NANOSIZED METALLO-ORGANIC DRUG AS A NEW TREND FOR HEPATOCELLULAR CARCINOMA CELL LINE(HepG-2)

MohamedI.Ayad[1],Ahmed S. Sharafeldin[1],Hytham M. Ahmed[2],Waheed M. Salem[3]and Mohammed H. H. Abu-Setta[*1]

[1]*Department of Chemistry, Faculty of Science, Menoufia University, Shebin El -Kom, Egypt.*
[2]*Department of Pharmaceutical analysis, Faculty of Pharmacy, MenoufiaUniversity,Egypt.*
[3]*Department of medical laboratories, Faculty of applied sciences health technology,Menoufia University, Egypt.*

,.

Abstract

Metallo-organic drugs of Cu(II) and Ag(I) with (E)-N'-(2-hydroxybenzylidene)-4-(4-((Z)-(2-hydroxybenzylidene)amino)phenoxy)benzohydrazide Schiff Base ligand have much potential as therapeutic and cytotoxic agents against hepatocellular carcinoma cell line -2. Specific examples involving the design of metal complexes as anticancer agents are discussed. These complexes have been synthesized and characterized by transmission electron microscope (TEM), Scanning electron microscope with EDEX, (mass, IR, UV-VIS and ESR) spectroscopy, XRD, as well as magnetic moments, conductance, elemental and thermal analyses. Molar conductance in DMF solution indicates that, the complexes are non-electrolytes. The ESR spectra of solid Cu(II) complexes **(2)** showed isotropic type indicating an octahedral geometry with covalent bond character. X-ray Diffraction Spectroscopy XRD has been done. The XRD reveal that $[(H_2L)CuSO_4.3H_2O].2H_2O$;Complex **(2)**, $[(HL)Ag].4H_2O$;Complex **(3)**these complexes have the average crystallite sizes of 19.21 and 21.25 nm respectively. It was suggested that the complexes are nanocrystalline. Cytotoxic evolution against hepatocellular carcinoma cell line -2 of the ligand and its complexes have been carried out. Complexes showed enhanced activity in comparison to the parent ligand or standard drug applied with IC_{50} = *13.70 – 30.60 µg/ml* range.

Keywords:

Schiff Baseligand, complexes, analysis,magnetism, ESR,XRD, cytotoxicity, Hepatocellular carcinoma.

INTRODUCTION

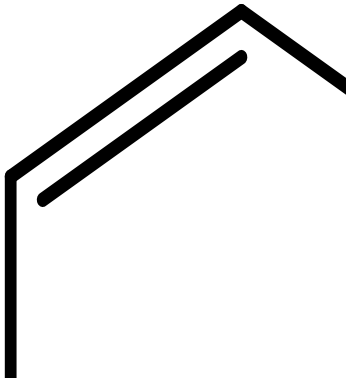
Schiff bases have played a key role in the development of coordination chemistry. Schiff bases complexes due to not only for coordination chemistry but for pharmacological applications, were, due to their good complexes properties and significant biological activity [1, 2]. Chemistry of transition metal complexes of Schiffbases became largely appealing because of their broad profile of pharmacological activity that provides a diverse variety of compounds with different activities[3]. Death from cancer,infectious diseases and newly emerging infectious diseases have remained a global health threat. Therefore, the continuous search for new and effective transition metal complexes as biological agents is important. Ag(I) complexes of Schiff bases are gaining more recognition recently due to their high bio-activity at low concentrations, low toxicity, strong

cytotoxicity, high selectivity, an effective scavenger of free radicals and ability to interact with biomolecules. Schiff bases are an important class of ligands in coordination chemistry due to their ease of synthesis, their diverse modes of coordination with Ag(I) ion and other transition metals and their wide therapeutic applications. As such, providing an avenue for possible drug design [3].we reported herein, synthesis and characterization of new metallo-therapeutic candidates derived from the novel ligand 1, (E)-N'-(2-hydroxybenzylidene)-4-(4-((Z)-(2-hydroxybenzylidene)amino)phenoxy)benzohydrazide.The cytotoxic activity of synthesized compounds has been also investigated.

MATERIALS AND METHODS

All the reagents employed for the preparation of the ligand and its complexes were synthetic grade and used without further purification. TLC is used to confirm the purity of the compounds. C, H, N and Cl analyses were determined at the Analytical Unit of Cairo University, Egypt. A standard gravimetric method was used to determine metal ions [4-6]. All metal complexes were dried under vacuum over P_4O_{10}. The IR spectra were measured as KBr pellets using a Perkin-Elmer 683 spectrophotometer (4000-400 cm-1). Electronic spectra (qualitative) were recorded on a Perkin-Elmer 550 spectrophotometer. The conductance(10^{-3}M) of the complexes in DMF were measured at 25°C with a Bibby conduct meter type MCl. Mass spectra were recorded using JEULJMS-AX-500 mass spectrometer provided with data sys-tem. The thermal analyses (DTA and TGA) were carried out in air on a Shimadzu DT-30 thermal analyser from 27 to 800°C at a heating rate of 10°C per minute. Magnetic susceptibilities were measured at 25°C by the Gouy method using mercuric tetrathiocyanatocobalt(II) as the magnetic susceptibility standard. Diamagnetic corrections were estimated from Pascal's constant[7]. The magnetic moments were calculated from the equation: The ESR spectra of solid complexes at room temperature were recorded using a varian E-109 spectrophotometer, DPPH was used as a standard material.Transmission electron microscopic samples were prepared by dropping the colloids onto carbon-coated TEM grids and allowed the liquid carrier to evaporate in air then assayed by a JEOL1230transimishion electron microscope(120KV). The TLC of all compounds confirmed their purity.

