Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Phytochemical Composition of Ethanol Extract of *Bryophyllum pinnatum* leaves (EEBP) with its Effects on Haematopoietic Indices and Bone Marrow Histology of Cadmium-intoxicated Rats

Doris Akachukwu[*1], Charles N. Chukwu[1], Philippa C. Ojimelukwe[2], Anthony C.C. Egbuonu[1], Inemesit C. Ubiom[3], Rosemary I. Uchegbu[4]

[1]*Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria*

[2]*Department of Food Science and Technology, College of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Nigeria*

[3]*Department of Biochemistry, Faculty of Sciences, University of Uyo, Uyo, Nigeria*

[4]*Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria*

(Received: 5 August 2022 Accepted: 6 July 2024)

KEYWORDS

Bone marrow;
Bryophyllum pinnatum;
Cadmium-challenged;
GC-MS;
Haematology;
Histology

ABSTRACT: Cadmium intoxication may arise from environmental pollution and cause bone and nervous system disorders. The phytochemical composition of ethanol extract of *Bryophyllum pinnatum* leaves (EEBP) with its effects on haematopoietic indices and bone marrow histology of cadmium-intoxicated rats was investigated. Twenty-four male albino rats were grouped for four treatments: Group 1 - normal control, Group 2 - 5 mg kg^{-1} bodyweight $CdCl_2$, Groups 3 and 5 - 200 and 400 mg kg^{-1} body weight EEBP, while Groups 4 and 6 received 5 mg kg^{-1} bodyweight $CdCl_2$ and treated with 200 and 400 mg kg^{-1} bodyweight EEBP respectively for 14 days. Feed and water were given ad libitum. Six bioactive compounds were obtained with 2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans-13-octadecanoic acid methyl ester (35.81%) being the most abundant. The extract-treated groups showed a significant increase in haemoglobin (from 10.61 to 10.78 mmol L^{-1}), packed cell volume (41.75%), red (6.36-6.42 × 10^6 mm^3), and total white blood cells counts (17.30-19.25 × 10^6 mm^3). The cadmium-intoxicated groups treated with 200 and 400 mg kg^{-1} body weight of EEBP showed a mild reduction in progenitor cells in the bone marrow. The results suggest that EEBP possesses potent bioactive compounds and nutrients that could improve hematological properties and attenuate bone marrow degeneration in cadmium-intoxicated rats.

INTRODUCTION

Cadmium (Cd) intoxication has been reported from several parts of the world as one of the global health challenges, which has deleterious effects on several organs and in some cases, can cause death [1]. The human system is vulnerable to harmful effects of toxicants including drugs and heavy metals [2]. Residents or workers near waste sites or

*Corresponding author: dorisakachukwu@yahoo.com (D. Akachukwu)
DOI: 10.60829/jchr.2024.1599

industrial areas that emit cadmium into the air have been shown to develop impaired health, including injured lungs, diarrhea, stomach aches and serious nausea, broken bone, disorders of the reproductive system, central nervous system damage, mental disorder, genetic damage or cancer development [3]. Cadmium toxicity also affects the blood and bone systems [4]. The hematopoietic system comprises the bone marrow, bood cells, stem cells, lymphoid tissues and circulatory system. It is responsible for the production, development and regulation of different blood cells that are vital for transporting oxygen, immune defence and blood clotting. According to El-Boshy et al. [5] cadmium disrupts hematopoietic activities by inducing genetic damage of stromal and hematopoietic cells, thereby upsetting cytokine balance. Kocak [6] findings indicate that cadmium induces a type of anaemia where red blood cells are smaller than the normal size in rats. Cadmium damages the structure and genetic material of the blood and bone marrow cells blood and bone marrow cells. One of the major deleterious effects of cadmium is that it temporarily increases white blood cells [7]. Cadmium has been reported to reduce red blood cell shelf-life and induce hemolysis that leads to anemia in animals and humans [8].

