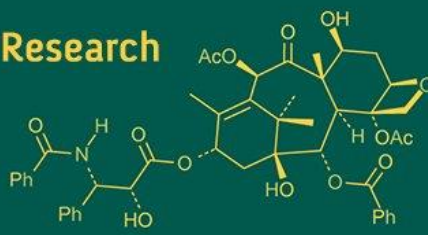


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Evaluation of dose-dependent hepatic injury induced by copper oxide nanoparticles in male Wistar rats

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Abstract

This study was conducted to evaluate the hepatotoxic effects of copper oxide (CuO) nanoparticles following repeated oral exposure in male Wistar rats. Seventy-two healthy male rats were randomly divided into four groups (n = 18). Group I served as control, whereas Groups II, III, and IV received 1/10th, 1/20th, and 1/40th of the LD50 dose of CuO nanoparticles, respectively, for 45 days. Six animals from each group were sacrificed at 15, 30, and 45 days of exposure for liver-related evaluation. Body weight, serum biochemical markers of liver function, oxidative stress indices, inflammatory mediators, and liver histopathology were assessed. CuO nanoparticle exposure produced dose- and duration-dependent toxic effects. Rats of Groups II and III showed reduced body weight gain during the later stages of exposure. Serum biochemical analysis showed significantly increased AST and ALT levels, particularly in Groups II and III at 30 and 45 days.

Oxidative stress analysis demonstrated increased malondialdehyde levels and decreased superoxide dismutase activity in treated animals, indicating enhanced lipid peroxidation and reduced antioxidant defense. The levels of TNF-alpha and IL-1 were also elevated in treated groups, suggesting activation of inflammatory pathways.

Histopathological examination of the liver revealed congestion, hepatocyte degeneration, bile duct dilatation, periportal fibrosis, focal necrosis, and inflammatory cell infiltration, with more severe lesions in higher dose groups. The findings of the present study indicate that repeated oral exposure to CuO nanoparticles induces significant hepatic injury in male Wistar rats through oxidative stress and inflammatory mechanisms. The severity of liver damage increased with higher dose and longer duration of exposure.

Keywords: Copper oxide nanoparticles, hepatotoxicity, oxidative stress, Wistar rats, liver pathology, and inflammation

1. Introduction

Nanotechnology has become an important component of modern medicine, agriculture, food processing, electronics, and pharmaceutical industries [1, 2]. Among different metallic nanoparticles, copper oxide nanoparticles are widely utilized because of their antimicrobial, catalytic, semiconducting, and antifungal properties [3, 4]. Among various nanoparticles, copper oxide (CuO) nanoparticles are extensively used due to their antimicrobial, catalytic, and industrial applications. However, their increasing use has raised concerns regarding their potential toxic effects on living systems.

Copper is an essential trace element involved in various physiological functions, but excess copper can lead to toxicity through the generation of reactive oxygen species (ROS) [5]. Due to their small size and increased surface area, CuO nanoparticles can penetrate biological membranes, accumulate in tissues, and induce cellular damage [6]. The liver is considered one of the major target organs for nanoparticle accumulation because it plays a central role in metabolism, detoxification, and excretion [7].

The liver is one of the principal organs involved in metabolism, detoxification, and excretion; therefore, it is highly susceptible to xenobiotic-induced injury. Nanoparticles entering the circulation are commonly accumulated in hepatic tissue, where they may trigger oxidative damage, inflammatory reactions, and cellular degeneration.

Copper oxide nanoparticles can generate excessive reactive oxygen species, alter membrane permeability, disturb mitochondrial function, and induce lipid peroxidation [8, 9].

These changes may ultimately result in hepatocyte degeneration, necrosis, fibrosis, and altered liver function. Although previous studies have reported the toxic effects of CuO nanoparticles, limited information is available regarding the sequential progression of liver injury following prolonged exposure.

Therefore, the present study was designed to investigate the clinicopathological, biochemical, oxidative, inflammatory, and histopathological changes induced by repeated oral administration of CuO nanoparticles in male Wistar rats. However, information regarding the dose-dependent and time-dependent hepatic alterations following prolonged exposure remains limited. Therefore, the present investigation was undertaken to study the toxicopathological effects of CuO nanoparticles on the liver of male Wistar rats.

