Copper Sulphate-Induced Nephrotoxicity in Adult Wistar Rats: Therapeutic Potential of Vitamin E

[1]Obayuwana, E.; [1]Imafidon, E.O

[1]Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

***Corresponding author**
Email: endurance.imafidon@uniben.edu

Abstract:
Background: Heavy metal contamination, particularly by copper, poses significant environmental and health concerns due to anthropogenic activities. Anthropogenic activities have substantially elevated heavy metal concentrations, leading to toxicity in various ecosystems. Copper sulphate, a common heavy metal, adversely affects human health, primarily targeting soft tissues such as the kidneys, leading to neurological disorders and multiorgan failure. Animal studies have revealed oxidative stress, hepatic, renal, and testicular toxicity upon exposure to copper sulphate. Vitamin E, a potent antioxidant, is explored for its potential protective role against copper sulphate-induced renal damage. The aim of this experiment was to investigate the ameliorative potential of Vitamin E on copper sulphate induced nephrotoxicity in adult Wistar rats. **Materials and Methods**: Twenty-five (25) adult Wistar rats weighing between 160g and 220g were separated into five (5) groups of randomized patterns with five (5) rats in each group. subjected to different treatments over 60 days. A, B, C, D, and E with each group having five Wistar rats which were all weighed prior to the administration. **Results:** The rats were administered with Copper Sulphate for 30 days and Vitamin E for 30 days totalling 60 days. Copper sulphate administration induced significant changes in body weights, electrolyte levels, and oxidative stress biomarkers. Histological analysis revealed interstitial congestion and tubular necrosis indicative of renal damage. However, co-administration of Vitamin E mitigated these effects, demonstrating its protective role against copper sulphate toxicity. **Conclusion**: Vitamin E mitigates copper sulphate-induced oxidative stress, dysregulation of renal electrolytes, and histological alterations in the kidney of adult Wistar rats.

Keywords: Copper sulphate, nephrotoxicity, vitamin E, Wistar rat, Kidney

Introduction

All areas of the ecosystem consist of relative anthropogenic amounts of copper and other heavy metals. However, human activity has increased the concentrations of these heavy metals in the environment to exceptionally high levels, especially in regions where the metals are mined, processed, and used industrially (1). Heavy metals come into contact with people in industrial, manufacturing, pharmaceutical, residential, or agricultural contexts through food, water, air, or skin absorption (2). When heavy metals accumulate in soft tissues without being metabolized by the organism, they become poisonous. Copper sulphate causes severe damage to all human tissues and is not a normal component of the body (3). The kidneys absorb lead when inhaled as minute particles, and some lead is consumed in food. About 10 % of adults and up to 53 % of young children are absorbed by the gastrointestinal system and mostly taken up by red blood cells (4). Over 94 % of it is deposited in the bones of adults, but only about 64 % in children. Copper sulfate toxicity is manifested in soft tissues, with the Kidney serving as the primary target organ. Copper sulfate toxicity in the kidney can cause several neurological disorders, including mental retardation, nerve damage, behavioural disorders It can be absorbed through gastrointestinal tract, lungs, and skin causing both systemic and local toxicity including stupor, coma, convulsion, hypertension, respiratory failure, pallor and jaundice (5).

Ingestion of significant quantity of copper sulphate carries a risk of multiorgan failure. Animals exposed to copper sulphate suffer from oxidative stress, hepatic, renal and testicular toxicity (5). Vitamin E (Vit E) was discovered by Evans and Bishop in 1922 and it was isolated from wheat germ oil in 1936 (6). Vitamin E is a fat-soluble vitamin that acts as a free radical scavenger for blocking lipid peroxidation from polyunsaturated fatty acids and preventing nitrosamine formation (7). Vitamin E is an essential biological system antioxidant that is abundant in tissues, particularly in membrane-rich sections (7). Administration of vitamin E can be used with other vitamins to support activities of antioxidant enzymes such catalase (8). It has a main role in protecting the body against harmful effects of metabolically active types of oxygen that can have a strong antioxidant function (9). Vit E, as a membrane stabilizer, is well known as free radical scavenger (8) and protects critical cellular structures against oxidative damage caused by oxygen free radicals and reactive products of by utilizing Wistar rats, known for their metabolic activity, the study aims to simulate real-world scenarios and provide insights into the interplay between copper sulfate exposure, renal damage, and the protective effects of Vitamin E. The kidneys, as crucial regulators of homeostasis, serve as a focal point in understanding the systemic impact of copper sulfate and the potential of Vitamin E to mitigate its adverse effects. This study aims to investigate the effect of vitamin E on copper sulphate-induced renal damage in Wistar rats including the body weight, urea, creatinine, potassium ion, sodium ion, chloride ion and bicarbonate ion, oxidative stress parameters including total protein, malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase and the histology of the kidney tissue.

Materials and Methods

Experimental Animals: Twenty-five Wistar rats of weight between 180g and 200g were used for this experiment. The rats were allowed to acclimatize for two-weeks period before the administration method begun. The rats were given free access to conventional feed and water. The Research Ethics Committee's Guidelines for animal care at the University of Benin's College of Medicine were followed. The experimental design is presented in Table 1.

Administration: Copper sulphate and Vitamin E were given using a gavage with an orogastric tube. The rats were carefully handled to minimize oral or oesophageal injuries. All administrations were done by gavage and lasted thirty (30) and sixty (60) days respectively.

Tissue collection, processing and staining, histopathology: The rats were sacrificed and the kidneys were taken at the end of thirty days and sixty days study. Blood (5 mL) was collected in sterile bottle for serum analysis of Na^+, K^+, Cl^-, HCO^{2-}, urea and creatinine levels, a section of the kidney was fixed in a plain bottle and preserved with formal saline for oxidative stress biomarkers.

The kidney tissues were preserved for 24 hours in 10% buffered formalin before being histologically processed and stained with Haematoxylin and Eosin using standard procedure (10). The sections obtained were examined and photomicrographs were taken using a Leica DM750 research microscope with a digital camera (Leica CC50). The tissues were photographed digitally at a magnification of x100.

Statistical analysis: Results obtained were expressed as Mean ± SEM (standard error of mean). Differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value is less than 0.05 ($p < 0.05$). LSD Post Hoc test was used to determine where the significance lay. Statistical package Graphpad Prism version 9 for Windows (Graphpad Software Inc.) was used to analyze the data obtained in the study.

Results and Discussion

Copper sulphate is known to cause oxidative stress by generating free radicals, which can lead to cellular damage and affect various biological parameters, including weight (11). The oxidative stress can result in liver damage, anaemia, and other systemic effects that might contribute to weight loss or poor weight gain (12).

