Journal of Pharmacy & Allied Medicine

JPAM, 4(1), 13-19, 2026
https://doi.org/10.58985/jpam.2026.v04i01.40
ISSN (Print): 3005-2726

Research Article

Renal tissue and biochemical responses to polyherbal consumption in Wistar rats

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Article Information
Received: 27 January 2026
Revised: 16 February 2026
Accepted: 17 February 2026
Published: 25 February 2026

Academic Editor
Prof. Dr. Christian Celia

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Keywords
Tetrapleura tetraptera, *Jatropha curcas*, polyherbal, renal function, electrolytes, urea, creatinine, Wistar rats.

Abstract

Polyherbal preparations are widely consumed for perceived therapeutic synergy, yet their renal safety is often poorly characterized. The kidneys are particularly vulnerable to xenobiotic exposure, and renal impairment may be detected using biochemical indices (urea, creatinine, and electrolytes) alongside histological assessment. This study evaluated renal biochemical indices and kidney tissue morphology following subacute oral administration of aqueous extracts of *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaf, administered singly and in combination, in Wistar rats. Twenty-four adult female Wistar rats were randomly assigned to four groups (n=6). Group A received distilled water (control Group). Group B received *T. tetraptera* fruit extract (200 mg/kg), Group C received *J. curcas* leaf extract (400 mg/kg), and Group D received a combination of both extracts (200 mg/kg + 400 mg/kg). The treatments were administered orally once daily for 28 days. At termination, serum urea, creatinine, sodium, potassium, chloride, and bicarbonate levels were analyzed using standard biochemical methods. Kidneys were excised, fixed, processed, and stained with hematoxylin and eosin for histological evaluation. Serum creatinine, sodium, and potassium levels showed no significant differences across the groups ($p > 0.05$). Urea, chloride, and bicarbonate levels differed significantly among the groups ($p < 0.05$), however, the values remained within the physiological range and were not accompanied by creatinine elevation. Kidney and body weight changes were not significantly altered in the present study. Histological findings demonstrated preserved renal architecture with normal glomeruli and tubules and no evidence of necrosis or inflammatory infiltration in any group. Subacute oral administration of a polyherbal mix did not produce biochemical or histological evidence of nephrotoxicity in Wistar rats at the tested doses.

1. Introduction

The kidneys play a critical role in maintaining internal homeostasis through regulation of electrolyte balance, acid–base equilibrium, fluid volume, and excretion of metabolic waste products. Disruption of renal structure or function often manifests as altered serum levels of urea, creatinine, and electrolytes, which are widely accepted indicators of renal impairment in experimental and clinical settings [1, 2]. Histological assessment of renal tissues provides direct evidence of glomerular and tubular integrity, complementing biochemical findings and improving the reliability of toxicity and safety evaluations [3].

The use of medicinal plants for disease management remains widespread, particularly in low- and middle-income countries, where herbal remedies are commonly consumed alone or in combination. Polyherbal formulations are often preferred in traditional practices based on the belief that

combining multiple plant extracts enhances therapeutic efficacy while reducing adverse effects [4, 5]. Despite their popularity, concerns persist regarding the renal safety of prolonged or repeated exposure to herbal products, especially when formulations are inadequately characterized or consumed without standardized dosing [6, 7].

Tetrapleura tetraptera is a West African medicinal plant that is traditionally used as a spice and therapeutic agent. Phytochemical investigations have demonstrated that its fruits contain bioactive compounds with antioxidant and metal chelating properties that may influence renal oxidative balance and tissue integrity [8, 9]. Experimental studies have reported the protective effects of *T. tetraptera* against chemically induced tissue damage, suggesting its potential benefits in preserving renal architecture under oxidative stress conditions [10, 11]. However, data on the renal effects of repeated oral exposure remain limited.

Jatropha curcas is another widely used medicinal plant with documented pharmacological activities, including antioxidant, antidiabetic, and anti-inflammatory effects. Several experimental studies have assessed both renal and systemic responses to *J. curcas* extracts, demonstrating nephroprotective properties as well as dose-dependent toxicological effects, which vary according to the plant part used, extraction method, and duration of exposure [12-15]. While controlled administration has been associated with the preservation of renal biochemical indices and tissue structure, inappropriate dosing and prolonged exposure have been linked to renal dysfunction, emphasizing the need for careful evaluation [6, 16].

