Open Access

Original Article

Renal Tubular Injury in Albino Rats after using Heavy Metal Copper Dosage: A Histopathological Study

Hira Kareem, Amna Jahan, Sobia Jahangir*, Roop e Zahra, Arslan Haider, Romman ul Haq*****

Department of Pathology, Services Institute of Medical Sciences, Lahore Pakistan, *Department of Pathology, University of Health Sciences, Lahore Pakistan, **Department of Pediatric, Lahore General Hospital, Lahore Pakistan, ***Department of Radiology, Services Institute of Medical Sciences, Lahore Pakistan

ABSTRACT

Objective: To compare the impact of doses of heavy metals like copper on renal tubules in albino rats.
Study Design: Comparative Cross-sectional study.
Place and Duration of Study: Pathology Department of Services Institute of Medical Science, (Animal House) University of Health Sciences, Lahore Pakistan, from Nov 2018 to May 2019.
Methodology: Two groups of Wistar Albino rodents were categorized as control and study groups consisting of ten rodents. Only standard pellets diet of mice with tap water was given to all control group members. On the contrary, the heavy metal dose was calculated according to the bodyweight of the rodents in the study group. The dose was given to the rodents every alternate day for up to eighteen weeks. At the end of the experiment, changes in renal tubules were compared microscopically and statistically analyzed in both categories, keeping the *p*-value of <0.05 as significant.
Results: The results showed that morphological changes in renal tubules in tubular epithelial necrosis and dilatation were significantly different in all members of both categories'., epithelial necrosis; (n=10, 100%, $p<0.001$), followed by dilatation (n=10, 100%, $p<0.001$), casts (n=10, 100%, $p<0.001$), basement membrane thickening (n=10, 100%, $p<0.001$), and vacuolization (n=10, 100%, $p<0.001$).
Conclusion: This study found that heavy metals like copper can cause renal tubular necrosis, dilatation, and inflammation.

Keywords: Copper, Diet, Dilatation, Epithelial Cell, Inflammation, Necrosis, Rats, Renal Tubules.

How to Cite This Article: *Kareem H, Jahan A, Jahangir S, Zahra R, Haider A, Haq R. Tubular Injury in Albino Rats after using Heavy Metal Copper Dosage: A Histopathological Study. Pak Armed Forces Med J 2025; 75(Suppl-6): S974-S978. DOI: https://doi.org/10.51253/pafmj.v75iSUPPL-6.8052*

INTRODUCTION

In prehistoric times many heavy metals have been used in medicine, but they have potentially toxic effects on humans. Consumption of various foods and water is also the primaeval cause of exposure to human beings; indeed, copper from food is a prime source.[1] Human health is reported in the danger zone after close and prolonged contact with these heavy metals, such as cadmium, copper, lead, nickel, and zinc.[2] Some heavy metals products treat tuberculosis, arthritis, sexual weakness, growth failure, and asthma. Unfortunately, these modes of treatment are quite popular in Southeast Asia for their quick and prompt action. Critical oxidative renal dysfunction has been clocked in after extended use of copper metal in various research studies.[3,4] These heavy metals preparations are in powder form, well absorbed after oral intake and are deposited mainly in the liver and kidney, likely to be damaged.[5]

Proximal renal tubules in kidneys are the first target site for accumulating an excess of copper compounds. They primarily cause hyaline drop and hydropic dystrophy in various tubular cells. The glomeruli remained unchanged at first, but epithelial cell necrosis appeared in the tubules. Tubular necrosis in kidney tissue is caused by damage to the basal tubule membrane.[6]

The primary site of heavy metal toxicity is the renal tubules. On the other hand, these metals Congestion of the glomeruli and denudation of the tubular cells can result in a prolonged usage of these heavy metals, especially copper compounds.[7] Spotted tubular epithelium edematous medulla and Eosinophilic casts are developed as the most familiar renal morphological changes.[8] Vulnerable morphological changes in the renal tubules of Wistar albino rodents after consumption of heavy metal copper are discussed in this manuscript. There is only a little scientific research on these products, mainly due to the lack of communication among traditional healers, physicians, and scientists. We need to conduct this histopathological study to assess the changes in renal tubular injury in albino rats after using heavy metal copper dosage. The study will help to know if

Correspondence: Dr Hira Kareem, Department of Pathology, Services Institute of Medical Sciences, Lahore Pakistan
Received: 25 Jan 2022; revision received: 03 Jun 2022; accepted: 05 Jul 2022

there is any significant difference in the severity of renal tubular injury in albino rats after using different dosages of copper.