Bryophyllum pinnatum (Lam.) is a perennial therapeutic herb, that naturally grows in Madagascar, and is widely found in a, Africa, Asia, Australia, and New Zealand [9]. In Nigeria, *B. pinnatum* is commonly called 'Never Die', Hausa – '*Karan maslach*', Igbo – '*Oda opue*' or 'Alupu', and Yoruba – '*abamoda*'. It is helpful in treating various ailments such as rheumatism, arthritis, body pain, heartburn, skin ulcers, diabetes, infections and high blood pressure [10]. In Nigeria, the plant is known for its wound healing properties and assist in the detachment of infants' umblical cords. The extract of *B. pinnatum* has been shown to raise white blood cells, lower neutrophil count, while maintaining lymphocyte count and packed cell volume [9]. It has also been shown to possess therapeutic and biological effects including anti-inflammatory, antihypertensive, immunomodulatory, antitumor, antioxidant properties, etc. [11-15]. Several phyto-constituents including alkaloids, triterpenes, glycosides, flavonoids and steroids [11, 16, 17] have been reported to be responsible for these activities. In a recent study, Kısadere et al. [18] reported the protective effect of melatonin in hematological characteristics of cadmium-induced rats. There is no available literature on the possible effect of ethanol extract of *B. pinnatum* leaves in rats under cadmium toxicity and this has necessitated this study. The study was therefore aimed at investigating the GC-MS composition of the ethanol extract of *B. pinnatum* leaves (EEBP) and its effect on hematological indices and bone marrow histology of cadmium-challenged rats.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh matured *Bryophyllum pinnatum* leaves were harvested from the premises of the National Soil, Plant and Water Laboratory which operates under the Federal Ministry of Agriculture and Rural Development, Umuahia North LGA, Abia State, Nigeria. The plant was identified and authenticated by the Taxonomic unit of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike. The plant leaves were sorted, dirt removed, and washed in running tap water. Thereafter, they were kept in a container to drain off water and left to air-dry. A quantity of 626.13 g of *Bryophyllum pinnatum* leaves was then weighed and ground using a hand-milling machine.

Extraction of plant sample

The ground sample was soaked with 1 L of ethanol (99.8%) with periodic shaking for 48 hours for efficient extraction. It was then filtered using Whatman No 1 filter paper. The filtrate was dried using a rotary evaporator and subsequently placed in the oven to concentrate it to obtain the methanol extract of *Bryophyllum pinnatum* (MEBP), while the residue was discarded. The extract was weighed and stored in a small beaker and kept in the refrigerator until it was ready for use.

GC-MS

Gas chromatography-mass spectra (GC-MS) analysis was conducted using an Agilent 7890A GC-MS system equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J & W Scientific, Folsom, CA). The initial oven temperature was 60 °C. Helium was used as the carrier gas at the rate of 1.0 mL/min. The eluent from the GC column was introduced directly into the MS via a transfer line maintained at 250 °C. The ionization voltage was 70 eV and the ion source temperature was 230 °C. The scan range was 41- 450 amu. The components were identified by comparing their retention times to the reference in the National Institute of Standards and Technology (NIST, ver. 2.0, 2008) mass spectral database.

Experimental animal

A total of 24 male albino rats (weighing 104-185 g) were procured from the Animal Breeding House of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The animals were transported to the Animal House of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, and acclimatized for 2 weeks in clean cages.

Experimental Design

The animals were randomly divided into six (6) groups of four (4) animals each. The animal groupings were as follows:

Group 1: Control (Feed + water only)

Group 2: 5 mg kg^{-1} body weight b.w $CdCl_2$

Group 3: 200 mg kg^{-1} b.w EEBP only

Group 4: 5 mg kg^{-1} BW $CdCl_2$ co-treated + 200 mg kg^{-1} b.w EEBP

Group 5: 400 mg kg^{-1} b.w EEBP

Group 6: 5 mg kg^{-1} BW $CdCl_2$ co-treated + 400 mg kg^{-1} b.w EEBP

The animals had free access to feed and water. The experiment was for 14 days, after which they were weighed and sacrificed through cervical dislocation on the 15^{th} day. The blood was collected into EDTA (Ethylenediaminetetraacetic acid) bottles with capillary tubes from the optical plexus for hematological assays. The bone marrows of the rats were collected by cutting their femur bone and a smear of the bone marrow was made on labeled slides according to their respective groups. The bone marrow slides were stored with a methanol solution.