2. Materials and Methods

2.1 Experimental location

The study was conducted under controlled laboratory conditions using healthy male Wistar rats maintained according to standard animal husbandry practices.

2.2 Preparation and dose selection of CuO nanoparticles

A pilot study was conducted before initiation of the experiment to determine the LD50 of CuO nanoparticles. Based on the LD50 value, three sublethal dose levels were selected for repeated oral administration.

2.3 Experimental animals

A total of 72 healthy adult male Wistar rats were used in the study. The animals were maintained under standard laboratory conditions with free access to feed and water throughout the experiment.

2.4 Experimental design

The rats were randomly divided into four groups:

- Group I: Control group
- Group II: Received 1/10th LD50 dose of CuO nanoparticles (320 mg/kg)
- Group III: Received 1/20th LD50 dose of CuO nanoparticles (160 mg/kg)
- Group IV: Received 1/40th LD50 dose of CuO nanoparticles (80 mg/kg)

- Group III: Received 1/20th LD50 dose of CuO nanoparticles (160 mg/kg)
- Group IV: Received 1/40th LD50 dose of CuO nanoparticles (80 mg/kg)

The CuO nanoparticles were administered orally for 45 days.

2.5 Parameters studied

The following parameters were evaluated at 15, 30, and 45 days of exposure:

- Clinical signs and body weight changes
- Organ weight of liver
- Serum biochemical parameters including AST, ALT, creatinine, and BUN
- Histopathological examination of liver tissue

2.6 Sample collection

At each interval of 15, 30, and 45 days, six rats from each group were sacrificed. Blood samples were collected for hematological and biochemical analysis. Liver tissue samples were collected for oxidative stress studies and histopathological examination.

2.7 Statistical analysis

The data obtained were analyzed statistically to determine significant differences among the control and treated groups at different intervals of exposure.

3. Results

3.1 Clinical signs and body weight changes

No obvious clinical signs of toxicity were observed up to the third week of experimentation in any treatment group. However, from the fourth week onward, rats in Groups II and III exhibited decreased feed intake, emaciation, drowsiness, and rough body coat. Body weight gain was reduced significantly in Groups II and III during the later stages of exposure. The mean values of body weight (in grams) of rats in different Groups (I, II, III and IV) at various intervals are depicted fig no 1.

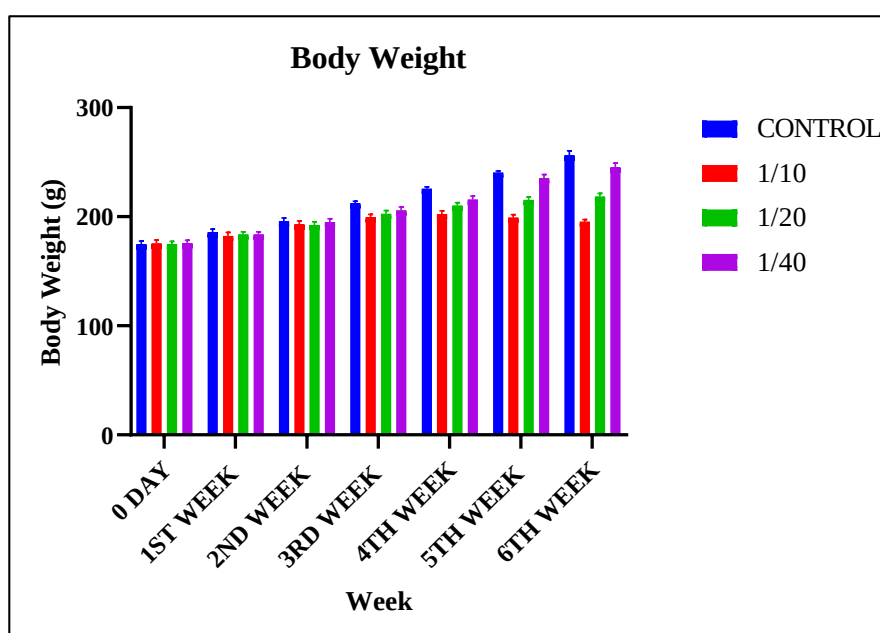


Fig 1: Effect of oral administration of CuO nanoparticles on general body weight in the rats of groups (II, III and IV) at 7 days intervals of experimentation