METHODOLOGY

This comparative cross-sectional study was conducted at the Pathology Department of Services Institute of Medical Science, (Animal House) University of Health Sciences (UHS), Lahore, from November 2018 to May 2019. Approval from the institutional review board has been taken vide letter no. IRB / 2021 / 886 / SIMS. The sample size of 61 was calculated while keeping a 20% expected ratio of hypertrophy, proliferation and swelling in the lining endothelium of the glomerulus, severe renal cortical congestion, 95% confidence interval, and 10% margin of error. However, due to the limited resources and financial constraints sample size of 20 was taken.[9]

Inclusion Criteria: Male and female rats of the six to eight weeks age group weighing 200 to 250 grams were included.

Exclusion Criteria: Underweight (less than 200 grams) and overweight animals (more than 250 grams) were excluded. Underage rodents less than six weeks were also excluded.

These Wistar albino rodents were taken from UHS. All twenty rodents were kept in separate cages in UHS' highly monitored animal nursery. The temperature of 220 to 250 degrees Celsius, with 65%±5 humidity, was maintained. The dark and light cycles were also kept to a 12-hour cycle each. On alternate days, the prescribed dose for each rodent was administered. After an acclimatization period of one week, rodents were divided into two groups control and study group.

Control Group: Ten healthy rodents were given tap water and a regular diet for 18 weeks.

Study Group: Ten healthy rodents received oral food on alternate days for 18 weeks. The food was given in the form of pellets prepared using Cu 0.15 mg/kg body weight, homogenized and mixed with wheat flour.

The guidance for the exact quantification of copper in the food of Wister albino rodents was taken from Biochemistry Department-UHS. According to their suggestion, rodents in the study group were given customized pellets. Oral LD50 of rats for copper metal has been reported as 2 mg/kg body weight, which is also supported by the extant research.[10] The dose of copper was adjusted according to the weight of each rodent to make sure that the rats received 0.15 mg/kg of copper metal in their food on the days they were given pellets.

At the end of the experiment, animals were sacrificed under anaesthesia (with carbon dioxide), necropsied and dissected according to proper procedure and ethics.[11] The kidneys were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylene, and embedded in paraffin; 4–6-micron thick sections were prepared and stained with haematoxylin and eosin. With more than five years of experience, two consultant pathologists evaluated all sections. There was no blinding, and all of the findings were discussed openly.

All the data were analyzed in Statistical Package for the social sciences (SPSS) version 23.00. The categorical variables, i.e., gender, presence, or absence of features, have been presented as frequency and percentages. The quantitative variables, i.e., weight, have been presented as mean and standard deviation. Fisher Exact Test has been applied to associate histopathological features among groups. A *p*-value of <0.05 was taken as significant.

RESULTS

A total of 4(20%) male and 16(80%) female rodents with an average weight of 227.54±13.05 grams (Range: 201.80-245.70 grams) were included. Histo-pathologically, there were significant differences in the degree of renal tubular injury in the copper-treated group compared with the control group. The most common finding in the copper-treated group was epithelial necrosis; an important feature of early toxicity (n=10, 100%), followed by dilatation (n=10, 100%), casts (n=10, 100%). There was also a significant increase in the number of basement membrane thickening (n=10, 100%) and vacuolization (n=10, 100%) (Table-I). The number of rats with epithelial necrosis ($p<0.001$), dilatation ($p<0.001$), casts ($p<0.001$), basement membrane thickening ($p<0.001$), and vacuolization ($p<0.001$) was significantly different between the two groups. These results indicate that exposure to copper is associated with an increased risk of renal tubular injury in albino rats.

Figure-I represents that the glomeruli and renal tubules in the control group do not show thickened basement membrane or any signs of inflammation (Figure-I). Figure-II and III represent that renal tubular epithelial cell cast reflects renal epithelial cell damage in the collecting ducts (formed by solidifying the

proteins within the tubular lumen, making it expand and fill) in the study group, and Figure-IV represents dilatation and thickened basement membrane in the study group. These findings indicate that the rats in the copper-treated group experienced renal tubular injury after exposure to heavy metal copper dosage, likely due to the toxicity of copper, which can damage the epithelial cells lining the renal tubules and cause inflammation and basement membrane thickening. Vacuolization may result from cell death or damage, and casts can form when renal tubules are damaged and cannot properly filter waste products from the blood.

