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Original Research Article

Effect of aqueous extract of *Bryophyllum pinnatum* on kidney and haematological indices of adult female Wistar rats

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Abstract

Purpose: *To investigate the effect of aqueous leaf extract of Bryophyllum pinnatum on haematological indices, biochemical parameters, and kidney histology using female Wistar rats.*
Methods: *Twenty female Wistar rats were divided randomly into four groups of five animals per group. Group A received 100 mg/kg, Group B 200 mg/kg, Group C 400 mg/kg of the extract and Group D (control) received feed and water ad libitum; all via the oral route. The animals were housed in clean, well-ventilated cages that provided sufficient lighting for the duration of the experiment.*
Results: *Findings revealed no significant differences in biochemical and haematological indices across the experimental groups and the control. Histological findings showed alterations in the microscopic architecture of the kidney, ranging from crescent-shaped glomeruli seen in Group A animals to glomerular atrophy in Group B and thrombosed glomerular vessels, haemorrhage, and interstitial oedema seen in Group C animals.*
Conclusion: *The findings of this study suggest that consumption of aqueous extract of B. pinnatum may have compromised the microarchitecture of the kidneys of Wistar rats. Hence, excessive and/or unregulated consumption of B. pinnatum extract is not totally safe in rats and should be closely monitored.*

Keywords: *Aqueous extract, Bryophyllum pinnatum, Medicinal plant, Kidney, Wistar rats*

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INTRODUCTION

Bryophyllum pinnatum (Lam.) Oken *(B. pinnatum)* is an extensively used medicinal plant for the treatment of a variety of conditions, such as diabetes mellitus, hypertension, diarrhoea, and wound healing in Africa, tropical America, and other countries like India, China, and Australia [1]. There has been significant demand for herbal medicine; however, many of the claims associated with it have not yet been scientifically validated [2]. Herbal medicines have long been regarded as a valuable source of treatment, and as a result, they are increasingly integrated into contemporary high-tech medical practices [3]. It is worthy of note that some medicinal plants and herbal products could as well be toxic to organs

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like the liver and kidneys when not taken in safe dosages [4].

Medicinal plants and herbal products have the potential to be harmful to organs such as the ovaries if consumed in excessive dosages [5]. The leaves of *B. pinnatum* are known to contain elevated levels of alkaloids and mild levels of saponins, which may be toxic to organs when ingested in high doses [5]. Leaves of *B. pinnatum* contain several active compounds such as alkaloids, flavonoids, steroids, triterpenes, bufadienolides and organic acids, such as malic acid, isocitric acid, and succinic acid [1,6]. The leaves also contain various vitamins, including ascorbic acid, riboflavin, thiamine, and niacin, as well as essential minerals like calcium, zinc and phosphorus [7].

According to Harlalka *et al* [8], the aqueous extract of leaves of the plant was found to possess potent nephroprotective activity against gentamicin-induced toxicity in nephrons of experimental rats. The aqueous extract of *B. pinnatum* leaves was also effective in the prevention and treatment of ethylene glycol-induced urolithiasis in Wistar rats [9]. The present study aimed at assessing the effect of aqueous leaf extract of *B. pinnatum* on histological and biochemical parameters of kidneys and haematological indices in Wistar rats.

EXPERIMENTAL

This study was conducted in the animal housing facility of the Human Anatomy Department, Nnamdi Azikiwe University (NAU), Okofia Campus. The animals were housed in cages that were regularly cleaned and well-ventilated, with 12 hours of light and 12 hours of dark, a relative humidity of the laboratory environment between 40 and 70 %, and a temperature between 18 and 26 °C.

Animals

Thirty-three female adult Wistar rats, with weights ranging between 100 and 200 g, were utilised. Of these, thirteen (13) rats were used for the determination of the median lethal dose (LD_{50}) using Dietrich Lorke's [10] method, while the remaining twenty (20) were used for the experiment. The rats were sourced from a private farm located in Nnewi, Nigeria, and were fed with a standard commercial rat diet along with free access to drinking water throughout the duration of the experiment.

Ethical statement

The study protocol was approved by the Research Ethics Committee of the Faculty of Basic Medical Sciences, NAU (Protocol Reference: NAU/CHS/NC/FBMS/140). It was carried out in compliance with the regulations of the Research Ethics Committee as well as "humane animal care" as stated in the Guide for the care and use of laboratory animals [11].

