



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

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### 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 CuSO<sub>4</sub> for two months. The second group was gavage by stomach tube 40 mg/kg BW CuSO<sub>4</sub> 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 1<sup>st</sup> 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 2<sup>nd</sup> 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 1<sup>st</sup> group (CuSO<sub>4</sub> group). The microscopic examination of the histopathological sections of livers and kidneys of 1<sup>st</sup> group animals showed high severe changes or lesions in both livers and kidneys due to the pathotoxic effect of CuSO<sub>4</sub> on these organs, whereas these microscopic changes showed marked decreased or mild severity in the 2<sup>nd</sup> group (CuSO<sub>4</sub> + ALA) due to the protective and ameliorative effect of ALA against the toxicity of CuSO<sub>4</sub>.

**Keywords:** Effect. ALA. CuSO<sub>4</sub>. Hepatorenal. Toxicity

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### Introduction

to 5 water molecules. Therefore, the most common hydrated form of it is CuSO<sub>4</sub>.5 H<sub>2</sub>O (2).

The copper sulfate is worldwide used as an algacide 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 CuSO<sub>4</sub>. Similarly, it has a molecular weight of 249.99 g mol<sup>-1</sup>. 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



### 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

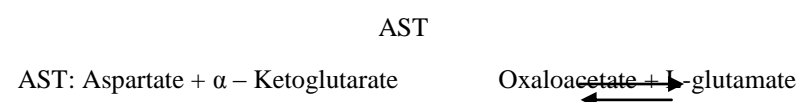
#### Alkaline phosphatase (ALP)

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 spectrophotometrically 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:-



#### ALT



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

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

Pyruvate + L-glutamate

### 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  $\mu$ m 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  $\pm$  standard error (SE) (25).

## RESULTS

### Biochemical Test

Alanine aminotransferase (ALT): Daily administration of Copper sulfate (CuSo4) ( $40\text{mg/kg B.W}$ ) 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 ( $58.31 \pm 7.89$ ) 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 $\pm$ SE)	AST IU /dl (Mean $\pm$ SE)	ALP IU/dl (Mean $\pm$ SE)
1 <sup>st</sup> group CuSo4 alone	58.31 $\pm$ 7.89 b	284 $\pm$ 12.48 b	423 $\pm$ 19.31 b
2 <sup>nd</sup> group CuSo4 + ALA	29.17 $\pm$ 2.89 a	170.3 $\pm$ 6.61 a	317.3 $\pm$ 61.49 a
3 <sup>rd</sup> group ALA alone	26.71 $\pm$ 3.03 a	162.3 $\pm$ 18.02 a	281.3 $\pm$ 24.1 a
4 <sup>th</sup> group Control	23.33 $\pm$ 0.71 a	161.6 $\pm$ 07.13 a	301 $\pm$ 16.76 a



second group (2<sup>nd</sup> 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 (3<sup>rd</sup> 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 (4<sup>th</sup> 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 ( $P \leq 0.05$ ), [CuSo4: Copper sulfate, ALA : alpha lipoic acid.**

