**Uludağ Üniversitesi Tıp Fakültesi Dergisi**
***Journal of Uludağ University Medical Faculty***
**52, 1886172, 2026**
**DOI: https://doi.org/10.32708/uutfd.1886172**

ORIGINAL RESEARCH

# The Effect of Copper Sulfate on Aquaporins in Kidney Tissue

**Selda KAHVECİ[1*], Tahsin SELVİ[2], Şaban VARİNLİ[2], Taha Berkay BOR[1], Züleyha DOĞANYİĞİT[1], Osman ÖZTÜRK[3], Aslı OKAN OFLAMAZ[1], Sümeyye UÇAR[4], Seher YILMAZ[5]**

[1] Department of Histology and Embryology, Faculty of Medicine, Yozgat Bozok University, Yozgat, Türkiye.
[2] Department of Histology and Embryology, School of Graduate Studies, Yozgat Bozok University, Yozgat, Türkiye.
[3] Department of Child Health and Diseases, Faculty of Medicine, Yozgat Bozok University, Yozgat, Türkiye.
[4] Department of Anatomy, Faculty of Medicine, Erciyes University, Erciyes, Türkiye.
[5] Department of Anatomy, Faculty of Medicine, Yozgat Bozok University, Yozgat, Türkiye.

**ABSTRACT**

Copper (Cu) is a cofactor for most enzymes and an essential trace element. However, elevated levels of Cu exposure cause toxic effects in various tissues. Aquaporins (AQP) are integral membrane proteins that play a key role in fluid transport, particularly in kidney tissues. In our study, we aimed to evaluate the expression of AQP-1, -2, and -3 in the kidneys of female and male rats following the administration of high-dose copper sulfate ($CuSO_4$). First, the control group (n=10) received saline via oral gavage, while the experimental group ($CuSO_4$) (n=10) received 100 mg/kg/day via oral gavage for 14 days. Histopathological, PAS staining, and immunohistochemical evaluation of AQP-1, -2, and -3 were then performed on kidney tissues from the control and experimental groups. $CuSO_4$ caused tubular vacuolization in the kidneys. $CuSO_4$ administration decreased AQP-2 levels while increasing AQP-1 and AQP-3 levels. These findings suggest that $CuSO_4$ may have re-modulated AQP-1 and AQP-3 levels due to the tubular vacuolization and degeneration it caused; however, the effect of $CuSO_4$ on estrogen levels in females and the vasopressin effect via AQP-2 need to be investigated in detail.

**Keywords: Aquaporins. Copper sulfate. Kidney. Rat.**

**Böbrek Dokusunda Akuaporinler Üzerine Bakır Sülfat Etkisi**

**ÖZET**

Bakır (Cu), çoğu enzimin kofaktörü ve temel bir eser elementtir. Ancak, yüksek düzeyde Cu maruziyeti çeşitli dokularda toksik etkilere neden olur. Akuaporinler (AQP), sıvı taşınmasında, özellikle böbrek dokularında önemli bir rol oynayan integral membran proteinleridir. Çalışmamızda, yüksek doz bakır sülfat ($CuSO_4$) uygulamasının ardından dişi ve erkek sıçanların böbreklerinde AQP-1, -2 ve -3 ekspresyonunu değerlendirmeyi amaçladık. İlk olarak, kontrol grubuna (n=10) oral gavaj yoluyla salin verilirken, deney grubuna ($CuSO_4$) (n=10) 14 gün boyunca oral gavaj yoluyla 100 mg/kg/gün $CuSO_4$ verildi. Daha sonra kontrol ve deney gruplarının böbrek dokularında AQP-1, -2 ve -3'ün histopatolojik, PAS boyama ve immünohistokimyasal değerlendirmeleri yapıldı. $CuSO_4$, böbreklerde tübüler vakuolizasyona neden oldu. $CuSO_4$ uygulaması, AQP-1 ve AQP-3 düzeylerini artırırken AQP-2 düzeylerini düşürdü. Bu bulgular, $CuSO_4$'ün neden olduğu tübüler vakuolizasyon ve dejenerasyon nedeniyle AQP-1 ve AQP-3 düzeylerini yeniden modüle etmiş olabileceğini düşündürmektedir; ancak $CuSO_4$'ün dişilerdeki östrojen düzeyleri üzerindeki etkisi ve AQP-2 aracılığıyla vazopressin etkisi ayrıntılı olarak araştırılmalıdır.