Plant source and identification

Clean fresh leaves of the plant were collected from a farm located in Awka, Anambra State, Nigeria. They were subsequently verified and authenticated at the herbarium unit of the Department of Botany, NAU, Awka, Nigeria, where they were assigned the voucher number NAU/BOT/609.

Preparation of extract

The fresh leaves of *B. pinnatum* obtained were thoroughly washed under clean, running water to remove any foreign matter. Subsequently, the leaves were chopped and shade-dried for two weeks. The dried leaves were ground into a coarse powder. A 300 g portion of powdered sample was soaked in 1.5 L of water and allowed to stand for 24 hours. Thereafter, the mixture was filtered through muslin cloth and then through Whatman filter paper to achieve a homogeneous extract. The resulting aqueous extract yielded a weight 90 g, which was stored in the refrigerator for the entire duration of the experiment.

Phytochemical analysis of plant extract

A small sample of the aqueous leaf extract was used for phytochemical analysis. This was carried out in the pharmaceutical laboratory of the Pharmaceutical Microbiology and Biotechnology Department, NAU, Agulu Campus, using standard procedures as described by Sofowora [12].

Extract for administration

The extract was prepared daily by dissolving 1 g of *B. pinnatum* extract in 10 mL of distilled water, resulting in a stock solution (5 mg/mL). The distilled water used was sourced from the Medical Biochemistry Department, NAU in Nnewi, Anambra, Nigeria. The extract was administered at dosages of 100, 200, and 400 mg/kg for groups A, B, and C.

Protocol

Twenty (20) rats were acclimatised for two weeks, with free access to feed and drinking water. At the end of acclimatisation, the rats were randomly divided into four groups: A, B, C, and D, with five rats each. Group D, the control received drinking water and feed freely, and the test groups were A, B and C. The rats were weighed before administration of the test substance, and this was recorded as the initial weight. Animals were also weighed weekly and their weights recorded. The test groups received the extract at different doses as follows: Group A (low dose) received 100 mg/kg, Group B (medium dose) received 200 mg/kg, and Group C (high dose) received 400 mg/kg. The extracts were given orally via a syringe without a needle once daily in the evening, specifically between 6:00 PM and 7:15 PM GMT+1. The administration always commenced with the rats in Group A and proceeded alphabetically through Groups B and C over a period of 56 days.

Twenty-four hours after the last administration, the rats were weighed, and body weight was recorded as the final body weight. The rats were anaesthetised with chloroform applied to cotton wool and placed in an enclosed jar. Following anaesthesia, blood samples were obtained from two (2) rats per group directly using a microcapillary tube into a plain bottle via ocular puncture (for biochemical analysis) [5] and an ethylenediaminetetraacetic acid (EDTA) bottle (for haematological analysis), after which the rats were sacrificed. Each of the sacrificed rats was placed on a flat board in a supine position, with limbs firmly fixed to the dissection board using thumb pins [5]; the abdomen was humanely dissected. Kidneys were harvested from each of the sacrificed rats, weighed in pairs on an electronic weighing balance following the suggestion of Sellers *et al* [13] that paired organs should be routinely weighed together, washed and cleared free of blood, then examined macroscopically. The relative weights of the organs were calculated as the absolute weight of the organ divided by the body weight of the rat on the day of sacrifice [14]. Thereafter, the organs were preserved in a specimen container with 10 % formalin as a fixative for histological procedures. Biochemical and haematological analyses were done using sera and whole blood collected from the rats. Systemx XE-2100 Hematology analyser manufactured by Systemx America, Batch No: 00127819 made in the United States of America was used for haematological analysis. Jaffe-Slot method was used for quantitative determination of creatinine while Diacetyl monoxime method was used for the quantitative determination of urea. For each blood sample, serum was separated through centrifugation and stored at −20 °C for biochemical analysis.

Tissue processing

Tissues of the kidneys were processed by immersing in 10 % formalin as a fixative for 24 hours immediately after harvest to preserve tissue structure. The fixed tissues were dehydrated to extract water by placing them in a series of increasingly concentrated alcohol baths (50, 70, 90, 95, and 100 % absolute alcohol, once for 2 hours each but twice in absolute alcohol). The dehydrated tissues were then dealcoholised with the clearing agent xylene for 1 to 2 hours, and placed in a bath of molten paraffin wax at a temperature of 60 °C for 2 hours for infiltration. The infiltrated tissues were embedded. Five-micron-thick sections were stained with haematoxylin and eosin.

