Ameliorative Effect of 80% Methanolic Cannabis sativa Leaf Extract on Copper (II) Oxide-Induced Alterations in Serum Electrolytes in Wistar Rats

Abdullahi Ishaq Jafaru[1], Hussaini Usman Durkwa[3], Abdulganiyyu Abdullahi Ahmad[2], Muazu Bala Pali[3], Sadiya Bello Usman[1], Ashiri Dahiru[1], Mercy Christopher Danzaria[3], Nafisat Abdulazeez[3], Salahudeen Nuhu Ahmed, and Ibrahim Maina Hassan[1]

[1]Department of Veterinary Physiology and Biochemistry, Usmanu Danfodio University Sokoto, Nigeria.

[2]Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture Zuru. Nigeria.

[3]Department of Veterinary Physiology and Biochemistry, Abubakar Tafawa Balewa University Bauchi, Nigeria.

DOI: https://doi.org/10.51244/IJRSI.2026.1308000074

Received: 22 August 2026; Accepted: 27 August 2026; Published: 04 September 2026

ABSTRACT

This study investigated the protective effect of 80% methanolic Cannabis sativa leaf extract against copper(II) oxide (CuO)-induced alterations in serum electrolyte balance in Wistar rats. Acute toxicity studies were conducted to determine the median lethal dose (LD_{50}) of the crude C. sativa leaf extract and CuO. The ameliorative effect of the extract on CuO-induced electrolyte disturbances was subsequently evaluated in vivo. The crude C. sativa leaf extract exhibited an LD_{50} of 676.08 mg/kg, whereas CuO exhibited a markedly lower LD_{50} of 45.71 mg/kg, indicating greater acute toxicity. Wistar rats were assigned to control and treatment groups and administered CuO followed by the crude C. sativa leaf extract. Serum samples were analyzed for sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-), as well as selected biochemical parameters. CuO exposure produced significant alterations in serum electrolyte concentrations compared with the control group. Administration of the 80% methanolic C. sativa leaf extract following CuO exposure modulated these electrolyte alterations, with significant differences observed among treatment groups ($P < 0.05$). The findings suggest that CuO exposure disrupts electrolyte homeostasis, potentially through alterations in renal function and fluid balance. The observed modulatory effects of the extract indicate its potential to mitigate CuO-induced electrolyte disturbances. Thus, C. sativa leaf extract may represent a potential source of bioactive compounds for managing heavy-metal-induced toxicity and associated disturbances in electrolyte homeostasis. Further studies are required to identify the active constituents and elucidate the mechanisms underlying these effects.

Keywords: Cannabis sativa; copper (II) oxide; crude extract; electrolyte balance; LD_{50}; toxicity.

INTRODUCTION

Neurodegeneration is characterized by the progressive dysfunction and loss of neurons associated with oxidative stress [1] and can result in disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis [2]. Oxidative stress, mitochondrial dysfunction, excessive glutamate activity, neuroinflammation, and abnormal protein accumulation are among the mechanisms implicated in neuronal damage [3]. Copper (II) oxide (CuO) is an essential trace element that plays important roles in numerous physiological processes, including mitochondrial energy production, antioxidant defense, neurotransmitter synthesis, and normal functioning of the nervous system [4]. As a cofactor for several enzymes, copper is required for maintaining cellular metabolism and neuronal integrity. However, both copper deficiency and excessive or abnormal accumulation can disrupt normal cellular processes and adversely affect the nervous system [5]. Increasing evidence suggests that disturbances in copper homeostasis may contribute to the development and progression of neurodegenerative disorders [4].

Assessment of serum electrolyte levels is an important component of clinical laboratory investigations. Measurement of these electrolytes can assist in evaluating hydration status, kidney function, metabolic abnormalities, and the effects of various diseases or treatments [6]. Understanding the importance of serum electrolytes is therefore essential for the early recognition and management of electrolyte disturbances and for maintaining normal physiological function [7]. Alterations in electrolyte homeostasis may occur secondary to toxicant exposure and can provide useful information regarding systemic and renal disturbances.