There was a significant decrease in Superoxide dismutase, Catalase and Glutathione peroxidase for rats treated with 200mg/kg of copper sulphate when compared with control. This suggests that copper sulphate at this dosage has a notable effect on the protein metabolism and Oxidative stress of the rats. Copper sulphate is known to be involved in various enzymatic reactions in the body. It is absorbed from the gut via high-affinity and low-affinity copper uptake proteins. Once inside the cells, copper is believed to be reduced to the Cu^1+ form before being transported (13). It plays a role in the function of many enzymes, including cytochrome c oxidase and superoxide dismutase (13). However, an excess of copper can lead to the production of hydroxyl radicals through reactions like the Fenton and Haber-Weiss reactions, resulting in oxidative stress, lipid peroxidation, and protein oxidation (14). Wang *et al*., (15) reported similar findings after administration of copper sulphate on intestines of juvenile *Epinephelus coioides* (15). This decrease was reversed following treatment with 200mg/kg of Vitamin E and Distilled water. Vitamin E, primarily in the form of alpha-tocopherol, acts as an antioxidant, protecting cell membranes from oxidative damage caused by free radicals (16). While the exact mechanism of action for many of Vitamin E's effects is still unknown, it is recognized for its role in preventing free radical reactions with cell membranes (17). In some cases, Vitamin E has been shown to have pro-oxidant activity, which might contribute to its complex interaction with copper sulphate (18). In the context of the study results, the decrease in total protein levels in rats treated with copper sulphate and Vitamin E compared to the control could be attributed to the oxidative stress induced by copper sulphate, which may lead to the degradation of proteins (19). The presence of Vitamin E seems to mitigate this effect, likely due to its antioxidant properties, which help protect proteins from oxidative damage. Conversely, the increase in total protein levels in rats treated with copper sulphate and Vitamin E or distilled water compared to those treated with copper sulphate alone suggests that Vitamin E and possibly distilled water may help maintain or restore protein levels, possibly by reducing the oxidative stress or stabilizing the cellular environment.

Malondialdehyde (MDA) is a by-product of lipid peroxidation and is commonly used as a biomarker for oxidative stress (20). Elevated levels of MDA indicate increased oxidative damage to cell membranes, which can lead to various pathological conditions (20). There was a statistically significant increase in MDA levels in rats treated with 200mg/kg of copper sulphate compared to the control group. This suggests that copper sulphate at this dosage induces oxidative stress in rats, leading to lipid peroxidation and an increase in MDA levels. This agrees with study conducted by Kirici, (2017) on toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish *Capoeta umbla* (21). Vitamin E and Distilled water was able to reverse the increased MDA activities caused by copper sulphate intoxication.

Result from this study showed a significant increase in urea, creatinine, and electrolyte levels upon administration of copper sulphate indicating its nephrotoxic potential. This aligns with known complications of copper toxicity, which include direct damage to renal tubular cells, leading to acute kidney injury (22). Elevated urea and creatinine levels are classic biomarkers of renal impairment. Their increase suggests that copper sulphate at the given dosage can cause significant renal dysfunction (23). Vitamin E and distilled water was able to mitigate the increased levels of urea, creatinine and kidney electrolyte caused by copper sulphate administration. The observed decrease in urea, creatinine, and electrolyte levels with the co-administration of Vitamin E suggests its protective role against nephrotoxicity. Vitamin E is known for its antioxidant properties, which can mitigate oxidative stress-induced damage in the kidneys (24). The protective effect of Vitamin E could be attributed to its ability to scavenge free radicals and reduce lipid peroxidation, thereby preserving the integrity of cellular membranes in the kidneys (25).

Histological results revealed interstitial congestion and focal tubular necrosis after

administration of copper sulphate at 200mg/kg. Interstitial Congestion indicates an accumulation of fluid in the interstitial space within the kidney, which can be a response to injury or inflammation caused by copper toxicity (26). Focal Tubular Necrosis suggests that localized areas of the renal tubules have undergone cell death. Copper is known to induce oxidative stress and inflammation, leading to cellular damage and necrosis (27). Vitamin E was able to reverse the effect caused by copper sulphate intoxication. The return to normalcy in tubular and glomerular structures after initial copper-induced damage suggests that Vitamin E has a significant reparative or protective effect against copper toxicity. This aligns with Vitamin E's role as an antioxidant, helping to mitigate oxidative stress and promote recovery of renal tissues (28). Interstitial Congestion (CO) and Interstitial Infiltrates of Inflammatory Cells (IC) and the persistence of tubular necrosis indicate ongoing inflammation and suggest that distilled water alone may not be sufficient to counteract the copper-induced damage (29).

Conclusion

In conclusion, the study demonstrates that copper sulphate administration at a dosage of 200mg/kg induces oxidative stress, evidenced by significant changes in various biological parameters including weight, protein metabolism, and lipid peroxidation, as well as nephrotoxic effects such as elevated urea, creatinine, and electrolyte levels along with histological evidence of renal damage. However, co-administration of vitamin E appears to mitigate these effects, likely through its antioxidant properties, suggesting a potential therapeutic role in counteracting copper-induced toxicity.

Table 1: Experimental Design

Groups	Treatments
A	Served as control and were fed with Animal feed and water ad libitum.
B	Received 200mg/kg of copper sulphate daily for 30 days.
C	Received 200mg/kg of Vitamin E daily for 30 days.
D	Received 200mg/kg of copper sulphate daily for 30 days followed by 200mg/kg of vitamin E daily for 30 days
E	Received 200mg/kg of copper sulphate daily for 30 days followed by distilled water daily for 30 days.

