Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 22 No.3; pp. 2420-2431 (2024). ISSN (print):1596-7409; eISSN (online):2705-3822

Kidney function assessment in wistar rats: The role of *Dalium guineense* and *Annona muricata* in modulating water balance, urinary biomarkers, and kidney histology

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Abstract

This study assessed the renal effects of *Dialium guineense* and *Annona muricata* extracts on female Wistar rats, exploring their potential roles in kidney health. Given limited research on these extracts' impacts on kidney biomarkers and tissue structure, this study investigated water balance, renal biomarkers, and kidney histology after 30 days of treatment. Rats were divided into four groups: *Dialium guineense* (100 mg/kg), *Annona muricata* (100 mg/kg), a combination (50 mg/kg each), and a control. Following 30 days of exposure, water and urine output were measured, with urine samples analyzed both qualitatively and quantitatively for protein, creatinine, and protein/creatinine ratios. Blood samples were collected, and kidney tissue was obtained following standard methods for histological examination. Results showed an increased urinary protein across all treated groups, with *Dialium guineense* showing the highest levels. Urinary creatinine levels decreased, especially in the *Dialium guineense* group, leading to elevated protein-creatinine ratios in both *Dialium guineense* and *Annona muricata* groups. Blood analysis showed slight but non-significant increases in uric acid and magnesium, a slight calcium increases in the Annona muricata group, and a significant sodium decrease in the combination treatment group. Histological examination revealed intact kidney structure, indicating no nephrotoxicity from the treatments. These findings suggest that, while the extracts affect specific renal biomarkers, they do not compromise kidney structure, supporting their potential as safe options in traditional medicine. Further studies are recommended to explore their long-term safety and mechanisms of action.

Keywords: *Annona muricata*, *Dalium guineense*, Renal impact, Urinary biomarkers, Water balance

Received August 27, 2024; **Revised** October 30 2024; **Accepted** November 4, 2024

https://dx.doi.org/10.4314/br.v22i3.1
Journal Homepage: https://www.bio-research.com.ng

Publisher*: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria*.

INTRODUCTION

Kidneys are critical to maintaining physiological balance, regulating electrolytes, fluid, and acid-base homeostasis, and excreting metabolic wastes (Smith *et al*., 1951). The growing prevalence of chronic kidney disease (CKD) has brought global attention to the limitations of conventional treatments, which primarily include pharmacological interventions like ACE inhibitors and ARBs. These treatments often incur high costs, carry side effects, and may not halt disease progression, creating an urgent need for alternative or complementary therapies (Brenner & Rector, 2011). Consequently, there is a significant interest in plant-based therapies with nephroprotective properties, such as *Dialium guineense* and *Annona muricata* (Bork & Schumann, 2010). Extensive research has documented the pharmacological potential of *Annona muricata* (soursop) in renal protection. Studies have demonstrated that *A. muricata* exhibits protective effects in conditions involving renal toxicity, hyperglycemia, and oxidative stress. The plant's nephroprotective actions are largely attributed to its antioxidant-rich profile, particularly acetogenins, alkaloids, and flavonoids, which mitigate oxidative stress and inflammation (Moghadamtousi *et al*., 2015; Hamizah *et al*., 2017). Despite these findings, much of the literature has focused on *A. muricata*'s general antioxidant and anti-inflammatory effects rather than on specific kidney functions. Few studies, for instance, have assessed parameters like water balance or urinary biomarkers, which are crucial indicators of kidney function. These gaps underscore the need to examine *A. muricata*'s impact on renal physiology in a more comprehensive manner, including parameters such as electrolyte balance and structural integrity of kidney tissues.

Dialium guineense, known as Velvet Tamarind, has been widely used in West African traditional medicine for various therapeutic purposes, including digestive health, fever reduction, and microbial infections (Adekunle & Oyesola, 2017). While the plant's rich antioxidant content, such as phenolic compounds and flavonoids, suggests potential protective effects against oxidative damage, research specifically targeting renal health is scarce (El-Sayed & Ibrahim, 2019). Limited studies have evaluated *D. guineense*'s impact on specific renal parameters, leaving a gap in understanding how it might influence kidney functions beyond its known antioxidative properties. Therefore, while traditional uses suggest kidney-protective potential, there is limited evidence regarding its effects on urinary biomarkers, electrolyte regulation, or histological integrity. Although both *A. muricata* and *D. guineense* show promising nephroprotective effects, there remains a lack of comprehensive studies that systematically evaluate their impact on kidney function parameters within an in vivo model. Prior research has largely focused on their roles in mitigating oxidative stress and inflammation but has not extended to a detailed examination of kidney function parameters such as water consumption, urinary protein, creatinine levels, serum electrolytes, or kidney histology. Since these factors are early indicators of renal dysfunction, assessing them is essential to fully understand the therapeutic potential of *A. muricata* and *D. guineense* in kidney health.

To address these gaps, this study specifically examines the effects of *D. guineense* and *A. muricata* on critical renal parameters, including water balance, urinary biomarkers, and structural tissue integrity, using a Wistar rat model. Although this study does not explore specific biochemical mechanisms, it provides valuable groundwork by documenting functional impacts that can be expanded in future studies to investigate detailed pathways, such as antioxidative or anti-inflammatory actions, through which these plants may exert renal effects.

