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Research Article

Toxicity Assessment of Aqueous Leaf Extract of *Bryophyllum pinnatum* on Body and Organ Weights, and Haematologic Parameters in Rodents

Yemitan Omoniyi K[1,*], Akinyemi Akinsuyi O[2], Sunday Olayemi O[2], Ayorinde Esther O[1]
[1]Department of Pharmacology, Therapeutics & Toxicology, Lagos State University College of Medicine, P.M.B. 21266 Ikeja, Lagos, Nigeria. [2]Department of Pharmacology, Therapeutics & Toxicology, College of Medicine of the University of Lagos, P.M.B. 12003 Idi-Araba, Lagos, Nigeria.

[*]***Author for Correspondence:***
Yemitan O. K.
E-mail: kayodeyemitan@yahoo.com

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SUMMARY

Objective: This study assessed the acute and subchronic toxicities of the aqueous leaf extract of *Bryophyllum pinnatum* (ALBP) on the bodyweight, vital organs and haematologic parameters in rodents, with a view to predicting its safety in humans.

Methods: To five groups of albino mice (n = 5), ALBP was given at doses up to 5000mg/kg either as oral or intraperitoneal injections for 1-14 days during which they were observed for morphologic changes or mortality. For the 90-day subchronic study, male rats (16/group) received daily administration of 10, 100, 1000 mg/kg extract doses or distilled water (control); body weights were measured weekly; after 90 days, some rats were sacrificed (10/group) for vital organs weights and haematology. Remaining rats (6/group) were retained, untreated, for reversibility study.

Results: For acute toxicity test, ALBP, up to 5000 mg/kg, did not cause mortality, but caused sedation, bradycardia and hypercapnia; whereas, for i.p. administration the LD_{50}was1650 ± 8.7 mg/kg. Subchronic test caused significant ($P<0.05$) weight reduction at end of study. At 1000 mg/kg, ALBP significantly caused increased weights of kidneys, liver & spleen, but reduction in weights of lungs and testes at all doses. Reversibility test resulted in reversal of weights of the liver and spleen; but not the lungs and testes. At 100 mg/kg, increases in PCV % & WBC, but at 1000 mg/kg, significant reduction of RBC, Hb concentration, PCV % and platelets were recorded. Significant WBC elevation was recorded at 100 & 1000 mg/kg of ALBP; all haematologic parameters except WBC were reversed.

Conclusion: At high doses, ALBP has potential to cause degrees of toxicity to the lungs and testes, as well as anaemia, in humans, thereby requiring monitoring during long-term use.

INTRODUCTION

Qualitative healthcare needs of a soaring world population, largely unmet by existing orthodox healthcare givers, has warranted a shift toward the use of complementary and alternative medicines of which herbal medicines are considerable. The acceptance for herbal medicines is largely encouraged by being readily available all year round, affordable, and with the cultural belief that, unlike the synthetic orthodox medicine, they are safe and devoid of harmful side effects.

According to a World Health Organization report, in Africa, up to 80% of the population depends on herbs; In India, Canada and France, 65%, 50% and 75% of the population, respectively, use herbal alternatives alone or to supplement orthodox pharmaceuticals. Moreover, in Japan, it has been reported that 85% of doctors prescribe both modern and traditional herbal medicines concurrently.[1]

However, the classes of positive contribution of plants to medicine, has not been without side effects at normal doses, or toxic effects at larger doses. To benefit fully from the pharmacological uses of plants, the safety potentials need to be fully studied and reported as well.

The plant *Bryophyllum pinnatum*(Family-Crassulaceae) is a perennial herb growing widely (Figure 1) and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia. Where cultivated, it is used as a divine herb.[2] Some of its common names include 'life plant', 'air plant', 'maternity plant', 'love plant', 'Canterbury bells', 'Cathedral bell', 'Parnabija', 'miracle leaf', 'Katakataka'.