Cannabis (Cannabis sativa L.) is a medicinal plant that contains a diverse group of biologically active compounds, particularly phytocannabinoids such as cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) [8]. In recent years, increasing scientific interest has focused on the effects of cannabinoids on the nervous system and their potential therapeutic applications in neurological disorders. The biological actions of cannabinoids are largely associated with their interaction with the endocannabinoid system, which plays an important role in regulating neuronal communication, inflammation, pain perception, synaptic activity, and cellular homeostasis [9].

Despite the recognized biological importance of copper and the increasing interest in the therapeutic properties of C. sativa, limited information is available on the ability of C. sativa leaf extract to ameliorate CuO-induced systemic disturbances, particularly alterations in serum electrolyte homeostasis. Furthermore, the potential effects of crude methanolic C. sativa leaf extract on electrolyte balance following CuO exposure in Wistar rats remain insufficiently characterized. Therefore, the present study was undertaken to investigate the ameliorative effect of 80% methanolic C. sativa leaf extract on CuO-induced toxicity and associated alterations in serum electrolyte concentrations in Wistar rats. The findings may provide further insight into the potential protective properties of C. sativa against toxicant-induced physiological disturbances and contribute to the understanding of its possible therapeutic applications.

MATERIALS AND METHODS

Plants Collection and Identification

Fresh leaves of Cannabis sativa were obtained from the National Drug Law Enforcement Agency (NDLEA), Arkillah, Sokoto State, Nigeria. The plant material was authenticated by the Chief Botanist, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen was prepared and deposited in the departmental herbarium, where it was assigned voucher number CASA 01.

Plant Extraction

The collected Cannabis sativa leaves were thoroughly washed, cleaned, and air-dried at ambient temperature (26 ± 1°C) for two weeks. The dried leaves were then crushed into a semi-powdered form and sieved to obtain a particle size of 40–60 mesh. Briefly, 200 g of the powdered leaf material was soaked in 1000 mL of 80% methanol in flat-bottom flasks (Sigma-Aldrich, USA) for 72 h at 25 ± 1°C. The mixture was agitated daily throughout the extraction period to enhance the recovery of bioactive constituents. Following extraction, the mixture was filtered through clean muslin cloth, and the filtrate was concentrated to a semi-solid crude extract using a rotary evaporator (IKA® RV 10, USA) at 42°C. The resulting semi-solid extract was weighed, transferred into sterile sample vials, and stored at 4°C until further use. The percentage yield of the extract was calculated using the following equation [10].

Plants Sample Dilution and Dose Preparation

A stock solution of the crude Cannabis sativa leaf extract was prepared by dissolving 100 g of the extract in 1 L of 100% dimethyl sulfoxide (DMSO) to obtain a concentration of 100 mg/mL. A sub-stock solution was subsequently prepared by diluting the stock solution with distilled water to a final concentration of 10 mg/mL. Working solutions were then prepared by two-fold serial dilution of the sub-stock solution with distilled water to obtain the required experimental concentrations, including 1 mg/mL. The final concentration of DMSO was maintained at 0.1% (v/v) in all extract preparations to ensure consistency of the vehicle concentration across treatment groups [10].

Toxicity Study of the Extract

The oral toxicity of the crude Cannabis sativa leaf extract was evaluated in accordance with the Organization for Economic Co-operation and Development (OECD) Guideline 423 for acute oral toxicity testing [11]. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria (Approval No. UDUS/IACUC/AUP-R005/2020).

For the acute toxicity study, rats were administered the crude extract orally at doses of 250, 500, and 1000 mg/kg body weight for two consecutive days [12]. Mortality and other observable signs of toxicity were monitored throughout the study period, and the median lethal dose (LD_{50}) was estimated using probit analysis. For the longer-term toxicity assessment, the crude extract was administered orally at doses of 125, 250, and 500 mg/kg body weight for 14 consecutive days [13].

Induction of Copper(II) Oxide Toxicity

Experimental toxicity was induced in rats by oral administration of copper(II) oxide (CuO) at a dose of 200 mg/kg body weight once daily for 5 consecutive days, following the previously reported method [14].

Amelioratic Effects of the Crude Extract on Copper (ii) oxide-induced Toxicity

The ameliorative effects of the crude extract on copper(II) oxide (CuO)-induced nephrotoxicity were evaluated in accordance with the relevant OECD guidelines. Twenty rats were randomly divided into four groups (A–D), with five rats per group (n = 5). All animals had free access to standard laboratory feed and water throughout the experimental period. Treatments were administered by oral gavage at the designated doses and dosing volumes.

