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Blood Cells Formative Properties of *B. pinnatum* in Chronic Inflammatory Disorders: An Experience with Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The use of medicinal plants to resolve many ailments especially inflammatory diseases is gaining global attention. *Bryophyllum pinnatum*(Crassulaceae) called '*Oda-opue*' in Igbo, '*Eru-odundun* in Yoruba and '*Abomoda'* in Hausa languages is one of the plants widely used as food and medicines in tropical Africa, America, India and China. This study investigated the effect of ethanol leaf extract of *B. pinnatum* on haematological parameters in Wistar rats induced with chronic inflammation. Fresh green leaves of *B. pinnatum* were collected from International Center for Ethno-medicine and Drug Development (InterCEDD), Nsukka, Enugu-State, Nigeria. Identification and authentication of the plant was carried and a voucher specimen was deposited at the InterCEDD herbarium. The plant material was then shredded, air-dried under shade and pulverized. The fine powders obtained was weighed and extraction was done via solvent combination of water and ethanol (3:7) for 72 hr via maceration. The filtrate gotten was evaporated to dryness to obtain the ethanol extract which was used for further bioassay study. Chronic inflammation was induced intraperitoneally using cotton pellet and hematological parameters were analyzed using mindray hematology auto analyzer. Results showed a significant increase ($p<0.05$)

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in the total white blood cell (TWBC) as well as the red blood cell, haemoglobin and packed cell volume levels in the extract treated groups compared with the control group. The findings of this study showed the reversibility of the induced and suppressive effects of chronic inflammatory disorder on blood cell progenitors. It therefore becomes possible for the adjunct use of *B. pinnatum* in the management of anemia in chronic inflammatory disorders.

Keywords: Chronic disease; inflammation; health; medicinal plants.

1. INTRODUCTION

The use of medicinal plants in the treatment of diseases has span through ages and currently gaining wide acceptance globally [1], as about 80 to 90 % of primary healthcare is sourced from traditional medicine worldwide [2]; hence, they remain a crucial part for drug development [3]. Several researchers have reported that plant metabolites are effective in the treatment of many ailments especially inflammatory diseases [4,5]. The ease of availability, low cost, and least side effects of plant based treatment therapies made it a focus of several available therapies most importantly in developing world [6].

Several medicinal plants have been reported to have diverse phytochemicals that can enhance erythropoiesis, protein synthesis and immune defense [7,8], lower blood glucose, triglyceride and cholesterol levels [9]; possess anti-inflammatory, anti-oxidative, antimicrobial, renal and hepatic protective potentials [5,10,11].

Findings from traditional medical practitioners showed that *Bryophyllum pinnatum* is one of the promising plants useful in ameliorating several disease conditions especially chronic inflammatory diseases.

Bryophyllum pinnatum popularly called life plant belongs to the family of Crassulaceae. It is a crucial ethno-medicinal plant widely distributed in many parts of the world such as Europe, Madagascar, America, India, China, Asia and Africa [12]. It is a fast-growing, succulent perennial plant found in temperate, tropical and subtropical areas. It is also grown around houses and in gardens for both ornamental and medicinal purposes. The plant can grow to about 1.5 meter in height with leaves arranged in opposite direction [13]. Leaves have a wide spectrum of therapeutic potentials attributed to the rich phytochemicals such as flavonoids, triterpenes, alkaloids, steroids, saponins, glycosides, tannins, bufadienolides [5,14]. Although *B. pinnatum* is used traditionally to treat many illnesses including diabetes, liver and kidney diseases, dyslipidaemia, obesity, cough, wound, ulcer, infection and anaemia [15,16], there is a dearth of literature reports of its importance on haematological profiles in a biological system. Hence, this study was carried out to evaluate the possible effects of the leaf extract of *B. pinnatum* on haematological parameters in Wistar rats induced with chronic inflammation.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Materials

Fresh green leaves of *B. pinnatum* were collected from International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu-State, Nigeria. Identification and authentication of the plant was carried out by a taxonomist and a voucher specimen was deposited at the InterCEDD herbarium (specimen number: BDCP/INTERCEDD-78). The plant material was shredded with a knife and air-dried under shade for 21 days.

