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Toxicology of the renal indices of Wistar rats induced with leaf extracts of *Acalypha wilkesiana*

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Abstract

Acalypha wilkesiana has been used in time past to treat various ailments that were not limited to skin infections, inflammation, or stomach ache. However, the safety and toxicity of the plant cannot be over emphasized. This study aimed to assess the toxicological effects on renal indices in Wistar rats induced with *A. wilkesiana* leaf, extracted with aqueous and methanol solvents using maceration method. Lorke's method was employed to determine oral toxicity (LD_{50}) of both extracts. Sixty-five rats (150 - 180 g) were divided into seven groups of five rats each for toxicity study while thirty-five rats were placed in four groups of five rats each for biochemical analysis: the control designated group 1, were not given extract, groups 2, 3, and 4, were orally feed different doses of the leaf extracts each at 1000, 1500, and 2000 mg/kg body weight, respectively. The experiment lasted for 28 days and blood recovered for analysis. Changes in body weight and kidney biochemical parameters were tracked as markers of toxicity. The results showed no signs of toxicity, significant changes in body weight, or death in any of the treated groups. Rats given leaf extracts had no discernible negative effects, as seen by changes in plasma non-protein nitrogen and electrolytes. This suggests that *A. wilkesiana* is relatively safe at the tested doses but may have influence on renal function. However, no significant adverse effects were observed in the treated groups. Further studies are necessary to reveal any adverse effects in renal indices.

Keywords: *Acalypha wilkesiana*, Electrolyte, Renal Indices, Toxicity, Wistar rats

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INTRODUCTION

The rationale behind medicinal plants is that as natural products, they are safe for discretional uses. The ecosystem is full of wide arrears of plants which may be useful for the identification of pharmaceutical lead compounds and drug discovery (Harvey *et al.,* 2015). In traditional medicine, there is no standardized dosage or precise quantity of material administered to patients, and the nature or type of active ingredients in the mixtures is often unknown. This increases the risk of administering toxic doses (Musa *et al*., 2005).

Drug discovery is a complex and intricate process, requiring not only information about the pharmacodynamics and pharmacokinetics of a compound, but also emphasizing the critical importance of its safety (Thomford *et al.*, 2018). *Acalypha wilkesiana* (Figure 1), a member of the Euphorbiaceae family, is commonly known by various names, including copper leaf, fire dragon, beefsteak plant, and Jacob's coat (Riley, 1963). The plant is widely distributed across tropical regions of Africa, America, and Asia (Ogundaini, 2005).

Figure 1: *Acalypha wilkesiana*

Acute toxicity studies are crucial, as scientific validation of drugs by medical practitioners is essential, and drug regulatory authorities require that all potential drugs undergo thorough research and evaluation (Abdulrahman, 2004). Sub-acute toxicity tests are performed to assess a new drug's potential side effects after a 2-4-week therapy term. To choose the dosage levels to be employed in later sub-chronic and chronic toxicity tests, range-finding sub-acute toxicity experiments are carried out. Additionally, preliminary clinical trials with treatment periods of up to 4 weeks may be supported by sub-acute toxicity investigations. These investigations are intended to evaluate the development and remission of drug-induced lesions, but they typically last too little time to fully characterize all potential side effects that can manifest during prolonged clinical usage or during chronic toxicity and carcinogenicity testing (Colerangle 2017). The adult human kidneys are positioned on the left and right outside the peritoneal cavity. They measure approximately 12 centimeters in length (Mescher, 2016). Their high susceptibility to xenobiotics is likely due to the large volume of circulating blood (about 25% of cardiac output) that reaches the kidneys, carrying xenobiotics or their substances for filtration. Additionally, the role of nephrons in urine formation increases the concentration of toxic substances in the tubular fluid of the kidney (Schnellman, 2008). While ongoing efforts aim to identify specific urinary biomarkers to assess the effects of potential drug candidates on the kidneys and monitor renal function—with some progress made—the U.S. Food and Drug Administration continues to recommend using blood urea nitrogen and serum creatinine in rats as reliable indicators of kidney toxicity. (Dieterle *et al.,* 2010). The leaves of *A. wilkesiana* contain secondary metabolites used for various ailments and Some researches suggests that *Acalypha* species may be non-toxic and safe at relatvely low doses (Olukunle *et al.,* 2015; Omage *et al.,* 2017; Olubodun *et al.,* 2021a). However, very little consideration has been given to the safety of the plant in relation to kidney functions. The experiment sought to investigate the safety of aqueous and methanol leaf extracts of *A. wilkesiana* in Wistar rats by assaying for changes in weight and some basal biochemical parameters.

