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Effect of 2-Thioxo Imidazolidin-4-Ones derivative copper complex on the oxidative state on the male rats exposed to hydrogen peroxide

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***Abstract*---**In this work, a new thioxoimidazolidine derivative had been prepared by the reaction of bromo*benzaldehyde* with thiosemicarbazide then the resulting product reacted with ethelchloroacetate to produce the ligand, L= (3{(E)[(4-bromophenyl) methyldenamino-2-sulfanylideneimidazo lidin-4-one, in an attempt to synthesis its copper complex. The new ligand and its copper complex was isolated, studied and characterized in solid states by studying the analytical and physicochemical properties. It was found that ligand coordinate to copper ion as a bidentate ligand through nitrogen atom of Azomethine groups and sulfur atoms of the thione group. The outcomes recorded that copper complex could be founds in "[1:2] (M:L) molar ratio". From study of geometrical shape, the copper ion has a square planer shape with Ligand. Twenty-four adult male rats were divided into three equal groups, with age 2-3 months old. The first group returned to the control group, as it was given the necessary food and water, second, third group was given 0.5% hydrogen peroxide in drinking water. The third group was dosage by orally copper complex of 2-Thioxo Imidazolidin-4-Ones by Cavage Needle. after thirty and sixty days of experimental period, blood sample was drawn from each animal. The antioxidant study of the prepared copper complex of ligand showed a decrease in GSH, Catalase and total antioxidant concentrations of animals of selected groups when

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treated with 0.5% v/v of hydrogen peroxide, while a significant elevation in serum GSH, Catalase and total antioxidant concentrations in another group after treatment with copper complex of ligand.

Keywords---Oxidative stress, Antioxidant, 2-Thioxo Imidazolidine, copper complex, & Catalase.

1. Introduction

Essentially, oxidation takes place at the cellular level and throughout the body. While oxidative stress happens when there is an imbalance between radical and antioxidant activity, it is an oxygen reaction that occurs within the body and produces byproducts known as free radicals.. free radicals such as singlet oxygen $O_2\bullet^-$ hydrogen peroxide H_2O_2, hydroxyl radicals (•OH), are the types of oxidative stress substances (ROS); They are formed during metabolism by biological systems (Sato et al.,2013: Navarro et al,.2014)

Environmental stressors (such as UV rays, ionizing radiation, pollutants, heavy metal) and xenobiotic (anti-plastic drug) significantly contributes to the increased in ROS productions. (Pizzino et al,.2017). It is impossible to completely avoid exposure to free radicals and oxidative stress. However, there are things you can do to reduce the effects of oxidative stress on the body by either increasing antioxidant levels or decreasing the formation of free radicals. One way to prevent oxidative stress is to get enough antioxidants in the diet such as eating fruits and vegetables . Several antioxidants were exploited recently as they having supposed and actual beneficial effects against oxidative stresses, such as vitamin E, flavonoid, and polyphenol (Pizzino et al,.2017).

The potential benefits of antioxidants in preventing disease and promoting health have led to a significant expansion of antioxidant research. In biological systems such cell cultures, animal models, and clinical trials, researchers have thoroughly examined the antioxidant activity of pure substances, meals, and supplements. (Lotito & Frei,.2006: Williamson & Manach,.2005). They include synthetic antioxidants such butyl hydroxyl toluene (BHT, E320), propyl gallate (PG, E310), tertiary butyl hydroquinone (TBHQ), and butyl hydroxyl anisole (BHA, E320), as well as natural antioxidants like ascorbic acid (AA, E300) and tocopherol (E306) (BHT, E321). Usji and Sleno 2020).

Imidazole is among the heterocyclic compounds having antioxidants and anti-inflammatory effect, anticancer effect and cancer prevention property (El-Salam et al,.2013) was used in treatments of different disease included rheumatoid arthritis, diabetes, Alzheimer and cancer. Imidazole, five membered hetero-cyclic rings contained 2 nitrogen and 3 carbon atoms at position 1and 2 were present in various natural compound and bioactive compound (Rahman et al,.2012). Imidazoles are founds in structures of various important biologic component of body such as histidine's, as well as numbers of active pharmaceutical compound (Nagalakshmi. 2008). In the present study, we investigate effects of 2-Thioxo imidazolidine-4-one (2-Thiohydantion) and the copper derivative of the compound on some biomarkers of rat exposed to oxidative stresses by hydrogen peroxides.

