THE EFFECT OF Hg-POLLUTED DRINKING WATER ON THE LEVEL OF HORMONES IN MALE RATS AND THE POTENTIAL THERAPEUTIC ACTION OF THE THIOSEMICARBAZONE AND ANTIPYRINE LIGANDS AND THEIR COMPLEXES

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Novel Hg(II) complexes of d^{10} configuration have been synthesized and characterized by elemental analysis, IR, UV-VIS spectra and thermal analysis. The analytical and spectral data reveal that the ligands (H_2L^1- HL^3) behave as neutral or mono basic bidentate in nature, coordinating via C=O or C-O and NH or C=N. The harmful effect of Hg-polluted drinking water on male sex hormones, kidney function as well as oxidative status biomarkers of male rats was investigated. Meanwhile, the potential protective effects of synthesized complexes and their ligands were studied. Results showed that orally administration of $HgCl_2$ for 30 days caused a significant disruption of male sex hormones and kidney function. Further, the level of lipid peroxidation was elevated and activities of antioxidant enzymes were markedly declined in kidney and testes homogenates. The co-administration of $HgCl_2$ with antipyrine and thiosemicarbazone as well as their complexes for four weeks led to amelioration in the kidney and testes functions as the levels of male sex hormones and kidney function tests were recovered. Meanwhile, these compounds showed ameliorative effects on the oxidative status of rats. It can be concluded that drinking of Hg-polluted water induces oxidative stress pathways that may lead to deterioration in kidney and testes function. The findings also suggest the curative action of antipyrine and thiosemicarbazone as well as their complexes since they exhibited the ability to resist the harmful action of mercury and to protect the organs from the action of free radicals.

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INTRODUCTION

Mercury is one of the oldest chemical elements used in human applications. It is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders.[1] Its wide industry-related effects on human and animal biosystems have been well documented[2] and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food.[3]

Inorganic mercury is widely used in certain types of batteries and continues to be an essential component of fluorescent light bulbs.[4] Inorganic mercury is the most common form that is present in drinking water but is not considered to be very harmful to human health, in terms of the levels found in drinking water.[5]

The endocrine system is one of the three important integrating and regulatory systems in the human body. The other two are the nervous and immune systems. The major endocrine glands include the hypothalamus, pituitary, thyroid, parathyroid, adrenal gland, pancreas, and gonads (ovary and testis). Hormones are natural secretory products of the endocrine glands and travel via the blood to exert their effects at distant target tissues or organs by binding to specific cell surfaces or nuclear receptors.

Many researchers reported that mercury promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxide. ROS enhance the subsequent iron- and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical.[6] These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell. Inorganic mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase and glutathione peroxidise.[7]

Thiosemicarbazone is an emerging moiety with wide spectrum of biological activity and having sound scope in research and developing process in pharmaceutical and medicinal chemistry.[8-11] Thiosemicarbazones are of considerable interest because of their chemistry and potentially beneficial biological activity, such as antibacterial antifungal,[12] antiviral,[13] antiamoebic,[14] antimalarial[15,16] and antitumor activity.[17] The biological activities of thiosemicarbazones are considered to be due to their ability to form chelates with metals. Biological activities of metal complexes differ from those of either ligands or the metal ions and increase and/or decreased biological activities are reported for several transition metal complexes. Thiosemicarbazone are versatile compounds, two structural isomers (E and Z forms) are possible and they can co-ordinate to the metal either as a neutral ligand or as a

deprotonated ligand through the N, S atoms. Thiosemicarbazones have been frequently employed for the quantitative determination of inorganic ions.[18]

Pyrazoles is a five-membered heterocyclic system.[19] Many synthetic compounds containing pyrazole moiety are active in the field of medicinal chemistry.[20] One of the pyrazole derivatives, 4-aminoantipyrine has played an important role in inorganic chemistry; it forms stable complexes with many transition metal ions. 4-aminoantipyrene and its complexes have found applications in analytical, biological and clinical areas.[21,22] Antipyrine derivatives are used as anti-inflammatory[23,24] and chemotherapeutic agents.[25] 4-aminoantipyrine is an intermediate in the synthesis of antipyretic and analgesic drugs[26] and it is also active against a wide range of microorganisms viz *E.coli, Pseudomonas aeuruginosa, Staphylococcus aureus* and *Candida albicance.*

The target of this work is to synthesis and characterization of new mercury complexes of thiosemicarbazones and antipyrine ligands. The potential protective effects of the synthesized compounds against mercury disruption of male sex hormones in rats were evaluated.

EXPERIMENTAL

All organic compounds and the solvents were purchased from Fluka or Merck (Nasr City, Egypt) and used without further purification.