In Nigeria, particularly, the plant has been classified as weed,[3] and flourishes throughout the southern part of the country,[4] where it is applied in folkloric uses for hypertension, sedation, wound-healing, cough suppression, inflammation and diabetes. Others are in the treatment of skin ulcer, boils, intestinal parasite, and pneumonia. In other parts of Africa, the plant is used in traditional medicine in the treatment of diarrhea, dysentery, and cholera. In Bangladesh, the plant is used for treating cough, fever, epilepsy and constipation. The leaf of the plant has also found various

topical applications which include treatment of dislocation and callosities, asthma, urinary bladder stones and fever, and to relieve symptoms of headache.

Recently, researchers have corroborated the justification for the use of the plant in folkloric traditional medicine. These include antihypertensive,[5] antibacterial, antifungal,[6-7] anti-toxin,[7] anti-nociceptive, anti-inflammatory and anti-diabetic.[8] neurosedative and muscle relaxant,[9] among others. However, despite the therapeutic successes of this plant, mortalities have been reported due to use of the plant or its extracts in rabbits[5] and cattle.[10] Particularly quoting a scientist, "one must be wary of ingesting the extract of this herb because of its potential to be cardiotoxic".[5]

Currently, particularly across Nigeria, the leaf part of *B. pinnatum*is fast becoming popular for use as mono- or poly-herbal tea in the management of chronic diseases such as hypertension, diabetes, gastric ulcer, kidney stones, chronic wound, convulsion, asthma, cancer etc. In view of these health conditions, there may be need to use this plant extract for a long period in the treatment of these ailments. Therefore, during use, toxicity to organs and tissues of the body may occur, or silently manifesting much later in the life of the user, particularly since there is often no regulation on dosages, especially when intended for the treatment of chronic conditions.

Although some toxicities have been reported during unrestricted consumption by livestock, there is dearth of information on the potential toxicities that might arise in humans, despite its broad traditional medicinal applications in acute and chronic conditions. This study therefore assessed the toxicological effects which acute 24 h – 14 days, and 90-day subchronic administration of the aqueous extract of *B. pinnatum* would produce on the body viz the weight, vital organs and blood of rodents, with a view to providing information for likely human users of the leaf decoction of the plant. The information therefrom would enrich users with dosage pattern and untoward expectations during long-term use.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Bryophyllum pinnatum* was purchased from local market at Dopemu, Lagos State, Nigeria between July and November, 2016. It was confirmed by the Botany and Microbiology Department, University of Lagos, Nigeria, it was compared with a voucher specimen and was deposited for reference purpose.

Extract Preparation

Freshly harvested leaves of *B. pinnatum* was rinsed, air-dried under shade for 24h and weighed. Then 200 g of it was pulverized with an electric blender with addition of 400 mL of distilled water. The macerated leaves were filtered with Whatman filter paper No 1, and the leaf residue weighed to determine the quantity in solution. The extract was stored in the refrigerator at 4°C until it was ready for use each day. Fresh extract was prepared every 48 h.

ANIMALS

The animals used for these experiments were young adult male Sprague-Dawley rats (140-150g), which were obtained from, and kept at the Laboratory Animal Centre of the Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. The animals were maintained under standard environmental conditions, being fed with standard rodent feed obtained from Livestock Feed, Nigeria Ltd., and given water *ad libitum*. All the animals were kept at room temperature in cross-ventilated room, without illumination at night to achieve 12 hours light /12 hours dark period. The animals were acclimatized in the laboratory condition for 14 days prior to the experiment, during which they were given free access to food and water. The care and the use of animals were conducted in accordance with the National Institute of Health Guild for the care and use of Laboratory Animals. Moreover, Ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria.

TOXICITY STUDIES

Acute Toxicity test

Five groups of adult male Swiss albino mice (n = 5) were fasted for 12 h, and then administered with the extract (10 mg/kg - 5000 mg/kg), orally. In the same manner, the extract (10 - 2000 mg/kg) was administered to another set of mice, intraperitoneally (i.p.). The control mice were given distilled water (10 ml/kg). Mice were closely observed for toxic symptoms and behavioral changes for the first two hours of administration and mortality recorded within 24 h. The medial lethal dose, LD_{50} was calculated using the method of Miller and Tainter.[11] Mice orally administered with extract were observed for 14 days, further, to investigate for any sub-acute toxic effects. Test was repeated twice. Same procedure was carried out for the i.p.- administered groups.