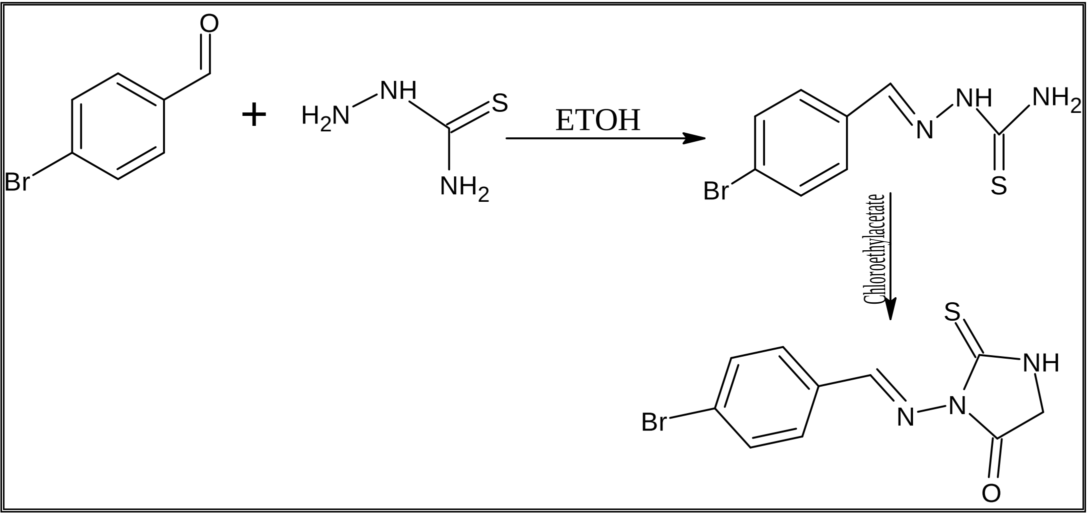
All living things include copper, a trace element that is essential for growth, development, and redox chemistry. Numerous enzymes and proteins, including cytochrome oxidase, superoxide dismutase, ascorbate oxidase, and tyrosinase, which are involved in energy metabolism, respiration, and DNA synthesis, depend on it for proper operation. The primary biological functions of copper include oxidation-reduction processes in which they directly react with molecules of oxygen to form free radicals. (Tisato et al 2010). Saczewski et al. developed and studied a series of bidentate chelating ligands derived from benzimidazoles that are coordinated to Cu (II). The imidazolate-bridged Cu (II)-Zn (II) hetero binuclear complexes and the best low molecular weight Cu, Zn-SOD mimics have been characterized in the literature as having significant Cu, Zn-SOD activity in vitro with IC50 values of 0.09 mM and 0.14 mM, respectively. 191 A moderate cell growth inhibitory Activity was observed in in vitro cytotoxicity experiments using seven distinct human tumor cell lines.(Sączewski et al,.2006).

2. Materials and Methods

All chemical were of highest purity and were used as received.

Synthesis of ligand (L)

A solution of thiosemicarbazide (0.58gm, 0.0054 mol) was added to the solution of (1g, 0.0054 mol in 10mL of absolute ethanol) of 4-bromobenzaldehyde. The resulting mixture were reflexed for few minutes and then drops of concentrated acetic acid was added. The mixture heated under reflux for (6 h.), then the mixture cooled and poured into crushed ice, the resulting solid produced was filtered, dried and recrystallized from ethanol. (Mp = 224-226C^{0}). Scheme (1). **(**0.00168mol, 0.5g) of pervious product was dissolved in absolute ethanol, then (0.205g, 0.00168mol) of ethyl chloroacetate was added. This mixture was heated under reflux for (3h.), the resulting solid product was filtered, dried and recrystallized from ethanol, (M.p =264-262C). Scheme (1).