Synthesis of ligands and Hg(II) complexes

Preparation and characterization of 2-phenyl-aminoacetyl-N-phenylhydrazine-carbothioamide (H_2L^1), 4-formylazohydrazoaniline antipyrine (H_2L^2) and [2-(2-(2,5-dihydro-2,3-dimethyl-1-phenyl-1H-pyrazol-4-yl-5-one)hydrazone)malononitrile] (HL^3) have been reported.[27-29] The Hg(II) complexes of the ligands were prepared by adding stoichiometric amount of the Hg (II) chloride, sulphate and nitrate in EtOH to the ligands in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at 60°C for 5-9 h. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10}.

Physical measurements

Elemental analysis (C, H and Cl) was performed at microanalytical unit of the Cairo Univerisity, Egypt. FT-IR measurements were performed (4000–400 cm^{-1}) in KBr with Nenexeus-Nicolidite-640-MSAFT-IR (Thermo-Electronics Co.) Spectrometer at the Central Lab., Minufiya University, Egypt. ^{1}HNMR spectra were recorded in DMSO-d_6 using 300 MHz Varian NMR spectrometer (Microanalytical Lab., Cairo University, Egypt). The molar conductivity measurements were made in DMF solution (10^{-3}M) using a Tacussel conductometer type CD6N. The electronic spectra were carried out as solution (10^{-3}M) in DMF using a Perkin-Elmer Lambada 4B spectrophotometer.

Thermal analysis (DTA/TG) were obtained out by using a Shimadzu DTA/TG-50 Thermal analyzer (Central Lab, Minufiya University, Egypt) with a heating rate of 10°C min^{-1} in nitrogen atmosphere with a following rate 20°C min^{-1}, in the temperature range 25 – 600°C using platinum crucibles.

Preparation of compounds and mercury-poisoned water

Newly synthesized derivatives of pyrimidine complexes were dissolved in DMSO to obtain the concentration of 1 mM. These stock solutions were stored at 4°C for further use.

To prepare a stock solution of 1000 ppm of mercury in drinking water, 1.35 g of $HgCl_2$ was dissolved in 1 litre of water. One milliliter of this solution was mixed with 10 litres of distilled water to obtain water containing mercury at a concentration of 1 ppm.

Animals grouping

Adult male albino rats, weighing about 160 ± 10 g, were housed at 23 ± 2°C and in daily dark/light cycle. They were caged in the animal house of College of Medicine, Qassim University and under standard condition and fed standard chow and water ad libitum.

All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee. After acclimatization, rats were divided into twelve groups each comprising of eight animals. Normal group (N) in which rats were maintained only on standard pellet diet and water ad libitium. $HgCl_2$-intoxicated drinking water group, in which, rats were maintained on drinking water intoxicated with 0.5 ppm of $HgCl_2$ for 30 days. The groups number 3 to 12 include animals co-treated with 0.5 ppm of $HgCl_2$-poisoned drinking water and 0.1mM of newly synthesized compounds for 30 days. During the course of the 30-day long experiment no animal was died.

Collection of blood and tissues' specimens

At the end of experiments, animals were sacrificed using a sharp razor blade. The blood was collected in prechilled heparinized centrifuge tubes. Plasma specimens were then obtained by centrifugation for 10 minutes at 4000 rpm at 4°C and were kept in clean well-stoppard vials at -20°C until assayed. The kidney and testes were removed and cut into pieces.

Preparation of testes and kidney homogenate

Kidney and testes homogenates were prepared by using a mechanical homogenizer (Potter-Elvehjem) in a 10-fold volume of ice-cold of 20 mM tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 5000 rpm for 30 minutes at 4°C to remove cell debris and nuclei. The supernatant liquid was collected, aliquoted and kept frozen at -20°C for further investigations.

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activities in kidney and testes homogenates were estimated according to the procedure of Nishikimi et al.[30] and by following the manufacturer's procedure (Biodiagnostics, Egypt).