MATERIALS AND METHODS

Plant materials

The leaves of *A. wilkesiana* were gathered from gardens inside and outside of Benin City. The leaves were verified at the University of Benin's Department of Plant Biology and Biotechnology in Benin City. The leaves were carefully chosen to remove any unwanted components, air dry at room temperature, grind into a fine powder, and then weighed in order to prepare them for future usage.

Preparation of methanol extract

Three hundred grams (300g) of the pulverized *A. wilkesiana* leaves were soaked in 95% methanol for 72 hours (3 days). To guarantee complete mixing, the mixture was periodically agitated with a magnetic stirrer throughout this time. A sintered funnel, which is similar to employing four folds of cheesecloth or gauze, was then used to filter the homogenate. The resulting filtrate (extract) was then concentrated using a rotary evaporator and subsequently weighed.

Preparation of aqueous extract

Following a 72-hour (3-day) soak in distilled water, the 300g of ground leaves were processed as previously mentioned for methanol extract (Omage and Azeke 2014).

Experimental rats

A total of sixty-five adult male Wistar rats weighing between 150g to 180g were used for this study. The experimental rats were obtained from a local breeder within Benin City metropolis. The rats were kept in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-hour light and dark cycle in clean disinfected cages. They were allowed free access to feed (standard pelleted growers feed from Vital Feed, Benin City, Edo State) and water ad libitum. Before the commencement of the study, the rats were acclimatized for a period of one week. The use of rats for the study was according to the ethical guidelines involving whole animal testing of the Animal Ethics Committee, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. After one week of acclimatization, the rats were divided into seven groups of five rats each for the phase 1 stage of acute toxicity study. The first three groups of the rats each were given the aqueous extracts, while the next three groups were given the same dose of the methanol extracts of *A. wilkesiana.* The last group (control) was given only feed and water.The doses given for the phase one stage are as follows: group one, 10mg/kg of body weight of aqueous and methanol extracts, group two, 100mg/kg of body weight for aqueous and methanol extracts and the third group, 1000mg/kg of body weight for aqueous and methanol extract. The fourth group was the control.For phase two of the acute toxicity studies. The first three groups of five rats each were given the aqueous extracts, while the next three groups were given the same dose of the methanol leaf extracts of *A. wilkesiana.* The last group was given only feed and water as the control. The different doses given to the rats for the phase two stage are as follows: group one 1600mg/kg of body weight of aqueous and methanol extract. Group two, 2900mg/kg of body weight for aqueous and methanol extract and the third group was given 5000mg/kg of body weight for aqueous and methanol extract. The fourth group was the control. For sub-acute toxicity study, the rats were then randomized with group 1 having 3 rats and groups 2-4 having 5 rats each for the aqueous and methanol sub-groups respectively.

Preparation and dosage regimen of *Acalypha wilkesiana* extracts

Doses were calculated and prepared daily before administration in dimethyl sulfone ($DMSO_2$).

Acute toxicity using Lorke's method

Lorke (1983) method with slight modifications, was adopted for acute toxicity (LD_{50}) study. Thirty-five rats were divided into seven groups of five rats each. Methanol and aqueous extracts of the plant was feed to the rats in groups 2 - 7 in doses (10, 100, 1,000, 1,600, 2900, and 5,000 mg/kg) according to their body weight. Control/Group (1) received distilled water. The rats were allowed to feed ad libitum, and observed regularly for two days (48 hours) for any changes that may occur physically or in behaviour, in coordination and/or mortality.

Sub-acute toxicity using Lorke's method

The methanol and aqueous extracts of the plant was delivered to thirty (30) rats in groups 2 - 7 comprising of five rats each, in doses of 1,000, 1,500, and 2,000 mg/kg body weight of both the aqueous and methanol extracts for 28 days and allowed to feed ad libitum. Control/group (1), made of five (5) rats, were given distilled water for 28 days.

Administration of extracts

A gavage was used to give the extracts, serving as an oro-gastric tube. Extreme caution was used

to avoid causing the rats any harm to their mouths or oesophagus.

Sub-chronic toxicity assessment

During the investigation, the extracts were given to the rats in estimated doses depending on their body weight (equivalent volume). For the sub-chronic studies, these dose calculations were performed weekly, taking into account the rats' changing weights. The weight of the five rats in each group was recorded weekly on day 0, day 7, day 14, day 21, and day 28 to ensure accurate dosing throughout the experiment. Untreated rats (group 1) served as the control and were administered 2 ml of distilled water (Pongri and Igbe, 2017).