Sub-Chronic Toxicity Testing

Young adult male rats were randomly allotted to four groups (16/group), consisting of the control and three extract-treated groups, given 10 mg/kg, 100 mg/kg and 1 g/kg, respectively. The doses were administered daily through drinking water, throughout 90-day test and 21-day reversibility period. Rats in different groups were observed closely for any behavioral changes, feeding and drinking habits, as well as weekly measured body weight and general morphological changes.

After 90 days, some rats were sacrificed (10/group) for internal macroscopic, and measurements of weights of heart, kidneys, liver, lungs, spleen and testes. Haematologic parameters including red blood cells, packed cell volume, haemoglobin and white blood cells were also estimated. Other rats were retained (6/group) for reversibility of test effects.

Body Weight Measurements

Sixty-four purpose-bred male Sprague-Dawley rats (7-8 weeks old, mean weight, 150 g approx.) were housed (8/cage) in eight large cages (dimension, 60 x 75 cm). They were divided into four groups. Each group of rats consisted of 16 animals: Group 1 rats were given tap water only, while group 2-4 were given 10 mg/kg, 100 mg/kg and 1g/kg of *B. pinnatum* extract in tap water, respectively. Body weight of each member of the group was measured individually by aid of a sensitive digital balance on day 0, then once every 7th day

till day 90. Mean weight ± SEM was recorded, accordingly.

Vital Organs Measurement

At the end of the study, qualitative data on the weights of vital organs (heart, kidneys, liver, lungs, spleen and testes) were assessed by carefully dissecting each organ from sacrificed animals into 10 % formol saline solution contained in a Petri-dish. Isolated organs were dried with a blotting paper and weighed on a sensitive balance. Each weighed organ was then standardized for 100 g body weight of each rat.

Reversibility of significant toxicity effects was tested following 21 days of treatment withdrawal in the *B. pinnatum* treated groups for only those groups and treatment where significance was recorded for organ weight change, following the 90-day study.

Haematologic Parameters

Blood samples were collected through heart puncture from each diethyl ether-anaesthetized rat into different EDTA sample bottles. The blood samples were analysed for red blood cells (RBC), haemoglobin (Hb), platelet, packed cell volume (PCV), white blood cells (WBC) and differential WBC (neutrophil, eosinophil, basophil, lymphocyte and monocyte. After blood film was mixed with necessary diluents and introduced into improved Neubauer counting chamber as reported for standard haematological methods[12-15]. Visual count was done by aid of light microscope connected to a CCTV (WT/CP410/G) Panasonic camera monitoring system.

Reversibility of significant toxicity effects was tested following 21 days of treatment withdrawal in the *B. pinnatum* treated groups for only those groups and treatment where significance was recorded for haematologic parameters.

Statistical Analysis

Results are presented as mean ± S.E.M or percentages. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using SPSS statistical software. A value of < 0.05 was considered significant.

RESULTS

Acute toxicity effects of aqueous leaf extract of *B. pinnatum*

Orally administered doses of the extract did not produce any observable toxicity or mortality in mice when administered up to 5000 mg/kg, except for dose-dependent sedation and visible decreases in heart, but increases in breathing rates which were observed with increasing doses.

Intraperitoneally administered doses of extract, however, produced dose-related mortalities, estimating a median lethal dose, LD_{50} of 1650 ± 8.7 mg/kg.

Effect of aqueous leaf extract of *B. pinnatum* on body weights of rats in the 90-day study

After 90-day subchronic treatment, control as well as 10 mg/kg & 100 mg/kg groups of rats began gaining significant weight from day 28; however, 1000 mg/kg BPproduced first significant weight gain at day 42. At the end of the study, there were significant (P<0.05, ANOVA) and dose-dependent reduction in body weights among the all extract treated groups (Table 1).

Effect of aqueous leaf extract of *B. pinnatum* on vital organs weights in the 90-day study

There was no significant (i.e. $P \geq 0.05$; ANOVA) change in the weights of the heart at all doses. However, at 1000 mg/kg, there were significant increases in the weights of the kidneys, liver and spleen, but significant reduction in weight of lungs. The testis, however, reduced in weight at all the extract doses tested (Table 2).