Given the increasing consumption of combined herbal preparations, there is a need for a systematic assessment of renal responses to polyherbal exposure using integrated biochemical and histological approaches. The measurement of serum electrolytes, urea, and creatinine provides insight into the functional renal status, whereas histological examination allows the detection of structural alterations at the tissue level [2, 3]. Therefore, this study investigates renal tissue morphology and biochemical indices following single and combined administration of *T. tetraptera* fruit and *J. curcas* leaf aqueous extracts in Wistar rats, with the aim of providing evidence-based data on the renal safety of this polyherbal combination.

2. Materials and methods

2.1. Plant materials

Fresh fruits of *T. tetraptera* and fresh leaves of *J. curcas* were obtained from southwestern Nigeria. The plant materials were authenticated by a plant taxonomist at our institution. Voucher specimens were deposited in a recognized herbarium under the reference numbers UBH-T472 (*T. tetraptera*) and UBH-J404 (*J. curcas*). Plant collection and handling were performed in accordance with established ethical and research guidelines.

2.2. Preparation and extraction of plant materials

The collected fruits and leaves were washed thoroughly with clean water and air-dried at ambient temperature to preserve the heat-sensitive constituents. The dried materials were separately milled into fine powders using a mechanical grinder. Aqueous extraction was performed by cold maceration. Five hundred grams of each powdered sample were soaked separately in 1.5 L of distilled water for 24 h with intermittent stirring. The mixtures were homogenized, refrigerated overnight at 4 °C, and filtered through a muslin cloth. The filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator and freeze-dried to obtain powdered extracts. The extracts were stored in airtight containers at 4 °C until use.

2.3. Acute toxicity information

Information on the acute toxicity and median lethal dose of *T. tetraptera* fruit and *J. curcas* leaf extracts was obtained from previously published studies [1-3,17]. Aqueous extracts of *T. tetraptera* fruit have been reported to be non-toxic at doses of up to 5000 mg/kg body weight in rodents. Similarly, detoxified aqueous leaf extracts of *J. curcas* have shown good tolerability at doses below 2000 mg/kg body weight. Therefore, the doses used in the present study were selected within the reported safe ranges.

2.4. Experimental animals

Twenty-four apparently healthy adult female Wistar rats weighing between 107 g and 155 g were used. The animals were obtained from a registered laboratory

https://doi.org/10.58985/jpam.2026.v04i01.40

animal facility and housed in well-ventilated wire mesh cages under standard laboratory conditions. Ambient temperature was maintained at 22-25 °C with a 12 h light/12 h dark cycle, while clean bedding was provided and changed regularly. The rats were acclimatized for two weeks and had unrestricted access to standard laboratory feed and clean drinking water ad libitum. The animals were observed daily for general health, behavior, and signs of distress throughout the study. Only female rats were used to minimize biological variability associated with sex-dependent differences in renal physiology, electrolyte handling, and xenobiotic metabolism, thereby improving group homogeneity and statistical robustness in subacute toxicity assessment. The limitation regarding the extrapolation of findings across sexes has been acknowledged in the limitations section. All procedures complied with the internationally accepted guidelines for animal care and use.

2.5. Experimental design

The rats were randomly assigned into four groups of six animals each. Group A served as the control and received distilled water and standard feed only. Group B received *T. tetraptera* fruit extract at a dose of 200 mg/kg body weight. Group C received *J. curcas* leaf extract at a dose of 400 mg/kg body weight. Group D received a combination of *T. tetraptera* fruit extract (200 mg/kg) and *J. curcas* leaf extract (400 mg/kg). All treatments were administered orally once daily for 28 consecutive days using an oral gavage. The administered volume did not exceed 1 mL per 100 g body weight.