Experemintal



Scheme 1: Preparation of LigandH_2L (1)

As shown in the scheme 1 the mixture of H_2L**;(1)**was stirred for 30 minutes. After cooling, the solvent was removed under reduced pressure to give crude product which was crystallized in ethanol to yield pure ligand (**1**). Ligand (1): Yield 78 %; m.p. 285; color is green; Anal. Calcd. (%)for$C_{27}H_{21}N_3O_4$ (FW = 451.47): C, 71.83; H, 4.69; N, 9.31; Found (%) C, 71.67; H, 4.54; N, 9.29; IR (KBr, cm^{-1}), 3451, υ(OH), 3262, υ(NH), 1622 υ(C=N), 1476, 758 $\upsilon(C=C)_{Ar}$, 1615 $\upsilon(C=C)_{Al}$, 1325υ(C-OH) .

Synthesis of metal complexes (2)-(3): The metal complexes **(2)** and **(3)** wereprepared by refluxing with string a suitable amount (1 mmol) of a hot ethanolic solution of the following metal salts $CuSO_4.5H_2O$ and Ag(CH3COO) to prepare complexes **(2)** and **(3)** with molar ratio (1 metal: 1 ligand) respectively. The refluxing times varied from 2 to 4 hours according to the depending to nature of metal ion. 3 mL of diethyl amine were added to the reaction mixture in order to initiate precipitation of complex. The precipitates, were filtered off, washed with ethanol then by diethyl ether and dried in vacuum desiccators over P_4O_{10}.

Fig.1. Structure representation of complex (2)

Fig.2. Structure representation of complex (3)

Complex (2),[$(H_2L)Cu(SO_4).3H_2O].2H_2O$**:** Yield: 73 %; m.p.>300^oC; color: green; molar conductivity (Λ_m): 5.76 $ohm^{-1}cm^2mol^{-1}$.Anal.Calcd. (%)for$C_{27}H_{31}CuN_3O_{13}S$ (FW = 701.16): C, 46.25; H, 4.46; N,5.99, Cu, 9.06; Found (%) C, 46.30; H, 4.48; N, 5.95, Cu, 9.09; IR (KBr, cm^{-1}), 3268, υ(NH), 1610 υ(C=N), 1502, 750 $\upsilon(C=C)_{Ar}$, 1622 $\upsilon(C=C)_{Al}$, 1315 υ(C-O) , 1029υ(N-N) 560υ(M←O), 459υ(M←N), 1153, 1110, 897, 680$\upsilon(SO_4)$.λ_{max}(nm) 275,305.,450,500,600,621;μ_{eff} inB.M. 1.73.

Complex (3),[(HL)Ag(CH3COO)].$4H_2O$: Yield: 69%; m.p.>300 ^oC; color: yellow; molar conductivity (Λm): 9.7 $ohm^{-1}cm^2mol^{-1}$.Anal.Calcd. (%)forC30H25N4O6Ag (FW = 601.38): C, 51.93; H, 4.53; N, 6.49, Ag, 17.94; Found (%) C, 51.98; H, 4.61; N, 6.59, Ag, 17.88; IR (KBr, cm^{-1}), 3408 υ(OH), 3050 υ(NH), 1610 υ(C=N), 1700,1680 υ(C=O), 1248 υ(C-O), 528 υ(M←O), 470 υ(M←N), 1500, 1380υsymCH3COO, υasymCH3COO (Δ=120 cm-1).λ_{max} (nm) 285,288,305,330,420,620;μ_{eff} inB.M. diamagnetic.

BIOLOGICAL ACTIVITY

Cytotoxic activity:

Mammalian cell lines: HepG-2 cells (human Hepatocellular carcinoma) were obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation: The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivatedfetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method [8].

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.The relation between surviving cells and drug concentration isplotted to get the survival curve of each tumour cell line after treatment with thespecified compound.The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) [9].

Magnetic moments: The magnetic moments of the metal complexes (**2**)-(**12**) at room temperatureswere shown in (Table 1). Cu(II) complexes (**8**)-(**10**) showed values in the 1.67-1.70

B.M, range corresponding to one unpaired electron in an octahedral structure.Manganese(II) complexes (**2**) and (**3**) showed values 6.22 and 6.42 B.M, indicating high spin octahedral geometry around the Mn(II) ion. Co(II) complex (**5**) showed value 4.34 B.M, indicating high spin octahedral Co(II) complex.Ni(II) complexes (**6**) and (**7**) showed value 3.21 and 2.98 B.M , indicating an octahedral Ni(II) complex. Fe(II) complex (**4**) showed value 5.73 B.M, indicating high spin octahedral geometry around the Fe(II) ion .Zn(II) complex (**11**), Cd(II) complex (**12**) showed diamagnetic property [10].

