Protective Effect of Alpha - Lipoic Acid against Copper Sulfate Hepatorenal Toxicity in Albino Rats

Khalil Gazar Chelab Al-Nailey, Atiaf Ghanim Rhyaf AL-Na'ely, Noora Kadhim Hadi Alyasari

*Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq.
** Department of Soil Science & Water Resources, College of Agriculture, University of Al-Qadisiyah, Diwaniyah, Iraq.
khalil.Chelab@qu.edu.iq

Abstract

The study was conducted on 60 albino rats, approximately one month old, of the same weight (200-220gms), and they were divided equally into 4 groups as follows: the first group was drenched by stomach tube 40 mg/kg BW CuSo4 for two months. The second group was gavage by stomach tube 40 mg/kg BW CuSo4 and injected with alpha - lipoic acid (ALA) (100 mg/kg BW intraperitoneally once daily) for two months. The third group was injected with alpha lipoic acid (100 mg/kg BW intraperitoneally once daily) for two months and the fourth group as the control group received only I/P 0.2 ml of normal saline once daily for two months. After two months all animals were sacrificed after the collection of blood for biochemical tests and livers and kidneys were taken for histopathological examination. In the biochemical study, the results showed that 1st group showed a significant increase ($P \leq 0.05$) in the levels of the liver enzymes (ALT, AST, and ALP) in comparison with the control group, whereas 2nd group showed a non-significant increase ($P > 0.05$) in comparison with the control group and a significant decrease ($P \leq 0.05$) as compared with 1st group (CuSo4 group). The microscopic examination of the histopathological sections of livers and kidneys of 1st group animals showed high severe changes or lesions in both livers and kidneys due to the pathotoxic effect of CuSO4 on these organs, whereas these microscopic changes showed marked decreased or mild severity in the 2nd group (CuSo4 + ALA) due to the protective and ameliorative effect of ALA against the toxicity of CuSo4.

Keywords: Effect. ALA. CuSo4. Hepatorenal. Toxicity

DOI Number: 10.14704/nq.2022.20.5.NQ22637 **NeuroQuantology 2022; 20(5):3359-3373**

Introduction

to 5 water molecules. Therefore, the most common hydrated form of it is CuSO4.5 H2O (2).

The copper sulfate is worldwide used as an algaecide and a fungicide in aquaculture and agriculture (3). It is mainly used for agricultural purposes, as an insecticide and bactericide, as a food additive for plants, and as a soil additive (4). It is commonly used as a pesticide (5). Also it is also used as a basic material in the manufacture of other copper compounds, as well as a reagent in analytical chemistry (6), and as an

Copper sulfate is commonly referred to as Bluestone or Blue Vitriol. It's an inorganic compound that combines sulfur with copper and we use it in dyeing, so it works great as a dyeing agent. Likewise, it also plays the role of a catalyst in some organic reactions. Mostly it works as a fungicide to treat fruit and vegetable diseases (1). The chemical formula for copper sulfate is CuSO4. Similarly, it has a molecular weight of 249.99 g mol-1. Furthermore, this compound is generally found as a hydrated salt with between 1

powerful antioxidant in the body, with several other benefits. This acid is considered an antioxidant, thus, it can eliminate free radicals in the body (17). There is also enough scientific evidence to show that ALA can be recycled and recycled into other antioxidants, such as glutathione, vitamin E and vitamin C. It helps remove toxins in the liver as a result of mineral pollutants and tries to protect the liver and lungs in case of smoking. It stimulates the body to produce glutathione (18), which helps absorb coenzyme Q10 (19), which are also considered antioxidants. Thus, our main objective is to evaluate the possible protective effect of ALA on CuSo4-induced hepatorenal toxicity in albino rats.

Ethics Statment

The study was conducted according to the national guidelines for the Care and Use of Laboratory Animals. All protocols were approved by the High Committee for Review and Approval of Research Proposals of the Faculty in the University of Qadisiyah college of Veterinary Medicine

MATERIALS AND METHODS

Animal models

Sixty healthy adult rats (1 month old and weighing 200-250 g) albino rats were obtained from Animal House of Veterinary Medicine college/ University of Al-Qadisiyah, Iraq. All rats were fed ad libitum and were grouped (n = 15 per cage) in standard plastic cages in an air-conditioned room with temperature maintained at 25 ±2 C. Before experimentation, the animals were acclimatized for 7 days at 12 h light/dark cycle.

electrolyte for batteries and electroplating basins (7), and in medicine it is used as a fungicide and bactericide (8). This substance can cause harm through contact with eyes or skin. And contact with the skin may lead to itching or eczema, and to the eye with conjunctivitis, inflammation of the lining of the eyelid, ulceration and hypertrophy of the cornea (9). When ingested, it is moderately toxic to an adult, and due to its irritating effect on the digestive system, vomiting, diarrhea, abdominal pain and loss of appetite begins spontaneously (10). If it is retained in the stomach, symptoms may be severe such as a burning sensation in the chest, nausea, diarrhea, vomiting, headache, and injury to the brain, stomach, liver or kidneys may occur (11).

Copper sulfate is a powerful oxidizing agent, which is corrosive to mucous membranes. Concentrated solutions are acidic with pH 4. Cellular damage and cell death may result from excessive copper accumulation through which free reduced copper in the cell binds to sulfhydryl groups and inactivates enzymes such as glucose-6-phosphate dehydrogenase and glutathione reductase (12).

Alpha lipoic acid (ALA), it is an organosulfur compound derived from octanoic acid (Caprylic acid). It contains two sulfur atoms (at C6 and C8) connected by a disulfide (dithiolane) bond chain (13). Alpha lipoic acid is a naturally occurring compound that is produced in small amounts by plants, animals and humans (14), but it is also found in a variety of foods and as a supplement, and research suggests that it may play a role in weight loss (15) and help treat diabetes and other health conditions (16).

ALA has gained a lot of attention in recent years, and it is an organic compound that acts as a

eISSN 1303-5150

Study groups and treatment of animals

All rats were randomized into four groups of 15 rats each and were treated for two months. The first group gavages by stomach tube 40 mg/kg BW Copper sulfate (CuSo4) (20) for two months. The second group was gavage by stomach tube 40 mg/kg BW CuSo4 and injected with ALA (100 mg/kg BW intraperitoneally once daily) (21) for two months. The third group was injected with alpha lipoic acid (100 mg/kg BW intraperitoneally once daily) for two months and the fourth group as the control group it received only I/P 2.0 ml of normal saline once daily for two months.

Blood collection

The rats were sacrificed under ethyl ether anesthesia after 24 hours of the last dose. Blood collection was done at two months of experiment via abdominal vein and then centrifuged at 2000 rpm for 15 minutes after allowing the blood to clot for 2 hours at room temperature. The sera from all rats were kept at –20°C until the proceeding of the biochemical tests (AST, ALT, and Alkaline phosphatase (ALP)).