2.2 Extraction of Plant Materials

The dried plant (leaves) was pulverized using a laboratory grinder and the fine powder obtained was stored in an air tight container at room temperature until further use. Weighed powdered sample was extracted with 70% ethanol (by maceration) for 72 hours. The yield of extracts was calculated according to the method of Nkafamiya et al., [17] using the formula below:

$$\text{Percentage yield} = \frac{\text{Mass of extract after rotary evaporation (g)}}{\text{Mass of powdered sample (g)}} \times 100$$

2.3 Procurement of Experimental Animals

Wistar rats (30) weighing between 180 g - 250 g were obtained from Chris Farm Ltd Mgbakwu, Awka, Anambra State. They were sorted, housed in standard cages with housing conditions of 12:12 light: dark cycles and fed with standard rat pellet and water *ad libitum*.

2.4 Induction of Inflammation

Cotton Pellet was used to induce chronic inflammation (granuloma) in the animals. One sterile cotton pellet weighing 20 mg each was implanted subcutaneously into the groin region of each anaesthetized rat and was allowed to stay for seven days.

2.5 Dose Preparation and Treatment

The hydro-ethanol leaf extract of *B. pinnatum* was prepared with distilled water in three divided dose (100, 200, and 400) mg / kg and Dexamethasone (25 mg/kg) used as a reference drug while distilled water was administered to the control group. The experimental animals were separated into five (5) groups (A - E) of six (6) animals per group, per cage. The animals were administered the extract and drug for seven consecutive days with water *per os* and feed *ad libitum* [18] as shown in the Table 1.

2.6 Collection of Blood Sample and Assay of Haematological Parameters

At the end seventh day, the experimental animals were anaesthetized with chloroform vapor, and sacrificed. A 5ml sterile syringe with needle was used for collection of blood via cardiac puncture and was used for bioassay studies. Collected blood sample were analyzed at WeCare diagnostic center, Zik Avenue, Awka, Anambra State using mindray hematology auto analyzer (BC 5300).

2.7 Data Analysis

The results were expressed as Mean ± Standard error of mean (S.E.M). One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$.

3. RESULTS

The results of this study are presented in Tables 2 and 3.

$$\text{Percentage yield} = \frac{\text{Mass of extract after rotary evaporation (g)}}{\text{Mass of powdered sample (g)}} \times 100$$

Mass of extract after rotary evaporation = 45 g
Mass of powdered sample = 500 g

$$\text{Percentage yield} = \frac{45\ g}{500\ g} \times 100$$

Percentage yield = 9 %

The effect of *B. pinnatum* leaf extract on hematological parameters (Total white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil and platelet) is presented in Table 2. There was a significant increase ($p<0.05$) in the TWBC levels of group C and D animals compared with the control group. Group D animals showed significant increase in the neutrophil count compared with the control group. The lymphocyte count of group C animals was found to be highest and significantly different from the control group.

The effect of *B. pinnatum* leaf extract on RBC, HGB, PCV, MCV, MCH and MCHC is presented in Table 3. There was a significant increase ($p<0.05$) in the RBC,HGB, PCV, MCV, MCH and MCHC levels of extract treated groups compared with the control group.

Table 1. Grouping and dose administration for animals

Group	Treatment
A	Inflammation plus 100 mg/kg extract sample
B	Inflammation plus 200 mg/kg extract sample
C	Inflammation plus 400 mg/kg extract sample
D	Inflammation plus 25 mg/kg Dexamethasone
E	Inflammation plus distilled water

4. DISCUSSION

The significant roles of plants in drug discovery cannot be overemphasized; and these have been associated with the bioactive compounds present in them [5]. Since phytochemical analysis is very useful in the evaluation of some active biological compounds of medicinal plants, the quantitative phytochemical analysis of the leaves of *Bryophyllum pinnatum* have been previously studied and documented [5].

The assessments of hematological parameters are useful guide to ascertaining the effect of foreign substances including plant extracts in a biological system. They are used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histopathology of the organs [19,20].