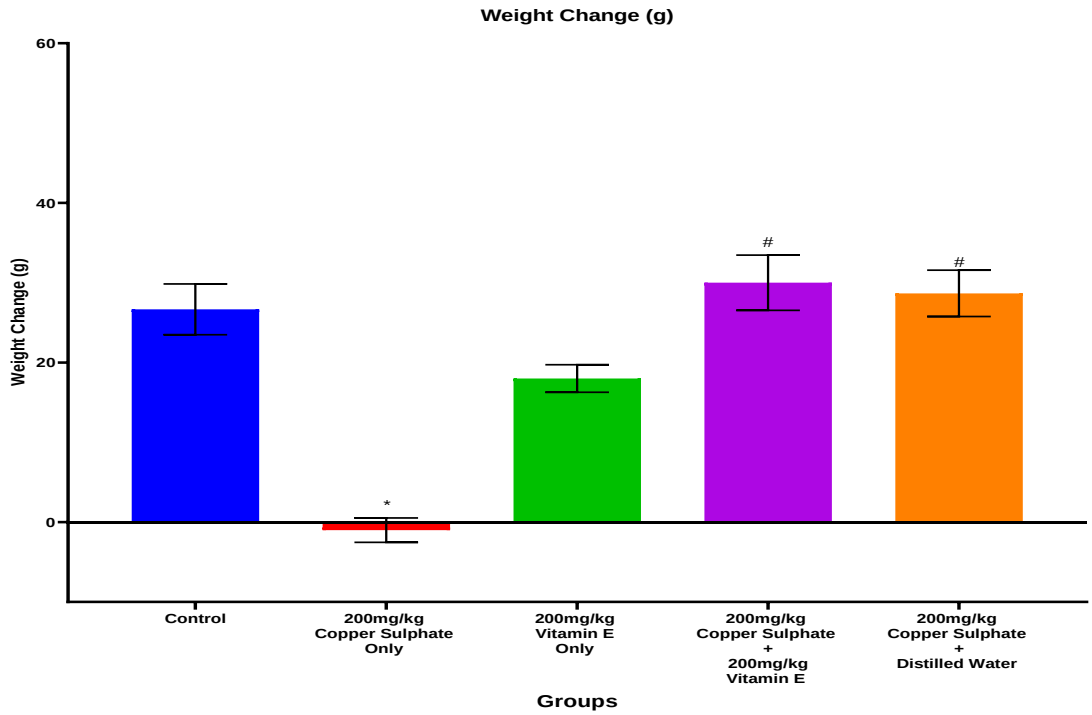

Fig 1: Changes in body weight (g) of rats in control and treated groups

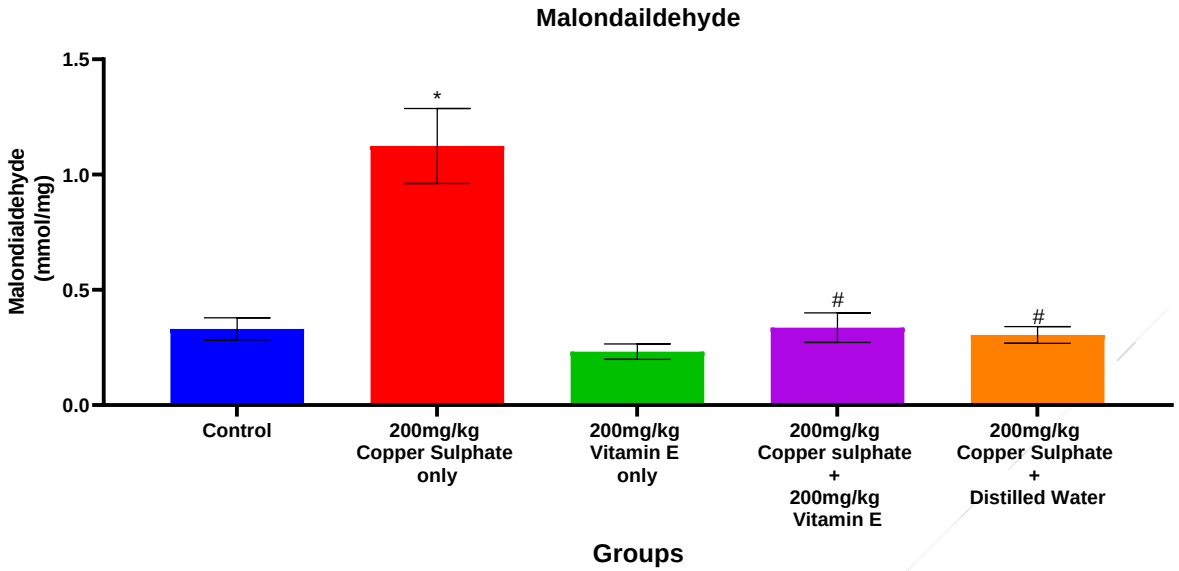

Fig 3: Changes in Malondialdehyde level (mmol/mg) of rats in control and treated groups.

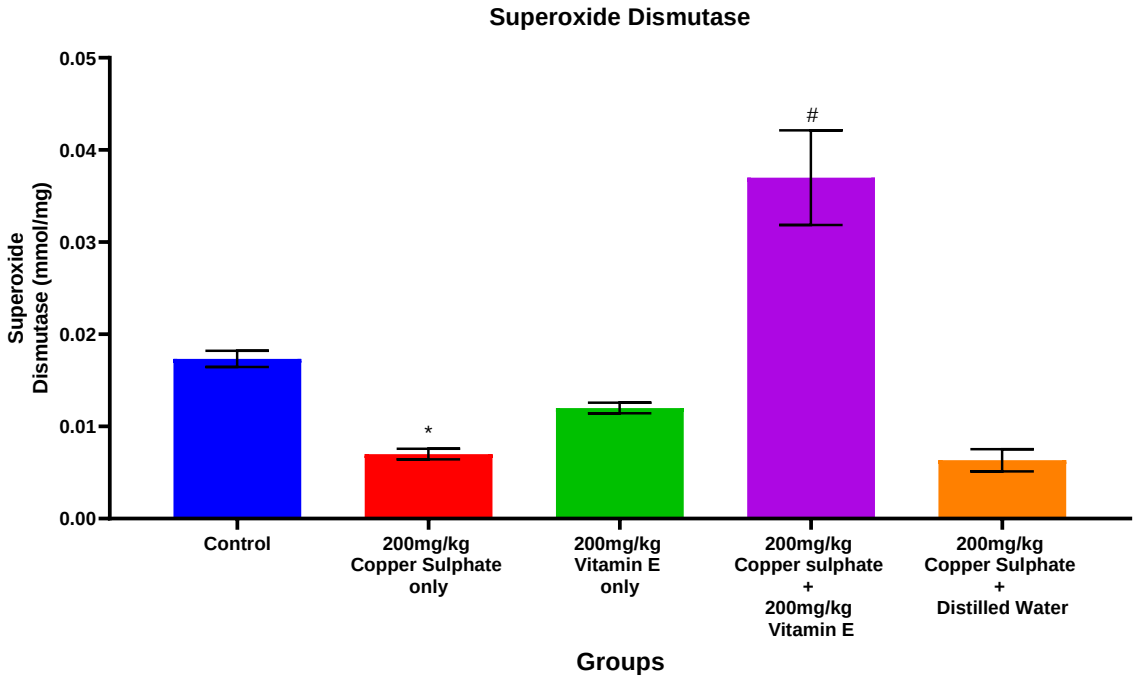

Fig 4: Changes in Superoxide Dismutase level (mmol/mg) of rats in control and treated groups.

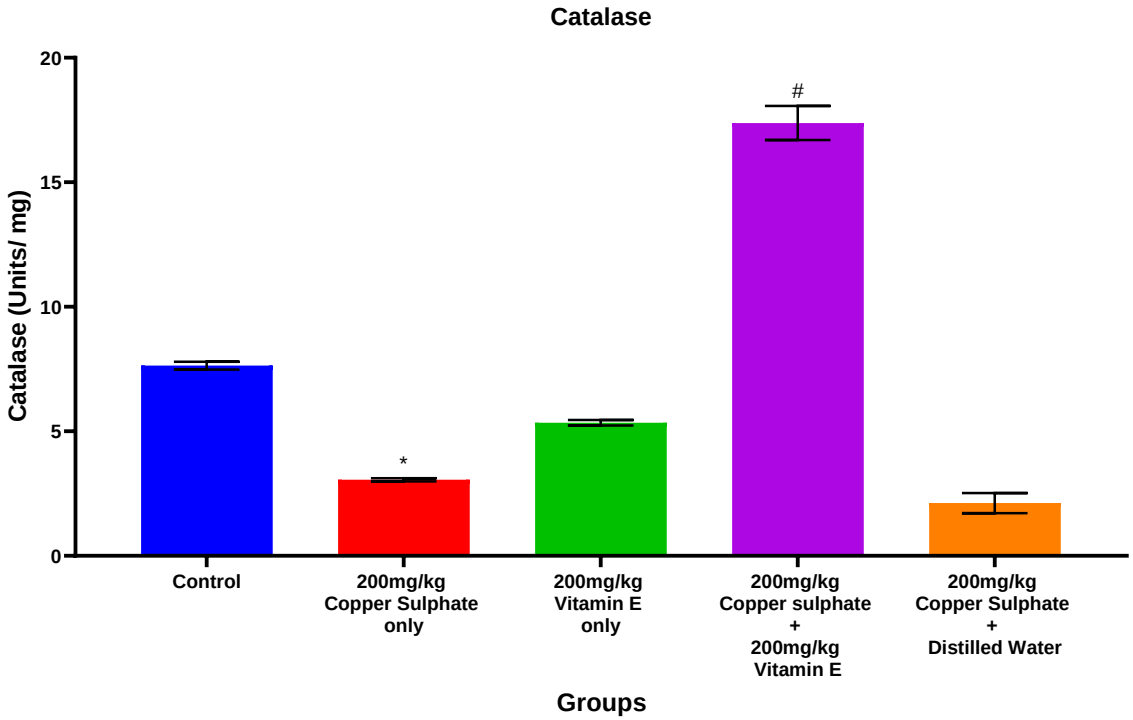

Fig 5: Changes in Catalase level (units/mg) of rats in control and treated groups.

