Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.083

RESEARCH ARTICLE

Unveiling the Oxidative Stress and Genotoxic Effects of Copper Oxychloride in Mice

Nouf Aldawood[1], Ahmed Aljazzar[2*], Aiman A. Alsaegh[3], Fahad Alhizab[4], Mahmoud Elalfy[5], Ahmed MA Meligy[6], Khalid Alkhodair[7], Ahmed Ezzat Ahmed[8,9], Yehia Hazzazi[10] and Nady Kh. Elbarbary[11*]

[1]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [2]Department of Pathology, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Hofuf, Al-Ahsa, 31982, Saudi Arabia; [3]Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah- Saudi Arabia; [4]Department of Pathology, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Hofuf, Al-Ahsa, 31982, Saudi Arabia; [5]Department of Clinical Science, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Hofuf, Al-Ahsa, 31982, Saudi Arabia; [6]Department of Clinical Science, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Hofuf, Al-Ahsa, 31982, Saudi Arabia; [7]Department of anatomy, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Hofuf, Al-Ahsa, 31982, Saudi Arabia; [8]Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia; [9]Prince Sultan Bin Abdelaziz for Environmental Research and Natural Resources Sustainability Center, King Khalid University, Abha 61421, Saudi Arabia; [10]Department of Biology, College of Science, Jazan University, Jazan 45142, Kingdom of Saudi Arabia; [11]Food Hygiene and Control Department, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.
*Corresponding author: Ajazzar@kfu.edu.sa (AA); nadykhairy@vet.aswu.edu.eg (NKE)

ARTICLE HISTORY (26-265)

Received: March 19, 2026
Revised: April 05, 2026
Accepted: April 21, 2026
Published online: April 27, 2026

Key words:
Copper oxychloride
Genetic toxicity
Mice
Oxidative stress
Histopathology

ABSTRACT

Copper oxychloride is extensively and persistently used due to its broad-spectrum antifungal actions to control the different fungal problems. This experimental study investigated the hemato-biochemical alterations, oxidative stress, DNA damage and histopathological changes in mice exposed to different doses of copper oxychloride. A total of twenty-five sexually mature male mice were obtained from local animal house and were randomly placed in wire cages in five groups and each group contained five mice. The mice in groups C_0, C_1, C_2, C_3 and C_4 were fed different doses of copper oxychloride mixed in the diet at 5, 10, 15 and 20 mg/kg/day for a period of two months. Group C_0 served as the control group. Blood was collected directly from the heart of each animal via cardiac puncture for serum separation. The results revealed red blood cells, hemoglobin and hematocrit values were significantly lowered in treated mice whereas leukocyte counts significantly increased compared to normal mice. Serum ALT, AST, glucose, and cholesterol levels considerably increased ($P<0.05$) and serum total proteins and albumin levels decreased significantly ($P<0.05$). Oxidative stress markers, including reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), were significantly increased, whereas antioxidant enzymes (SOD, POD, CAT and GSH) were significantly decreased in the liver, kidneys, and heart of treated mice compared to controls. Light microscopic analysis of different sections of liver, kidneys and heart of treated mice indicated moderate to severe histopathological alterations in a dose dependent pattern. The results on genotoxic effects of copper oxychloride indicated significantly increased percentile rate of DNA damage by comet assay in isolated cells of liver, kidneys and heart of mice treated with higher doses.

To Cite This Article: Aldawood N, Aljazzar A, Alsaegh AA, Aljazzar F, Elalfy M, Meligy AMA, Alkhodair K, Ahmed AE, Hazzazi Y and Elbarbary NKH, 2026. Unveiling the oxidative stress and genotoxic effects of copper oxychloride in mice. Pak Vet J, 46(4): 888-898. http://dx.doi.org/10.29261/pakvetj/2026.083

INTRODUCTION

Copper oxychloride (COC) is a widely used copper-based fungicide in agriculture, valued for its broad-spectrum efficacy against plant pathogens. However, its extensive and persistent application has raised growing concerns regarding environmental accumulation and potential toxicological effects in non-target organisms

(Qiao *et al*., 2021; Li *et al*., 2021). Once released into the environment, copper compounds can accumulate in soil and water systems, leading to increased bioavailability and biological uptake (Qiao *et al*., 2021). Although copper is an essential trace element involved in various enzymatic processes, excessive exposure disrupts redox homeostasis, induces oxidative stress, and impairs cellular functions. Therefore, understanding the toxicological impact of copper oxychloride, particularly under realistic exposure conditions, is critical for evaluating its ecological and health risks (Pelosi *et al*. 2024).

Copper play important role in physiological functions of mitochondria, clotting of blood formation of hemoglobin, bone mineralization and several other metabolic pathways (Qiao *et al*., 2021; Mujahid *et al*., 2021). Reports have highlighted that copper-based pesticides such as copper oxychloride, copper carbonate and copper sulphate are frequently used in different fields and may trigger allergy responses, irritate alimentary tract, induce abnormalities around the eyes and adverse effects in visceral organs and blood components (Ullah *et al*. 2024). Copper oxychloride is a widely used copper-based fungicide in agriculture. Although copper is an essential trace element involved in numerous metabolic and enzymatic processes, excessive exposure can disrupt cellular homeostasis and induce oxidative stress (Elalfy *et al*., 2021). Heavy metals, including copper, are well known to enhance the generation of reactive oxygen species (ROS), leading to oxidative damage in biological systems (Javed and Usmani, 2019; Iqbal and Irfan, 2024).

Copper-induced toxicity has been associated with multi-organ dysfunction, particularly affecting the liver, kidneys, and heart. Renal tissues are especially vulnerable due to their role in filtration and excretion, with studies reporting DNA damage, impaired antioxidant defenses, and structural degeneration following copper exposure (Ren *et al*., 2025). Similarly, chronic copper accumulation in the liver may exceed its storage capacity, resulting in oxidative injury and hemolytic complications (Yamkate *et al*., 2021; Kanwal *et al*., 2024). Cardiac tissues are also susceptible to copper-induced oxidative stress, which has been linked to altered enzyme activity, structural damage, and increased risk of cardiovascular disorders (Cui *et al*., 2022; Wang *et al*., 2024). Experimental studies in mice have further demonstrated that copper compounds can induce dose-dependent oxidative stress, antioxidant depletion, and histopathological alterations across multiple organs, highlighting oxidative stress as a key mechanism of toxicity (Mustafa *et al*., 2025).

Copper oxychloride, as a widely used copper-based fungicide, has raised concerns regarding its potential systemic toxicity beyond its agricultural benefits. Similar to other copper compounds, COC exposure may disrupt cellular redox balance, leading to oxidative stress and subsequent tissue injury. Emerging evidence suggests that copper-induced mechanisms, including oxidative stress and cuproptosis, contribute to cellular dysfunction in vital organs such as the heart. Although most mechanistic insights are derived from studies on other copper forms, these pathways are likely relevant to COC due to shared copper ion-mediated toxicity (Carvalho *et al*., 2021). In experimental models, exposure to copper-based compounds has been associated with hematological alterations, including reductions in hemoglobin levels and erythrocyte integrity, as well as oxidative damage in multiple tissues (Afzal *et al*., 2024). However, studies specifically addressing copper oxychloride remain limited and sometimes inconsistent, particularly regarding hematological indices (Sattanathan *et al*., 2019). Therefore, this study aimed to evaluate the toxicological effects of copper oxychloride in mice by assessing hemato-biochemical parameters, oxidative stress biomarkers, genotoxicity, and histopathological alterations under sub-chronic exposure conditions.

MATERIALS AND METHODS

This experiment was carried out at the Faculty of Veterinary Medicine, Aswan University, Egypt, during the time from December 2024 to April 2025. Twenty-five adult mice with the same age and equal mean body weights were obtained from the local animal house. The animals were housed in metal cages with good sanitation practices and were provided with fresh water and well-balanced food with 21% crude protein diet for two weeks prior to starting the experiment. Throughout the study, the animals were closely monitored for any behavioral or clinical problems. All experiments were conducted following the Ethical Committee's Guidelines for the Care and Use of Laboratory Animals set forth by the Aswan University Ethics Committee, Faculty of Veterinary Medicine (Protocol No.: ASWU/VM 18-1-2024).

Experimental Design: A total of twenty-five mice were randomly assigned to five equal groups (n = 5 per group): C0 (control, 0 mg/kg/day) and four treatment groups (C1–C4) receiving copper oxychloride at 5, 10, 15, and 20 mg/kg/day, respectively, for a period of two months.

Blood collection analysis: Blood was collected from each mouse of all treated and normal group by using sterile syringe via cardiac puncture in test tubes containing anticoagulant. Different hematological parameters were measured such as haemoglobin (Hb) amount, haematocrit (Hct), red blood cell (RBC) counts, total and differential white blood cell (WBC) counts, lymphocytes, and neutrophils with the help of automated hematology analyzer (Qayyum *et al*., 2025).