Table: Renal Histopathology Parameters in terms of Renal Tubules (n=20)

		Control Group	Study Group	*p*-value
Epithelial necrosis	Present	0(0%)	10(100%)	<0.001
	Absent	10(100%)	0(0%)	
Dilatation	Present	0(0%)	10(100%)	<0.001
	Absent	10(100%)	0(0%)	
Casts	RBCs	0(0%)	10(100%)	<0.001
	WBCs	0(0%)	10(100%)	
	Proteins	0(0%)	10(100%)	
Crystals	Present	0(0%)	0(0%)	--
	Absent	10(100%)	10(100%)	
Basement Membrane	Normal	10(100%)	0(0%)	<0.001
	Thickened	0(0%)	10(100%)	
	Thickened+ Focally Thick	0(0%)	0(0%)	
Vacuolization	Present	0(0%)	10(100%)	<0.001
	Absent	10(100%)	0(0%)	

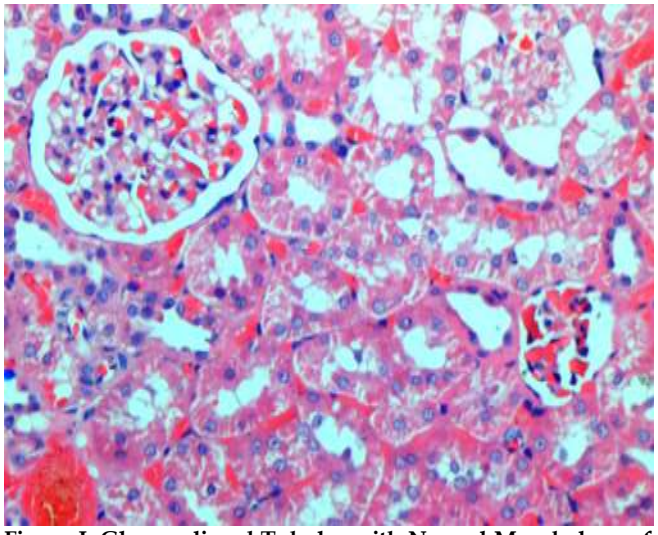

Figure-I: Glomeruli and Tubules with Normal Morphology of Group One Hematoxylin and Eosin Stain (10 Power field)

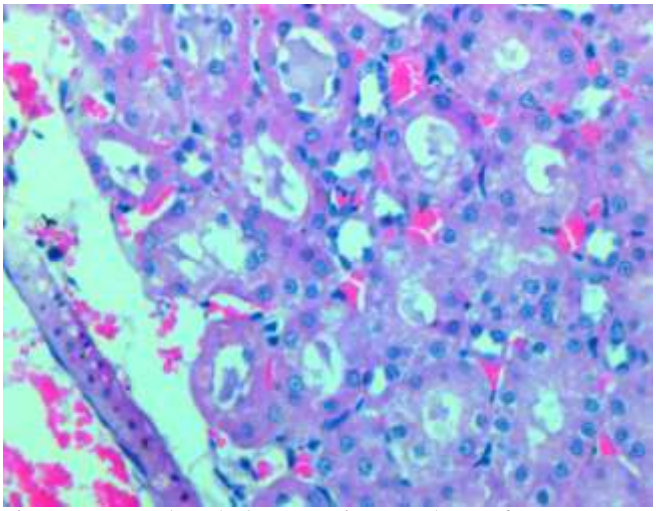

Figure-II: Renal Tubular Cast in Members of Group Two Hematoxylin and Eosin Stain (40 Power field)

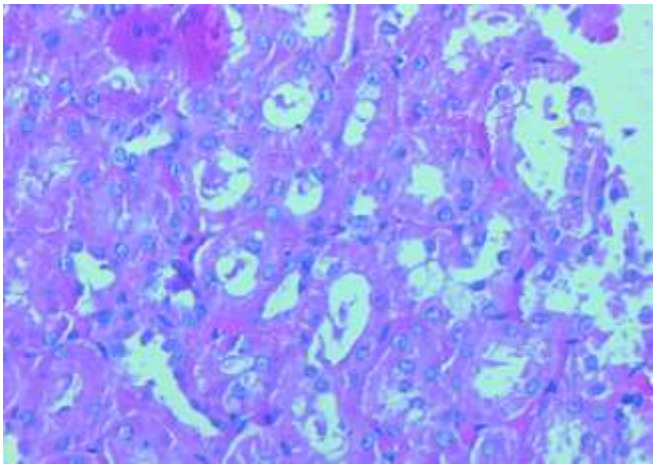

Figure-III: Renal Tubular Cast in Members of Group Two Hematoxylin and Eosin Stain (20 power field)