**Anahtar Kelimeler: Akuaporinler. Bakır Sülfat. Böbrek. Rat.**

**Date Received:** 11.February.2026
**Date Accepted:** 9.March.2026

Dr. Selda KAHVECİ
E-mail: selda.kahveci@yobu.edu.tr
Yozgat Bozok University Faculty of
Medicine Department of Histology and Embryology
66100 Azizli/Yozgat/Türkiye
ORCID: 0000-0002-0734-6720

**AUTHORS' ORCID INFORMATION**
Selda KAHVECİ: 0000-0002-0734-6720
Tahsin SELVİ: 0009-0004-4944-5091
Şaban VARİNLİ: 0000-0003-0621-5726
Taha Berkay BOR: 0009-0002-2437-9257
Züleyha DOĞANYİĞİT: 0000-0002-6980-3384
Osman ÖZTÜRK: 0000-0003-1156-7419
Aslı OKAN OFLAMAZ: 0000-0001-8152-7338
Sümeyye UÇAR: 0000-0003-3378-3745
Seher YILMAZ: 0000-0003-4551-995X

Copper (Cu) is an essential trace element that functions as a cofactor for many enzymes in living organisms[1]. Copper is an essential element involved in the mitochondrial respiratory chain, antioxidant defense, and iron metabolism, acting as a cofactor for redox-regulatory enzymes, and it also plays a crucial role in nerve myelination and endorphin function[2-5]. However, elevated levels of Cu can be dangerous as it can cause oxidative stress in cells and damage intracellular biomolecules[6,7]. Copper exerts toxic effects in multiple organs such as the liver[8], kidneys[9], and brain[10], where it alters Kir channel expression in the prefrontal cortex[11]; these neurotoxic effects are particularly evident in Wilson's disease[5,12].

Copper exposure leads to glomerular and tubular impairments in the kidney, which are further aggravated by proteinuria, reduced glomerular filtration, aminoaciduria, and increased urinary phosphate loss[13]. Exposure to copper sulfate has been shown to cause renal tubular necrosis and degeneration, interstitial tissue damage, and structural changes associated with ER stress[14]. Peng et al. (2021) reported that repeated copper sulfate exposure in mice results in pronounced tubular damage, impaired renal function, and activation of oxidative and inflammatory pathways[15]. Another study reported that chronic copper sulfate exposure causes significant kidney damage characterized by glomerular impairment and tubular epithelial degeneration. The findings indicate that oxidative stress is the underlying cause of this pathology, as antioxidant supplementation alleviated most of the observed tissue damage[16].

The kidney is a vital organ responsible for maintaining the body's water balance[17]. Aquaporins (AQPs), which play a key role in facilitating fluid transport in the kidney, are a family of small, integral membrane proteins that transport small, neutral solutes across various biological membranes[18]. Each AQP functions as a tetrameric structure consisting of six transmembrane helical domains that enter the cell membrane. AQPs can be divided into three subtypes based on their transport capacity, and a wide varieties are found in the kidney[19]. Among these, Aquaporin 1 (AQP-1) is highly expressed in the kidney, where it regulates water reabsorption in the apical and basolateral membranes of the proximal tubules, thin descending limb of Henle, and the descending vasa recta[19,20]. Aquaporin 2 (AQP-2), on the other hand, is primarily expressed in the main cells of the collecting ducts in the kidney and, unlike most other aquaporins, AQP-2 is retained more in the cytoplasmic compartment[21]. Aquaporin 3 (AQP-3), on the other hand, is localized in the basolateral membrane of the primary cells in the collecting duct of the kidney[22,23].

This study investigated whether copper sulfate application affects the distribution of AQPs in different sexes. Accordingly, morphological changes in the kidney tissues of female and male rats, and the differences in AQP distribution and density based on sex, were evaluated. Therefore, morphological evaluation using hematoxylin-eosin and immunohistochemical methods were used to demonstrate changes in the expression distribution of AQP-1, AQP-2, and AQP-3 proteins in female and male kidney tissues.

## Materials and Methods

*Experimental Groups*

The Animal Ethics Committee of Kayseri Erciyes University (HADYEK) approved the experimental protocols for this study (decision 25/209) on October 01, 2025. 10 male/10 female *Sprague dawley* rats were obtained from the Erciyes University Hakan Çetinsaya Experimental and Clinical Research Center (DEKAM). Rats were housed in cages at 24°C±2°C, 12h light/dark cycles, with food and water in the cages. At the end of the experimental procedure, rats were killed under anesthesia induced by ketamine (60 mg/kg i.p.) - 2% xylazine (15 mg/kg i.p.).

Experimental model induced by copper sulphate ($CuSO_4$):

1) Control groups (n=10) receiving saline by oral gavage (male; n= 5, female; n=5),
2) $CuSO_4$ groups (n=10) receiving $CuSO_4$ dissolved in saline at a dose of 100 mg/kg/day by oral gavage (male; n=5, female; n=5).