MATERIALS AND METHODS

Collection, identification and laboratory analysis of plant

Fresh leaves of *Dialium guineense* (black velvet) and *Annona muricata* (soursop) were collected from the junior staff quarters of the University of Benin and Upper Sakponba, Benin City, respectively. The taxonomic identities of the plants were confirmed by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. The phytochemical constituents of the leaf powders were identified using standard procedures as outlined by Sofowora (1982), Trease and Evans (1989), Kokate *et al*. (2008), and Harborne (1988). Additionally, a proximate analysis to determine the ash content, crude fiber, crude protein, fat, dry matter, moisture content, and nitrogen-free extract of the leaf

powders was conducted following the methods described by Olomu (2011).

Preparation of Leaf Extract

Plate 1: Fresh leaves of *Anonna muricata*

The fresh leaves were dried in the laboratory at a temperature of approximately 30 ± 2°C until they became crisp, a process that took two weeks. Following drying, the leaves were ground into a coarse powder using a pestle and mortar and then finely pulverized with a Viking Exclusive Joncod machine (Model: YLH2M2-4). A total of 30 grams of the powdered leaves from each plant was extracted with 200 mL of water for 48 hours. The extract was filtered using sterile Whatman paper No. 1, and the filtrate was dried to a solid form with a freeze dryer. This dried extract was then dissolved in distilled water to obtain the required concentrations for the study. Previous research by Akinmoladun *et al*. (2007) and Ibrahim *et al*. (2011) has shown that the LD50 of *Dialium guineense* and *Annona muricata* leaf extracts is greater than 2000 mg/kg in rats, suggesting a low level of acute toxicity. These results informed the selection of an appropriate dosage for this study.

Collection and acclimatization of experimental rats

All experimental animals were conducted in accordance with standard guidelines (Council for International Organizations of Medical Sciences (CIOMS), 2008) on use of animals for experimental toxicology study. Male and female Wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. They were acclimatized for 2 weeks until they were 8-9 weeks and their weights taken. The animals were housed (males and females) in

The leaf extracts of *Dialium guineense* (black velvet tree) and *Annona muricata* (soursop) were utilized in this study.

Plate 2: Fresh leaves of *Dalium guineense*

wooden cages with wire mess covers. The animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water *ad labium*. After acclimatization, the rats were distributed randomly into four groups and labelled as described below. The rats were administered different treatment protocol as stated below.

Group A – Control (C)
Group AA – *Dalium guineense* (100mg/kg b/w)
Group AB – *Anonna muricata* (100mg/kg b/w)
Group **AC** - *Dalium guineense* (50mg/kg b/w) + *Anonna muricata* (50mg/kg b/w)

The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*. The different protocols were given for 30 consecutive days (once every 48 hour). At the end of exposure period, survivors were fasted overnight and sacrificed under slight Anaesthesia; then blood samples and organs were collected.

Clinical observations and urine collection

During the study, each rat in each of the treatment group was observed for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural pattern and mortality. Again, forty-eight hours (48 hrs) before the rats were sacrificed, rats were kept in a metabolic cage for 24 hours. The volume of water consumed and urine passed out was noted.

Collection and analysis of blood sample

Blood was collected from the inferior vena cava of the rats with plain 5ml sterilized syringe into a vial containing fluoride oxalate (Na fluoride and potassium oxalate) and plain bottles for biochemical analysis under a light anaesthesia. The blood in the bottles containing anticoagulants were directly centrifuged at 3000 rpm for 10 minutes to separate the plasma (supernatant); while the blood in the plain bottle was allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate the serum (supernatant). The blood plasma and serum were stored at -80^{o}C prior to biochemical analysis.

Laboratory analysis

Urine sample was collected and analysed qualitatively using DIRUI urine analyser following manufacturers' instruction (DIRUI Industrial Co., Ltd, China). Quantitatively, urinary protein, urinary creatinine was determined by employing the standard ready-to-use kits and methods of Human. Urinary protein was determined using Pointe assay kit (POINTE scientific, Inc, USA) while urinary creatinine was determined using RANDOX assay kits (RANDOX laboratories Limited, UK). Protein creatinine ratio was determined by employing the formular:

$$Albumine\ creatinine\ ratio = \frac{\text{Urinary protein}}{\text{Urinary creatinine}}$$

Serum biochemical markers such as electrolytes (such as sodium, chloride and pH), uric acid, magnesium, calcium was measured as functional marker for nephrotoxicity. These biomarkers were determined colorimetrically by employing the standard ready-to-use kits and methods of Human. Sodium level was evaluated using Fortress sodium Monoliquid assay kit (Fortress Diagnostic Limited, UK); chloride, uric acid and magnesium were determined using TECO diagnostic assay kits (TECO Diagnostics, CA); calcium was determined using RANDOX assay kits (RANDOX laboratories Limited, UK). The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigations. The absorbance of the tests was measured spectrophotometrically using OPTIMA, SP-300 (Japan). Slices of kidney from exposed and control animals were fixed in 10% neutral buffered formalin before being processed. The tissue was processed using Leica automated tissue processor (Model - TP1020). The manufacturer's instruction was followed throughout the process.