Hematological investigation

The counts of red and white blood cells, packed cell volume, and hemoglobin concentration were determined using standard protocols. Red blood cell (RBC) count was determined using the Haematocrit method [19]. Total white blood cell (WBC) count and packed cell volume (PCV) were determined by hematocytometry following the method described by Ochei and Kolhatkar [20]. Packed cell volume (PCV) was estimated as described by [20] while hemoglobin (Hb) concentration determination was done using Cyanmethemoglobin method as described by [20]. The Hb concentration was determined by multiplying the absorbance of the sample by a calibration factor (36.8) which was derived from the absorbance and concentration of the standard [21].

Histological investigation

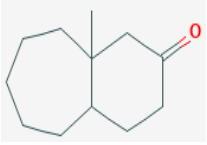
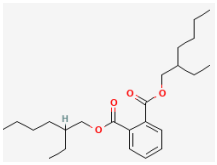
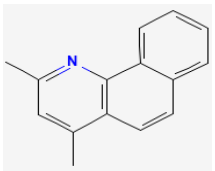
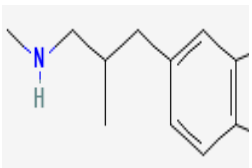
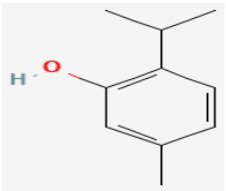
This was done using the paraffin technique as described by Bancroft and Stevens [22]. In this technique, the bone marrow tissues were fixed and embedded in paraffin wax. This hardens the tissues and makes it easier to cut its sections. The sections were then stained and examined under a microscope. The samples were placed individually into labelled sample bottle and immersed in a 10% formal saline solution for fixation. Thereafter, the samples were dehydrated through ascending grades of alcohol viz: 70% (1 hour); 95% twice (1 hour each); absolute alcohol twice (1 hour 30 minutes each); and another absolute alcohol for 2 hours; equal volumes of absolute alcohol and xylene, i.e. 50/50 (overnight); then cleared in two changes of xylene for one hour each.

Paraffin wax infiltration was carried out with two changes of paraffin wax at intervals of one hour each, in an electric hot air oven at 60°C. The samples were then embedded in paraffin wax, trimmed, and mounted in a wooden chuck. Each sample was sectioned on the microtome at 5 µm and floated on the floatation bath. The floated sections were picked out with clean albumenized microscopic slides; the slides were stained using Haematoxylin and Eosin (H & E) protocol and cover-slipped with a Depex mountant. The slides were later viewed with a binocular Olympus microscope (Zeedo, 5MP internal camera, e-PLAN objective). nLabelling was done with Photoscape v3.7.

RESULTS AND DISCUSSION

Table 1 summarizes the *GC-MS* analysis of *ethanol extract of B. pinnatum* (EEBP) while Figure 1 is the chromatogram showing 6 peaks. As presented in the table, six (6) compounds were detected namely: 2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans- (35.81%), Heptadecanoic acid, 16-methyl-, methyl ester (16.22%), Bis (2-ethylhexyl) phthalate (14.31%), MDMA methylene homolog (24.28%) and thymol (9.39%). These constituents obtained from GC-MS analysis have several biological activities, which can be exploited for therapeutic purposes. 2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans- 13-Octadecenoic acid, and methyl ester have been reported to possess anti-angiogenic and anti-tumor effects [23]. Heptadecanoic acid, 16-methyl-, methyl ester is a fatty acid methyl ester with anti-microbial properties. Bis (2-ethylhexyl) phthalate is a major bioactive metabolite with potent antibacterial, antimicrobial, and cytotoxic activities [24-26]. Benzo[h]quinoline, 2,4-dimethyl- and other quinolone analogs and derivatives have been reported to possess diverse biological effects such as seizure-preventing, anticancer, anti-inflammatory, blood pressure-lowering properties, antimicrobial, antimalarial and so on [27]. Several reports have shown that thymol possesses various pharmacological properties including antioxidant, free radical scavenging, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal, antiseptic, and antitumor activities [28]. These properties of thymol are attributed to hydroxyl group in its phenolic structure, which confers protection by mutual absorption or neutralization of free radicals or by supplementing endogenous antioxidants [29].