Table 1:-ESR data Cu(II) complex (2):-

No.	$g_{\parallel}$	$g_{\perp}$	g_{iso} [a]	$A_{\parallel}$ (G)	$A_{\perp}$ (G)	A_{iso} [b] (G)	G [c]	ΔE_{xy}	ΔE_{xz}	$K_{\perp}^2$	$K_{\parallel}^2$	K	K^2	$g_{\parallel}/A_{\parallel}$	α^2	$ß^2$	$ß_1^2$	-2 ß	a
(2)	2.2	2.06	2.11	95	5	35	3.33	17391	21505	0.75	0.52	0.91	0.84	200	0.61	1.23	0.85	206.3	

X-ray Diffraction Spectroscopy (XRD)

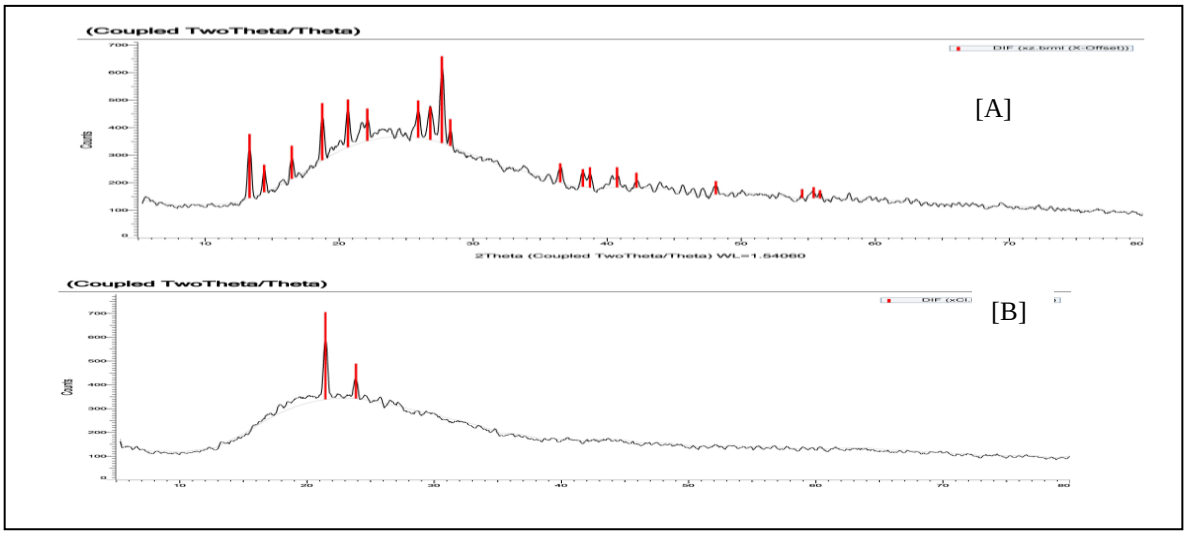


Fig. 3:X-ray Diffraction Spectroscopy XRD pattern of the Cu(II) complex (2):[A], Ag(I) complex (3);[B]

$[(H_2L)CuSO_4.3H_2O].2H_2O$;Complex (2), $[(HL)Ag].4H_2O$;Complex (3)these complexes have the average crystallite sizes of 19.21 and 21.25 nm respectively. It was suggested that the complexes are nanocrystalline.

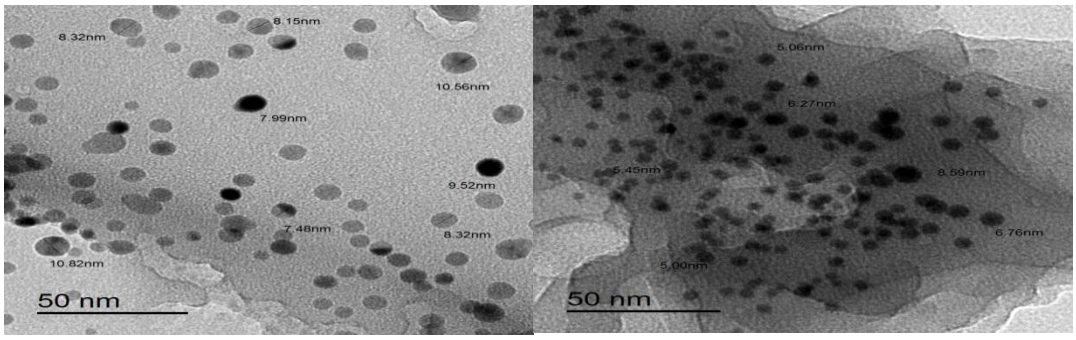


Fig.4. Transmission electron Microscope images: [A] TEM Image of the Cu(II) complex (2) ,[B]TEM Image of the Ag(I) complex (3)

Transmission electron microscope characterization (TEM): The average diameter of the complex particles Cu(II), complex **(2)** and Ag(I) ;complex(3) was determined to be 7.48-1082 nmand 5.0-859 nm respectively.All complexes are present in nano size particles i.e., their particles present in a diameter between 1 and 100 nm in size. complexes **(2)** and **(3)** show sign with ratio that exhibit new or enhanced size-dependent properties compared with larger particles of the same material with many advantages such as: Increased bioavailability, dose proportionality, decreased toxicity, smaller dosage form (i.e., smaller tablet), stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-Nano particulate dosage forms, increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body, faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity and reduction in fed/fasted variability.