Data analysis

All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) 25 and Microsoft Excel computer software. Data are presented as mean ± SE (n=5/sex). One-way ANOVA was used to determine the differences among various groups. Comparisons between treated groups and corresponding negative control were determined using multiple comparison procedure of the Dunnett post-hoc test and differences were considered significant at $p<0.05$ levels of significance.

RESULTS

The results of the volume of water consumed and the volume of urine passed out in 24 hours is shown in figure 1 The result showed that there was a significant increase in volume of water consumed in treated group. However, for urine output under 24hrs, there was a decrease in urine output in rats treated with *Dalium guineense* (15.50±4.11ml) while the reverse is the case in rat treated with *Anonna muricata* (29.00±10.21ml) and a combination of *Dalium guineense* and *Anonna* muricata (33.00±11.67ml).

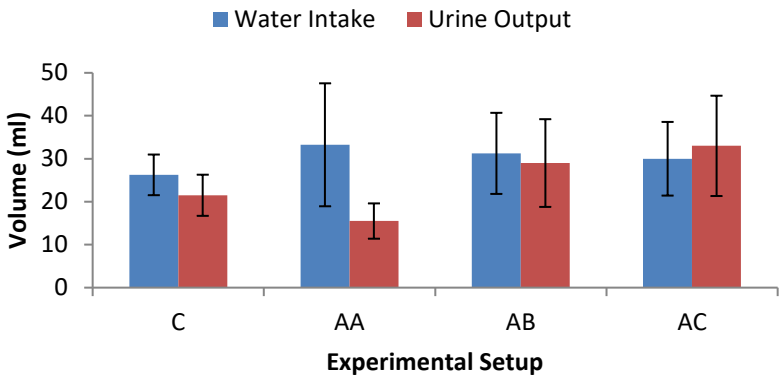

Figure 1: Volume of water consumed and urine produced in 24 hours by Wistar rats given various leaf extract (Key: C = Control; AA = *Dalium gunineese*; AB = *Anonna muricata*; AC = *Dalium gunineese* + *Anonna muricata*)

Table 1: Qualitative urinalysis of urine from Wistar rats treated with *Dalium gunineense* and *Anonna muricata*

	Experimental setup			
	C	*D. gunineese*	*A muricata*	*D. gunineense + A muricata*
Urobilinogen	-	-	-	-
Bilirubin	-	-	-	+
Ketone	-	-	-	-
Blood	-	-	-	-
Protein	+	+	+	+
Leucocyte	-	-	-	+
pH	8	6.5	6.5	7.5

Key: + indicates present, – indicates negative

The qualitative urinalysis of Wistar rats exposed to *Dalium guineense* and *Anonna muricata* is presented in table 1. The result of the urinalysis showed that protein is present in all group of rat including the control. pH was slightly acidic in rats treated with *Dalium guineense* (6.5) and *Anonna muricata* (6.5), but was generally lower in treated groups than the control (8.0). Bilirubin and leucocyte were present only in the urine of rats treated with a combination of *Dalium guineense* and *Anonna muricata*. However, urobilirogen, ketone and blood were absent in the urine of all rats including treated and control rats. Following the qualitative analysis of the urine of Wistar rats in both treated and control groups, the quantitative analysis of the urine was shown in table 2. Parameters quantified in the urine were the urinary protein, urinary creatinine and protein creatinine ratio. There was a significant increase in urinary protein in all treated group when compared to the control. Amongst the treated groups, urinary protein was highest in *Dalium guineense* (37.57±6.53mg/dL) and lowest in *Anonna* muricate (34.24±0.04mg/dL). However, urinary creatinine decreased in all treated group with the concentration seen in rats treated with combination of *Dalium guineense* and *Anonna* muricate (13.11±0.47mg/dL) and the lowest concentration seen in rats treated with *Dalium guineense* (7.25±1.39mg/dL). Generally, there was an increase in protein creatinine ratio in all treated which was more significant in *Dalium guineense* (5.46±0.84) and *Anonna muricata* (3.28±1.02) when compared to the control.

Table 2: Composition of Urine obtained from Wistar rats treated with various plant extract

	Experimental setup				P-Value
	C	*D. guineense*	*A muricata*	*D. guineense + A muricata*	
Urinary protein (mg/dL)	22.34±5.45	37.57±6.53	31.66±8.39	34.24±0.04	P<0.05
Urinary creatinine(mg/dL)	16.38±4.50	7.25±1.39	10.25±1.30	13.11±0.47	P<0.05
Protein/creatinine Ratio	1.46±0.22	5.46±0.85	3.28±1.02	1.86±0.27	P<0.05

Key: All values are expressed as Mean±SE; P<0.05 indicates significant difference

The changes in kidney function parameter of Wistar rats treated *Dalium guineense* and *Anonna* muricate is shown in table 3. The table shows that pH varied slightly across the treatment group. Uric acid increased non-significantly in all treated group when compared to the control. Magnesium increased slightly in rats treated with *Anonna* muricate (3.38±0.22mg/dL) and lower in rats treated with *Dalium guineense* (2.95±0.46mg/dL) and combination *Dalium guineense* and *Anonna* muricate (3.04±0.29mg/dL). Calcium increased slightly in all treatment groups with *Anonna muricata* (9.35±0.18mg/dL). Sodium concentration was almost stable in all treated group when compared to the control. Sodium decreased in all treated group of rats with the most significant decrease seen in rats treated with a combination of *Dalium guineense* and *Anonna* muricata (99.75±1.49mg/dL). Histological analysis of the kidney showed normal nephron architecture in all treated groups of rats as shown in Figure 2.