Table 1. Chemical Composition (GC-MS) of the EEBP

Peak No.	Name of compound	RT (Mins)	Exact mass (g)	Conc. (%)	Formula	Structure
1	2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans-13-Octadecanoic acid, methyl ester	16.03	180.15	35.81	$C_{12}H_{20}O$	
2	Heptadecanoic acid, 16-methyl-, methyl ester	16.30	298.29	16.22	$C_{19}H_{30}O_2$	
3	Bis (2-ethylhexyl) phthalate	18.95	390.28	14.31	$C_{24}H_{38}O_4$	
4	Benzo[h]quinoline, 2,4-dimethyl-	20.09	207.10	1.80	$C_{15}H_{13}N$	
5	MDMA methylene homolog	20.32	207.13	24.28	$C_{12}H_{17}NO_2$	
6	Thymol, TMS	20.67	150.10	9.39	$C_{10}H_{14}O$	

EEBP - Ethanol Extract of *Bryophyllum pinnatum* leaves

Figures 1, 2, and 3 show the results of the impact of ethanol extract from *Bryophyllum pinnatum* on hemoglobin concentration, red blood cell count, and packed cell volume of cadmium-challenged rats. As depicted in Figure 1, the group treated with 400 mg kg^{-1} of EEBP exhibited a significantly ($p < 0.05$) higher hemoglobin concentration compared to both the control and cadmium-intoxicated groups. Moreover, the groups intoxicated with cadmium and simultaneously treated with EEBP (at doses of 200 and 400 mg kg^{-1}) showed a significant increase in Hb concentration compared to both the normal control and cadmium control groups. The Hb concentration in the extract control group (200 mg kg^{-1}) was not significantly different ($p > 0.05$) from that in the normal control groups. The same trend of the effect of EEBP on hemoglobin concentration was observed in PCV (Figure 3). As shown in Figure 2 There was a significantly ($p < 0.05$) lower blood cell (RBC) count of the cadmium control group when compared to the normal control group and the groups intoxicated with cadmium and co-treated with EEBP (200 and 400 mg kg^{-1}). There was no significant ($p > 0.05$)

difference in the RBC count of the cadmium control group compared to the 200 mg kg^{-1} EEBP group.

Multiple studies have associated cadmium toxicity with various types of anemia, which can result from the destruction of peripheral red blood cells (RBCs), iron deficiency, and inadequate erythropoietin production [5, 30]. This was evident in this study as shown by the significant suppression of Hb, RBC count, and packed cell volume in the cadmium group. However, treatment with ethanol extract of *B. pinnatum* leaves (EEBP) significantly improved the hematological parameters (Hb, RBC count, and PCV). This aligns with the report of Ufelle et al. [31] who reported increased Hb and PCV levels in rats administered crude methanol leaf extract of *B. pinnatum.* The observed marked elevation in Hb concentration and PCV could be due to the stimulatory effect of *B. pinnatum* on the bone marrow to produce more hemoglobin and red blood cells. The presence of important phytochemical constituents in *B. pinnatum* such as tannin, ascorbic acid and phenol could boost erythropoiesis [30-33]. Flavonoid, zinc, riboflavin and niacin contents of *B. pinnatum*, as earlier reported by [34, 35] could be responsible for the improvement of the hematological parameters observed in this study. Also, benzo[h]quinoline, 2,4-dimethyl-, one of the constituents discovered from the GC-MS analysis was also present in the stem bark extract of *Mangefera indica* L. and showed significant anti-anemic property in hemolytic anemic rats [36]. Hence, *B. pinnatum* could serve as a hematinic product in terms of improving blood volume and erythropoietic processes.