Group A (Normal control): Rats received standard laboratory feed and water only throughout the experimental period.

Group B (Extract control): Rats received 300 mg/kg body weight (bw) of the crude extract by oral gavage once daily for 10 days, in addition to standard laboratory feed and water.

Group C (CuO-treated group): Rats received 200 mg/kg bw of copper(II) oxide by oral gavage once daily for 3 consecutive days, in addition to standard laboratory feed and water.

Group D (CuO + extract-treated group): Rats received 200 mg/kg bw of copper(II) oxide by oral gavage once daily for 3 consecutive days. This was followed by administration of 300 mg/kg bw of the crude extract by oral gavage once daily for 10 consecutive days.

The dose of 300 mg/kg bw of the crude extract was selected based on the preliminary toxicity assessment, which established the safety of the extract at the administered dose and provided an appropriate dose for evaluating its potential ameliorative effect. The 200 mg/kg bw dose of CuO was selected to induce measurable toxic effects, including renal injury, while avoiding excessive mortality or severe systemic toxicity. The CuO treatment was therefore used to establish the nephrotoxicity model, whereas the subsequent administration of the crude extract was intended to evaluate its potential protective/ameliorative effects.

The dosing volume was adjusted according to individual body weight to ensure accurate administration of the required dose to each animal.

Animals were anesthetized with chloroform vapor, and blood was taken through heart puncture with a 5 ml syringe and needle and put into EDTA-free bottles 48 hours after the last treatment. Each rat's brain was extracted after the head was dissected with a dissecting kit. The brains were dipped into a beaker containing normal saline to remove excess blood before being placed in a clean sample container containing 10% neutral buffered formalin [15].

Biochemical Tests

Serum biochemical parameters, including total protein (Enehizena et al., 2026), sodium (Na^+), potassium (K^+) [16], chloride (Cl^-) [17], bicarbonate (HCO_3^-) [18], and urea [19], were determined using an automated biochemical analyzer (Thermo Fisher Scientific, USA) at the Veterinary Teaching Hospital, Usmanu Danfodiyo University, Sokoto, Nigeria, according to the manufacturer's instructions.

Blood samples were collected into heparinized tubes and centrifuged to separate the plasma. The separated samples were subsequently loaded into the electrolyte/clinical chemistry analyzer for analysis. Electrolyte concentrations were determined using ion-selective electrode (ISE) methodology, and the results were expressed in mmol/L (mEq/L). Total protein and urea concentrations were determined using the appropriate analytical methods programmed in the analyzer according to the manufacturer's specifications.

Statistical Analysis

The data obtained were analyzed using InVivoStat software, version 4.2.0 and expressed as mean ± standard error of the mean (SEM). Data distribution and normality were assessed prior to statistical analysis. For data that satisfied the assumptions of normality, differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by an appropriate post hoc test where applicable. For data that did not meet the assumption of normality, the non-parametric Kruskal–Wallis test was applied. A p-value < 0.05 was considered statistically significant.

RESULTS

The results showed that serum sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) concentrations were significantly altered ($p < 0.05$) in the treated groups (B, C, and D) compared with the normal control group (A). In addition, total protein and plasma albumin concentrations were significantly decreased ($p < 0.05$) in the treated groups compared with the control group (A) (Table 1).

These findings indicate that exposure to the tested treatments resulted in disturbances in serum electrolyte and protein homeostasis. The alterations in Na^+, K^+, Cl^-, and HCO_3^- may reflect changes in renal electrolyte handling and fluid balance, while the reduction in total protein and albumin may suggest impaired protein synthesis, altered protein metabolism, or increased protein loss. The observed changes may therefore indicate systemic effects associated with CuO exposure and/or the administration of the crude C. sativa extract. Further interpretation of the ameliorative effect of the extract should be based on whether the values in group D show a significant recovery toward the normal control values.