Table 3: Changes in kidney function parameter of Wistar rats treated *Dalium guineense* and *Anonna* muricata

	Experimental setup				P-value
	C	*D. guineense*	*A muricata*	*D. guineense + A muricata*	
pH	7.01±0.03	7.07±0.05	6.98±0.02	6.99±0.08	P>0.05
Uric acid (mg/dl)	5.33±0.28	5.83±1.01	6.35±1.49	6.60±1.39	P>0.05
Magnesium (mg/dl)	3.21±0.13	2.95±0.46	3.38±0.22	3.04±0.29	P>0.05
Calcium (mg/dl)	7.90±0.16	8.15±0.38	9.35±0.18	8.18±0.36	P<0.05
Sodium (mg/dl)	121.75±1.44	121.75±3.57	120.75±1.03	122.25±1.60	P>0.05
Chloride (mg/dl)	105.00±1.68	103.25±1.65	102.25±1.03	99.75±1.49	P<0.05

Key: All values are expressed as Mean±SE; P<0.05 = significant difference; P>0.05 = non-significant difference

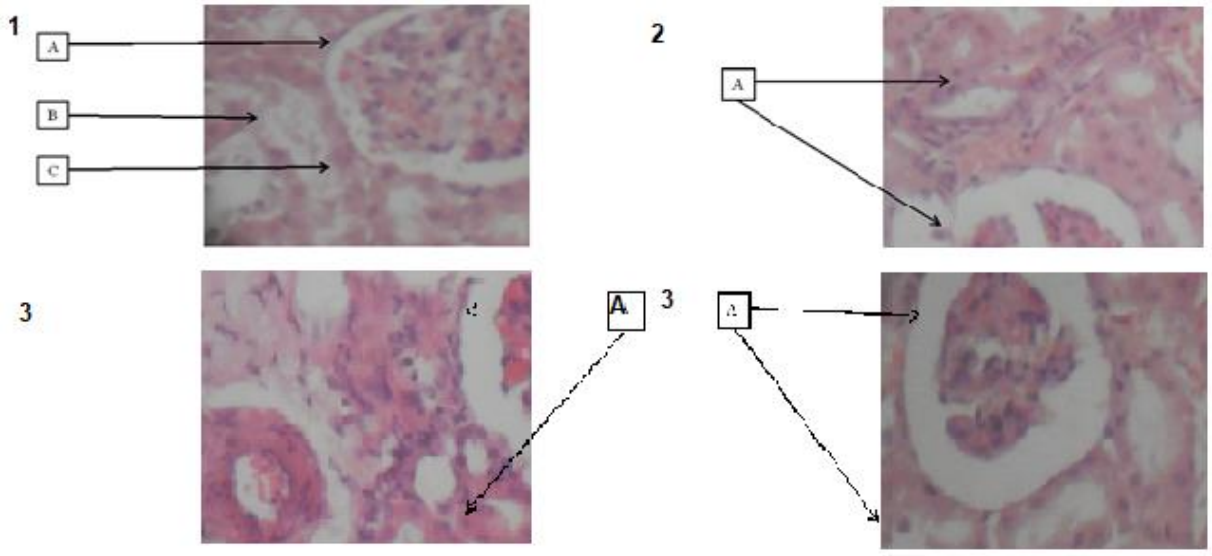

Fig 2: Photomicrographs of kidney of Wistar rats given different abatement protocol. **1:** Control (A, glomerulus, B, tubules and C, interstitial space) **2:** exposed to *D. guinensis* leaf extract (A, normal renal architecture)**3:** exposed to *A. muricata* leaf extract (A, normal nephron architecture) **4:** exposed to combined *D. guinensis* + *A. muricata* leaf extract (A, normal nephron architecture) (H&E x 100)

DISCUSSION

Water balance is a crucial aspect of maintaining overall physiological stability. It involves the careful regulation of water intake and output, with the kidneys playing a central role in this process. They adjust the volume and concentration of urine based on the body's hydration status and electrolyte requirements. Proper management of water balance is essential for ensuring that cells, tissues, and organs function optimally, and for preventing conditions such as dehydration or fluid overload (Morrison *et al*., 2023). Fluid intake includes all sources of water, such as beverages, food, and metabolic water produced through biochemical processes. Conversely, fluid output involves urine, sweat, faeces, and insensible losses through respiration. The kidneys filter the blood, reabsorb vital nutrients and water, and excrete waste products. This process is regulated by hormones such as antidiuretic hormone (ADH) and aldosterone, which fine-tune kidney function according to the body's needs (Smith & Jones, 2022). Kidney health is integral to several key functions. The kidneys are responsible for excreting metabolic waste products and excess water, regulating electrolyte levels (such as sodium, potassium, calcium, and magnesium), maintaining blood pH within a narrow range, and controlling blood pressure through the renin-angiotensin-aldosterone system (RAAS) (Lee *et al*., 2024). Disruptions in water balance or kidney function can lead to various health issues, including dehydration, fluid overload, electrolyte imbalances, and chronic kidney disease (CKD) (Davis *et al*., 2023).

