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RENAL SAFETY EVALUATION OF ETHANOLIC ROOT EXTRACT OF *Icacina trichantha* IN MALE WISTAR RATS

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ABSTRACT

Icacina trichantha is widely utilised in traditional medicine in several parts of West Africa for the management of various ailments. However, information regarding its effects on renal function following repeated exposure remains limited. This study evaluated the effects of repeated administration of I. trichantha root ethanolic extract (ITREE) on renal function and kidney histology in male Wistar rats. Twenty adult male Wistar rats were randomly assigned into four groups (n = 5). Group I served as the control and received distilled water, while Groups II, III, and IV received 200, 500, and 1000 mg/kg body weight (bw) of ITREE respectively by oral gavage for 28 days. Serum electrolyte concentrations (Na^+, K^+, Cl^-, and HCO_3^-), urea, and creatinine were determined using standard biochemical methods. Kidney tissues were examined histologically using haematoxylin and eosin staining. Data were analysed using one-way analysis of variance (ANOVA), and significance was accepted at $p < 0.05$. Significant reductions in serum potassium and chloride concentrations were observed in all ITREE-treated groups compared with the control group. Serum sodium concentration was significantly reduced in the 200 mg/kg bw and 1000 mg/kg bw groups, while bicarbonate concentration decreased significantly at 500 mg/kg bw and 1000 mg/kg bw. Serum creatinine concentrations were significantly lower in all treatment groups compared with the control, whereas serum urea showed a modest reduction only at 500 mg/kg bw. Histological examination revealed preserved renal glomerular and tubular architecture in most treatment groups, although mild interstitial lymphocytic infiltration with congestion was observed in the 200 mg/kg bw group. These findings indicate that repeated administration of ITREE altered serum electrolyte concentrations without producing marked renal structural injury within the duration of the study. Further studies involving longer exposure periods, larger sample sizes, and additional renal biomarkers are recommended to further clarify the renal effects associated with prolonged ITREE exposure.

INTRODUCTION

Medicinal plants remain an important component of healthcare delivery systems worldwide and are widely utilised for the prevention and management of numerous diseases. In many developing countries, particularly in Africa, herbal medicines are commonly used because they are affordable, culturally accepted, and readily accessible. The World Health Organization estimated that approximately 80% of populations in some developing regions depend partly on traditional herbal medicine for primary healthcare needs (World Health Organization [WHO], 2013). Despite their extensive use, many medicinal plants have not undergone comprehensive scientific evaluation regarding their safety following prolonged or repeated exposure. Consequently, there is increasing interest in establishing the toxicological and physiological effects of medicinal plants commonly used in traditional medicine (Ekor, 2014).

Icacina trichantha, a perennial shrub belonging to the family Icacinaceae, is one of the medicinal plants widely utilised in African ethnomedicine. The plant is distributed across tropical West and Central Africa and has traditionally been employed in the management of several ailments including fever, gastrointestinal disorders, infections, and inflammatory conditions (Burkill, 2004). In Nigeria, the roots of *I. trichantha* are incorporated into herbal preparations for the treatment of asthma (Ajibesin *et al.*, 2008), constipation, malaria, poisoning, toothache, rheumatism (Asuzu & Abubakar, 1995; Ariwaodo *et al.*, 2012), mumps (Ubom, 2010), and soft tumours (Burkill, 1985; Burkill, 1994). The plant has also been reported to possess aphrodisiac properties (Burkill, 1985; Burkill, 1994; Quattrocchi, 2012).

Phytochemical studies have shown the presence of several bioactive phytochemical constituents in medicinal plants including diterpenoids, alkaloids, flavonoids, and saponins, which are associated with antimicrobial, antioxidant, anti-inflammatory, and other pharmacological and biological activities (Hostettmann *et al.*, 2000; Izunya *et al.*, 2024).

Some of these phytochemicals, particularly flavonoids and antioxidant compounds, have been reported to exert protective effects on biological tissues through the modulation of oxidative stress and maintenance of cellular integrity (Kumar & Pandey, 2013; Panche *et al.*, 2016). However, prolonged exposure to herbal preparations containing bioactive compounds may also influence physiological functions, including renal handling of electrolytes and metabolic waste products (Ekor, 2014). Therefore, scientific evaluation of the renal effects of *I. trichantha* remains important, especially considering its repeated use in traditional medicine.