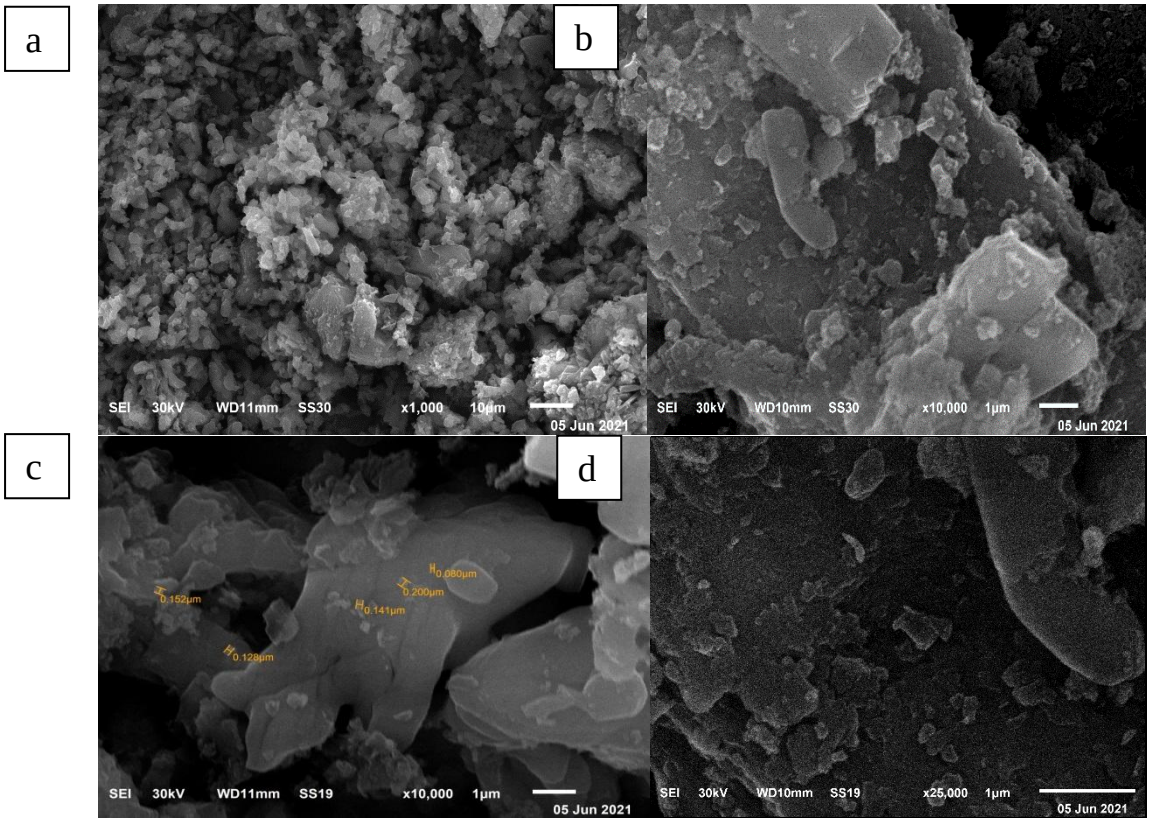


Fig. 5. SEM images for Cu(II) complex (2); a,b and Ag(I) Complex(3); c,d with different magnification

SEM

The sem images revealed that the surface of compounds are uniformly covered by the Cu(II) complex (2) and Ag(I) complex (3) . The layers were amorphous and crystaline structures appeared. The dimention of these crystalites ranged from 0.08 to 0.152 μmFig.5.

Biological Application

The biological activity of the ligand **(1)** and its metal complexes **(2)** and **(3)** were evaluated against HepG-2 cell lines figures **(6-9)**. In this study, we try to know the chemotherapeutic activity of the tested complexes by comparing them with the standard drug (Cisplatin). It seems that, changing the anion, coordination sites and the nature of the metal ion has effect on the biological behaviour by altering the binding ability of DNA [11-15]. Gaetke and Chow had reported that, metal has been suggested to facilitate oxidative tissue injury through a free-radical mediated pathway analogous to the Fenton reaction. It was showed that, the Cu(I) complexes are able to form GSSG (Glutathione disulfide) via the following reactions:

$$[Cu(I)\ (ligand)] + GSH \rightarrow [Cu(I)\ (GS)] + ligand + H^{+} \qquad (11)$$

$$[Cu(I)\ (GS)] + GS + [Cu(II) + GSSG \qquad (12)$$

These reactions lead to depletion of intracellular GSH pools, which has been frequently observed in cells after treatment with diverse Cu compounds [17-19]. In the presence of $H_2\ O_2$, the DNA-