Observations in sub-chronic assessment (clinical signs and mortality)

The rats were closely observed for indicators of weaknesses, alterations in appetite (boosted or diminished), weight loss, and other physiologic abnormalities, including mortality. Clinical observations were made at specific intervals: before dosing, immediately after dosing, and 4 hours post-dosing. These assessments included monitoring restlessness, changes in stool consistency, urine, and eye color, presence of worms in excretion, diarrhea, haematuria, and uncoordinated muscle movements, among other indicators of health and toxicity. The animals were observed for toxic symptoms such as weakness or aggressiveness, anorexia, weight loss, diarrhoea, discharge from the eyes and ears, stridor, and mortality (Suanarunsawat *et al*, 2009).

Protocol for sub-chronic toxicity studies

The extracts of the leaves were administered at increasing doses from 1000 mg/kg (group 2), 1500 mg/kg (group 3) and 2000 mg/kg (group 4). Distilled water (No extract) was given to the group 1 rats, which acted as the control. There were five (5) rats in each group.

Sacrifice of the rats

Before the animals were sacrificed, they were deprived of food and water, and their body weights were documented. The rats were anesthetized with chloroform and before they were sacrificed, blood samples were collected by cardiac puncture into labeled bottles. The abdominal cavities were opened, and the kidneys were carefully harvested and placed in plain sample containers containing a normal saline solution prepared with a phosphate buffer at pH 7.0. The kidney tissues were then homogenized in the phosphate buffer (pH 7.0) at a ratio of 0.5g of tissue per 5ml, using a mortar and pestle. The homogenates were centrifuged at 10,000g for five minutes, and the supernatant was collected and stored in a freezer for subsequent biochemical analyses.

Biochemical analysis

Plasma electrolyte, urea, and creatinine determination

The rats were weighed and put down at the end of the 28-day experimental treatment. Sterile syringes were used to draw blood samples, which were then placed in heparinized sample vials for biochemical analysis. Potassium and sodium levels were measured using a flame photometer (Eppendorf Flame Photometer AFM 5051, Germany), following the method outlined by Tietz (1987). Chloride concentration was determined using the method of Skeggs and Hochstrasser (1964), while bicarbonate levels were measured according to Van-Slyke et al. (1925). Urea was determined using the Urease Berthelot method described by Weatherburn (1967). Creatinine was measured according to the protocol by Bartels and Bohmer (1972). Chloride, bicarbonate, urea, creatinine, and protein assays were conducted using standard assay kits from Randox® (Randox Laboratory, UK).

Statistical analysis

The data were expressed as the mean ± standard error of the mean (± SEM) and analyzed using Duncan's multiple range test for analysis of variance (ANOVA). The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS®), Version 21.0, IBM Corp., Armonk, NY, USA. Values of $p < 0.05$ were considered significant.

RESULTS

Acute toxicity

The acute toxicity studies of the aqueous and methanol leaf extracts in the rats revealed in an earlier report that the LD_{50} was greater than 5000 mg/kg (Olubodun *et al*, 2023). Both extracts were safe at 5,000 mg/kg body weight and there was no mortality.

Sub-acute toxicity

Body weight and percentage change

The body weight of rats treated with different doses of *A. wilkesiana* are shown in Table 1 and Table 2 shows the effects of aqueous and methanol leaf extracts of *A. wilkesiana* on percentage change in body weight of the rats. Daily administration of aqueous and methanol leaf extracts of *A. wilkesiana* at 1,000, 1,500 and 2,000 mg/kg body weight did not result in significant differences in the body weight of the rats after 28 days when compared with the control. However, as time went on, the percentage change in body weight increased in the groups given 1,000 mg/kg leaf extracts when compared with other groups given higher doses.

