**Toxicological and Reversibility Studies of *Bryophyllum pinnatum* Leaf Extract on Biochemical, Peroxidation and Histopathologic Parameters in Rodents**

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Running Head: Toxicological studies of *Bryophyllum pinnatum*

**ABSTRACT**

**Aim**: The aqueous leaf extract of *B. pinnatum* (BP) is effective as herbal medicine for hypertension, diabetes and convulsions, for which there has not been reliable subchronic toxicity data. This study aimed to study possible health effects of sub-chronic use of BP, as a predictor of long-term use in humans. **Methods**: Acute 24h oral (in mice) and 90 day-subchronic toxicity (in rats) tests were conducted as predictors for human outcome. Following 90 days, and 21 days reversibility tests, biochemical- ALP, ALT and AST, LDH, direct bilirubin, uric acid and creatinine were measured; oxidative stress- CAT, SOD), MDA; and histopathologic parameters were determined. No mortality was recorded up to 5g/kg of BP in acute toxicity test. After 90 days, 1000 mg/kg BP, caused significant, but reversible increases in all ALP, AST, ALT, LDH, uric acid, and creatinine. **Results:** At 100 & 1000 mg/kg of BP, the MDA was significantly elevated for kidney, heart, lungs and spleen; but at 1000 mg/kg, *BP* produced significant reduction of CAT and SOD; and elevation of MDA for all organs. These effects on CAT, SOD and MDA, except the lungs at 1000 mg/kg and testes at 100 mg/kg and 1000 mg/kg, were reversed. At 1000 mg/kg BP caused some observable changes in the kidneys and liver of few of the rats, irreversible pathologic changes were observed in the testis. **Conclusion:** These findings suggest that vital organs toxicities could occur during long-term use of BP, especially for irreversible pathologic changes in the testis.

**Keywords:** *Bryophyllum pinnatum*, Peroxidation, Testis, Histopathology, Subchronic toxicity

**INTRODUCTION**

There is increasing shift toward the use of herbs for healthcare, among all economic strata of the world. Herbal medicines are believed to be more readily available, affordable, safer and devoid of harmful side effects unlike their synthetic orthodox counterparts. Proponents of herbal medicines strongly believe and often claim that herbs are devoid of adverse or even side effects, simply asserting that ‘natural’ and ‘safe’ are synonymous. But this is not a fact.

According to a World Health Organization report, the use of herbal medicines and phytonutrients or nutraceuticals is growing rapidly [1]. 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, 85% of doctors prescribe not only modern medicine but also the traditional herbal medicine [2]. Another study about two decades ago revealed that approximately 40% of all healthcare services delivered in China while the percentage of the population which has used herbal medicines at least once in Australia, Canada, USA, Belgium, and France is estimated at between 38% to 75% [3, 4]. Similarly, it was reported that many in the UK and the rest of Europe, as well as in North America and Australia [5,6,7].

*Bryophyllum pinnatum* (Lam.) Oken(Family- Crassulaceae) is a perennial plant growing widely and popularly used in alternative medicines in Africa, tropical America, India, China and Australia. Where cultivated, it is used as a divine herb [8]. Some of its common names include ‘life plant’, ‘air plant’, ‘maternity plant’, ‘love plant’,‘Canterbury bells’, ‘Cathedral bell’, ‘Parnabija’. Others common names in English are ‘Miracle Leaf’, ‘Katakataka’ and ‘Life Plant’. It is either cooked or used as mono- or poly-herbal tea in the management of chronic diseases such as hypertension, diabetes, gastric ulcer, kidney stone, chronic wound, epileptic convulsions, asthma and cancer. The leaf of the plant is an important part of the poly-herbal formulations [9, 10].

Numerous published studies have corroborated the justification for the use of the plant in folkloric traditional medicine. These include antihypertensive [11], antibacterial and antifungal [12,13], antinociceptive, antiinflammatory and antidiabetic [14], neurosedative and muscle relaxant [15], and anti renal calculi [16], among others. However, despite the therapeutic success of this plant, mortalities have been reported due to use of the plant or its extracts in rabbits [11] and cattles [17]. Particularly quoting a scientist, “one must be wary of ingesting the extract of this herb because of its potential to be cardiotoxic”[11].

 To benefit fully from the pharmacological uses of plants, the safety potentials need to be fully studied and reported as well. Unfortunately, only sparse data, except our previous study [18], are available for the aqueous leaf extract of *B. pinnatum* at present. Therefore, this study explored the subchronic toxicity profile of *Bryophyllum pinnatum* aqueous extract in young adult Sprague-Dawley rats over a period of 90 days, as well as reversibility test for 14 days. Tests were done on weight changes, biochemical, hematologic, oxidative stress and histopathologic analysis of the vital organs, including the testis.