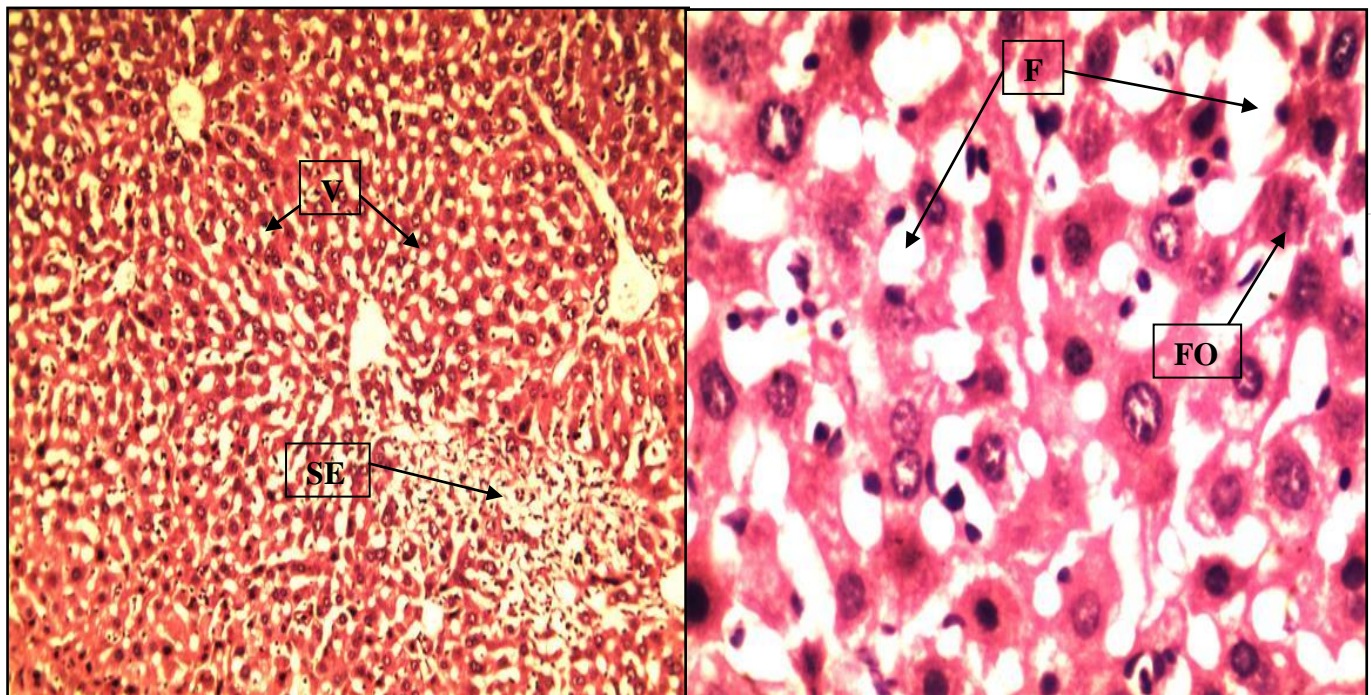
### **Histopathological Examination:**

#### **Livers:**

The first group (1<sup>st</sup> 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

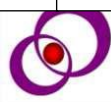
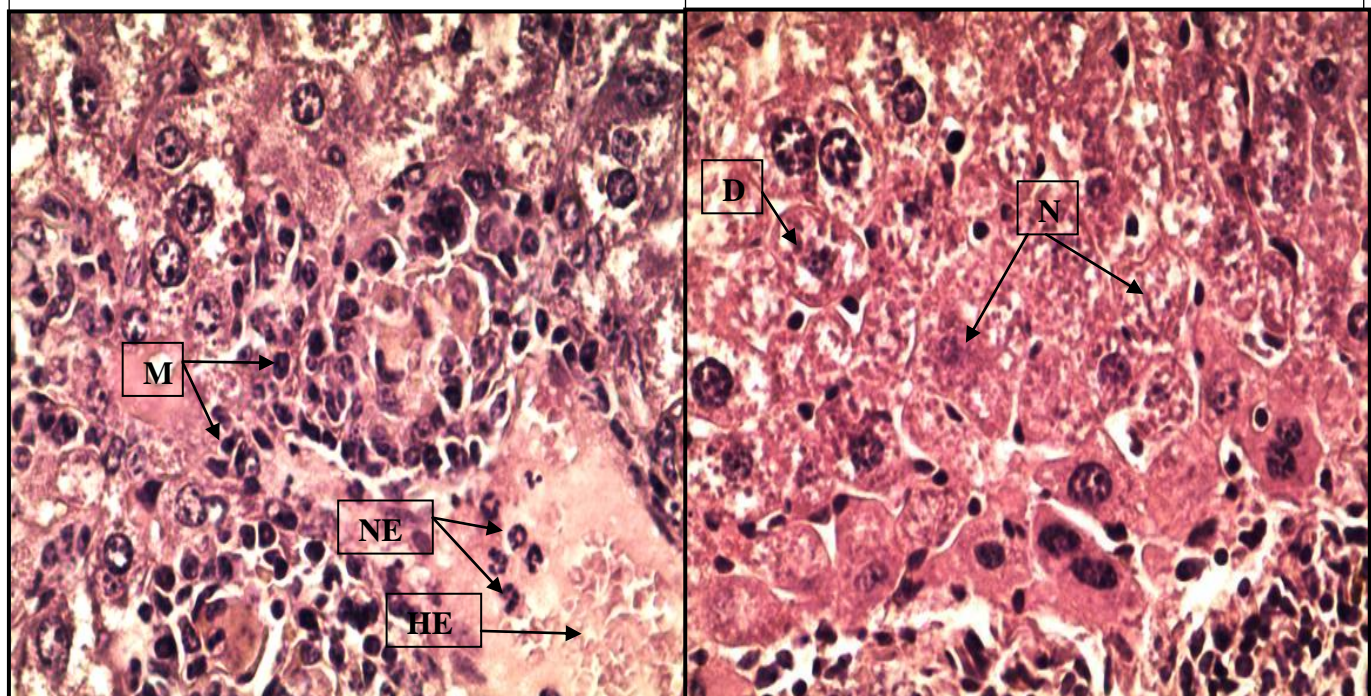
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Fig (1): Liver of rat (1<sup>st</sup> group). There is inflammatory cellular infiltration with suppurative exudate (SE). Also there is severe vacuolation (V) and degeneration of hepatocytes. 10X H&E.