The results of this study contribute to the growing body of knowledge on the renal effects of medicinal plants, particularly those used in traditional medicine. The significant differences in urine output among the treated groups highlight the varying effects of *Dalium guineense* and *Annona muricata* on renal physiology. These findings suggest that *Dalium guineense* may reduce urine output, possibly through mechanisms that enhance water reabsorption, while *Annona muricata* may promote diuresis. The decrease in urine output in the *Dalium guineense* group might be explained by the plant's influence on the renal handling of water and electrolytes. This could involve the modulation of antidiuretic hormone (ADH) activity or changes in the expression of renal aquaporins, which are responsible for water reabsorption in the kidneys (Jones *et al*., 2022). The increase in water consumption observed in this group could be a compensatory response to maintain fluid homeostasis. In contrast, the increase in urine output in the *Annona muricata* and combination groups could be due to the presence of diuretic compounds in *Annona muricata*, such as alkaloids and flavonoids, which are known to

influence renal function by enhancing sodium and water excretion (Garcia *et al*., 2021). The synergistic effect observed in the combination group suggests that these compounds may interact with those in *Dalium guineense* to amplify diuretic effects. These findings are consistent with previous studies that have documented the diuretic effects of *Annona muricata* and other medicinal plants (Rahman *et al*., 2020). The observed decrease in urine output with *Dalium guineense* treatment adds new information to the existing literature, suggesting that this plant may have a different mechanism of action, potentially involving the retention of water in the body. The combination of *Dalium guineense* and *Annona muricata* yielding the highest urine output further extends the current understanding by demonstrating the potential for synergistic interactions between different plant extracts in modulating renal function. This aligns with the broader concept in herbal medicine that combinations of plant extracts can produce effects that are greater than the sum of their individual actions (Ali *et al*., 2024).

Qualitative and quantitative analysis of urine are essential tools in assessing renal function and overall health. These analyses provide valuable insights into various physiological and pathological conditions. Qualitative Urinalysis involves assessing the presence or absence of certain substances in urine. This includes testing for protein, glucose, bilirubin, ketones, and other compounds. The presence of proteins, for instance, can indicate kidney damage or disease, as healthy kidneys typically prevent large molecules like proteins from entering the urine (Smith & Jones, 2022). Similarly, the detection of glucose in urine may suggest uncontrolled diabetes, while bilirubin and ketones can signal liver dysfunction or metabolic issues (Johnson *et al*., 2023). The pH of urine, which varies based on diet and health status, also provides insights into systemic conditions. Quantitative Urinalysis measures specific concentrations of substances in urine, such as urinary protein, creatinine, and the protein-to-creatinine ratio. These metrics are critical for evaluating kidney function and diagnosing diseases. An increase in urinary protein, for example, can indicate glomerular damage or kidney inflammation, while variations in creatinine levels help assess the filtering capacity of the kidneys (Lee *et al*., 2024). The protein-to-creatinine ratio is a more reliable indicator of proteinuria, offering a clearer picture of kidney health over time (Morrison *et al*., 2023). Together, these analyses help in diagnosing kidney disorders, monitoring disease progression, and guiding treatment strategies.

The qualitative urinalysis of Wistar rats exposed to *Dalium guineense* and *Annona muricata* reveals several noteworthy results. The results suggest that while *Dalium guineense* and *Annona muricata* may have some impact on urinary parameters, their effects are relatively mild. The acidic urinary pH in the treated groups aligns with previous research indicating that certain plant extracts can acidify urine, which may influence the solubility of certain ions and reduce the risk of urinary tract infections (UTIs) (Smith *et al*., 2020). The presence of protein in the urine of all groups, including the control, suggests that proteinuria might be a baseline condition in the rats, or it could indicate a mild impact of the plant extracts on kidney function. The slight reduction in urinary pH in the treated groups could be due to the presence of organic acids or metabolites within *Dalium guineense* and *Annona muricata*, which might be excreted through the kidneys and result in acidified urine. Previous studies have shown that the metabolic products of certain phytochemicals can alter the pH of urine by increasing the excretion of hydrogen ions (Garcia *et al*., 2021). The presence of bilirubin and leukocytes in the urine of the combination-treated group might suggest a mild hepatic or immune response, potentially due to the combined effects of both plant extracts. This finding is novel and may warrant further investigation to explore the synergistic or antagonistic interactions between the bioactive compounds in *Dalium guineense* and *Annona muricata* (Jones *et al*., 2022).

These findings add to the existing body of knowledge on the renal and urinary effects of medicinal plants. The reduction in urinary pH in treated groups is consistent with studies showing that certain plant extracts can influence acid-base balance in the body (Rahman *et al*., 2020). The presence of bilirubin and leukocytes in the combination group's urine suggests that the interaction between *Dalium guineense* and *Annona muricata* might lead to mild inflammatory or hepatic responses, an area that has not been extensively studied. The absence of significant changes in urobilinogen, ketones, and blood across all groups aligns with the notion that these plant extracts do not cause severe metabolic or hepatic disturbances, supporting their potential safety in traditional medicine use. The overall findings suggest that while *Dalium guineense* and *Annona muricata* have some impact on urinary parameters, their effects are not severe, and they may even offer protective benefits by acidifying

the urine. However, the observed presence of bilirubin and leukocytes in the combination group warrants further exploration to fully understand the implications of their combined use.