The required dosage of $CuSO_4$ (copper sulphate pentahydrate - purity > 98.0%,CAS number: 7758-99-8, purchased from Isolab chemicals, Germany) was 100 mg/kg/day for 14days following the standard protocol of Liu et al.[24]. After the rats were killed, kidney tissues were removed, and the left kidney tissues were subjected to histological analysis, while the right kidney tissues were subjected to immunohistochemical analysis.

*Histological Analysis*

*Hematoxylin and Eosin (H-E) staining*

The kidney tissues were fixed in 10% formaldehyde, then washed and cleaned under running water overnight. The tissues were placed in increasingly concentrated alcohol solutions to remove the water inside them. The tissue was dehydrated by removing water, rinsed with xylene (equal to the tissue volume), and then embedded in paraffin blocks at an appropriate depth. Sections 5 µm thick were cut from the tissue blocks using a microtome. The tissue

sections were then placed on poly-L-lysine-coated slides for immunohistochemical analysis and on regular slides for H-E staining. Following all staining procedures, tissue integrity, edema, and histological changes in the kidney tissue morphology were evaluated using a Zeiss Axiocope-5 light microscope[11].

*Periodic Acid-Schiff (PAS) Staining*

PAS (Bio-Optica, Milona) staining was performed on sections taken from paraffin blocks of kidney tissue[25]. Following PAS staining procedures, glomerular structure, tubular degeneration, and basement membrane structure were evaluated using a Zeiss Axiocope-5 light microscope.

*Immunohistochemical Analysis*

Immunoreactivity of Aquaporin 1 (Affinity, AF5231), Aquaporin 2 (Affinity, DF7560), and Aquaporin 3 (Affinity, AF5222) proteins in kidney tissues were determined using the avidin-biotin peroxidase complex (ABC) method[11], with each applied at a 1:100 dilution ratio[26]. Sections of 5 µm thickness were deparaffinized, and antigen retrieval was performed using citrate buffer (pH: 6.0; Thermo Fischer Scientific, UK, AP-9003-500). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol. To prevent nonspecific binding, sections were incubated with a blocking serum (Thermo Fischer Scientific, UK, TA-125-UB), followed by overnight incubation at 4°C with the primary antibodies. The sections were washed several times with phosphate-buffered saline (PBS), then treated with secondary antibody (Thermo Fischer Scientific, UK, TS-125-BN) for 2 hours at 37°C, and after washing again, incubated with streptavidin peroxidase (Thermo Fischer Scientific, UK, TS-125-HR) at 37°C for 30 minutes. The antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (Thermo Fisher Scientific, UK, TA-125-HD). The sections were counterstained with Gill's hematoxylin (Merck, Germany, 1.05174.1000), dehydrated in a graded alcohol series, and mounted with Entellan. Images were acquired using a light microscope (Zeiss Axiocope-5).

*Statistical analysis*

All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Data were expressed as mean ± standard deviation (SD) for normally distributed variables. The effects of treatment and sex on renal parameters were analyzed using two-way ANOVA. When significant main effects were detected, multiple comparisons were performed using Šídák's post hoc test to compare group means within rows or columns, as appropriate. For non-normally distributed data, non-parametric tests were applied. A $p$ value <0.05 was considered statistically significant.

## Results

*Histological Analysis*

H-E staining of kidney tissues revealed intense vacuolization in the proximal tubules located in the cortex of the kidney tissues in the $CuSO_4$ group. Vacuolization was also observed in the collecting ducts of the cortex and in the medullary distal and proximal tubules (Figure 1). In particular, due to vacuolization in the proximal tubules, the lumen is greatly narrowed, making it impossible to distinguish the microvillus structures (Figure 1).

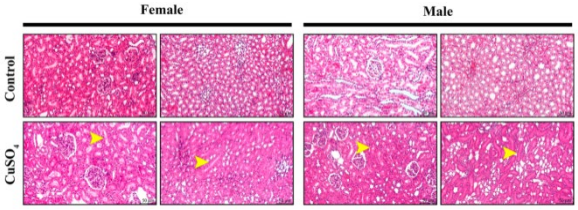


***Figure 1.***
*Hematoxylin and eosin staining of kidney tissue. Yellow arrowhead: Coiled proximal tubule Magnification: 20X.*

*PAS Analysis*

In PAS staining of kidney tissue, areas rich in glycoprotein in the cortex region of the control group were particularly dense in the basolateral region of the proximal and distal tubules. Similarly, PAS staining was observed in the CuSO4 group (Figure 2). On the other hand, PAS staining was also observed in the parietal layer of the Bowman's capsule in both groups. Furthermore, in the $CuSO_4$ group, PAS staining was intensely retained in the apical regions of the proximal and distal tubules (Figure 2).