bound Cu(II) complex is oxidized to form presumably Cu(II) (oxo/ hydroxo) species [13-18], thus, the reaction of Cu(II) complex with nucleic acid is occurred via Cu-oxo/ hydroxo intermediate. Cu(II) complexes are well known for their redox activity, which seems to be responsible for biological activities [14]. The redox cycling of Cu complexes is based on the reduction of Cu(II) to Cu(I) by intracellular thiols such as GSH (glutathione, nonenzymatic antioxidant under oxygen-contaning conditions" reaction pathway for [Cu(II) (ligand)] complexes was given in equations (13 - 15). Briefly, Cu(II) schematically, the underlying complexes rapidly form adducts with GSH[53], leading to Cu(I) complexes and GS+. In the presence of oxygen, this Cu(I) complex is able to generate a superoxide anion, which can induce ROS via a fentonlike reaction:

$$[Cu(II)\ (ligand)] + GSH \rightarrow [(GS)\ Cu(II)\ (ligand)] + H^{+} \quad (13)$$

$$[(GS)\ Cu(II)\ (ligand)] \rightarrow [GS^{*} + [Cu(I)\ (ligand)] \quad (14)$$

$$[Cu(I)\ (ligand)] + O2 \rightarrow O_2 + [Cu(II)\ (ligand)] \quad (15)$$

The treatment of the different complexes in DMSO showed similar effect in the tumoral cell line used as it was previously reported[15]. The solvent dimethyl sulphoxide (DMSO) showed no effect in cell growth. The ligand **(1)** showed a moderate inhibition effect at ranges of concentrations used, however, the complexes showed better effect against HepG-2 cell lines. The data obtained indicated the surviving fraction ratio against HepG-2 tumour increasing with the decrease of the concentration in the range of the tested concentrations[16]. Cytotoxicity results indicated that the tested complexes **(2),** and **(3)** demonstrated potent. Copper (II) complex **(2)** showed the cytotoxicity effect against cell line with IC_{50} value of 13.7 *µg/ml*, and then complex **(3)** with IC_{50} value 15.4 *µg/ml*.TheIC_{50} of complex **(2)** and **(3)** comparing with standard drug (Cisplatin) which is 17.97 at all concentrations complex **(2)** and **(3)** may be showed a good drug for treatment of HepG-2 cell lines .This can be explained as Cu (II) ion bonded to DNA. It observed that, changing the anion and the nature of the metal ion has effect on the biological behaviour, due to alter Binding ability of DNA binding, so testing of different complexes is very interesting from this point of view. Chemotherapeutic activity of the complexes may be attributed to the central metal atom which was explained by Tweedy's chelation theory[17, 18]. Also, the positive charge of the metal increases the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhance the biological activity[19, 20].