Biochemical Tests

Alkaline phosphatase (ALP)

ALP is an enzyme originated mainly in the bone, liver and placenta, with some activity in the kidney and intestine, It is called alkaline because it functions best at a pH of 9 (22). Serum ALP concentration was enzymatically measured using standard assay (ALP-kit) (Biomerieux/France) alkaline phosphates activity in serum samples was estimated spectrohotometrically by employing (king and Armstrong) method, in which the disodium phenyl phosphate is hydrolyzed with liberation of phenol and formation of sodium phosphate.The amount of liberated Phenol is read on absorbance at 510 Nm, by using the following equation:

$$\text{ALP} = \frac{\text{Test - blank}}{\text{Standard - blank}} \times 20 = \text{IU\dl}$$

AST and ALT:

AST and ALT concentrations were enzymatically measured using standard assay (AST, ALT-Kit) (Biomerieux /France) (23). AST and ALT enzymes catalyze the transfer of the amino group of glutamic acid to oxaloacetic acid and pyruvic acid in reversible reaction. The transaminase activity is proportional to the amount of oxaloacetate or pyruvate formed over a definite period of time and is measured by a reaction with 2,4- Dinitrophenhydrazin (DNPH) in alkaline solution at wave length of 510 Nm. The following formula indicated these reactions:-

$$\text{AST: Aspartate} + \alpha - \text{Ketoglutarate} \xrightleftharpoons{\text{AST}} \text{Oxaloacetate} + \text{L-glutamate}$$

$$\xrightleftharpoons{\text{ALT}}$$

eISSN 1303-5150

ALT: Alanine + α - Ketoglutarate ⇌ Pyruvate + L-glutamate

normal values (P< 0.05) (23.33 ±0.71). However, there is no significant difference (P> 0.05) in the 2nd group (CuSo4 + ALA group) (29.17±2.89) in comparison with that of the control group. Also, the result of ALT of 3rd group (received alpha lipoic acid alone for 2 months), showed non–significant change (P> 0.05) (26.71±3.03) compared to the control group.

Aspartate aminotransferase (AST): The result of AST values increased significantly (P< 0.05) in the CuSo4 group (284±12.48) in relation to the control group. But there is non significant difference (P> 0.05) in the AST values of the 2nd group (CuSo4 + ALA group) (170.3±6.61) in comparison with the control group (161.6±07.13), whereas the AST values of the 3rd group showed a non significant difference (P> 0.05) (162.3±18.02) in comparison with the control group as listed in the table (1). The values of Serum Alkaline Phosphatase (ALP) showed a significant difference (P< 0.05) in the 1st group animals (423±19.31) in comparison with the control group (301±16.76). The ALP values of 2nd group animals were showed non – significant change (P> 0.05) (317.3±61.49) relative to the control group. Also the ALP values of 3rd group animals demonstrated non significant change (P>0.05) (281.3±24.1) in comparison with the control group as listed in the table (1).

Histopathological Examination:

For light microscopic examination, liver and kidney specimens from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4 µm thickness and stained with hematoxylin and eosin (24).

Statistical analysis

All the grouped data were statistically read by SPSS program, Version 17 software (2010).Testing methods including one way ANOVA for comparisons among groups followed by least significant differences (LSD) test for comparison between two groups. P values of $p \leq 0.05$ were considered to record statistical significance. All data were expressed as means ± standard error (SE) (25).

RESULTS

Biochemical Test

Alanine aminotransferase (ALT): Daily administration of Copper sulfate (CuSo4) (40mg\kg B.W) for 2 months on the ALT (IU/ dL) in CuSo4 group rats are shown in table (1). The ALT values increased significantly (P< 0.05) in the CuSo4 group (58.31±7.89) in comparison with that of the control group which showed

TABLE 1: Serum liver enzyme tests (ALT, AST and ALP) in different rat groups

Groups	ALT IU/dl Mean ± SE)	AST IU /dl (Mean ± SE)	ALP IU/dl (Mean ± SE)
1st group CuSo4 alone	58.31±7.89 b	284±12.48 b	423±19.31 b
2nd group CuSo4 + ALA	29.17±2.89 a	170.3±6.61 a	317.3±61.49 a
3rd group ALA alone	26.71±3.03 a	162.3±18.02 a	281.3±24.1 a
4th group Control	23.33 ±0.71 a	161.6±07.13 a	301±16.76 a

second group (2nd group): The liver section showed radially arranged hepatocytes as hepatic cords around the central vein which showed slightly congested and also there is a proliferation of Kupffer cells and mild hemorrhage of sinusoids. Few hepatocytes showed steatosis (fatty change) as fig. (5). Mild degeneration of hepatocytes. But most of these cells showed normal hexagonal shape with little proliferation of Kupffer cells. Hyperplasia of hepatocytes which showed as binucleated cells as fig. (6). The third group (3rd group): There is a central vein with radially arranged cords of hepatocytes with blood sinusoids lined by flat endothelial and Kupffer cells located between them. There are hepatocytes with acidophilic cytoplasm. Some hepatocytes were binucleated as in fig. (7). The fourth group (4th group) as (control group): It showed no histopathological changes in the liver sections of this group of animals and this microscopic appearance were radially arranged of hepatic cords around the normal central vein. The hepatocytes showed acidophilic cytoplasm with prominent and central nuclei as in fig. (8).

The similar letters refer to the non–significant differences while the different letters refer to the significant differences at (P≤ 0.05), [CuSo4: Copper sulfate, ALA : alpha lipoic acid.

Histopathological Examination:

Livers:

The first group (1st group): Examination of liver sections of copper sulfate-exposed rats showed that the liver had inflammatory cellular infiltration with suppurative exudate. Also, there is severe vacuolation and degeneration of hepatocytes as in fig. (1). Severe fatty change (steatosis) was observed in the hepatocytes in which it appeared as a signet-like shape due to the peripheral site of their nuclei and presence of foamy Macrophages (Macrophages engulf fat) as fig. (2). There is inflammatory cellular infiltration mainly macrophages and lymphocytes. Also there is severe vacuolation of hepatocytes as fig. (3). Also, there is severe necrosis, vacuolation, and degeneration of hepatocytes as fig. (4). The

eISSN 1303-5150

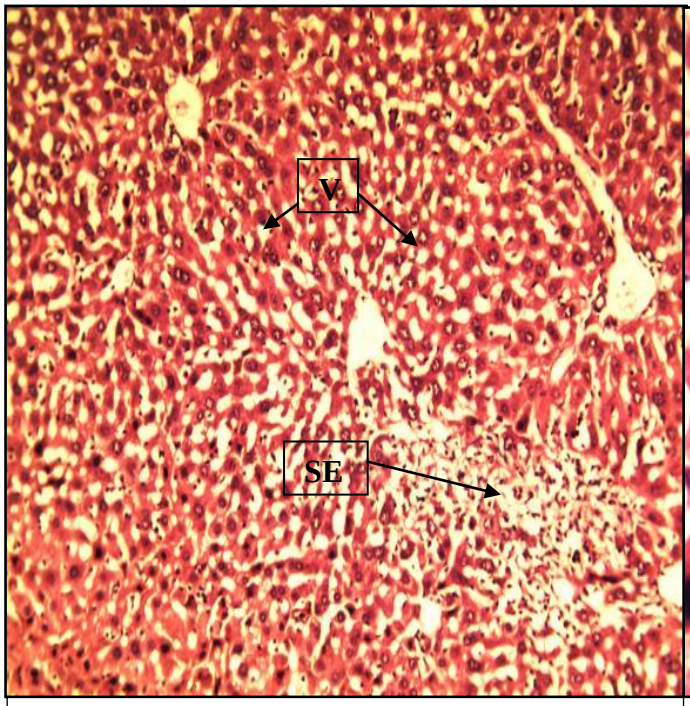

Fig (1): Liver of rat (1st group). There is inflammatory cellular infiltration with suppurative exudate (SE). Also there is severe vaculation (V) and degeneration of hepatocytes. 10X H&E.