Table 1. Effects of aqueous and methanol leaf extracts of *Acalypha wilkesiana* on body weights of normal Wistar rats

Assays	Groups	Mean body weights of rats (g)				
		Day 1	Day 7	Day 14	Day 21	Day 28
Aqueous extracts						
Control	1	158.44±1.70	160.92±1.17	165.05±1.74	167.84±2.50	170.27±1.90
1, 000mg/kg AE	2	171.50±1.76	171.96±1.69	173.27±1.32	174.51±3.30	175.65±5.22
1, 500mg/kg AE	3	165.60±1.63	164.40±0.96	165.65±1.19	167.18±1.91	167.65±1.43
2, 000mg/kg AE	4	173.84±1.60	174.33±1.81	174.39±1.50	174.56±1.13	176.11±1.51
Methanol extracts						
Control	1	158.44±1.70	160.92±1.17	165.05±1.74	167.84±2.50	170.27±1.90
1, 000mg/kg ME	5	177.00±1.59	178.40±1.60	178.65±0.91	179.65±0.39	183.11±0.63
1, 500mg/kg ME	6	174.16±1.61	174.81±1.43	175.84±2.10	176.42±0.25	177.09±0.37
2, 000mg/kg ME	7	171.81±0.52	171.40±5.44	173.16±3.30	174.62±5.50	174.42±8.25

AE: Aqueous leaf extract, ME: Methanol leaf extract, Body weights of rats are represented as means ± SEM of five replicates.

Table 2. Percentage change in body weight in normal Wister rats treated with aqueous and methanol leaf extracts of *Acalypha wilkesiana*

Assays	Groups	Initial body weight (g)	Final body weight (g)	Change in body weight (%)
Aqueous Extracts				
Control	1	158.44±1.70	170.27±1.90	7.47
1, 000mg/kg AE	2	171.50±1.76	175.65±5.22	2.52
1, 500mg/kg AE	3	165.60±1.63	167.65±1.43	1.24
2, 000mg/kg AE	4	173.84±1.60	176.11±1.51	1.31
Methanol Extracts				
Control	1	158.44±1.70	170.27±1.90	7.47
1, 000mg/kg ME	5	177.00±1.59	183.11±0.63	3.45
1, 500mg/kg ME	6	174.16±1.61	177.09±0.37	1.68
2, 000mg/kg ME	7	171.81±0.52	174.42±8.25	1.50

AE: Aqueous leaf extract, ME: Methanol leaf extract, Body weights of rats are expressed as means ± SEM of five replicates.

Electrolytes study

The results of the electrolytes of Wistar rats treated with aqueous leaf extracts of *A. wilkesiana* is shown in Table 3**.** The plasma sodium (Na^+) increased in group 2 with decreases in groups 3 and 4 whose decrease was significant ($p < 0.05$). Plasma potassium (K^+) level decreased but a non-significant ($p > 0.05$) increase in group 4 relative to the control value was observed. Chloride ion increased non-significantly but decreased significantly in group 4, while bicarbonate ion increased significantly in group 2, there was significant ($p < 0.05$) decrease in group 4 when compared with the control values (group 1) (Table 3).

Table 4 shows the results of the electrolytes of Wistar rats treated with methanol leaf extracts of *A. wilkesiana***.** The plasma sodium (Na^+) level in group 2 revealed a significant decrease while groups 3 and 4 reflected non-significant increases. Plasma potassium (K^+) and bicarbonate levels revealed non-significant increases except in group 4 where bicarbonate level recorded a significant increase. Chloride ion recorded significant increase in group 2 and significant decrease in group 4 when compared with control values (group 1) (Table 4).

Urea, creatinine and tissue protein study

Tables 3 and 4 shows the effects of aqueous and methanol leaf extracts of *A. wilkesiana* on urea, creatinine and tissue protein of Wister rats. The results showed that plasma urea level decreased in all the groups in both extracts when compared with control ($p > 0.05$). Plasma creatinine showed varied pattern in both extracts. While plasma creatinine showed significant reduction in aqueous extracts (Table 5), plasma creatinine in methanol leaf extracts showed non-significant increases in the rats when compared with control values (Table 6). Generally, the extracts results showed that both extracts significantly influenced a decrease in the rats' plasma urea/creatinine ratio, particularly at the maximum dose.

Table 3. Electrolytes of Wistar rats treated with aqueous extracts of *A. wilkesiana*

Groups	Na^+ (mmol/L)[10]	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (No extract)	43.17 ± 0.96^a	4.24 ± 0.11	12.36 ± 0.90^a	3.66 ± 0.50^a
Group 2 (1000 mg/kg)	44.70 ± 2.72	3.96 ± 0.19	12.47 ± 6.00	4.20 ± 3.00^b
Group 3 (1500 mg/kg)	38.57 ± 1.38^b	3.90 ± 0.29	12.69 ± 4.00	3.97 ± 2.00
Group 4 (2000 mg/kg)	40.98 ± 2.20	4.47 ± 0.01	11.36 ± 3.00^b	3.20 ± 2.00^c