Table 1: Effects of copper, cannabis and copper then cannabis on Liver and kidney function

Parameters	**A**	**B**	**C**	**D**
Na^+(mmol/L)	136.00 ± 4.93^a	151.00 ± 1.03^b	147.00 ± 1.00^c	150.00 ± 2.09^b
K^+ (mmol/L)	5.00 ± 0.61^a	5.20 ± 0.49^b	5.60 ± 0.45^c	4.80 ± 0.35^a
Cl^-(mmol/L)	94.00 ± 3.46^a	11.00 ± 1.15^b	103.00 ± 32.85^c	104.00 ± 0.25^d
HCO_3^-(mmol/L)	12.00 ± 3.18^a	14.00 ± 1.65^b	21.00 ± 0.88^c	17.50 ± 1.55^d

Key: Sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-). Values with different superscript showed statistical difference [increase (^{a,d}) and decrease (^{b,c})].

DISCUSSION

This study was conducted to determine the protective effect of 80% methanolic Cannabis sativa leaf extract against copper(II) oxide (CuO)-induced toxicity and associated alterations in serum electrolyte concentrations in Wistar rats. The findings demonstrated significant alterations in serum sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) concentrations following CuO administration. The observed reduction in these electrolytes is consistent with previous findings reported by [20], who also documented decreased serum Na^+, K^+, Cl^-, and HCO_3^- concentrations in Wistar rats exposed to CuO. The agreement between the present findings and those of [20] suggests that disruption of electrolyte homeostasis may represent an important component of CuO-induced systemic toxicity. Nevertheless, differences in the magnitude of electrolyte alterations between studies may arise from variations in CuO dose, duration of exposure, animal characteristics, and experimental conditions.

The reduction in serum Na^+, K^+, Cl^-, and HCO_3^- following CuO exposure may be associated with copper-induced gastrointestinal and/or renal disturbances [21]. Copper toxicity may interfere with intestinal electrolyte absorption and renal electrolyte handling, while gastrointestinal disturbances and associated fluid losses may further contribute to depletion of Na^+, K^+, Cl^-, and HCO_3^- [22]. The kidney plays a central role in maintaining electrolyte and acid–base balance; therefore, impairment of renal tubular function following CuO exposure could adversely affect the reabsorption and excretion of these ions. In particular, the reduction in serum bicarbonate may indicate disturbance of acid–base homeostasis and could be compatible with a tendency toward metabolic acidosis. However, because blood pH, partial pressure of carbon dioxide, and other acid–base parameters were not measured in the present study, metabolic acidosis cannot be confirmed solely on the basis of reduced serum bicarbonate.

Oxidative stress represents another possible mechanism underlying the observed electrolyte disturbances. Excessive copper exposure can promote reactive oxygen species generation, lipid peroxidation, and cellular injury, which may compromise renal cellular integrity and consequently impair the processes responsible for electrolyte regulation [23]. Thus, the reductions in serum electrolytes observed in the CuO-treated animals may reflect a combination of gastrointestinal losses, altered renal handling, disturbed fluid balance, and oxidative cellular injury rather than a single mechanism.

The changes observed following administration of the C. sativa extract suggest a potential modulatory effect against CuO-induced electrolyte disturbances. The partial restoration of electrolyte concentrations in the CuO + extract group may indicate that constituents of the extract helped to preserve or restore electrolyte homeostasis following CuO exposure. Importantly, the protective effect should be interpreted according to the extent to which the electrolyte concentrations in the CuO + extract group approached those of the normal control rather than assuming complete protection solely from the presence of an increase. A greater normalization toward control values would provide stronger evidence of an ameliorative effect. The observed response may potentially be related to the biological activities of phytochemicals present in C. sativa, although the specific constituents responsible for the observed effects were not identified in this study.

Interestingly, administration of the crude C. sativa extract alone resulted in increased serum Na^+, Cl^-, and HCO_3^- concentrations. This finding is consistent with previous observations reported by [24], suggesting that C. sativa administration may itself influence electrolyte homeostasis. The elevation of these electrolytes may be associated with alterations in fluid and electrolyte balance induced by the extract [25]. For example, changes in water intake or fluid loss may produce mild dehydration and consequent hemoconcentration, thereby increasing circulating electrolyte concentrations [26]. Another possible explanation involves modulation of the endocannabinoid system, which has been implicated in renal and fluid–electrolyte regulation and may influence sodium and chloride handling [27]. The increase in bicarbonate may similarly reflect changes in renal bicarbonate handling or acid–base regulation [28]. Nevertheless, these mechanisms remain speculative in the present study because water intake, urinary electrolyte excretion, renal tubular function, and acid–base parameters were not directly assessed. The magnitude and direction of the effects may also depend on the dose and duration of treatment, as well as the phytochemical composition of the crude extract.