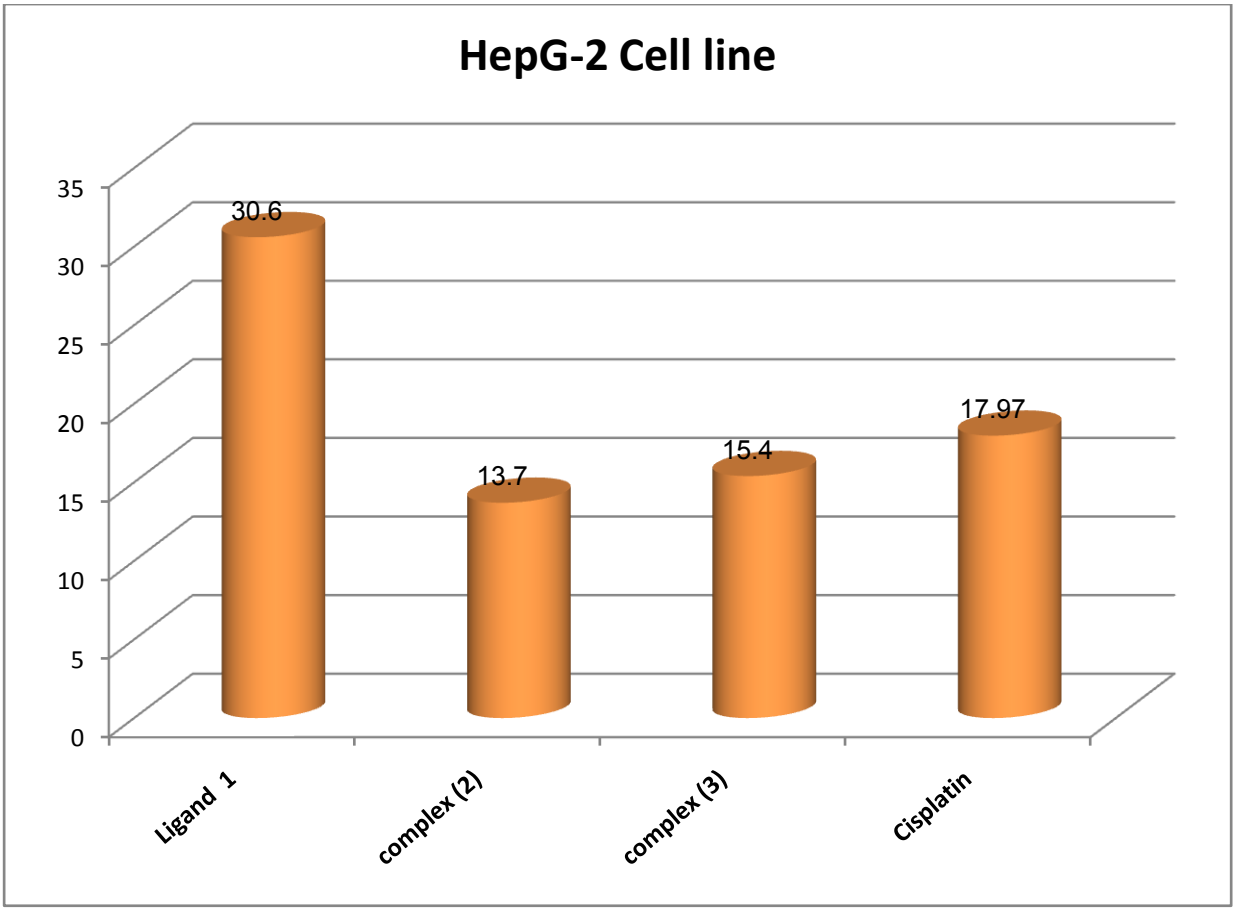
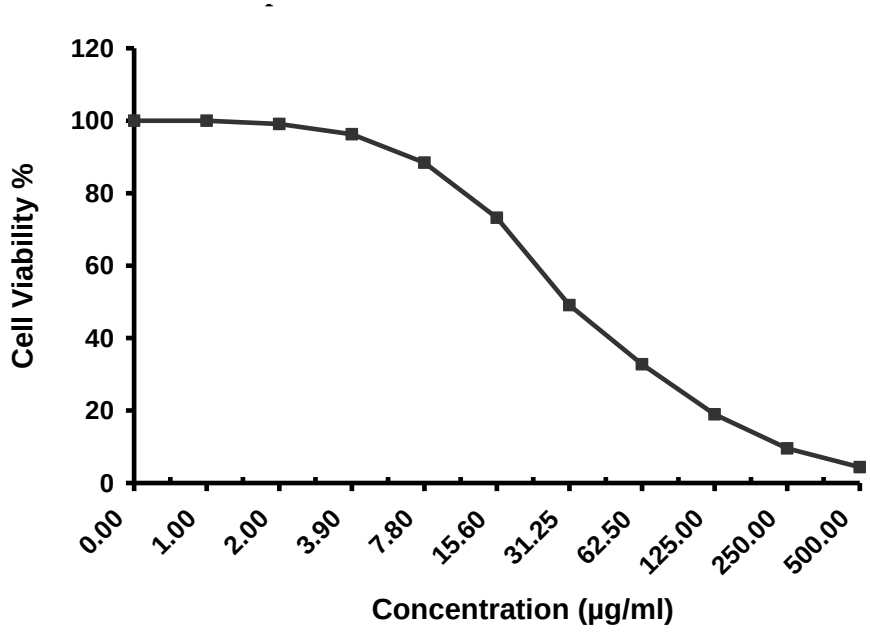


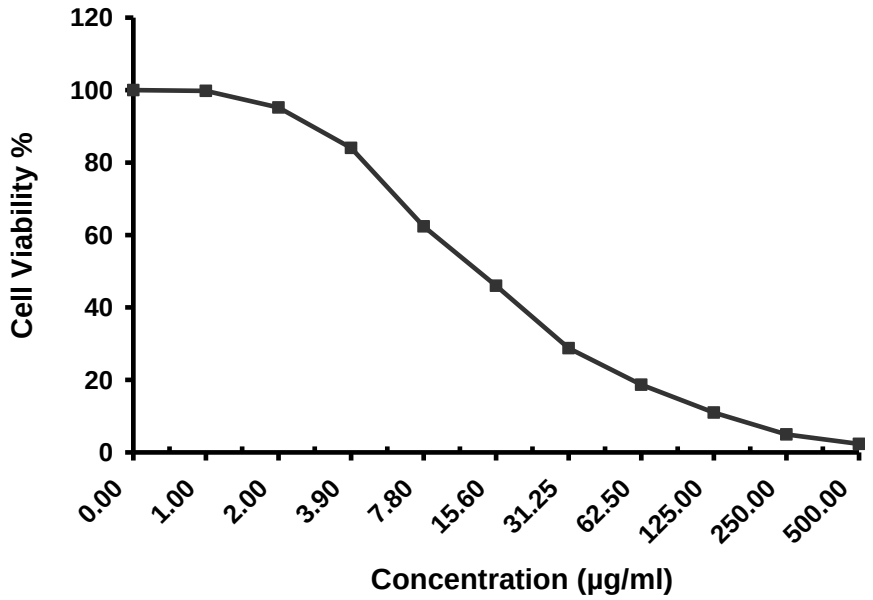
Fig. 6.IC_{50} values of the H_2L; (1),Cu(II) complex ;(3) , Ag(I) complex; (3) andcisplatin standard drug usedagainst HepG-2 cells