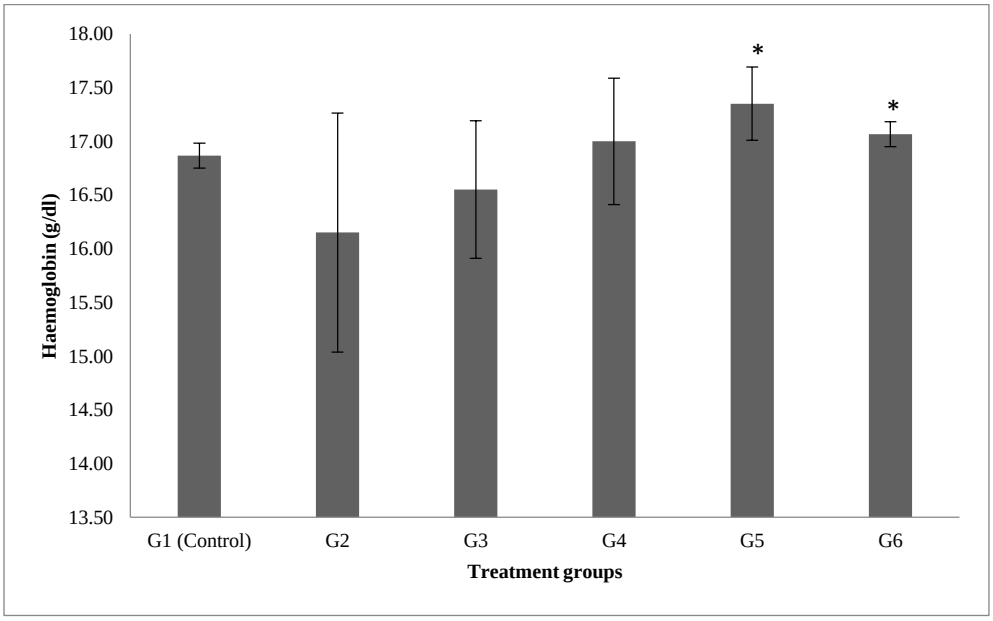


Figure 1. Effect of EEBP on Haemoglobin Concentration of Cadmium-Challenged Rat. Bars are mean ± standard deviation (n=4). Bars with an asterisk (*) are significantly ($p < 0.05$) different compared with $CdCl_2$-treated group. BP = *Bryophyllum pinnatum.*

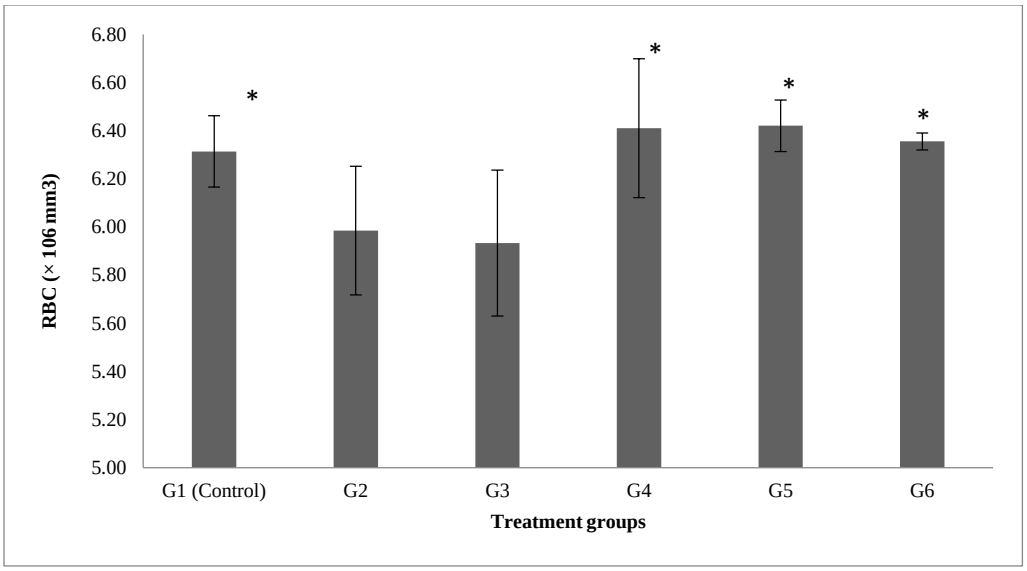


Figure 2. Effect of EEBP on Red Blood Cell Concentration of Cadmium-Challenged Rat. Bars are mean ± standard deviation (n=4). Bars with asterisk (*) are significantly ($p < 0.05$) different compared with $CdCl_2$-treated group. BP = *Bryophyllum pinnatum*