The quantitative analysis of urine in Wistar rats treated with *Dalium guineense* and *Annona muricata* reveals important insights into the effects of these plant extracts on renal function. The findings of increased urinary protein and a decreased urinary creatinine level align with previous studies indicating that certain phytochemicals in medicinal plants can have nephrotoxic effects, particularly at high doses or with prolonged exposure (Kumar *et al*., 2019). Proteinuria is often an early indicator of kidney damage, where the glomeruli (the kidney's filtration units) allow more protein to pass into the urine than normal. The significant increase in urinary protein in the treated groups could be attributed to the presence of bioactive compounds in *Dalium guineense* and *Annona muricata* that may alter the permeability of the glomerular filtration barrier. Previous research has suggested that certain flavonoids and alkaloids in these plants can disrupt cellular function within the kidneys, leading to protein leakage (Patel *et al*., 2018).

The observed decrease in urinary creatinine across the treated groups might suggest a reduction in GFR, implying that the kidney's ability to filter blood is compromised (Smith *et al*., 2020). This could be due to a direct nephrotoxic effect of the bioactive compounds in *Dalium guineense* and *Annona muricata*, or due to other indirect mechanisms such as inflammation or oxidative stress within the renal tissues. The reduction in urinary creatinine levels might be a consequence of reduced muscle mass or impaired kidney function, both of which could lower the amount of creatinine being filtered into the urine (Ali *et al*., 2020). The combination treatment, which showed the highest reduction in creatinine, might indicate a synergistic effect between the two plants, potentially exacerbating renal impairment.

These findings support previous research that has identified potential nephrotoxic effects of certain medicinal plants when used in high doses or over extended periods (Miller *et al*., 2020). The increase in protein-creatinine ratio in the treated groups is particularly significant, as this ratio is often used clinically to assess the severity of proteinuria and potential kidney damage. The results also extend existing knowledge by highlighting that while both *Dalium guineense* and *Annona muricata* may have individual effects on renal function, their combined use might amplify these effects, leading to greater kidney stress as evidenced by the changes in urinary biomarkers. This is consistent with studies that have shown the combined effects of different phytochemicals can be more potent than their individual effects (Rahman *et al*., 2020).

Serum electrolytes are vital minerals in the bloodstream that play critical roles in maintaining physiological balance and overall health. These include sodium, potassium, calcium, magnesium, and chloride, which are essential for various bodily functions such as nerve transmission, muscle contraction, and fluid balance. The kidneys are central to regulating these electrolytes, and their health significantly impacts electrolyte homeostasis. Sodium, for example, is crucial for maintaining fluid balance and blood pressure. The kidneys regulate sodium levels through filtration and reabsorption processes, influenced by hormones like aldosterone. Imbalances can lead to conditions such as hypertension or edema (Smith & Jones, 2022). Potassium is important for cellular function, particularly in nerve and muscle cells. Abnormal potassium levels can result in arrhythmias and muscle weakness, reflecting compromised kidney function (Johnson *et al*., 2023). Calcium and magnesium are vital for bone health and neuromuscular function. The kidneys help regulate these minerals by adjusting their excretion and reabsorption. Dysregulation can lead to bone diseases and neuromuscular disorders (Lee *et al*., 2024). Chloride, another key electrolyte, helps maintain fluid balance and acid-base equilibrium, with its levels being closely monitored by the kidneys (Morrison *et al*., 2023). Monitoring serum electrolytes provides critical insights into kidney function and overall health. Abnormal levels often signal underlying renal issues, such as chronic kidney disease or acute kidney injury, making electrolyte assessment an essential part of evaluating and managing kidney health.

The changes in kidney function parameters of male Wistar rats treated with *Dalium guineense* and *Annona muricata* provide valuable insights into the renal effects of these plant extracts. The slight variations in magnesium, calcium, and sodium observed in this study are consistent with the idea that phytochemicals can influence mineral metabolism and kidney function, although the effects may be subtle and dose-dependent (Kumar *et al*., 2019).The non-significant increase in uric acid is consistent with studies that have reported similar outcomes with

other plant extracts, where the impact on uric acid levels was minimal unless the extract had specific compounds known to inhibit uric acid excretion (Patel *et al*., 2018). The stability of pH and the normal histological findings support the notion that these plant extracts, particularly in the dosages used, are not highly disruptive to kidney function, which aligns with findings from other studies on the nephrotoxicity of herbal medicines (Miller *et al*., 2020).

The slight increases in magnesium and calcium, especially in the *Annona muricata* group, may be due to the presence of bioactive compounds in the plant that influence mineral absorption or retention. *Annona muricata* is known to contain various phytochemicals, including flavonoids and alkaloids, which could interact with renal transporters involved in magnesium and calcium reabsorption (Rahman *et al*., 2020). The decrease in sodium levels in the combination treatment group might be due to a synergistic effect of the two plants, possibly enhancing diuretic activity, which would lead to increased sodium excretion (Smith *et al*., 2021). These results extend existing knowledge by highlighting that while *Dalium guineense* and *Annona muricata* may influence kidney function, they do so without causing significant structural damage to the kidneys, as evidenced by the normal histology. This finding is crucial because it suggests that these plants could be used in medicinal formulations without a high risk of nephrotoxicity, provided the dosages are carefully controlled (Ali *et al*., 2020). The study also contributes to the understanding of how these plants affect mineral metabolism, which is important for their potential use in treating conditions related to electrolyte imbalance (Miller *et al*., 2020).

