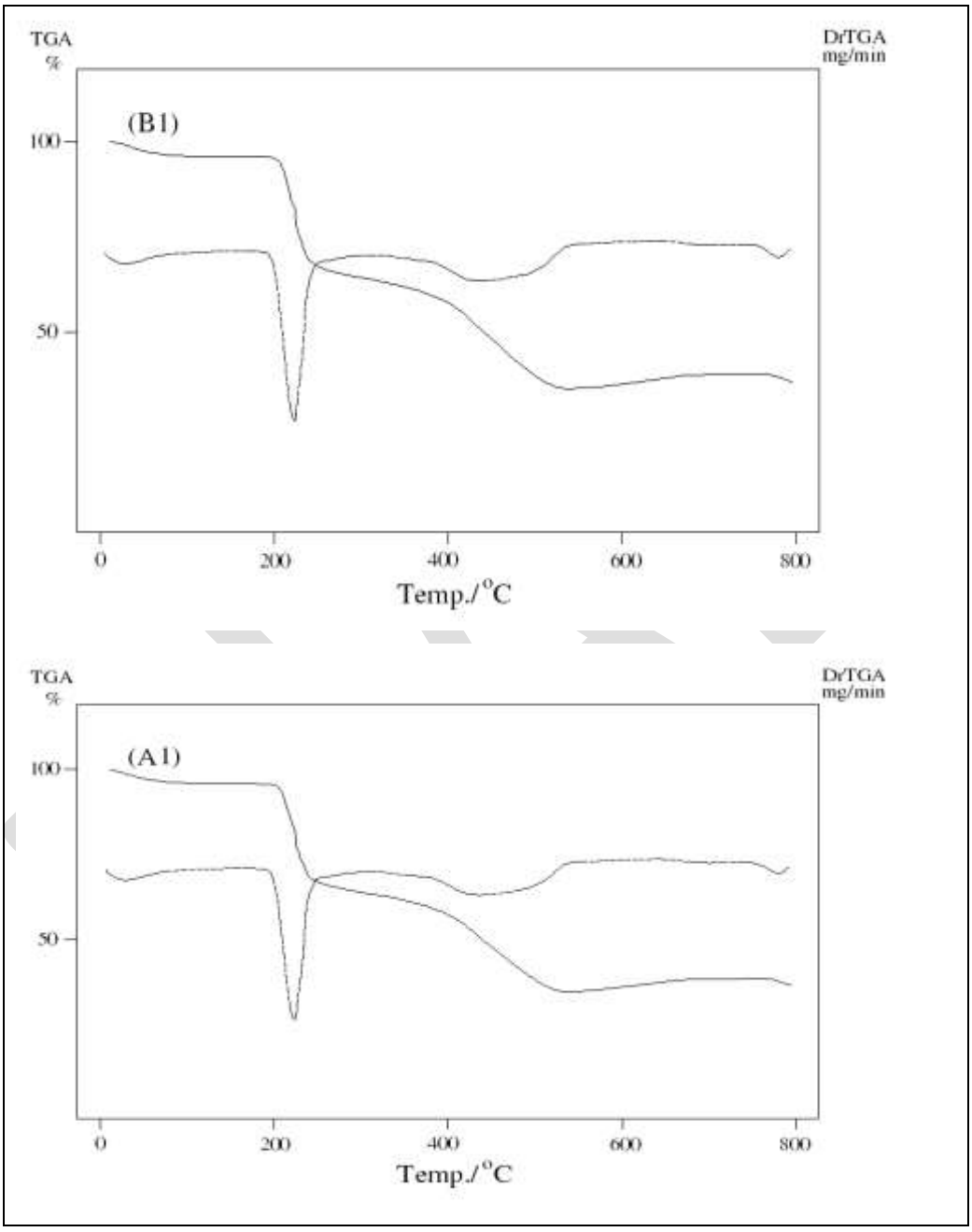

Fig. 2: TGA/ DTG curves of copper complexes before irradiation (B_1), after irradiation (A_1)

Antibacterial Activity:

The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases in table(3) showed antibacterial activity against the tested microbes. Generally, it was found that the antibacterial activity of both the synthetic ligand and Cu(I) complex was proportionally

increased with increased concentration. The tested compounds are found to have remarkable biological activity. For 1 µg/ml concentration of both synthetic ligand and Cu(I) complex, the antibacterial activity of the tested compounds was found to follow the order : Cu(I) complex < ligand in case of *E. coli* as shown in figure (4). On the other hand, a higher antibacterial activity was recorded when using the ligand with *S. pyogenes* as shown in figure (3) with the same concentration. (**Elboraey et al., 2016**).

Antibacterial activity of 5 µg/ml concentration for both the free acyclic ligand and its complex followed the order ligand complex when compounds were used with both *S. pyogenes* and *E. coli* **(Sönmez et al., 2010).** Results in Figure (4) suggested that in case of 1 µg/ml Cu(I) complex, the chelation could facilitate the ability to cross the cell membrane of *E. coli* and can be explained by Tweedy's chelation theory. Chelation complication could enhance the lipophilic nature of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane **(Tweedy, 1964).** Complex when used with both concentrations (1 µg/ml and 5 µg/ml) in case of the gram-positive *S. pyogene* bacterium. It also has been observed that some moieties such as N(2)H linkage introduced into such compounds exhibits extensive biological activity **(Singh et al., 2013)** . The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases.

Table (3): Antibacterial activity of ligand and its metal complex

Compound	Inhibition %			
	E. coli		*S. pyogenes*	
	1µg/ml	5µg/ml	1µg/ml	5µg/ml
H_2L_2	48.76	56.23	75.32	91.24
H_2L_2*	52.11	66.76	74.40	95.32
Cu(H_2L)I	86.44	92.13	57.66	88.3
Cu(H_2L)1*	82.8	86.50	83.41	94.31