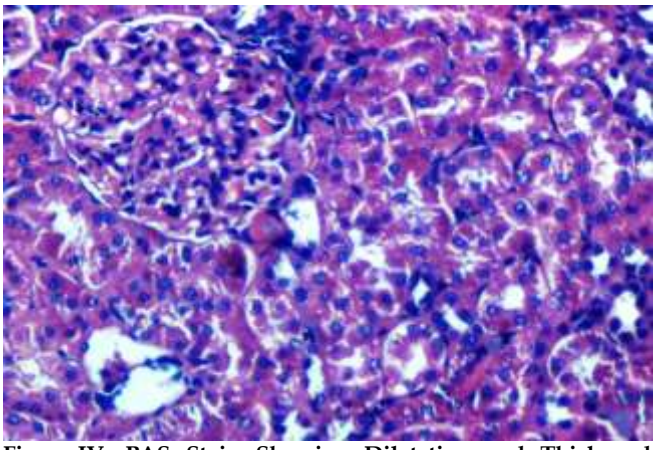

Figure-IV: PAS Stain Showing Dilatation and Thickened Basement Membrane (20 power field)

DISCUSSION

Copper compounds can harm renal epithelial cells either directly (by producing reactive oxygen and peroxidation) or indirectly (by depleting cellular glutathione). In epithelial necrosis dilatation, cast formation, and vacuolization, the present study supports the metallic toxicity of kidney tubules.[12,13] Numerous studies indicate tubular necrosis is frequently caused by intravascular hemolysis and direct copper action on the kidneys. Human health is harmed by copper accumulation in proximal tubules, which leads to tubular necrosis in Wilson's disease.[14,15]

Tubular and glomerulus necrosis and eosinophilic intranuclear inclusions in the lining epithelium of some renal tubules were observed in all toxicated rats.[16] Similarly, Akintunde et al. found that 20% of exposed rats had severe renal cortical congestion; hypertrophy, proliferation, and swelling in the lining endothelium of the glomerulus, and 40% had glomerular tubular degeneration with degeneration in the lining epithelial cells of renal tubules, and 60% had cortical congestion with protein cast.[9] According to a recent Iranian study by Shahsavari et al., the highest concentrations of metals were found in the kidney. In renal tissue samples, hyperemia, haemorrhage, necrosis, degenerative damage to tubule epithelial cells, and the presence of hyaline casts and, in one case, mononuclear leukocyte infiltration were observed.[17]

Researchers studied copper toxicity on thirty male Wistar rats, and they found deranged renal parameters, especially serum creatinine levels indicating the reversible renal parenchymal damage. In our study, it is proved by histopathological changes within renal tissue.[18] Most of the studies are in accordance with our findings that there is a direct relationship between heavy metal intoxication and renal injury. This damage may be due to the production of free radicals that overwhelm the antioxidant defence system, causing oxidative stress.

LIMITATION OF STUDY

This study has a few limitations. Firstly, it is a small study with only 20 rats. This study cannot conclude the toxic effects of heavy metals on other body organs. Studies on a more significant number of rats with different treatment durations are warranted to elucidate further the renal tubular injury associated with copper exposure. Secondly, this is a histopathological study, and the degree of renal tubular injury was only scored semi-quantitatively. A more detailed quantitative study is required to determine the exact extent of renal tubular injury caused by copper exposure.

CONCLUSION

In conclusion, exposure to heavy metal copper dosage is associated with an increased risk of renal tubular injury in albino rats, as evidenced by epithelial necrosis, dilatation, casts, basement membrane thickening and vacuolization on histopathological examination. These findings suggest that caution should be exercised when using copper-containing products and that further research is needed to understand the mechanisms by which copper causes renal tubular injury.

