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Effect of gamma irradiation on the antitumor activity of newly synthesized of copper (II) complexes of thiosemicarbazone derivatives

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ABSTRACT

Cancer is undoubtedly one of the main health concerns facing our society and one of the primary targets regarding medicinal chemistry. Thiosemicarbazones and their metal complexes are compounds that possess antitumor, antibacterial, antifungal and antiviral properties. In this study, a series of copper complexes of 2-anilinophenyisothiocyanate semicarbazone has been prepared and phyico-chemical characterized by elemental analysis, infrared spectroscopy (IR), Electronic spectra, magnetic moment, molar conductance measurements and X-ray diffraction pattern. The IR data before and after γ- irradiation revealed that the ligand behaves as neutral, monobasic bidentate coordination of copper ion via the carbonyl group or enolic oxygen group, NH group and thiol sulphr atom group in complex B_2. The molar conductance data revealed that the chelates are nonelectrolytes. From the spectra and magnetic moment data, the complexes were found to have square planar geometrical structures. The antitumor activities of these compounds were investigated against solid tumor induced in mice by injection of Ehrlich Ascites Carcinoma (EAC) cell line. Results revealed that tested compounds significantly reduced the tumor size. Gamma-irradiated compounds showed potent antitumor activities when compared to that of non-irradiated compounds. In addition, tested compounds exhibited stimulatory effect on the level of catalase and superoxide dismutase activities and glutathione content in liver of tumor bearing mice, while the level of lipid peroxidation was significantly reduced. It is concluded that thiosemicarbazone complexes and lignad are considered as promising anticancer drugs candidate. Moreover, the γ-irradiation evokes the antitumor activity of the tested compounds.

Key words: Antitumor activity, Antioxidant enzymes, γ-irradiation Thiosemicarbazone complexes.

INTRODUCTION

Cancer continues to be a devastating disease that affects all societies. It is multifactorial disease that develops over a long period of time and progresses through different stages [1]. Chemistry plays a critical role in research on cancer diagnosis, prevention, and treatment.

The synthesis and structural investigations of thiosemicarbazone and their metal complexes are of considerable centre of attention because of their potentially beneficial pharmacological properties and a wide variation in their modes of bonding and stereochemistry [2]. Thiosemicarbazones have emerged as an important sulfur containing ligands in the last two decades. They have been found to possess a variety of physiological properties including antibacterial, antifungal, antineoplastic, antiulcer, antiviral and enzymatic inhibition [3]. In addition, they have been screened for their medical properties because they possess some cytotoxic effect. They also stabilize uncommon oxidation states; generate a different coordination number in transition metal complexes in order to participate in various redox reactions [4]. As a result of the significant pharmacological effects of thiosemicarbazone derivatives, there is an increasing interest in synthesizing and biotesting of these derivatives [5]. The metal ion chelating activity

of thiosemicarbazone derivatives has been reported [6]. Some of substituted benzaldehyde thiosemicdarbazones are described along with their anticancer and antifungal activity [7-12].

The current research proposal aims to design and synthesis new thiosemicarbazones complexes with bivalent copper. Also, to study the antitumor activities of these complexes before and after gamma irradiation against solid tumor induced in experimental animals by using Ehrlich ascitis carcinoma

EXPERIMENTAL SECTION

2.1. Preparation of the ligand

The ligand 2-anilino-phenyl isothiocyanate (H_2L) was prepared by mixing equimolar amount of desired hydrazide (0.01mol) in 10ml the phenyl isothiocyanate (0.01mol) in 10 ml of absolute ethanol [13,14]. The reaction mixture was refluxed for 3 hrs. The reaction mixture was recrystalized several times from ethanol.

2.2. Preparation of the metal complexes

Copper (II) complexes of the ligand were prepared by adding stoichiometric amount of the copper (II) acetate, bromide and nitrate in EtOH to 2-phenylaminoacetyl-N-phenyl hydrazine carbthioamide (H_2L) in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at 60^oC for 5hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10}.

2.3. γ-Irradiation of Complexes

Energetic γ-irradiation exposure was undertaken using a γ-Co^{60} unit at Atomic Energy Establishment, Egypt; at an accumulated dose of 1 Mega rad in air.

2.4. Characterization of Complexes

Elemental analyses (C, H and Cl) were performed in the Microanalytical Unit, Cairo University, Egypt. IR absorption spectra before and after γ-irradiation were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometer. ^1H NMR spectra were recorded in d^6-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. Magnetic susceptibilities were measured at 27^oC using a modified Gouy method with Johnson Mattey balance.

X-ray powder diffraction before and after γ-irradiation was measured using a Schimadzu XD-3 diffractometer (Japan) using CuKα radiation and Ni- Filter.

2.5. Preparation of compounds solutions

Tested complexes were dissolved in DMSO/H_2O (7:3) to give a final concentration of 1mM and kept at 4^oC.