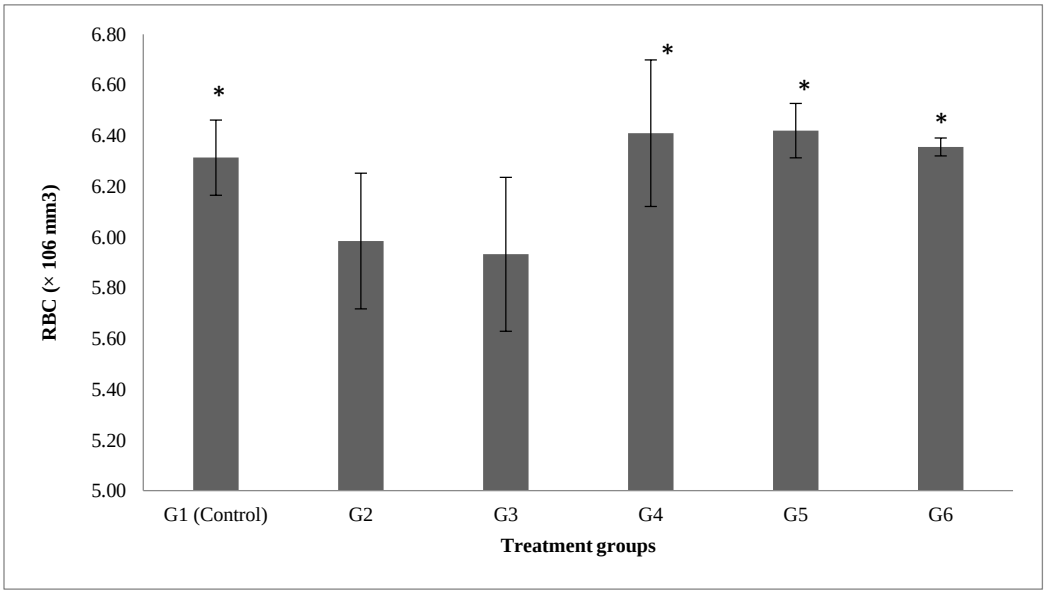


Figure 3. Effect of EEBP on Packed Cell Volume of Cadmium-Challenged Rat. Bars are mean ± standard deviation (n=4). Bars with asterisk (*) are significantly ($p < 0.05$) different compared with $CdCl_2$-treated group. BP = *Bryophyllum pinnatum*

As shown in Table 2, the cadmium control group had the least TWBC count when compared to the rest of the groups – showing statistical significance ($P<0.05$) regarding extract-treated (200 mg kg^{-1} EEBP) and cadmium-extract co-treated (5 mg kg^{-1} Cd + 400 mg kg^{-1} EEBP) groups. The percentage lymphocyte and neutrophil concentrations of the extract-only and cadmium-extract co-treated groups (Groups 3 – 6) were significantly ($P<0.05$) elevated compared to those of the normal and cadmium control groups. No significant ($P > 0.05$) difference in the percentages of monocytes and eosinophils was observed.