When extract was withdrawn (reversibility test) from rats for 21 days after 90-day treatment, the changes in weight on the liver and spleen were reversed to normal; but weight changes in the lungs and testes were not reversed within the 21 days of reversibility study (Table 3).

Effect of aqueous leaf extract of *B. pinnatum* on haematological parameters in the 90-day study

At 100 mg/kg, the extract produced significant ($P<$ 0.05) increases in PCV % and WBC. However, at 1000 mg/kg, significant reduction of RBC, Hb concentration, PCV % and platelets concentration were recorded. For WBC, significant elevation was recorded at 100 mg/kg and 1000 mg/kg of BP (Table 4).

When extract was withdrawn (reversibility test) from rats for 21 days after 90-day treatment, the effects on all haematologic parameters except that of WBC, were reversed to values comparable to those of the control (Table 5).

Effect of aqueous leaf extract of *B. pinnatum* on white blood cells differentials in the 90-day study

Further studies of WBC differential count of neutrophil, eosinophil, basophil, lymphocyte and monocyte did not manifest any significant alterations by extract across the groups during the 90-day subchronic toxicity study (Table 6.

Table 1: Body weights of rats during 90 days of treatment with aqueous leaf extract of *B. pinnatum*.

Treatment (mg/kg)	Mean Body Weight (g) S.E.M													
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 90	Weight gain/ wk
Control	150.3 ± 1.1	149.7 ± 1.2	151.5 ± 1.1	152.4 ± 1.3	164.4 ± 1.4	175.5 ± 1.3	191.6 ± 1.5	202.7 ± 1.4	207.5 ± 1.7	211.9 ± 1.6	217.6 ± 2.1	221.4 ± 2.0	225.6 ± 2.3	230.6 ± 2.1	6.02 ± 0.31
BP 10	148.4 ± 1.3	149.6 ± 1.2	144.2 ± 1.3	148.2 ± 1.6	157.6 ± 1.4	167.4 ± 1.6*	177.5 ± 1.7*	186.8 ± 2.0	193.3 ± 1.7	198.5 ± 2.1	204.5 ± 2.0	211.7 ± 2.3	217.4 ± 2.4	222.2 ± 2.2	5.67 ± 0.33[b]
BP 100	153.7 ± 1.0	147.6 ± 1.3	143.8 ± 1.2*	153.6 ± 1.4	161.3 ± 1.3	166.5 ± 1.4*	173.7 ± 1.8*	175.4 ± 1.6*	179.7 ± 1.9*	183.3 ± 1.6*	89.2 ± 1.8*	210.0 ± 2.1*	215.3 ± 2.3	220.4 ± 2.2	5.13 ± 0.42[b]
BP 1000	147.2 ± 1.1	142.8 ± 1.3	138.7 ± 1.4	138.8 ± 1.4	142.5 ± 1.5*	149.5 ± 1.6*	158.4 ± 1.7*	170.7 ± 1.4*	177.1 ± 1.5*	182.4 ± 1.7*	185.4 ± 1.6*	193.5 ± 1.6*	198.7 ± 1.8*	203.7 ± 2.0*	4.34 ± 0.38[b]

Table showing weekly body weight changes in rats during the 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control). * Significant (P<0.05; ANOVA) decrease in body weight among the groups. Number of rats used = 16 male per group.

Table 2: Organ weights (per 100 g body weight) of rats after 90-day subchronic treatment with *B. pinnatum.*

Treatment (mg/kg)	Mean organ weight per 100 g body weight S.E.M					
	Heart	Kidneys	Liver	Lungs	Spleen	Testis
Control	0.36 ± 0.01	0.57 ± 0.03	2.31 ± 0.46	0.66 ± 0.03	0.23 ± 0.01	2.36 ± 0.01
BP 10	0.34 ± 0.02	0.60 ± 0.05	2.50 ± 0.19	0.65 ± 0.04	0.22 ± 0.0	21.86 ± 0.11$^{\beta}$
BP 100	0.33 ± 0.03	0.67 ± 0.04*	2.55 ± 0.18	0.57 ± 0.05	0.19 ± 0.03	1.82 ± 0.12$^{\beta}$
BP 1000	0.32 ± 0.03	0.68 ± 0.03*	2.65 ± 0.24*	0.54 ± 0.03$^{\beta}$	0.31 ± 0.03*	1.81 ± 0.11$^{\beta}$

Table showing weights of vital organs of rats after 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control). *Significant increase; $^{\beta}$ Significant decrease (Two-way ANOVA) at p< 0.05. Number of rats used = 10 male per group.