Biochemical serum investigation: Serum was separated from blood samples by centrifugation and stored at −20 °C until analysis. Alanine aminotransferase (ALT; Cat. No. 2650052), aspartate aminotransferase (AST; Cat. No. 260002), total protein (Cat. No. 310001), albumin (Cat. No. 2110001), creatinine (Cat. No. 2370002), triglycerides (Cat. No. 314001), cholesterol (Cat. No. 230001), and bilirubin (Cat. No. 226001) were measured using commercial kits (Spectrum Diagnostics, Egypt) according to the manufacturer's instructions. All biochemical analyses were performed using a chemistry analyzer (Ali *et al*., 2025).

Sample Collection Tissue preparation: All mice were humanely euthanized in accordance with institutional ethical guidelines and approved protocols. After euthanasia, viscera (liver, kidneys and heart) were quickly harvested during postmortem examination. Samples of the tissues were immersed in 10% buffered formalin for histopathological investigation, whereas other samples were washed with distilled water and reserved for biochemical study (Elbarbary *et al*., 2024).

For biochemical analyses homogenates of each tissue was subjected to centrifugation at 5000 rpm for 5 minutes. After that, supernatants were obtained and kept at −20 °C for further investigation (Ali *et al*., 2025). Different biomarkers of oxidative stress such as reduced glutathione (GSH), reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) were measured using spectrophotometer. The contents of various antioxidant enzyme including peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) were recorded using a UV spectrophotometer at appropriate wavelengths for each assay (Li *et al*., 2022; Zaki *et al*., 2023).

Histopathological Examination: Visceral organs from each experimental mice were taken at necropsy for histopathological examination. For histopathological examination, each organ was preserved in 10% formaldehyde solution (Liu *et al*., 2021; Aldawood *et al*., 2026). Using a rotary microtome, thin microscopic slices (4–5 µm thick) were cut, processed, and stained with haematoxylin and eosin (H&E). A light microscope (Nikon Eclipse 80i, Nikon Co., Tokyo, Japan) was used to examine visceral organs (Elsayed *et al*., 2025; Gareh *et al*., 2026).

Assessment of Genotoxic effects: The genotoxic potential of copper oxychloride in isolated cells of visceral organs such as liver, kidneys and heart was determined according to previous established protocol (Singh *et al*., 1988) using comet assay/alkaline assay. Approximately 250 cells from each visceral organ of each experimental mice were examined and genotoxic effects were observed in terms of DNA material fluorescing around the nucleus and or making a tail.

Statistical Analysis Data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics (Version 16, IBM Corp., Armonk, NY, USA). Differences among groups were assessed using Tukey's post hoc test, and results were considered statistically significant at $P<0.05$.

RESULTS

Physical parameters: No mortality was observed in mice administered different doses of copper oxychloride throughout the experimental period. However, mice receiving higher doses exhibited moderate clinical and behavioral changes, including lethargy, dullness, hair loss, and watery diarrhea.

Hematological parameters including red blood cells Counts, hemoglobin values, and hematocrit decreased significantly in treated mice compared to untreated normal mice (Figure 1). The hematological analysis revealed that the total white blood cells, and neutrophil count increased significantly while monocyte and lymphocyte count decreased significantly compared to control mice. Serum biochemistry analysis indicated significantly increased quantity of various liver functions tests (ALT, AST and bilirubin), kidney function tests (urea, creatinine) and different cardiac biomarkers (cholesterol, cardiac enzymes, triglyceride) compared to healthy mice. The results on oxidative stress in visceral organs such as liver, kidneys and heart indicated significantly increased contents of biomarkers of oxidative stress (ROS and TBARS) while significantly lower contents of antioxidant enzymes (SOD, POD, CAT and GSH) when compared to normal mice.

Oxidative stress and antioxidant enzyme test on heart of mice: This study evaluates the effects of various concentrations of copper oxychloride on oxidative stress parameters and serum biochemistry in mice. The investigation focused on the impact of exposure on glucose, cholesterol, triglycerides, CPK, and CKMB levels in serum, as well as on oxidative stress parameters including TBARS, GSH, SOD, CAT, POD, and ROS in heart tissue over a period of two months.

Serum biochemical analysis: Figures 2-3 present the serum biochemical parameters with respect to varying concentrations of copper oxychloride. An increase in the copper oxychloride concentration from C0 to C4 results in an increase in the glucose level, indicating glucose imbalance. In addition, there is a marked increase in the cholesterol and triglycerides concentration, indicating a possible imbalance in the metabolic process of lipids in the heart. Additionally, the presence of high CPK and CKMB values indicate injury to the heart, as both these enzymes are cardiac markers.

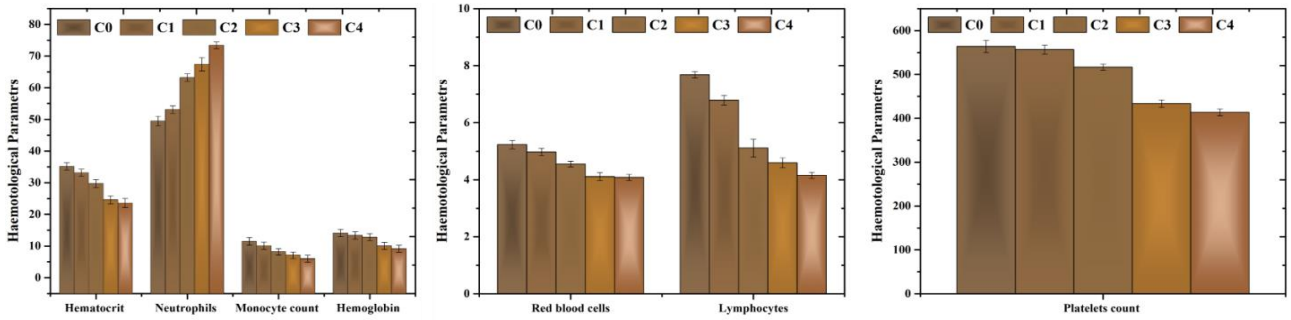

Fig. 1: Photograph showing comparison of different liver function testes in copper oxychloride treated and untreated mice.

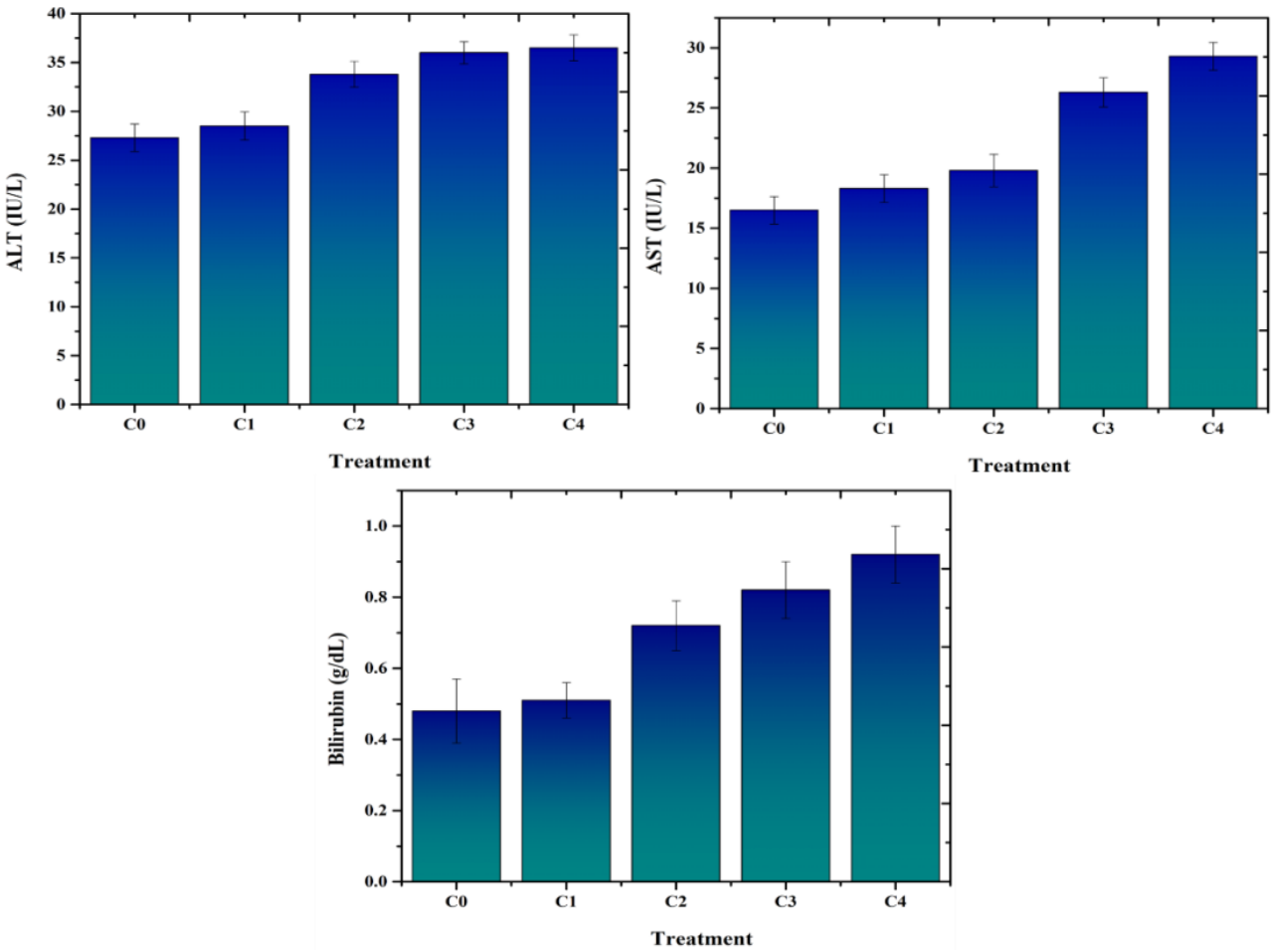

Fig. 2: A photograph comparing the liver function tests of mice treated and untreated with copper oxychloride.