2.6. Induction of solid tumor in experimental animals

A model of solid tumor was induced in female Swiss albino mice, weighing 18 to 20 g, by injecting of $1x10^6$ Ehrlich Ascetic Carcinoma (EAC) cell line subcutaneously into the right thigh of the lower limb of the mice [15]. A total of 60 mice were injected with EAC and divided into 10 groups, 6 animals per group. Animals of groups 1 to 8 were I.P. injected with 0.1 mM of tested compounds daily for 15 days after tumor implantation. Animals of group 9 were I.P. injected with dissolving solution (DMSO/H_2O) daily for 3 days after tumor implantation and served as sham group. Group 10 served as tumor control group. In addition to normal control group

2.7. Determination of solid tumor size

Tumor size was estimated according to the method of Geran et al. [16]. The resultant solid tumor was considered to be prelate ellipsoid with one long axis and two short axes. The two short axes were measured with vernier caliper. The tumor size was calculated using the following formula: Size = Length (cm) × $width^2$ (cm)/2

2.8. Preparation of liver homogenate

A tissue sample from a known portion of the liver was accurately weighed and homogenized (Potter-Elvehjem) in a 10-fold volume of ice-cold (20mM) tris-HCl buffer pH 7.4. The homogenates were divided into aliquots kept at -20°C for future measurements.

2.9. Estimation of hepatic oxidative status

Level of hepatic lipid peroxidation was estimated colorimetrically by measuring malondialdehyde (MDA) using the method of Ohkawa et al. [17] and by following the manufacturer's procedure (Biodiagnostics, Egypt). Hepatic catalase activity was estimated by the method of Aebi [18] and according to the manufacturer's procedure (Biodiagnostics, Egypt). Superoxide dismutase activity in liver homogenate was estimated according to the procedure of Nishikimi et al. [19] and by following the manufacturer's procedure (Biodiagnostics, Egypt). Level of reduced glutathione in liver homogenate was estimated by using the method mentioned by Beutler et al. [20].

2.10. Lymphoproliferation assay

Polymorphonuclear cells (PMNC) were isolated from spleen according to the method described by Om Ali et al. [21]. Lymphoproliferation assay was conducted according to the method of [22]. Briefly, cells were suspended in complete RPMI media supplemented with 5% human AB serum and cultured in 96-well round-bottom plates at $3x10^5$ per well. Cells were stimulated with phytohemagglutinin (PHA; Sigma, St Louis, MO). Cells were incubated in 5% CO_2 incubator at 37^{o}C for 72 hr. Eighteen hours before the end of incubation, 20 mM of BrdU solution (Pharmingen, San Diego, CA) was added to the cells. A BrdU proliferation kit (5-bromo-20-deoxy-uridine (BrdU) Labeling and Detection Kit III, Roche) was used according to the manufacturer's instructions and the reaction was quantified by reading in a microplate photometer at a test wavelength of 405 nm. Absorbances (optical density, OD) were determined. The proliferation index to PHA was calculated by dividing OD of PHA-stimulated cells by OD of unstimulated cells.

2.11. Statistical analysis

All data were expressed as Mean values ± SD. *t*-test was used for comparison between groups. *P* values less than 0.05 were regarded as statistically significance.

RESULTS AND DISCUSSION

3.1. ^{1}H NMR Spectra

The ligand of 2-[aniline phenyl isothiocyanate (H_2L) was confirmed by elemental analysis (Table 1), infrared (Table 2) and ^{1}HNMR spectroscopy. The ^{1}HNMR spectrum of H_2L in chloroform, which would produce more information concerning intra molecular hydrogen bonding [23], was not possible due to their low solubility, so they have recorded as d^6-DMSO solution. The resonance for the amido N(4)H attached to phenyl group is located in the 9.7 ppm spectral region, including that hydrogen bonding with d^6-DMSO does not occur, in agreement with previous results [24,25] . The ^{1}H signals due to the hydrazido group for N(1)H occurs at 10.0 ppm indicating the involvement of these hydrogens through intramolecular hydrogen bonding with the carbonyl oxygen of -C-NH group. The other hydrazido group N(2)H appears at 9.5 ppm. A singlet at 3.8 ppm and multiplet at 7.6 ppm are attributed to the protons CH_2 and aryl groups respectively.

The stiochiometries of the isolated complexes of thiosemicarbazide are shown in table (1). Copper complex of the neutral ligand are formed with nitrate. Copper complex of the monobasic ligand are formed with acetate and bromide. The reaction of the ligand with different salts of Cu(II) acetate, bromide and nitrate produce complexes of the general formulae. $Cu(H_2L)(OAc).H_2O$, $Cu(H_2L)Br.H_2O$ and $Cu(H_2L)(NO_3)_2.3H_2O$. These air stable complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities in DMF (10^{-3}M) solution (Table 1) show that the complexes are non-electrolytes, indicating coordination of the ligand anions [26].