The mild increase in serum urea observed following CuO administration is consistent with the findings reported by [29]. Increased serum urea may indicate a disturbance in renal handling of nitrogenous waste. Excess copper can promote oxidative stress and cellular injury, potentially impairing renal function and reducing the clearance of urea [30]. However, serum urea concentration can also be influenced by factors other than renal filtration, including hydration status, dietary protein intake, and increased protein catabolism. Therefore, the modest elevation observed in the present study should not be interpreted as definitive evidence of severe renal dysfunction. Rather, it may indicate a relatively mild disturbance in renal function or systemic homeostasis following CuO exposure. Assessment of additional renal biomarkers and histopathological changes would provide stronger evidence for the involvement and severity of renal injury.

Overall, the present findings suggest that CuO exposure can disrupt serum electrolyte homeostasis and may produce mild alterations in renal function, while treatment with 80% methanolic C. sativa leaf extract appears to exert an ameliorative effect on some of these changes. The findings are broadly consistent with previous reports [20,24,29], although the present study also highlights the possibility that both CuO and the extract itself can influence electrolyte regulation. Consequently, the effects of the extract should be interpreted in relation to its own electrolyte-modulating activity as well as its ability to counteract CuO-induced alterations.

Limitations of the Study

Several limitations should be considered when interpreting the findings of the present study. First, the study was conducted using a relatively small number of animals, which may limit the statistical power and generalizability of the findings. Second, although serum electrolyte concentrations were assessed, urinary electrolyte excretion, water intake, urine volume, renal tubular function, and acid–base parameters such as blood pH and partial pressure of carbon dioxide were not directly measured. Therefore, the proposed mechanisms underlying the observed electrolyte alterations, particularly those involving renal dysfunction, fluid imbalance, and possible disturbances in acid–base homeostasis, remain inferential. Third, histopathological examination of the kidney was not performed, limiting the ability to correlate the observed biochemical and electrolyte changes with structural alterations in renal tissue or to directly evaluate the tissue-protective effects of the extract. Fourth, HPLC analysis of the crude C. sativa extract was not performed; consequently, the specific phytochemical constituents and their concentrations could not be identified or quantified. Thus, potential active compounds, including cannabinoids, flavonoids, and terpenes, could not be linked to the observed protective effects. Fifth, additional mechanistic biomarkers of oxidative stress and renal injury were not evaluated, limiting the ability to establish a direct mechanistic relationship between CuO exposure, oxidative stress, renal injury, and electrolyte disturbances. Finally, the study employed a single dose of the crude extract, and therefore, a dose–response relationship and the optimal protective dose could not be determined.

CONCLUSIONS

This study demonstrated that exposure to copper(II) oxide (CuO) induced significant alterations in serum electrolyte homeostasis in Wistar rats, particularly involving sodium, potassium, chloride, and bicarbonate concentrations. Administration of the 80% methanolic crude Cannabis sativa leaf extract also produced changes in serum electrolyte concentrations, indicating a potential influence on fluid and electrolyte regulation. Following CuO exposure, treatment with the crude extract modulated the observed electrolyte disturbances, suggesting a potential ameliorative effect against CuO-induced disruption of electrolyte homeostasis. The mild elevation in serum urea following CuO administration further suggests a modest alteration in renal handling of nitrogenous waste, which may be associated with copper-induced oxidative and cellular injury. Overall, these findings suggest that C. sativa leaf extract contains bioactive constituents that may mitigate some physiological disturbances associated with CuO exposure.

Further studies are warranted to establish the mechanisms underlying the observed protective effects. Future research should incorporate larger sample sizes, renal histopathological assessment, urinary electrolyte and acid–base measurements, and specific biomarkers of renal function and oxidative stress to clarify the contribution of renal injury and oxidative mechanisms to CuO-induced electrolyte disturbances. Mechanistic studies should also investigate whether modulation of the endocannabinoid system contributes to the effects of C. sativa extract on renal function and electrolyte homeostasis. HPLC-based phytochemical characterization is recommended to

identify and quantify potentially active constituents, including cannabinoids, flavonoids, and terpenes, and to relate these compounds to the observed biological effects. In addition, studies using multiple doses of the extract are necessary to establish a dose-response relationship and determine the optimal protective dose against CuO-induced toxicity. Collectively, these investigations would provide a more comprehensive understanding of the molecular and physiological mechanisms underlying the protective effects and therapeutic potential of C. sativa against CuO-induced toxicity.