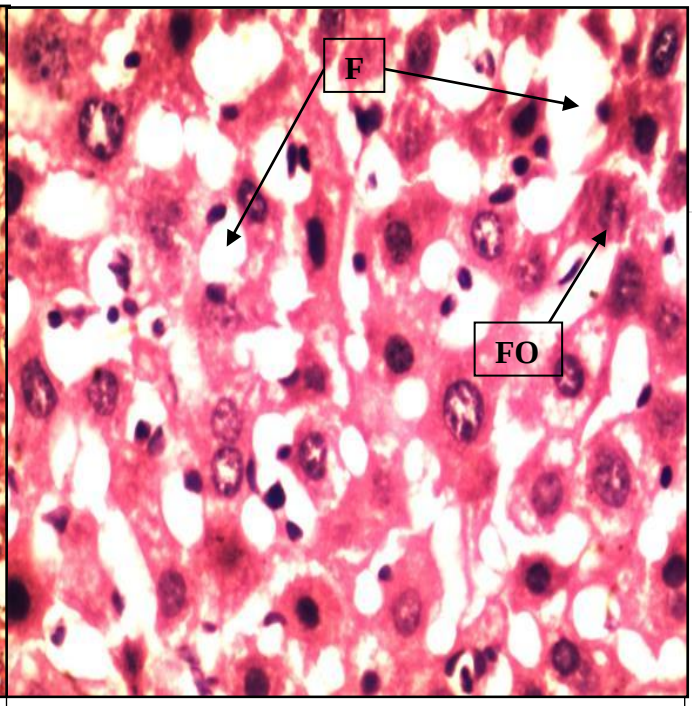

Fig (2): Liver of Rat (1st group). There is sever fatty change (steatosis) (F) were observed in the hepatocytes in which it appeared as signet-like shape due to the peripheral site of their nuclei. Presence of foamy Macrophage (FO).40X H&E.

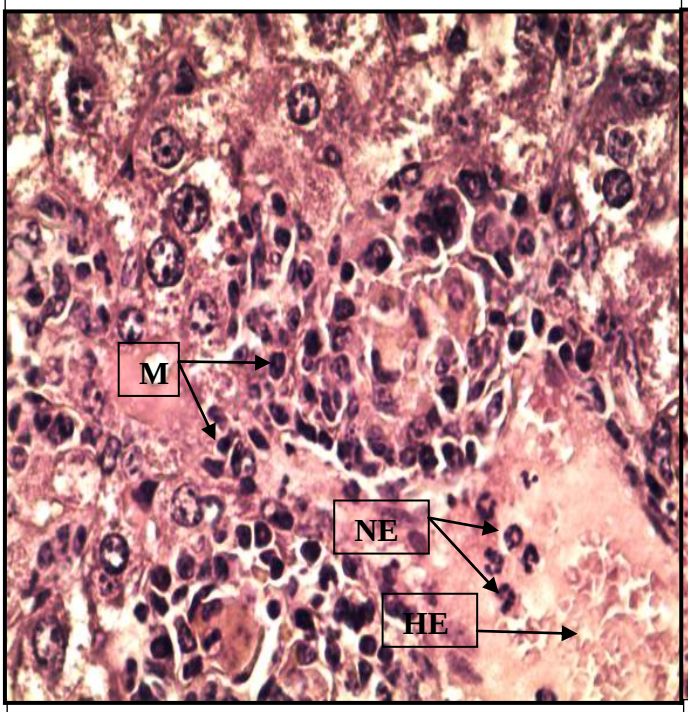

Figure (3): Liver of rat (1st group). There is inflammatory cellular infiltration mainly macrophages (M) and lymphocytes. Also there is severe vaculation of hepatocytes. Severe hemorrhage (HE) with pavementing neutrophils (NE). 40X H&E.

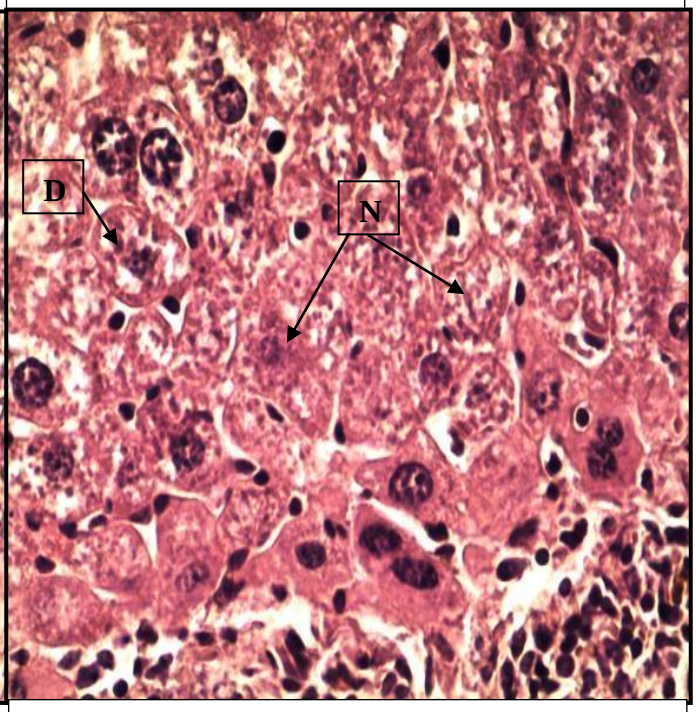

Figure (4): Liver of rat (1st group). There is inflammatory cellular infiltration (M). Also there is severe necrosis (N) and degeneration (D) of hepatocytes. 10X H&E.