Table 3: Organ weights per 100 g body weight of rats after 90-day subchronic treatment with *B. pinnatum* and 21-day reversibility test.

Treatment (mg/kg)	Mean organ weight per 100 g body weight ± S.E.M					
	Heart	Kidneys	Liver	Lungs	Spleen	Testis
Control	NT	0.57 ± 0.03	2.31 ± 0.46	0.66 ± 0.03	0.23 ± 0.01	2.36 ± 0.11
BP 10	NT	NT	NT	NT	NT	1.90 ± 0.12*
BP 100	NT	0.60 ± 0.05	NT	NT	NT	1.83 ± 0.12*
BP 1000	NT	0.68 ± 0.03*	2.42 ± 0.30	0.56 ± 0.03*	0.24 ± 0.02	1.80 ± 0.13*

Table showing weights of vital organs of rats after 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control). *Significant (Two-way ANOVA) at P < 0.05. Number of rats used = 6 male per group. NT = Not tested (because there was no significant difference in values compared with control in the 90 days subchronic toxicity study).

Table 4: Haematological parameters of rats after 90-day subchronic treatment with aqueous leaf extract of *B. pinnatum*

Treatment (mg/kg)	RBC (x 10^6) ± S.E.M	Hb (g/dl) ± S.E.M	PVC (%) ± S.E.M	Platelet (x 10^6) ± S.E.M	WBC (x 10^3/µL) ± S.E.M
Control	9.21 ± 0.10	17.20 ± 0.25	41.38 ± 1.20	472 ± 12.17	3.75 ± 0.06
BP 10	9.27 ± 0.09	16.84 ± 0.30	42.50 ± 23	469 ± 10.33	3.88 ± 0.07
BP 100	9.72 ± 0.06	18.03 ± 0.31	44.00 ± 1.16$^{\beta}$	478 ± 10.81	4.11 ± 0.07$^{\beta}$
BP 1000	7.45 ± 0.13$^{\alpha}$	14.64 ± 0.34$^{\alpha}$	38.61 ± 1.31$^{\alpha}$	448 ± 9.02$^{\alpha}$	4.51 ± 0.09$^{\beta}$

Table showing measured values of haematological parameters in rats after 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control); n = 10 male rats. $^{\alpha}$Significant reduction, $^{\beta}$elevation among the groups (p<.05; Two-way ANOVA). RBC = red blood cells, Hb = haemoglobin, PCV = packed cell volume, WBC = white blood cells.

Table 5: Haematological parameters of rats after 90-day subchronic treatment with aqueous leaf extract of *B. pinnatum* and 21-day reversibility test.

Treatment (mg/kg)	RBC (x 10^6) ± S.E.M	Hb (g/dl) ± S.E.M	PVC (%) ± S.E.M	Platelet (x 10^6) ± S.E.M	WBC (x 10^3/µL) ± S.E.M
Control	9.21 ± 0.10	17.20 ± 0.25	41.38 ± 1.20	472 ± 12.17	3.75 ± 0.06
BP 100	NT	NT	41.03 ± 1.37	NT	3.87 ± 0.10
BP 1000	9.03 ± 0.15	16.84 ± 0.27	40.52 ± 1.6	8469 ± 11.06	4.05 ± 0.12$^{\beta}$

Table showing measured values of haematological parameters in rats after 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control); n = 6 male rats. $^{\beta}$elevation among the groups (p<.05; Two-way ANOVA). RBC = red blood cells, Hb = haemoglobin, PCV = packed cell volume, WBC = white blood cells. NT = Not tested (because there was no significant difference in values compared with control in the 90 days subchronic toxicity study).