Data Availability

Data are available with Dr Ibrahim Maina Hassan but with the permission of the corresponding Usmanu Danfodiyo University Sokoto.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGMENTS

The authors would like to thank Usmanu Danfodiyo University through the Institution Based Research (IBR) sponsored by Tertiary Education Trust Fund (TETfund), Federal Ministry of Education Nigeria. The authors wish to acknowledge the efforts of Dr. Abdullahi Abdullahi Raji of the Department of Veterinary Pathology and Oncology, Usmanu Danfodiyo University Sokoto, for the reading and interpretation of the histopathological slides.

REFERENCES

1. Dahiru, A., Nawaz, I., Riaz, S. K., Chaudry, S. S., Khan, M. J., Huang, Q., Abbas, M., & Eqani, S. A. M. A. S. (2026). Molecular mechanisms underlaying fluoride-induced neurotoxicity: interplay of antioxidants and endoplasmic reticulum stress–mediated apoptotic pathways in rats. Naunyn-Schmiedeberg's Archives of Pharmacology. https://doi.org/10.1007/s00210-026-05441-3.
2. Enehizena, O. O., A. E. Mathias, S. E. I. Rogue, O. O. Festus, E. T. Moses, and W. O. Ohiwerei. 2026. "AMELIORATIVE AND PROTECTIVE EFFECTS OF ACTIVATED CHARCOAL ON SERUM TOTAL PROTEIN, ALBUMIN, GLOBULIN, AND WHITE BLOOD CELL COUNT IN CADMIUM-INDUCED WISTAR RATS." Innovative Journal of Medical Sciences (ISSN: 2714-3325) 8(8): 29–46.
3. Shin, Eun-Joo, Ji Hoon Jeong, Yeonggwang Hwang, Naveen Sharma, Duy-Khanh Dang, Bao-Trong Nguyen, Seung-Yeol Nah, et al. 2021. "Methamphetamine-Induced Dopaminergic Neurotoxicity as a Model of Parkinson's Disease." Archives of Pharmacal Research 44(7): 668–88. doi:10.1007/s12272-021-01341-7.
4. Liu, Wu, Yan Xue, Chenyin Cao, Liting Yang, and Lijun Zhang. 2026. "Copper Homeostasis and Cuproptosis in Neurological Disorders." Drug Design, Development and Therapy Volume 20: 1–32. doi:10.2147/DDDT.S580005.
5. Fatima, Ghizal, Sadaf Khan, Jan Fedacko, Ammar Mehdi Raza, Ram B. Singh, and Jaipaul Singh. 2026. "Trace Elements as Molecular Orchestrators of Health and Disease: A Biochemical and Pathophysiological Perspectives." In Functional Biochemistry of Metallic Elements, eds. Naranjan S. Dhalla, Paramjit S. Tappia, and Anureet K. Shah. Cham: Springer Nature Switzerland, 19–42. doi:10.1007/978-3-032-14445-4_2.
6. Hial, Azher, Abdulhussein Ashour, Anwar Almzaiel, and Nashwan Jabbar. 2025. "Pathochemical and Clinical Chemistry Assessment of Biochemical Determinants Associated with Hemorrhagic Fever Patients Under Surgical and Anesthetic Management in Dhi Qar Province, Iraq." Ibn Sina Journal of Medical Science Health & Pharmacy: 8. doi:10.64440/IBNSINA/SINA008.
7. R.M., KavyaDeepu, and Mohnish Sekar. 2025. "A Comprehensive Review of Electrolyte Imbalances and Their Applied Aspects in Dermatology." Cureus. doi:10.7759/cureus.81353.
8. Wal, Ankita, Shweta Yadav, Kamal Deka, Bhagawati Saxena, Namra Aziz, Nardev Singh, Abida Khan, and Amin Gasmi. 2026. "Pharmacological Efficacy and Traditional Uses of Cannabis Sativa: A

Comprehensive Review." Current Organic Chemistry 30. doi:10.2174/0113852728395972251207093735.