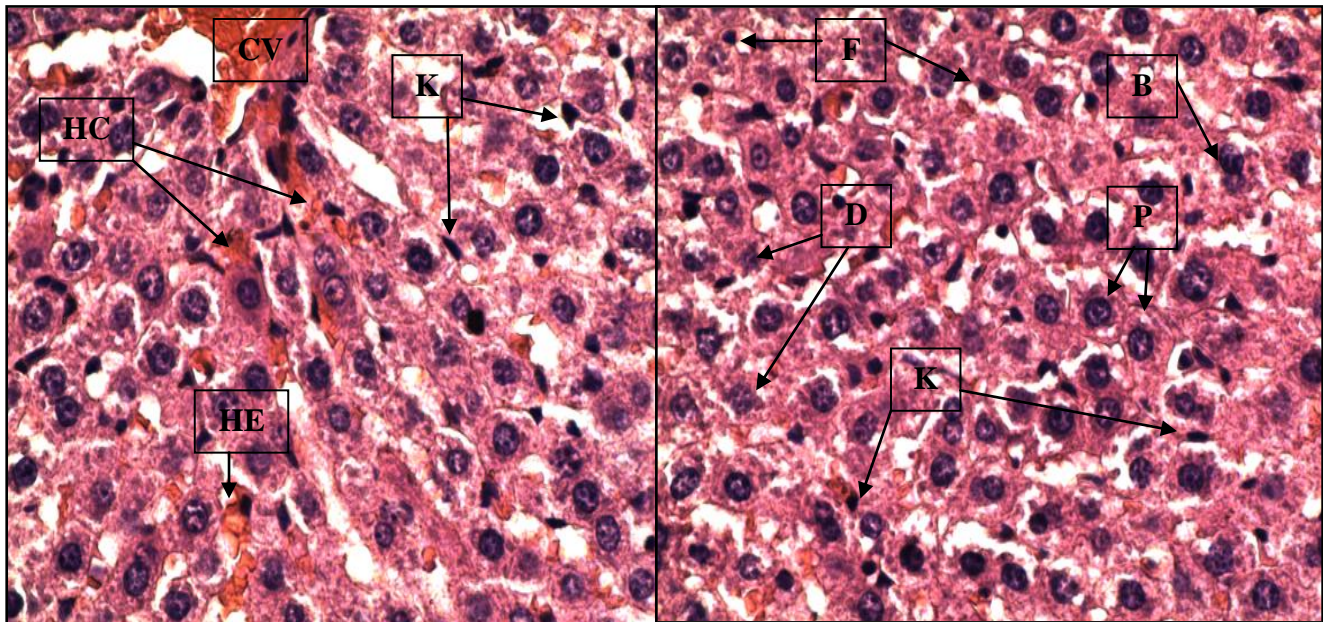
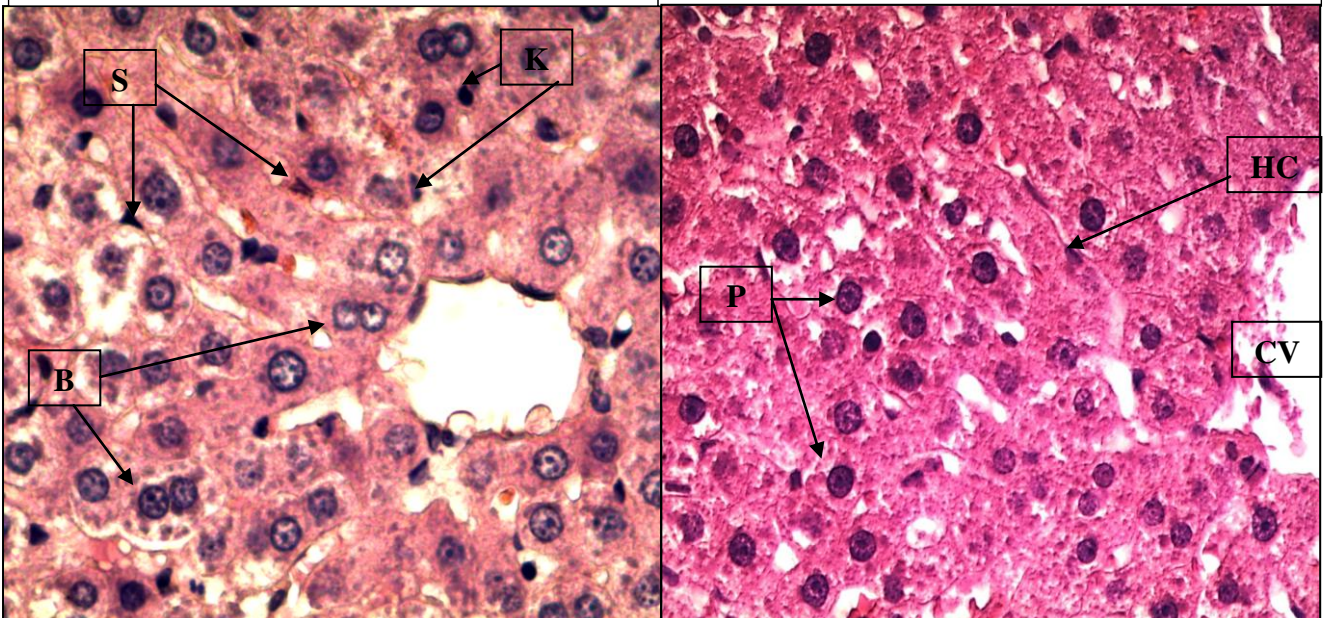