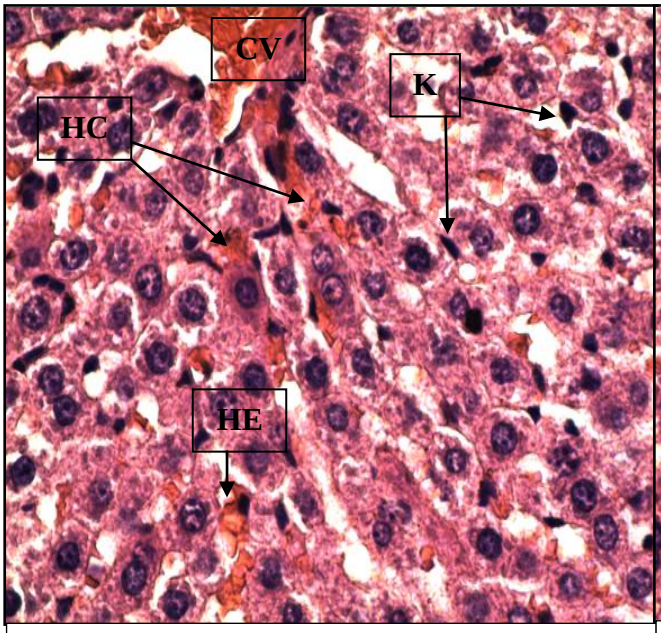

Fig (5): Liver of rat (2nd group). Radially arranged hepatocytes as hepatic cords (HC) around the central vein which showed slightly congested (CV). Also there is proliferation of Kupffer cells (K) and mild hemorrhage of sinusoids (HE). Few hepatocytes showed steatosis (fatty change) (F). 40X H&E.

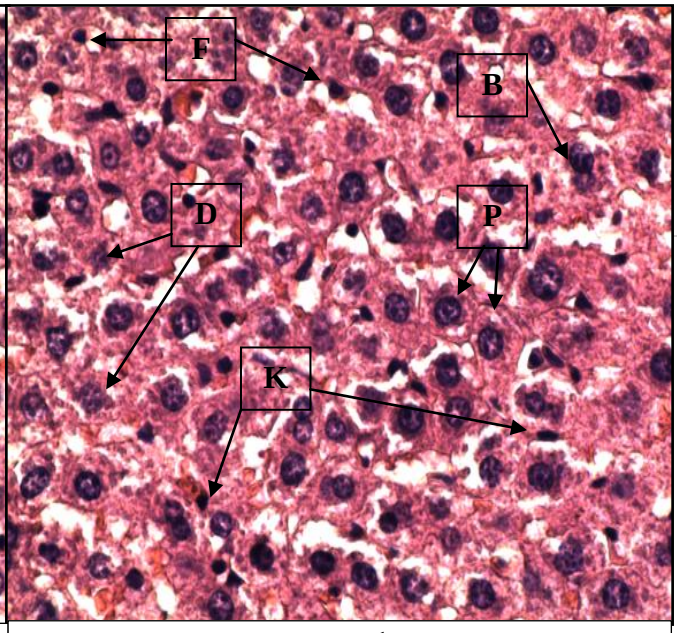

Fig (6): Liver of rat (2nd group). Note mild degeneration of hepatocytes (D). But most of these cells showed normal hexagonal shape (P) with few proliferation of Kupffer cells (K). Hyperplasia of hepatocytes which showed as binucleated cells (B) with mild steatosis (F). 40X H&E.

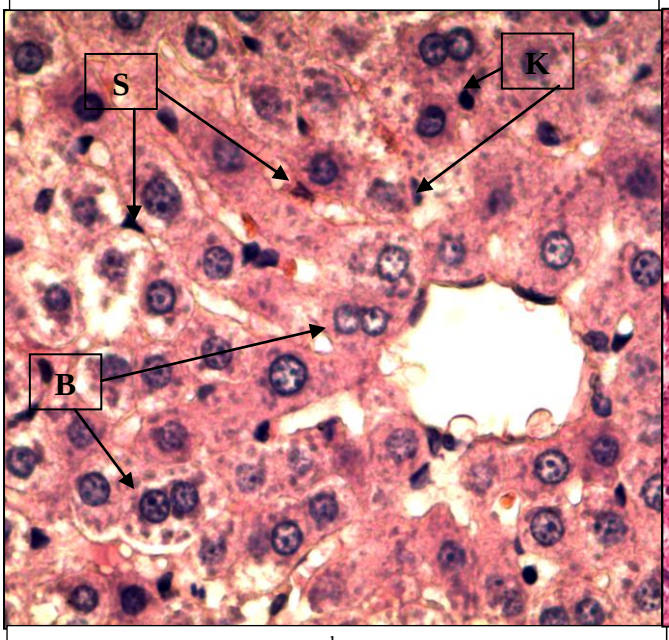

Fig (7): Liver of rat (3rd group). There is central vein with radially arranged cords of hepatocytes with blood sinusoids lined by flat endothelial (S) and Kupffer cells located between them (K). There are hepatocytes with acidophilic cytoplasm. Some hepatocytes were binucleated (B). 40 H&E.

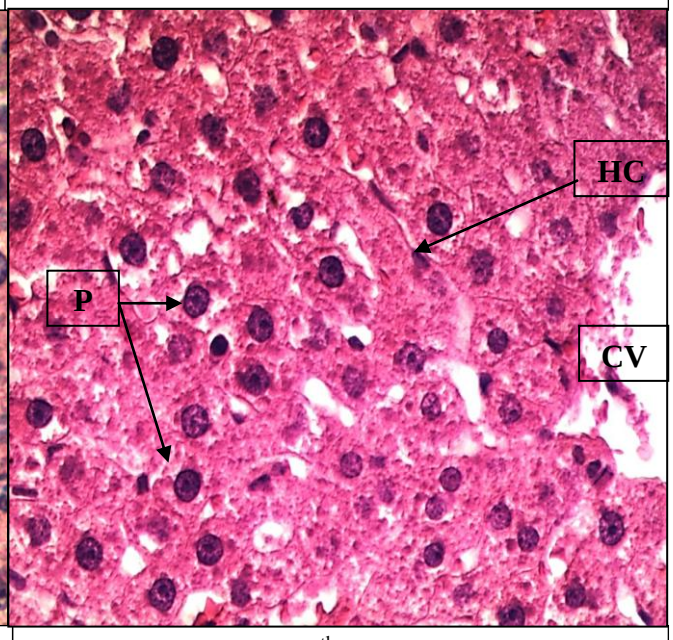

Fig (8): Liver of rat (4th group) as control group. There is radially arrangement of hepatic cords (HC) around normal central vein (CV). The hepatocytes showed with acidophilic cytoplasm with prominent central nuclei (P). 40X H&E.

eISSN 1303-5150

epithelial cells of renal convoluted tubules as in fig (12).

The third group (3rd group): the results of this group of animals showed normal glomeruli with high cellularity and normal renal convoluted tubules as in fig (13) and normal cuboidal cells which line these tubules as in fig (14).

The fourth group (4th group) as a control group: It showed no histopathological changes in the kidney sections of this group of animals and this microscopic appearance were a normal arrangement of cortical and medullary elements which characterized by the presence of high cellularity glomeruli and normal, narrow and small proximal convoluted tubules as in fig (15) & (16).