Table 2. Effect of *B. pinnatum* leaf extract on hematological parameters

Treatment group	TWBC (cells/L)	Neut. (cells/L)	Lymp. (cells/L)	Mon. (cells/L)	Eos. (cells/L)	Bas. (cells/L)	PLT (cells/L)
Group A	7.95±0.28^{c}	4.35±0.53^{c}	3.80±0.42^{c}	0.24±0.14^{a}	0.04±0.01^{c}	0.03±0.01^{b}	640.33±1.53^{c}
Group B	8.60±1.15^{b}	4.39±0.18^{d}	4.00±0.59^{b}	0.29±0.02^{c}	0.06±0.02^{c}	0.04±0.05^{a}	646.67±2.89^{a}
Group C	10.35±1.41^{a}	4.55±0.22^{d}	6.01±1.30^{a}	0.31±0.06^{b}	0.14±0.07^{b}	0.07±0.05^{a}	682.67±2.08^{b}
Group D	10.49±1.28^{a}	5.59±2.08^{a}	5.31±0.53^{b}	0.33±0.13^{a}	0.39±0.19^{a}	0.07±0.02^{b}	782.67±1.15^{d}
Group E	7.63±0.28^{c}	3.78±0.77^{b}	2.57±0.40^{c}	0.20±0.13^{a}	0.02±0.01^{c}	0.01±0.01^{b}	579.00±2.65^{b}

Values are means ± standard error of mean. Values on the same column with different alphabet superscript are significantly different at P< 0.05

TWBC: Total white blood cell; Neut.: Neutrophil; Lymp.: Lymphocyte; Mon.: Monocyte; Eos.: Eosinophil; Bas.: Basophil; PLT: Platelet

Table 3. Effect of *B. pinnatum* leaf extract on RBC, Hgb, PCV, MCV, MCH and MCHC

Treatment group	RBC (million/mm^3)	HGB (g/dL)	PCV (mL)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Group A	6.97± 0.01^{d}	131.33± 2.08^{b}	4.23± 0.15^{a}	49.04±0.04^{b}	20.02±0.14^{b}	31.12±0.06^{c}
Group B	7.41± 0.04^{d}	134.00± 2.00^{c}	4.33± 0.04^{d}	54.22±0.12^{a}	21.12±0.18^{a}	31.02±0.09^{b}
Group C	7.70± 0.13^{c}	142.30± 2.52^{a}	4.69± 0.10^{b}	56.28±0.02^{b}	24.22±0.06^{c}	32.16±0.18^{a}
Group D	7.47± 0.62^{a}	143.67± 1.15^{d}	4.93± 0.08^{c}	59.03±0.14^{a}	25.02±0.06^{c}	32.05±0.10^{b}
Group E	6.21± 0.24^{b}	119.33± 2.08^{b}	3.40± 0.07^{c}	48.00±0.00^{c}	18.23±0.04^{d}	31.00±0.04^{c}

Values are means ± standard error of mean. Values on the same column with different alphabet superscript are significantly different at P< 0.05

RBC: Red blood cell; HGB: Heamoglobin; PCV: Packed cell volume; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration

Tables 2 and 3 showed the effect of *B. pinnatum* leaf extract on Hematological parameters. As evident in Table 2, there was significant increase ($p< 0.05$) in a dose dependent manner in the total white blood cell (TWBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil and platelet counts of the treated groups when compared with the control group. This result is in agreement with the findings of Ahumibe and Braide [21] and also supports the observation made by Nwankpa et al. [22] who suggested that increase in white blood cells helps to stimulate cytokine erythropoietin. *B. pinnatum* extract therefore, might have plausibly stimulated cytokine erythropoietin which consequently may have stimulated blood cell synthesis.

White blood cell (WBC) and its differentials (lymphocytes and neutrophils) and other haematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function [23]. WBC's are essential for the protection of the animal against foreign invaders [8]. Elevation in their levels is indicative of response to an immunological challenge. Neutrophil are important phagocytic cells normally elevated in the early inflammatory response [24], while lymphocytes are subtypes of leucocytes critically essential for providing cell mediated immunity. In addition, increase in WBC and neutrophil counts suggest the ability of the leaf extract of *B. pinnatum* to boost the cells immune system since they function as active phagocytic agents against foreign compounds [25]. This may possibly explain its use in the management of inflammation and other related ailments.