Table 1. Analytical and physical data for the ligands and Hg(II) complexes

No.	Complexes	Color Yield (%)	Mol. Wt.	Found (Calc.) %					Λ_M [a]
				C	H	N	M	Cl	
1	$[Hg(H_2L^1)Cl_2(H_2O)_2].\ H_2O$	Pale brown (60)	625.6	28.6 (28.8)	3.3(3.5)	8.9(9.0)	32.3(32.0)	11(11.3)	24
2	$[Hg(H_2L^1)SO_4(H_2O)]$	Pale yellow (65)	613.6	29.5 (29.3)	3.0(2.8)	9.2 (9.1)	32.8(32.6)	-	15
3	$[Hg(HL^1)_2]$	Pale yellow (60)	798.6	45.0 (45.1)	3.9(3.8)	13.9(14.0)	25.2(25.1)	-	18
4	$[Hg_2(HL^2)(OH)_2Cl]$	Yellow (70)	870.5	29.2 (29.0)	2.5(2.3)	11.4(11.3)	46.2(46.0)	4.2(4.1)	10
5	$[Hg(HL^2)(NO_3)(H_2O)]$	Brown (70)	680.6	37.2 (37.0)	3.0(3.0)	14.4(14.4)	29.7(29.5)	-	20
6	$[Hg(HL^3)(SO_4)]$	Yellow (70)	594.6	28.4 (28.5)	2.2(2.4)	14.3(14.1)	33.6(33.7)	-	Insol.
7	$[Hg(HL^3)(NO_3)_2]$	Brown (60)	604.6	27.7 (27.8)	1.8(2.0)	14.2(13.9)	33.0(33.2)	-	18

[a] Λ_M = molar conductivity $ohm^{-1}\ cm^2\ mol^{-1}$ in 10^{-3} M DMF

Table 2. Fundamental IR spectral bands (cm^{-1}) for the ligands and Hg(II) complexes

Compound	$\nu_{(O-H)}/\nu_{(N4-H)}$	$\nu_{(N1-H)}$	$\nu_{(N2-H)}$	$\nu_{(C=O)}$ [a]	$\nu_{(C=O)}$ [b]	$\nu_{(C=N)}$ [c]/ $\nu_{(C=S)}$ [d]	$\nu_{(M-O)}$	$\nu_{(M-N)}$
H_2L^1	3463	3340	3250	1677	-	747[d]	-	-
$[Hg(H_2L^1)Cl_2(H_2O)_2].\ H_2O$	3393	-	3100	1700	-	752[d]	614	507
$[Hg(H_2L^1)SO_4(H_2O)]$	3390	-	3100	1698	-	751[d]	615	510
$[Hg(HL^1)_2]$	3402	-	3108	-	-	749[d]	607	506
H_2L^2	3430	-	-	1645	1635	1536[c]	-	-
$[Hg_2(HL^2)(OH)_2Cl]$	3435	-	-	-	1625	1552[c]	593	536
$Hg(HL^2)(NO_3)(H_2O)$	3438	-	-	-	1612	1534[c]	640	454
HL^3	3410	-	-	-	1630	1587	640	477
$[Hg(HL^3)(SO_4)]$	3434	-	-	-	1609	1496	574	443
$Hg(HL^3)(NO_3)_2$	3429	-	-	-	1607	1495	582	445

[a] (C=O) of side chain, [b] (C=O) of pyrazolone ring, [c] (C=N), [d] (C=S)

Determination of catalase activity

Antioxidant enzyme catalase (CAT) activities in kidney and testes homogenates were determined according to the method of Bergmayer[31] as described in the manufacturer's procedure (Biodiagnostics, Egypt).

Determination of lipid peroxidation level

The levels of lipid peroxides (LPO) in kidney and testes homogenates were estimated colorimetrically by measuring malondialdehyde (MDA) using the method of Ohkawa et al and by following the manufacturer's procedure (Biodiagnostics, Egypt).[32]

Determination of testosterone level

Level of testosterone in testes homogenate was processed by using Fertigenix Testo-ELISA kit (Biosource, Belgium) in accordance with the protocol described by Park et al.[33]

Determination of follicle-stimulating hormone level

Follicle stimulating hormone (FSH) concentration was estimated in testes homogenate with IMMULITE analyzer according to the method of Odell et al[34] using IMMULITE FSH kit purchased from EURO/DPC Ltd., USA.

Determination of leutinizing hormone level

Leutinizing hormone (LH) concentration was estimated in testes homogenate according to the method of Knobil[35] using LH kit purchased from Ameritek (USA) with Vmax ELISA reader

Determination of fructose level

Fructose concentration was estimated in testes homogenate spectrophotometrically according to the method of Karvonen and Malm.[36] Briefly, fructose in presence of hydrochloric acid forms a pink colored complex with indole-3-acetic acid. The complex has maximum absorbance at 500-530 nm.