ACKNOWLEDGEMENT

We thank Almighty Allah, the Compassionate and Most Merciful, who bestowed the potential and ability to complete the research Project. Glory and praise to our Last Prophet Hazrat Muhammad (Peace be upon Him), who is forever a symbol of direction and knowledge for the whole of humanity. We also want to acknowledge the role of an institutional review board which approved this study.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

HK & AJ: Data acquisition, data analysis, critical review, approval of the final version to be published.

SJ & RZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AH & RH: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Xu X, Nie S, Ding H, Hou FF. Environmental pollution and kidney diseases. Nat Rev Nephrol 2018; 14(5): 313-324.
2. Sall ML, Diaw AKD, Gningue-Sall D, Efremova Aaron S, Aaron JJ. Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. Environ Sci Pollut Res Int 2020; 27(24): 29927-42.
3. Taylor AA, Tsuji JS, Garry MR, McArdle ME, Goodfellow WL, Adams WJ, et al. Critical Review of Exposure and Effects: Implications for Setting Regulatory Health Criteria for Ingested Copper. Environ Manage 2020; 65(1): 131-159.
4. Padrilah SN, Sabullah MK, Shukor M, Yasid NA, Shamaan NA, Ahmad SA. Toxicity effects of fish histopathology on copper accumulation. Pertanika J Trop Agric Sci 2018; 41(2): 519-540.
5. Bind A, Goswami L, Prakash V. Comparative analysis of floating and submerged macrophytes for heavy metal (copper, chromium, arsenic and lead) removal: sorbent preparation, characterization, regeneration and cost estimation. Geology, Ecology, and Landscapes 2018; 2(2): 61-72.

6. Abhijit G, Jyoti J, Aditya S, Ridhi S, Candy S, Gaganjot K. Microbes as potential tool for remediation of heavy metals: a review. J Microb Biochem Technol 2016; 8(4): 364-372.
7. Rana MN, Tangpong J, Rahman MM. Toxicodynamics of Lead, Cadmium, Mercury and Arsenic- induced kidney toxicity and treatment strategy: A mini review. Toxicol Rep 2018; 5: 704-713.
8. Kurisaki E, Kuroda Y, Sato M. Copper-binding protein in acute copper poisoning. Forensic Sci Int 1988; 38(1-2): 3-11.
9. Akintunde JK, Oboh G. Nephritic cell damage and antioxidant status in rats exposed to leachate from battery recycling industry. Interdiscip Toxicol 2016; 9(1): 1-11.
10. Pregel P, Scala E, Bullone M, Martano M, Nozza L, Garberoglio S, et al. Radiofrequency Thermoablation On Ex Vivo Animal Tissues: Changes on Isolated Swine Thyroids. Front Endocrinol (Lausanne) 2021; 12: 575565.
11. Garcês A, Pires I. Necropsy Techniques for Examining Wildlife Samples: Bentham Science Publishers; 2020.
12. Bugata LSP, Pitta Venkata P, Gundu AR, Mohammed Fazlur R, Reddy UA, Kumar JM, et al. Acute and subacute oral toxicity of copper oxide nanoparticles in female albino Wistar rats. J Appl Toxicol 2019; 39(5): 702-716.
13. Anant JK, Inchulkar S, Bhagat S. An overview of copper toxicity relevance to public health. EJPMR 2018; 5(11): 232-237.
14. Hashimyousif E, Obaid HM, Karim AJ, Hashim MS, Naimi RAA. Toxico pathological study of copper sulfate modulate by zinc oxide and Coriandrum sativum plant treatment in mice. Plant Archives 2019; 19(1): 299-308.
15. Baruah S, Goswami S, Kalita D. Haematobiochemical and pathological alterations of chronic copper toxicity in ducks. J Anim Res 2018; 8(2): 283-287.
16. Goma A, Tohamy H. Impact of some heavy metals toxicity on behaviour, biochemical and histopathological alterations in adult rats. Adv Anim Vet Sci 2016; 4(9): 494-505.
17. Shahsavari A, Tabatabaei Yazdi F, Moosavi Z, Heidari A, Sardari P. A study on the concentration of heavy metals and histopathological changes in Persian jirds (Mammals; Rodentia), affected by mining activities in an iron ore mine in Iran. Environ Sci Pollut Res Int 2019; 26(12): 12590-604.
18. Babaknejad N, Moshtaghie AA, Shahanipour K. The toxicity of copper on serum parameters related to renal functions in male wistar rats. Zahedan J Res Med Sci 2015; 15: 29-31.

.....