Scheme (1): Synthesis of Ligand (L)

Preparation of metal complexes (N_1-N_5)

Copper complex had been synthesized by the reaction $CuCl_2.2H_2O$ with prepared ligand (0.5g, 0.00168mol) with was added to the ethanolic solution of prepared ligand solution (0.143g, 0.00084mol), the reaction mixture was reflux for (4h.) through this time the precipitate was formed, then, filtered and washed several times with hot absolute ethanol, and then by cold distilled water, dried under the oven, the prepared copper complex was thermally stable. Can be stored up at room temperature and unaffected by moisture. (Rashed and Abdullaha, 2021).

Table (1): physical properties and yield percentage of synthesized compounds

Compound symbol	Molecular	Melting point (C^0)	Color	Yield %	Recryst. Solvent
A	$C_8H_8BrN_3S$	224- 226	Off white	98	Acetone
L	$C_{10}HOBrN_3S$	272-274	yellow	91	Aceton +ethanol
S	$[Cu(L_2)Cl_2].2H_2O$	286 dec	green	80	Methanol

dec* = *decompose*

Preparation of Hydrogen peroxide solation

50MN of hydrogen peroxide was prepared by diluting absolute hydrogen peroxide with normal drinking water to 0.5%. The solution was prepared daily and given to experimental animals in special bottles The trial period is 60 days**.**

Design of the experiment:

In this study, thirty-two adult albino male rats were used, and the experiment lasted for sixty days from Date twenty-three of January until twenty-three of

March 2022. The animals were randomly divided into three equal groups. The first group returned to the control group, as it was given the necessary food and water, as for the second, third groups, the study was conducted on them for two months.

Second, third group was given 0.5% hydrogen peroxide in drinking water. The third group was dosage by orally 2-Thioxo Imidazolidin-4-Ones and its copper complex by Cavage Needle. after thirty and sixty days of experimental period at 10 o'clock, blood sample was drawn from each animal, after synthesized by mixture of ketamine in a dose of 90 mg/Kg B.W. and xylazine, 40 mg/Kg B.W. an intramuscular rout injectable that is safe (jiheel, 2015). Following completion of laboratory testing, they were compared with animals in the control group, and after sixty days, the same processes were performed blood samples placed in a centrifuge at a power of 3000 cycles/ After the centrifugation process, the serum layer was isolated from the rest of the blood components. The serum was withdrawn by a fine pipette and then placed in abendrov tubes for the purpose of conducting functional tests. After that, the serum samples were kept at a temperature of -20°C to preserve them from damage and until use.

Results

Physical properties of ligand and its copper complex

The reaction of copper ions with synthesized ligand produce new complexes have general formula $[[Cu(L)_2]Cl_2.2H_2O$. The copper complex was stable and unchanged at room temperature, insoluble in polar solvent but soluble only in DMSO and DMF. The physico-chemical properties data of the synthesized ligand (L) and its copper ion complex were given in Table (2). The outcomes attained from the elemental analysis are reach agreement with the calculated value**.**

Table (2) Physical properties of ligand and its copper complex

Comp. Symbol	**General formula**	**Mwt. g.mol^{-1}**	**Color**	**M.P (°C*)**	**Elemental analysis(%) Found(calc.)**				
					C	**H**	**N**	**S**	**M**
L	$C_{10}H_8$ OBr N_3S	298.15	Yellow	274-272	40.28 (40.25)	2.70 (2.72)	14.09 (14.06)	10.75 (10.71)	-
S	$[Cu(L_2)\ Cl_2].2H_2O$	766.84	Green	283dec*	31.29 (31.31)	3.12 (3.11)	10.95 (10.88)	8.34 (8.30)	8.28 (8.31)

dec* = decompose

Mass spectrum of Ligand

The mass spectrum of Ligand, Fig. (6) showed the mother ion peak at (m/z =299) as a base peak which is corresponds to (M+) [63]