Sample conc. (µg/ml)	Viability %
500	4.37

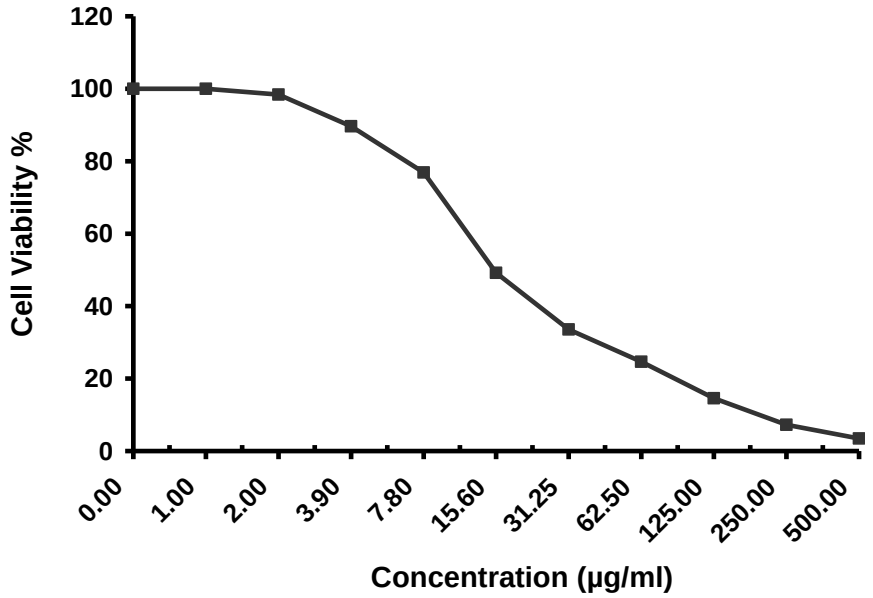
250	**9.56**
125	**18.94**
62.5	**32.75**
31.25	**49.07**
15.6	**73.18**
7.8	**88.41**
3.9	**96.23**
2	**99.08**
1	**100**
0	**100**

Fig. 7. Cell viability ofand cytotoxic evaluation of H_2L; (1) against HepG-2 cells



Sample conc. (µg/ml)	Viability %
500	**2.34**
250	**4.96**
125	**10.97**
62.5	**18.65**
31.25	**28.76**
15.6	**45.98**
7.8	**62.37**
3.9	**84.06**
2	**95.21**
1	**99.78**
0	**100**

Fig. 8.Cell viability and cytotoxic evaluation of Cu(II) complex ;(3) against HepG-2 cells



Sample conc. (µg/ml)	Viability %
500	3.45
250	7.27
125	14.58
62.5	24.67
31.25	33.54
15.6	49.21
7.8	76.89
3.9	89.63
2	98.40
1	100
0	100

Fig. 9. Cell viability ofand cytotoxic evaluation of Ag(I) complex ; (3) against HepG-2 cells

CONCLUSION

In the present study, new metal complexes of the ligand were prepared. Structural and spectroscopic properties revealed that, the ligand adopted a hexadentate ligand fashion; on the other hand, the metal complexes adopted a distorted octahedral geometry around metal ions. All the complexes were non-electrolytic in nature as suggested by molar conductance measurements. The ligand coordinated to the central metal ion forming six membered rings including the metal ions. The antitumor activities against (HepG-2cell line)of the ligand as well as its metal complexes were assessed that, the toxicity of both ligand and metal complexes was found to be concentration dependent, the cell viability decreased with increasing the concentration of complexes.

ACKNOWLEDGMENT

We thankthe laboratory of materials and renewable energy and the central laboratory, Faculty of Science, Menofia University for doing Physical measurements.

FUNDING

Theauthor(s)receivednofinancialsupportfor theresearch or authorship.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

References

1. El Saied, F. A., Abd-Elzaher, M. M., Abdou, S., Shakdofa, M. M., &Rasras, A. J. (2017). Synthesis, physicochemical studies and biological evaluation of unimetallic and heterobimetallic complexes of hexadentatedihydrazone ligands. *Beni-Suef University Journal of Basic and Applied Sciences*, *6*(1), 24-32.

2. Emam, S. M., Abdou, S., Ahmed, H. M., &Emad, E. A. (2017). Synthesis, structural characterization, electrochemical and biological studies on divalent metal chelates of a new ligand derived from pharmaceutical preservative, dehydroacetic acid, with 1, 4-diaminobenzene. *Arabian Journal of Chemistry*, *10*, S3816-S3825.

3. Abdou, S., Abd-El Wahed, M. M., & Abu-Setta, M. H. (2018). Metallo-bioactive compounds as potential novel anticancer therapy. *International Journal of Advances in Chemistry*, *4*(1), 17-37.

4. Capano, G., &Howlett, M. (2020). The knowns and unknowns of policy instrument analysis: Policy tools and the current research agenda on policy mixes. *Sage Open*, *10*(1), 2158244019900568.

5. Varshochi, M., Hasani, A., Derakhshanfar, S. J., Bayatmakoo, Z., Ghavghani, F. R., Poorshahverdi, P., &Nouri-Vaskeh, M. (2019). In Vitro Susceptibility Testing of Rifampin Against AcinetobacterBaumannii: Comparison of Disk Diffusion, Agar Dilution, and E-test. *Erciyes Medical Journal*, *41*(4), 364-369.