Statistical analysis

Results are expressed as mean ± S.D. The data for various biochemical parameters were analyzed using analysis of t-test and the group mean was compared by one-way ANOVA. Values were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Physical properties

The reaction of the ligands with Hg(II) chloride, sulphate and nitrate give complexes of general formulae $[Hg(H_2L^1)Cl_2(H_2O)_2].H_2O$, $[Hg(H_2L^1)SO_4(H_2O)]$, $[Hg(HL^1)_2]$, $[Hg_2(HL^2)(OH)_2Cl]$, $[Hg(HL^2)(NO_3)(H_2O)]$and $[Hg(HL^3)XY]$,where X = SO_4 or NO_3 and Y = 0 or NO_3 (Table 1). While the reaction of H_2L^2 with Hg(II)sulphate and HL^3 with Hg(II)chloride produce decomposed products. All Hg(II) complexes are freely soluble in DMF and DMSO except complex (**6**), which is insoluble in DMF and DMSO. The molar conductivity of all Hg(II) complexes in DMF solution (10^{-3} M) at room temperature indicate that all complexes are non-electrolyte.[37]

FT-IR For H_2L^1 ligand and Hg(II) complexes

The diagonostic IR bands for ligand 2-phenylaminoacetyl-N-phenylhydrazine-carbothioamide (H_2L^1) and Hg(II) complexes (**1-3)** are listed in Table 2. The most important four bands, exhibited by ligand (H_2L^1) at 3463 cm^{-1}, 3340 cm^{-1}, 1677 cm^{-1} and 747 cm^{-1} are assigned to ν(N4-H), ν(N1-H), ν(N2-H), and ν(C=S) vibrations, respectively. The bands at 1500 cm^{-1}, 1440 cm^{-1} and 1280 cm^{-1} may be due to ν(N-C=S).[38]

The bands at 3100 cm^{-1}, 1700 -1698 cm^{-1} and 752 cm^{-1} seen in the complexes (**1**) and (**2**) have been assigned to ν(N2-H), ν(C=O) and ν(C=S) respectively. The IR spectrum of the complex **(3)** shows that bands due to ν(C=O) and ν(N2-H) disappear up on complexation and a new band appeared at 1600 cm^{-1}, which has been assigned to ν(C=N).

For H_2L^2, HL^3 and Hg(II) complexes (**4-7**) the IR data are presented in Table 2. The IR spectra of the free ligands (H_2L^2 and HL^3) show four bands at 3430 cm^{-1}, 3434 cm^{-1}; 2210 cm^{-1}, 2205 cm^{-1}; 1645 cm^{-1}, 1630 cm^{-1}; and 1610-1587 cm^{-1}, assigned to ν(N–H), ν(C≡N), ν(C=O) of side chain, ν(C=O) of pyrzolone ring and ν(C=N) respectively. The infrared spectra of complexes (**6** and **7**) show a decrease in the energy of ν(C=O) of side chain, ν(C=O) of pyrazolone ring and ν(C=N) up on complex formation, indicating that carbonyl oxygen of C=O of side chain, C=O of pyrazolone ring and C=N participate in coordination. While in complexes(**4** and **5**) the bands corresponding to ν(C=O) of side chain and ν(N–H) disappear indicating that the ligand is in enolimino form and new bands appears at 1552 cm^{-1}, 1537 cm^{-1}, assigned to ν(C=N) up on complexation.

The IR spectra of all Hg (II)complexes show new two bands at 640 -574 cm^{-1} and 536-443 cm^{-1}, assigned to ν(Hg-O) and ν(Hg-N).[39,40] However, complexes (**1** and **4**) exhibit medium bands at 320 and 325 cm^{-1} due to ν(Hg-Cl).[41] Also in the complexes (**2** and **6**) strong band appears at 1110-1178 cm^{-1}, assigned to unidentate sulphate moiety.[42]

The IR spectra of complexes (**5** and **7**) show strong bands at 1379- 1390 cm^{-1}, assigned to monodentate of nitrate group. While complexes (**1**, **2**, **4** and **5**) reveal broad bands at 3390–3435 cm^{-1} and 765- 878 cm^{-1} assigned to ν (Hg-O) of coordinated water except in complex (**4**) only appears a band at 3435 cm^{-1}, assigned to coordination of Hg(II) with the hydroxy group.

Table 3. Electronic spectral bands of the Hg(II) complexes

No.	Complexes	λ_{max} nm
1	$[Hg(H_2L^1)Cl_2(H_2O)_2].H_2O$	270
2	$[Hg(H_2L^1)SO_4(H_2O)]$	268
3	$[[Hg(HL^1)_2]$	267
4	$[Hg_2(HL^2)(OH)_2Cl]$	268, 402
5	$[Hg(HL^2)(NO_3)(H_2O)]$	266, 401
6	$[Hg(HL^3)(SO_4)]$	268, 389
7	$[Hg(HL^3)(NO_3)_2]$	267, 387

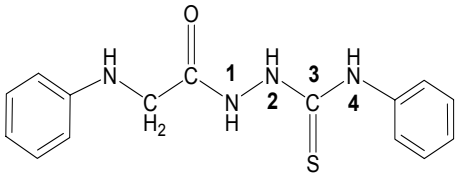
Electronic spectra

The data of electronic spectra of Hg(II) complexes (**1-7**) are given in Table 3. The absorption spectra of Hg(II) complexes were recorded in DMF solutions (10^{-3} M) in the range 190-800 nm using a quartz cuvette of 1 cm path length.