Similarly, a significant increase ($p<0.05$) in RBC, Hgb, PCV, MCV, MCH and MCHC were observed in the treated groups compared to the control (Table 3). The result of this study is similar to that of Esenowo et al. [26] and Okon et al. [27] in their separate studies. Although the result on RBC, Hgb, and PCV in this study is in agreement with the findings of Ogbonnia et al. [28] and Nwankpa et al. [22], the result of MCV concentration which showed a slight increase on administration of leaf extract of *B. pinnatum* is in contrast with their result.

This hematopoietic condition may be due to different mechanisms which include increase in rate of blood cell synthesis and or decrease in rate of blood cells destruction. Any of the two mechanisms may have been responsible for the increase in the red cell indices. The plant extract may have the potential to stimulate erythropoietin release which consequently increases the synthesis of red cells [29] and or help fight against infections and microbial invasions [27] which destroys blood cells both of which may culminate to increases in blood cells as observed in this study.

5. CONCLUSION

The observations from the present study showed that the extract of this plant is capable of stimulating blood cell formation and act as active phagocytic agent against foreign compounds. This could partly be attributed to many bioactive compounds present in the extract which could be synthesized to produce new plant based product to fight inflammatory disorders with fewer side effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Animal Research Ethics Committee of the Nnamdi Azikiwe University, Awka with institutional animal ethics committee (IAEC) number NAU/AREC/PERM/2021/00017

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oladejo AA, Onwubuya EI, Ogbunugafor HA, Okafor CS, Ogbodo UC. Inhibitory effects of *Bryophyllum pinnatum* leaf extract on inflammatory biomarkers. Journal of Advances in Medical and Pharmaceutical Sciences. 2021;23(2):7-13.
2. Mafimisebi TE, Oguntade AE. Preparation and use of plant medicines for farmers' health in Southwest Nigeria: Socio-cultural, magico-religious and economic aspects. Journal of Ethnobiology and Ethnomedicine. 2010;6(1):1-9.
3. Salehi B, Kumar NVA, Şener B, Sharifi-Rad M, Kılıç M, Mahady GB, Vlaisavljevic S, Iriti M, Kobarfard F, Setzer WN. Medicinal plants used in the treatment of human immunodeficiency virus. International Journal of Molecular Science. 2018;19:14-59.
4. Katsiari CG, Bogdanos DP, Sakkas LI. Inflammation and cardiovascular disease. World Journal of Translational Medicine. 2019;8(1):1-8.
5. Onwubuya EI, Ogbunugafor HA, Okafor CS, Oladejo AA. Acute and chronic anti-inflammatory effect of *Bryophyllum pinnatum* leaf extract on wistar rats. European Journal of Medicinal Plants. 2021;32(6):6-14.
6. Onwubuya EI, Oladejo AA. Possible effects of free radical scavengers on dissolution of inflammatory granuloma: An experience with wistar rats. Current Research in Complementary & Alternative Medicine. 20225:149.
7. Udo EF, Olantunji OI, Abayomi FT, Idowu AM, Abiodun AO. Comparative haematological and serum biochemical evaluation of rats fed with hydro-ethanolic leaves extracts of *Moringa oleifera* and *Telfairia occidentalis*. Covenant Journal of Physical & Life Sciences. 2017;5(1):17-26.
8. Oladejo AA, Osukoya O. Hematological profiles of naturally infected pigs treated with *Bridelia ferruginea* leaf extracts. Asian Hematology Research Journal. 2021;4 (2):1-10.
9. Howida S, Abou S. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni-Suef University Journal of Basic and Applied Sciences. 2016;5(2):134-146.
10. Feng Y, Li S, Tan H, Wang N, Zhang Z, Lao L, Wong W. The role of oxidative stress and antioxidants in liver diseases. International Journal of Molecular. Science. 2015;16(1):26087- 20124.
11. Kyrian-Ogbonna EA, Ekwealor IA, Eze EN, Eluba RA, Nworji EC, Ogah EC, Oladejo AA. Antibiotic resistance patterns of *Escherichia coli* detected in pig faecal samples from Anambra state. Microbiology Research Journal International. 2021;31 (5):56-63.
12. Seema VP. Kalanchoe pinnata: Phytochemical and pharmacological profile. International Journal of Pharmaceutical science and Research. 2018;3(4):993 -1000.
13. Anuradha KP, Shankul K, Iyeri SV, Rahul JS, Vaidya SK. Pharmacognostical, phytochemical and pharmacological review on *Bryophyllum pinnata*. International Journal of Pharmaceutical and Biological Archives. 2012;3(3):423-433.
14. Aprioku JS, Igbe I. Effects of aqueous *Bryophyllum pinnatum* leaf extract on haematological, renal and sperm indices in wistar rats. Indian Journal of