Table 1: Elemental analyses and molar conductivities of 2-[anilinophenyl isothiocyanate] ligand (H_2L, $C_{15}H_{16}N_4OS$) ligand and copper complexes

	Complex	Colour	Mol. Wt.	Found (Calc)%		
				C	H	^M
B	H_2L	Pale brown	300	60.2(60.1)	5.6(5.3)	-
B_1	$Cu(H_2L)(OAc).H_2O$	Dark green	439	46.6(46.5)	4.5(4.5)	19
B_2	$Cu(H_2L)Br.H_2O$	Green	460	38.7(38.5)	3.5(3.6)	28
B_3	$Cu(H_2L)(NO_3)_2.3H_2O$	Dark green	841	42.6(42.8)	4.3(4.5)	20

Where: B=Before γ-irradiation, M=molar conductivity ohm^{-1} cm^2 mol^{-1} in 10^{-3}M DMF

3.2. The infrared spectra of the ligand and copper complexes

The most important assignments of free ligand are shown in table (2). The existence of two strong bands at 1677 and 747 cm^{-1} assigned to ν(C=O) and ν(C=S) vibrations. The absence of any bands above 3500 cm^{-1} or the region 2600-2550 cm^{-1} due to the bands of ν(OH) and ν(SH), respectively; and the lack of any signals in the NMR spectra of the free ligands due to the protons of the -OH or -SH, confirms that the ligand exist entirely in the keto form. The three bands at 3340, 3290 and 3250 cm^{-1} in the spectra of the ligand are assigned to ν(N4-H), ν(N2-H) and ν(N1-H), while

the ν(N-N) [27] vibration is observed at 925 cm^{-1} as medium sharp band. Also, the bands at 1500, 1440 and 1280 cm^{-1} may be due to ν(N-C=S) [28]. These bands are assigned as coupled modes consisting principally of ν(NH) and ν(CN).

The IR spectra of the complexes $Cu(H_2L)(OAc).H_2O$ and $Cu(HL)Br.H_2O$ show that the ligand of (H_2L) behaves as monobasic bidentate ligand coordinating via the (N-2H) in two complexes, enolic oxygen in the first and thiolsulfur atom in the second complex. This mode of chelating show that the disappearance of the ν(C=O) with the appearance of new band at 1580 cm^{-1} and 1180 cm^{-1} assigned to ν(O-C=N) [29] and ν(C-O) [30], ν(N-N) shifts to higher wave number at 1050 cm^{-1} in the first complex. On the other hand, the disappearance of ν(C=S) with the appearance of new bands due to ν(S-C=N) [31] and ν(C-S) in the second complex [32]. While the IR spectra of the complex $Cu(H_2L)(NO_3)_2.3H_2O$ shows that the ligand behaves as neutral bidentate, coordinating via the carbonyl oxygen ν(C=O) and (N-2H) group. The mode of complexation is suggested by the shift of both ν(C=O) and (N-2H) group to lower wave number.

The new bands appeared at 450-405 cm^{-1} and 502-515 cm^{-1} assigned to ν(Cu-O) andν(Cu-N) [33,34], respectively. In complex $Cu(H_2L)(OAc).H_2O$ appears two bands at 1560 and 1440 cm^{-1} assigned to ν_a(COO) and ν_s(COO), respectively [35].

While in complex $Cu(H_2L)(NO_3)_2.3H_2O$, two strong bands were appeared at 1285 and 1385 cm^{-1} corresponding to ν_1 and ν_4 modes of monodentate nitrate group [36]. The absence of coordinated water molecules from the hydrated complexes $Cu(H_2L)(OAc)$. H_2O and $Cu(H_2L)(NO_3)_2.3H_2O$ are confirmed by the absence of rocking, twisting and wagging vibrational modes which are normally activated at 970-930 cm^{-1} and 660-600 cm^{-1}, as well as the presence of medium and broad band at 3430-3445 cm^{-1} indicating that the water in these complexes are lattice rather than coordinated [37]. While complex $Cu(H_2L)Br.H_2O$ appeared coordinated water [38].

A study and comparison of the IR spectra before and after γ-irradiation of 2-[phenyl amino acetyl-N-phenyl hydrazine carbothioamide ligand (H_2L) and Cu(II) complexes imply that the ligand is bidentate in nature, with carbonyl oxygen and NH, as two coordinates sites. The IR spectrum of the free ligand shows five bands at 3340, 3290, 3250, 1677 and 747 cm^{-1} assigned to ν(N4-H), ν(N2-H), ν(N1-H), ν(C=O) and ν(C=S); respectively. General feature of the bands of ligand (**A**) after γ-irradiation are observed. As a result of γ-irradiation broadening of complex (**A**) after γ-irradiation are observed. Results display that thus complex ($\mathbf{A_1}$) is the most γ-radiation resistant material. Since complex ($\mathbf{A_2}$) undergoes no noticeable radiation damage, thus this material is the most stable one. As general feature in common, IR the bands of N(4)H, N(1)H and some bands disappear as a result of γ-irradiation damage. For complex ($\mathbf{A_3}$) the band of N(4)H weaken by γ-irradiation. This is due to partial damage of material.