3.2 Absolute organ weight

3.2.1 Absolute Liver weight

The mean values of absolute weight (in grams) of liver depicted that there was no significant ($p>0.05$) variations was noticed in absolute organ weight of liver on day 15th of experimental period. At the interval of days 30th, there was significant change was observed in the liver of group II as

compared to the liver of groups (I, III and IV). Mean value of absolute organ weights (g) of liver rats of different groups at(I, II, III and IV) at the intervals of 15, 30 and 45 days exposure intervals of CuO nanoparticles at three different doses @ 320 mg/kg (1/10th LD50), 160 mg/kg(1/20th LD50) 80 mg/kg(1/40th LD 50) for 45 days in the rats of groups(I,II,III and IV) is depicted in fig no.2

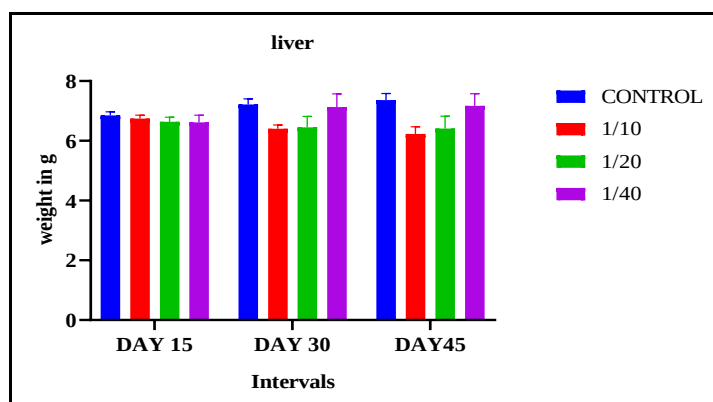


Fig 2: Effect of oral administration of CuO nanoparticles on weight of liver in the rats of groups (II, III and IV) at the intervals of 15, 30 and 45 days of experimentation.

3.3 Serum biochemical changes

Serum AST and ALT levels showed non-significant elevation on day 15 but increased significantly in Groups II and III by day 30. By day 45, all treatment groups showed significantly elevated AST and ALT levels compared to

controls, indicating liver injury. The mean values of AST, ALT, creatinine and BUN, on day in serum of rats of groups (I, II, III and IV) on the day of 15, 30 and 45 of experimentation are presented in fig no 3-6.

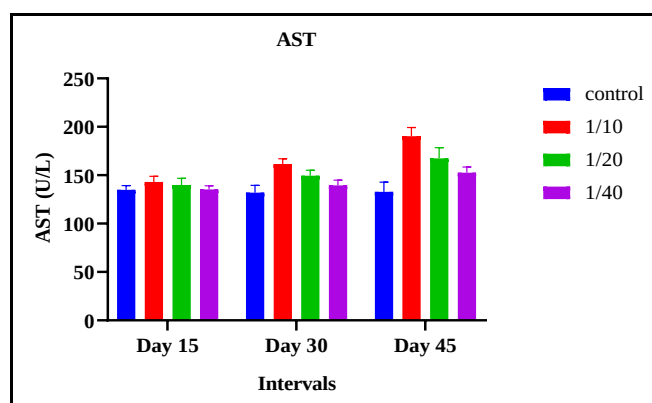


Fig 3: Effect of oral administration of CuO nanoparticles on AST level in the rats of groups (II, III and IV) at the intervals of 15, 30 and 45 days of experimentation

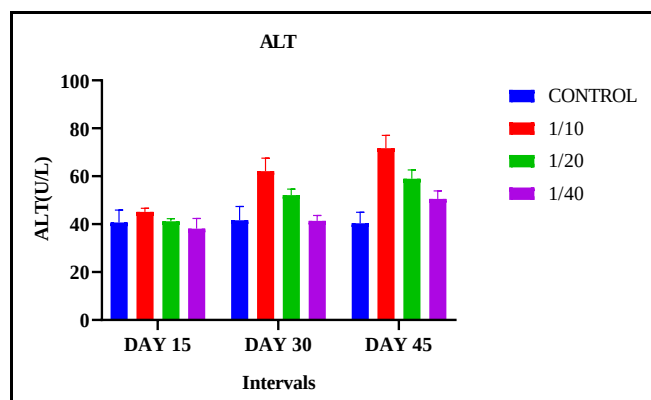


Fig 4: Effect of oral administration of CuO nanoparticles on ALT in the rats of groups (II, III and IV) at the intervals of 15, 30 and 45 days of experimentation