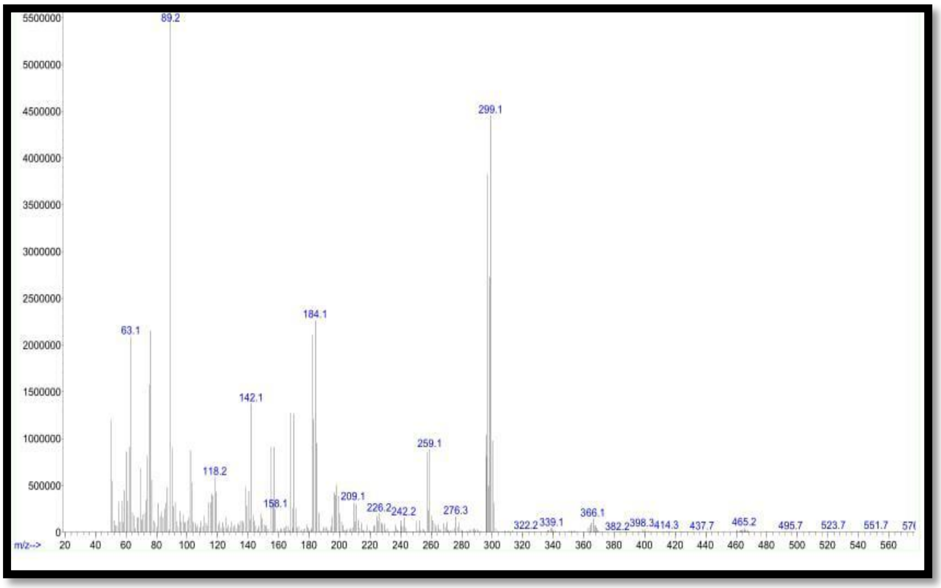
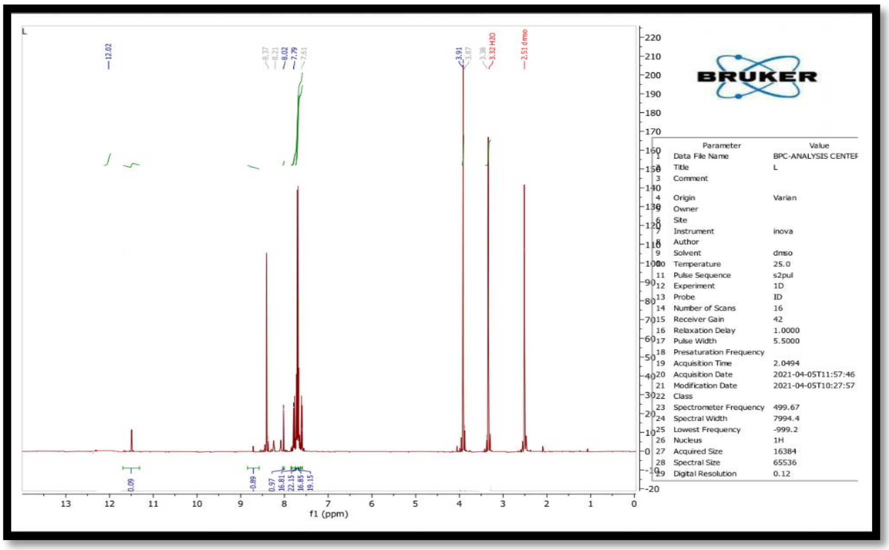


Figure (6): mass spectra of prepared Ligand

H-NMR spectra of prepared ligand

H-NMR spectrum of prepared Ligand, Fig.(8) in (DMSO-d6) showed the signals at (11.5ppm) 1H for (NH) group , and (8ppm) 4H related benzene ring and (4ppm) 1H proton of Schiff base , and signals at (2.5ppm) 2H for (CH_2) group of thioxoimidazolidine ring[65].



Figure(8) H-NMR spectrum of L

FT-IR spectra of complex

The FTIR spectrum of free ligand was showed many bands including ($3437cm^{-1}$) due to ν(NH) group and ($3059cm^{-1}$) related to ν(C-H) aromatic, while the band at ($2929cm^{-1}$) due to ν(C-H) aliphatic, also band at ($1710cm^{-1}$) belonged to ν(C=O) group, this band undergoes a small variation in shape and position because of formation of copper complex this mentions to that this group was not take part in the coordination process ((Silverstein, Robert M.; Bassler, G. Clayton, 2005.