Oxidative stress and antioxidant enzyme activity: Figure 4 provides an illustration of oxidative stress indicators and antioxidant enzyme activities in the heart after being exposed to copper oxychloride. The outcomes from the TBARS analysis reveal that there is a remarkable increase in the rate of lipid peroxidation in relation to an increase in copper concentration, which represents oxidative stress. On the other hand, there is a remarkable decrease in the level of reduced glutathione (GSH) and the activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT). This shows that the antioxidant mechanisms are compromised due to the high concentration of copper oxychloride. It is clear that there is a marked reduction in peroxidase activity. There is a significant increase in ROS.

In conclusion, all these results have shown the physiological effects caused by copper oxychloride, which include changes in serum biochemistry and poor antioxidant status in the heart, leading to oxidative stress in mice.

Oxidative Stress and Antioxidant Enzyme Activity in the Liver of Mice: The results that are illustrated by Figure 5 describe the oxidative stress markers and the antioxidant enzymes' activity in the liver of mice that have been treated under different conditions (C0-C4). Importantly, the levels of thio-barbituric acid reactive substances (TBARS), which act as indicators of lipid peroxidation, increase progressively from 1.38±0.11 nmol TBARS/h/mg protein (C0) to 2.25±0.12 nmol TBARS/h/mg protein (C4). It is evident that there is increased oxidative stress due to the drugs. In addition, reduced glutathione (GSH), an important antioxidant, decreases from 1.37±0.12 µmol GSH/mg protein (C0) to 0.61±0.11 µmol GSH/mg protein (C4), showing that the important protective substance has been depleted due to oxidative stress. There is also considerable decrease in the activity levels of superoxide dismutase (SOD) and catalase (CAT), where SOD drops from 1.85±0.10 units/mg protein (C0) to 1.01±0.13 units/mg protein (C4) the CAT from 1.93±0.17 units/mg protein (C0) to 1.19±0.11 units/mg protein (C4). These observations indicate reduced efficiency to cope with the presence of oxidative stress. In addition, there is a fall in peroxidase activity from 0.83±0.07 units/mg protein (C0) to 0.39±0.07 units/mg protein (C4). This observation supports the hypothesis that there are reduced antioxidant capabilities. There is an increased level of reactive oxygen species (ROS) activity, which represents the degree of oxidative stress from 0.95±0.06 units/mg protein (C0) to 1.88±0.07 units/mg protein (C4). This observation demonstrates oxidative stress damage during this experiment. Regarding serum biochemistry, ALT (alanine aminotransferase) increases from 27.3±1.41 IU/L (C0) to 36.5±1.34 IU/L (C4), predicting potential liver injury. In the same way, the AST (aspartate aminotransferase) values range from 16.5±1.15 IU/L (C0) to 29.3±1.15 IU/L (C4), indicating that there is indeed some form of liver

malfunction. It is also worth mentioning that the amount of bilirubin increases from 0.48±0.09 g/dL (C0) to 0.92±0.08 g/dL (C4), showing increased stress in the liver. In conclusion, the findings presented in this paper clearly show that there is an increasing oxidative stress and liver malfunction in the mice exposed to different treatments. Highlighting the necessity of additional research into the underlying mechanisms and possible treatments.

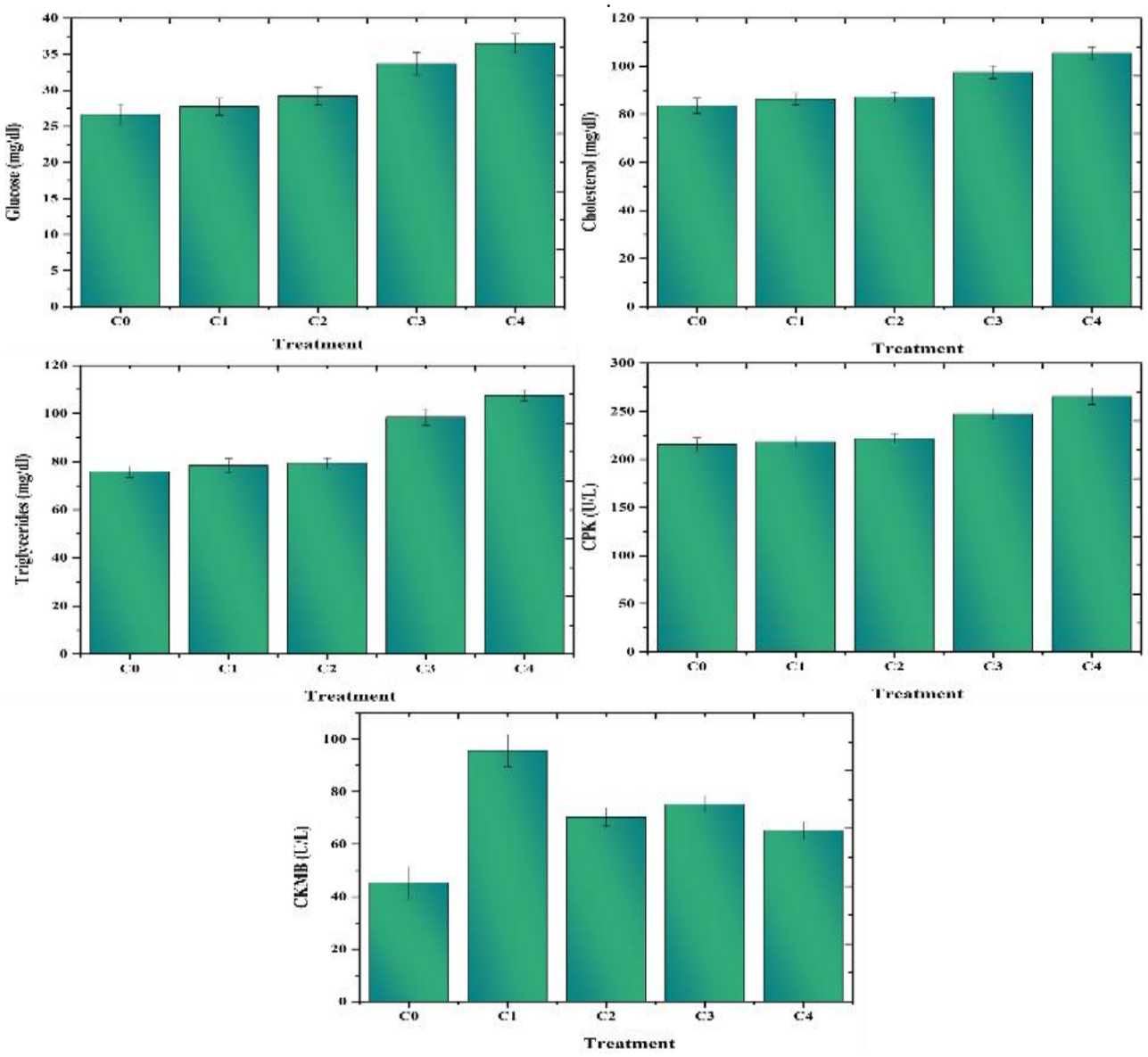

Fig. 3: Photograph depicting comparison of various types of kidneys and heart activity testis of copper oxychloride and control group of mice.

