

# Effects of Aqueous *Bryophyllum pinnatum* Leaf Extract on Haematological, Renal and Sperm Indices in Wistar Rats

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## Aprioku and Ighodaro: Toxicity Profile of *Bryophyllum pinnatum* Leaf Extract

*Bryophyllum pinnatum* is a perennial herb, widely used in the treatment of several conditions in folklore medicine. The effects of sub-acute treatment of the aqueous extract of *B. pinnatum* leaf on haematological, renal and testicular functions were investigated in the Wistar rat. Adult rats were randomly distributed into three groups of six rats each and administered 0, 62.5 or 125 mg/kg of extract daily for 28 days by oral gavage. Blood levels of urea, creatinine and haematological parameters, as well as epididymal sperm parameters were measured. Kidney and testis of animals were removed and organ weight to body weight ratios were determined. Histology of the organs was also analysed. The extract elevated white blood cell count, reduced neutrophil count ( $P < 0.05$ ) without affecting lymphocyte count and packed cell volume, when compared to control. The extract did not alter sperm count, motility, morphology and viability as well as blood urea and creatinine levels. The extract did not alter histology and relative weights of the kidney and testis. It can be concluded that sub-acute administration of the aqueous extract of *B. pinnatum* did not produce any renal and testicular toxicity, but may depress neutrophil action.

**Key words:** *Bryophyllum pinnatum*, flavonoids, neutrophil, sub-acute, testis

*Bryophyllum pinnatum* (Lam.), syn. *Kalanchoe pinnata* (Lam.), and *B. calycinum* (Salisb.) is a widely distributed perennial medicinal herb. It is native to Madagascar, but has been naturalized in several other regions, including the temperate regions of Asia, Australia, and New Zealand. *B. pinnatum* is known by some common names including, life plant, air plant, maternity plant, love plant, miracle leaf, cathedral bells, mother of thousands, leaf of resurrection plant, and *Lao di Sheng gen*. The plant is locally called "Never Die" in Nigeria, and is very popular in folklore medicine. It has been used for the treatment of a variety of conditions in tropical America, India, China, Australia and Africa, including, rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcer, diabetes mellitus, microbial infections, and hypertension<sup>[1-6]</sup>. In Nigeria, the plant is particularly known for its effective wound healing properties and detachment of the umbilicus of infants. Pharmacological studies on *B. pinnatum* reported several biological activities some of which could authenticate the plant's traditional uses including, immunomodulatory<sup>[7]</sup>, CNS depressant<sup>[8]</sup>, analgesic,

antiinflammatory<sup>[9]</sup>, antimicrobial<sup>[10,11]</sup>, antitumor<sup>[12]</sup>, antiulcer<sup>[13]</sup>, insecticidal<sup>[14]</sup>, antidiabetic<sup>[15]</sup>, anticonvulsant<sup>[16]</sup>, antioxidant<sup>[17]</sup>, and antihypertensive properties<sup>[18]</sup>. Studies have also reported a wide range of active phytochemicals such as alkaloids, triterpenes, glycosides<sup>[18]</sup>, flavonoids<sup>[16,19]</sup>, steroids<sup>[9]</sup>, bufadienolides<sup>[20]</sup>, lipids and organic acids<sup>[21]</sup>. These compounds have been considered to be responsible for the plant's diverse pharmacological activities.

Although, folklore claims of many herbal remedies are yet to be authenticated scientifically, *B. pinnatum* has been reasonably studied with justification of most of the claims. This has enhanced the promotion of the use of *B. pinnatum* and other plants either as alternatives or to complement orthodox medicines. Additionally,

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cost of orthodox medications and the development of resistance to most orthodox chemotherapeutic agents has resulted in the use of herbal medications like *B. pinnatum* leaf as alternatives, especially in underdeveloped countries<sup>[22,23]</sup>. In many cases, *B. pinnatum* leaves were soaked in cold water overnight, boiled in water, squeezed or roasted and the extracts obtained are used to treat fevers, headache, joint pains, arthritis, body pains, asthma, cancers, tonsillitis, diarrhoea, cough, and many other conditions<sup>[2-4,6]</sup>. Aside from the earlier stated factors, *B. pinnatum* is also a popular herbal medicine due to local belief that natural extracts are free of adverse effects<sup>[23,24]</sup>. This makes safety evaluation of frequently used medicinal plants imperative. The present study was thus aimed at investigating the effects of sub-acute administration of aqueous *B. pinnatum* leaf extract on haematological, renal and testicular indices in rats.