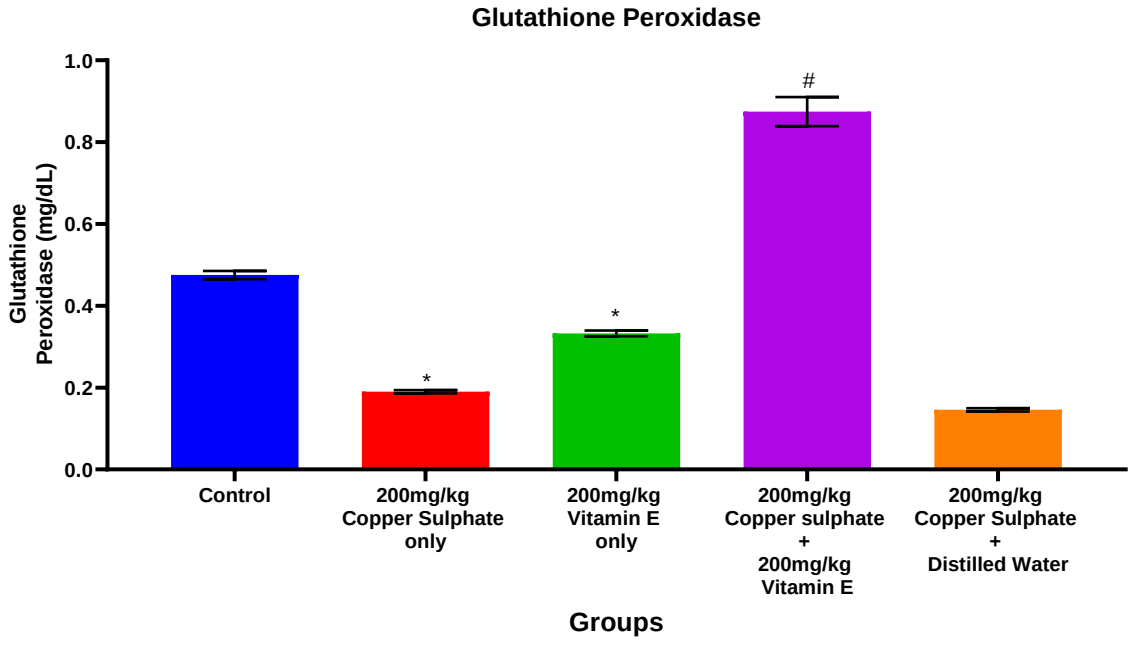

Fig 6: Changes in Glutathione peroxidase level (mg/dL) of rats in control and treated groups.

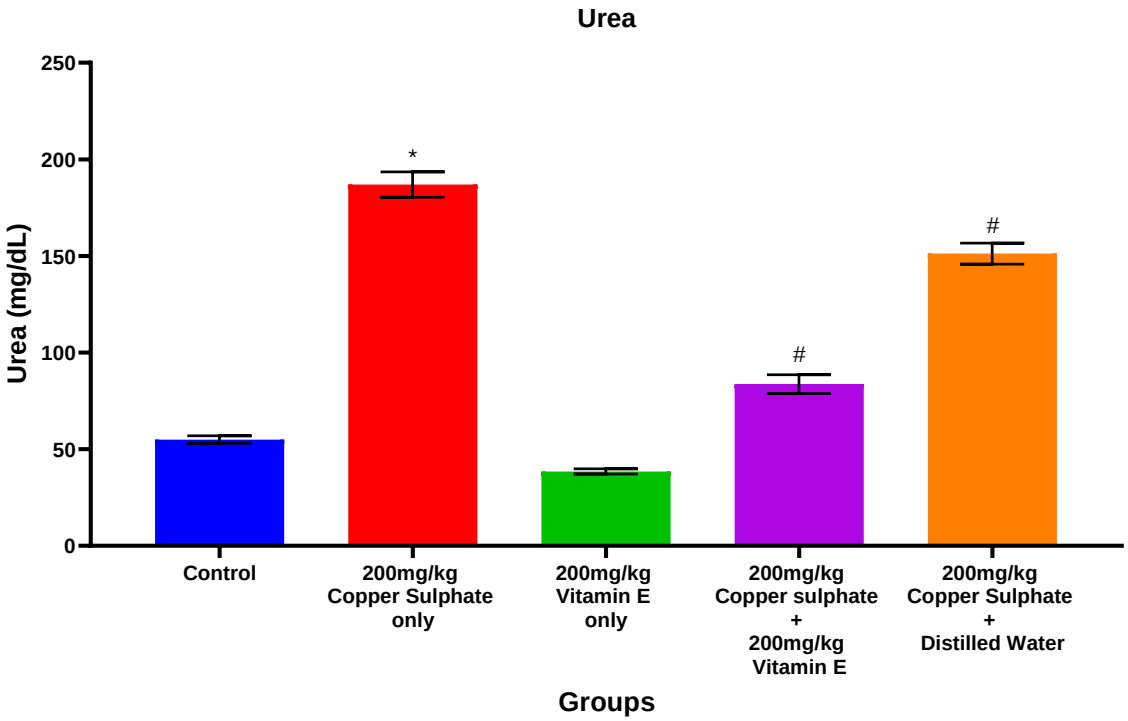

Fig 7: Changes in Urea level (mg/dL) of rats in control and treated groups.

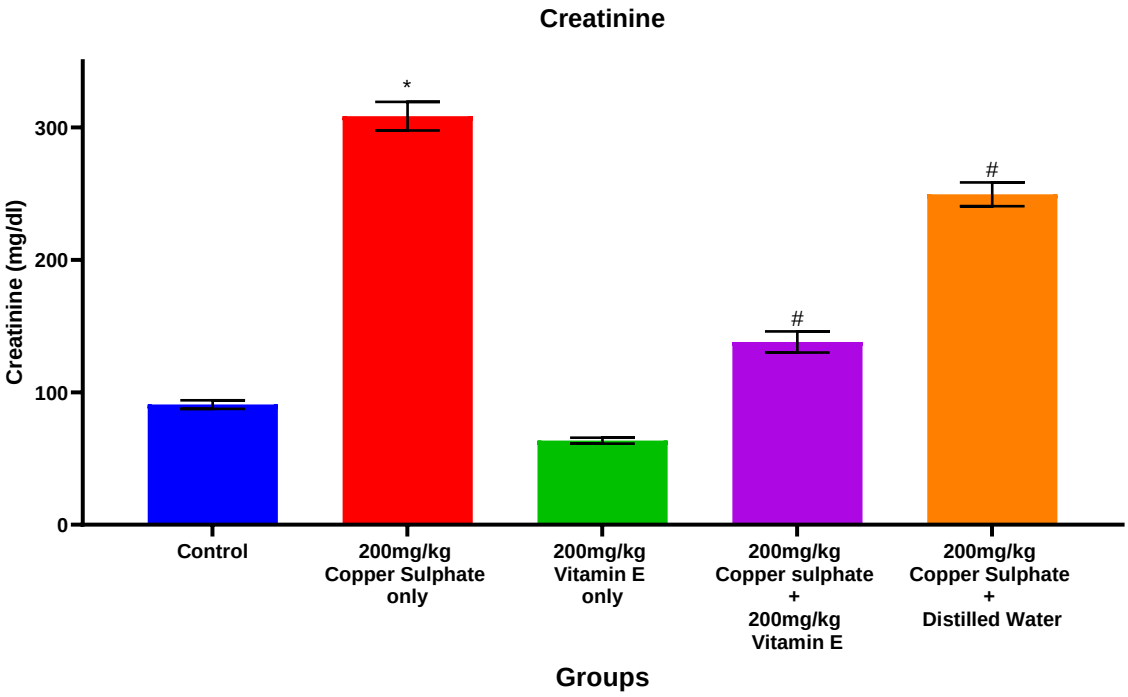

Fig 8: Changes in Creatinine level (mg/dL) of rats in control and treated groups.