Values are represented as mean ±SEM (n=5). Means with different superscripts are significantly different ($p < 0.05$) down the column by one-way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 4. Electrolytes of Wistar rats treated with methanol extracts of *A. wilkesiana*

Groups	Na^+ (mmol/L)[10]	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (No extract)	43.17 ± 0.96^a	4.23 ± 0.11	12.36 ± 0.90^a	3.66 ± 0.50^a
Group 2 (1000 mg/kg)	37.31 ± 0.36^b	4.92 ± 0.04	14.08 ± 2.00^b	4.80 ± 1.00
Group 3 (1500 mg/kg)	43.51 ± 1.68	4.80 ± 0.10	12.67 ± 4.00	4.73 ± 2.00
Group 4 (2000 mg/kg)	43.96 ± 0.71	4.05 ± 0.23	11.97 ± 6.00^c	4.83 ± 1.00^b

Values are represented as mean ± SEM (n=5). Means with different superscripts are significantly different ($p < 0.05$) down the column by one-way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 5. Urea, creatinine and tissue protein of rats treated with aqueous leaf extracts of *A. wilkesiana*

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (No extract)	42.75 ± 0.37^a	0.96 ± 0.53^a	1.83 ± 0.08^a	44.53 ± 0.45^a
Group 2 (1000 mg/kg)	34.88 ± 0.29^b	0.91 ± 0.79^b	1.85 ± 0.04	38.33 ± 0.54^b
Group 3 (1500 mg/kg)	26.25 ± 0.84^c	0.97 ± 0.54	1.89 ± 0.05^b	27.06 ± 0.69^c
Group 4 (2000 mg/kg)	15.96 ± 0.18^d	0.90 ± 0.45^b	1.82 ± 0.02	17.73 ± 0.32^d

Values are represented as mean ±SEM (n=5). Means with different superscripts are significantly different ($p < 0.05$) down the column by one-way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 6. Urea, Creatinine and Tissue Protein of rats treated with methanol leaf extracts of *A. wilkesiana*

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (No extract)	42.75 ± 0.37[a]	0.96 ± 0.53	1.83±0.08[a]	44.53 ± 0.45[a]
Group 2 (1000 mg/kg)	19.92 ± 0.25[b]	1.05 ± 0.24	1.88±0.06	18.96 ± 0.25[b]
Group 3 (1500 mg/kg)	19.96 ± 0.25[b]	1.05 ± 0.18	1.95±0.06[b]	19.01 ± 0.22[b]
Group 4 (2000 mg/kg)	32.42 ± 1.61[c]	0.96 ± 0.39	1.92±0.05[b]	33.77 ± 1.00[c]

Values are represented as mean ±SEM (n=5). Means with different superscripts are significantly different ($p < 0.05$) down the column by one-way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

DISCUSSION

The acute toxicity study of the aqueous and methanol leaf extracts of *Acalypha wilkesiana* on normal Wistar rats, as previously reported by Olubodun *et al*. (2023), revealed an LD50 value greater than 5000 mg/kg. However, Olukunle *et al.,* (2015) reported LD_{50} less than 5000 mg/kg for aqueous extracts of the same plant species. This variation may be due to species or seasonal variation. The toxicity tests showed that the rats did not exhibit any noticeable symptoms of toxicity, including decreased feeding, water intake, shivering, hair erection, respiratory problems, or decreased mobility. The rats' behaviour, physical appearance, and overall activity remained unchanged after administration of either the aqueous or methanol leaf extracts, and they continued to move normally in their cages. The study recorded that the plant may be considered non-toxic since no observable deleterious effects were preceded by clinical signs of toxicity of physical or/and behavioural changes in the rats (Olubodun *et al.*, 2023).

Chemical toxicology has been shown to induce changes in organisms. Body weight changes are general indicators of toxicity (Olubodun *et al*, 2021b; Olubodun *et al*, 2023). The relatively non-significant increase in body weight observed in the treated groups may be attributed to the minerals or electrolytes present in the extracts which may have improved the absorption of food and water (Okpo and Amoo, 2016; Olubodun *et al*, 2021b, Olubodun *et al*, 2023).

Groups 2 - 7 rats recorded non-significant increase of 2.52, 1.24, 1.31, 3.45, 1.68 and 1.50% respectively with control having the highest percentage increase of 7.47%. The percentage change in body weight that each rat showed (treated and untreated) is expected since they were given food ad libitum. However, the nutrients present in the extracts may have contributed to the non-significant increase observed in the treated groups when compared to control. The study agrees with Iserhienrhien and Okolie who reported no significant increase in body weight when leaf extracts of *Geophila obvallata* was used to treat Wister rats (Iserhienrhien and Okolie, 2020) but is at variance with Olubodun *et al*, (2021b) and Kinuthia *et al*, (2023) who reported significant increases in body and organ weight of rats treated with other plant species. The reason for the discrepancies may be because of the higher doses used and the length of time the extracts were administered.