## MATERIALS AND METHODS

Fresh leaves of *B. pinnatum* were collected from the botanical garden of the University of Port Harcourt, Port Harcourt. The leaves were air dried, pulverized (400 g) and extracted exhaustively in distilled water for 72 h by cold maceration. The filtrate was subsequently evaporated to obtain the dry extract using a rotary evaporator. The dry extract was weighed (28 g) and the percentage yield was determined (7%). The extract was then tested for the presence of phytochemicals using standard methods<sup>[25]</sup>.

### Experimental animals:

Eighteen adult male Wistar rats of 12 w of age, obtained from the animal house of the University of Port Harcourt, Nigeria were used for the study. The animals were given standard rodent chow and clean drinking water *ad libitum*. The animals were kept in a well-ventilated room with a 12 h light/dark cycle at room temperature. All animal experiments were approved by the Animal Research Ethics Committee of the University, in accordance to the guide for care and use of laboratory animals<sup>[26]</sup>.

### Experimental design:

The animals were randomly distributed into three groups of 6 rats per group. The first and second groups were administered 62.5 and 125 mg/kg of aqueous *B. pinnatum* leaf extract by oral gavage (1 ml/kg) daily for 28 d, respectively. The animals in the third group served as control and were given only distilled water (1 ml/kg) daily for 28 d. At the end of extract

administration, the rats were sacrificed by cervical dislocation under deep diethyl ether anaesthesia. Blood samples were collected separately into labelled plain and EDTA bottles, for measurement of urea, creatinine and haematological parameters, respectively. Animals were dissected and sperm was extracted from cauda epididymis for sperm analysis. The kidney and testis were also removed, weighed and routinely processed for histological evaluation.

Blood sample was centrifuged for 15 min at 3000 rpm to separate serum and the serum was stored at  $-80^{\circ}$ . Serum levels of urea and creatinine were then assayed. Creatinine was measured using alkaline picrate method<sup>[27]</sup>, and urea was assayed using urease-Berthelot method<sup>[28]</sup>. Whole blood collected into EDTA bottle was used to assay packed cell volume (PCV), and total white blood cell (WBC), lymphocyte and neutrophil counts, using standard laboratory techniques<sup>[29]</sup>.

### Sperm analysis:

The method of Amelar *et al.*<sup>[30]</sup> was used in collecting sperm cells from the epididymis. Briefly, the testis was excised and the caudal epididymis was carefully isolated and placed in a petri dish containing 3 ml of sodium bicarbonate ( $\text{NaHCO}_3$ ) buffered Tyrodes' solution. Several (1 mm) incisions were made on it and sperm was gently drawn into a plastic transfer pipette and transferred into 5 ml test tubes and vigorously shaken for homogeneity and dispersal of sperm cells. Sperm was then analysed to determine sperm motility, sperm count, percentage of abnormal sperm cells (sperm morphology) and percentage of viable sperm cells (sperm viability) following standard procedures<sup>[29]</sup>.

### Histopathological analysis:

The kidney and testicular tissues were fixed in 10% buffered formalin. The tissues were embedded in paraffin and tissue sections (5-7  $\mu\text{m}$ ) were stained with haematoxylin and eosin (H and E) and examined with light microscope (Nikon Eclipse E400). All alterations from the normal structures were registered and histopathological changes between control and experimental rats were noted. The images were photographed with an Olympus Model BX51 microscope at a magnification of 400x.

### Statistical analysis:

The results are presented as mean $\pm$ SEM for each group. Differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Dunette's multiple comparison test. Data were

analysed using GraphPad Prism Version 5 and values were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The doses used in the present study were selected with the specific aim of evaluating the effect of lower biologically active doses of the extract. The  $LD_{50}$  of *B. pinnatum* (the aqueous extract) has been reported to be 1.8 g/kg (i.p.) in rats; while no obvious toxicological signs were observed at 5 g/kg dose administered orally<sup>[31]</sup>. In many previous animal studies, doses of *B. pinnatum* used range from 150 mg/kg to as high as 2000 mg/kg. The toxicological profile of sub-acute treatment of the aqueous extract of *B. pinnatum* leaf (62.5-125 mg/kg) on renal, haematological and testicular indices in the rat is herein reported.

Phytochemical analysis of aqueous *B. pinnatum* leaf extract showed the presence of alkaloids, carbohydrates, flavonoids, saponins, triterpenoid, tannins/phenols, anthraquinone and steroids (Table 1). Administration of the extract did not cause any significant change ( $P > 0.05$ ) in serum levels of urea and creatinine when compared to the control (Table 2). Sperm counts and motility obtained in extract treated rats were not significantly different ( $P > 0.05$ ) from those of control animals (Table 3). Percent viable sperms (sperm viability) and abnormal sperms (sperm morphology) in extract-treated rats were also not altered, when compared to control values (Table 3).

**TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF AQUEOUS *B. PINNATUM* LEAF EXTRACT**

Phytochemical constituents	Results
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Saponins	+
Triterpenoid	+
Tannins/phenols	+
Cardenolide	-
Anthraquinone	+
Steroids	+

‘+’ Indicates present, ‘-’ indicates absent

**TABLE 2: EFFECTS OF SUB-ACUTE ADMINISTRATION OF AQUEOUS *B. PINNATUM* LEAF EXTRACT ON SERUM LEVELS OF UREA AND CREATININE IN WISTAR RATS**

Dose	Urea (mmol/l)	Creatinine ( $\mu$ mol/l)
Control	3.38 $\pm$ 1.2	32.00 $\pm$ 3.67
62.5 mg/kg	3.37 $\pm$ 1.41	36.00 $\pm$ 4.50
125 mg/kg	3.18 $\pm$ 1.32	35.50 $\pm$ 2.57

Data expressed as mean $\pm$ SEM, n=6 animals per group

**TABLE 3: EFFECTS OF SUB-ACUTE ADMINISTRATION OF AQUEOUS *B. PINNATUM* LEAF EXTRACT ON SPERM PARAMETERS IN WISTAR RATS**

Dose (mg/kg)	Sperm count ( $\times 10^6$ )	Sperm motility (%)	Sperm viability (%)	Sperm morphology (%)
Control	63.83 $\pm$ 5.02	60.83 $\pm$ 3.96	70.25 $\pm$ 6.18	9.17 $\pm$ 0.83
62.5	58.50 $\pm$ 4.77	57.50 $\pm$ 3.35	54.78 $\pm$ 3.06	6.67 $\pm$ 1.05
125	65.83 $\pm$ 2.87	51.67 $\pm$ 2.79	62.27 $\pm$ 1.78	7.50 $\pm$ 1.12

Data expressed as mean $\pm$ SEM, n=6 animals per group

The results indicated that *B. pinnatum* administration would have no adverse effect on the kidney over the time and dose range used in this study as serum levels of urea and creatinine remained unchanged after treatment with the extract. Organ-to-body weight ratio is a useful indicator often employed in the assessment of organ toxicity<sup>[32]</sup>. There was no significant ( $P > 0.05$ ) change in kidney-to-body weight-ratio in all extract treated when compared to control rats (Table 4), neither did the plant cause any alteration in the micro structure of the kidney. Kidneys of treated and control rats showed normal architecture and structure with numerous glomeruli and tubules, and blood vessels lined by endothelial cells (fig. 1A, B and C). The implication of this observation was that sub-acute treatment of the plant extract had no adverse effect on the kidney, showing a positive correlation with the biochemical parameters measured. Previous studies have reported that aqueous extract of *Kalanchoe pinnata* (*B. pinnatum*) possessed protective effects on chemical-induced nephrotoxicity<sup>[33]</sup>.

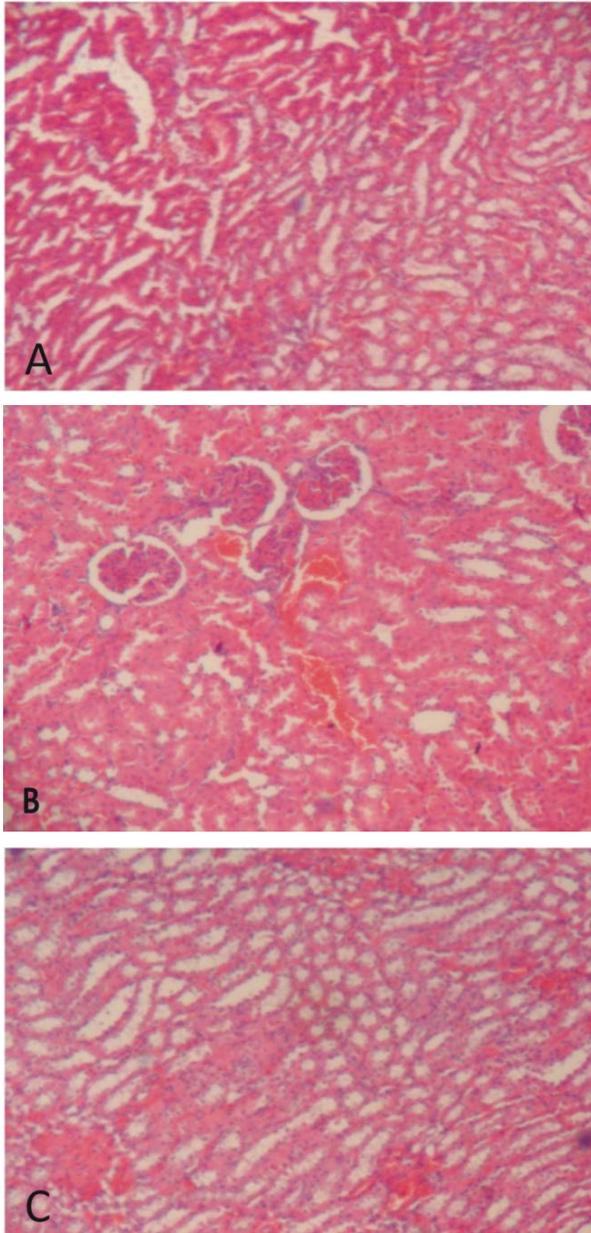
The testis is highly susceptible to toxicity by chemicals and many medicinal plants have been reported to adversely affect testicular function in animals<sup>[34,35]</sup>. In the present study, the plant had no effect on sperm count, motility, morphology and viability (Table 3). Additionally, testes of control and extract-treated rats showed normal histology with numerous seminiferous tubules containing spermatozoa concentrated in the lumen (fig. 2A, B and C). Testicular organ-to-body weight ratio was also not altered by the extract (Table 4). These results provided evidence that sub-acute administration of the plant extract exerted no adverse effect on testicular function and fertility in rats.