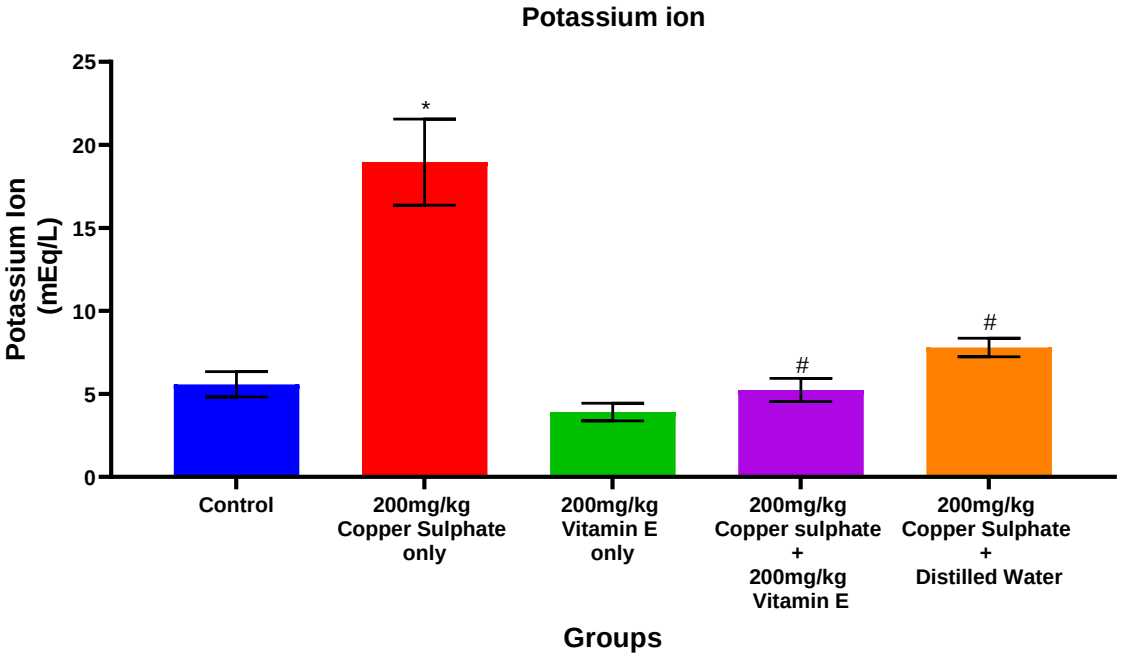

Fig 9: Changes in Potassium ion level (mEq/L) of rats in control and treated groups.

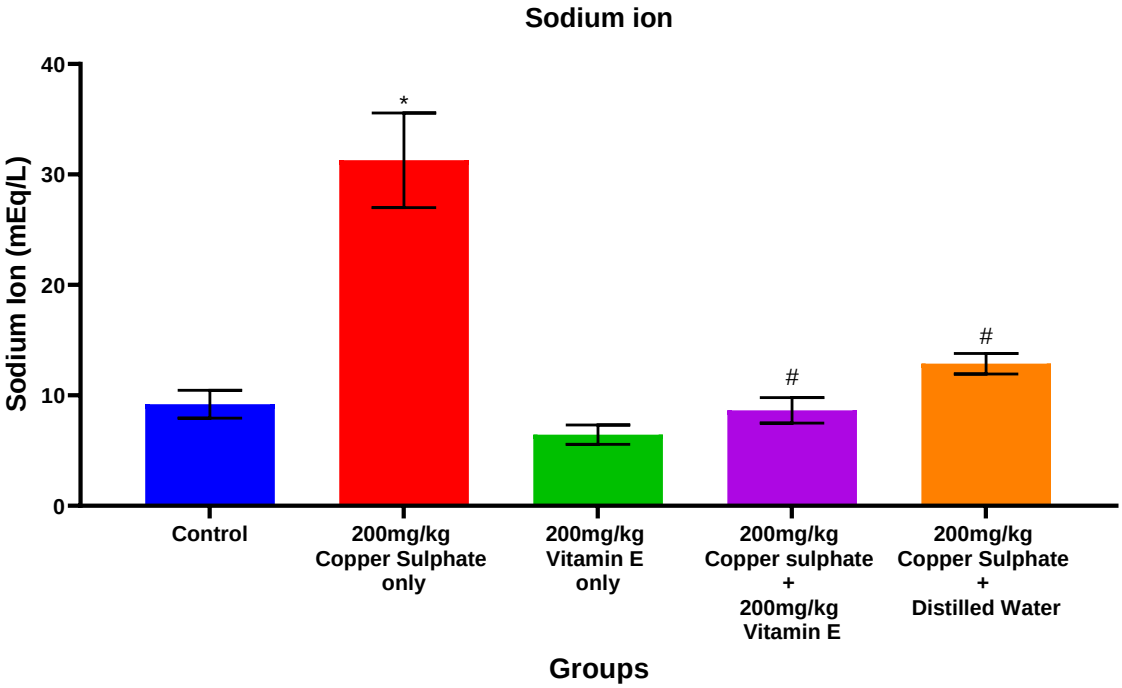

Fig 11: Changes in Sodium ion level (mEq/L) of rats in control and treated groups.