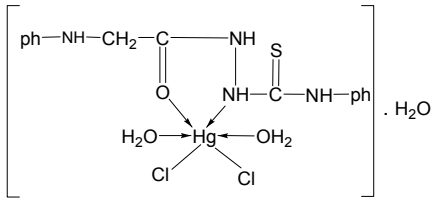
The complexes show only the charge transfer transitions which can be assigned to charge transfer from the ligand to the metal and these ions have the d^{10} configuration and therefore, their complexes should not exhibit any d-d transition. All of complexes of these Hg(II) ions were found to be diamagnetic.[43] The absorption bands of Hg(II) complexes observed listed in Table 3. Probable structures of the ligands and complexes are given in Scheme 1.

Thermal studies for Hg(II) complexes

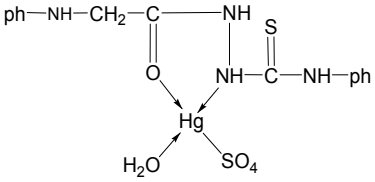
Thermal analyses have been carried out using differential thermal analysis (DTA) and thermogravimetric analysis (TGA) techniques. The thermal behaviour carried out in temperature range 25-600°C. DTA and TGA curves recorded for the complexes in an atmosphere of nitrogen and important data are summarized in Table 4. The various steps of the decomposition of the compound with the corresponding mass loss in terms of the proposed formulas for the complexes are given. The Hg(II) complexes (**1** and **5**) show the three exothermic peaks each. While, complexes (**2**, **3** and **4**) exhibit two borad exothermic and one endothermic DTA peaks.



H_2L^1



(1)



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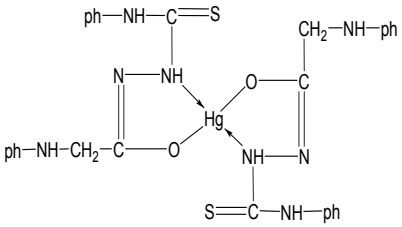

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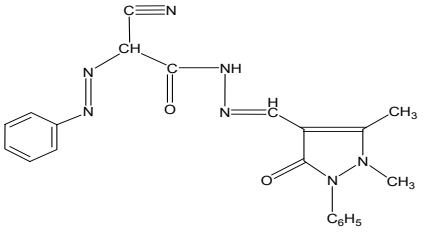
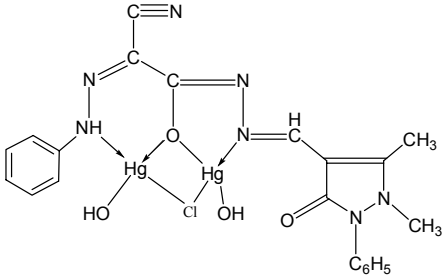
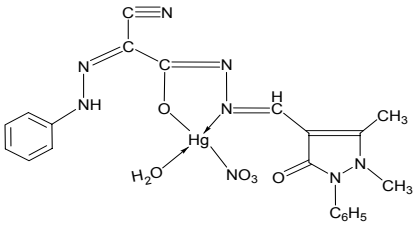


H_2L^2



(4)



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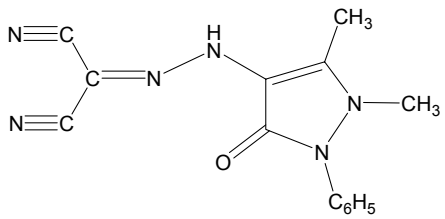
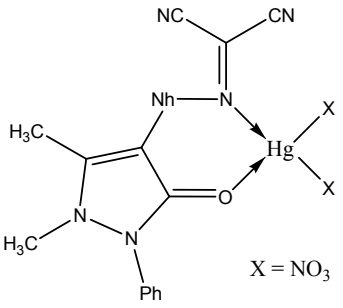


HL^3



(7)

Scheme 1. Chemical structure of ligands and their Hg(II) complexes

However, in Hg(II) complex (**7**) one strong exothermic DTA curve in temperature range 200-233°C appears, which has been assigned to loss of (($0.8L + 2HNO_3$) and ($2CO + 3C$),[44] as shown from TG mass loss in that temperature range. The initial decomposition temperature has been used as an indicator of the thermal stability of the complexes. The results of the thermal analysis of the mercury complexes indicated that complex (**3**) is thermally more stable compared to the rest of mercury(II) complexes.