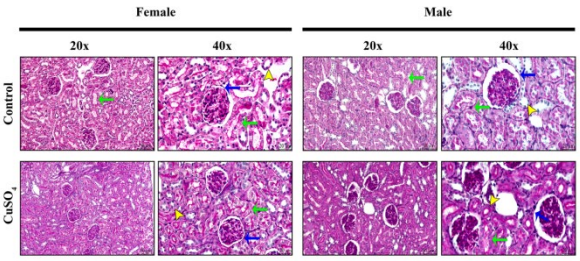


***Figure 2.***
*PAS staining of kidney tissue. Blue arrow: Bowman's capsule. Yellow arrowhead: Distal tubule. Green arrow: Proximal tubule. Magnification: 20X, 40X.*

*Immunohistochemistry Analysis*

Immunohistochemical analysis of kidney tissue revealed intense AQP-1 expression in the apical and basolateral membranes of proximal tubular epithelial cells. In control groups, AQP-1 immunoreactivity was observed in the proximal tubules of both female and male kidneys, and no significant gender-related difference was observed ($p$ = 0.3709) (Figure 3a, b). After $CuSO_4$ administration, AQP-1 expression in the proximal tubules of female kidneys increased in both apical and basolateral regions (Figure 3a); however, this marked increase was not statistically significant. It was found that $CuSO_4$ had no significant main effect ($p$ = 0.3007) and that there was no significant interaction between treatment and sex on AQP-1 expression ($p$ = 0.4151) (Figure 3b).

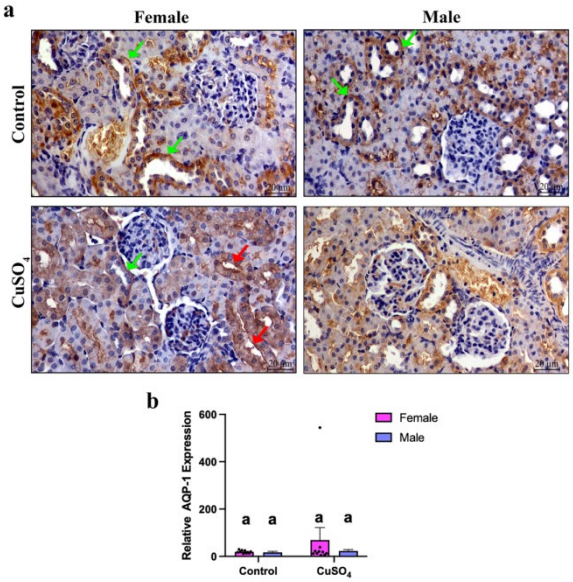


***Figure 3.***
*AQP-1 expression in kidney tissue. a. Green arrow: Proximal tubule basolateral membrane, red arrow: Proximal tubule apical. b. Relative AQP-1 expression levels. Magnification: 40X*

When examining AQP-2 expression in kidney tissue, no interaction between treatment and sex was detected ($p$=0.3360). Immunohistochemically, AQP-2 staining was predominantly localized to the apical region of collecting duct epithelial cells (Figure 4a). Although AQP-2 expression appeared lower following $CuSO_4$ exposure compared with controls, the main effect of treatment was not statistically significant ($p$=0.5838) (Figure 4a, b). In contrast, a significant main effect of sex was observed, with overall higher AQP-2 expression in females than in males when averaged across treatment groups ($p$=0.0372) (Figure 4a, b).

AQP-3 immunoreactivity was predominantly localized in the basolateral membrane of the outer medullary collecting duct epithelium in kidney tissue (Figure 5a). It revealed no significant interaction between treatment and sex ($p$ = 0.3647). Although AQP-3 expression showed a tendency to increase in $CuSO_4$-treated female kidneys, this difference was not statistically significant compared to female controls (p = 0.0683). A significant main effect of sex was detected, with females exhibiting higher overall AQP-3 expression than males (p = 0.0030), which was primarily driven by a significant sex difference in the $CuSO_4$-treated group (p = 0.0126) (Figure 5a, b).