Kidneys:

The first group (1st group): Examination of kidney sections of copper sulfate-exposed rats showed that marked necrosis and sloughing of epithelial cells of proximal convoluted tubules. Massive infiltration of inflammatory cells mainly macrophages and lymphocytes and there is congestion within the kidney tissue as in fig (9). Dilation of the renal convoluted tubules and severe necrosis with degeneration of epithelial cells that line these tubules. The glomerulus was atrophied as in fig (10).

The second group (2nd group): There is mild dilation of renal convoluted tubules, few hemorrhage, normally organized glomeruli, and regeneration of renal convoluted tubules epithelium as in fig (11) with hyperplasia of

*e*ISSN 1303-5150

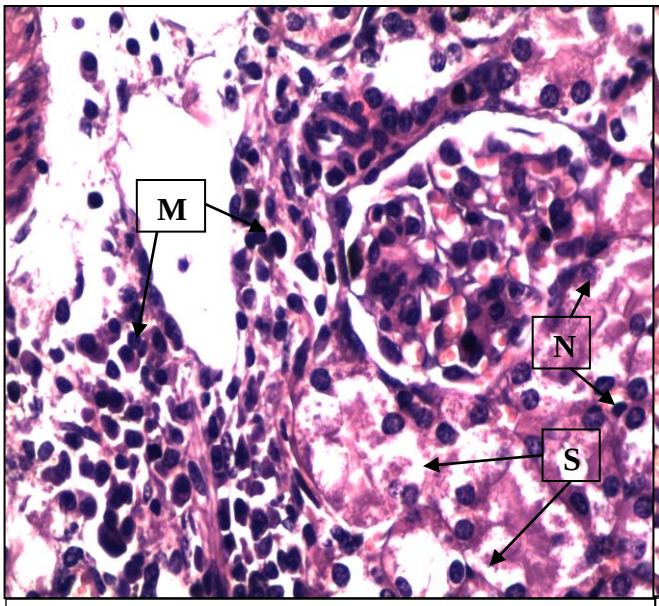

Fig (9): Kidney of rat (1st group). Note marked necrosis (N) and sloughing of epithelial cells of proximal convoluted tubules (S). Massive infiltration of inflammatory cells mainly macrophages and lymphocytes (M). 40X H&E.

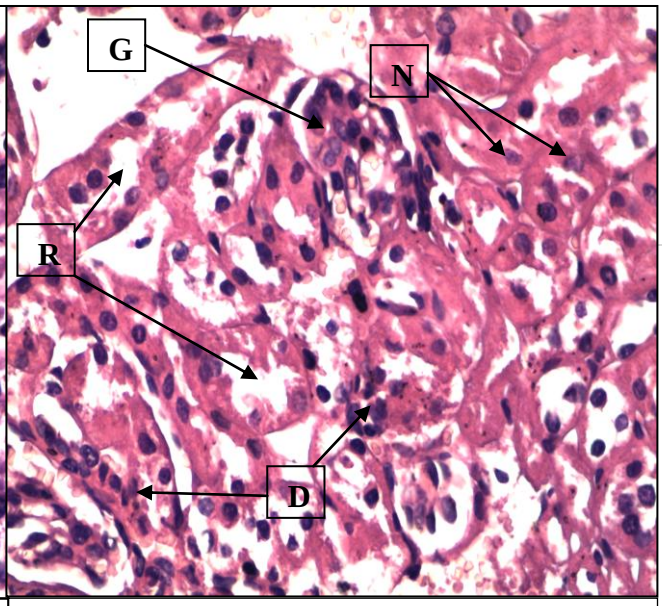

Fig (10): Kidney of rat (1st group). Dilation the renal convoluted tubules (R) and severe necrosis (N) with degeneration of epithelial cells that line these tubules (D). glomerulus was atrophied (G). 40X H&E.

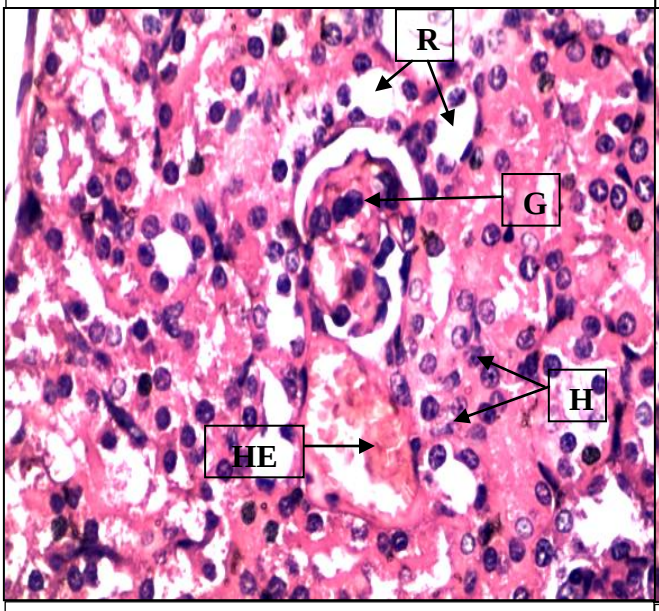

Fig (11): Kidney of rat (2nd group). Note mild dilation of renal convoluted tubules (R), few hemorrhage (HE), normal organized glomeruli (G) and regenerative with hyperplastic of renal convoluted tubules epithelium (H). 40X H&E.

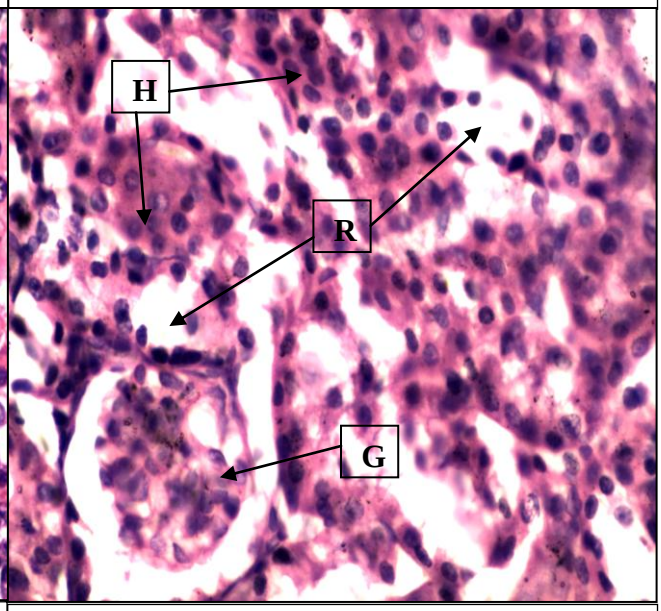

Fig (12): Kidney of rat (2nd group Proliferating and circled glomerulus (G). Mild dilation of renal convoluted tubules (R) and hyperplasia of epithelial cells of renal convoluted tubules (H). 40X H&E.