Pharmaceutical Sciences. 2017;79(4):521-526.
15. Okwu DE, Nnamdi FU. Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. Journal of Chemistry and Pharmacology Research. 2011;3(2):1-10.
16. Muhammad A, Imran K, Ruqaiyah K, Rajbala S, Mohit C, Tanvi B, Firoz A. *Bryophyllum pinnatum:* A review. International Journal of Research in Biological Sciences. 2012;2(4):143-149.
17. Nkafamiya II, Maina HM, Osemeahon SA, Modibbo UU. Percentage oil yield and physiochemical properties of different groundnut species (*Arachis hypogaea*). African Journal of Food Science. 2010;4 (7):418-421.
18. Goldstein SA, Shemano L, Daweo R, Betler JM. Cotton pellet granuloma pauchmethod for evaluation anti-inflammatory activity. Archives Internationales de Pharmacodynamie et de Therapie. 1967;165:294-301.
19. Magalhaes P, Appell H, Duarte J. Involvement of advanced glycation and products in the pathogenesis of diabetes complication; the protective role of regular physical activity. European Review of Aging and Physical Activity. 20085:17-29.
20. Oyedemi SC, Adewusi EA, Aiyegoro CA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic wistar rats. Asian Pacific Journal of Tropical Biomedicine. 2011;1:353-358.
21. Ahumibe AA, Braide VB. Effect of gavage treatment with pulverized *Garcina kola* seeds on erythrocyte membrane integrity and selected haematological indices in male albino wistar rats. Nigerian Journal of Physiological Sciences. 2009;24:47-52.
22. Nwankpa P, Chukwuemeka OG, Ekweogu CN. Investigation of ethanol stem bark extract of *treculia africana* on the haematological parameters of albino wistar rats. Journal of Biochemistry and Analytical Biochemistry. 2017;6(4):347-351.
23. Aprioku JS, Obianime, AW. Evaluation of the effects of *Citrus aurantifolia* (Lime) juice in lead-induced haematological and testicular toxicities in rats. Pharmacologia. 2014;5:36-41.
24. Zhang W, Fievez L, Cheu E, Bureau F, Rong W, Zhang Y. Antiinflammatory effects of formoterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats. The European Journal of Pharmacology. 2010;628:171-8.
25. Teguia A, Telefo PB, Fosto RG. Growth performances, organ development and blood parameters of rats fed raded levels of steeped and cooked taro tuber (*Esculenta*) meal. Livestock Research for Rural Development. 2007;19:1-8.
26. Esenowo GJ, Sam SM, Bala DN, Ekpo BA, Edung EM. Phytochemical screening and the haematological effect of *Peristrophe bicalycuta* (RETZ) Nees diet preparation in albino rats. World Journal of Applied Science and Technology. 2010;2:277-281.
27. Okon JE, Esenowo GJ, Afaha IP, Umoh, NS. Hematopoietic parameters of ethanolic fruits extract of musa accumulate on albino rats. Bulletin of Environment, Pharmacology and Life Sciences. 2013;2:22-26.
28. Ogbonnia SO, Anyika EN, Mbaka GO, Utah P, Ugwu D. Antihyperglycaemic and antiheperlipidaemic effects of aqueous ethanol extract of *Tapinanthus globiferus* leaves and *Treculia africana* root bark and their mixture on alloxan diabetic rats. Agriculture and Biology Journal of North America. 2012;3:237-246.
29. Mbaka GO, Adeyemi OO, Oremosu AA. Acute and sub-chronic toxicity studies of the ethanol extract of the leaves of *Sphenocentrum jollyanum* (menispermaceae). Agriculture and Biology Journal of North America. 2010; 1:265-272.

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