Table 2: Infrared spectral bands (cm^{-1}) for 2-[anilino phenyl isothiocyanate] ligand (H_2L,$C_{15}H_{16}N_4OS$) ligand and copper (II) complexes

No.	Compound	ν(N4-H)	ν (N2-H)	ν (N1-H)	ν (C=O)	ν (C=S)	ν (Cu-N)	ν (Cu-O)
B	H_2L	3340(m)	3290(w)	3250(w)	1677(s)	747(s)	-	-
A	H_2L*	3448(br)	3295(w)	3290 (w)	1679(s)	748(s)	510(s)	450(m)
B1	$Cu(H_2L)(OAc).H_2O$	3450(w)	3256(w)	-	-	692(m)	515(m)	410(w)
A1	$Cu(H_2L)(OAc).H_2O$*	3450(m)	3260(w)	-	-	692(m)	510(w)	405(w)
B2	Cu (H_2L)Br.H_2O)	3428(br))	3288(m)	3185 (m)	1602(m)	695(m)	504(m)	432(m)
A2	Cu (H_2L)Br.H_2O*	3433(br)	3289(m)	3184 (m)	1602(m)	755(s)	502(m)	430(w)
B3	$Cu(H_2L)(NO_3)_2.3H_2O$	3436(br)	3292(s)	3203(m)	1600(s)	755(s)	506(m)	420(w)
A3	$Cu(H_2L)(NO_3)_2.3H_2O$*	3445(w)	3292(s)	3205(m)	1600(s)	756(s)	502(m)	410(w)

Where: B=Before γ-irradiation, A= After γ-irradiation (*)

3.3. Electronic absorption spectroscopy

The electronic spectral bands of the Cu(II) complexes as well as the spectra of the ligand in solution DMF are shown in table (3). The π - π* transition band is observed at 33000 cm^{-1} for H_2L. Compared to the free ligand, in the Cu(II) complexes, this band is shifted to longer wave length (Red shift) which is consistent with an increase in the degree of Pi- cloud conjugation [39]. The electronic spectra of Cu(II) complex display one broad band at the 15560 cm^1 range due to the $^2B_{2g} \rightarrow {}^2A_{2g}$ transition with a square planar geometry [40]. The band at 15950, 15960 cm^{-1}, assigned to charge transfer band. The magnetic moment value of the Cu(II) complexes (Table 1), lie in the range observed for the Cu(II) complexes with one unpaired spin (1.73 B.M).

Table 3: Solution DMF electronic spectra (Cm^{-1}) of H_2L and their Cu(II) complexes.

Compounds	Intraligand and charge transfer		d-d bands	μ_{eff} (B.M)
H_2L	33000	-	-	-
$Cu(H_2L)(OAc).H_2O$	32250	25710	15950	1.8
$Cu(H_2L)Br.H_2O$	32020	25310	15560	1.5
$Cu(H_2L)(NO_3)_2.3H_2O$	32480	25575	15960	1.82

3.5. X-ray diffraction patterns

The X-ray diffraction patterns of the ligand before and after γ-irradiation show that: (a) The identity of the material is still reserved; (b) Displacement of longer interplanar spacing, (c) Some peaks newly appeared.

Complex $Cu(H_2L)(OAc).H_2O$ displays that new peaks appear and some peaks weaken and displaced to shorter interplanar spacings [41]. Thus, these due to partial γ-irradiation damage [42]. While in the complex $[Cu(H_2L)Br.H_2O]$ show that the X-ray diffraction pattern before and after γ-irradiation appear peaks as in figure (2). Also, complex $[Cu(H_2L)(NO_3)_2.3H_2O]$ showed an X- ray diffraction pattern before and after γ-irradiation that appeared as peaks (Figure1). This material show γ-irradiation induced decreased degree of crystallinity. This is a result of partial γ-irradiation damage.