Another bands in FT-IR spectrum of ligand was showed band at ($1639cm^{-1}$) related to Azomethine group ν(C=N), this group was shifted by ($10cm^{-1}$) to lower wave number in FTIR spectrum of the metal ion complex, while band at ($1030cm^{-1}$) that related to thione group ν(C=S) was shifted by ($28cm^{-1}$) to lower frequency in FTIR spectrum of the copper ion complex, this changing in FTIR spectrum of the synthesized ligand deduced that the ligand coordinated to copper ion from (C=N) of Azomethine group and from ν(C=S) of thion group(Nakamoto, 1997). figure (6).

Copper complex also showed a broad band in FTIR spectrum in the region between (3460 and $3431cm^{-1}$) this refers to presence of coordinated water molecule in this complex

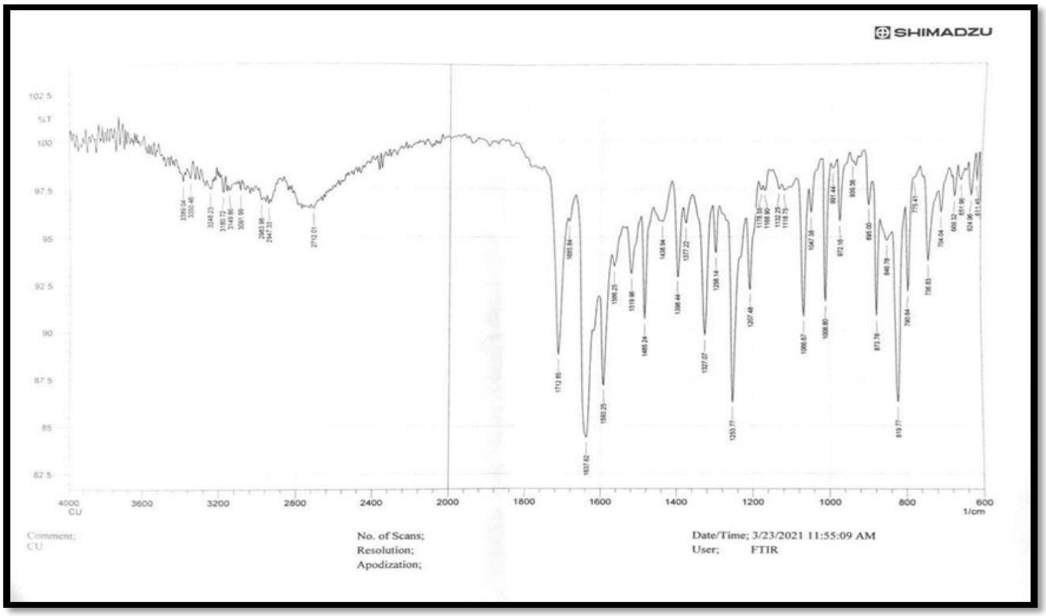


Figure (6) FT-IR spectrum of copper complex

Electronic spectra of copper complex

Three absorption bands at (225 nm, 44444 cm-1), (290 nm, 34482 cm-1), and band at (325 nm, 30769 cm-1) were ascribed to ((((*) transitions in the (U.V.-Vis) spectrum of the ligand in absolute ethanol. While one broad-band was captured in the cupper complex's electromagnetic spectrum at (750nm,13333cm-1), which correspond to ($^2B1g" \rightarrow {}^2B2g+ {}^2Eg$) and shoulder transition band at (490nm, 20408.16cm^{-1}), which was assigned to($^2B1g \rightarrow ^2A1g$) transitions. Band at (340nm, 29411cm^{-1}) may be related to charge transfer transition, the positions of band was in good approve with the described for highly distorted octahedral geometry, the value of magnetic moment at room temperature was found to be (1.83 B.M) , which agree well with (square planer geometry) around Cu(II) complex, conductivity measurement in DMF showed that the complex has electrolytic nature (Ballhausen, 1962), figure (7) and figure (8).