Total white blood cell (TWBC) count and its differentials are quantifiable parameters of the blood that are commonly used to appraise hematopoietic function [37]. White blood cells (WBC) are a vital in conferring protection to living organisms against foreign invaders and a rise in their concentration could denote an immunological challenge. Conversely, neutrophils are crucial phagocytic cells that typically increase during the initial stages of the

inflammatory response [38, 39] whereas lymphocytes are a subtype of WBC that play a crucial role in cell-mediated immunity. The observed elevation in total white blood cell (TWBC) count in the EEBP-treated groups of the rats is in tandem with earlier findings [31,40] which is suggestive of the fact that *B. pinnatum* possesses antimicrobial activity. The observed significant elevation of TWBC count and lymphocytes by the extract as seen in this study (Table 2) suggests that *B. pinnatum* could serve as a potent antimicrobial agent and immune booster against cadmium-induced toxicity. These effects could have been enhanced by the presence of the notable antimicrobial constituents (heptadecanoic acid, 16-methyl-, bis (2-ethylhexyl) phthalate, benzo[h]quinoline, 2,4-dimethyl- and thymol) shown by the GC-MS analysis (Table 1).

Furthermore, the significant decrease in neutrophils (Table 2) observed in this study aligns with the findings previously reported Ufelle et al. [31]. This strongly suggests that the plant has the ability to weaken primary immune response, including inflammation. Previous reports showed that the aqueous extract of *B. pinnatum* possesses anti-inflammatory [41,42] and immunosuppressive activities [38] which could in part relate to the observed neutrophilic effect.

Table 2. The Effect of Ethanol Extract of *B. pinnatum* Leaves on Total White Blood Cell (TWBC) Count and Differentials of Cadmium-Challenged Rats.

Group	TWBC ($x10^3$ mm^{-3})	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophil (%)
Normal Control	9.73 ± 1.16	54.67 ± 1.76	37.67 ± 2.02	4.67 ± 0.67	3.00 ± 0.58
$CdCl_2$	12.13 ± 0.26	54.25 ± 0.63	38.00 ± 0.58	5.25 ± 0.48	2.50 ± 0.65
200mg kg^{-1} BP	19.25±1.06*	60.00 ± 0.41*	31.00 ± 0.91*	5.75 ± 0.48	3.25 ± 0.48
400mg kg^{-1} BP	14.70 ± 0.66	59.00 ±0.71*	32.25 ± 1.25*	5.50 ± 0.64	3.25 ± 0.48
Cd+200mg kg^{-1} BP	14.20 ± 2.07	58.25 ± 0.85*	33.50 ± 0.96*	5.25 ± 0.48	3.00 ± 0.81
Cd+400mg kg^{-1} BP	17.30 ± 0.14*	57.33 ± 11.45*	33.33 ± 1.86*	5.67 ± 0.67	3.67 ± 0.67

Values are mean ± standard deviation. Values with an asterisk (*) are significantly ($P < 0.05$) different compared with the $CdCl_2$-treated group. TWBC = Total white blood cells; BP = *Bryophyllum pinnatum*

Result of histological investigation

Figure 6 show the photomicrographs of the bone marrow of the animals in the study showcasing various stages of normal development in erythroid and granulocytic progenitor cells, with a notable presence of metamyelocytes (MM), erythroblasts (EB), lymphoblasts (LM), megakaryocyte (MK), occasional macrophages (M) and band forms of neutrophils (NB) among others. Occasional mitotic figures (MF) are also seen. The group induced with $CdCl_2$ showed moderate to severe reduction of the different progenitor cells compared to the normal control group. However, there was a mild reduction of the different progenitor cells in the cadmium-challenged groups treated with 200 and 400 mg kg^{-1} b.w of *B. pinnatum* compared to the normal control. There were no pathological alterations in the groups treated with only the ethanol extract (200 and 400 mg kg^{-1} b.w) of *B. pinnatum* compared to the normal control.