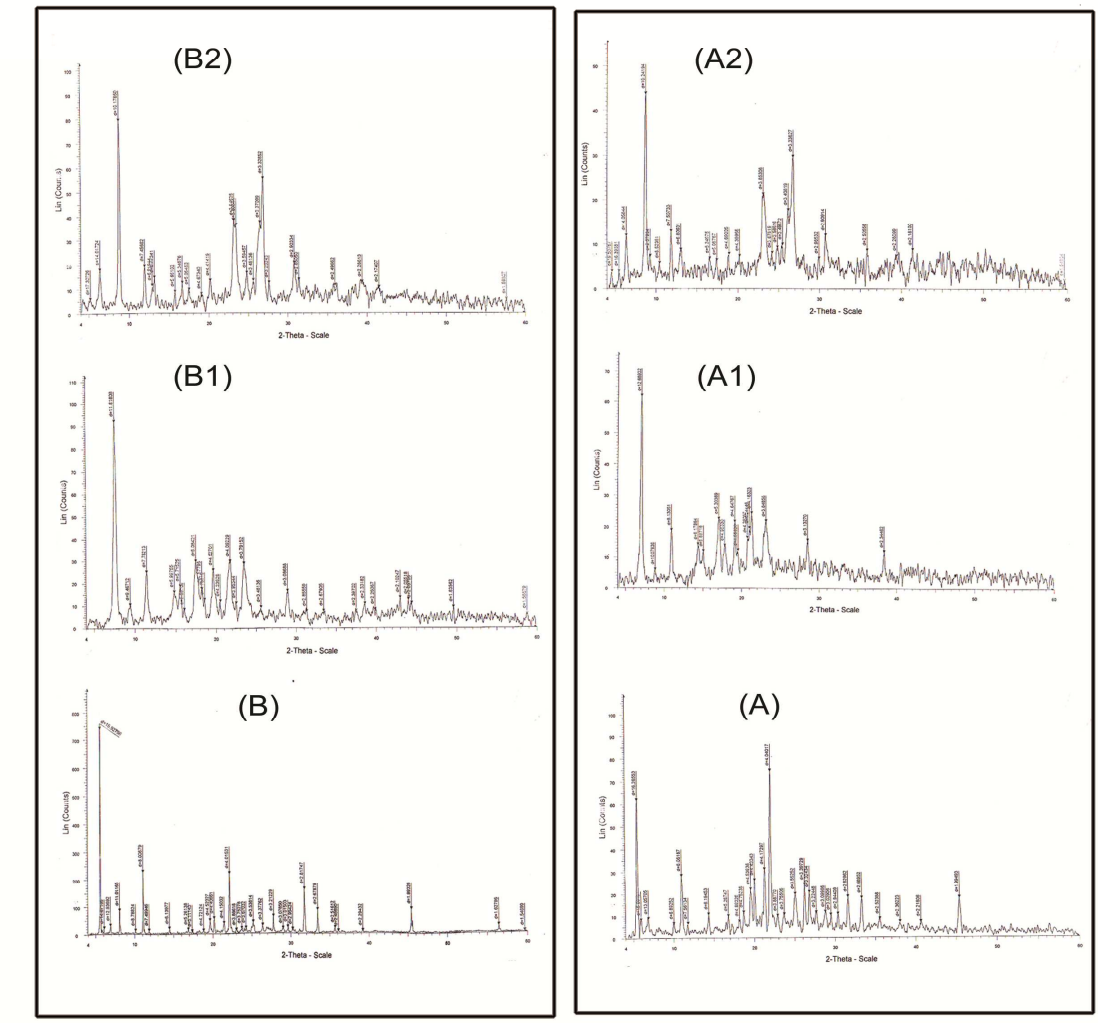
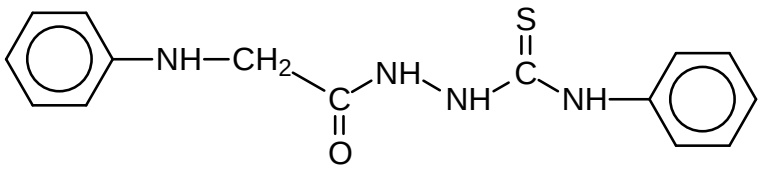
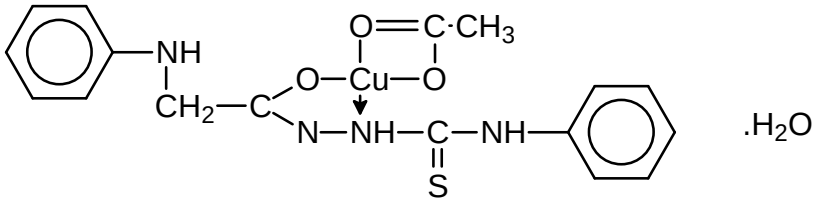


Fig (1): X-Ray Diffraction Pattern Of Ligand and Copper (II) Complexes

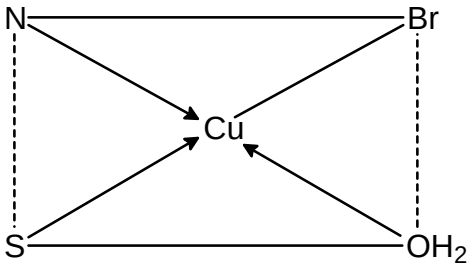


H_2L



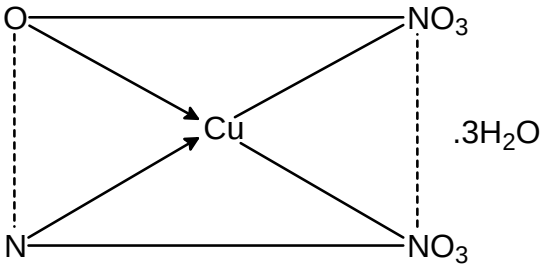
$Cu(HL)(OAc).H_2O$

B_1



$Cu(HL)Br(H_2O)$

B_2



$Cu(H_2L)_2(NO_3)_2.3H_2O$

B_3

Scheme (1): The chemical structure of ligand and copper(II) complexes

3.7 Effect of copper (II) complexes of thiosemicarbazone on tumor size

Results show that the treatment of tumor bearing mice with Cu(II) complexes of thiosemicarbazone resulted in a varied inhibitory effect on the tumor growth. As shown in table (4), significant decreases in solid tumor size were observed after treatment tumor bearing mice with non-irradiated complexes for 15 days as compared to that of tumor bearing mice group. Yousof et al. [43] reported that thiosemicarbazide complexes exhibited antitumor activity against EAC in mice. In contrast to non-irradiated complexes, γ-irradiated complexes exhibited more potent inhibitory action on the tumor size.