Table 6: White Blood Cell differentials in rats after 90 days subchronic treatment with the aqueous leaf extract of *B. pinnatum*

Treatment& dose	Mean WBC differentials (%) ± S.E.M				
	Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte
Control	43.7 ± 0.81	15.9 ± 0.51	6.8 ± 0.32	31.0 ± 0.8	2.6 ± 0.23
BP 10	44.0 ± 0.39	15.5 ± 0.45	6.6 ± 0.37	31.2 ± 0.37	2.7 ± 0.24
BP 100	43.6 ± 0.32	16.1 ± 0.49	6.6 ± 0.24	30.9 ± 0.49	2.8 ± 0.20
BP 1000	42.8 ± 0.97	16.0 ± 0.51	6.9 ± 0.58	32.0 ± 1.58	2.3 ± 0.18

Table showing measured values of white blood cells differentials in rats after 90 days treatment with different doses of aqueous leaf extract of *B. pinnatum* (BP) or distilled water (control); n = 16 male rats. No significant changes were recorded among the groups (P<.05, ANOVA).

Fig. 1: *Bryophyllum pinnatum* plant in a natural habitat

DISCUSSION

During the evaluation of the toxic characteristics of medicinal plants, the determination of LD_{50} is usually an initial step to be conducted.[16] Data from the acute toxicity study have been reported to serve as the basis for classification and labelling, provide initial information on the mode of toxic action of a substance, help in dose determination in animal studies; and/or help determine LD_{50} values that provide many indices of potential types of drug activity.[17] In this study, orally administered doses of the aqueous leaf extract of *B. pinnatum* did not produce any observable toxicity or mortality in mice when administered up to 5000 mg/kg within 24 hours of dosage, even when observed for further 14 days. According to the chemical labelling and classification of acute systemic toxicity recommended by Organisation for Economic Cooperation and Development (OECD), the orally administered aqueous leaf extract of *B. pinnatum* would be assigned class 5 status (LD_{50}> 5000 mg/kg), which depicts the lowest toxicity class. Substances with LD_{50} values higher than 5000 mg/kg by oral route are regarded as being safe or practically nontoxic.[18] The only observable effects were hard stools and dose-dependent sedation and visible increases in heart and breathing rates which occurred, but reversed within five hours of treatment, dose-dependently. These cardiac and respiratory effects require monitoring and further tests. Albeit, the lack of mortality is a good indication that the extract could be safe when given orally up to the test limit of 5 g/kg body weight above which toxicity arising from a substance is not considered relevant.[19] Based on this, the extract could be said to be non-lethal when used through the oral route.[20] The hard stools observed in the extract-treated animals persisted, and is suggestive of constipation. This supports folkloric use of the aqueous extract of the plant for the treatment of diarrhoea, of which mechanism is yet to be determined. The intraperitoneally administered doses of *B. Pinnatum,* however, produced dose-related mortalities, with a median lethal dose, LD_{50} of 1650 mg/kg. The mortalities recorded could have resulted from direct toxic action of the extract, which was not attenuated by entero-hepatic metabolic biotransformation (*first-pass effect*) that the orally-administered extract might have offered.[20]

The subchronic toxicity test was performed following the protocol described by the OECD guideline 408 for testing chemicals.[21] In this study, the daily exposure of the plant extract for a period of 90 days through the drinking water did not produce detectable physical abnormalities. Worthy of mention, however, was a detectable morphological abnormality noted during dissection of the animals in the 1000 mg/kg treatment group, where the right lung of one animal was grossly deformed containing pus. While another animal from same treatment group had abnormally large kidney containing mainly clear fluid.