Statistical analysis

Data from the research were analysed using the Statistical Package for the Social Sciences (SPSS) software version 21.0, IBM Corp., (Armonk, NY, USA). Statistical analysis of variance was carried out using one-way ANOVA to determine the difference among the groups. Post-hoc analysis was done to determine the specific nature of each group using the least significant difference test. A value of $p < 0.05$ was used as the level of significance. The results are expressed as mean ± standard deviation (SD) and mean difference for the least significant difference test.

RESULTS

Acute toxicity

The median lethal dose (LD_{50}) procedure was conducted in two distinct phases, with animals divided into specific groups during each phase. Upon completion of the procedure, the LD_{50} for *B. pinnatum* was 3807.87 mg/kg.

Physical observation

There were no obvious physical or behavioural changes observed in the rats across all test groups following administration of aqueous extract of *B. pinnatum*.

Phytochemical results

Qualitative phytochemical constituents of the extract, following analysis, showed mild presence

(+) for tannin, saponin, terpenoid, cardiac glycoside; moderate presence (++) for steroid, and markedly high presence (+++) of alkaloid and flavonoid.

Organ weight (OW) and relative organ weight (ROW) of the rats

There was no significant difference in organ weight and relative organ weight of the rats across test groups (Table 1).

Haematological analysis

There was no significant difference ($p > 0.05$) in haematological parameters across the groups (Table 2).

Biochemical analysis

There was no significant difference in renal function parameters ($p > 0.05$) across the groups (Table 3).

Histological examination

The animals in the control group showed normal histology with normal-appearing glomeruli (Figure 1 A). However, microscopic analysis of the kidneys of animals in the test groups revealed pathological lesions. A crescent-shaped glomerulus was observed in the kidney of animals in Group A (low dose; Figure 1 B). Animals in Group B that received medium doses of *B. pinnatum* showed normal tubules with glomerular atrophy (Figure 1 C). However, the histology of the kidneys of animals that received high doses of *B. pinnatum* (Group C) showed a series of distortions, including a lymphocytic infiltrate around the tubules, thrombosed glomerular vessels, interstitial oedema, and haemorrhage (Figure 1 D).

DISCUSSION

Medicinal plants are frequently regarded as safe, often without a thorough examination of the potential toxicities that may be linked to them [15]. While medicinal plants play a role in disease treatment, this does not guarantee their safety for public use [16]. Researchers investigated the effects of the aqueous extract of *B. pinnatum,* a plant commonly used in folk medicine to address various disorders in Nigeria and other regions, specifically focusing on its impact on the kidneys and haematological indices of female Wistar rats. This study employed biochemical and histological methods over a duration of 56 days.

Table 1: Organ weight (OW), Relative organ weight (ROW) of test and control groups

Parameter	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)	Control	*P*-value
OW (g)	0.97±0.27	1.35±0.02	1.13±0.07	1.34±0.09	0.157
ROW (g/g)	0.006±0.00	0.007±0.00	0.007±0.00	0.008±0.00	0.625

Table 2: Haematological parameters in test and control groups

Parameter	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)	Control	F-Ratio	*P*-value
HB (g/dL)	19.00±1.41	18.50±0.71	19.00±2.83	20.00±0.00	0.302	0.824
PCV (%)	58.50±2.12	56.00±1.41	58.00±5.66	60.50±0.71	0.701	0.599
WBC (10^9/L)	7.95±1.91	6.25±0.92	5.75±0.21	4.80±0.85	2.652	0.185
PLAT (10^9/L)	313.50±50.20	365.00±93.34	288.00±80.61	243.00±21.21	1.141	0.434
Neutr	24.50±13.44	29.50±12.02	45.00±7.07	43.50±6.36	1.997	0.257
Lymph	70.00±16.97	64.50±13.44	51.00±7.07	52.00±2.83	1.336	0.381
Eosino	4.00±1.41	4.00±1.41	3.50±0.71	3.50±2.12	0.074	0.971
Mono	2.50±0.71	2.50±0.71	1.00±0.00	2.00±0.00	4.000	0.107

The data presented in Table 2 are means ± standard deviations. The table showed no significant difference ($p > 0.05$) in HB (haemoglobin) $p > 0.05$, PCV (packed cell volume) $p > 0.05$, WBC (white blood cells) $p > 0.05$, PLAT (platelets) $p > 0.05$, Neutr (neutrophils) $p > 0.05$, Eosino (eosinophils) $p > 0.05$ and Mono (monocytes) $p > 0.05$ across the groups