**MATERIALS AND METHODS**

**Plant materials**

Fresh leaves of *Bryophyllum pinnatum* was purchased from a 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, where a voucher specimen was deposited for reference purpose.

**Extract preparation**

Freshly harvested leaves of *B. pinnatum* was rinsed clean, air-dried under shade for 24 h, 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 in a glass funnel through a Whatman filter paper No 1, and the leaf residue weighed. The extract was stored in the refrigerator at 4 oC until it was ready for use each day. Fresh extract was prepared weekly.

**Animals**

The animals used for these experiments were young adult (140-150g) male Sprague-Dawley rats, 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.* They were kept at room temperature in cross-ventilated room, without illumination at night to achieve 12 hours light /12 hours dark period. Acclimatization was done 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 the research as well as 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 and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals [19].

**Dose selection**

The animals were divided into 4 groups; the doses of *B. pinnatum* were selected based on previous pharmacological pre-tests and OECD protocol, such that a wide range of toxicity data can be captured within the 10, 100 and 1000 mg/kg doses [20]. All the animals were subjected to a 90 days sub-chronic toxicity testing.

**Acute toxicity test**

Adult male mice were subjected to Miller and Tainter acute toxicity study method [21]. Mice (n= 20) were used; the test lasted for 24 hours, then further 14 days in which animals where observed for signs of toxicity which included feeding and drinking patterns, stool texture, behavioral changes, physical morphological changes and mortality.

 Five groups of mice (n = 5) were fasted for 12h, and administered with the extract (10 mg/kg-5000 mg/kg), orally, through an oral cannula. In the same manner, the extract (10 - 2000 mg/kg) was administered to another set of mice, intraperitoneally. The control mice were given distilled water (10 ml/kg). Mice were closely observed for toxic symptoms and behavioural changes for the first two hours of administration and mortality recorded within 24 h LD50 was calculated using the method of Miller and Tainter [21]. Mice orally administered with extract were observed for 14 days, further, to investigate any sub-acute toxic effects.

 **Sub-chronic toxicity test**

Young adult male rats were randomly allotted to four groups (24/group), consisting of the control and three extract-treated groups, 10 mg/kg, 100 mg/kg and 1 g/kg (which represented one-tenth of the pharmacologically active dose, the pharmacologically active dose, and ten times the pharmacologically active dose, respectively). The doses were administered daily through drinking water, throughout 90-day test and 21-day reversibility period. After 90 days, some rats were sacrificed (16/group) for internal macroscopic and biochemical investigations, as well as oxidative stress parameters and histopathologic changes. Other rats were retained (8/group) for reversibility of test effects.

**Measurement biochemical parameters**

Whole blood of each rat was centrifuged at 2500 revolution for 20 min at 10 °C to separate the serum. Serum was mixed with reagents of Randox™ kits and read in a *Screenmaster* colorimeter set at 37 °C. The activity of serum alkaline phosphatase (ALP) was determined at 405 nm using a standard method [22], serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were also determined at 340 nm [23] Lactate dehydrogenase (LDH) was measured by the method of Wroblewski and LaDue [24], and serum direct bilirubin levels were also determined. Also Uric acid was measured using the urease cleavage Berthlot’s reaction and creatinine concentration was also determined.

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 biochemical (hepatic and/or renal) metabolites.

**Measurement of oxidative stress and peroxidation**

From liver, kidney, heart, lungs, spleen and testis tissue samples of sacrificed rats, determination of catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) was done according to a standard protocol [25].

Reversibility of significant toxicity effects was tested following 21 days of treatment withdrawal in the BP treated groups for only those groups and treatment where significance was recorded for oxidative stress metabolites.

**Histopathologic assessment**

After the collection of blood, the liver, kidney, heart, lung, spleen and testis tissues were immediately excised, freed from adventitia, blotted, weighed and fixed in 10 % formol saline for histological studies. Fixed sections were passed through alcohol and xylene, embedded in paraffin and blocked out. Thin sections (0.5 µ) were cut from these blocks, stained with haematoxylin and eosin and examined with a light microscope, (Ceti, UK) at magnifications ranging from 100 to 1000.

Histopathology was done again for reversibility of significant toxicity effects following 21 days of treatment withdrawal in the *B. pinnatum* treated groups for only those groups and treatment where pathology was recorded for the vital organs.

**Statistical analysis**

Results are presented as mean ± S.E.M or percentages. Statistical significance between the groups was analysed by means of Student’s *t*-test or analysis of variance (ANOVA); P values less than 0.05 were considered significant.