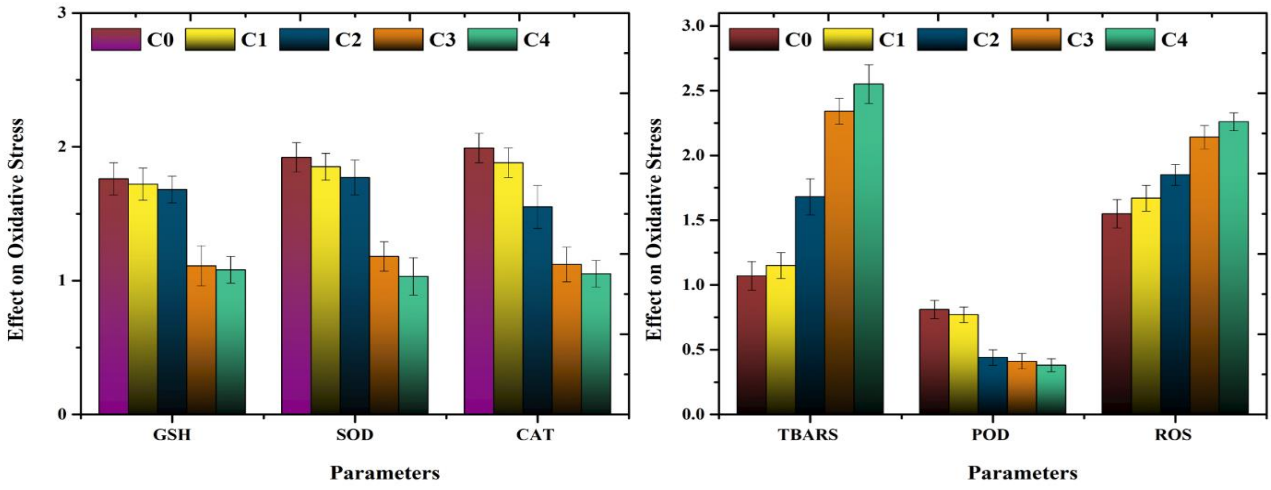

Fig. 4: Photograph depicting comparison of oxidative stress and antioxidant stress markers in the heart of copper oxychloride and control group of mice.

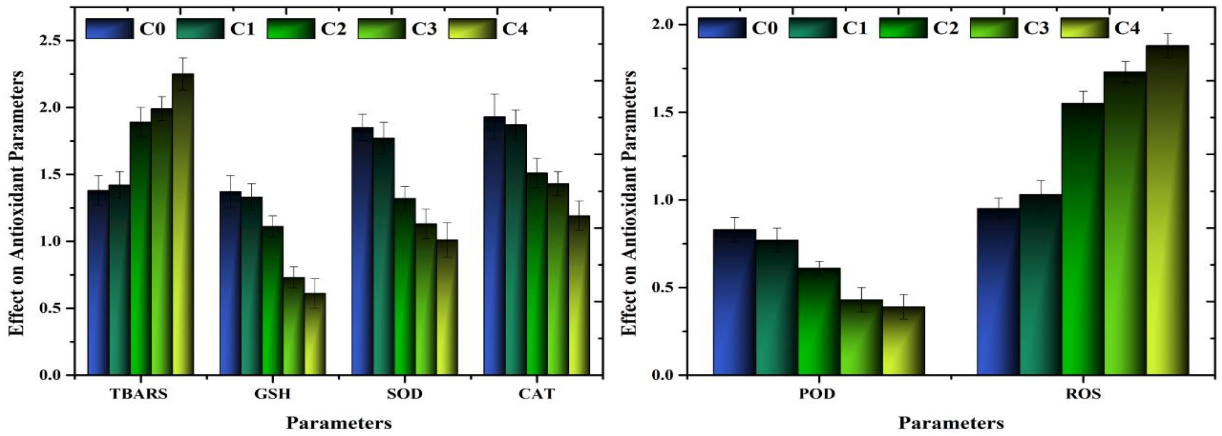

Fig. 5: An image comparing the oxidative and antioxidant stress profile indicators in the livers of mice treated and untreated with copper oxychloride.

Oxidative stress and antioxidant enzyme test on kidney of mice: The study focuses on the examination of oxidative stress and antioxidant status in the kidney tissue of mice exposed to different treatment regimens for a period of two months (Figure 6). The main variables examined in the experiment are Thio-barbituric Acid Reactive Substances (TBARS), reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), and Reactive Oxygen Species (ROS).

TBARS content, which is a biomarker for lipid peroxidation, exhibited a marked elevation among groups C0-C4, where TBARS concentrations in the control group (C0) amounted to 1.11 ± 0.11 nmol TBARS/h/mg protein while TBARS levels in group C4 were 1.93 ± 0.11.

On the other hand, the concentrations of GSH, which is an important non-enzymatic antioxidant, were found to be significantly reduced from 1.15 ± 0.11 µmol GSH/mg protein in C0 to 0.65 ± 0.10 µmol GSH/mg protein in C4, indicating that under oxidative stress, cellular antioxidants are being depleted. Enzymatic antioxidant activity was evaluated using SOD, CAT, and POD. There was a reduction in SOD activity, which started at 1.83 ± 0.11 units/mg protein in C0 and declined to 1.29 ± 0.13 units/mg protein in C4. Moreover, there was also a reduction in the activities of CAT and POD, illustrating the damage caused by the impaired kidney antioxidant defense system, which saw CAT activity decrease from 1.53 ± 0.11 units/mg protein to 0.67 ± 0.10 units/mg protein and POD from 1.33 ± 0.10 units/mg protein to 0.66 ± 0.11 units/mg protein. The assessment of ROS indicated an increasing pattern with values ranging from 1.28 ± 0.10 units/mg protein in C0 to 2.25 ± 0.11 units/mg protein in C4, suggesting a rise in oxidative stress. Overall, the observations suggest that oxidative stress negatively impacts kidney tissues, causing lipid peroxidation accompanied by a reduction in non-enzymatic and enzymatic antioxidants.

Hematological parameter: The observed decreases in hemoglobin and red blood cell (RBC) counts, together with decreases in monocytes and lymphocytes, are caused by the detrimental effects of copper oxychloride.

Specifically, hemoglobin levels declined from 14.11 ± 1.12% in the control group (C0) to 9.13 ± 1.11% in the treatment group (C4), while RBC counts fell from 5.23 ± 0.15 in C0 to 4.08 ± 0.11 in C4. Copper oxychloride has the potential to cause oxidative stress and impair erythropoiesis, causing a reduction in RBC production and consequently leading to reduced hemoglobin concentration. Furthermore, the toxicity of copper oxychloride might possess immunosuppressive activity, leading to a decrease in lymphocyte percentage from 7.68 ± 1.11% in C0 to 4.15 ± 1.11% in C4 and monocyte counts from 11.48 ± 1.21 in C0 to 6.01 ± 1.09 in C4. This reveals that copper oxychloride adversely affects hematological values and immune cell numbers, requiring further research for its specific mechanism of action.

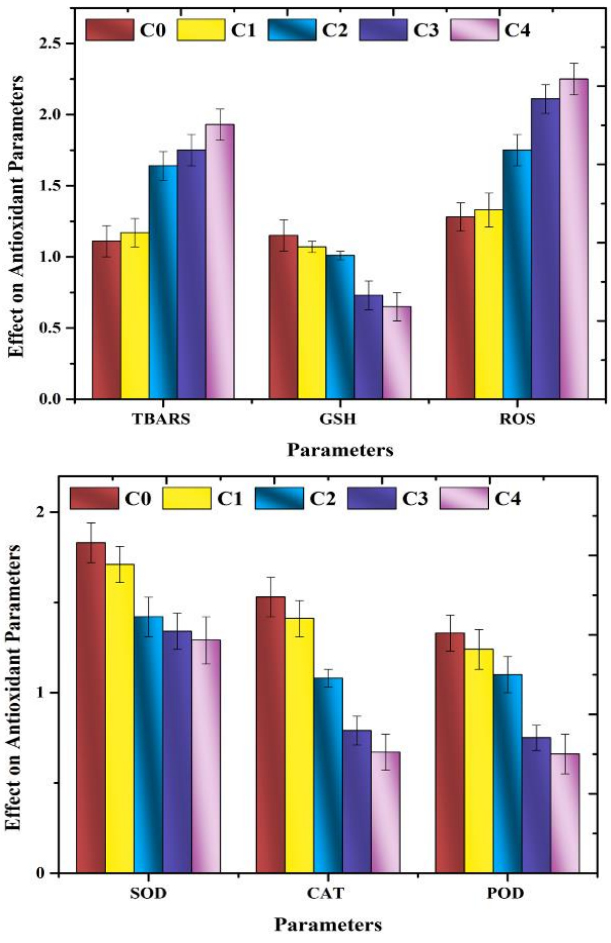

Fig. 6: Photograph comparing the oxidative and antioxidant stress profile indicators in the kidneys of mice treated with and not treated with copper oxychloride.