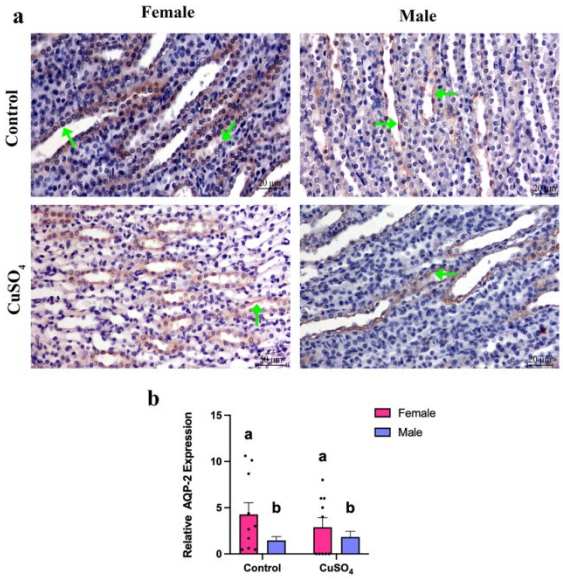


***Figure 4.***
*AQP-2 expression in kidney tissue. a. Green arrow: Apical membrane of the collecting duct epithelial cell. b. Relative AQP-2 expression levels. Magnification: 40X*

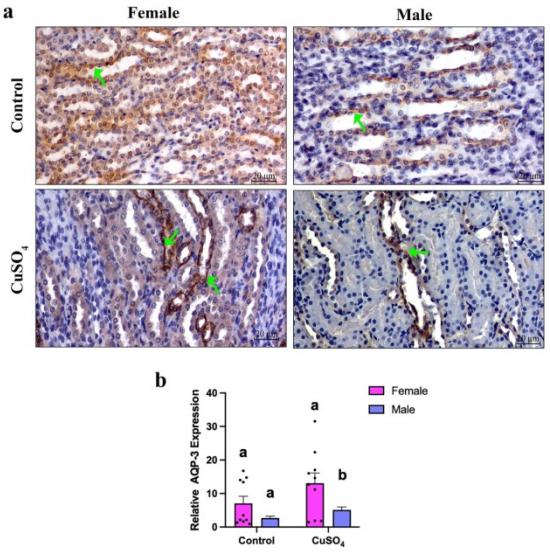


***Figure 5.***
*AQP-3 expression in kidney tissue. a. Green arrow: Basolateral membrane of the outer medullary collecting duct epithelium. b. Relative AQP-3 expression levels. Magnification: 40X*

## Discussion and Conclusion

In this study, we examined histopathological changes in the kidney tissues of mice administered Copper Sulfate ($CuSO_4$), using the Periodic acid-Schiff (PAS) reaction, and the immunohistochemical expression of Aquaporin (AQP) 1, 2, and 3, specifically in the areas where they are expressed. H-E staining; $CuSO_4$ application caused intense vacuolization in kidney tissues, particularly in the distal and proximal tubules, and the microvillus structure could not be observed due to luminal narrowing. PAS staining is frequently used to evaluate renal histopathology and provides information about changes in tubular structure and glycogen accumulation that may be caused by toxic exposures such as $CuSO_4$[27]. PAS staining has been noted to highlight damaged areas within the renal structure, including sclerosis and inflammation[28]. In our study, PAS staining revealed that copper sulfate causes glycogen accumulation in the apical region of the proximal and distal tubules in kidney tissue and leads to vacuolization in the tubules.

Copper pollution/poisoning causes multiple-organ damage in humans and animals[2,29]. Excess Cu causes redox imbalances, toxic events (e.g., cell apoptosis, necrosis, inflammation), and can damage the liver, kidneys and brain[30-32]. In chicken kidney tissues treated with copper sulfate and arsenic for 12 weeks, necrosis and degeneration were observed in tubular cells, swelling was observed in glomeruli, and it was demonstrated that this caused nephrotoxicity by increasing oxidative stress[33]. A recent study showed that copper sulfate administration to fetal rats increased tubular vacuolization in kidney tissues, caused degeneration in the glomerular structure, and increased inflammatory cells. In addition, malondialdehyde (MDA) and total oxidant status (TOS) values, which are oxidative markers, were found to be high in the copper sulfate groups [30]. Another study demonstrated that copper accumulation in rats can vary depending on dose and time in the kidneys, brain, and liver, and also indicated that glutathione (GSH) and total antioxidant capacity (TAC) values decreased, while MDA levels increased, depending on dose and time. The maximum effect was observed on day 60, and the maximum oxidative stress was demonstrated at a dose of 100 mg/kgBWt/day[34]. In our study, by using high doses of copper sulfate, we observed vacuolization in the proximal and distal tubules and deformation in the microvillus structure of the proximal tubules in particular during the histopathological evaluation of kidney tissue. Additionally, in the $CuSO_4$ group, PAS staining was found to be intensely preserved in the apical regions of the proximal and distal tubules.