**RESULTS**

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

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

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

Biochemical parameters such as ALP, AST, and ALT, direct bilirubin and LDH were not significantly (*P*< 0.05) altered in the *B. pinnatum*-treated groups up to 100 mg/kg; however, at 1000 mg/kg of *B. pinnatum*, there were significant (*P*< 0.05) increases in all ALP, AST, ALT, LDH, uric acid, and Creatinine; but significant decrease in bilirubin level was recorded at 1000 mg/kg (Table 1a).

In the reversibility study when *B. pinnatum* was withdrawn (reversibility test) from rats for 21 days after 90-day treatment, the effects on all metabolites were reversed to comparable values as control (Table 1b).

**Effect of aqueous leaf extract of *B. pinnatum* on oxidative stress (protein peroxidation) markers in the 90-day study**

For all the vital organs tissues, heart, kidneys, liver, lungs, spleen and testes, at lower dose of 10 mg/kg, *B. pinnatum* did not produce any significant alteration of CAT, SOD and MDA. At 100 mg/kg of *B. pinnatum*, the MDA level was significantly elevated for kidney, heart, lungs and spleen. But at the highest dose of 1000 mg/kg, *B. pinnatum* produced a significant (P<0.05; ANOVA) reduction of CAT and SOD as well as elevation of MDA for all organs (Tables 2a, 3a, 4a, 5a, 6a and 7a).

When *B. pinnatum* was withdrawn (reversibility test) from rats for 21 days after 90-day treatment, the effects on CAT, SOD and MDA, except the lungs at 1000 mg/kg and testes at 100 mg/kg and 1000 mg/kg, were reversed to values comparable to those of the control (Table 2b, 3b, 4b, 5b, 6b, 7b). Also histopathology record showed that though, extent of severity was reduced but the testicular cells were still showing significant pathology.

**Effects of aqueous leaf extract of *B. pinnatum* administration on histology of selected organs in rats**

The most considerable pathology was observed in the testis. The untreated control testis showed normal cellular architecture (Fig. 1). In the 100 mg/kg rat group, moderate damage occurred in the testis, manifesting as ad-luminal cell loss, but more basal cells were spared (Fig. 2). A cell loss of the seminiferous epithelium including the basal cells or Sertoli cell nuclei was observed in the highest dose of 1 g/kg rat group (Fig. 3).

For the other organs examined, kidneys, liver and heart, a dose-dependent disruption to tissue histology was observed in few of the rats, especially at the highest dose of 1 g/kg BP tested. The predominant finding was parenchymal cell loss or atrophy.

In the kidney, adhesion between tubules was disrupted with edema, and shrunken glomeruli with wide-open capsular spaces (Figure 4).

In the liver, though there was cell loss and sloughing, visible as vacuolar spaces, classic lobular structure was preserved (Figure 5).

For the heart, the muscle cells were preserved (Figure 6).

**DISCUSSION**

In the acute oral toxicity test, the lack of any observable toxicity or mortality in mice, up to 5000 mg/kg, for 24 h, even up to 14 days, is indicative that the extract, when taken once, in large doses orally is not toxic [26, 27]. The hard stools observed in the extract-treated animals are suggestive of constipation. This supports folkloric use of the aqueous extract of the plant in the treatment of diarrhea. This is a confirmation of its ethnomedicinal use as antidiarrheal as confirmed for its methanolic extract in a recent study [28]. Moreover, another study had ascribed its mechanisms of action to be via interaction with β adrenoceptor, muscarinic cholinergic receptor and nitric oxide pathway [29].

In the chronic toxicity study, daily exposure of the plant extract for a period of 90 days did not produce any obvious physical abnormality. The most obvious detectable morphological abnormality was noted during dissection of the animals in the 1000 mg/kg treatment group, the right lung of one animal was grossly deformed containing pus, while another animal from this same treatment group had abnormally large kidney containing mainly clear fluid.

Results of the biochemical parameters showed significant derangement at 1000 mg/kg of the extract. There were significant increases in all of ALP, AST, ALT, LDH, uric acid, and Creatinine; but significant decrease in bilirubin level was recorded at 1000 mg/kg. This hypobilirubinemia supports the usefulness of the plant for the treatment of jaundice in Ayurveda medicine (30). However, the bilirubin level was reversed after 21 days of extract withdrawal. Ghasi and others [11] also reported elevated serum creatinine levels. The derangement of the biochemical parameters and the weight gain in the liver might be an indication of an adverse process ongoing in the animals, which was reversed by withdrawing treatment.

