Histopathological Assessment of Hepato-Nephrotoxicity Induced by Nano Copper Particles in Adult Male Albino Rats and the Potential Protective Effect of Alpha Lipoic Acid: A Chronic Study

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Abstract

Background: Copper nanoparticles (CNPs) have unique physical and chemical properties for this reason it was widely used in various medical and non-medical applications. This study aimed to study the toxic effect of copper nanoparticles on liver and kidney through histopathological examination of liver and kidney tissues and if there is improvement of hepato-nephrotoxicity after cessation of exposure and to study the potential protective effect of alpha lipoic acid (ALA) against hepato-nephrotoxicity induced by copper nanoparticles.

Finding: Histopathological examination of liver and kidney tissues in rats received CNPs showed significant alteration, cessation of exposure can cause slight improvement and ALA can cause significant improvement.

Conclusion: Copper Nanoparticles have hepato-nephrotoxic effect and ALA can be safely used as a protective agent against CNPs toxicity.

Keywords: *Copper nanoparticles, Nanotoxicity, Hepatotoxicity, Nephrotoxicity, Histopathology.*

Background

Nanotechnology is highly promising technology that is concerned with understanding the behavior of nanoparticles and its properties and how to control particles at nanoscale range.[1]

Copper nanoparticles have unique physical and chemical properties such as high electrical conductivity, high melting point, low electrochemical migration behavior. [2]

The increasing production and use of metal nanoparticles for different applications lead to many adverse effects on health.[3]

The main target organs for toxicity with CNPs are kidney, liver and blood. Liver is the main damaged organ.[4]

Alpha lipoic acid is a potent antioxidant. It has antioxidant activity in both fat and water-soluble mediums.[5]

There for the aim of this study is to identify the toxic effect of CNPs on liver and kidney through histopathological examination and to study the potential protective effect of ALA against hepato-nephrotoxicity induced by CNPs.

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Methodology

Chemicals

1. **Copper Nanoparticles**

Copper Nano Particles (CNPs) 25 nm at 99.5% purity, mineral in nature and spherical in shape, was purchased from Sigma Aldrich, co. (St. Louis, Missouri, USA).

2. **ALPHA LIPOIC ACID**

Alpha lipoic acid (ALA) at 99% purity, was purchased from Sigma Aldrich, co. (St. Louis, Missouri, USA).

Study Design

The study design was approved from the local ethical committee of faculty of medicine, Cairo university and from Cairo University Institutional Animal Care and Use Committee (CU- IACUC), Medical Science Sector.

Animal grouping:

100 adult male albino rats of body weight 150–170 g was supplied and housed by the National Instituted of Ophthalmology. All animals have free access to water and food and exposed to 12 hours light/ dark cycle. After acclimatization for 2 weeks, 100 adult male albino rats were randomized into four main groups and were treated daily for 90 consecutive days as follows:

Group I act as controls (30 rats):

Group **I-A** (negative control group of 10 rats),

Group **I-B** (10 rats): receive deionized water (2 ml) daily,

Group **I-C** (10 rats): receive olive oil (2 ml) daily.

Group II (30 rats):

Group **II-A** (20 rats)**:** receive Copper Nano Particles (40mg/kg bw) for 90 days,

Group **II-B** (follow up group of 10 rats): receive Copper Nano Particles (40 mg/kg bw) for 90 days then no treatment for the following 4 weeks.

Group III (20 rats)**:** receive ALA (100 mg/kg bw) daily.

Group IV (20 rats): receive Copper Nano Particles (40 mg/kg bw) and ALA (100 mg/kg bw) as a prophylactic agent.

After 90 consecutive days following the last dose administration, rats were anaesthetized and sacrificed by decapitation. The follow up group were allowed for recovery with no treatment for 30 days then scarified as before.