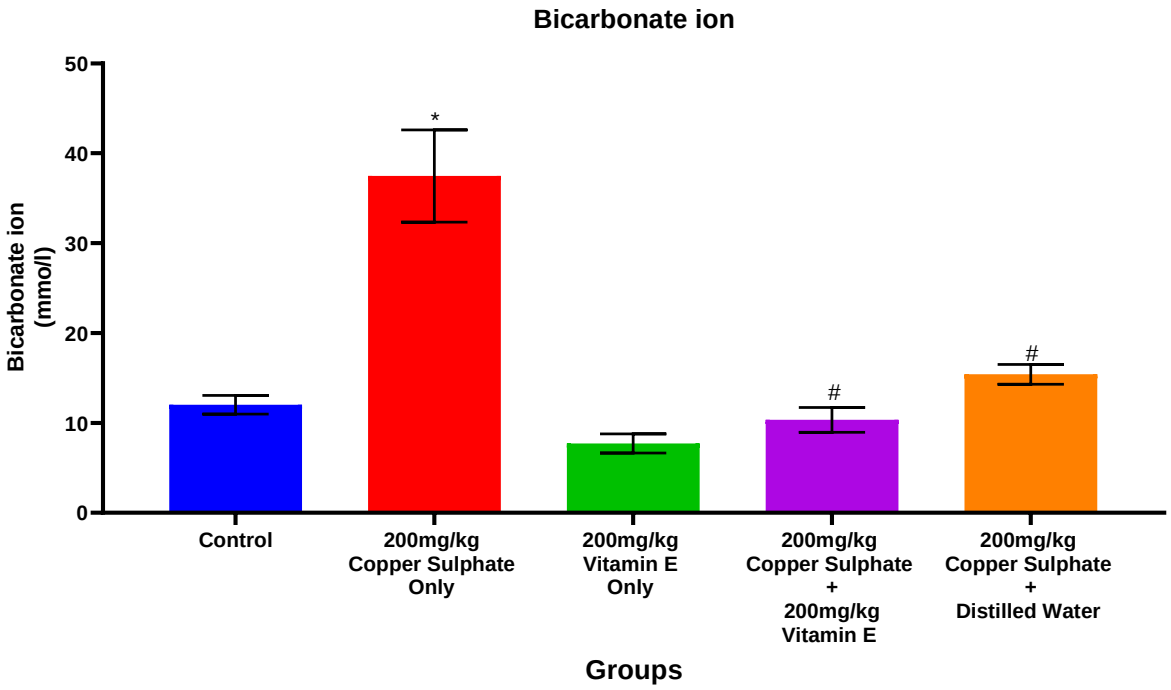

Fig 12: Changes in Bicarbonate ion level (mmol/L) of rats in control and treated groups

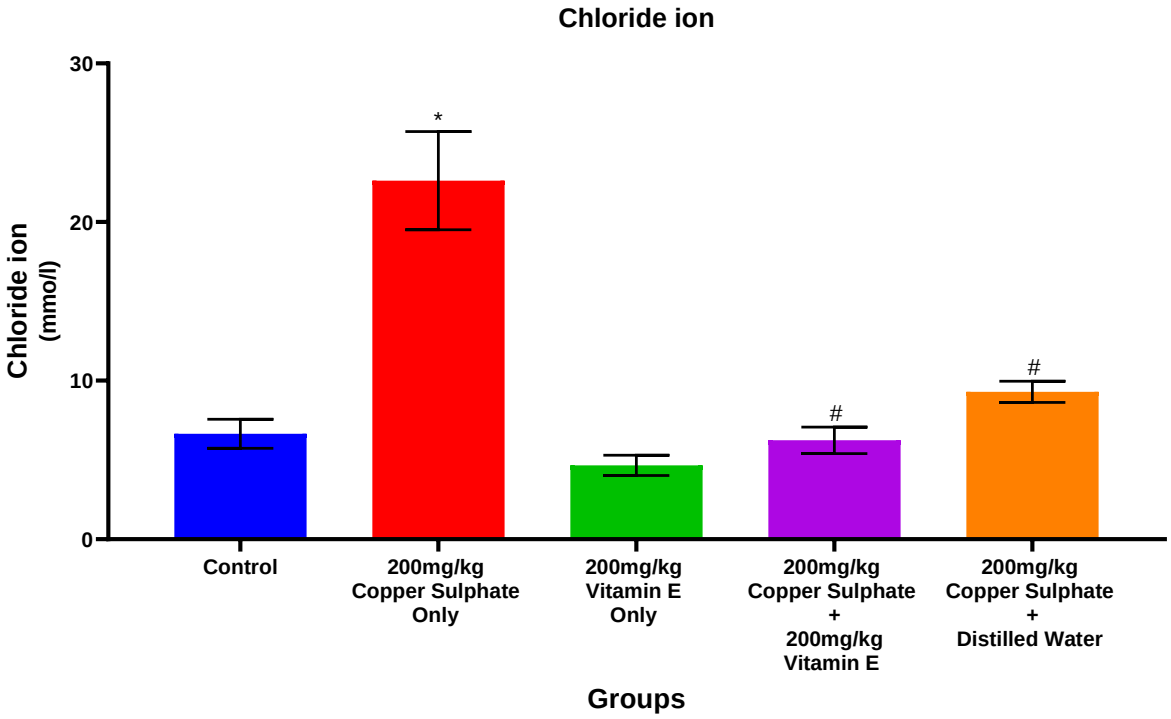

Fig 13: Changes in Chloride ion level (mmol/L) of rats in control and treated groups

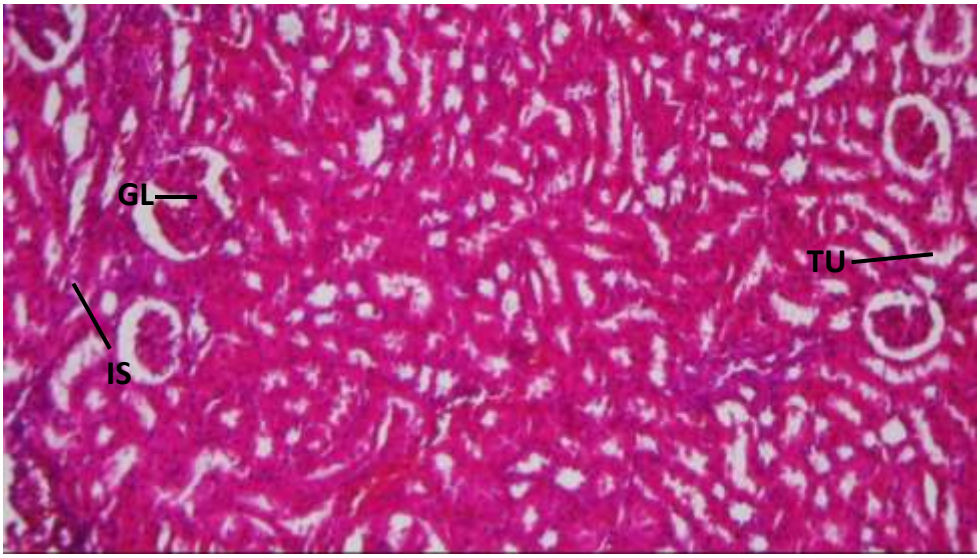

Plate 1. Rat kidney, Control. Showing: tubules (TU), interstitial space (IS) and glomeruli (GL), all; normal: (H&E x 100)