The kidney plays a central role in maintaining fluid and electrolyte balance and in the excretion of metabolic waste products and xenobiotics. Because of its high perfusion rate and active transport systems, the kidney is sensitive to physiological and biochemical alterations induced by endogenous and exogenous substances (Levey & Coresh, 2012; Hall & Guyton, 2021). Assessment of renal function in experimental studies commonly involves the evaluation of serum electrolytes, urea, and creatinine concentrations, which provide useful information regarding glomerular filtration and renal tubular function (Gowda *et al.*, 2010). Histopathological examination of renal tissue also provides valuable insight into possible structural alterations associated with exposure to medicinal plant extracts (Slaoui & Fiette, 2011).

Although several studies have documented the pharmacological activities of *I. trichantha*, information regarding its effects on renal function and electrolyte balance following repeated administration remains limited. Existing studies have focused largely on its ethnomedicinal applications and general pharmacological properties, with relatively little attention given to sub-chronic renal evaluation. Since herbal remedies are often

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consumed repeatedly over extended periods, sub-chronic studies are essential for establishing their renal safety profiles and identifying possible physiological alterations associated with long-term exposure (Ezejiofor *et al.*, 2013).

Therefore, the present study hypothesised that repeated exposure to ethanolic root extract of *I. trichantha* could influence renal histology, function and electrolyte balance in Wistar rats and so was designed to evaluate the effects of repeated-dose oral administration of ethanolic root extract of *I. trichantha* on renal function, electrolyte concentrations, and kidney histology in Wistar rats. The study assessed serum electrolyte levels (Na^+, K^+, Cl^-, HCO_3^-), urea, and creatinine concentrations alongside histopathological examination of kidney tissues in order to provide scientific evidence regarding the renal safety profile of this widely used medicinal plant.

MATERIALS AND METHODS

Plant Material Collection and Identification: Fresh roots of *I. trichantha* were obtained from Idunwele-Ewu in Esan Central Local Government Area of Edo State, Nigeria on the 22nd of November, 2024. Botanical authentication of the plant material was carried out by at the Department of Botany, Ambrose Alli University, Ekpoma, Edo State, where the specimen was examined and confirmed by Prof B. O. Obadoni, a Botany Professor then of the Department of Botany, Ambrose Alli University, Ekpoma, Edo State. A voucher specimen was subsequently prepared and deposited in the departmental herbarium under the voucher number 1046 for future reference.

Preparation of Plant Extract: The collected roots were thoroughly washed with clean water to remove adhering soil and debris and subsequently air-dried for two weeks at ambient laboratory temperature away from direct harsh sunlight with periodical turning. The dried roots were then pulverised into coarse powder using Kenwood BLK-829A heavy duty dry blender. A measured quantity of the powdered root material (408g) was macerated in 40% ethanol at a plant-to-solvent ratio of 1:5 (w/v)**.** 40% ethanol was chosen for extraction in order to mimic the extraction done by indigenous people using Seaman's Aromatic schnapps which contains 40% alcohol by volume (ABV) or 40% v/v ethanol. The mixture was kept on a mechanical shaker at room temperature for 72 hours to ensure adequate extraction of phytoconstituents. After maceration, the extract was filtered using Whatman filter paper (Olumese *et al.* 2015) to remove plant residues. The filtrate obtained was concentrated under reduced pressure of 120 mbar using a rotary evaporator at 40°C to remove the organic solvent while preserving any heat-sensitive compounds. The concentrated extract was subsequently freeze-dried using a lyophilizer under pre-freezing temperature of ≈ −40°C, primary drying temperature of ≈ −20°C and secondary drying temperature of ≈ 25°C as well as a pressure of ≈ 0.10 mbar. The freeze-drying process took a cumulative period of 168 hours resulting from several incidences of electrical power shortage. This was done to obtain the dry *I. trichantha root* ethanol extract (ITREE). The dried extract was stored in an airtight desiccator until further use. The percentage yield of the extract was determined to be 5.12%. This was calculated using the expression of Ngamkhae *et al.* (2022) as shown below:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100$$

$$= \frac{20.87g}{408g} \times 100 = 5.12\%$$

Experimental Animals: Twenty adult male Wistar rats aged approximately six weeks and weighing 150 g to 180 g were used for the study. Male Wistar rats were used exclusively in this study in order to minimise physiological variability associated with the oestrous cycle in female rats, which may influence biochemical, hormonal, and renal parameters (Westwood, 2008; Morse *et al.*, 2023). The use of male animals therefore provided a more stable experimental model for evaluating the effects of repeated administration of *I.*

trichantha root extract on renal function, electrolyte balance, and kidney histology.