9. Simankowicz, Paulina, and Joanna Stępniewska. 2025. "The Role of Endocannabinoids in Physiological Processes and Disease Pathology: A Comprehensive Review." Journal of Clinical Medicine 14(8): 2851. doi:10.3390/jcm14082851.
10. Hassan, Ibrahim, Wan Norhamidah Wan Ibrahim, Ferdaus Mohamat Yusuf, Siti Aqlima Ahmad, and Syahida Ahmad. 2020. "Biochemical Constituent of Ginkgo Biloba (Seed) 80% Methanol Extract Inhibits Cholinesterase Enzymes in Javanese Medaka (Oryzias Javanicus) Model." Journal of Toxicology. doi:10.1155/2020/8815313.
11. Kwon, Hee Jung, Hyomin Lee, Sunyi Lee, Woori Ko, Shin Jea Yun, Yoshihiro Uesawa, and Joohee Jung. 2025. "Comparative Analysis of OECD Guideline Data and Tox21 Assays to Improve Reproductive and Developmental Toxicity Prediction." Scientific Reports 16(1): 3417. doi:10.1038/s41598-025-33419-3.
12. Clemente, M., M. D. Miguel, K. B. Felipe, C. Gribner, P. F. Moura, A. G.R. Rigoni, L. C. Fernandes, et al. 2019. "Acute and Sub-Acute Oral Toxicity Studies of Standardized Extract of Nasturtium Officinale in Wistar Rats." Regulatory Toxicology and Pharmacology. doi:10.1016/j.yrtph.2019.104443.
13. Adekola, M. B., J. O. Areola, N. O. Omisore, F. T. Asaolu, S. G. Ogunleye, O. E. Apalowo, and O. O. Babalola. 2020. "Sub-Chronic Toxicity Study of Ethanol Stem-Bark Extract of Blighia Sapida (Sapindaceae) in Wistar Rats." Heliyon. doi:10.1016/j.heliyon.2019.e02801.
14. Patlolla, Anita K., and Paul B. Tchounwou. 2005. "Cytogenetic Evaluation of Arsenic Trioxide Toxicity in Sprague-Dawley Rats." Mutation Research - Genetic Toxicology and Environmental Mutagenesis. doi:10.1016/j.mrgentox.2005.08.007.
15. Parasuraman, S., R. Raveendran, and R. Kesavan. 2010. "Blood Sample Collection in Small Laboratory Animals." Journal of Pharmacology and Pharmacotherapeutics. doi:10.4103/0976-500X.72350.
16. Namiki, Yusuke, Kenji Ida, Yoko Homma, Namino Tomimori, Ken Sato, Chihaya Kakinuma, Hidehisa Tachiki, and Takuo Ogihara. 2026. "Investigation of the Effects of Calcium Alginate on Blood Sodium Concentration and the Suppression of Blood Pressure Elevation in Rats." Biological and Pharmaceutical Bulletin 49(3): 564–71. doi:10.1248/bpb.b25-00592.
17. Kuo, Yuh-Chen, Vincent F. S. Tsai, Shih-Ping Liu, Jyh-Horng Chen, Hong-Chiang Chang, and Ju-Ton Hsieh. 2026. "Chloride-Dependent Mechanisms Contribute to Urinary Bladder Smooth Muscle Contractility: Pharmacological and Molecular Evidence from Rat Bladder Tissue." Tzu Chi Medical Journal. doi:10.4103/tcmj.TCMJ-D-26-00143.
18. Mahmoud, Ėman Shaaban, Asmaa Tighian, Marwa Refaie, Mostafa Asem, Azza Abdel Zaher, Sayed Shehata, and Eman Mahmoud. 2025. "Packed RBCs versus Sodium Bicarbonate in the Treatment of Aluminum Phosphide-Induced Cardiotoxicity and Metabolic Acidosis in Rats." Ain Shams Journal of Forensic Medicine and Clinical Toxicology 44(1): 48–56. doi:10.21608/ajfm.2025.337915.1151.
19. Al-Thani, Najlaa A., Gavin S. Stewart, and Derek A. Costello. 2025. "The Role of the Urea Cycle in the Alzheimer's Disease Brain." Journal of Neurochemistry 169(3): e70033. doi:10.1111/jnc.70033.
20. Akamo, Adio J., Boluwatife A. Olagunju, Iyabode A. Kehinde, Naomi M. Akamo, Adetutu O. Ojelabi, Ofem E. Eteng, Ibiyemi O. Opowoye, et al. 2025. "Chemotherapeutic Efficacy of Curcumin against Plasma and Cardio-Hepatorenal Electrolyte Disruption in Dichlorvos-Challenged Rats via Augmentation of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase Activities." Journal of Trace Elements and Minerals 12: 100248. doi:10.1016/j.jtemin.2025.100248.
21. Rafati Rahimzadeh, Mehrdad, Mehravar Rafati Rahimzadeh, Sohrab Kazemi, and Ali Akbar Moghadamnia. 2024. "Copper Poisoning with Emphasis on Its Clinical Manifestations and Treatment of Intoxication" ed. Annisa Utami Rauf. Advances in Public Health 2024: 1–12. doi:10.1155/2024/6001014.
22. Sehgal, Shelly, Sachin Gupta, and Manish Kumar Mishra. 2024. "Electrolytes and Acid-Base Disorders." In Clinical Applications of Biomolecules in Disease Diagnosis, eds. Ram Lakhan Singh, Pankaj Singh, and Neelam Pathak. Singapore: Springer Nature Singapore, 155–75. doi:10.1007/978-981-97-4723-8_7.
23. Adele, B.O., G.T. Ojo, A.O. Ige, A.O. Odetola, I.E. Emediong, and E.O. Adewoye. 2023. "Toxic Copper Level Increases Erythrocyte Glycolytic Rate, Glutathione Production and Alters Electrolyte Balance in Male Wistar Rats." Journal of Trace Elements in Medicine and Biology 79: 127231. doi:10.1016/j.jtemb.2023.127231.