WBC and its differentials (like lymphocytes and neutrophils) and other haematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function<sup>[34]</sup>. WBC's are essential for the protection of the animal against foreign invaders. Elevation in their levels is indicative of response to an immunological challenge. Neutrophils

**TABLE 4: *B. PINNATUM* LEAF EXTRACT CAUSES NO EFFECT ON KIDNEY- AND TESTIS-BODY WEIGHT RATIOS IN WISTAR RATS**

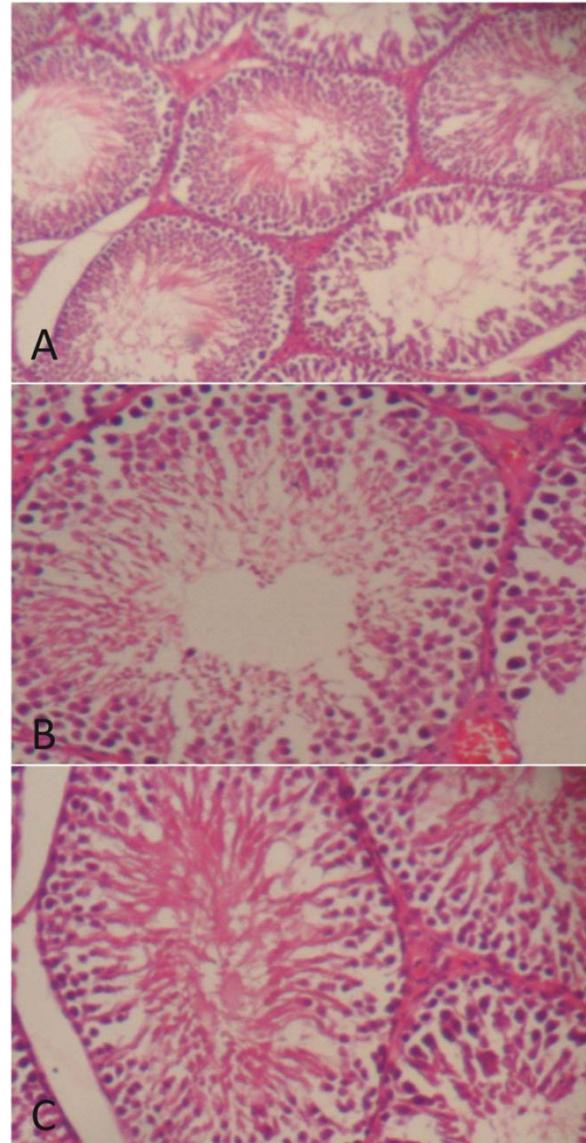
Dose (mg/kg)	Organ-to-body weight ratio ( $\times 10^{-3}$ )	
	Kidney	Testis
Control	713.30 $\pm$ 71.49	753.30 $\pm$ 192.90
62.5	838.00 $\pm$ 169.10	796.00 $\pm$ 168.40
125	746.00 $\pm$ 70.47	938.00 $\pm$ 185.50