Evaluation of kidney function

Serum creatinine level is one of the traditional screening indices for kidney function and renal structure epithelium.[45] The effects of mercuric chloride on the biochemical tests of renal function in the animals are presented in table 5. After drinking water poisoned with 0.5 ppm of $HgCl_2$ for 30 days, a statistically significant increase of creatinine and urea concentrations in plasma was observed as compared with control groups. The elevation in creatinine level after exposure to inorganic mercury is accordance with the report of Oriquat et al. in rats.[46] The rise in creatinine level might be due to damage caused to kidney tubules by inorganic mercury. Co-treatment with different ligands and their complexes caused amelioration on renal function. Results showed that ligands H_2L^1, H_2L^2 and HL^3 and their complexes, except complex (**4**), significantly reduced ($P<0.05$) the elevated renal function markers.

Evaluation of oxidative status

The level of malodialdehyde (MDA) is widely used as a marker of free radical mediated lipid peroxidation (LPO). The results of the LPO assays in the kidney and testes homogenates are shown in Table 6. LPO level increased significantly in the kidney and testes homogenates of rats after exposure to 0.5 ppm of mercury for 30 days as compared to the normal group. The mercuric chloride toxic effect is due to its ability to adhere or to form link with cell enzymes of the respiratory chain and proteins, which alter the metabolism of target cells in organs participating in its elimination. Mercury also provokes a reactive oxygen species (ROS)-dependent vascular damage producing large scale haemorrhage in many organs like kidney. This ROS was significantly reduced when animals were supplemented with various ligands and their complexes showing the ameliorative effects of these compounds.

Results shown in Table 6 revealed that ligands H_2L^1, H_2L^2 and HL^3 significantly reduced the elevated levels of LPO in testes and kidney tissues showing their ability to scavenge free radicals. Meanwhile, their complexes exhibited varied effects on LPO in testes and kidney, where complexes (**3, 6** and **7**) showed no antioxidative activities as compared to $HgCl_2$- treated group.

Oxidative stress defines an imbalance between the formation of ROS and antioxidative defence mechanisms. Drinking of $HgCl_2$-poisoned water for 30 days generated the ROS and caused oxidative stress in intoxicated animals. In oxidative stress, LPO is occurred due to excessive free radical production and is considered a primary mechanism of cell membrane destruction and cell damage. Malondialdehyde (MDA) is the end product of lipid peroxidation.

The toxicity with inorganic mercury increased the testicular MDA and simultaneously decreased the CAT and SOD activities in this study. CAT and SOD were estimated in the kidney and testes homogenates. Results showed that administration of 0.5 ppm of $HgCl_2$ in drinking water for 30 days significantly decreased the activities of CAT and SOD as compared to those of the normal control group (Table 6).

Table 4. Thermal data of Hg(II) complexes

No.	Complex	DTA/°C	TGA/°C	Mass loss %Cal. (F.)	Leaving species
1	$[Hg(H_2L^1)Cl_2(H_2O)_2].H_2O$	97- 201	87 -173	2.8(2.9)	$-H_2O$
		288- 337	193-272	11.5(11.3)	$-(2H_2O+HCl)$
		338-447	294-355	17.8(18.0)	-(HCl+0.25L)
			405- 468	17.0(17.2)	$-(C_6H_5NHCH_2)$
2	$[Hg(H_2L^1)SO_4(H_2O)]$	130-180	111- 165	2.9(2.7)	$-H_2O$
		227-274	188-335	39.8(40.3)	$-(0.6L+SO_2)$
		293 -329	394-424	9.1(9.5)	-2CO
3	$[Hg(HL^1)_2]$	210-257	111 -117	3.6(3.3)	$-(CH_2NH)$
		413 -450	200-289	14.9 (14.8)	$-(C_6H_5+C_2H_2O)$
		505 -550	340-411	13.3 (13.5)	$(C_6H_5NHCH_2)$
			459-509	14.8 (14.4)	$(C_6H_5NH+C_2H_2)$
4	$[Hg_2(HL^2)(OH)_2Cl]$	229- 256	176 -247	16.9(17.0)	$(2OH + HCl + C_6H_5)$
		280-322	263- 335	50..6(50.3)	(0.6L+Hg)
		504-550	Above 335		Thermal stability
5	$Hg(HL^2)(NO_3)(H_2O)$	232-271	192-280	18.1 (18.4)	$(H_2O+$
		301- 335	297 -379	40.8 (40.4)	$HNO_3+C_2H_2O)$
		370-408	Above 379		$-(0.5L+C_6H_5)$
7	$Hg(HL^3)(NO_3)_2$	200-238	170-238	57.9 (57.7)	$-(0.8L+2HNO_3)$
			344 -410	13.9 (13.6)	- (3CO)