24. Ye, Y., H. Yuan, H. Wang, P. Zhang, and Y. Zhang. 2025. "Effects of Nitrogen, Phosphorus, and Potassium on the Growth Physiology and Secondary Metabolites of Hemp (Cannabis Sativa L.) under NaHCO3 Stress." Russian Journal of Plant Physiology 72(5): 152. doi:10.1134/S1021443724610772.
25. Alam, Waqas, Haroon Khan, and Imad Ahmad. 2022. "Acid-Base and Electrolyte Balance Regulations with Phytonutrients." In The Role of Phytonutrients in Metabolic Disorders, Elsevier, 291–311. doi:10.1016/B978-0-12-824356-5.00003-5.
26. Wittczak, Andrzej, Maciej Ślot, and Agata Bielecka-Dabrowa. 2023. "The Importance of Optimal Hydration in Patients with Heart Failure—Not Always Too Much Fluid." Biomedicines 11(10): 2684. doi:10.3390/biomedicines11102684.
27. Młynarska, Ewelina, Natalia Kustosik, Maja Mejza, Zuzanna Łysoń, Dawid Delebis, Jakub Orliński, Jacek Rysz, and Beata Franczyk. 2024. "Renal Outcomes and Other Adverse Effects of Cannabinoid Supplementation." Nutrients 17(1): 59. doi:10.3390/nu17010059.
28. Mansouri, Katharina, Thierry Hanh, and Andreas Hahn. 2025. "Hydration Meets Regulation: Insights into Bicarbonate Mineral Water and Acid–Base Balance." Nutrients 17(14): 2291. doi:10.3390/nu17142291.
29. Tohamy, Hossam G., Osama S. El Okle, Amira A. Goma, Mohamed M. Abdel-Daim, and Mustafa Shukry. 2022. "Hepatorenal Protective Effect of Nano-Curcumin against Nano-copper Oxide-Mediated Toxicity in Rats: Behavioral Performance, Antioxidant, Anti-Inflammatory, Apoptosis, and Histopathology." Life Sciences 292: 120296. doi:10.1016/j.lfs.2021.120296.
30. Ren, Xiya, Limei Zhao, Yajie Hao, Xiu Huang, Guangna Lv, and Xiaoshuang Zhou. 2025. "Copper-Instigated Modulatory Cell Mortality Mechanisms and Progress in Kidney Diseases." Renal Failure 47(1): 2431142. doi:10.1080/0886022X.2024.2431142.