During long term treatment, body weight changes serve as a sensitive indication of the general health status of animals. [21]The weekly measurement of body weight resultantly yielded significant and dose-dependent reduction in body weights among the *B. Pinnatum*-treated, compared with the control group. This could suggest increase in metabolic activity in body of the animals attributable to the extract; alternatively, it could be attributable to appetite suppression or distaste; this gives room for study of the mechanism of this effect in future. Interestingly, the plant has been reported to be used as a weight reduction regimen in traditional medicine.[22]

The usefulness of weighing organs in toxicity studies includes their sensitivity to predict toxicity, enzyme induction, physiologic perturbations, and acute injury; any organ(s) showing marked weight changes could predictably be a target organ of toxicity, and often correlates well with histopathological changes.[23] In this study, the effects of the plant extract on vital organs weights showed no significant changes in the weight of the heart, at all doses. However, at 1000 mg/kg of the extract, there were significant increases in the weights of the kidneys, liver and spleen which might be suggestive of an ongoing inflammatory process, but significant reduction in weight of the lungs also requires further investigations. The testis, however, showed a reduction in weight at all the extract doses tested. This is therefore indicative of the plant extract being selective and potent in its toxic action to the testes, irrespective of the dosage, and this could be a predictor of possible effect of infertility in males, due to long term consumption of the aqueous leaf extract of *B. pinnatum*. Further studies will ascertain this observation. For the purposes of clarity, histopathologic assessment of these organs, including the testis is presently being explored in our laboratory. Nevertheless, the reversibility tests, after withdrawal of extract treatment for 21 days, showed that the significant changes in weights of the liver and spleen were reversed to normal, whereas the weight changes in the kidneys, lungs and testes were not reversed after 21 days of treatment withdrawal.

Analysis of haematologic parameters following chronic administration of a plant extract is relevant to risk evaluation as changes in the haematologic system have higher predictive value for human toxicity when the data are translated from animal studies.[24] In this study, the

haematologic parameters of animals treated with 100 mg/kg of the extract showed significant increases in packed cell volume (PCV) and white blood cells (WBC), whereas the 1000 mg/kg- treated group showed significant reduction of red blood cells (RBC), PCV and haemoglobin (Hb) and platelet concentrations, suggestive of an ongoing haemolysis at the high doses. This may also reflect marrow suppression at higher concentration. The initial increase may be the result of its nutritional value.The WBC was significantly high in both 100 mg/kg and 1000 mg/kg- treated groups, suggesting some level of inflammatory changes or reaction to components of the plant, an effect which was not reversed after the reversibility test unlike RBC, PCV, and Hb & platelet concentrations. Despite this, there were no significant changes in the WBC differentials in all treated groups.

The results of phytochemical screening revealed presence of alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids, and organic-acids.

Bufadienolide has been reported to be poisonous with digitalis toxicity-type cardiac effects such as slowing of heart rate, heart blocks and potentially fatal ventricular arrhythmias, which was, perhaps, the cardiac effect reported as one of our gross observations during the acute toxicity study. It should be noted that the plant has been reported to have killed cattle fed with it; for which the bufadienolide has been implicated as likely cause of the toxicity.[2] Further chemical screening had reported presence of arachidic acid, astragalin, behenic acid, beta amyrin, benzenoids, bersaldegenin, beta-sitosterol, bryophollenone, bryophollone, bryophyllin, caffeic acid, ferulic acid, quercetin, steroids, and taraxerol; furthermore, Bryophyllum A, B and C, a potent cytotoxic bufadienolide orthoacetate,[25-26] has also been reported present in the leaf extract of the plant, including Bryophillin-A, a bufadienolide compound, that has shown anti-tumor promoting activity.[27] Any one or combination of these chemical constituents could be responsible for some of the toxicities recorded in this our study.

CONCLUSION

The results suggest that the aqueous leaf extract of *Bryophyllum pinnatum* has the potential to cause some degree of toxicity to the lungs, testes, and haematologic parameters, such as anaemia and elevated leucocytes counts indicative of immune activation which probably suppress rapidly growing tissues like haemopoiesis or spermatogenesis. Reversible increase in weight of the liver, spleen and kidneys may be due to short-lived congestion as a result of poor returns to the heart or cardiac lesions at high doses. Further studies are required to clarify these issues.

These observations suggest significant toxicities when the leaf extract of the plant is used for a long time at high doses. It is recommended that during oral use of the leaf extract of *B. pinnatum*, at least the haematologic parameters, lung function and male reproductive functions need to be monitored.

Conflict of interest

The authors declare no conflict of interest in this study.

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