Histopathological observations: Light microscopic examination revealed dose-dependent histopathological alterations in multiple organs of treated mice. In the liver (Figure 7), observed changes included hepatocellular pyknosis, increased sinusoidal spaces, vascular congestion, inflammatory cell infiltration, and hepatocyte degeneration. Kidney sections (Figure 8) showed moderate to severe lesions, including necrosis of renal tubular epithelial cells, tubular degeneration, and widening of the urinary space. In the heart (Figure 9), treated mice exhibited necrosis of cardiac myocytes, inflammatory reactions, and disruption of muscle fibers. These alterations were more pronounced at higher exposure levels.

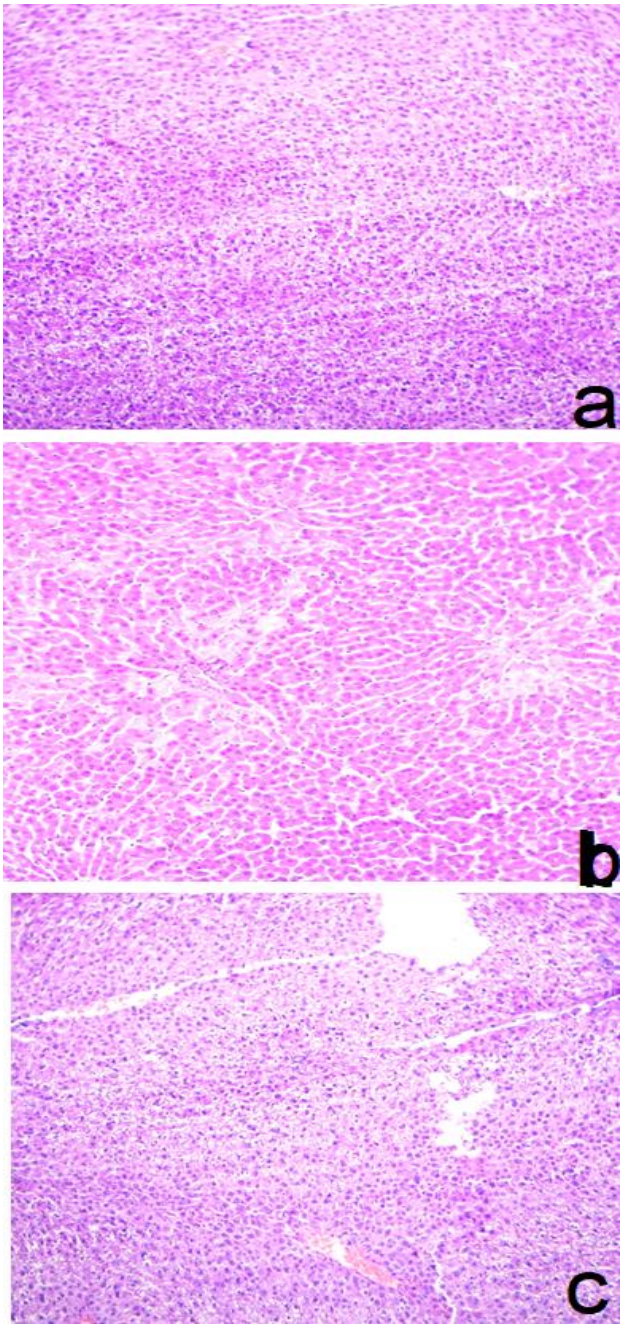

Fig. 7: Photomicrograph of different sections of liver of mice fed different doses of copper oxychloride showing various histopathological changes in a dose dependent manner. a-c) sections showing changes in liver of mice treated with copper oxychloride in groups (C2-C4) respectively. H&E stain; 400X

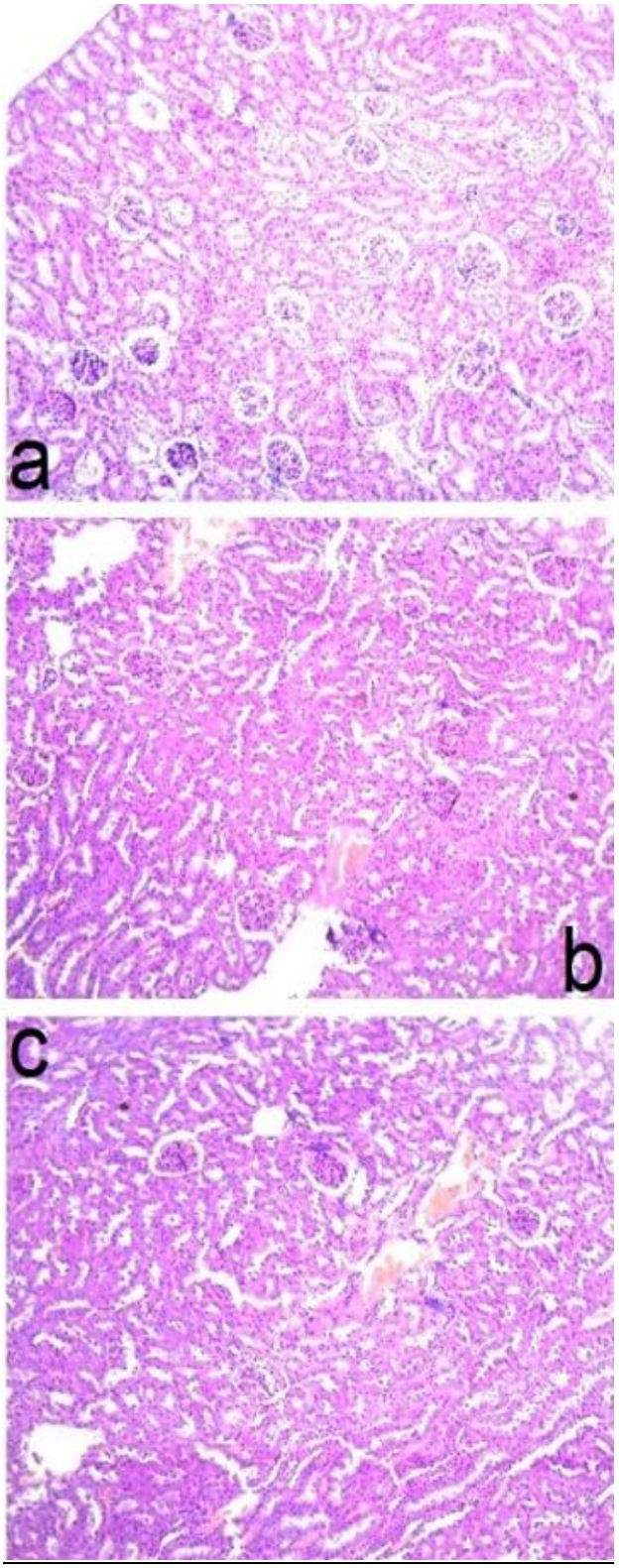

Fig. 8: Photomicrograph of different sections of kidneys of mice fed different doses of copper oxychloride showing various histopathological changes in a dose dependent manner. a-c) sections showing changes in kidneys of mice treated with copper oxychloride in groups (C2-C4) respectively. H&E stain; 400X.

Genotoxic studies: The results revealed significantly increased frequency of DNA damages in isolated cells of heart (Figure 10), liver (Figure 11), and kidneys (Figure 12), of mice treated with high doses of copper oxychloride compared to control mice in term of DNA material fluorescing around the nucleus and or making a tail.

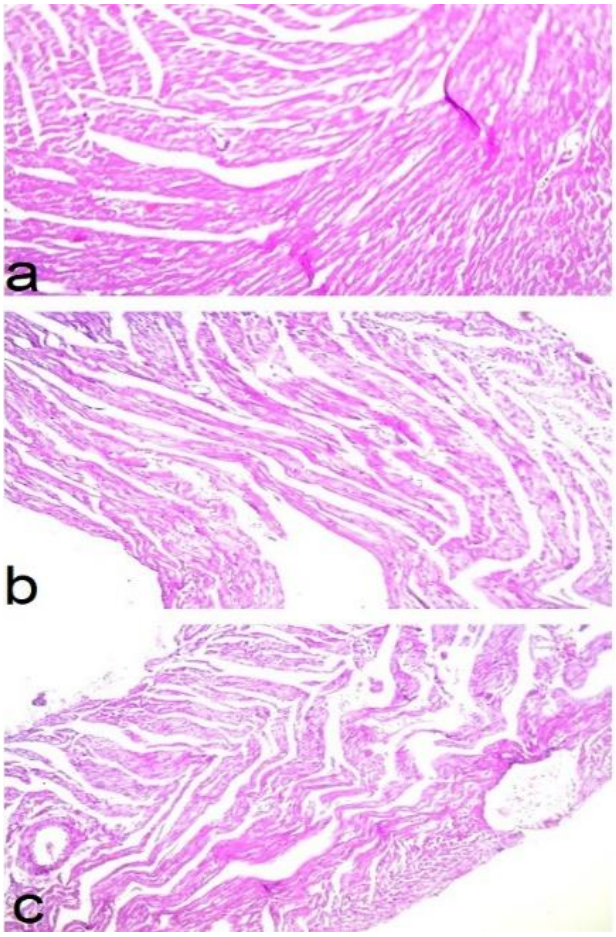

Fig. 9: Photomicrograph of different sections of heart of mice fed different doses of copper oxychloride showing various histopathological changes in a dose dependent manner. a-c) sections showing changes in heart of mice treated with copper oxychloride in groups (C2-C4) respectively. H&E stain; 400X.