Dose Selection and Preparation of Oral Suspension

- **Copper Nanoparticles Dose:**

LD50 for CNPs is 413 mg/kg body weight. The selected dose of CNPs was 1/10 LD50 which induce biochemical alteration in rats without morbidity.[6] Stock suspension of CNPs was prepared by dispersing CNP powder in deionized water followed by vigorous vertexing and sonication.

- **Alpha lipoic Acid Dose :**

The selected dose for ALA is (100 mg/kg bw) was reported to be hepatoprotective in rats.[7] Stock of ALA oral suspension was prepared by dissolving ALA in olive oil to improve its absorption by vigorous vertexing and sonication.

At the End of the Study Period:

After 90 consecutive days following the last dose administration, rats were sacrificed. The follow up group were allowed for recovery with no treatment for 30 days then scarified.

Histopathological Observation:

liver and kidney from each rat were cut rapidly, fixed in neutral purified formalin (10%), then dehydrated with grades of ethanol (70,80,90,95 and 100%). Dehydration is then followed by clearing the samples with 2 cycles of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 μm) were stained in hematoxylin and eosin and get examined under light microscope under high power (X100).

Stained sections of all groups were examined for:

1- **Liver:** alteration of architecture, portal inflammation, hepatocytes and for the presence of degeneration, necrosis and portal fibrosis.

2- **Kidney:** alteration of architecture of the cortex and medullary segments and the structure of glomeruli as well as proximal and distal tubules.

Statistical Analysis

Data were coded and entered using the statistical package of Social Sciences (SPSS). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test.[8] For comparing categorical data, Chi square ($\chi 2$) test was performed.[9]

Finding

Liver Histopathological Examination of the Studied Groups:

1. **Histopathological Examination of Group 1 & Group 3:**

Histopathological examination of sections of liver from rats of group 1 and group 3 showed normal hepatic parenchyma with preserved lobular architecture, normal arrangement and structure of the hepatocytes. The portal tract showed no inflammatory cells. No congestion was seen, and central vein was within normal ***(figures 1).***

2. **Histopathological Examination of Group 2A:**

Histopathological examination of sections of liver from rats of group 2A showed severe histopathological alterations characterized by dilatation of central vein with disruption of lobular architecture and disorganization of hepatic cords as well as necrobiotic changes in the form of vacuolar degeneration with vesicular nuclei of the hepatocyte and fat accumulation; steatosis. The portal tract is dilated with inflammatory exudate. Periportal fibrosis was detected and the central vein was dilated ***(figure 1).***

3. **Histopathological Examination of Group 2B:**

Histopathological examination of sections of liver from rats of group 2B revealed mild ballooning of the hepatocytes , however the portal area is dilated and showed inflammatory cell infiltrate, the central vein was dilated congested. The hepatocytes showed some disorganization ***(figure 2).***

4. **Histopathological Examination of Group 4:**

Histopathological examination of sections of liver from rats of group 4 revealed that portal tracks appeared normal in some sections other sections showed minimal to mild inflammation in occasional portal tracts, the central vein is of normal caliber and is not congested. The hepatocytes showed minimal to absent vacuolar changes ***(figure 2).***

Comparison Between Studied Groups using Chi-Square Tests:

Regarding severity of lesion in the four main studied group it was found that, 35% of liver sections of group 2A showed severe lesion and 54% of sections showed moderate lesion while 10 % of liver sections of group 2B showed sever lesion and 40 % was moderately affected compared to the three control groups 1A,1B & 1C as well as group 3 which were normal. While 65% of group 4 liver section were normal and 35% showed mild lesion with statistically significant difference between the 4 main groups (p value <0.001) ***(figure 3).***

Renal Histopathological Examination of the Studied Groups:

1. Histopathological Examination of Group 1& Group 3:

Histopathological examination of group 1& group 3 showed normal appearance of the glomeruli with normal cellularity. The tubules are not dilated with the lining cells are of normal appearance and the interstitial tissues showed no inflammation or congested blood vessels ***(figure 4).***