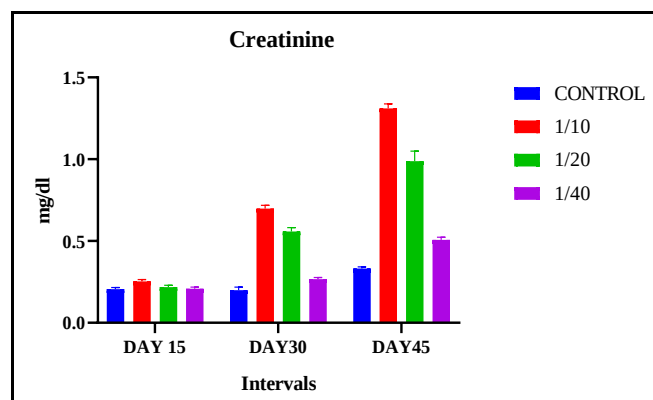


Fig 5: Effect of oral administration of CuO nanoparticles on Creatinine level in the rats of groups (II, III and IV) at the intervals of 15, 30 and 45 days of experimentation.

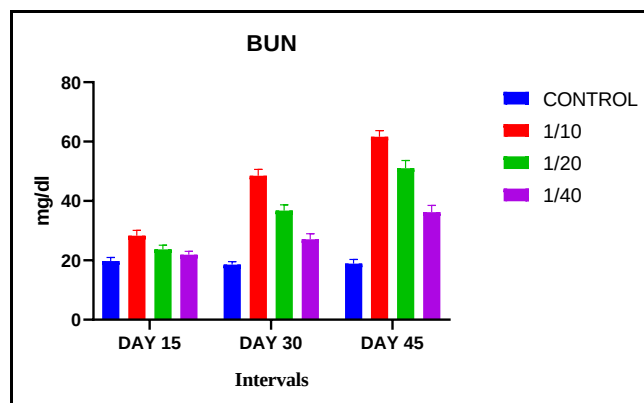


Fig 6: Effect of oral administration of CuO nanoparticles on BUN in the rats of groups (I, II, III and IV) at the intervals of 15, 30 and 45 days of experimentation.

3.4 Histopathological findings

The liver of control rats showed normal hepatic architecture throughout the study period. On day 15, Group II showed moderate congestion and inflammatory cell infiltration, while Groups III and IV exhibited normal histology. Histopathological finding of seminal vesicle in the rat of experimental groups (II, III and IV) are illustrated in Fig 7-9.. By day 30, Group II exhibited mild hepatocyte degeneration, bile duct dilation, and portal triad fibroplasia.

Group III showed congestion in the central vein and portal triad vessels, whereas Group IV remained largely normal.

At day 45, severe hepatic lesions were observed in Group II, including periportal fibrosis, severe multifocal necrosis, inflammatory infiltration, and marked hepatocyte degeneration. Group III showed focal hepatic necrosis, hepatocyte degeneration, and inflammatory cell infiltration. Group IV exhibited mild congestion of central veins and hepatic sinusoids.