Electrolytes are necessary for many bodily functions, such as the creation and conductivity of action potentials in the nerves and muscles and the preservation of electrical neutrality in cells. Abnormality in the body's electrolyte content may be caused by imbalance in electrolyte. Electrolyte imbalances can occur when blood levels are either too high or too low, depending on the situation (Hew-Butler *et al.,* 2017). Electrolyte abnormalities frequently result from excessive heat-related dehydration, vomiting, or diarrhea. The relative increase and decrease observed in the electrolyte levels of the rats treated when compared to the group 1 rats may be a result of methanol and aqueous extracts. Agu and Olubodun (2023), also observed the fluctuations in the electrolyte levels in rats treated with different parts of *Annona muricata* (Agu and Olubodun, 2023).

The elemental analysis of the *A. wilkesiana* aqueous extract revealed the presence of sodium, potassium, and calcium, which has an impact on cardiac output (Madziga *et al*., 2010). The kidneys are responsible for excreting the majority of potassium from the body (Tintinalli *et al.,* 2016) and they play critical role in preserving a healthy potassium balance in the blood. The increased potassium observed in the study may be as a result of the extracts ingested since food can contribute to high potassium in the blood (Walls *et al.,* 2018).

Undoubtedly sodium is one of the key electrolytes in the extracellular fluid, functioning as an osmotically active cation. It plays a crucial role in maintaining extracellular fluid volume and regulating cell membrane potential. Sodium and potassium are actively transported across cell membranes as part of active transport processes (Ferrannini, 2017). The kidneys regulate sodium levels through reabsorption, with aldosterone controlling sodium-chloride symporters responsible for salt transport (Palmer and Schnermann, 2015; Tintinalli *et al*., 2016).

Treatment with the methanol extract did not significantly affect plasma sodium levels compared to the control group ($p > 0.05$). However, at a dosage of 1000 mg extract per kg of body weight, there was a significant reduction in plasma sodium ion concentration compared to the control ($p > 0.05$). The fluctuations in plasma sodium and potassium levels in the treated rats, though not significant ($p > 0.05$), may be related to the effects of the ingested extracts and kidney function. Hew-Butler *et al*., (2017) noted that electrolyte imbalances may occur due to decreased kidney excretion, shifts of electrolytes into the extracellular space, or increased ingestion of electrolyte-rich foods, depending on the situation (Hew-Butler *et al.*, 2017; Walls *et al.*, 2018).

The decrease observed in plasma urea level in this study is at variance with increase urea level reported by Olukunle *et al*., (2015). Plasma urea and creatinine levels are critical biomarkers for evaluating renal function, with elevated levels often indicating renal dysfunction (Agu and Olubodun, 2023). Increases may also signal dehydration, leading to hemoconcentration, or be related to post-nutritional status following a high-protein diet. Toxicological assessments, therefore, examine the effects of plant extracts, natural plant compounds, and drugs on kidney biomarkers and renal function (El-Khasmi and Farh, 2022). Under normal conditions, these metabolic products remain at stable levels (Li *et al.*, 2022), and renal impairment reflect their decrease or increase (El-Khasmi and Farh, 2022). An increase in serum urea levels can indicate dehydration, electrolyte imbalance, hypoalbuminuria, nephropathy, and tissue catabolism (Li *et al*., 2022). However, since there was no significant variation in these parameters among the experimental rats exposed to the aqueous and methanol leaf extracts relative to controls, a relative normal kidney function was proposed.

CONCLUSION

The study results indicated changes in plasma electrolytes and non-protein nitrogen compounds, suggesting that *Acalypha wilkesiana* exhibits relatively safe toxicity at the administered doses but may impact renal functions. However, no significant adverse effects were observed in the rats treated with either the aqueous or methanol leaf extracts of *A. wilkesiana*. Further studies are necessary to reveal any adverse effects in renal indices.

Conflict of Interest

Authors have no conflict of interest to declare.

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Author contribution

OSO designed and supervised the study. IPE, HEU, OTI, IJA performed the experiments. IPE wrote part of the manuscript, while OSO completed the write up, proofread and prepared it for publication.

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