2.6. Kidney tissue collection and histological processing

At the end of the treatment period, the animals were humanely sacrificed by cervical dislocation. The abdominal cavity was opened, and both kidneys were carefully excised, rinsed with normal saline, and fixed in 10% neutral buffered formalin. The tissues were processed using standard histological procedures, including dehydration in graded alcohol, clearing with xylene, and embedding in paraffin wax. Sections (5 µm thick) were cut, mounted on glass slides, and stained with hematoxylin and eosin. Renal histology was examined under a light microscope, and photomicrographs were captured for evaluation. Histological assessment was performed without prior knowledge of the group allocation.

2.7. Blood sample collection and renal biochemical analysis

Blood samples were collected by cardiac puncture immediately after sacrifice and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 10 minutes and used for renal biochemical analysis. Serum electrolytes (sodium, potassium, chloride, and bicarbonate) were determined using ion-selective electrode (ISE) methods according to the manufacturer's instructions. Serum urea concentration was measured using an enzymatic urease-Berthelot colorimetric method, in which urea is hydrolyzed to ammonia and carbon dioxide to produce a measurable color change proportional to the urea concentration. Serum creatinine was determined using the kinetic Jaffe colorimetric method, in which creatinine reacts with alkaline picrate to form a colored complex. All biochemical analyses were performed using a Thermo Scientific™ Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, United States) following calibration according to the manufacturer's instructions. The results were calculated from standard calibration curves and expressed in standard international units.

2.8. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 9.5.1 (GraphPad Software, USA). Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

3. Results and discussion

The effects of polyherbal intake on renal biochemical parameters are presented in Table 1. Serum urea levels showed a statistically significant difference among the experimental groups ($p < 0.05$), with higher values observed in rats administered *T. tetraptera* fruit extract alone and in combination with *J. curcas* leaf extract. Despite this increase, the urea concentrations remained within the physiological limits reported for healthy Wistar rats, suggesting preserved renal excretory capacity. Serum urea is a sensitive indicator

https://doi.org/10.58985/jpam.2026.v04i01.40

Table 1. Effect of polyherbal intake on serum biochemical parameters of Wistar rats.

Parameter	Group A	Group B	Group C	Group D	p-value
Urea (mg/dL)	26.00 ± 1.78	32.00 ± 1.00	27.50 ± 0.65	32.00 ± 1.08	0.016*
Sodium (mmol/L)	134.00 ± 1.47	137.50 ± 0.50	135.50 ± 0.87	130.75 ± 2.14	0.095
Potassium (mmol/L)	3.80 ± 0.15	4.00 ± 0.10	4.25 ± 0.12	3.88 ± 0.06	0.075
Bicarbonate (mmol/L)	17.25 ± 1.11	16.00 ± 1.00	19.75 ± 0.48	19.50 ± 0.65	0.043*
Chloride (mmol/L)	94.25 ± 3.71	105.50 ± 3.50	97.00 ± 1.47	88.25 ± 0.48	0.011*
Creatinine (mg/dL)	0.43 ± 0.05	0.45 ± 0.05	0.53 ± 0.05	0.53 ± 0.03	0.283

Values are presented as mean ± standard error of mean (SEM) (n = 6 per group). Group A = Control (distilled water); Group B = *T. tetraptera* fruit extract (200 mg/kg); Group C = *J. curcas* leaf extract (400 mg/kg); Group D = Combined extracts (*T. tetraptera* 200 mg/kg + *J. curcas* 400 mg/kg).

*p < 0.05 indicates statistically significant difference compared with control (one-way ANOVA followed by Tukey's post hoc test).

Table 2. Effect of polyherbal intake on body weight and kidney weight of Wistar rats.