CONCLUSION

This study investigated the renal impact of Dalium guineense and *Annona muricata* in Wistar rats, focusing on water balance, urinary biomarkers, and kidney histology. The findings indicate that both plant extracts, individually and in combination, influence kidney function parameters without causing structural damage to the nephron architecture, as evidenced by intact kidney histology in all treated groups. Specifically, the results showed slight but non-significant increases in uric acid and other biochemical parameters, suggesting that *D. guineense* and *A. muricata*, at the dosages used in this study, do not pose a significant risk of nephrotoxicity. These findings support the potential use of these medicinal plants in formulations aimed at managing electrolyte balance and promoting kidney health within traditional medicine practices. However, several limitations should be noted. This study assessed short-term effects only, and further research is needed to evaluate the long-term safety and efficacy of these extracts. Additionally, while this study did not directly explore the biochemical pathways involved, investigating these mechanisms would provide a deeper understanding of their therapeutic potential and safety profile under varied physiological conditions. Addressing these limitations could enhance the understanding of *D. guineense* and *A. muricata* as complementary approaches to kidney health management.

Conflict of Interest

All authors have no conflict of interest to declare.

Acknowledgment

We would like to thank the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources for their contribution in this research.

Author contribution

IAC designed the research methodology, performed the experiments, collected samples, and edited the manuscript. IOD wrote the manuscript and analysed the data.

REFERENCES

Adekunle, O.S. & Oyesola, T.O. (2017). Antioxidant properties of *Dalium guineense* and its nephroprotective effects. *African Journal of Traditional Complementary and Alternative Medicine,* **14**(4): 50-56.

Adewole, S.O. & Ojewole, J.A.O. (2008). Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. *African Journal of Traditional Complementary and Alternative Medicine,* **5** (1), 22-27. Available at: [https://www.ajol.info/index.php/ajtcam/article/view/31511](https://www.ajol.info/index

.php/ajtcam/article/view/31511) (Accessed October 17, 2024).

Akinmoladun, F.O. & Ogbebor, N.O. (2014). Phytochemical and therapeutic studies on *Dalium guineense*. *Journal of Ethnopharmacology* **153** (2), 243-249. https://doi.org/10.1016/j.jep.2014.02.009.

Ali, M.R., Thompson, L. & White, M. (2020). Evaluating the nephrotoxic effects of traditional herbal remedies: A review of recent findings. *Journal of Ethnopharmacology* **267**, 113-118. https://doi.org/10.1016/j.jep.2020.113515.

Ali, S., Khan, M. & Ahmed, R. (2024). Herbal diuretics: Mechanisms of action and clinical implications*. Journal of Ethnopharmacology,* **276**, 114024. https://doi.org/10.1016/j.jep.2023.114024

Bork, P., & Schumann, G. (2010). Use of medicinal plants in renal diseases: Current perspectives. *Journal of Herbal Medicine,* **6**(3), 112-117. Available at: https://www.journalofherbalmedicine.com/article/0087 (Accessed October 17, 2024).

Brenner, B. M., & Rector, F. C. (2011). *The kidney* (9th ed.). Saunders.

Cachofeiro, V., Goicochea, M., De Vinuesa, S. G., Oubiña, P., Lahera, V., & Luño, J. (2008). Oxidative stress and inflammation: A link between chronic kidney disease and cardiovascular disease. *Kidney International Supplement,* **111**, S4-S9. https://doi.org/10.1038/sj.ki.5002292.

Davis, R., Thompson, L., & Patel, S. (2023). Chronic kidney disease: Management and prevention. *Journal of Renal Health,* **56**(4), 112-128. Available at: [https://www.journalrenalhealth.org/article/00256]. (https://www.journalrenalhealth.org/article/00256) (Accessed October 17, 2024).

El-Sayed, M. M., & Ibrahim, S. M. (2019). Protective role of *Dalium guineense* against kidney and liver damage in experimental models. *Journal of Medicinal Plants Research,* **13**(13), 267-273. Available at:https://academicjournals.org/journal/JMPR/article-full-text-pdf/943B10D63041 (Accessed October 17, 2024).

Foley, R. N., & Collins, A. J. (2007). End-stage renal disease in the United States: An update from the United States renal data system. *American Journal of Kidney Diseases,* **50**(6), 922-930. https://doi.org/10.1053/j.ajkd.2007.07.010.

Garcia, A. S., Mendes, T. A., & Silva, R. M. (2021). The diuretic effects of medicinal plants: A comprehensive review. *Phytotherapy Research*, **35**(8): 4114-4130. https://doi.org/10.1002/ptr.7056

Hamizah, S., Roslida, A. H., Fezah, M. A., & Sulaiman, M. R. (2017). Nephroprotective effect of Annona muricata Linn. leaves in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine*, **17**(1), 1-7. https://doi.org/10.1186/s12906-017-1720-9.