2. Histopathological Examination of Group 2A:

Histopathological examination of group 2A showed swollen glomeruli with thickened basement membrane. Both the glomeruli and the interstitial blood vessels are congested. Foci of fibrosis are seen. The tubular lining showed degenerative changes ***(figure 4).***

3. Histopathological Examination of Group 2B:

Histopathological examination of group 2B showed swollen glomeruli with thickened basement membrane with congested both glomerular and interstitial blood vessels however, the tubular lining showed no degenerative changes and there was no fibrosis seen ***(figure 4).***

4. Histopathological Examination of Group 4:

Histopathological examination of group 4 showed thin basement membrane of the glomeruli. Both the tubules and interstitial tissues showed no pathological changes apart from minimal degenerative changes of the tubular lining ***(figure 4).***

Comparison Between Studied Groups using Chi-Square Tests:

Regarding severity of lesion in the four main studied group it was found that, 40% of renal sections of both members of group 2 (2A &2B) showed moderate lesion and 60% of sections showed mild lesion compared to the three control groups 1A,1B & 1C as well as group 3 which were normal. While 75% of group 4 liver section were normal and 25% showed mild lesion with statistically significant difference between the 4 main groups (p value <0.001) ***(figure 5).***

Pairwise Comparison Between Studied Groups

Using Post hoc Pairwise Comparisons between group 1, group 2A,2B, group 3 and group 4 in histopathological examination of liver sections there was statistically significant difference between group 2 with groups 1,3,4 (p value <0.001) and group 4 with groups 2A & 2B (p value <0.001) while there was no statistical significant difference between groups 2A and 2B**.**

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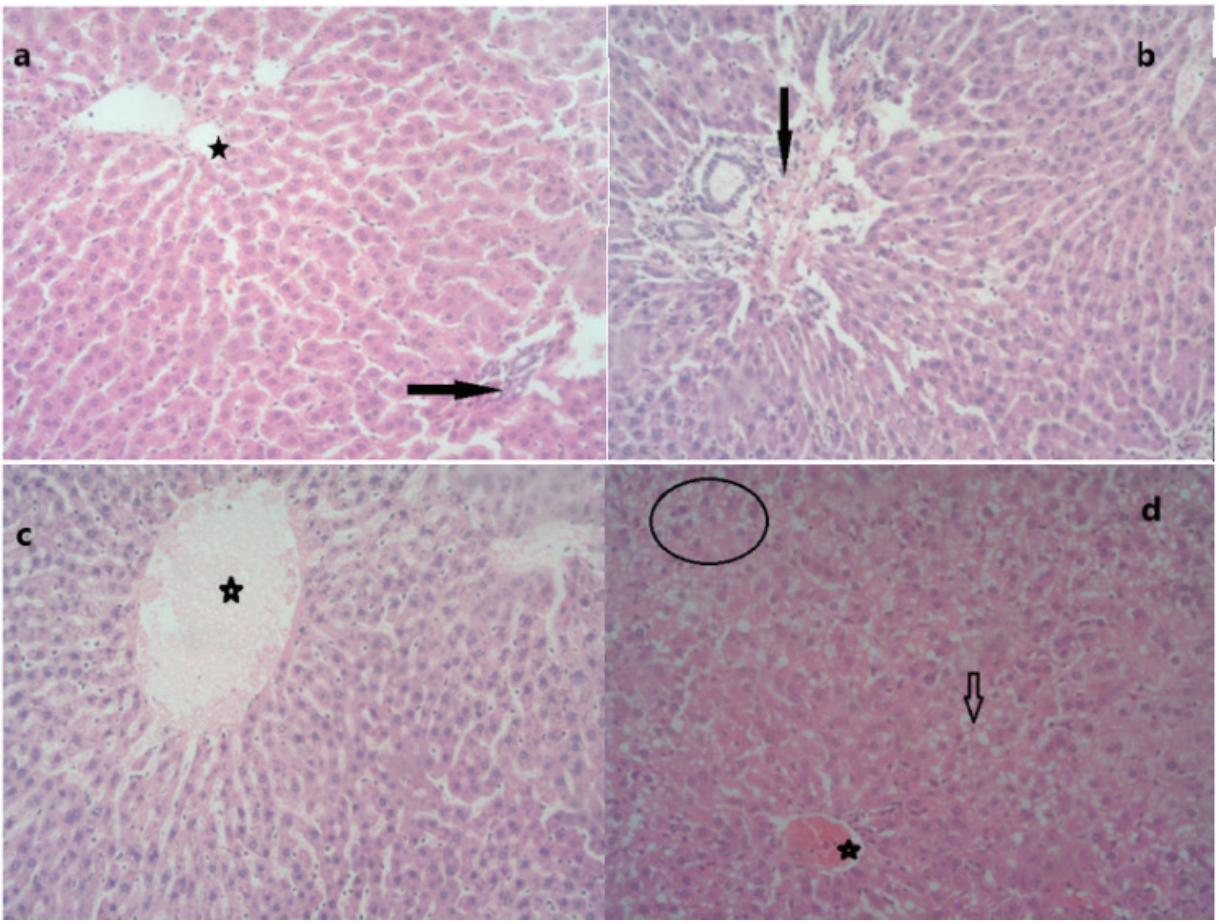