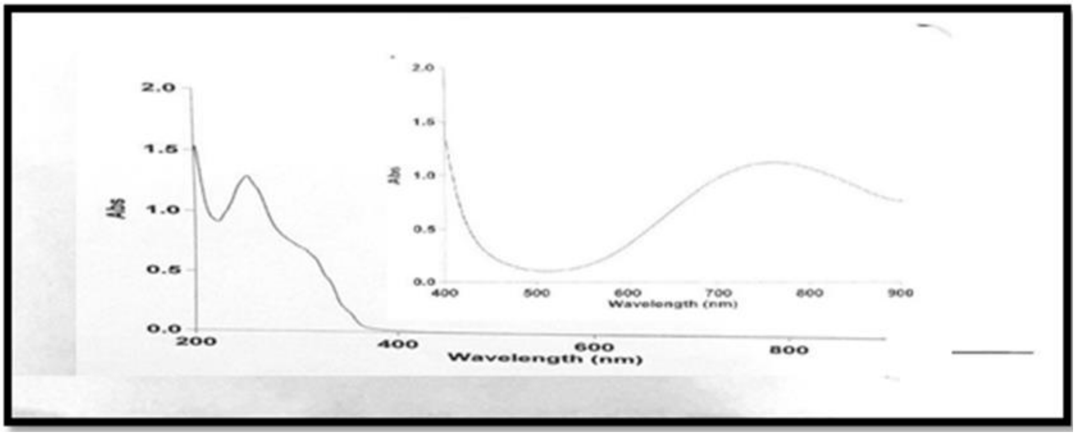


Figure (7) Uv.Vis spectrum of copper complex

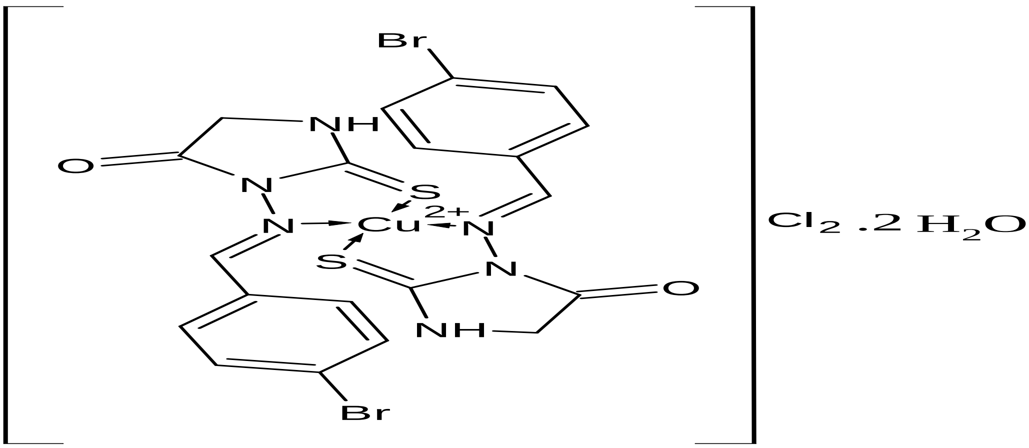


Figure (8) Proposed chemical structure of copper complex

Serum GSH

Table (3) demonstrated that values of serum GSH concentrations of animals of GII group that treatment by 0.5% hydrogen peroxide in drinking a significant reduction after thirty days and sixty days compared with control group. while a significant elevation in serum GSH concentrations in GIII group after treatment with copper complex of 2-Thioxo Imidazolidin-4-Ones as antioxidants.

Serum Catalase

Table (4) showed serum Catalase concentrations of animals of GII group that treatment by 0.5% hydrogen peroxide in drinking a significant reduction after thirty days and sixty days compared with control group, while a significant elevation in serum Catalase concentrations in GIII group after treatment with copper complex of 2-Thioxo Imidazolidin-4-Ones as antioxidants after sixty days of experimental Periods that means the antioxidant activity ability of extract in limited periods.

Serum total antioxidant

The results that show in table (5) after thirty and sixty days the serum antioxidant concentration levels at GII were appear a significant reduction when compared with control group. The result of antioxidant of GIII group after thirty and sixty days was appeared a significant elevtion when compared with GII and control group

Table (3) Effects of 2-Thioxo Imidazolidin-4-Ones and its copper complex on GSH (µg\ ml) in male rats exposed to 0.5% hydrogen peroxide in drinking

	30 Days			60 Days		
	Mean	±	SD	Mean	±	SD
Control	0.072	±	0.004 Aa	0.067	±	0.006 Ab
GII\H2O2	0.050	±	0.011 Ab	0.033	±	0.004 B c
GIII\cu	0.075	±	0.007 A a	0.078	±	0.008 A a