Parameter	Group A	Group B	Group C	Group D	p-value
Initial body weight (g)	127.50 ± 3.77	115.00 ± 2.97	139.25 ± 8.38	143.00 ± 4.64	0.185
Final body weight (g)	153.50 ± 6.66	132.75 ± 2.46	152.25 ± 7.78	158.50 ± 7.96	0.599
Right kidney weight (g)	0.48 ± 0.08	0.50 ± 0.07	0.55 ± 0.06	0.52 ± 0.09	0.912
Left kidney weight (g)	0.47 ± 0.07	0.50 ± 0.06	0.55 ± 0.06	0.51 ± 0.09	0.894

Values are presented as mean ± standard error of mean (SEM). Differences were considered significant at $p < 0.05$.

of renal function, and moderate variations without concurrent creatinine elevation are generally not indicative of overt renal impairment [1, 2]. Similar observations have been reported in experimental studies assessing the renal safety of *J. curcas* and *T. tetraptera* at moderate oral doses, where mild biochemical fluctuations were not associated with renal dysfunction [12,16,17].

Serum bicarbonate and chloride concentrations also differed significantly among the groups ($p < 0.05$). However, the absence of a consistent pattern of electrolyte imbalance across the treatment groups suggests that the tubular handling of electrolytes remained largely intact. Electrolyte regulation is a key renal function, and its disruption is often associated with tubular injury or impaired acid-base balance [1, 3]. Sodium and potassium levels did not differ significantly among the groups, indicating the maintenance of electrolyte homeostasis following the administration of plant extracts. The preservation of electrolyte balance following controlled exposure to *J. curcas* extracts has been documented in previous renal safety and toxicity studies [12,18].

Serum creatinine levels were not significant different among the experimental groups. Since creatinine is a reliable indicator of glomerular filtration efficiency, the absence of elevation indicates that glomerular function was not adversely affected by the single or combined administration of the extracts [2]. This finding is consistent with earlier experimental reports demonstrating that aqueous extracts of *J. curcas* and *T. tetraptera* do not impair renal filtration when administered within the established safety margins [13,17,19].

The changes in body weight and kidney weights are presented in Table 2. No statistically significant differences were observed in the initial or final body weights among the groups, suggesting that the treatments did not adversely affect the growth or general metabolic status of the animals. Similarly, right and left kidney weights did not differ significantly among the experimental groups. Organ weight assessment is a sensitive indicator of systemic and organ-specific toxicity. The absence of kidney weight alterations suggests that the extracts did not induce renal hypertrophy or atrophy. Comparable findings have been reported in subacute toxicity and renal safety studies involving *J. curcas* and other medicinal plants administered within controlled dose ranges [14,16, 20].

Histological evaluation of renal tissues, as shown in, Fig. 1 revealed preserved renal architecture across all

https://doi.org/10.58985/jpam.2026.v04i01.40

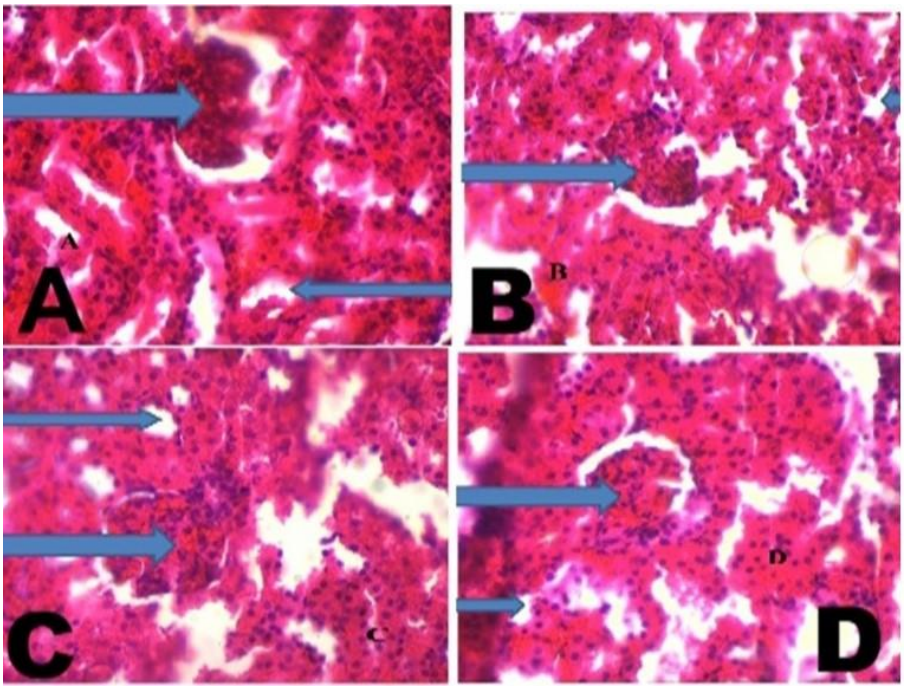

Figure 1. Representative photomicrographs of renal histology showing preserved glomeruli and renal tubules across groups (H&E, ×400).