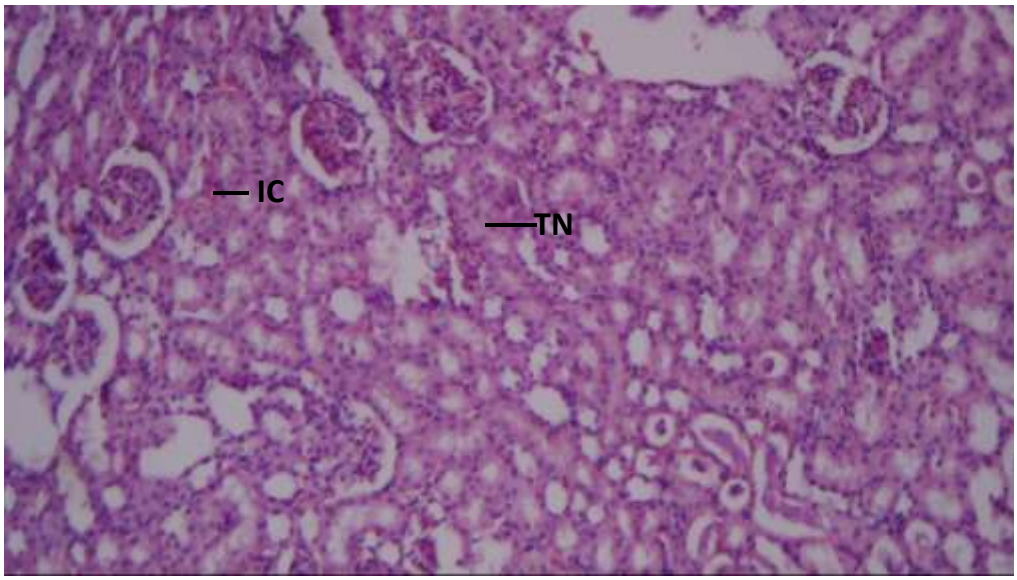

Plate 2. Rat kidney given Copper only showing: interstitial congestion (IC) and focal tubular necrosis (TN): H&E x 100

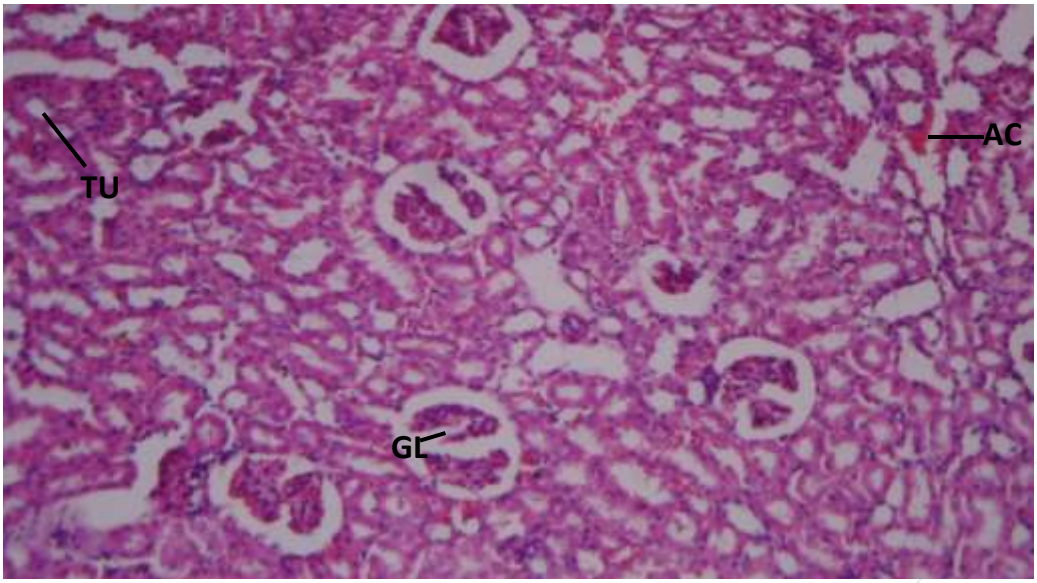

Plate 3. Rat kidney given Vit. E only showing: normal tubules (TU) and glomeruli (GL) and active interstitial congestion (AC): H&E x 100

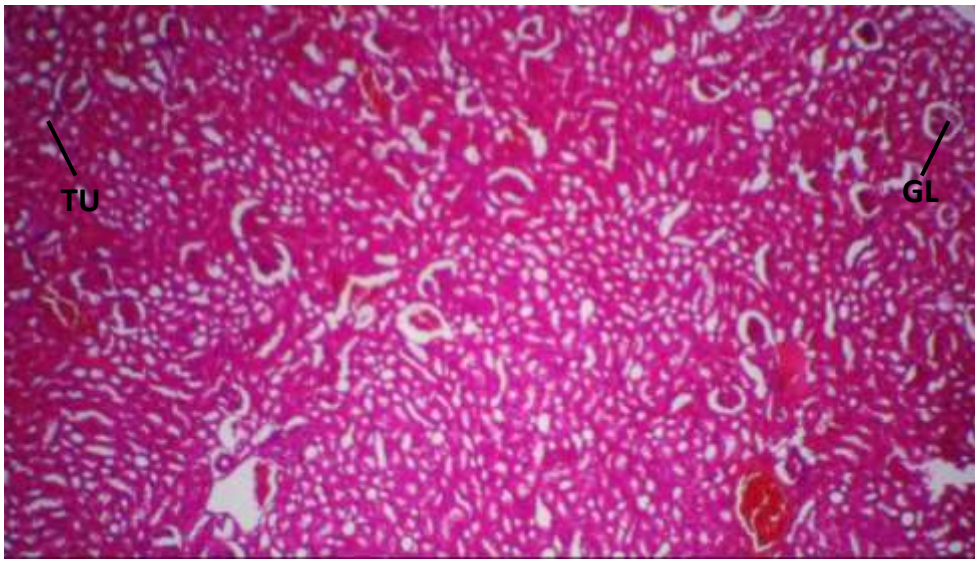

Plate 4. Rat kidney given Copper for 30 days, then Vit. E for 30 days showing: tubules (TU) and glomeruli (GL), all normal: H&E x 100

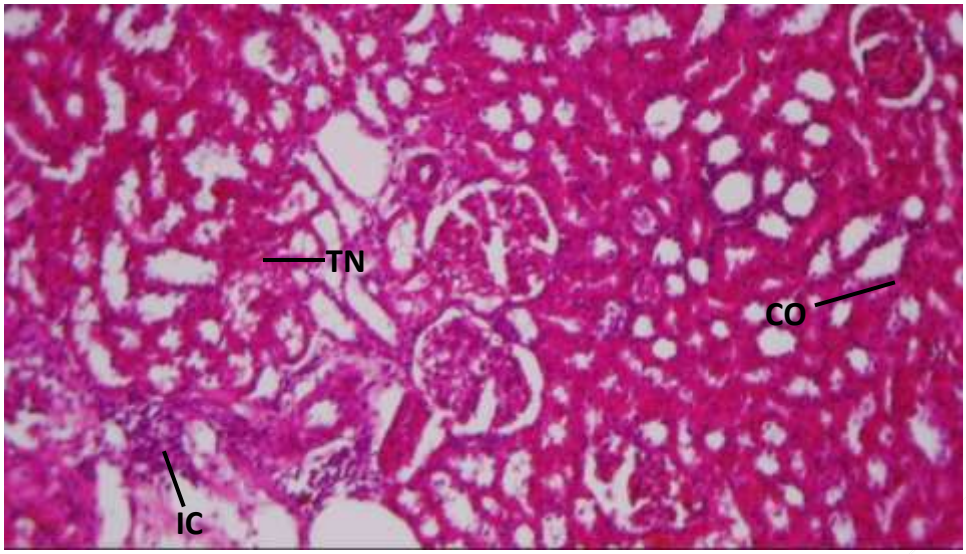

Plate 5. Rat kidney given Copper for 30 days, then distilled water for 30 days showing: interstitial congestion (CO), interstitial infiltrates of inflammatory cells (IC) and focal tubular necrosis (TN): H and E x 100.