In the kidneys, approximately 70% of filtered water is reabsorbed in the proximal tubule. AQPs are integral membrane proteins that facilitate water and, in some cases, other solute and ion movement[19]. AQP-1 is reported to be present in the apical and basolateral membranes of proximal tubule cells, accounting for 70% of total water absorption, thanks to its high water permeability[35]. Additionally, it has been reported that AQP-1 is expressed in the thin descending limb of Henle's loop (tDLH) and descending vasa recta in the kidney[19,20]. In their study, Lv et al. demonstrated that AQP-1 plays a protective role in modulating acute kidney injury (AKI), mitigating the inflammatory response, apoptosis, and fibrosis by downregulating p53 in HK-2 cells exposed to septic AKI or LPS[17]. Additionally, it has been reported that when the kidneys are exposed to the harmful effects of copper sulfate, a decrease in AQP-1 mRNA levels may occur, which could lead to impaired water reabsorption capacity. Decreased AQP-1 expression has been reported to be associated with renal dysfunction [36], which may be caused by the nephrotoxic effects of copper sulphate on aquaporin channels[18]. In a study demonstrating the mitigating effect of bee venom (BV) treatment on kidney damage caused by gentamicin (GM), it was noted that GM reduced the AQP-1 expression level[37]. In our study, it was observed that copper sulfate administration increased excretion in the kidneys of female rats, particularly in the apical and basolateral regions of the proximal tubules, but there was no significant difference compared to the control group. The increase in AQP-1 observed in the female $CuSO_4$ group in our study may be related to a compensatory adaptation mechanism mediated by estrogen. A study has shown that AQP-1 expression can be increased under estrogen-related stress conditions[38]. AQP-1 is highly concentrated in the proximal tubules and plays a critical role in water reabsorption. When $CuSO_4$ is applied, the kidney may have developed an adaptive response by increasing AQP-1 to maintain fluid balance during toxic damage.

AQP-2 is expressed in the principal cells of the connecting tubules and collecting ducts, which regulate body water balance and urine concentration[19]. AQP-2 is in the apical and subapical regions of the plasma membrane in the collecting duct epithelial cells, facilitating water entry into the cells. AQP-2 is under the control of antidiuretic hormone arginine vasopressin (AVP) and plays a critical role in water reabsorption in the kidney[39]. In our study, the AQP-2 transport channel was localized at the apical surface of epithelial cells, but its expression decreased in the group treated with $CuSO_4$. A recent study on pigs reported that AQP-2 levels in kidney tissue decreased as a result of chronic pancreatitis, potentially due to AVP antagonizing water permeability[40]. In another study, AQP-2 transcription and expression were reported to be reduced in LPS-stimulated cultured

collecting duct cells and native kidney tissues[41,42]. In one study, lithium treatment in hypokalemia was reported to cause a decrease in AQP-2 expression in the collecting ducts of rats' kidneys[43]. In our study, tubular damage/oxidative stress associated with $CuSO_4$ may be due to impaired vasopressin response in the collecting duct and reduced AQP-2 expression and/or membrane transport.

AQP-3 is localized in the basolateral membrane of principal cells in the collecting duct. A study has reported that AQP-3 deficiency in mice reduces the transepithelial osmotic water permeability of the collecting ducts, resulting in impaired urine concentration function[22,44]. In another study, deletion of AQP-3 in mice was reported to cause abnormalities in renal collecting ducts and worsen ischemia-reperfusion injury[23]. In recent years, it has been observed that AQP-2 expression decreased and AQP-3 expression increased in the kidney tissues of the experimental group with induced chronic pancreatitis[40]. As can be understood from the decrease in AQP-2 expression, it has been stated that this condition is not related to the increase in water flow through the basolateral membrane in pigs after cerulein injection[40]. These results are directly proportional to the decrease in AQP-2 levels and the increase in AQP-3 levels in our study. On the other hand, it has been suggested that estrogen may modulate AQP expression[45]. In our study, both AQP-1 and AQP-3 expression increased in the kidney tissues of females treated with copper sulfate. Estrogen may modulate AQP-1 and AQP-3 expression.

In conclusion, when evaluating the expression of AQP-1, -2, and -3 in female and male rats following copper sulfate administration, AQP-1 and AQP-3 levels increased in females, while AQP-2 levels decreased. The decrease in AQP-2 levels following tubular damage may have subsequently modulated AQP-1 and -3 levels. This provides a fundamental reference point for future studies investigating how copper sulfate may exert its effects, particularly through estrogen and vasopressin.