On the other hand, rats that were supplemented with ligands and their complexes together with $HgCl_2$ for 30 days experienced significant increase in CAT and SOD activities when compared to the $HgCl_2$-treated group. Ligands H_2L^1, H_2L^2 and HL^3 exhibited higher stimulatory effect on the activities of CAT and SOD in testes and kidney tissues as compared to their complexes.

Table 5. Effect of different ligands and their Hg complexes on renal function parameters

Group	Creatinine (mg dL^{-1})	BUN (mg dL^{-1})
Normal	0.7±0.1	10.4±1.0
$HgCl_2$	2.9±1.4	20.8±2.8
$HgCl_2 + H_2L^1$	1.6±0.5*	11.0±0.9*
$HgCl_2$ + 1	1.9±0.3*	17.6±2.1*
$HgCl_2$ + 2	1.3±0.5*	15.2±4.4*
$HgCl_2$ + 3	2.1±0.6*	21.8±1.9
$HgCl_2 + H_2L^2$	0.8±0.2*	11.7±3.1*
$HgCl_2$ + 4	2.1±0.6	26.0±5.7
$HgCl_2$ + 5	1.7±0.3*	16.8±1.9*
$HgCl_2 + HL^3$	0.8±0.3*	10.8±2.8*
$HgCl_2$ + 6	1.5±0.4*	15.0±3.8*
$HgCl_2$ + 7	2.3±0.9	20.2±2.4

These complexes showed diverse antioxidant actions on the activities of CAT and SOD, where, complexes (**3, 6 and 7**) did not show ameliorative actions on the activities of CAT and SOD.

Evaluation of some male sex hormones

The mean values of the serum hormones; testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) are shown in Table 7. Results showed that after drinking of 0.5 ppm of $HgCl_2$-poisoned water for 30 days led to significant decreases ($P<0.05$) in the levels of testosterone, LH and FSH as compared to the control group. The levels of male sex hormones were restored to control values after combination of $HgCl_2$ with studied ligands and some of their complexes. The tested complexes showed varied effects on the level of male sex hormones. In contrast to their complexes, ligands exhibited potent stimulatory actions on the levels of testosterone, LH and FSH.

Testosterone is essential for spermatogenesis completion because it stimulates the conversion of round spermatids into elongated spermatids between stages VII and VIII of the spermatogenic cycle. Thus, testicular testosterone deficiency as observed in this study after exposure to $HgCl_2$ for 30 days will impair the spermiation process.[47]

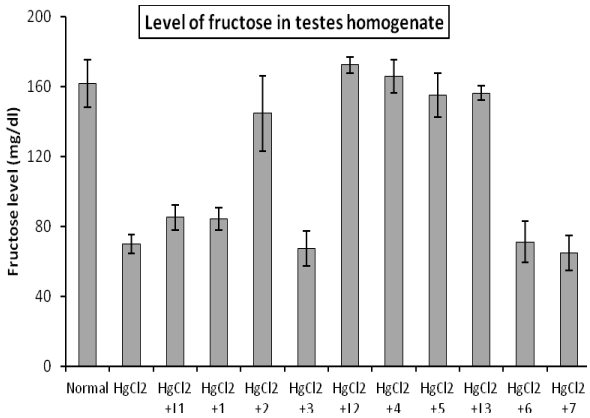


Figure 1. Effect of $HgCl_2$ and tested compounds on the levels of testicular fructose

Fructose provides energy for sperm motility.[48] As shown in Figure 1, level of fructose is significantly reduced ($P<0.05$) in rats after exposure to Hg-intoxicated water for 30 days as compared to that of the control group. Different effects of tested ligands and their complexes on the level of testicular fructose were observed. Results indicate that tested ligands restored the fructose levels to that of the normal animals, while, some complexes did not ameliorate the fructose levels.