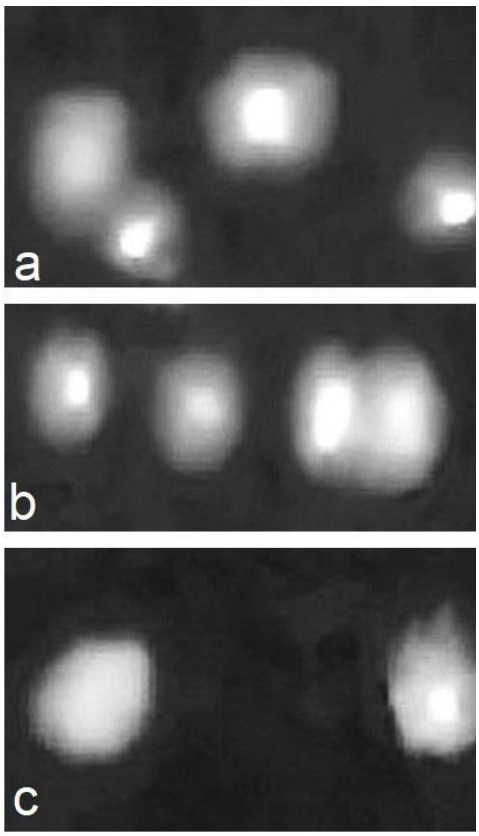

Fig. 10: Photomicrograph of exhibiting results on genetic damage in isolated cells of heart of mice fed different doses of copper oxychloride. a-c) showing DNA damages in heart of mice treated with copper oxychloride in groups (C2-C4) respectively. Ethidium bromide stain; 100X.

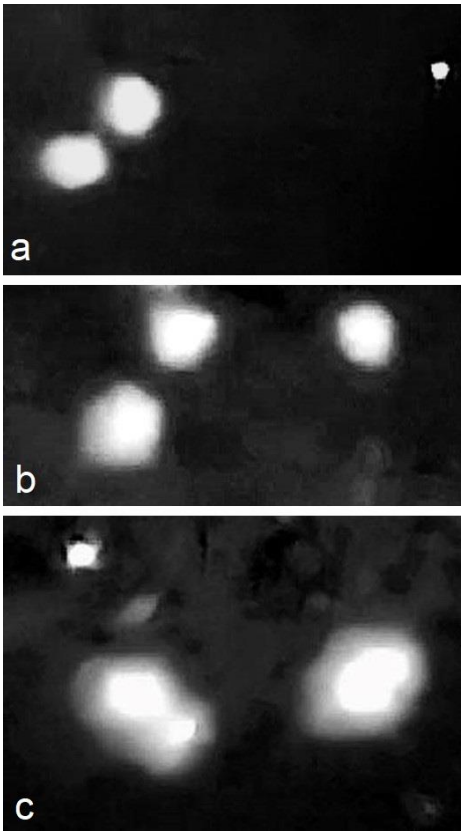

Fig. 11: Photomicrograph of exhibiting results on genetic damage in isolated cells of liver of mice fed different doses of copper oxychloride. a-c) showing DNA damages in liver of mice treated with copper oxychloride in groups (C2-C4) respectively. Ethidium bromide stain; 100X

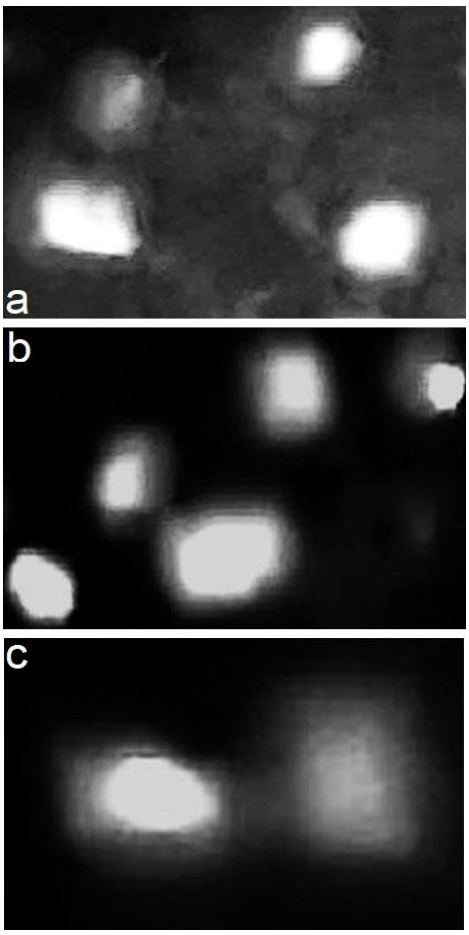

Fig.12: Photomicrograph of exhibiting results on genetic damage in isolated cells of kidneys of mice fed different doses of copper oxychloride. a-c) showing DNA damages in kidneys of mice treated with copper oxychloride in groups (C2-C4) respectively. Ethidium bromide stain; 100X

DISCUSSION

The present study demonstrates that subchronic exposure to copper oxychloride induces dose-dependent

toxic effects in mice, primarily mediated through oxidative stress. The observed clinical manifestations such as lethargy, alopecia, and diarrhea indicate further the occurrence of systemic toxicity. Such symptoms occur due to excessive copper deposition and may be linked to the interplay of oxidative stress and metabolic disturbances (Thompson *et al*., 2022). Consistent with the experimental design (0–20 mg/kg/day for 60 days), treated mice exhibited significant hematological alterations, including decreased erythrocyte count, hemoglobin, and hematocrit, alongside increased leukocyte counts, indicating systemic toxicity and possible inflammatory responses. Elevated serum concentrations of ALT, AST, glucose, and cholesterol, combined with a decrease in total proteins and albumin, were observed, indicating the presence of liver injury (Ouyang *et al*., 2021). This is consistent with earlier literature studies on the role of copper in hepatic injury (Kumar and Singh, 2022; Mustafa *et al*., 2025).

This study highlights the hazards associated with long-term exposure to copper oxychloride fungicides and emphasizes the importance of proper risk assessment in environmental and occupational settings. The induction of oxidative stress was evidenced through the increased ROS and TBARS, along with decreased SOD, CAT, POD, and GSH activities. This reflects the important role of oxidative stress in copper oxychloride toxicity (Fotouh *et al*., 2025). The histopathological observations showed evidence of prominent microscopic alterations in the liver, kidneys, and heart due to the toxic effects of copper oxychloride including cellular degeneration, necrosis and inflammatory infiltration (Al-Saeed *et al*., 2023; Ahmed *et al*., 2025). The administration of copper oxychloride caused toxic effects on multiple organs of mice via oxidative stress pathways. The results on hematological evaluation indicated a considerable adverse effect on the mice exposed to different doses of copper, as evident from lower hemoglobin concentration, erythrocyte count, and hematocrit levels. This observation is likely due to oxidative damage caused by copper to the erythrocytes' membrane, impairment in heme production, and erythropoiesis. Copper's role as a redox element allows for the production of ROS, causing lipid peroxidation and early destruction of erythrocytes (Hassan *et al*., 2021; Qiao *et al*., 2021). Furthermore, changes in leukocyte profile like reductions in lymphocyte and monocyte counts along with an elevation in neutrophil counts are suggestive of toxic and injurious stimuli in mice. The results imply immunosuppression along with inflammation. It is possible that lymphocytopenia is caused by oxidative stress-induced apoptosis, while the neutrophilia is a consequence of tissue damage. The findings are similar to those observed previously on the effects of copper-containing substances (Kumar and Singh, 2022).

Hepatotoxicity effects were recorded in terms of increased levels of aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mice exposed to copper oxychloride, together with increased levels of bilirubin, suggesting liver cell damage and malfunction (Ahmed *et al*., 2025; Khairy *et al*., 2026). This indicates the alterations in the integrity of liver cells due to exposure. The mode of action for toxicity caused by copper is mostly linked to the excessive accumulation of copper in the hepatocytes. The accumulation of copper beyond the ability of metallothionein and ceruloplasmin to bind leads to high concentrations of free copper ions, hence triggering oxidation processes and formation of ROS (Mustafa *et al*., 2025). In accordance with the proposed mechanism, the current study showed that there was an elevation in lipid peroxidation (TBARS) and ROS, along with a significant decline in antioxidant systems, such as GSH, SOD, CAT, and POD, indicating that oxidative stress was induced in liver tissue (Khairy *et al*., 2026).