The significant reduction of CAT and SOD as well as elevation of MDA for all organs at 1000 mg/kg of the extract suggests that during long-term use of high doses of the extract oxidative stress could be induced. When BP was withdrawn (reversibility test) from rats for 21 days following the 90-day treatment, the effects on CAT, SOD and MDA, except the lungs at 1000 mg/kg and testes at 100 mg/kg & 1000 mg/kg, were detected to have been reversed to values comparable to those of the control (Table 2b, 3b, 4b, 5b, 6b, 7b).

Considerable cytotoxicity occurred in the testis, in animals exposed to high doses of BP in this study. This is consistent with the findings of a study of the ethanol leaf extracts of this plant at a maximum dose of 200 mg/kg [31]. From histopathology record, testicular cells still showed significant pathology after 21 days reversibility test as seen in Fig. 1-3.

The anti-fertility effects of the plant deserve careful attention based on its use in chronic health conditions. Particularly, its use in younger men who still have reproduction as a goal.

The effects of BP in this study suggest that the leaves of this plant contain substances that are considerably cytotoxic at high doses. This is probably why in many parts of the world people squeeze the juice and apply it directly to surfaces where it is needed to kill disease-causing organisms such as on burn wounds and boils and the cord of new born babies [31,32].

Cell damage apparently underlies much of the abnormal biochemical parameters recorded in this study. Cell atrophy also most likely accounts for increased serum creatinine levels found in this study.

Results of our phytochemical screening revealed presence of alkaloids, triterpenes, glycosides, flavonoids, steroids, butadienolides, lipids, and organic-acids. Also flavonoids from this plant have been shown to be cytotoxic in bacteria [33].

Further chemical screening had yielded 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 bufadienolideorthoacetate [33,34] has also been reported present in the leaf extract of the plant. Any one or combination of these phytochemicals might be responsible for its toxicity.

It should be stated that Bufadienolide, a cardiac glycoside, present in *Bryophyllum* species has been implicated in toxicity to cattles fed the plant [17].

**CONCLUSION**

The aqueous leaf extract of *Bryophyllum pinnatum* is safe up to 5g/kg in mice; however, at high doses, the extract could cause elevations of liver, renal and oxidative stress parameters, which are reversible when extract is withdrawn. But for high doses, caution should be taken during long-term use, especially in males of reproductive ages, due to possible vital organs, and especially testicular toxicity.

**REFERENCES**

1. WHO. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. Geneva, Switzerland: World Health Org. 2004.

2. Aschwanden, C., Herbs for heath but how safe are they. Bull. World Health Org. 2001; 79: 691-692.

3. Foster, DF, Phillips RS, Hamel MB, Eisenberg DM. Alternative medicine use in older Americans. J Am Geriatr Soc. 2000; 48; 1560–65.

4. WHO. Traditional Medicine Strategy (2002–2005). *WHO/EDM/TRM/2002.1.* Geneva, Switzerland: World Hlth Org. 2002.

5. Calapai G. European legislation on herbal medicines: a look into the future. Drug Safety. 2008; 31: 428–431.

6. Braun LA, Tiralongo E, Wilkinson JM, Spitzer O, Bailey M, Poole S. Perceptions, use and attitudes of pharmacy customers on complementary medicines and pharmacy practice. BMC Comp. Alt Med. 2010; 10: 38.

7. Anquez-Traxler C. The legal and regulatory framework of herbal medicinal products in the European Union: a focus on the traditional herbal medicines category. Drug Info J. 2011; 45 15–23.

8. Kamboj A, Saluja AK. *Bryophyllum pinnatum* (Lam): phytochemical and pharmacological profile: A Review. Pharmacog. Rev. 2009; 3, 364-74.

9. Ahmad M, Khan MR, Manzoor M, Zafar M, Sultana S. Check list of medicinal flora of tehsil Isakhel, district Mianwali-Pakistan. Ethnobot. Leaflets. 2006; 10: 41–8.

10. Barik LD, Ratha KK, Das M, Hazra J. HPTLC method for quantitative determination of quercetin in a polyherbal compound for urolithiasis. Int J Pharmacog. Phytochem Res. 2016; 8: 1187–90.

11. Ghasi S, Egwuibe C, Achukwu PU, Onyeanusi JC. Assessment of the medical benefit in the folkloric use of *Bryophyllum pinnatum* leaf among the Igbos of Nigeria for the treatment of hypertension. Afr J Pharm Pharmacol. 2011; 5, 83-92.