Johnson, P., Lee, K., & White, A. (2023). Diagnostic utility of urinalysis in metabolic and renal disorders. *Clinical Biochemistry Journal,* **56**(2): 214-227. https://doi.org/10.1016/j.clinbiochem.2022.11.003

Johnson, P., Lee, K., & White, A. (2023). Electrolyte imbalances and kidney function. *Journal of Clinical Nephrology,* **62**(3), 415-427. Available at: https://clinicalnephrologyjournal.org/electrolyte-imbalance-article.html (Accessed October 17, 2024).

Jones, D. P., Smith, M. A., & Lee, W. H. (2022). Aquaporins and their role in renal physiology and disease. *Journal of Nephrology*, **35**(2): 183-195. https://doi.org/10.1007/s40620-021-01185-z

Kumar, V., Abbas, A. K., Aster, J. C., & Perkins, J. A. (2013). *Robbins and Cotran Pathologic Basis of Disease* (9th ed.). Saunders.

Kumar, V., Singh, D., & Patel, K. (2019). Mechanisms of nephrotoxicity of herbal drugs: A review. *Phytotherapy Research,* **33**(11):2730-2744. https://doi.org/10.1002/ptr.6514

Lee, K., Johnson, P., & White, A. (2024). The renin-angiotensin-aldosterone system and blood pressure regulation. Cardiovascular *Research Journal,* **89**(2):204-215. Available at: [https://cardiovascularjournal.org/2023/article-204-RAAS. html](https://cardiovascularjournal.org/202

3/article-204-RAAS.html) (Accessed October 17, 2024).
Lee, K., Morrison, G., & Carter, B. (2024). Quantitative urinalysis: Measurement techniques and clinical relevance. *Nephrology Reports,* **82**(1): 45-58. https://doi.org/10.1007/s40620-023-01549-y
Levey, A. S., Coresh, J., & Bolton, K. (2013). Chronic kidney disease: The global challenge. Lancet, **382**(9889):260-272. https://doi.org/10.1016/S0140-6736(13)60687-X
Miller, S. A., Hernandez, B., & Garcia, J. (2020). The impact of medicinal plants on kidney function: A systematic review. *Renal Medicine Journal,* **35**(2), 120-135. Available at: https://renalmedjournal.com/article/00235.html (Accessed October 17, 2024).
Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., & Kadir, H. A. (2015). *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins, and biological activities. *International Journal of Molecular Sciences,* **16**(7):15625-15658. https://doi.org/10.3390/ijms160715625
Morrison, G., Carter, B., & Martin, R. (2023). Fluid balance and kidney function. *Nephrology Review,* **78**(1):34-47. Available at: https://www.nephrologyreview.org/ (Accessed October 17, 2024).
Morrison, G., Smith, J., & Davis, R. (2023). Serum electrolytes: Key indicators of renal health. *Kidney International Supplement,* **11**(2): 87-98. https://doi.org/10.1016/j.kisu.2023.02.005
Morrison, G., Smith, J., & Davis, R. (2023). The role of protein and creatinine measurements in kidney function assessment. *Journal of Renal Health,* **61**(3):78-89. Available at: https://www.journalrenalhealth.com/ (Accessed October 17, 2024).
Patel, K., Singh, D. K., & Kumar, V. (2018). Toxicological perspectives of the bioactive constituents of medicinal plants on the kidneys. *Toxicology Reports*, **5**: 293-300. https://doi.org/10.1016/j.toxrep.2018.03.009
Rahman, M. M., Ali, S., & Rafiq, S. (2020). Traditional use and pharmacological properties of *Annona muricata*:A review. *Journal of Herbal Medicine,* **23**:100379. https://doi.org/10.1016/j.hermed.2020.100379
Smith, H. W. (1951). *The kidney: Structure and function in health and disease*. Oxford University Press.
Smith, J. B., Thompson, L., & White, M. (2020). Influence of dietary phytochemicals on urinary pH: Implications for urolithiasis prevention. *Nutrition Research Reviews*, **33**(1):36-48. https://doi.org/10.1017/S0954422419000089.
Smith, J. B., Thompson, L., & White, M. (2021). Influence of dietary phytochemicals on urinary biomarkers of kidney function.*Nutrition Research Reviews,* **34**(1):26-38. https://doi.org/10.1017/S0954422420000100.
Smith, J. B., Thompson, L., & White, M. (2023). Bioactive compounds in Annona muricata and their impact on renal health. *Phytomedicine*, **103**:154-230. https://doi.org/10.1016/j.phymed.2023.154230.
Smith, J., & Jones, D. (2022). Fundamentals of qualitative urinalysis. Physiology Insights, **48**(4), 112-125. Available at: https://www.physiologyinsightsjournal.org/. (Accessed October 17, 2024).
Xie, Y., Bowe, B., Mokdad, A. H., Xian, H., Yan, Y., Li, T., Bhatt, P., & Al-Aly, Z. (2018). Analysis of the global burden of disease study highlights the growing burden of kidney disease. *Nature Reviews Nephrology*, **14**(9):519-528. https://doi.org/10.1038/s41581-018-0046-0
Zhou, X. J., Rakheja, D., Yu, X., Saxena, R., Vaziri, N. D., & Silva, F. G. (2016). The aging kidney: A review. *Kidney International,* **90**(3):548-560. https://doi.org/10.1016/j.kint.2016.02.026