These results coincide with earlier research, where hepatotoxic effects induced by copper were noted due to increased levels of liver enzymes, oxidative stress, and liver pathology in the form of degeneration, necrosis, and inflammatory cell infiltration (Pal *et al*., 2023; Shaofeng *et al*., 2021). In addition, copper-induced liver damage has been associated with inflammation and apoptosis mechanisms activated via NF-κB pathway and increased levels of pro-inflammatory mediators such as TNF-α and IL-6 (Ali *et al*., 2025). Metabolic disturbances in the liver related to lipids and energy have also been observed after copper exposure (Zhang *et al*., 2021; Yang *et al*., 2021). Oxidative stress was one of the primary indicators of copper oxychloride-induced renal toxicity (Ahmed *et al*., 2026). This is because the increased levels of TBARS and ROS were associated with the reduced antioxidant status in the form of GSH, SOD, CAT, and POD. This shows that there was a shift in the redox balance, which made the renal tissues prone to oxidation (Riaz *et al*., 2025).

The kidneys are highly susceptible to heavy metal toxicity, especially when they are considered to have a higher metabolic rate, numerous mitochondria, and a filtration mechanism for xenobiotic accumulation (Abdeen *et al*., 2023; Riaz *et al*., 2025). High concentrations of copper in renal tissues increase oxidative stress through redox cycling and free radical formation, which leads to the oxidation of lipids and proteins and disrupts their functions.

These results are similar to the observations recorded in prior studies indicating nephrotoxicity resulting from copper exposure through oxidative stress and histological changes such as tubular necrosis, glomerular injury, and inflammation (Amara *et al*., 2021). At a cellular level, it has been demonstrated that copper can cause stress via stress signal transduction pathways, such as MAPK and ER stress, leading to inflammation and cell death (Li *et al*., 2022; Fotouh *et al*., 2025). Cardiotoxicity in copper oxychloride exposure was seen in terms of increased cardiac injury biomarkers such as CPK and CK-MB that denote myocardial injury. The increase in blood concentrations of cholesterol, triglycerides, and glucose further suggests that there is an imbalance in the cardiometabolic processes. Oxidative stress proved to be the major cause of cardiac injury through the increases in TBARS and ROS levels, coupled with the marked reduction in antioxidant levels such as GSH, SOD, CAT, and POD in treated mice (Martinez *et al*., 2023; Sharma *et al*., 2025).

The cardiac muscle is especially susceptible to oxidative damage, which results from high energy demands coupled with a high mitochondrial population

(Ahmed *et al*., 2025). The presence of too much copper will cause mitochondrial malfunction, interfere with proper calcium balance in cells, trigger inflammation, and apoptosis, which cause damage to the cardiomyocytes. The findings are in agreement with those published earlier that revealed cardiotoxicity mediated by copper toxicity through oxidative stress, cellular alterations, and dysfunction (Narula *et al*., 2023). Furthermore, copper toxicity has been linked to metabolic disorders and signaling via profibrotic and stress pathways that can lead to cardiotoxicity (Wang *et al*., 2021). The continuous elevation of the oxidative stress parameters (ROS and TBARS), accompanied by a decrease in antioxidants (GSH, SOD, CAT, and POD), signified systemic oxidative damage in the body and tissue-specific toxicity (Ouyang *et al*., 2021). The systemic effects of copper can be explained by the redox properties of copper; this metal is involved in the oxidation-reduction cycle and, hence, generates ROS and causes oxidative damage. The significant decrease in the level of GSH and the inhibition of antioxidant enzymes activity are also indicative of weakened defensive functions of cells, which can be attributed to the oxidation process and redox imbalance (Fotouh *et al*., 2025). The role of copper in the generation of oxidative stress and subsequent activation of stress response pathways, such as the Nrf2 pathway, was also confirmed by earlier investigations (Chen *et al*., 2023; Gill *et al*., 2025; Rashid *et al*., 2025). The significantly increased frequency of DNA damages in isolated cells of heart, liver and kidneys of mice treated with high doses of copper oxychloride in term of DNA material fluorescing around the nucleus and or making a tail might be due to activated DNase resulting in cleavage nuclear proteins (Hussain *et al*., 2014), lipid peroxidation, over release of intracellular nitrogenous and reactive oxygen species, nitration of proteins and oxidation of mRNA (Sharma *et al*., 2021).

Conclusions: The study demonstrates that there is strong evidence indicating that exposure to copper oxychloride causes severe multisystem toxicity in mice through oxidative stress pathways as shown by blood disorder, liver, kidney, and heart dysfunction. The dose-response pattern observed here and concurrence with current research literature further support the need for avoiding exposure to copper oxychloride and finding substitutes for it in order to reduce any harm caused to health.

Authors Contribution: NA, AA, AAA: Writing –original draft, Writing – review & editing, Investigation, Data curation, Conceptualization, Funding acquisition. AEA, YH, NKE: Methodology, Investigation, Supervision. FA, ME: Data curation, Resources. AMAM, KA: Writing – review & editing, Conceptualization, Project administration, Resources. All authors read and approved the manuscript.

Acknowledgements: The authors gratefully acknowledge the financial support provided by the Princess Nourah bint Abdulrahman University Researchers Supporting Project (PNURSP2026R367), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors also acknowledge the support of King Khalid University through the Large Research Grant Group (R.G.P2/189/47). In addition, this work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia grant number (KFU261507)

Declaration of competing interest: The authors have stated that they have no personal connections or conflicting interests.

Conflicts of interests: The authors declare that there are no conflicts of interest.

REFERENCES

Abdeen A, Elsabagh R, Elbasuni S, *et al.,* 2023. Microalgae (*Chlorella vulgaris*) attenuates the aflatoxin-associated renal injury. Frontiers in Pharmacology 14:1291965.

Afzal G, Ahmad HI, Hussain R, *et al*., 2022. Bisphenol A induces histopathological, hematobiochemical alterations, oxidative stress, and genotoxicity in common carp (Cyprinus carpio L.). Oxidative Medicine and Cellular Longevity 9:1-14.

Ahmed F, Elbarbary NK, Maha AM, *et al*., 2025. Hepatoprotective effects of mesenchymal stem cells in CCl_4-induced liver toxicity in rats: restoration of liver parameters and histopathological evaluation. American Journal of Veterinary Research 86(6):1-10.

Ahmed F, Swillam S, Radwan WA, *et al*., 2026. An updated review on avian liver pathology with special reference to chickens, ostriches, and pigeons. (Review). Comparative Clinical Pathology 35(1):38.

Aldawood N, Aljazzar A, Alsaegh AA, *et al.,* 2026. Quantitative prediction of azoxystrobin-induced genetic damage, histopathology, brain and testicular disparities in male albino rats. Pakistan Veterinary Journal 46(3): 662-669.

Ali NM, Hussein MK, Elbarbary NK, *et al.,* 2025. Saccharomyces cervisiae ameliorative impact combined with sulfaclozine on broiler chicken oxidative status. BMC Veterinary Research 21:507-521.

Al-Saeed FA, Naz S, Saeed MH, *et al.,* 2023. Oxidative stress, antioxidant enzymes, genotoxicity, and histopathological profile in Oreochromis niloticus exposed to lufenuron. Pakistan Veterinary Journal 43(1): 160-166.

Amara I, Hakim A, Troudi A, *et al.,* 2021. Protective role of selenium against copper-induced nephrotoxicity through modulation of oxidative stress and apoptotic pathways in rats. Biol Trace Element Research 199(6): 2234-2245.

Carvalho J. A, Boavida L, Ferreira R, *et al.,* 2021. Copper-induced haemolytic anaemia. European Journal of Case Reports in Internal Medicine *8*(9): 002785.

Chen L, Wang X, Zhang Y, *et al.,* 2023. Systemic toxicity of copper nanoparticles: Multiorgan oxidative stress and transcriptomic alterations in mice. Environmental Toxicology and Pharmacology 97:104012.

Cui X, Wang Y, Liu H, *et al*., 2022. The molecular mechanisms of defective copper metabolism in diabetic cardiomyopathy. Oxidative Medicine and Cellular Longevity, 1: 5418376. doi: 10.1155/2022/5418376.

Elalfy MM, Abomosallam MS, Sleem F, *et al.,* 2021. Copper and copper containing pesticide as copper oxychloride toxicity and its adverse effects on animal and human health. Medico Research Chronicle,8(2): 1-18.

Elbarbary NK, Darwish WS, Fotouh A, *et al.,* 2024. Unveiling the mix-up: investigating species and unauthorized tissues in beef-based meat products. BMC Veterinary Research 20: 380-398.

Elsayed AK, Abd-El Hamed AM, Khairy NE, *et al.,* 2025. Pathological investigation of late embryonic death in ostrich hatcheries and their economic impact in Egypt. American Journal of Veterinary Research 5: 1–9.