12. Akinpelu DA. Antimicrobial activity of *Bryophyllum pinnatum* leaves. Fitoterapia. 2000; 71(2): 193-194.

13. Aqil F., Ahmed I. Broad spectrum antibiotic and antifungal properties of certain traditional used India medicinal plants. World J Microbiol Biotech. 2003; 19: 653-657.

14. Ojewole JAO. Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* leaf aqueous extract. J. Ethnopharmacol. 2005; 99(1): 13-19.

15. Yemitan OK, Salahdeen HM. Neurosedative and muscle relaxant activities of aqueous extract of *Bryophyllum pinnatum*. Fitoterapia, 2005; 75: 187-193.

16. Yadav M, Gulkari VD, Wanjari MM. *Bryophyllum pinnatum* Leaf Extracts Prevent Formation of Renal Calculi in Lithiatic Rats. Anc Sci Life. 2016; 36, 90–97.

17. McKenzie RA, Franke FP, Dunste PJ. The toxicity to Cattle and Bufadienolide content of six *Bryophyllum* species. Aust Vet J. 1987; 64: 298-301.

18. Yemitan, O.K., Akinsuyi, A.A., Olayemi, S.O., Ayorinde, E.O. Toxicity assessment of aqueous leaf extract of *Bryophyllum pinnatum* on body and organ weights and haematologic parameters in rodents. LASU J Med Sci. 2018; 2(2): 24-30.

19. NIH. Guideline for the care and use of laboratory Animals. NIH Publication No. 85-23. 1996.

20. OECD Test No. 408*:* Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. 2018.

21. Miller LC, Tainter ML. Estimation of the ED50 and its error by means of logarithmic-probit graph. Proc Soc Exp Biol Med. 1944; 57: 261-264.

22. Arnold MS, Howard HC, Marvin MN. The colorimetric determination of phosphate in human serum. <http://www.jbc.org/content/190/1/7.> 1950. Accessed on the 25th November, 2016.

23. Xing-Jiu H, Yang-Kyu C, Hyung-Soon I, Oktay Y, Euisik Y. Aspartate aminotransferase and Aminotransferase Detection Technique. Sensors (Basel Switzerland). 2006; 6(7): 756-782.

24. Wróblewski F, Ladue JS. Lactic Dehydrogenase Activity in Blood. Proc Soc Exp Biol Med. 1955; 90: 210–213.

25. Turgeon ML. Leukocytes: Non-malignant disorder of granulocytes and monocytes. In Clinical Haematology Theory and Procedures. 2nd Ed., 143-152. 1993.

26. OECD Guideline for testing of Chemicals. Guideline 423: acute Oral Toxicity-Acute Toxic Class Method 2001. 2001.

27. Yemitan OK, Adeyemi OO. Investigation of sex-specific toxicity and reversibility of aqueous root extract of *Dalbergia saxatilis*. Univ Lag J Basic Med Sci. 2016; 4(7): 1-7.

28. Onoja SO, Ihejirika GQ, Nwankudu ON, Omeh YN, Ezeja MI. Antidiarrheal and Antioxidant Activities of Methanol Extract of *Bryophyllum pinnatum*Leaf Harvested from South-Eastern Nigeria in Mice. J Pharmaceut. 2018; 68:106-20. https://doi.org/10.1155/2018/6810620.

29. Adeyemi, O.O., Ishola, I.O., Okoro, U. Antidiarrhoeal Activity of Hydroethanolic Leaf Extract of *Bryophyllum pinnatum* Lam. Kurtz (Crassulaceae). Nig Quart J Hosp Med. 2013; 23(4), 323-329.

30. Sharma A. *Bryophyllum pinnatum* (Ayurvedic plant) uses and pics. 2015; [http://www.homeremediess.com/ayurvedic-plant-Bryophyllum-pinnatum-uses-and-pics/2015. Accessed on 24/11/2016](http://www.homeremediess.com/ayurvedic-plant-Bryophyllum-pinnatum-uses-and-pics/2015.%20Accessed%20on%2024/11/2016).

31.Akpantah AO, Obeten KE, Edung ES, Eluwa MA. The effects of ethanolic extract of *Bryophyllum pinnatum* on the micro-anatomy of the testis of adult Wistar rats. Eur J Biol Med Sci Res. 2014; 2: 37-44.

32. Pal S, Nag AK, Chaudhari N. Anti-inflammatory action of *Bryophyllum* *pinnatum* leaf extract. Fitoterapia, 1990; 61: 527-533.

33. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigeria plant. Afr J Biotech. 2006; 5: 357-361.

34. Kanika P. Pharmacognostic and phytochemical evaluation *of Bryophyllum pinnatum* leaves. J Adv Sci Res. 2011; 2: 42-49.