The animals were procured from the Department of Physiology, Ambrose Alli University and housed in clean polypropylene cages within a well-ventilated experimental animal facility at Poiema Analytical Laboratory**.** The rats were maintained under standard laboratory conditions with a 12-hour light and dark cycle**,** ambient room temperature of 26^{o}C to 28^{o}C, relative humidity of 70% to 80% and adequate ventilation. They were provided with commercial pellet feed (Chikun feed) and clean drinking water *ad libitum* throughout the experimental period. Nutritional composition upon typical analysis of Chikun Grower Pellet feed in Nigeria shows the presence of 18-20% crude protein, ≤ 6% crude fibre, ≥ 4% crude fat, 0.9-1.1% Calcium, 0.6% Phosphorus, 0.95% Lysine and 0.45% Methionine, ≥ 8.6% moisture content, and 37 - 40% carbohydrate (HTS Farms, 2025). Prior to the commencement of the study, the animals were acclimatized to the laboratory environment for 14 days**.**

Ethical Approval: All experimental procedures involving animals were conducted in accordance with established guidelines for the care and use of laboratory animals in biomedical research (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, & National Research Council, 2010). Ethical approval for the study was obtained from the Faculty of Life Sciences Research Ethics Committee, University of Benin, Benin City, Nigeria (Approval No. FLSREC-2023-016).

Experimental Design: The study employed an experimental design to evaluate the renal safety upon sub-chronic administration of ethanolic root extract of *I. trichantha* in consideration of renal function, electrolyte balance, and kidney histology in Wistar rats. Twenty male Wistar rats were randomly assigned into four groups comprising five animals per group (n = 5) as follows:

- Group I (Control): Received distilled water
- Group II: Received 200 mg/kg body weight of ITREE
- Group III: Received 500 mg/kg body weight of ITREE
- Group IV: Received 1000 mg/kg body weight of ITREE

The selected doses were chosen based on dose ranges commonly employed in experimental studies involving medicinal plant extracts. They represent low-, medium-, and high-dose exposure levels suitable for evaluating possible dose-dependent physiological and renal effects following repeated administration. Similar dose ranges have previously been employed in experimental toxicological studies involving medicinal plant extracts in Wistar rats.

A sample size of five rats per group was adopted based on established practices in experimental toxicology and renal-function studies involving Wistar rats, where similar group sizes have been used for biochemical and histopathological evaluations (Oyelowo *et al.*, 2016; Madueke *et al.*, 2021). The selected number was also consistent with the principle of reduction in animal research ethics, which recommends the minimum number of animals necessary to obtain scientifically reliable data (Organisation for Economic Co-operation and Development (OECD), 2002; Parasuraman, 2011).

The extract was freshly reconstituted in distilled water prior to administration at appropriate concentrations of 10 mL/kg body weight to ensure uniform dosing volume across treatment groups. Animals in the control group received distilled water at a volume equivalent to that administered to extract-treated groups of 10 mL/kg body weight, a standard oral gavage volume for aqueous preparations in rats consistent with commonly recommended oral gavage volumes

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for aqueous preparations in rats (Brown *et al.* 2000; Turner *et al.* 2012; Queen's University Animal Care Services, 2013). Administration was carried out orally using a gastric gavage once daily for 28 consecutive days (OECD, 2008) between 5:00 p.m. and 6:00 p.m. to minimise possible diurnal variations. Dosing was performed sequentially beginning with the control group followed by the treatment groups in ascending order of dose.

Collection of Blood and Tissue Samples: At the end of the 28-day treatment period, the animals were fasted overnight for approximately 12 hours prior to sacrifice. The rats were euthanized by cervical dislocation performed by trained personnel in accordance with institutional animal handling procedures. Cervical dislocation was selected in order to facilitate rapid blood collection while minimising possible interference of chemical anaesthetic or euthanasia agents with serum biochemical parameters and tissue morphology (Parasuraman *et al.* 2010). The animals were then placed in dorsal recumbency for blood collection. Blood samples were obtained via **cardiac puncture from the left ventricle** using sterile syringes and transferred into appropriately labelled plain sample tubes (Opeyemi *et al.*, 2021). The blood samples were allowed to clot and were subsequently centrifuged at **3000 rpm for 10 minutes** to obtain serum, which was carefully separated for biochemical analysis. Centrifugation was carried out at an ambient laboratory temperature of approximately 28 ± 2°C using a non-refrigerated bench-top centrifuge (Model 800-1, China) with maximum speed of 4000 rpm, capacity of 20 mL × 6 tubes, and relative centrifugal force of ~1790 × g. Following blood collection, the abdominal cavity was opened, and both kidneys were carefully excised (Yi *et al.*, 2017). The harvested left and right kidneys were rinsed in normal saline to remove residual blood and were then fixed in 10% buffered formalin for histological processing (Slaoui & Fiette, 2011).