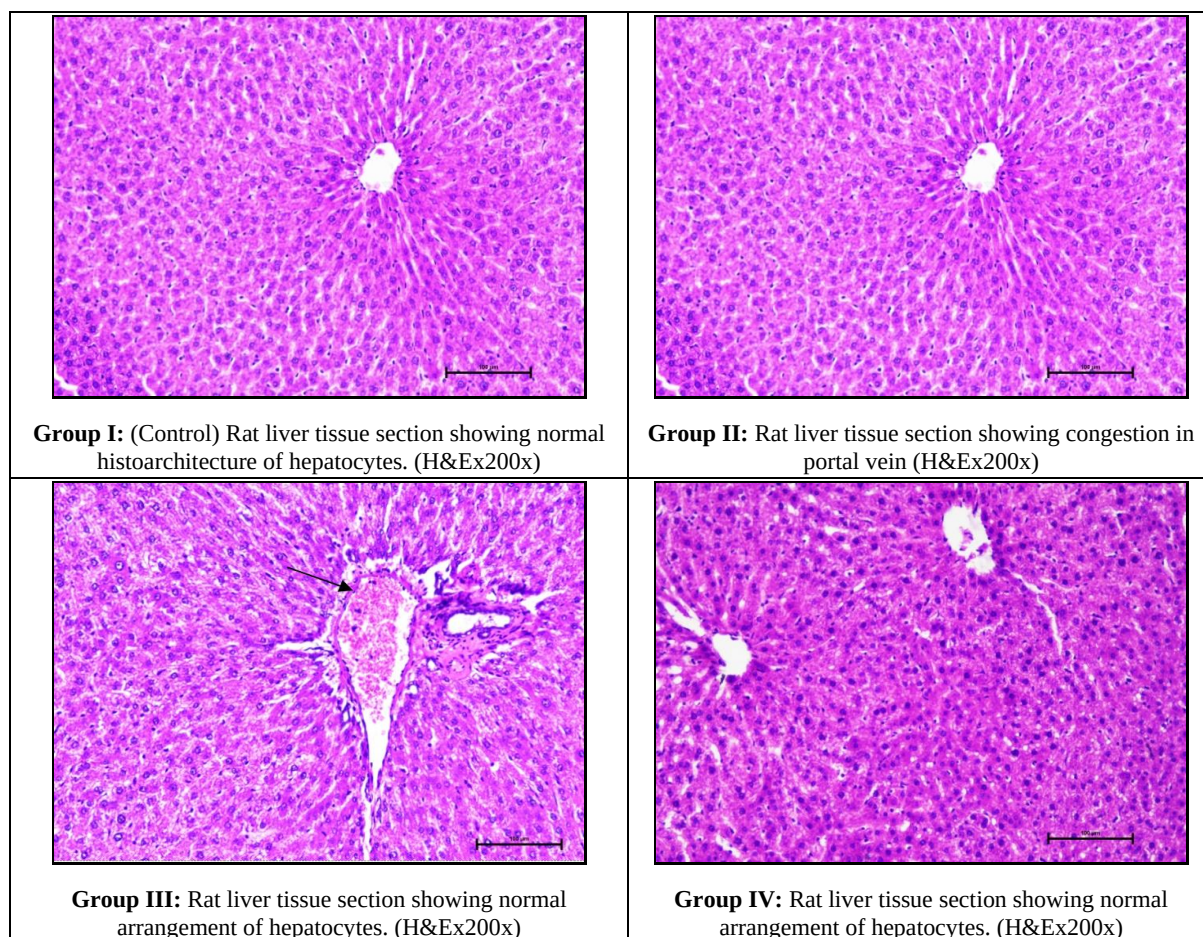
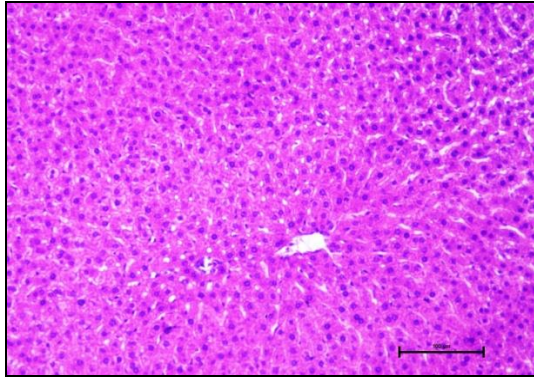
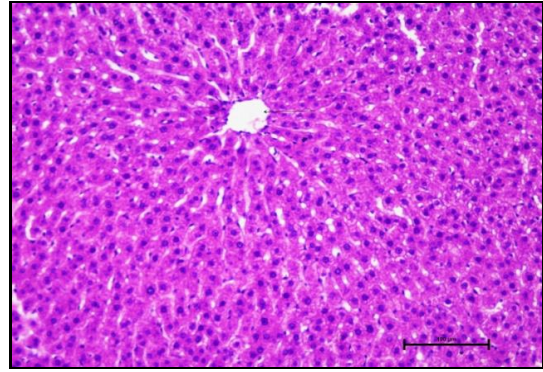