Figure 1: Sections of liver a) group 3 with normal appearance, central vein (the star) within normal b) group 2A showed vesicular nuclei of the hepatocyte with the portal tract is dilated showed inflammatory exudate (the arrow), c) group 2A showed dilated central vein (the star). d) group 2A showed mildly dilated congested central vein (the star). The hepatocytes showed disorganization, with hydropic changes and ballooning (the circle) and steatosis (the empty arrow). X100 H&E.

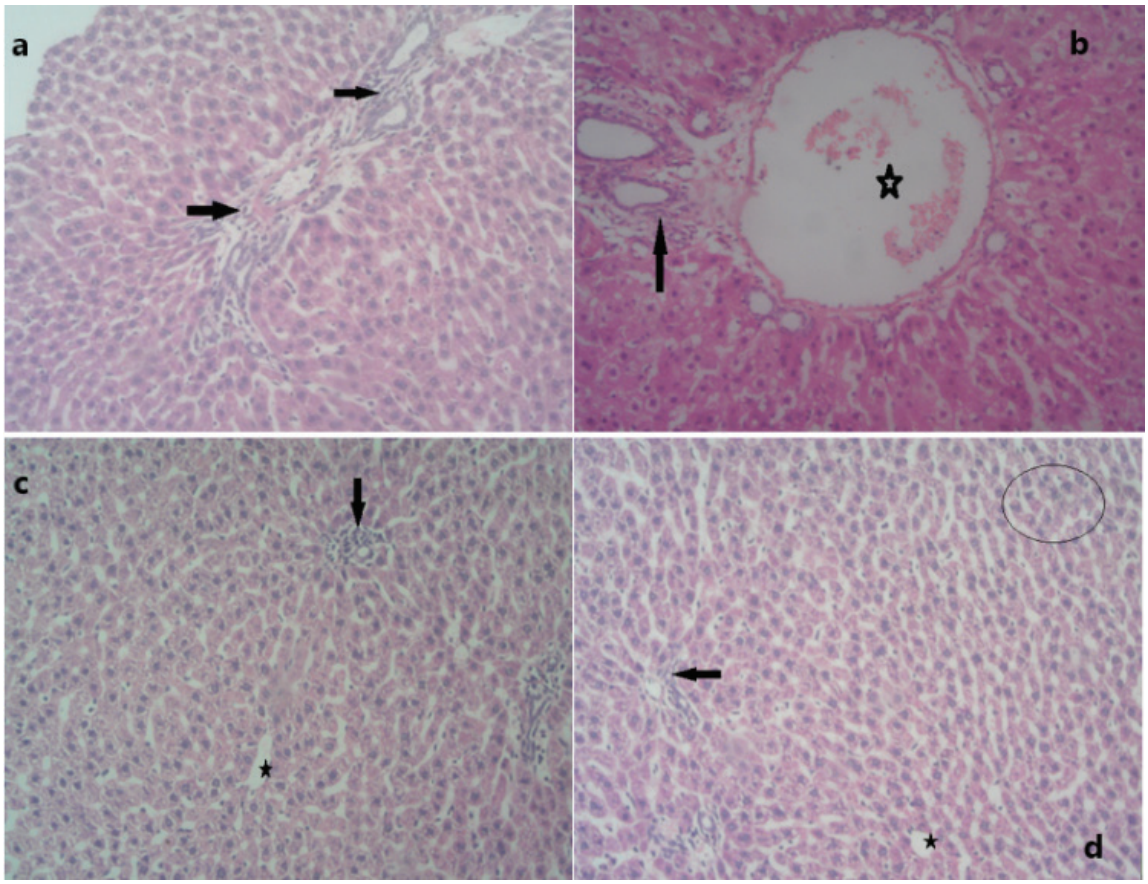

Figure 2 Sections from the liver: a) group 2B showed mild ballooning of the hepatocytes , the portal area is dilated and showed inflammatory cell infiltrate (the arrow), b) group 2B