Determination of Serum Electrolytes: Serum concentrations of sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-), expressed in mmol/L, were determined using the Ion Selective Electrode (ISE) method with a CBS-50 electrolyte analyser. The principle of the method is based on the generation of an electrical potential across an ion-selective membrane when the membrane comes into contact with a solution containing the target ion. The electrical potential generated is proportional to the concentration of the specific ion present in the sample (Külpmann, 1991). Prior to sample analysis, the analyser was calibrated according to the manufacturer's operational procedure using standard electrolyte calibrators. Selected serum samples were analysed in duplicate to ensure analytical reliability and reproducibility.

Determination of Serum Urea: Serum urea concentration, expressed in mg/dL, was determined using the Urease–UV (Liquizyme) enzymatic colourimetric method with a commercially available assay kit (Spectrum Diagnostics, Egypt; Catalogue No. 319 001). The principle of the assay is based on the hydrolysis of urea by urease to produce ammonia and carbon dioxide. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH), the liberated ammonia reacts with α-ketoglutarate to form L-glutamate with simultaneous oxidation of NADH to NAD^+. The rate of decrease in NADH concentration is directly proportional to the urea concentration in the sample and was measured spectrophotometrically at 340 nm (Patton & Crouch, 1977).

Working reagent was prepared according to the manufacturer's instructions by mixing reagent 1 and reagent 2 in a 9:1 ratio. For the assay, 1 mL of working reagent was mixed with 10 µL of serum sample, corresponding to a sample-to-reagent ratio of 1:100. The reaction mixture was incubated at 37°C, and absorbance readings were obtained at 30 seconds and again after 90 seconds using a UV-visible spectrophotometer. Commercial standard and quality control sera supplied with the kit were analysed alongside test samples to

ensure analytical reliability. Selected samples were analysed in duplicate to assess reproducibility.

Determination of Serum Creatinine: Serum creatinine concentration, expressed in mg/dL, was determined using the modified kinetic Jaffe method with a commercially available assay kit. The method is based on the reaction between creatinine and picric acid in an alkaline medium to form an orange-red coloured Janovski complex. The rate of formation of this complex is directly proportional to the creatinine concentration in the sample and was measured spectrophotometrically at 500 nm (Faust & Hampson, 1974).

For the assay, the reagent and serum sample were prepared according to the manufacturer's instructions. Briefly, a specified volume of serum sample was added to the working reagent containing alkaline picrate solution, mixed thoroughly, and incubated at room temperature. Absorbance readings were obtained at defined time intervals using a UV-visible spectrophotometer at 500 nm. Creatinine concentration was subsequently calculated from the change in absorbance relative to the standard provided with the kit. Commercial standards and quality control sera were analysed alongside test samples to ensure analytical reliability, while selected samples were analysed in duplicate to assess reproducibility.

Histological Examination: Kidney tissues fixed in 10% buffered formalin were processed for histological examination using standard haematoxylin and eosin (H&E) staining procedures as described by Drury *et al.* (1967). Briefly, the tissues were dehydrated through ascending grades of ethanol comprising 70%, 80%, and 90% alcohol for 1 hour each, followed by 95% alcohol for 1 hour 30 minutes and two changes of absolute alcohol for 2 hours each. The dehydrated tissues were subsequently cleared in two changes of xylene for 1 hour 30 minutes each and infiltrated with molten paraffin wax in two successive stages for 2 hours each. The processed tissues were embedded in molten paraffin wax, allowed to solidify, and refrigerated at 5°C for 15 minutes to facilitate block hardening. Paraffin blocks were trimmed and sectioned at approximately 4 µm to 5 µm thickness using a rotary microtome. The sections were floated on a water bath maintained at approximately 55°C, mounted on clean glass slides, and dried on a hot plate for 40 minutes to ensure proper tissue adhesion.