6. El-Tabl, A. S., Abd-El Wahed, M. M., Nofal, E. S., &Saad, A. A. Anticancer Agents; Synthesis, Structural Characterization of Novel Di-hydrazone Ether Complexes and Study their Anticancer Activities.

7.Bosch, F., &Rosich, L. (2008). The contributions of Paul Ehrlich to pharmacology: a tribute on the occasion of the centenary of his Nobel Prize. *Pharmacology*, *82*(3), 171-179.

8.Adeleke, A. A., Islam, M. S., Sanni, O., Mocktar, C., Zamisa, S. J., &Omondi, B. (2021). Aryl variation and anion effect on CT-DNA binding and in vitro biological studies of pyridinyl Ag (I) complexes. *Journal of Inorganic Biochemistry*, *214*, 111266.

9. Takeuchi, H., Baba, M., &Shigeta, S. (1991). An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. *Journal of virological methods*, *33*(1-2), 61-71.

10. Al-Mutabagani, L. A., Abdelrazek, F. M., Gomha, S. M., Hebishy, A. S., Abdelfattah, M. S., Hassan, S. M., ... &Elaasser, M. M. (2021). Synthesis and biological evaluation of thiazolyl-ethylidenehydrazino-thiazole derivatives: A novel heterocyclic system. *Applied Sciences*, *11*(19), 8908.

11. Resta, R., Biesecker, B. B., Bennett, R. L., Blum, S., Estabrooks Hahn, S., Strecker, M. N., & Williams, J. L. (2006). A new definition of genetic counseling: National Society of Genetic Counselors' task force report. *Journal of genetic counseling*, *15*, 77-83.

12. El-Tabl, A. S., Wahed, M. M. A. E., Wahba, M. A., El-assaly, S. A., &Saad, L. M. (1860). sugarhydrazone complexes; Synthesis, spectroscopic characterization and antitumor activity. *Journal of Advances in Chemistry, 9: 1837*.

13. Surati, K. R.andThaker, B. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy., 75, 235-242 (2010).

14. El-Tabl, A. S., Abd-Elwahed, M. M., & El-Saied, S. A. (2018). Design, Spectroscopic Characterization and Antitumor Action of Synthetic Metal Complexes of Novel BenzohydrazideOxime. *Chemistry and Chemical Sciences*, *8*, 1048-1072.

15. Abdou, S., Abd-El Wahed, M. M., Ashour, A. M., Abu-Setta, M. H., Hassanein, O. H., &Saad, A. A. (2019). Metallo-organic Copper (II) Complex in Nano Size as a New Smart Therapeutic Bomb for Hepatocellular Carcinoma. *Journal of Chemistry and Chemical Sciences*, *9*(1), 33-44.

16. Al Zoubi, W., Kandil, F., &Chebani, M. (2012). Synthesis of Macrocyclic Schiff Bases Based on Pyridine-2, 6-Dicarbohydrazide and their Use in Metal Cations Extraction, Organic Chem. *Current Res*, *1*(1).

17. Avaji, P. G., Patil, S. A., &Badami, P. S. (2008). Synthesis, spectral, thermal, solid state dc electrical conductivity and biological studies of Co (II), Ni (II) and Cu (II) complexes with 3-substituted-4-amino (indole-3-aldehydo)-5-mercapto-1, 2, 4-triazole Schiff bases. *Journal of Coordination Chemistry*, *61*(12), 1884-1896.

18. Opletalovà, V., Kalinowski, D. S., Vejsova, M., Kunes, J., Pour, M., Jampilek, J., ...& Richardson, D. R. (2008). Identification and characterization of thiosemicarbazones with antifungal and antitumor effects: cellular iron chelation mediating cytotoxic activity. *Chemical research in toxicology*, *21*(9), 1878-1889.

19. El-Tabl, A. S., Shakdofa, M. M., & El-Seidy, A. (2011). Synthesis, Characterization and ESR Studies of New Copper (II) Complexes of Vicinal Oxime Ligands. *Journal of the Korean Chemical Society*, *55*(4), 603-611.

20. Lima, L. M., Frattani, F. S., Dos Santos, J. L., Castro, H. C., Fraga, C. A. M., Zingali, R. B., & Barreiro, E. J. (2008). Synthesis and anti-platelet activity of novel arylsulfonate–acylhydrazone derivatives, designed as antithrombotic candidates. *European journal of medicinal chemistry*, *43*(2), 348-356.

21. Bernhardt, P. V., Mattsson, J., & Richardson, D. R. (2006). Complexes of cytotoxic chelators from the dipyridyl ketone isonicotinoylhydrazone (HPKIH) analogues. *Inorganic chemistry*, *45*(2), 752-760.

22. Ismail, H., Mirza, B., Haq, I. U., Shabbir, M., Akhter, Z., &Basharat, A. (2015). Synthesis, characterization, and pharmacological evaluation of selected aromatic amines. *Journal of Chemistry*, *2015*.

Corresponding author :**Mohammed H. H. Abu-Setta**

Department of Chemistry, Faculty of Science, El-Menoufia University, Shebin El-Kom, Egypt.

Email: **drmohammedhosnyabusetta@gmail.com**