Table 4: Effect of Cu(II) complexes of thiosemicarbazone before and after irradiation on tumor size

		Tumor size
Tumor bearing mice		1.8±0.2
Tumor + DMSO/H_2O		1.7±0.4
Tumor bearing mice	**+ B**	1.1±0.3 [b]
	+A	0.7±0.2 [b,c]
	+ B1	1.8±0.7 [b]
	+A1	0.6±0.1 [b,c]
	+ B2	1.1±0.4 [b]
	+A2	0.4±0.1 [b,c]
	+ B3	1.3±0.3 [b]
	+A3	0.4±0.1 [b,c]

(b) Significant when compared with tumor bearing mice group.
(c) Significant change when compared with its non-irradiated complex.

3.8. Effect of copper(II) complexes of thiosemicarbazone on the oxidative status

Table (5) demonstrates the effect of γ–irradiated and non-irradiated thiosemicarbazone complexes on some oxidative status parameters in liver of mice bearing malignant tumors. Results showed that all complexes exhibited antioxidative activities where, the levels of lipid peroxidation, measured as MDA, were markedly decreased (p<0.001) as compared to that of the tumor group. As shown in table (7), the γ–irradiated complexes exhibited higher antioxidative activities as compared to those of the non-irradiated complexes. Meanwhile, results indicate that the hepatic activities of catalase and SOD in tumor bearing mice were significantly reduced when compared to those of normal mice. On the other hand, treatment of tumor bearing mice with Cu(II) complexes restored the activities of SOD and catalase as compare those of the untreated mice. Previous study revealed the ability of thiosemicarbazone complexes to induce the activities of SOD and catalase as well as the level of GSH in mice [44]. In contrast to the effect of non-irradiated complexes, the γ–irradiated complexes exhibited potent stimulatory actions on the hepatic activities of catalase and SOD.

The level of hepatic GSH was significantly decreased in tumor bearing mice as compared to that of the control group (p<0.05). The treatment of tumor bearing mice with different thiosemicarbazone complexes resulted in amelioration in the level of hepatic GSH. Results also indicated that γ–irradiated complexes exhibited high stimulatory effect on the level of hepatic GSH as compared to non-irradiated complexes.

Table 5: Effect of copper complexes of thiosemicarbazones before and after irradiation on hepatic oxidative status

		CAT (U/g tissue)	SOD (U/g tissue)	GSH (mmol/g tissue)	MDA (nmol/g tissue)
Normal Control (NC)		4.2±0.5	8.5±0.3	7.8±0.4	0.4±0.04
Tumor bearing mice		1.2±0.2 [a]	1.9±0.2 [a]	2.4±0.2 [a]	3.4±0.1 [a]
Tumor + DMSO/H_2O		1.3±0.5 [a]	1.5±0.3 [a]	2.6±0.4 [a]	3.6±0.4 [a]
Tumor bearing mice	**+ B**	1.8±0.6 [b]	3.5±0.2 [b]	3.1±0.4 [b]	2.6±0.3 [b]
	+A	2.7±0.4 [b,c]	4.9±0.4 [b,c]	5.4±0.4 [b,c]	1.7±0.2 [b,c]
	+ B1	2.8±0.6 [b]	5.7±0.5 [b]	4.3±0.2 [b]	1.9±0.1 [b]
	+A1	3.6±0.4 [b,c]	6.5±0.1 [b,c]	6.1±0.2 [b,c]	0.85±0.1 [b,c]
	+ B2	2.1±0.4 [b]	4.8±1.3 [b]	4.2±0.2 [b]	1. 9±0.1 [b]
	+A2	3.6±0.4 [b,c]	6.6±0.3 [b,c]	6.4±0.4 [b,c]	0.75±0.2 [b,c]
	+ B3	2.3±0.3 [b]	5.4±0. 1 [b]	3.3±0.4 [b]	2.1±0.2 [b]
	+A3	4.6±0.4 [b,c]	7.2±0.3 [b,c]	7.4±0.4 [b,c]	0.6±0.1 [b,c]

(a) Significant change when compared with normal control group (NC).
(b) Significant change when compared with tumor bearing mice group.
(c) Significant change when compared with its non-irradiated complex.