Table 6. Effect of different ligands and their Hg complexes on the oxidative status in kidney and testes

Group	CAT U g $tissue^{-1}$		SOD U g $tissue^{-1}$		LPO nmol g $tissue^{-1}$	
	Kidney	Testes	Kidney	Testes	Kidney	Testes
Normal	3.5±0.6	5.9±0.7	122.5±11.4	155.2±13.8	135.3±16.7	152.2±9.1
$HgCl_2$	1.1±0.3	1.3±0.5	76.8±8.3	92.8±11.1	230.8±23.9*	252.8±16.5
$HgCl_2 + H_2L^1$	3.1±0.8*	6.1±0.5*	121.6±10.3*	151.6±12.2*	142.5±21.8*	150.0±12.1*
$HgCl_2$ + **1**	2.9±0.5*	5.9±0.4*	115.6±9.4*	155.6±10.2*	134.3±13.9*	150.2±21.9*
$HgCl_2$ + **2**	2.2±0.8*	4.2±0.2*	116.6±12.3*	156.6±15.5*	133.2±14.2*	154.2±4.5*
$HgCl_2$ + **3**	1.5±1.1	1.8±0.8	72.6±7.7	92.1±9.8	225.0±21.3	248.6±24.5
$HgCl_2 + H_2L^2$	3.2±0.7*	6.2±0.5*	117.8±10.3*	137.8±11.5*	152.6±15.3*	151.2±14.0*
$HgCl_2$ + **4**	2.2±0.8*	5.2±0.3*	108.0±13.1*	121.0±15.0*	159.2±14.2*	159.4±13.5
$HgCl_2$ + **5**	2.1±0.6*	5.1±0.2*	118.4±9.6*	133.4±8.8*	152.6±15.3*	165.7±16.3*
$HgCl_2 + HL^3$	4.2±1.4*	6.2±0.4*	119.6±7.7*	159.6±11.8*	142.6±15.3*	153.2±12.4*
$HgCl_2$ + **6**	1.1±0.3	2.1±0.2	63.2±9.8	83.2±10.8	222.6±18.9	245.6±10.1
$HgCl_2$ + **7**	0.9±0.1	1.5±0.3	62.5±5.5	92.5±9.9	220.6±17.9	253.3±11.0

Table 7. Levels of testosterone, luteinizing hormone and follicle-stimulating hormone in testes of animals of different studied groups

Groups	Testesterone (ng g^{-1})	LH (ng g^{-1})	FSH (ng g^{-1})
Normal	121.8±2.2	47.2±2.6	34.7±1.5
$HgCl_2$	85.6±8.6	20.2±1.9	15.7±1.5
$HgCl_2 + H_2L^1$	103.0±5.7*	34.8±7.4*	23.7±1.5*
$HgCl_2$ + **1**	93.4±9.4*	24.8±0.8	25.3±2.1
$HgCl_2$ + **2**	102.2±4.5*	20.6±2.4	30.7±4.0*
$HgCl_2$ + **3**	74.2±10.3	19.8±3.8	17.3±5.0
$HgCl_2 + H_2L^2$	99.2±7.9*	37.6±6.3*	29.3±1.5*
$HgCl_2$ + **4**	85.2±9.4	45.2±2.4*	21.3±0.6*
$HgCl_2$ + **5**	101.4±3.2*	16.4±3.2	32.7±1.5*
$HgCl_2 + HL^3$	126.8±4.4*	41.6±2.1*	30.3±2.1*
$HgCl_2$ + **6**	89.2±3.0	23.2±4.0	13.7±3.2
$HgCl_2$ + **7**	77.8±9.1	19.6±5.3	15.3±1.5

CONCLUSION

In our study, we characterized mercury(II) complexes of ligands (2-phenylaminoacetyl-N-phenylhydrazine carbothio-amide (H_2L^1), 4-formylazohydrazoanilinoantipyrine (H_2L^2) and [2-(2-(2,5-dihydro-2,3-dimethyl-1-phenyl-1H-pyrazol-4-yl-5-one)hydrazono)malononitrile] (HL^3) using different analytical and spectroscopic methods. The IR spectral show that the ligand of complexes **(2)** and **(5)** behave as mono basic bidentate, coordination take place by (C-O) and N(2)H or (C=N). While the ligand of complexes (**1, 6** and **7**) behave as neutral bidentate and coordination via (C=O) and N(2)H or (C=N) groups. On the other hand, the ligand for complexes (**3** and **4**) produce mono, dibasic tetradentate and chloro bridge of binuclear complex (**4**). All complexes are tetrahedral geometry except complex (**1**) is octahedral geometry and diamagnetic of d^{10} of Hg(II) ions. The thermal behavior study showed that complex (**3)** is more stable as compared of the rest of Hg(II) complexes.

Further it has been shown that mercury causes severe toxic tissue damage in the testis and kidney of rats. This damage may be caused by the ROS produced by mercury within the animals' body. The tested ligands and some of their Hg complexes showed varied effects against mercury toxicity. They interacted with mercury ions, neutralize them and prevent the ROS mediated oxidative damage in testes.

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