eISSN 1303-5150

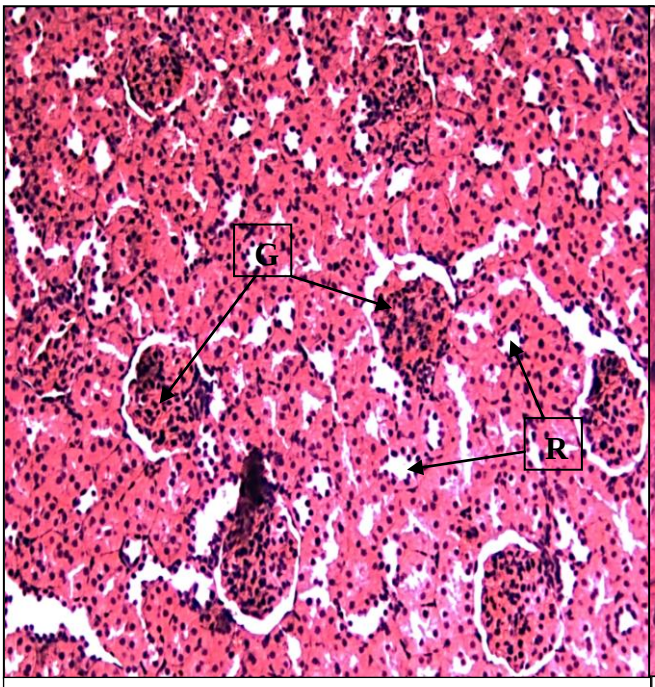

Fig (13): Kidney of rat of 3rd group. Note normal glomeruli with high cellularity (G) and normal renal convoluted tubules (R). 10X H&E.

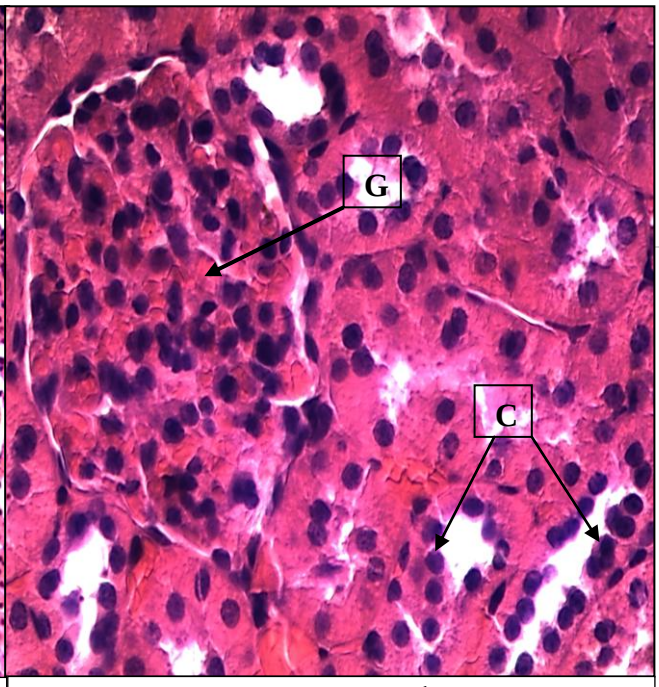

Fig (14): Kidney of rat of 3rd group. Normal renal convoluted tubules which lining with normal cuboidal cells (C). Normal glomeruli with high cellularity (G). 40X H&E

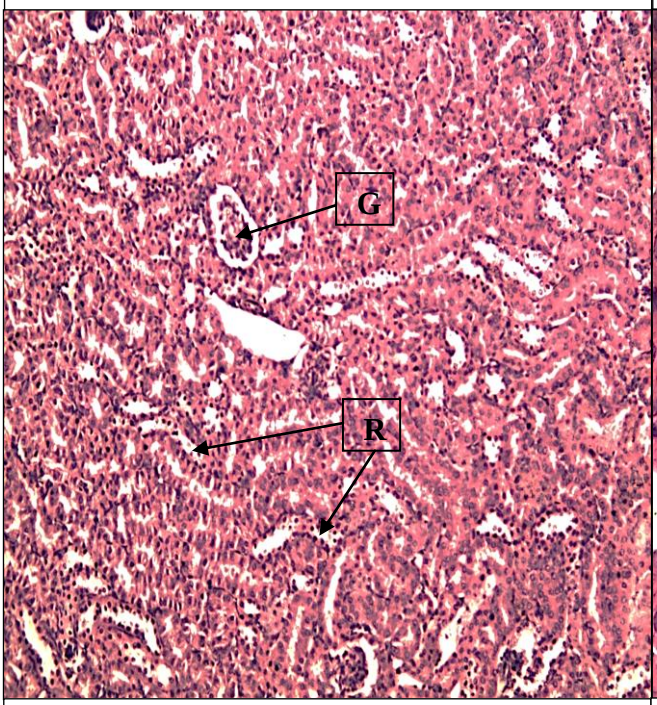

Fig (15): Kidney of rat as control group (4th group). Note normal arrangement of cortical and medullary elements (high cellularity glomeruli (G) and normal proximal convoluted tubules (R)). 10X H&E.

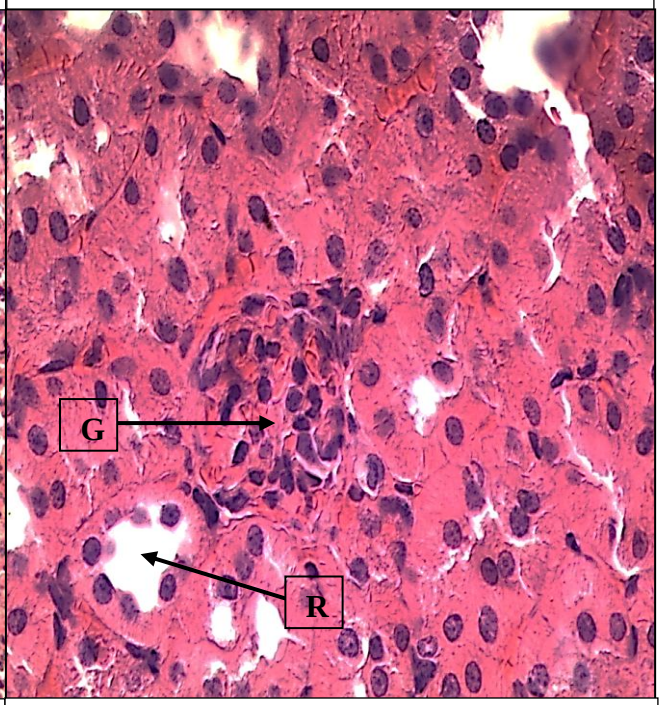

Fig (16): Kidney of rat as control group (4th group). Note normal glomerulus (G) and normal, narrow and small proximal convoluted tubules (R). 40X H&E.

eISSN 1303-5150

decreased (P≤ 0.05) compared with 1st group (CuSo4 alone group) in the levels of these liver enzymes (ALT, AST, and ALP) in the serum suggesting that it offers protection by preserving the structural integrity of the hepatocytes. Administration of ALA preserved the integrity of the hepatocellular membrane via decreased production of reactive oxygen species and amelioration of the oxidative damage i.e. the leakage of liver enzymes because of liver injury was preserved by the hepatocyte membrane stability via the action of ALA. These results coincide with previous studies (28) that reported the ALT and AST values increased significantly (P≤0.05) in copper sulfate-treated albino rats. Other studies indicate the ameliorative effect of ALA for the liver tissue (29) showed that ALA enhanced free radical scavenging and antioxidant status. Also (30) proved that ALA can protect or reduce liver toxicity after exposure to cisplatin via reducing the levels of liver enzymes like ALT and AST. Other findings showed that ALA and/or sesame oil (SO) administration can reduce the toxic effects of Diazinon (DZN) via their potent antioxidant and free radical-scavenging activities (31).