with dilated blood vessel (the star) , with the portal tract is dilated with inflammatory exudate (the black arrow). The hepatocytes showed some vascular degeneration, c) group 4 showed minimal to mild inflammation in occasional portal tracts (the black arrow), the central vein is of normal caliber and is not congested. The hepatocytes showed minimal to absent vacuolar changes, d) group 4 showed within normal portal tracts (the black arrow), the hepatocytes showed no hydropic degeneration(the circle), the central vein is of normal caliber and is not congested (the star) x100 H&E.

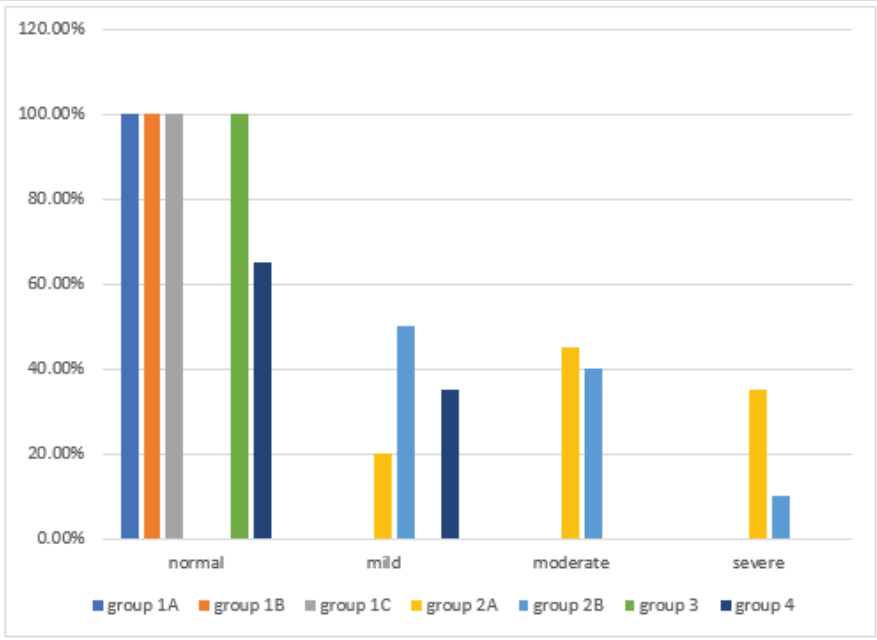

Figure 3: Histopathological grading of liver sections in the 4 studied groups.