For staining, tissue sections were deparaffinised in xylene, rehydrated through descending grades of alcohol, and stained routinely with Harris haematoxylin and eosin. The stained sections were subsequently dehydrated through ascending grades of alcohol, cleared in xylene, and mounted using dibutyl phthalate polystyrene xylene (DPX) mounting medium. Histological sections were examined under a light microscope for structural alterations in renal tissues including changes in glomeruli, renal tubules, and interstitial architecture. Photomicrographs of representative tissue sections were obtained using a digital photomicroscope at ×100 and ×400 magnifications. Histological assessment was qualitative in nature and focused on identification of observable morphological changes in renal tissue architecture.

Statistical Analysis: Data obtained from the study were expressed as Mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) post-hoc test for comparisons between treated groups and the control group. The LSD post-hoc approach was adopted due to the relatively small sample size and the exploratory nature of the study. Statistical significance was accepted at $p < 0.05$.

RESULTS

The effect of sub-chronic administration of ITREE on serum electrolyte concentrations in Wistar rats is presented in Table 1. Significant

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alterations in electrolyte levels were observed in the treated groups compared with the control group. Serum sodium (Na^+) concentration was significantly reduced in rats administered 200 mg/kg body weight (bw) and 1000 mg/kg bw of ITREE compared with the control group. No significant difference in sodium concentration was observed in the 500 mg/kg bw group. Serum potassium (K^+) concentration showed a significant decrease in all ITREE-treated groups relative to the control group. Similarly, chloride (Cl^-) concentrations were significantly lower in all treatment groups compared with the control. Serum bicarbonate (HCO_3^-) concentration also showed statistically significant decreases in the 500 mg/kg bw and 1000 mg/kg bw groups.

Table 1: Effect of administration of ITREE on serum electrolyte concentrations

Electrolyte	Group I (Control)	Group II	Group III	Group IV
Na^+ (mmol/L)	109.64±0.18	101.38±0.41*	111.21±5.41	95.79±0.78*
K^+ (mmol/L)	12.51±0.76	5.12±0.19*	0.65±0.12*	3.23±0.06*
Cl^- (mmol/L)	95.74±0.19	81.23±0.75*	85.13±1.96*	77.43±1.82*
HCO_3^- (mmol/L)	23.13±0.03	23.10±0.04	13.64±0.49*	14.62±2.14*

Values are expressed as mean ± SD (n = 5). *indicates statistically significant difference compared with control ($p < 0.05$).

The results of serum urea and creatinine assays are presented in Table 2. Serum urea concentration showed a significant decrease in rats administered 500 mg/kg bw of ITREE compared with the control group. However, no statistically significant differences were observed in the 200 mg/kg bw and 1000 mg/kg bw groups relative to the control. Serum creatinine concentration was significantly reduced in all ITREE-treated groups when compared with the control group.

Table 2: Effect of administration of ITREE on serum urea and creatinine concentrations

Parameter	Group I (Control)	Group II	Group III	Group IV
Urea (mg/dL)	61.94±2.22	60.19±1.75	58.06±0.91*	62.55±0.92
Creatinine (mg/dL)	0.44±0.23	0.12±0.06*	0.22±0.05*	0.11±0.07*

Values are expressed as mean ± SD (n = 5). *indicates statistically significant difference compared with control ($p < 0.05$).

Representative photomicrographs of kidney sections from control and ITREE-treated rats are presented in Figure 1. Histological examination revealed preserved renal architecture in both the control and treatment groups, characterised by normal glomeruli and renal tubules. However, kidney sections from rats administered 200 mg/kg bw of ITREE showed mild interstitial lymphocytic infiltration with congestion.