Data expressed as mean $\pm$ SEM, n=6 animals per group



**Fig. 1: Photomicrographs of kidney following sub-acute administration of aqueous leaf extract**

**A: (Control):** shows normal architecture and structure with numerous glomeruli and tubules, and blood vessels lined by endothelial cells; **B: (62.5 mg/kg AQBP treated group):** Shows normal architecture and structure with numerous glomeruli and tubules; **C: (125 mg/kg AQBP treated group):** Shows normal architecture and structure with numerous glomeruli and tubules; Magnification of ( $\times 400$ ), and stained with H and E



**Fig. 2: Photomicrographs of testis following sub-acute administration of aqueous *Bryophyllum pinnatum* leaf extract (AQBP)**

**A: (Control):** Shows normal histology of testis with normal seminiferous epithelium, normal spermatogenic cell differentiation, and numerous spermatozoa in the lumen; **B: (62.5 mg/kg AQBP treated group):** Shows normal architecture of testis with normal spermatogenic cell differentiation, and normal sperm production; **C: (125 mg/kg AQBP treated group):** Shows normal architecture of testis with normal seminiferous epithelium, and normal sperm production; aqueous *Bryophyllum pinnatum* leaf extract; Magnification of ( $\times 400$ ), and stained with H and E

are important phagocytic cells normally elevated in the early inflammatory response<sup>[36,37]</sup>, while lymphocytes are subtypes of leucocytes critically essential for providing cell mediated immunity. Our results showed that total WBC and lymphocyte counts were increased in extract (165 mg/kg) treated group, but only the effect on WBC was significant ( $P < 0.05$ ), when compared to

**TABLE 5: EFFECTS OF SUB-ACUTE ADMINISTRATION OF AQUEOUS *B. PINNATUM* LEAF EXTRACT ON SOME HEMATOLOGICAL PARAMETERS IN WISTAR RATS**

Dose (mg/kg)	PCV (%)	WBC ( $\times 10^6$ /ml)	Neutrophils (%)	Lymphocytes (%)
Control	42.25 $\pm$ 1.32	2.95 $\pm$ 0.37	50.00 $\pm$ 9.13	50.00 $\pm$ 9.13
62.5	42.25 $\pm$ 1.32	3.00 $\pm$ 0.40	27.50 $\pm$ 1.09	72.50 $\pm$ 11.09
125	39.50 $\pm$ 2.10	4.93 $\pm$ 0.22**	20.00 $\pm$ 5.74	75.00 $\pm$ 5.00

Data expressed as mean $\pm$ SEM, n=6 animals per group. \*\*Significant at P<0.01. PCV: packed cell volume, WBC: white blood cells

the control (Table 5). Neutrophil count was significantly reduced, while PCV was not altered in treated groups compared to control group (Table 5). Inhibition of neutrophils suggested that the plant might depress primary immune response, including inflammation. Previously, the aqueous extract of *B. pinnatum* has been demonstrated to possess antiinflammatory<sup>[7,13]</sup> and immunosuppressive activities<sup>[36]</sup>. These properties could partly be related to the neutrophilic effect observed in this study. Additionally, the results indicated that the plant might not cause anaemia as it did not affect PCV. Earlier, Ufelle *et al.*<sup>[37]</sup> had also reported elevation of WBC following sub-acute treatment of methanol extract of *B. pinnatum*, which is consistent with our results, but reported elevation of PCV. In another study, sub-acute oral treatment of a single large dose (2 g/kg) of the plant has been reported to produce similar haematological effects as obtained in the present study, except on WBC and neutrophils, which were reported to be unaffected<sup>[31]</sup>. We attributed these different results to different concentrations that were used in the studies; however, more studies are necessary to provide better understanding of the mechanisms involved. Further, non-characterization of the extracts is a limitation of this study and it is recommended that further studies to identify and establish the chemical structure(s) of the active components be taken up.

Overall, these results indicated that *B. pinnatum* could be considered to have relatively low toxicity profile, with respect to the organs evaluated in this study. Sub-acute administration of the plant might exert no adverse effect on the kidney and testis, but could potentially depress neutrophil activity in rats. The phytoconstituents of the plant obtained in this study included flavonoids, saponins, tannins and alkaloids, which were similar to those reported earlier<sup>[15,20]</sup>. Flavonoids and other important antioxidant constituents of the plant were known free radical scavengers, capable of preventing oxidative cell damage and reducing oxidative stress. Other components like saponins and triterpenoids could be toxic to certain cellular components. Different effects that were observed might therefore be related to the numerous phytochemical compounds contained in the extract.

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### Conflict interests:

The authors declare that there is no conflict of interest.

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