Examination of liver and kidneys sections of copper sulfate-exposed rats showed severe pathological lesions in the histological structure of these tissues, which were severe damage as previously mentioned in the results of the histopathological examination of these organs, compared to the control group. This explains the severe pathotoxic effect of copper sulfate on these organs because these organs are considered the main target organs for CuSo4. This evidence is supported with other studies, (32) indicated that

DISCUSSION

There are three liver enzymes that are commonly used in the diagnosis of liver disease; they are Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP). Injury to the liver, whether is acute or chronic by toxic substances eventually results in an increase in serum concentrations of aminotransferases (26). The abnormal elevation of liver enzymes such as ALT, AST and ALP can be taken as an index for liver injury or disease. These enzymes are normally present in the serum and tissues of the body; especially the tissues of liver, the researchers attributed increase serum activities of ALT, and AST to the increased cellular basal metabolic rate, irritability, and destructive changes of the liver and skeletal muscles cells (27). In the present study, Administration of intraperitoneally (40 mg\kg B.W) of copper sulfate for 2 months caused a significant increase (P≤ 0.05). In the levels of hepatic marker enzymes in serum (ALT, AST, and ALP), this may be due to the leakage of the enzymes to the bloodstream. The increase in the levels of these enzymes in the serum indicates the liver damage and alteration in the liver function due to exposure to copper sulfate, the exact mechanism of copper sulfate involved in the elevation of these enzymes may be due to the hepato-cellular damage with severe necrosis and vacuolation of hepatocytes, resulting in increased plasma membrane permeability led to leakage of these enzymes from the hepatic tissue into the bloodstream. This is because copper sulfate is known to produce oxidative damage in the liver tissue via production of reactive oxygen species (ROS). Treatment with ALA significantly

eISSN 1303-5150

formation of ceruloplasmin. The main target organs of CuSO4 are livers and kidneys which lead to hepatorenal toxicity (32).

expression of COX-2, lipid peroxide, and DNA fragmentation. In contrast, SOD and glutathione (GSH) levels were inhibited. Through this molecular mechanism, the copper sulfate is a catalyst for oxidative stress via the generation of ROS and causing damage to the activity of both free radicals and antioxidants in the body; this defect may lead to damage to the cells and tissues. Several previous studies showed that ALA possesses an opposite molecular mechanism to that of CuSo4, which was mentioned above. Several pieces of evidence indicate that ALA exerts potent antioxidant activity in vitro and in vivo (35).

ALA can cause reducing in the production of ROS which resulting from copper sulfate exposure via decreasing in the MDA levels and exhaled nitric oxide (eNO) expression then impaired anti-oxidative capacity and NO secretion (36). But it can cause increasing in SOD, CAT activities, GSH (38), G6PD, and GPx activities (39).

Conclusion

The present findings show that the administration of ALA has a significant effect in controlling hepatotoxicity and nephrotoxicity in copper sulfate-treated rats. Since it has an opposite mechanism to copper sulfate toxicity, it would make it a promising protective agent against the CuSo4-induced biochemical and histopathological toxicity.

the copper taken up from the gastrointestinal tract enters the systemic circulation and binds to plasma amino acids and albumin, where it is transported to the liver and participates in the

While the histopathological examination of liver and kidneys tissues of the second group showed less severe or mild changes. These regenerative changes in the livers and kidneys tissues greatly explained the protective and ameliorative effect of ALA against the toxicity of copper sulfate.

The livers and kidneys in both the 3rd and 4th (control) groups showed normal histological structures in the hepatic and renal tissues.

The mechanism by which copper sulfate caused pathotoxic changes in the tissues of the livers and kidneys is that the copper sulfate is able to induce oxidative stress by accelerating the production of highly reactive O2 species (ROS) and causing lipid peroxidation (33).

Several previous studies have shown that copper sulfate has a direct effect on the antioxidant enzymes, thus causing the generation of reactive oxygen species and lipid peroxidation. Ahmed et al., 2000 indicated that the CuSo4 can cause an increase in the Malondialdehyde (MDA) levels, superoxide dismutase (SOD), and catalase (CAT) activities. But it can cause decreasing in glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), and glutathione peroxidase (GPx) activities. Also (34) showed that the ingestion of a toxic dose of CuSO4 induced the elevation of serum biomarkers, including AST, Lactate Dehydrogenase (LDH), and the inflammatory marker hepatic nitric oxide (NO), C-Reactive Protein (CRP), protein

10- Xu Shiwen, Chi Yumei. Copper deficiency and livestock disease Heilongjiang animal husbandry and veterinary [J]. 1998.4 (3): 40-41.
11- James LP, Stowe CD, Argao E. Gastric injury following copper sulphate ingestion. Pediatr Emerg Care 1999;15:429-31.
12- Saravu K, Jose J, Bhat MN, Jimmy B, Shastry BA. Acute ingestion of copper sulphate: A review on its clinical manifestations and management. Indian J Crit Care Med 2007;11:74-80.
13- Yadav, V.; Marracci, G.; and Bourdette, D. N. (2008). Lipoic Acid-A Novel Therapeutic Approach for Multiple Sclerosis. *Mult Scler*.; pp:12-15.
14- Carreau J. P. (1979). Biosynthesis of lipoic acid via unsaturated fatty acids. *Methods Enzymol*.; 62:152-158.
15- Seo, E.; Ha, A.; and Kim, W. (2012). α-Lipoic acid reduced weight gain and improved the lipid profile in rats fed with high fat diet. *Nutrition Research and Practice;* 6:195-200.
16- Bajaj, S.; and Khan, A. (2012). Antioxidants and diabetes. *Indian Journal of Endocrinology and Metabolism;* 16:S267-S271.
17- Shay, KP; Moreau, RF; Smith, EJ; Hagen, TM (June 2008). "Is alpha-lipoic acid a scavenger of reactive oxygen species in vivo? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity". IUBMB Life. 60 (6): 362–7. doi:10.1002/iub.40. PMID 18409172. S2CID 33008376
18- Suh, J. H.; Shenvi, S. V.; and Dixon, B. M. (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci U S A*.; 101(10):3381-3386.
19- Bast, A.; and Haenen, G. R. (1988). Interplay between lipoic acid and glutathione in the protection against micro-somal lipid peroxidation. *Biochim Biophys Acta*.; 16;963(3):558-61.