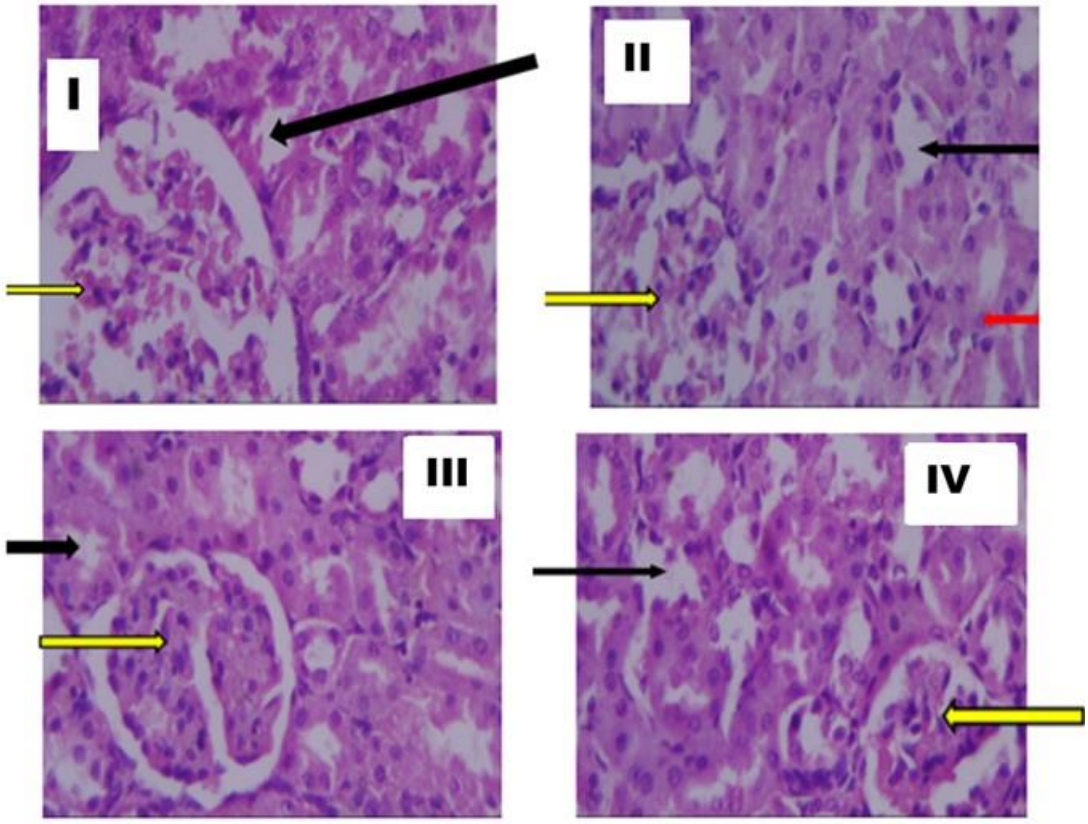

Figure 1: Photomicrographs of kidney sections from control and ITREE-treated Wistar rats stained with haematoxylin and eosin (H&E, ×400). The control and most treatment groups show preserved renal architecture. Mild interstitial lymphocytic infiltration with congestion is observed in the kidney section of the 200 mg/kg bw ITREE-treated group.

KEY:

=Normal architecture of the glomeruli (GL)

= Normal architecture tubules (TU)

= Active interstitial congestion (AC)

DISCUSSION

Sub-chronic administration of *I. trichantha* root ethanolic extract (ITREE) produced significant alterations in serum electrolyte concentrations in Wistar rats, particularly in sodium, potassium, chloride, and bicarbonate levels, while serum creatinine concentrations were reduced across treatment groups. Histological examination revealed largely preserved renal architecture in most treated groups, although mild interstitial lymphocytic infiltration with congestion was observed in the 200 mg/kg bw treatment group. Collectively, these findings suggest that repeated administration of ITREE influenced electrolyte regulation without evidence of severe structural renal damage within the 28-day study period.

The reductions observed in serum sodium, potassium, and chloride concentrations indicate that ITREE influenced electrolyte homeostasis. The kidneys play a central role in regulating electrolyte balance through selective filtration, tubular reabsorption, and excretion processes (Kamel & Halperin, 2017). Alterations in serum electrolyte concentrations may therefore reflect changes in renal handling of ions or systemic physiological responses to phytochemical exposure. The marked reduction in serum potassium concentration across all treatment groups suggests that potassium homeostasis

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was particularly sensitive to ITREE administration. Similarly, the reduction in serum chloride concentration in all treated groups and the decrease in bicarbonate concentration at 500 mg/kg bw and 1000 mg/kg bw indicate that repeated exposure to the extract affected acid–base-associated electrolyte regulation.