Fig (5): Liver of rat (2<sup>nd</sup> group). Radially arranged hepatocytes as hepatic cords (HC) around the central vein which showed slightly congested (CV). Also there is proliferation of Kupffer cells (K) and mild hemorrhage of sinusoids (HE). Few hepatocytes showed steatosis (fatty change) (F).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

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

The third group (3<sup>rd</sup> 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 (4<sup>th</sup> 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 (1<sup>st</sup> 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 (2<sup>nd</sup> 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

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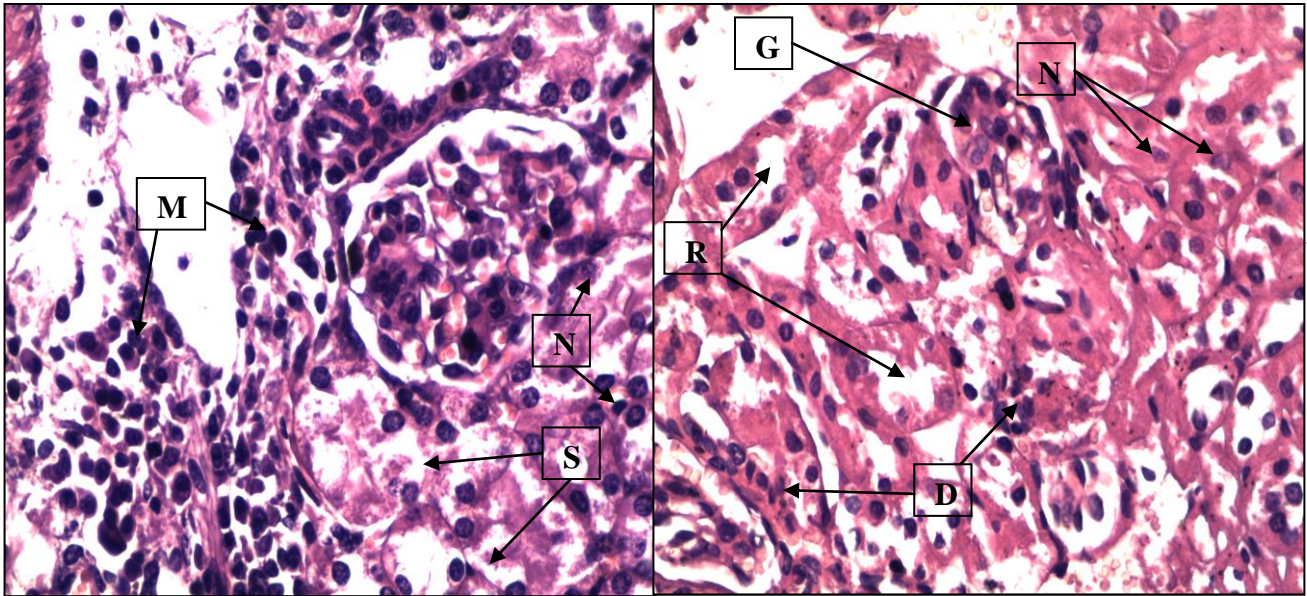
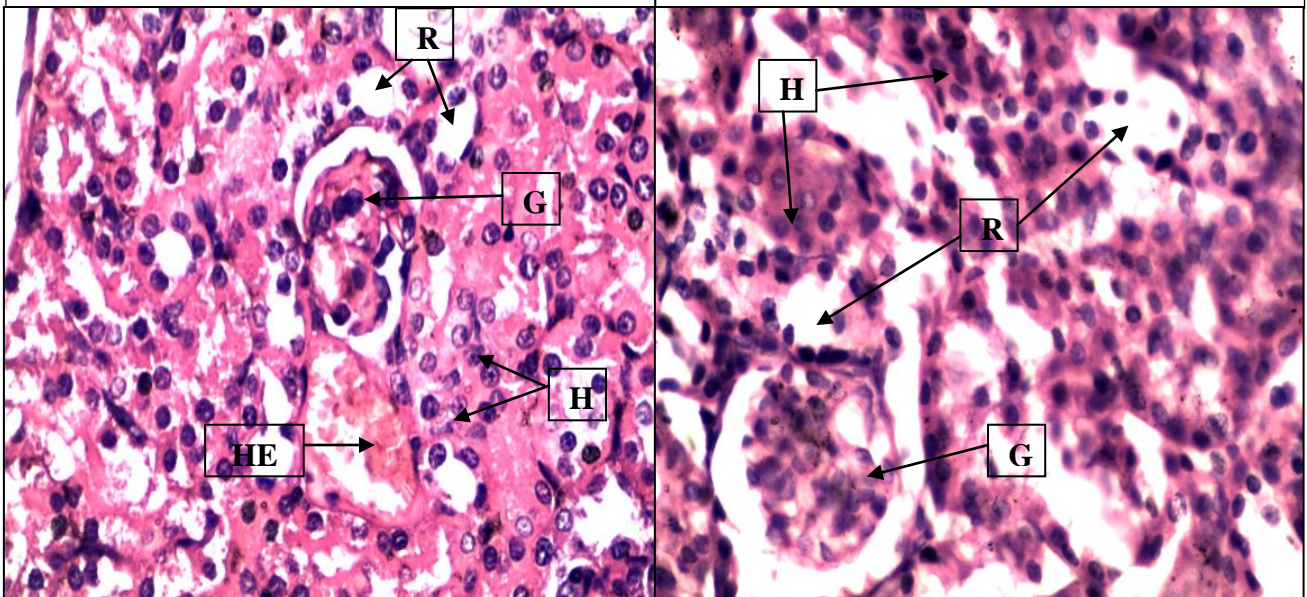