Fotouh A, Elbarbary NK, Moussa MA, Diab MS, Elshafae SM, *et al.,* 2025. Histopathological effects of azithromycin on broilers: immune system alterations and apoptotic changes. British Poultry Science 66(5):1-7.

Fotouh A, Elbarbary NK, Elshafae S, *et al.,* 2026. Unlocking the Pathological Insights of the Bacterial Infections of Free-Living Pigeons. Veterinary Medicine and Science 12(2):e70866.

Gareh A, Elbarbary NK, Fotouh A, *et al.,* 2026 Seromolecular and histopathological study on Toxoplasma gondii infection in ruminants in Aswan, Egypt. BMC Veterinary Research 22:49-62.

Gill H, Ali N, Tariq S, *et al.,* 2025. Assessment of antioxidant defense, histological modifications and metal bioaccumulation in tilapia fish brain and muscle exposed to aqueous Nickel and Chromium Co-Exposure. International Journal of Agriculture and Biosciences 14(6): 1316-1322.

Hassan MA, El-Sayed SM, Mohamed WA, *et al.,* 2021. Hematological and biochemical alterations induced by copper sulfate toxicity in rats: Protective role of quercetin. Journal of Trace Elements in Medicine and Biology 68: 126831.

Iqbal R and Irfan M, 2024. Impacts of ZnO nanoparticles on brain and spleen in male albino rats, A comprehensive exploration of diverse exposure routes. Continental Veterianry Journal 4:92–100.

Javed M and Usmani N, 2019. An overview of the adverse effects of heavy metal contamination on fish health. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 89(2): 389-403.

Kanwal N, Aziz S, Abdullah S, *et al.,* 2024. Studies on the changes in antioxidant enzyme activity induced by parathion in Hypophthalmichthys molitrix. Continental Veterinary Journal 4:40–45.

Khairy NE, Elbatawy RM, Ghania AA, *et al.,* 2026. Antioxidant chemistry and its role in mitigating oxidative stress in animals. (Review). Open Veterinary Journal 16(3):1426-1437.

Kumar A and Singh R, 2022. Immunotoxic effects of chronic copper exposure in mice: Alterations in lymphoid organs and immune cell populations. Immunopharmacology and Immunotoxicology 44(3): 412-423.

Li X. Naseem S, Hussain R, *et al.,* 2022. Evaluation of DNA Damage, Biomarkers of Oxidative Stress, and Status of Antioxidant Enzymes in Freshwater Fish (Labeo rohita) Exposed to Pyriproxyfen. Oxidative Medicine and Cellular Longevity 5859266,

Li Y, Chen H, Wang L, *et al.,* 2022. Copper-induced nephrotoxicity involves endoplasmic reticulum stress and MAPK pathway activation in renal tubular cells. Toxicology 465: 153043.

Liu B, Zeng Qi, Chen H, *et al.,* 2021. The hepatotoxicity of altrazine exposure in mice involves the intestinal microbiota. Chemosphere 272: 129572.

Martinez E, Rodriguez P, Santos L, *et al.,* 2023. Antioxidant therapy in heavy metal toxicity: N-acetylcysteine attenuates copper-induced oxidative damage in experimental models. Toxicology and Applied Pharmacology 461: 116398.

Mujahid Q, Khan A, Qadir MF, *et al.,* 2021 Allethrin induced toxicopathological alterations in adult male albino rats. Agrobiological Records 5:8-14.

Mustafa G, Hussain R, Afzal G, *et al.,* 2025. Adverse impacts of copper oxide nanoparticles on Rattus norvegicus: Provocation of oxidative stress and depletion of antioxidant enzymes in multiple visceral organs. Biological Trace Element Research 204(3):1201-1212.

Narula S, Sharma R, Kumar V, *et al.,* 2023. Cardiac dysfunction and oxidative stress induced by copper oxide nanoparticles in rats: Histopathological and molecular insights. Cardiovascular Toxicology 23(2): 145-158.

Ouyang Z, Yang B, Yi J, *et al.,* 2021. Exposure to Fluoride induces apoptosis in liver of ducks by regulating Cyt-C/Caspase 3/9 signaling pathway, Ecotoxicology and Environmental Safety 224: 112662

Pal S, Ghosh M, Mondal S, *et al*. (2023). Hepatotoxicity of copper oxide nanoparticles: Oxidative stress, inflammation, and apoptosis in rat liver. Journal of Applied Toxicology 43(4): 567-579.

Pelosi C, Gavinelli F, Petit-dit-Grezeriat L, *et al.,* 2024. Copper toxicity to earthworms: A comprehensive review and meta-analysis. Chemosphere 362:142765.

Qayyum A, Shafique S, Ghori MT, *et al.,* 2025. Evaluation of clinico-hematological and biochemical changes in camels with trypanosomiasis in the Cholistan region, Bahawalpur. International Journal of Veterinary Science 14(6): 1162-1169.

Qiao N, Yang Y, Liao J, *et al.,* 2021. Metabolomics and transcriptomics indicated the molecular targets of copper to the pig kidney. Ecotoxicology and Environmental Safety 218:112284.

Rashid M, Gill H, Ali N, *et al.,* 2025. Effects of aqueous cadmium and nickel Co-exposure on antioxidant defense mechanisms, histological changes and metal bioaccumulation in the brain and muscle of Tilapia fish. International Journal of Veterinary Science 14(5): 1023-1029.

Ren X, Zhao L, Hao Y, *et al.,* 2025. Copper-instigated modulatory cell mortality mechanisms and progress in kidney diseases. Renal failure 47(1): 2431142.

Riaz, R., M. Asif, G. Afzal, *et al.,* 2025. Investigation of oxidative stress and antioxidative enzymes in erythrocytes and bone marrow of albino rats treated with different concentrations of copper ferrite nanoparticles. International Journal of Agriculture Bioscience 14:1035–1041.

Sattanathan G, Amsath A, Senthilmurugan S, *et al.,* 2019. Toxicity of copper oxychloride (fungicide) in Oreochromis mossambicus on haemato-immunological and biochemical alterations and recovery assessment by marine algae Chaetomorpha aerea. International Journal of Zoology and Applied Biosciences 4(4): 184-194.

Shaofeng W, Zhong G, Wan F, *et al.,* 2021. Evaluation of toxic effects induced by arsenic trioxide or/and antimony on autophagy and apoptosis in testis of adult mice. Environmental Science and Pollution Research 28: 54647–54660 (2021).

Sharma V, Kumar P, Singh A, *et al*. (2021). Epigenetic modifications in copper-induced toxicity: DNA methylation and histone changes affecting antioxidant gene expression. Toxicological Sciences 184(1): 112-125.

Sharma VA and Frishman WH, *et al.,* 2025. Copper dysregulation and cardiovascular disease: A review of underlying mechanisms and therapeutic targets. Cardiology in Review 6: 1-14.

Singh NP, Mccoy MT, Ticer RR, *et al.,* 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research 175:184–191

Thompson DJ, Williams RE, Anderson KL, *et al.,* 2022. Clinical manifestations of copper toxicosis: A comprehensive review of experimental and human case studies. Journal of Toxicology and Environmental Health Part B, 25(7): 321-340.

Ullah N, Khan A, Hussain I, *et al.,* 2024. Determination of commonly used pesticides and lethal concentrations of copper oxychloride to Cyprinus carpioat District Swat. Planta Animalia 3(2): 59-65.

Wang H, Liu Z, Chen Q, *et al.*, 2021. Copper-induced cardiac fibrosis through TGF-β signaling pathway activation and oxidative stress. Toxicology Letters 350: 45-56.

Wang YM, Feng LS, Xu A, *et al.,* 2024. Copper ions: The invisible killer of cardiovascular disease (Review). Molecular Medicine Reports 30(5): 210.

Yamkate P, Gold R. M, Xenoulis P. G, *et al.,* 2021. Assessment of copper accumulation in archived liver specimens from cats. Journal of Feline Medicine and Surgery 23(6), 526-533.

Yang B, Liu Y, Li Y, *et al.,* 2021, Exposure to the herbicide butachlor activates hepatic stress signals and disturbs lipid metabolism in mice, Chemosphere 283:131226.

Yang CC, Wu ML, Deng JF, *et al.,* 2004. Prolonged hemolysis and methemoglobinemia following organic copper fungicide ingestion. Veterinary and Human Toxicology 46(6): 321–323.

Zaki RS, Neveen MA, Reda AG, *et al.,* 2023. Impact of thawing techniques on the microstructure, microbiological analysis, and antioxidants activity of Lates niloticus and Mormyrus kannume fish fillets, The Egyptian Journal of Aquatic Research 49(4): 530-536.

Zhang X, Li M, Wang J, *et al.,* 2021. Metabolomic analysis reveals copper-induced disruption of hepatic metabolism in rats. Chemosphere 283: 131176.