However, the biochemical alterations observed in this study were not accompanied by widespread structural renal damage. Histological examination showed preserved glomerular and tubular architecture in the 500 mg/kg bw and 1000 mg/kg bw groups, with only mild interstitial lymphocytic infiltration and congestion observed at 200 mg/kg bw. Importantly, tubular necrosis, glomerular degeneration, and extensive inflammatory lesions were not observed. This suggests that the electrolyte changes occurred in the absence of overt renal tissue destruction during the study duration. Similar observations have been reported in studies involving medicinal plant extracts in which biochemical alterations occurred without marked histopathological injury, particularly during short-term or exploratory exposure studies (Fuchs & Hewitt, 2011).

The inverse dose-response pattern observed in the present study deserves consideration. Mild inflammatory changes were identified in the 200 mg/kg bw group, whereas the higher dose groups demonstrated largely preserved renal histology. Non-monotonic responses of this nature have been reported in experimental toxicology and phytomedicine studies and may reflect adaptive physiological responses, differential phytochemical bioavailability, hormetic effects, or variability associated with small experimental sample sizes (Calabrese & Baldwin, 2003). Since the inflammatory changes observed were mild and not progressive with increasing dose, the findings do not support a clear dose-dependent nephrotoxic effect of ITREE under the conditions of the present study.

The significant reduction in serum creatinine concentration across all treatment groups is notable because elevations in serum creatinine are more commonly associated with impaired renal filtration function (Levey *et al.*, 2014). In the present study, creatinine concentrations decreased rather than increased following ITREE administration. Although serum creatinine alone cannot be used to directly estimate glomerular filtration rate without creatinine clearance measurements, the absence of creatinine elevation suggests that severe impairment of renal filtration did not occur during the exposure period. Similar reductions in serum creatinine have been reported in experimental studies involving plant-derived extracts and have been interpreted as evidence of preserved renal excretory function or altered creatinine metabolism rather than renal injury (Perrone *et al.*, 1992; Delanaye *et al.*, 2017).

Likewise, serum urea concentration remained relatively stable across most treatment groups, with a modest but significant reduction observed only at 500 mg/kg bw. Elevated serum urea is typically associated with impaired renal excretion or increased protein catabolism (Burtis & Bruns, 2015). Therefore, the absence of increased urea concentrations further supports the observation that ITREE administration did not produce marked impairment of renal excretory function within the duration of this study.

The renal effects observed in the present study may be related to the phytochemical constituents previously reported in medicinal plants, including alkaloids, diterpenoids, flavonoids, and saponins (Hostettmann *et al.*, 2000). Some phytochemicals, particularly flavonoids and phenolic compounds, have been reported to modulate oxidative balance, membrane permeability, and cellular physiological processes in biological tissues (Kumar & Pandey, 2013; Panche *et al.*, 2016).

In addition, several plant-derived flavonoids possess antioxidant and anti-inflammatory properties that may contribute to preservation of tissue architecture despite biochemical alterations. The preserved histological appearance observed in most treatment groups

may therefore reflect an absence of marked renal structural injury during the exposure period. This study possesses some limitations that should be considered during interpretation of the findings. The sample size was relatively small, and the duration of exposure was limited to 28 days. Furthermore, assessment of oxidative stress biomarkers, urinalysis, renal clearance indices, and mechanistic molecular markers was not performed. Such investigations would provide a more comprehensive understanding of the renal effects and physiological mechanisms associated with prolonged ITREE exposure. Additional chronic toxicity studies involving larger sample sizes, assay of specific biomarkers such as KIM-1, NGAL, cystatin C and extended exposure periods are therefore recommended.

Overall, the findings from the present study indicate that sub-chronic administration of ethanolic root extract of *I. trichantha* altered serum electrolyte concentrations in Wistar rats while largely preserving renal histological architecture. The observed biochemical alterations were not associated with progressive dose-dependent renal lesions or marked increases in conventional renal injury biomarkers within the duration of the study, suggesting mild functional effects on electrolyte regulation without overt renal structural damage. Given the mild electrolyte alterations and limited histological changes observed at 200 mg/kg bw, further studies involving chronic exposure, larger sample sizes, and more sensitive renal biomarkers such as cystatin C, urinary indices, and oxidative stress parameters are recommended to further clarify the long-term renal effects of ITREE. In addition, detailed phytochemical characterisation and mechanistic investigations are necessary to identify the specific bioactive constituents responsible for the observed renal effects. Caution is advised in the prolonged or unregulated traditional use of *I. trichantha* until more comprehensive chronic safety data become available.

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Declaration of Conflict of Interest

The authors declare no conflicts of interest

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