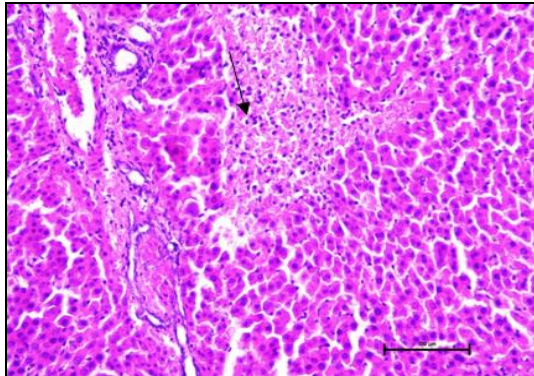
Fig 7: Effect of oral administration of CuO NP (320 mg/kg BW, 160mg/kg BW &80mg/kg BW) at the intervals of 15 days on histoarchitecture of Liver in rats of different treatment groups (II, III and IV).



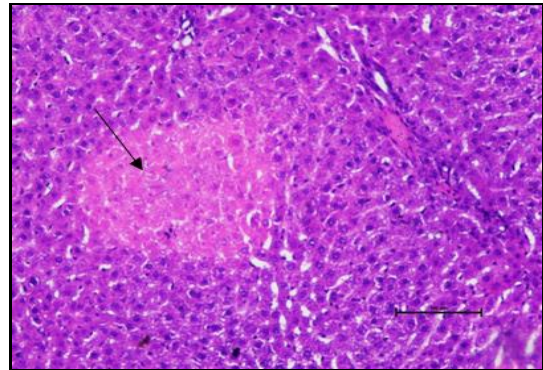
Group-I: (Control) Rat liver tissue section showing normal histoarchitecture of hepatocytes. (H&Ex200x)



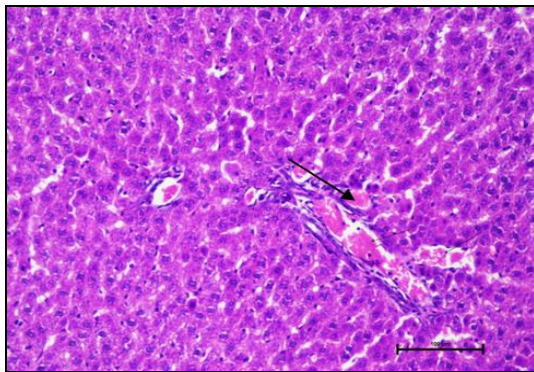
Group-I: (Control) Rat liver tissue section showing normal histoarchitecture of hepatocytes. (H&Ex200x)



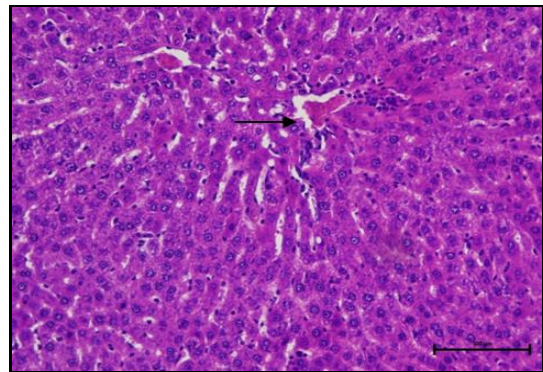
Group-II: Rat liver tissue section showing infiltration of mononuclear cells in liver parenchyma (H&Ex200x)



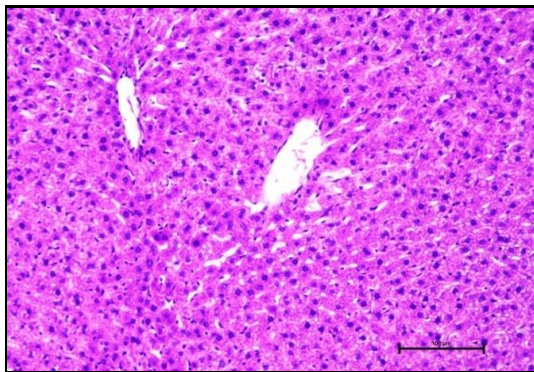
Group-II: Rat liver tissue section showing focal area of necrosis in liver parenchyma (H&Ex200x)