groups. The glomeruli appeared normal with intact mesangium, capillary loops, and epithelial lining. Renal tubules were well organized and lined by intact cuboidal epithelium, with no evidence of tubular degeneration, necrosis, or inflammatory infiltration. The histological preservation of renal tissues is a critical indicator of nephro-safety and complements biochemical findings in toxicity assessment [3]. The preservation of renal histoarchitecture following exposure to *Jatropha* species and *T. tetraptera* has been reported in previous experimental studies, particularly where antioxidant and anti-inflammatory phytoconstituents are implicated [8-11,17, 21].

Fig. 1 show preserved renal architecture across all groups, characterized by normal glomeruli with intact mesangium, capillary loops, and epithelium, as well as well-organized renal tubules lined by cuboidal epithelium, with occasional luminal pale eosinophilic material. No evidence of glomerular distortion, tubular degeneration, necrosis, or inflammatory infiltration was observed (H&E, ×400).

Overall, the concordance between the renal biochemical indices, organ weight measurements, and histological findings suggests that subacute oral administration of aqueous extracts of *T. tetraptera* fruit and *J. curcas* leaves did not induce nephrotoxic effects at the investigated doses. The minor biochemical variations observed did not translate into functional or structural renal damage, indicating physiological adaptation rather than pathological alteration. These findings are consistent with broader evidence indicating that medicinal plants, when appropriately processed and administered within controlled dose ranges, may exhibit renal tolerance and safety in experimental models [6, 7, 22, 23].

3.1. Limitations of the study

Despite the strengths of the present study, it has certain limitations. The experimental duration was limited to 28 days, which may not fully reflect the renal effects associated with the long-term or chronic consumption of polyherbal formulations. In addition, the assessment of renal function was restricted to conventional biochemical indices and routine histological evaluation. Therefore, subtle molecular alterations involving oxidative stress pathways, inflammatory mediators, or apoptotic signaling were not investigated. The use of only female Wistar rats may also limit the extrapolation of the findings across sexes. Furthermore, dose-response relationships beyond the selected treatment doses were not investigated.

https://doi.org/10.58985/jpam.2026.v04i01.40

4. Conclusions

The findings of this study indicate that subacute oral administration of aqueous extracts of *T. tetraptera* fruit and *J. curcas* leaves, administered singly or in combination, did not adversely affect renal biochemical indices or tissue architecture in Wistar rats. The results suggest preserved renal function and structural integrity at the tested doses, supporting the short-term renal safety of this polyherbal formulation under controlled conditions.

Ethical statement

All experimental procedures were performed in accordance with internationally recognized ethical standards for animal research and were approved by the accredited Institutional Ethics Review Committee (approval number: MAFSAEC: 025-08/25/0043).

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The authors acknowledge the use of ChatGPT (OpenAI) exclusively for language refinement, formatting, and alignment with the journal's author guidelines. Artificial intelligence tools were not utilized for data generation, statistical analysis, interpretation of results, or scientific decision-making. The authors retain full responsibility for the integrity and content of the manuscript.

Authors' contributions

Conceptualization, B.E.O., E.B.O.; methodology, B.E.O.; software, B.E.O.; validation, B.E.O., E.B.O.; formal analysis, B.E.O.; investigation, B.E.O.; resources, E.B.O.; data curation, B.E.O.; writing - original draft and preparation, B.E.O.; writing review and editing, B.E.O., E.B.O.; visualization, B.E.O.; supervision, E.B.O.; project administration, E.B.O.

Acknowledgements

The authors declare no acknowledgements.

Funding

This research was conducted without external financial support.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

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