references

1- Naha K, Saravu K, Shastry BA. Blue vitriol poisoning: a 10-year experience in a tertiary care hospital. Clin Toxicol (Phila) 2012;50:197-201.
2- Haynes, William M., ed. (2011). CRC Handbook of Chemistry and Physics (92nd ed.). Boca Raton, FL: CRC Press. ISBN 978-1439855119.
3- Lasiene, K., Straukas, D., Vitkus, A., Juodziukyniene, N. (2016): The influence of copper sulphate pentahydrate (CuSO4·5H2O) on the embryo development in the guppies (*Poecilia reticulata*). Italian Journal of Animal Science 15: 529-535.
4- Copper Development Association. "Uses of Copper Compounds: Table A - Uses of Copper Sulphate". Copper. Copper Development Association Inc. Retrieved 12 May 2015.
5- ACGIH (American Conference of Governmental Industrial Hygienists) (1986). Copper. In: Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. ACGIH, Cincinnati, OH, p. 146.
6- Estridge, Barbara H.; Anna P. Reynolds; Norma J. Walters (2000). Basic Medical Laboratory Techniques. Thomson Delmar Learning. p. 166. ISBN 978-0-7668-1206-2.
7- Hazardous Substances Data Bank; *Copper (II) Sulfate, Pentahydrate*, CASRN: 7758-99-8. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~7rxy67:2 (accessed Dec 2009), updated 2007.
8- Wang Zongyuan. Animal nutrition metabolic diseases and poisoning [J]. Beijing. China Agricultural Publishing House. 1997, 2
9- Reregistration Eligibility Decision (RED) for Coppers; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2009.

IN ALBINO RATS. BENHA VETERINARY MEDICAL JOURNAL, VOL. 27, NO. 1:146-156.

29- Ying Li, Yaping Zhao*, Wenli Yu, Siyuan Jiang. Scavenging ability on ROS of alpha-lipoic acid (ALA). Food Chemistry 84 (2004) 563–567

30- Pınar N, Çakırca G, Hakverdi S, Kaplan M. Protective effect of alpha lipoic acid on cisplatin induced hepatotoxicity in rats. Biotech Histochem. 2020 Apr;95(3):219-224. doi: 10.1080/10520295.2019.1667025. Epub 2019 Nov 5. PMID: 31687838.

31- Abdel-Daim MM, Taha R, Ghazy EW, El-Sayed YS. Synergistic ameliorative effects of sesame oil and alpha-lipoic acid against subacute diazinon toxicity in rats: hematological, biochemical, and antioxidant studies. Can J Physiol Pharmacol. 2016 Jan;94(1):81-8. doi: 10.1139/cjpp-2015-0131. Epub 2015 Jul 19. PMID: 26550680.

32- Hassan S, Shaikh MU, Ali N, RiazM. Copper sulphate toxicity in a young male complicated by methemoglobinemia, rhabdomyolysis and renal failure. J Coll Physicians Surg Pak. 2010;20(7):490-491.

33- Qu, R., Feng, M., Wang, X., Qin, L., Wang, C., Wang, Z., Wang, L. (2014): Metal accumulation and oxidative stress biomarkers in liver of freshwater fish Carassius auratus following in vivo exposure to waterborne zinc under different pH values. - Aquatic Toxicology 150: 9–16.

34- Ahlam Alhusaini, Laila Fadda, Iman Hassan, Hanaa M. Ali, Njood Alsaadan, Nouf Aldowsari, Azizah Aldosari, and Bshayer Alharbi. (2018).Liposomal Curcumin Attenuates the Incidence of Oxidative Stress, Inflammation, and DNA Damage Induced by Copper Sulfate in Rat Liver. Dose-Response: An International Journal. 1-7

35- T. W. Nichols Jr., "α-lipoic acid: biological effects and clinical implications," *Alternative Medicine Review*, vol. 2, no. 3, pp. 177–183, 1997.

20- Yousif , Eman Hashim; Obaid, Hashim M.; Karim, Abdulkarim Jafar; Hashim, Muna Sachit and Al.Naimi, Rajiha A. (2019). TOXICO PATHOLOGICAL STUDY OF COPPER SULFATE MODULATE BY ZINC OXIDE AND *CORIANDRUM SATIVUM* PLANT TREATMENT IN MICE. *Plant Archives* Vol. 19, Supplement 1, pp. 299-308.

21- Ahmed, F.; and Khan, Z. (2012). Hepatoprotective effect of Lipoic Acid in Ethanol Intoxicated Chicks on Liver Biochemical Parameters Like LDH, SDH, & Glucose-6-Phosphatase. *International Journal of Pharmaceutical Research & Allied Sciences*. Vol. 1; issue 2:12-22.

22- Lieberman, D.; and Phillips, D. (1990). "Isolated" elevation of alkaline phosphatase: significance in hospitalized patients". *J Clin Gastroenterol*.;12:415–9.

23- Bergmeyer, H.U. and E. Bernt, 1974. Methods of Enzymatic Analysis. Academic Press, New York, Pages: 735.

24- Luna, L. G. (1968). Manual of histologic staining methods of the armed forces institute of pathology, 3rd edn. McGraw- Hill, New York, NY.

25- Leech, N. L.; Barrett, K. C.; and Morgan, G. A. (2011). IBM SPSS For Intermediate statistics.4th ed.Taylor and Francis Group. LLC.USA.

26- Senior JR. Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury-past, present, and future. Clin Pharmacol Ther. 2012 Sep;92(3):332-9. doi: 10.1038/clpt.2012.108. Epub 2012 Aug 8. PMID: 22871997.

27- Pettersson J, Hindorf U, Persson P, et al. Muscular exercise can cause highly pathological liver function tests in healthy men. Br J Clin Pharmacol. 2008;65(2):253-259

28- Saida A. Mohammed; Bakery H. H.; Abuo Salem M. E.; Nabila, A. M. and Elham,A. E. (2014). HEPATOTOXIC EFFECT OF COPPER SULPHATE AND COBALT CHLORIDE AS FEED ADDITIVES

supplementation improved antioxidant enzyme activities in hemodialysis patients. *Int J Vitam Nutr Res*. 89(3-4):161-167. doi: 10.1024/0300-9831/a000552.

38- E. Dulundu, Y. Ozel, U. Topaloglu, et al., "Alpha-lipoic acid protects against hepatic ischemia-reperfusion injury in rats," *Pharmacology*, vol. 79, no. 3, pp. 163–170, 2007.

36- Smith AR, Hagen TM. Vascular endothelial dysfunction in aging: loss of Akt-dependent endothelial nitric oxide synthase phosphorylation and partial restoration by (R)-alpha-lipoic acid. Biochem Soc Trans. 2003 Dec;31(Pt 6):1447-9. doi: 10.1042/bst0311447. PMID: 14641086.

37- Reza Mahdavi, Tannaz Khabbazi, Javid Safa. (2019). Alpha lipoic acid

*e*ISSN 1303-5150