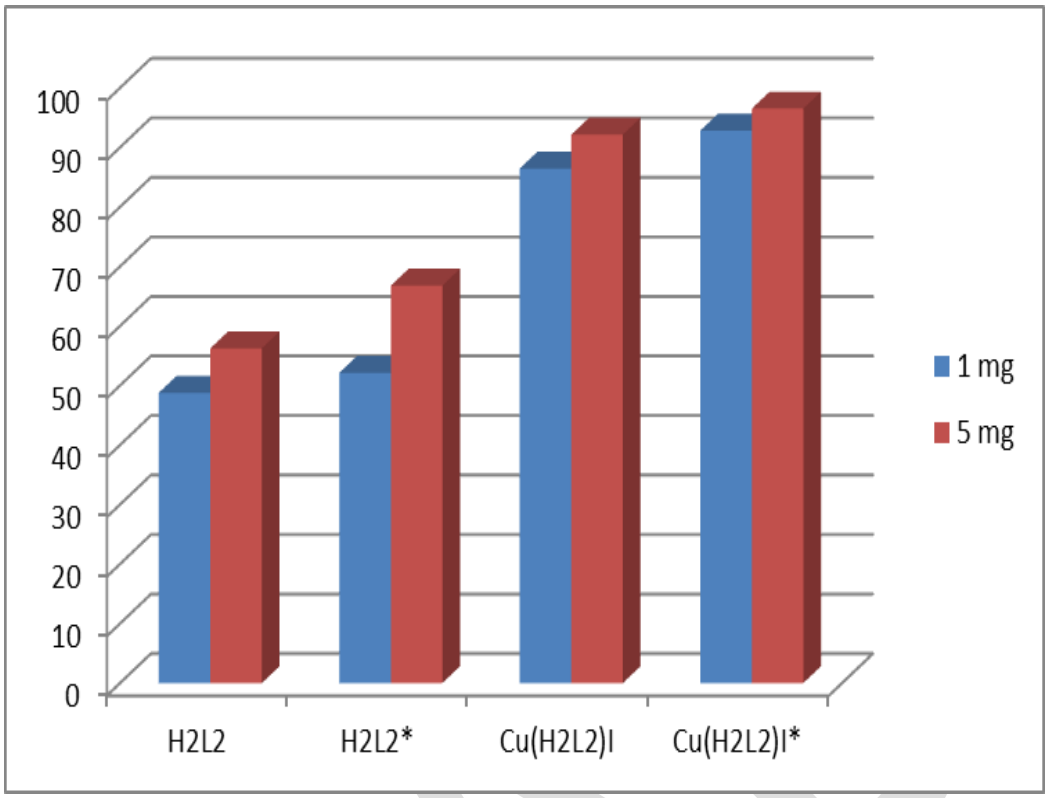

Fig.3: antibacterial activity of ligand and copper(I) complex against S. Pyogenes before and after irradiation.

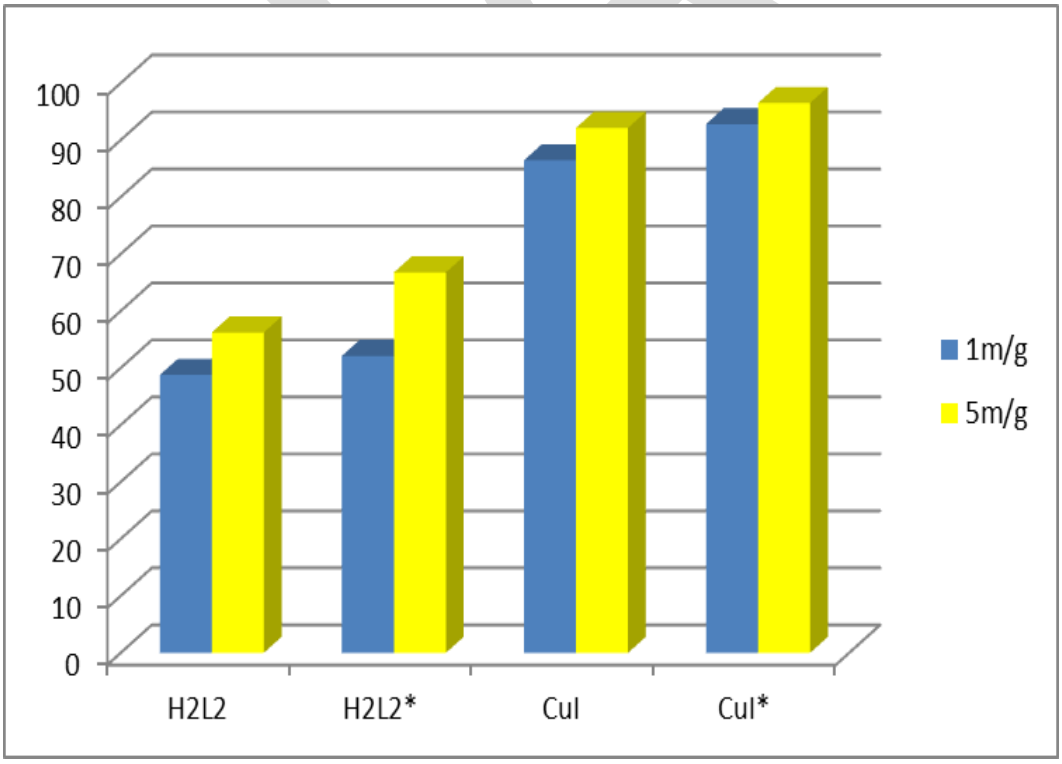

Fig. 4: Antibacterial activity of ligand and copper (I) complex against *E.coli* before and After irradiation.

CONCLUSION

In this study we prepared Cu(I)complex which is in the agreement with the proposed structure and study the effect of gamma irradiation on Cu(I) complex by using physicochemical and spectral measurements before and after gamma irradiation and the study proved that:

1- The spectral analysis of infrared using γ-ray showed that effective bands to groups growing larger and stronger than before irradiation.

2- Low wave length of complex after gamma irradiation than before gamma irradiation

3- The activity of antibacterial of Cu(I) after gamma irradiation was increased than before gamma irradiation.

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