Table (4) Effects of 2-Thioxo Imidazolidin-4-Ones and its copper complex 0n Catalase (U/mL). in male rats exposed to 0.5% hydrogen peroxide in drinking

	30 Days			60 Days		
	Mean	±	SD	Mean	±	SD
Control	0.063	±	0.005 A b	0.066	±	0.003 A b
GII\H2O2	0.045	±	0.008 A c	0.037	±	0.006 B c
GIII\cu	0.068	±	0.005 A ab	0.074	±	0.003 A a

Table (5) Effects of 2-Thioxo Imidazolidin-4-Ones and its copper complex on antioxidant (µmol/mL) in male rats exposed to 0.5% hydrogen peroxide in drinking water for 60 days

	30 Days			60 Days		
	Mean	±	SD	Mean	±	SD
Control	0.061	±	0.007 A b	0.060	±	0.007 A b
GII\H2O2	0.040	±	0.006 A c	0.028	±	0.004 B c
GIII\cu	0.074	±	0.008 B a	0.082	±	0.009 A a

3. Results and Discussion

Results of our study show a significant reduction of serum GSH concentrations of animals of GII group that treatment by 0.5% hydrogen peroxide in drinking water, The reason maybe dueto the vital roles of GSH inn reduction and oxidation reaction, and that givingghydrogen peroxides in drinking waters works to empty "GSH" into the tissue and blood, Martin and his group indicated that states of oxidative stresses lead to increasing in oxidations of "GSH" to theedisulfide form offGSSG by inhibitinggthe pathways of its conversion to pentoseephosphate shunts, which determine the productions of NADPH necessaryyfor the activity of theeglutathione reductases enzyme too restore "GSH" synthesis (Martins et al,.1985). The reason for the decrease in catalase is due to the fact that it removes hydrogen peroxide molecules into water and oxygen (Clavel et al.,1985). Giving them hydrogen peroxide in the drinking water, which caused the generations of free radical and increase in freeeradicals leads to a decrease in the concentrations of antioxidant in theebody that leads to damages to various tissues of the body (Dalle-Donne et al,.2006). Weak antioxidant defense systems are a significant indicator that cells are in a state of oxidative stress. In many pathological conditions that cause oxidative stress, the activity of free radicals increases. High levels of ROS in mitochondria can result in free radical attack of membrane phospholipids and cause mitochondrial membrane depolarization (Zhou et al,.2009).

With treatment of copper complex of 2-Thioxo Imidazolidin-4-Ones as antioxidants, antioxidant levels were significantly increased to (0.074) and it was observed that GSH was increased to (0.075), Catalase was increased to (0.068), These results are in accordance with (Nafie et al.,2021) antioxidant levels were significantly increased, Because the treatment caused the regulation of apoptosis. Apoptosis usually occurs via the mitochondrial (intrinsic) pathway and/or the death receptor (extrinsic) pathway. Since GSH and Catalase deficiency is associated with mitochondrial dysfunction and cell death, antioxidant levels increased.

On another hand the Study showed that the compounds Cu (II) have relatively high antioxidant activity (Patel et al.,2012), so serum antioxidant concentrations of animals of GIII record significant elevtion and these results are in compatible with studies by (Aburas et al,.2013), that mentioned Copper complexes showed a significantly higher antioxidant activity.

4. Conclusions

1) In general, the present work describes the reaction of 4-bromobenzaldehyde with thiosemicarbazide to produce (2*E*)-2- [(4 bromophenyl) methylidene] hydrazine-1-carbothioamide; this product reacted with chloroethylacetate to form thioxoimidazolidine, the final products (L).
2) These ligands were characterized by (UV-Vis, IR and 1HNMR).
3) From this ligand, copper complex has been prepared and characterized by available measurements like ―elementan analyses‖ "(CHNS)", spectral (UV-Vis, IR and 1HNMR), molar conductivity, atomic absorption spectroscopy and magnetic susceptibility to determine the coordination behavior.
4) Copper complex of ligand has an anti-oxidative activity that revealed by an increase in serum GSH, Catalase and total antioxidants.

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