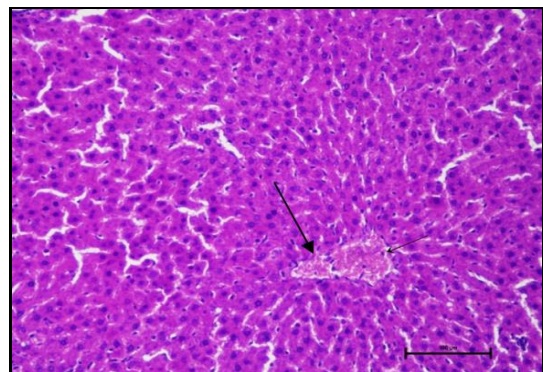
Group-III: Rat liver tissue section showing mild congestion in liver parenchyma. (H&Ex200x)



Group-III: Rat liver tissue section showing congestion and mild infiltration of mononuclear cell. (H&Ex200x)



Group-IV: Rat liver tissue section showing normal arrangement of hepatocytes. (H&Ex200x)



Group-IV: Rat liver tissue section congestion in liver parenchyma. (H&Ex200x)

Fig 8: Effect of oral administration of CuO NP (320mg/kg BW, 160mg/kg BW & 80mg/kg BW) at the intervals of 30 days on histoarchitecture of Liver in the rats of different treatment groups (II, III and IV).

Fig 9: Effect of oral administration of CuO NP (320mg/kg BW, 160mg/kg BW & 80mg/kg BW) at the intervals of 45 days on histoarchitecture of Liver in the rats of different treatment groups (II, III and IV).

4. Discussion

The present investigation demonstrated that oral administration of copper oxide nanoparticles caused progressive and dose-dependent liver injury in male Wistar rats. The toxic changes became more prominent with increasing dose and duration of exposure.

The reduction in feed intake and body weight gain in treated groups may be attributed to reduced appetite, altered metabolism, and general systemic stress associated with nanoparticle exposure. Such findings indicate that prolonged exposure to copper oxide nanoparticles adversely affects normal physiological functions.

Serum biochemical analysis revealed marked increases in AST and ALT activities in treated animals ^[10, 11]. These enzymes are normally present within hepatocytes, and their elevated concentration in blood indicates membrane damage and leakage from injured liver cells. Therefore, the rise in these enzymes suggests that CuO nanoparticles produce direct hepatocellular injury.

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Histopathological examination of liver tissue confirmed the biochemical findings. Congestion of blood vessels, hepatocyte degeneration, inflammatory infiltration, focal necrosis, bile duct alterations, and periportal fibrosis were observed in treated groups, especially in animals receiving higher doses. These lesions indicate progressive structural damage to hepatic tissue following repeated nanoparticle exposure.

The severity of hepatic lesions increased with dose and duration of exposure, suggesting cumulative toxic effects of CuO nanoparticles in the liver. The observed microscopic lesions correspond well with the increased AST and ALT levels, indicating that biochemical and histopathological alterations occurred simultaneously in treated animals.

Histopathological examination of liver tissue confirmed the biochemical and oxidative findings. Congestion of blood vessels, hepatocyte swelling, inflammatory infiltration, focal necrosis, bile duct alterations, and periportal fibrosis were observed in treated groups, especially in animals receiving higher doses. These lesions indicate progressive structural damage to hepatic tissue following repeated nanoparticle exposure.

Overall, the study indicates that CuO nanoparticles can produce severe hepatic injury after prolonged administration and that oxidative stress and inflammation are the principal mechanisms involved in the pathogenesis of toxicity.

Conclusion

The present study concluded that CuO nanoparticles induce significant hepatotoxicity in male Wistar rats following repeated oral exposure. The toxic effects were more severe at higher doses and longer exposure durations.

CuO nanoparticles caused alterations in body weight, hematological parameters, liver function biomarkers, oxidative stress markers, inflammatory mediators, and histopathological architecture of the liver. The major mechanism involved in hepatic toxicity appears to be

excessive production of reactive oxygen species leading to oxidative stress, inflammation, and cellular damage.

Therefore, prolonged exposure to CuO nanoparticles should be carefully monitored due to their potential to induce serious liver injury.

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