**Researcher Contribution Statement:**
Idea and design: S.K., T.S., T.B.B., Z.D., A.O.O., S.U., S.Y.; Data collection and processing: S.K., S.Y.; Analysis and interpretation of data: S.K., T.S., S.V., T.B.B., Z.D.; Writing of significant parts of the article: S.K., T.S., S.V., T.B.B., Z.D.
**Support and Acknowledgement Statement:**
Non-applicable
**Conflict of Interest Statement:**
No conflicts of interest.
**Ethics Committee Approval Information:**
Approving Committee: Animal Ethics Committee (HADYEK) of Erciyes University
Approval Date: October 01, 2025
Decision No: 25-209

## References

1. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med. 2005;26(4-5):235-244.
2. Kumar V, Kalita J, Bora HK, Misra UK. Relationship of antioxidant and oxidative stress markers in different organs following copper toxicity in a rat model. Toxicol Appl Pharmacol. 2016;293:37-43.
3. Valko M, Jomova K, Rhodes CJ, Kuca K, Musilek K. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Arch Toxicol. 2016;90(1):1-37.
4. Shim H, Harris ZL. Genetic defects in copper metabolism. J Nutr. 2003;133(5 Suppl 1):1527S-1531S.
5. Scheiber IF, Mercer JF, Dringen R. Metabolism and functions of copper in brain. Prog Neurobiol. 2014;116:33-57.
6. Arnal N, Dominici L, de Tacconi MJ, Marra CA. Copper-induced alterations in rat brain depends on route of overload and basal copper levels. Nutrition. 2014;30(1):96-106.
7. Kozlowski H, Janicka-Klos A, Brasun J, et al. Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation). Coordination Chemistry Reviews. 2009;253(21-22):2665-2685.
8. Toyokini S OS, Hamazaki S, Fujoka M, Li JL, Midorikawa O. Cirrhosis of the liver induced by cupric nitrilotriacetate in Wistar rats. An experimental model of copper toxicosis. Am J Pathol. 1989;134:1263-1274.
9. Zhao H, Wang Y, Fei D, et al. Destruction of redox and mitochondrial dynamics co-contributes to programmed cell death in chicken kidney under arsenite or/and copper (II) exposure. Ecotoxicol Environ Saf. 2019;179:167-174.
10. Waggoner DJ BT, Gitlin JD. . The role of copper in neurodegenerative disease. Neurobiol Dis. 1999;6:221-230.
11. Kahveci S, Ozturk O, Ucar S, et al. Effect of copper sulphate in rat brain tissue: Kir channels. Turkish Bulletin of Hygiene and Experimental Biology. 2024;81(4):419-430.
12. Scheinberg IH, Sternlieb I. Wilson disease and idiopathic copper toxicosis. Am J Clin Nutr. 1996;63(5):842S-845S.
13. Fuentealba ICH, S.; Foster, J. Cellular mechanisms of toxicity and tolerance in the copper-loaded rat. III. Ultrastructural changes and copper localization in the kidney. . Br J Exp Pathol 1989;70:543-556.
14. Dai C, Liu Q, Li D, et al. Molecular Insights of Copper Sulfate Exposure-Induced Nephrotoxicity: Involvement of Oxidative and Endoplasmic Reticulum Stress Pathways. Biomolecules. 2020;10(7):1010.
15. Peng X, Dai C, Zhang M, Das Gupta S. Molecular Mechanisms Underlying Protective Role of Quercetin on Copper Sulfate-Induced Nephrotoxicity in Mice. Front Vet Sci. 2020;7:586033.
16. Ghara AR. GF. Effect of purslane on kidney failure following copper toxicity in a rat model. Iranian Journal of Health Sciences 2018;6(1):25-32.
17. Lv W, Liao J, Li C, et al. Aquaporin 1 is renoprotective in septic acute kidney injury by attenuating inflammation, apoptosis and fibrosis through inhibition of P53 expression. Front Immunol. 2024;15:1443108.
18. Hua Y, Ying X, Qian Y, et al. Physiological and pathological impact of AQP1 knockout in mice. Biosci Rep. 2019;39(5).
19. Su W, Cao R, Zhang XY, Guan Y. Aquaporins in the kidney: physiology and pathophysiology. Am J Physiol Renal Physiol. 2020;318(1):F193-F203.
20. Pallone TL KB, Nielsen S, Agre P, Knepper MA. . Evidence that aquaporin-1 mediates NaCl-induced water flux across descending vasa recta Am J Physiol Renal Physiol 1997;272:F587-F596.