Table 3: Kidney function test parameters

Parameter	Group A 100 mg/kg	Group B 200 mg/kg	Group C 400 mg/kg	Control	F-Ratio	*P*-value
Urea (mmol/L)	5.20±0.00	5.30±0.14	5.15±0.49	4.95±0.07	0.642	0.627
Creatinine (µmol/L)	83.20±1.41	76.00±15.56	74.50±13.44	66.50±3.54	0.839	0.539

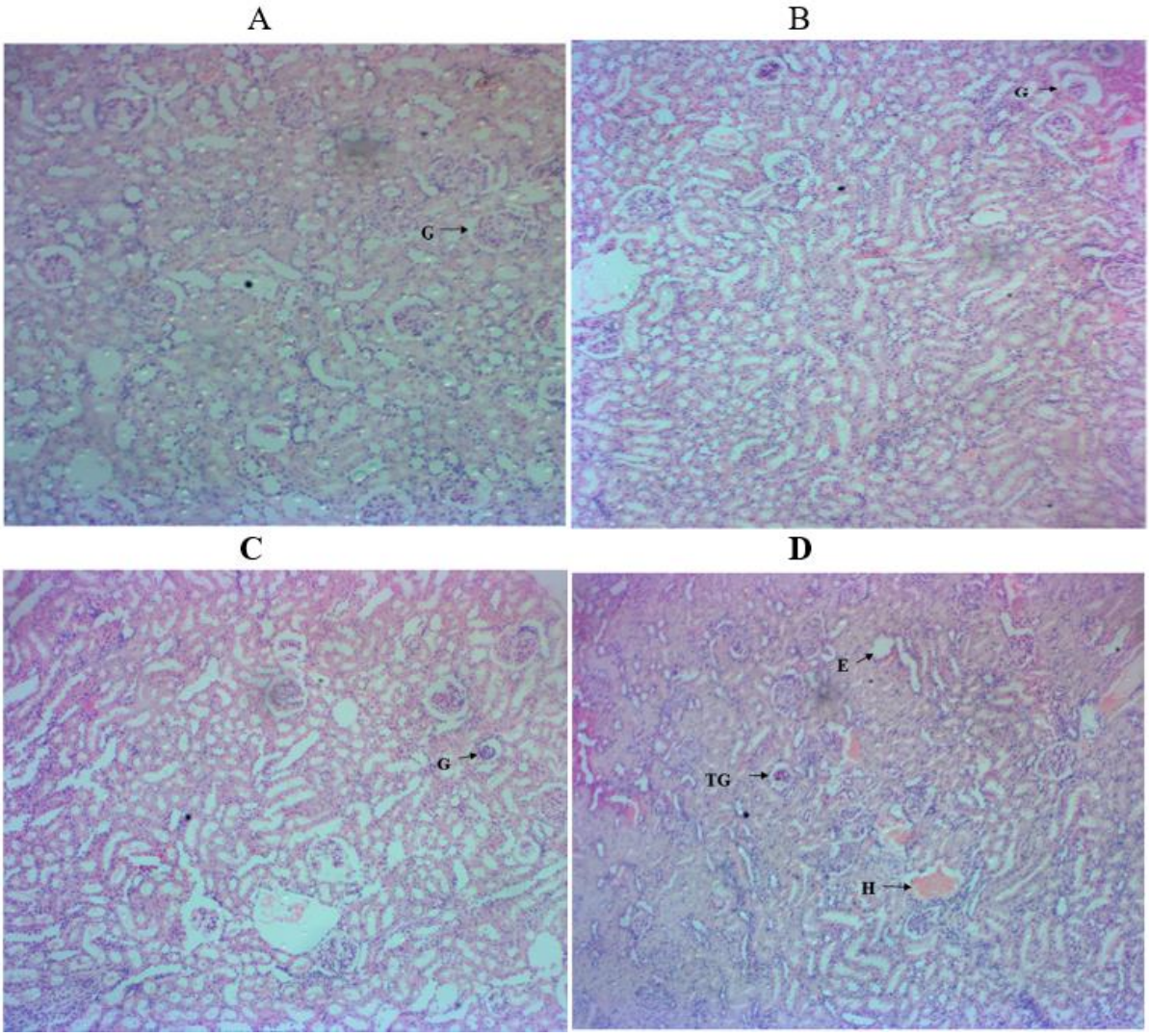


Figure 1: H & E stain of kidney (A) control group showing normal histology with normal glomeruli; (B) Group A of adult female Wistar rat showing crescent-shaped glomerulus; (C) Group B of adult female Wistar rat showing glomerular atrophy (G) and normal tubules; (D) Group C of adult female Wistar rat showing thrombosed glomerular vessels (TG), interstitial oedema (E), and haemorrhage (H) ×100