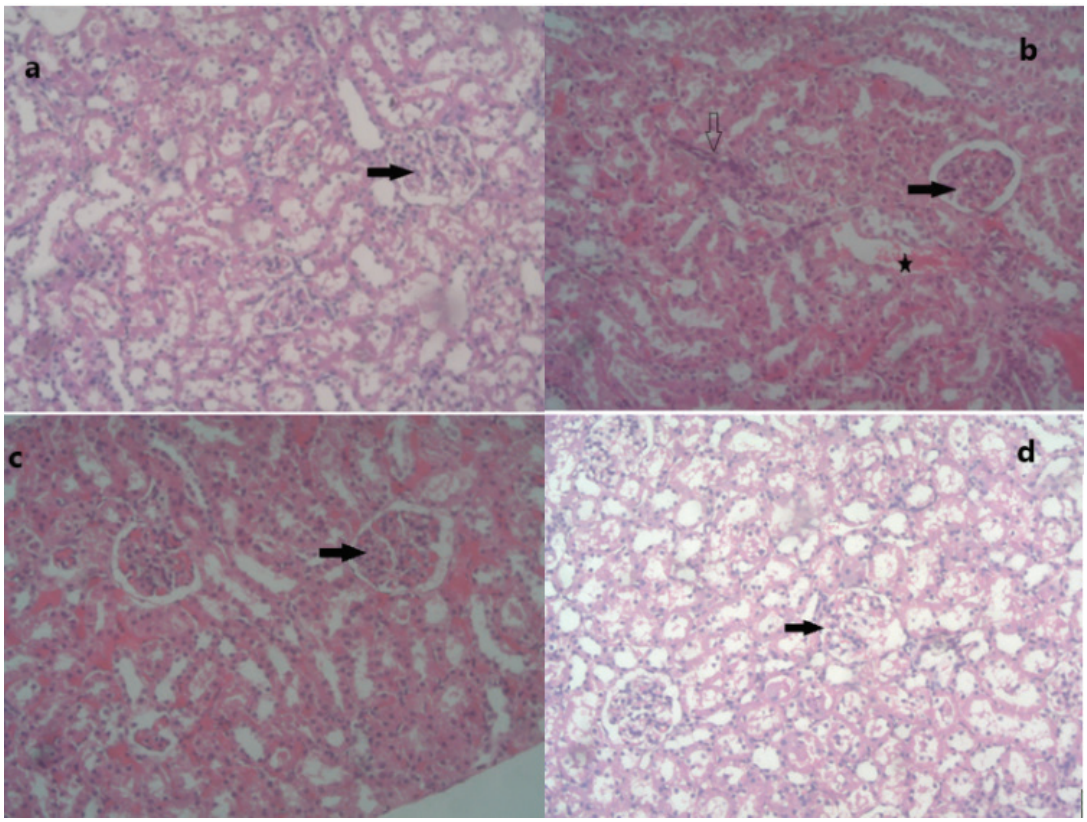

Figure 4: Section from kidney a) group 3; showed normal appearance of the glomeruli with normal cellularity (the arrow). The tubules and the lining cells are of normal appearance, b) group 2A showed swollen glomeruli with thickened basement membrane (the black arrow). Both the glomerular and the interstitial blood vessels are congested (the star). Foci of fibrosis are seen (empty arrow). The tubular lining showed degenerative changes, c) group 2B showed swollen glomeruli with thickened basement membrane (the arrow) with congested both glomerular and interstitial blood vessels, d) group 4 showed thin basement membrane of the glomeruli (the black arrow). Both the tubules and interstitial tissues showed no pathological changes apart from minimal degenerative changes of the tubular lining. X100 H&E.