References

1. ATSDR, Agency for Toxic Substances and Disease Registry (2014). Toxicological Profile for Cadmium. Atlanta, Georgia. 1998. www.atsdr.cdc.gov/cxcx3.html. 8th March 2014.
2. Radostitis, O.M., Blood, D.C. and Gay, C.C. (1994). Veterinary medicine. A text book of the diseases of cattle, sheep, pigs, goat and horses. Ed 8th, 31: 1469-1471.
3. Ani, M., Moshtaghie, A. A., and Akbarzadeh, S. (2007). The effect of lead on red blood cell sodium-lithium counter transport and induction of hypertension. *Journal of Cell and Tissue Research*, *7*(1), 981.
4. Alexander, F. W., Delves, H. T., and Clayton, B. E. (1973). The uptake and excretion by children of lead and other contaminants. *Environmental Health Aspects of Lead. Commission of the European Communities Directorate General for Dissemination of Knowledge Centre for Information and Documentation, Luxemburg*, 319.
5. Olugbami, J. O., Damoiseaux, R., France, B., Onibiyo, E. M., Gbadegesin, M. A., Sharma, S., ... and Odunola, O. A. (2017). A comparative assessment of antiproliferative properties of resveratrol and ethanol leaf extract of Anogeissus leiocarpus (DC) Guill and Perr against HepG2 hepatocarcinoma cells. *Complementary and Alternative Medicine*, 17, 1-11.
6. Brigelius-Flohé, R. (2021). Vitamin E research: Past, now and future. *Free Radical Biology and Medicine*, 177, 381-390.
7. Sinbad, O. O., Folorunsho, A. A., Olabisi, O. L., Ayoola, O. A., and Temitope, E. J. (2019). Vitamins as antioxidants. *Journal of Food Science and Nutrition Research*, 2(3), 214-235.
8. Hamza, R. Z., Al-Harbi, M. S., and El-Shenawy, N. S. (2017). Ameliorative effect of vitamin E and selenium against oxidative stress induced by sodium azide in liver, kidney, testis and heart of male mice. *Biomedicine and Pharmacotherapy*, 91, 602-610.
9. Panda, A. K., and Cherian, G. (2014). Role of vitamin E in counteracting oxidative stress in poultry. *The Journal of Poultry Science*, 51(2), 109-117.
10. Drury
11. Azeez, O. I., and Braimah, S. F. (2020). Mitigating Effect of Vitamin-E on Copper Sulphate-Induced Toxicity in African Catfish (Clarias gariepinus). *European Journal of Medical and Health Sciences*, *2*(4).

12. Bissinger, R., Bhuyan, A. A. M., Qadri, S. M., and Lang, F. (2018). Oxidative stress, eryptosis and anemia: a pivotal mechanistic nexus in systemic diseases. *The FEBS Journal*, *286*(5), 826–854.
13. Collins, J. F. (2021). Copper nutrition and biochemistry and human (patho)physiology. *Advances in Food and Nutrition Research*, *96*, 311–364.
14. Kanti , T. D., Wati, M. R., and Fatima-Shad, K. (2014). Oxidative Stress Gated by Fenton and Haber Weiss Reactions and Its Association With Alzheimer's Disease. *Archives of Neuroscience*, *2*(3), e60038.
15. Wang, T., Long, X., Liu, Z., Cheng, Y., and Yan, S. (2015). Effect of copper nanoparticles and copper sulphate on oxidation stress, cell apoptosis and immune responses in the intestines of juvenile Epinephelus coioides. *Fish and Shellfish Immunology*, *44*(2), 674–682.
16. Azzi, A. (2018). Many tocopherols, one vitamin E. *Molecular Aspects of Medicine*, *61*, 92–103.
17. Galli, F., Azzi, A., Birringer, M., Cook-Mills, J. M., Eggersdorfer, M., Frank, J., Cruciani, G., Lorkowski, S., and Özer, N. K. (2017). Vitamin E: Emerging aspects and new directions. *Free Radical Biology and Medicine*, *102*, 16–36.
18. Liao, S., Omage, S. O., Börmel, L., Kluge, S., Schubert, M., Wallert, M., and Lorkowski, S. (2022). Vitamin E and Metabolic Health: Relevance of Interactions with Other Micronutrients. *Antioxidants*, *11*(9), 1785.
19. Husak, V. (2015). Copper and copper-containing pesticides: Metabolism, toxicity and oxidative stress. *Journal of Vasyl Stefanyk Precarpathian National University*, *2*(1), 39–51.
20. Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S., and Minciullo, P. L. (2023). Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules*, *28*(16), 5979.
21. Kirici, M. (2017). Toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish Capoeta umbla (Heckel, 1843) tissues. *Applied Ecology and Environmental Research*, *15*(3), 1685–1696.
22. Gamakaranage, C. S., Rodrigo, C., Weerasinghe, S., Gnanathasan, A., Puvanaraj, V., and Fernando, H. (2011). Complications and management of acute copper sulphate poisoning; a case discussion. *Journal of Occupational Medicine and Toxicology*, *6*, 34.
23. Sabra, M. S., Hemida, F. K., and Allam, H. (2023). Adenine model of chronic renal failure in rats to determine whether MCC950, an NLRP3 inflammasome inhibitor, is a renopreventive. *BMC Nephrology*, *24*(1), 377.
24. Erdemli, M. E., Zayman, E., Erdemli, Z., Gul, M., Gul, S., and Bag, H. G. (2020). Protective effects of melatonin and vitamin E in acetamiprid-induced nephrotoxicity. *Environmental Science and Pollution Research International*, *27*(9), 9202–9213.
25. Samsami, M., Shabani, M., Hajiesmaeili, M., Tavakoli-Ardakani, M., Ardehali, S. H., Fatemi, A., Barati, S., Moradi, O., and Sahraei, Z. (2021). The effects of vitamin E on colistin-induced nephrotoxicity in treatment of drug-resistant gram-negative bacterial infections: A randomized clinical trial. *Journal of Infection and Chemotherapy*, *27*(8), 1181–1185.
26. Tasić, D., Opačić, M., Kovačević, S., Nikolić Kokić, A., Dimitrijević, M., Nikolić, D., Vojnović Milutinović, D., Blagojević, D., Djordjevic, A., and Brkljačić, J. (2022). Effects of Fructose and Stress on Rat Renal Copper Metabolism and Antioxidant Enzymes Function. *International Journal of Molecular Sciences*, *23*(16), 9023.
27. Khevana , A. T., Amit , R. B., Abdulkadir , M., Swati , S. P., Raj , S. D., Harshad, B. P., and Dhaval , T. F. (2024). Nephrotoxic effect of copper oxide nanoparticles in Wistar rats (Rattus norvegicus). *International Journal of Advanced Biochemistry Research*, *8*(4), 431–440.
28. Baltušnikienė, A., Stanevičienė, I., and Jansen, E. (2023). Beneficial and adverse effects of vitamin E on the kidney. *Frontiers in Physiology*, *14*, 1145216.

29. Alerna Kidney Health. (2023). *Is Drinking Distilled Water Good for Your Kidneys?* Alerna Kidney Health. https://alernakidneyhealth.com/blogs/kidney-health/distilled-water-and-kidney-health

How to Cite this article: **Obayuwana, E. & Imafidon, E. O. (2024).** Copper Sulphate-Induced Nephrotoxicity in Adult Wistar Rats: Therapeutic Potential of Vitamin E. *Journal of Basic and Applied Medical Sciences*, 4(1&2), 35-48.