The qualitative phytochemical analysis of the leaf of *B. pinnatum* from this study showed the marked presence of alkaloid and flavonoid, and a moderate to mild presence of steroid, tannin, saponin, terpenoid, and cardiac glycoside. These findings are consistent with results from several previous studies [1,4,7]. The active compounds identified in *B. pinnatum* may account for its considerable medicinal significance.

The kidney is an important organ in homeostasis and in waste product elimination [17]. Organ weight is one of the most sensitive indicators of the effect of a test substance, as significant differences in organ weight between animals in test groups and control can occur in the absence of any morphological changes [18, 19]. There was no significant difference ($p > 0.05$) in the weight of kidneys across the groups (tests and control) as represented in the results of this research. This may suggest that the test substance had no toxic effect on the kidneys. However, toxicity evaluation of the liver, kidney, and heart can be best predicted using the organ-to-body weight ratio [14]. The statistical analysis of organ body weight (relative organ weight) in this study likewise showed no significant difference ($p > 0.05$) in kidney relative organ weight across the groups, suggesting that, based on the toxicity evaluation, there was no toxic effect on the organ. The result is similar to that of Ozolua *et al* [20] and Aprioku and Igbe [1].

The result of the haematological parameters showed that there was no significant difference ($p > 0.05$) across the groups. This is an indication that there was no toxic effect of the extract on these blood parameters. The study by

Ozolua *et al* [20] of rats administered with 2 g/kg body weight of *B. pinnatum* orally for 35 days reported no significant difference ($p > 0.05$) in haematological indices across the study groups.

Biochemical analysis of renal indices, such as urea and creatinine, can be used to evaluate nephron function in animals, with elevated values indicative of a defective functional state [22]. Urea and creatinine are end products of protein (amino acids) and muscle metabolism, respectively. Serum urea and creatinine levels showed no significant difference ($p > 0.05$) across the groups. The mean value of serum creatinine level showed a non-significant increase. This change, though not significant, does not necessarily reflect kidney dysfunction or impairment, as other factors, such as muscle metabolism, may contribute to an elevated serum creatinine level. The study by Shukla *et al* [9] reported significantly improved serum creatinine and blood urea levels in animals with ethylene glycol-induced renal calculi that were treated with the aqueous extract of *B. pinnatum.* The difference in the results of this research may be due to the route of administration used in the experiment (intraperitoneal). Another study also observed significantly lower serum creatinine levels in the treatment groups compared with the control group [4]. Study by Aprioku *et al* [1] reported that administration of the aqueous *B. pinnatum* leaf extract did not alter blood urea and creatinine levels [1].

The histological assessment of this study indicated that there were signs of abnormality in the structure of the kidney across the test groups. This is derived from the structural glomerular compromise shown by the crescent-shaped glomerulus observed in Group A, to a few atrophied glomeruli evident in Group B, to thrombosed glomerular vessels, interstitial oedema, and haemorrhage observed in Group C. All these may imply that the kidneys were most likely undergoing compromise, even though this was not reflected in the relative organ weight, organ weights, and biochemical parameters of the kidneys. The change in kidney histomorphology may be attributed to the plant's bufadienolide content, which has been reported to be toxic [6]. A previous study suggested that an aqueous extract of *B. pinnatum*, administered intraperitoneally at a dose of 125 mg/kg for 8 days, reduced glomerular congestion, accumulation of inflammatory cells, epithelial desquamation, and necrosis of kidney cells induced by gentamicin nephrotoxicity in Wistar albino rats [8]. The study by Ghasi *et al* [4] reported mild changes in kidney histomorphology, including shrunken glomerulus, increased urinary space, mild tubular necrosis, and hyaline droplets following treatment with *B. pinnatum*.

CONCLUSION

This study has demonstrated that *B. pinnatum* aqueous leaf extract showed no toxic effects on haematological and biochemical indices of the kidney but appeared to compromise the histology of the kidney. This suggests that using this plant may not be a completely safe herbal remedy.

DECLARATIONS

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None.

Ethical approval

It is stated in the Experimental section.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

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

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors contributed to the conception, execution as well as writing and editing of this manuscript.

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