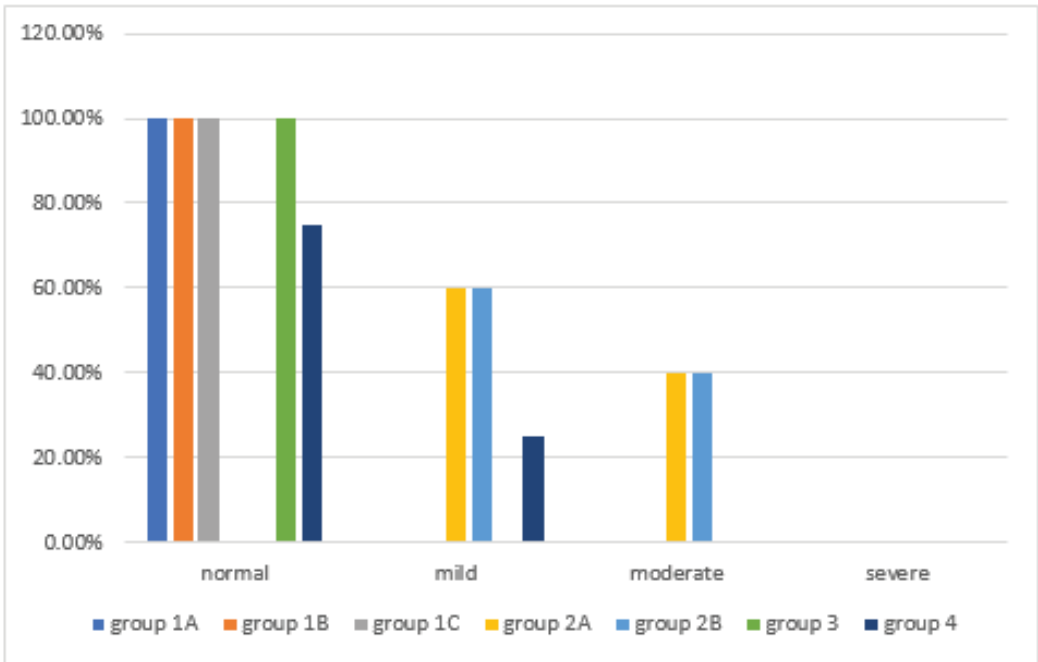

Figure 5: Histopathological grading of renal sections in the 4 studied groups.

Discussion

Histopathological alteration of liver tissue could be explained by accumulation of CNPs in the liver which can cause discontinuous endothelia that allow the passage of NPs from the blood into the liver parenchyma. This accumulation is responsible for the sever histopathological alterations.[10]

In accordance with our study, CNPs caused various histopathological alterations in the liver tissues and the hepatocytes.[6,11]

The protective effect of ALA against liver toxicity could be explained as ALA was considered as chelator compound as it can chelate metals ions.[12] ALA has a protective effect of on kidney and the histopathological result showed marked improvement in the group of rats received ALA.[13]

CNPs caused severe damage to the kidney tissues as the strong ionization potential of copper ions lead to its accumulation in the renal tissue which lead to inflammation in the renal tissues.[14] In accordance, CNPs caused widespread tubular necrosis[15] and can cause damage to the renal tissue[14.]

Conclusion and Recommendations

- CNPs caused histopathological alterations in liver and kidney tissues.
- Discontinuation of CNPs exposure in the follow up group caused non-significant improvement in liver tissues and did not improve the kidney tissues.
- Alpha lipoic acid can cause improvement in the histopathological changes caused by CNPs toxicity.
- Results of this study recommend periodic medical examination as well as Liver and kidney function tests in case of continuous exposure to CNPs.
- In case of deterioration of liver functions due to continuous exposure to CNPs; a period of cessation of exposure for at least 4 weeks is recommended.
- Alpha lipoic acid can be safely used as a prophylactic agent in case of Nano copper exposure.
- Confirmation of this study results regarding the period of cessation of exposure to CNPs is needed with prolongation of the follow up period.

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Ethics Approval: The study work was conducted after the approval of Ethical Committee, Faculty of medicine, Cairo University and the approval of Cairo University Institutional Animal Care and Use Committee (CU- IACUC), Medical Science Sector.

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