21. Tajika Y MT, Suzuki T, Aoki T, Hagiwara H, Tanaka S, Kominami E, Takata K. . Immunohistochemical characterization of the intracellular pool of water channel aquaporin-2 in the rat kidney. Anato Sci Int. 2002;77:189-195.
22. Ma T SY, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. . Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. PNAS. 2000;97:4386-4391.
23. Lei L, Wang W, Jia Y, et al. Aquaporin-3 deletion in mice results in renal collecting duct abnormalities and worsens ischemia-reperfusion injury. Biochim Biophys Acta Mol Basis Dis. 2017;1863(6):1231-1241.
24. Liu JY, Yang X, Sun XD, et al. Suppressive Effects of Copper Sulfate Accumulation on the Spermatogenesis of Rats. Biol Trace Elem Res. 2016;174(2):356-361.
25. Nangaku M, Alpers CE, Pippin J, et al. A new model of renal microvascular endothelial injury. Kidney Int. 1997;52(1):182-194.
26. Doganyigit Z, Okan A, Taheri S, et al. Evaluation of linagliptin and insulin combined therapy on unfolded protein response in type 1 diabetic mouse heart. Chem Biol Drug Des. 2023;102(5):1085-1096.
27. Yulug E, Turedi S, Yildirim O, et al. Biochemical and morphological evaluation of the effects of propolis on cisplatin induced kidney damage in rats. Biotech Histochem. 2019;94(3):204-213.
28. Ghosh SS MH, Krieg R, Fazelbhoy ZA, Ghosh S, Sica DA, Fakhry I, Gehr TW. Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. Am J Physiol Renal Physiol 2009;296(5):F1146-1157.
29. Kumar V, Kalita J, Bora HK, Misra UK. Temporal kinetics of organ damage in copper toxicity: A histopathological correlation in rat model. Regul Toxicol Pharmacol. 2016;81:372-380.
30. Uçar S, Yılmaz S, Ateş Ş, et al. Prenatal Exposure to Copper Sulfate: Effects on Fetal Development in Rats. Bratislava Medical Journal. 2026.
31. Erfanizadeh M, Noorafshan A, Naseh M, Karbalay-Doust S. The effects of copper sulfate on the structure and function of the rat cerebellum: A stereological and behavioral study. IBRO Neurosci Rep. 2021;11:119-127.
32. Kalita J, Kumar V, Misra UK, Bora HK. Movement Disorder in Copper Toxicity Rat Model: Role of Inflammation and Apoptosis in the Corpus Striatum. Neurotox Res. 2020;37(4):904-912.
33. Wang Y ZH, Shao Y, Liu J, Li J, Xing M. Copper or/and arsenic induce oxidative stress-cascaded, nuclear factor kappa B-dependent inflammation and immune imbalance, trigging heat shock response in the kidney of chicken. Oncotarget. 2017;8:98103-98116.
34. Kumar V, Kalita J, Misra UK, Bora HK. A study of dose response and organ susceptibility of copper toxicity in a rat model. J Trace Elem Med Biol. 2015;29:269-274.
35. Feraille E, Sassi A, Olivier V, Arnoux G, Martin PY. Renal water transport in health and disease. Pflugers Arch. 2022;474(8):841-852.
36. Vallon V, Verkman, A.S. and Schnermann, J. Luminal hypotonicity in proximal tubules of aquaporin-1-knockout mice. Am J Physiol Renal Physiol. 2000(278):1030-1033.
37. Abdelrahaman D, Shanab O, Abdeen A, et al. Bee venom ameliorates gentamicin-induced kidney injury by restoring renal aquaporins and enhancing antioxidant and anti-inflammatory activities in rats. Front Pharmacol. 2025;16:1525529.
38. Marrone J, Lehmann GL, Soria LR, et al. Adenoviral transfer of human aquaporin-1 gene to rat liver improves bile flow in estrogen-induced cholestasis. Gene Ther. 2014;21(12):1058-1064.
39. Nguyen H, Huynh NV, Hyndman KA. Diurnal function and expression of aquaporins in the mouse kidney. Am J Physiol Renal Physiol. 2025;329(5):F601-F614.
40. Michalek K, Oberska P, Murawski M, et al. Kidney morphology and renal expression of aquaporins 2, 3 and 4 during cerulein - Induced chronic pancreatitis in pigs. Adv Med Sci. 2023;68(2):306-313.
41. Mariajoseph-Antony LF, Kannan A, Panneerselvam A, et al. Role of Aquaporins in Inflammation-a Scientific Curation. Inflammation. 2020;43(5):1599-1610.
42. Hasler U, Leroy V, Jeon US, et al. NF-kappaB modulates aquaporin-2 transcription in renal collecting duct principal cells. J Biol Chem. 2008;283(42):28095-28105.
43. Marples D FJ, Dørup J, Knepper MA, Nielsen S. Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. J Clin Invest. 1996;97(08):1960-1968.
44. Hussein AA, El-Dken ZH, Barakat N, Abol-Enein H. Renal ischaemia/reperfusion injury: possible role of aquaporins. Acta Physiol (Oxf). 2012;204(3):308-316.
45. Oliveira dV, Schaefer J, Abu-Rafea B, et al. Uterine aquaporin expression is dynamically regulated by estradiol and progesterone and ovarian stimulation disrupts embryo implantation without affecting luminal closure. Mol Hum Reprod. 2020;26(3):154-166.