3.9. Effect of copper(II) complexes of thiosemicarbazone on lymphocytes proliferation

Effects of Cu(II) complexes of thiosemicarbazone on the lymphoproliferative response to phytohemagglutinine (PHA) were investigated and presented in figure (2). Results indicated that induction of solid tumor in albino mice resulted in a significant reduction in lymphocytes' response to PHA when compared to that of the normal mice. Treatment of tumor bearing mice with non-irradiated and γ–irradiated Cu (II) thiosemicarbazone complexes led to non-significant increases in the lymphoproliferative response to PHA in vitro. Also, results indicated that there are no significant changes between non-irradiated and γ–irradiated complexes as regard to their effects on the lymphoproliferative response to PHA.

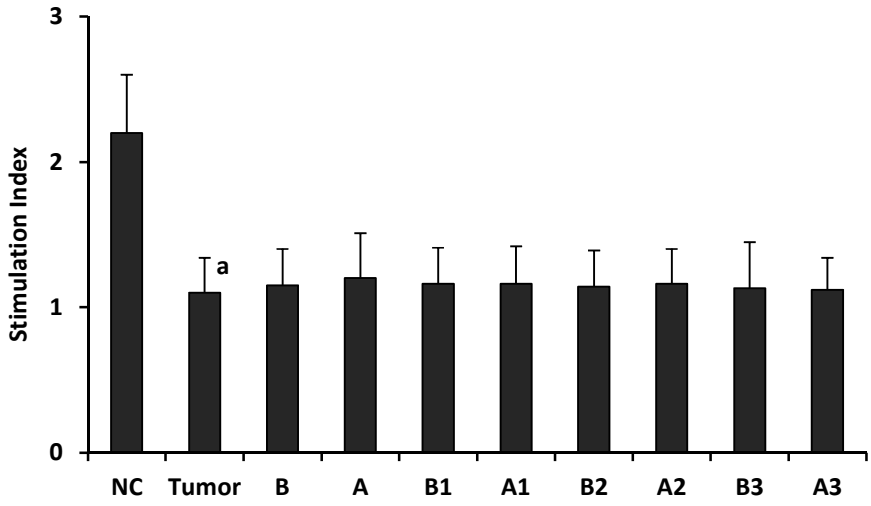


Figure 2: Lymphoproliferative response to PHA

(a) Significant change as compared to the normal controls (NC)

CONCLUSION

A series of copper complexes of thiosemicarbazones were prepared and their plausible structures were supported by IR,^{1}H NMR, X-ray diffraction and Electronic spectral data. The ligand and their complexes were irradiated with γ-radiation. All compounds were tested for their anticancer activity against solid tumor bearing mice induced by Ehrlich Ascitis Carcinoma (EAC) cell line before and after γ-irradiation. Copper complexes exhibited antioxidant activities evidenced by significant increasing in the level of antioxidant enzymes, catalase and superoxide dismutase, and reducing the level of lipid peroxidation in the liver of mice with solid tumor. As compared to their ligand, complexes demonstrated potent antioxidant activities indicating the ability of metal ion to induce the antioxidant potential activity. Meanwhile, the γ-irradiation enhanced the antioxidant activity of tested compounds. On the other hand, the tested compounds failed to induce the lymphocyte proliferation in vitro. These findings revealed that the tested compounds exert their antitumor activity through induction of the cellular antioxidant capacity, not via immunological pathway. Also, the γ-radiation evokes the antitumor activity of tested compounds.

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REFERENCES

[1] C Jeong; A Bode; A Pugliese; Y Cho; H Kim; J Shim; Y Jeon; Li H Jiang; Z Dong; *J. Cancer Res*., **2009**, 1, 69 (13), 5584-91.
[2] H Beraldo; D Gambino; *Mini-Rev Med Chem*., **2004**, 4, 159–165.
[3] S Pandeya; S Smitha; M Jyoti; S Sridhar; *Acta Pharm*., **2005**, 55, 27–46.
[4] R El-Shazly; G Al-Hazmi; S Ghazy; M El-Shahawi; A El-Asmy; *Mol. Biomol. Spect*., **2005**, 61,243–252.
[5] H Pervez; M Iqbal; M Tahir; F Nasim; I Choudhary; K Khan; *J Enz Inh & Med Chem*., **2008**, 23(6), 848–854.
[6] P Bernhardt; P Sharpe; M Islam; D Lovejoy; D Kalinowski; D Richardson; *J Med Chem*., **2009**, 52(2), 407-15.
[7] J Jampilek; R Musiol; J Finster; M Pesko; J Carroll; K Karlova; M Vejsova; J Mahony; A Coffey; J Dohnal; J Polanski; *Molecules*, **2009**, 14, 4246-4265.
[8] J Jampilek; R Musiol; M Pesko; K Kralova; M Vegsova; J Carroll; A Coffey; J Finster; D Tabak; H Niebala ; *Molecules*, **2009**,14,1145-1159.
[9] R Musiol; M Serda; S Hensel-Bielowka; J Polanski; *Curr. Med.Chem*., **2010**, 17, 1960- 1973.
[10] R Musiol; J Jamphlek; L Buchta Silva; H Niedballa; B Poeszwa; A Palka; K Magerz-Manieck; B Oleksyn; J S Polanski; *Bioorg. Med. Chem*., **2006**, 14, 3592- 4598.
[11] R Musiol; JE Jampilek Nycz; M Pesko; K Carrol Kralova; M Vejsova; O Mahony; J Coffey; A Mrozek; J Polanski; *Molecules*, **2010** ,15, 288 -304.
[12] M Serda; D S Kalinowski; A Mrozek- Wilekiewiewicz; R Musiol; A Szurko; N Ratuszna Pantarat; Z Kovacevic; A M Merlot; D R Richarson; J Polanski; Bioorg. Med. Chem. Lett., **2012**, 22, 552.
[13] A M Aal; W A El-Sayed; AHA Aleem; E SH El-Ashry*; J Pharamazie*, **2003**, 58 (11), 788-792.