Fig (9): Kidney of rat (1<sup>st</sup> group). Note marked necrosis (N) and sloughing of epithelial cells of proximal convoluted tubules (S). Massive infiltration of inflammatory cells mainly macrophages and lymphocytes (M). 40X H&E.

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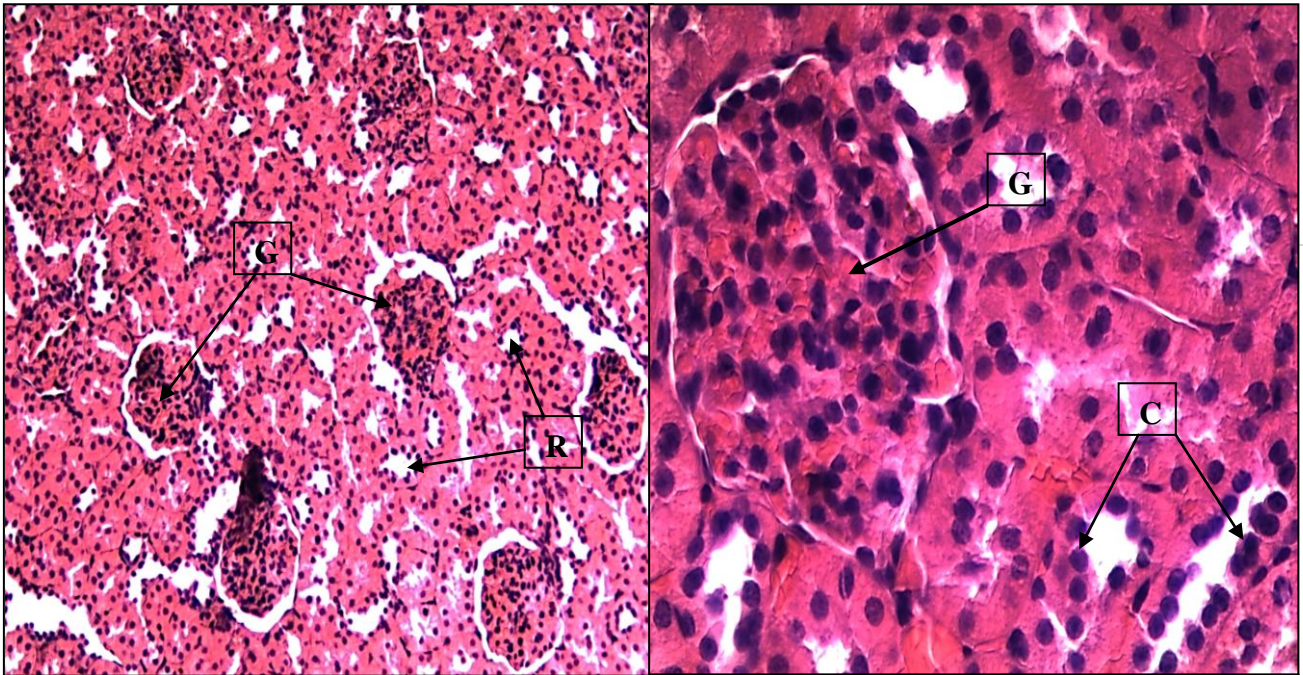
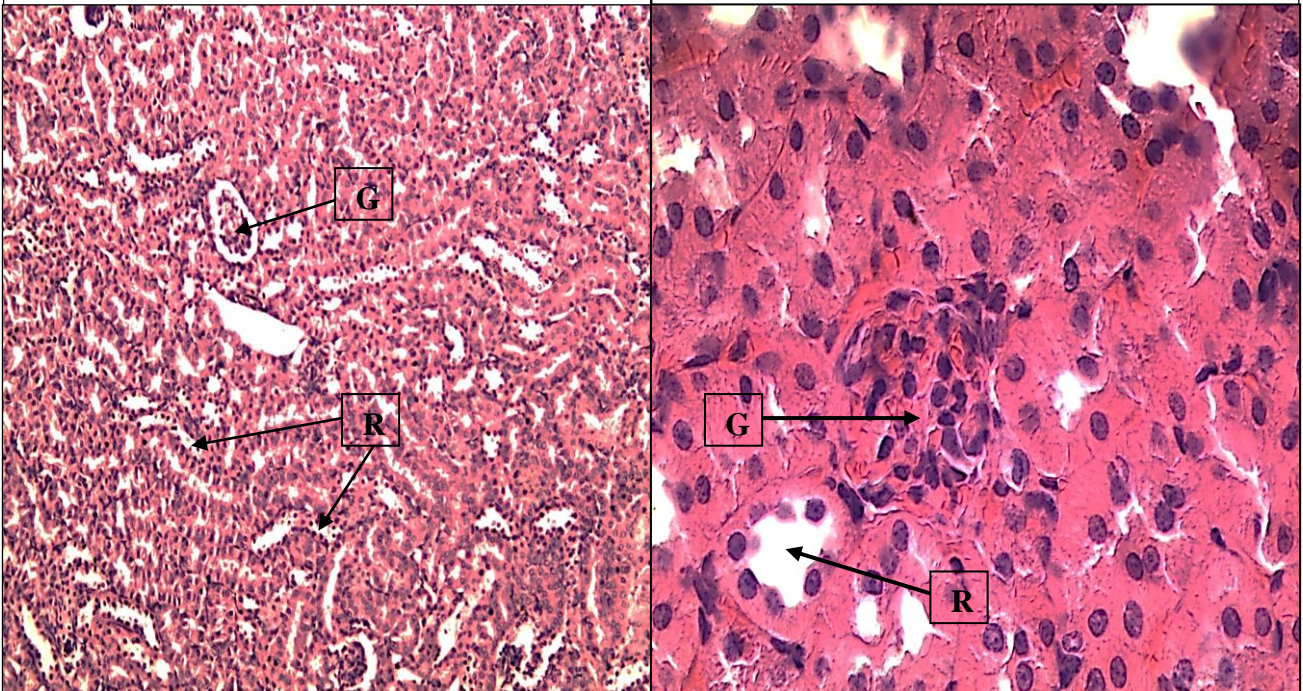


Fig (13): Kidney of rat of 3<sup>rd</sup> group. Note normal glomeruli with high cellularity (G) and normal renal convoluted tubules (R). 10X H&E.



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decreased ( $P \leq 0.05$ ) compared with 1<sup>st</sup> 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 ( $P \leq 0.05$ ) 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 ( $P \leq 0.05$ ). 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



formation of ceruloplasmin. The main target organs of CuSO<sub>4</sub> 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 CuSO<sub>4</sub>, 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 CuSO<sub>4</sub>-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 3<sup>rd</sup> and 4<sup>th</sup> (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 O<sub>2</sub> 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 CuSO<sub>4</sub> 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 CuSO<sub>4</sub> induced the elevation of serum biomarkers, including AST, Lactate Dehydrogenase (LDH), and the inflammatory marker hepatic nitric oxide (NO), C-Reactive Protein (CRP), protein



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