[14] S P Hirmath; J S Biradar; S M Kudari; *Indian J. Chem. Soc*., **1984,** 61 (1) 746.
[16] R I Geran; H M Greenberg; M McDonald; B J Abbott; *Cancer. Chemoth. Rep*. **1972,** 33, 1–17.
[17] H Ohkawa; A Wakatsuki; C Kaneada; *Anal. Biochem*., **1982,** 95, 351-358.
[18] H Aebi; *Meth. Enzmol*., **1984**, 105, 121 - 126.
[19] M Nishikimi; N A Rao; K Yog; *Bioch. Biophys. Res. Commun*., **1972**, 46, 849-851.
[20] E Beutler; O Duron; M B Kelly; *J. Lab. Clin. Med*., **1963**, 61, 882 - 885.
[21] Y Om-Ali El-khawaga; A Tarek Salem; F Mohamed Elshal; *Clinica Chimica Acta*, **2004**, 338 (1-2), 11-6.
[22] J A Sakai; M Nagai; M B Brennan; C A Mora; S Jacobson; *Blood*, **2001**, 98,1506-1511.
[23] D X West; A M Stark; G A Bain; A E Liberate; *Transition Met*., **1996**, 21, 289.
[24] D X West; M M Salberg; G A Bain; A E Liberate., *Transition Met.*, *Chem*., **1977**, 22, , 180.
[25] D X West; Y H Yang; T L Klein; K I Goldbery; A E Liberate; J Valdez Martinez; S Hemdrdez; *Polyhedron*, **1995**, 14, 305 .
[26] H Youhong; I Zienglu; Z Yulan; W Shozu; *Syn. React. Inorg. Met. Org. Chem*., **1995**, 25, 349.
[27] D N Sathyanarayana; D Nicholls; *Spectro Chim.Acta*, **1978**, 34A, 263.
[28] C N R Rao; R V Enkataraghavan; *Spectrochim. Acta*, **1962**, 18, 541.
[29] N B Colthup; L Daly; S E Wiberley; Introduction to Infrared and Raman Spectroscopy, Academic press, New York, **1975**,30, 327.
[30] N S Biradar; B R Patil; V H Kulkarni*; J. Inorg. Nucl. Chem*., **1975**, 37, 1901.
[31] R C Aggarwal; N K Singh; L Prasad; *Indian. J. Chem*., **1975**, 14, 325.
[32] D X West; LN Panel; *Transition Met. Chem*., **1989**,14, 457
[33] N T Akinchan; D X West; Y H Yang; M M Salberg; T L Kelin; *Transition Met. Chem*., **1995**, 70, 48.
[34] El-Sawaf A K; West D X; El-Saied F A; El-Bahnasawy R M; *Transition Met.*, *Chem.*, **1998**, 23, 565.
[35] G M Abou; KM El-Reash, R E Ibrahim; El-Shadily; M M Bekheit; *Acta Chem*., Hung, **1990**, 127, 21.
[36] A C Faberge; G C Fenhini; G Peyronel; *Transition Met. Chem*., **1978**, 3 , 363.
[37] S V Tatwawadi; A P Sing; K K Narang; *J. Sci., Res., Banners Hindu Univ*., **1980**, 3, 143.
[38] M Teito; J N Garth; V B Rama; *Inorg. Nucl., Chem*., **1987**,42, 821.
[39] A W Ainscough; R A Plowman; *Aust. J. Chem.*, **1970**, 23, 699.
[40] M Pulauniandavar; C Natajon; *Aust. J.Chem*., **1985**, 37, 337.
[41] M A Sekkina; El-Sayed El-Shereafy; A Mashaly; M El-Ashary; *J. Radioanal. Nucl. Chem*., **1998**, 237 (2) , 113- 119.
[42] T Sherertz; K Wallner; G Merrick; W Cavanagh; W Butler; D Reed; L True; *Cancer*, **2004**, *10*, 301.
[43] Yousef T A; F Badria; S E Ghazy; O A El-Gammal; G M Abu El-Reash; *Int J Med. Med Sci*., **2011**, 3(2), 37-46.
[44] D T Nguyena; T H Lea; T T Bui; *Eur J Med Chem*., **2013**, 60 , 199-207.