The sole administration of the extract did not induce any changes in the histological structure of the bone marrow. This is suggestive of the fact that *B. pinnatum* was not harmful to the bone marrow, and hence, may not affect hematopoietic process. Cadmium toxicity has been reported to hinder the transformation of bone marrow mesenchymal stem cells (BMSCs) into osteoblasts and leads to apoptosis of BMSCs directly. Cadmium toxicity can affect the skeletal system via direct interaction with bone cells thereby causing demineralization [43]. Cadmium triggers oxidative stress and cause damage to osteoblasts, leading to

DNA damage, impaired mitochondria function, and endoplasmic reticulum stress, ultimately causing apoptosis [44]. This was evident in this study as shown by the moderate to severe reduction of the different progenitor cells of the cadmium-intoxicated group relative to the normal control group. This effect could have also possibly contributed to the significantly lower red and white blood cells' counts and PCV in the cadmium group (Plate 2). This is because the bone marrow is the primary hematopoietic organ responsible in generating erythrocytes, leucocytes, and platelets [45]. The mild reduction in the different progenitor cells by the *B. pinnatum* extract suggests that it could ameliorate the degeneration of the progenitor cells. An earlier study by Cruz et al. [46] also highlighted the potential of *B. pinnatum* to suppress the release of granukles and cytokines from mast cells derived from bone marrow following IgE/FcRI crosslinking [46].

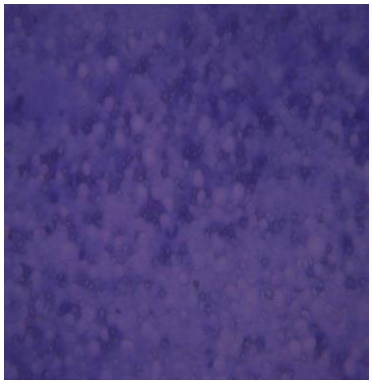


Figure 4a. Photomicrograph of the bone marrow of normal control group

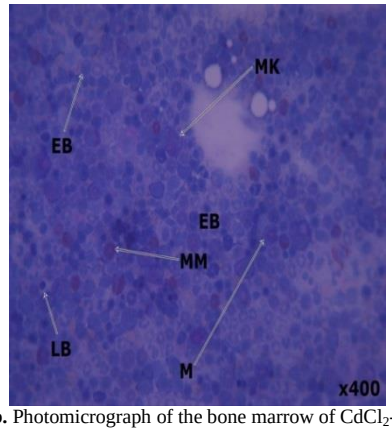


Figure 4b. Photomicrograph of the bone marrow of $CdCl_2$-challenged group

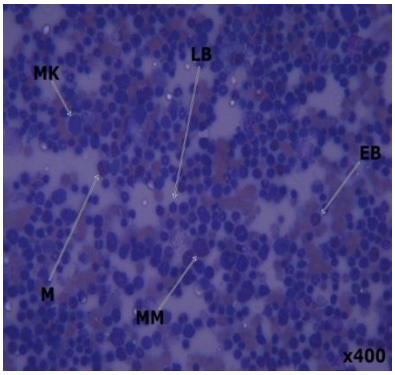


Figure 4c. Photomicrograph of the bone marrow of *B. pinnatum* extract group (200 mg kg^{-1} b.w)

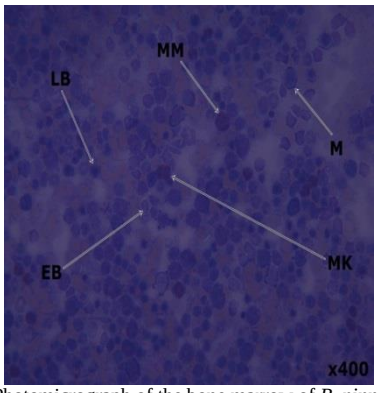


Figure 4d. Photomicrograph of the bone marrow of *B. pinnatum* extract group (400 mg kg^{-1} b.w)

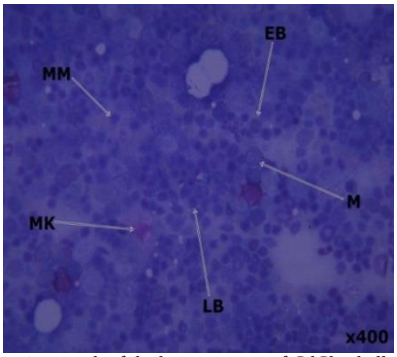


Figure 4e. Photomicrograph of the bone marrow of $CdCl_2$-challenged rats co-treated with 200 mg kg^{-1} b.w of *B. pinnatum* extract

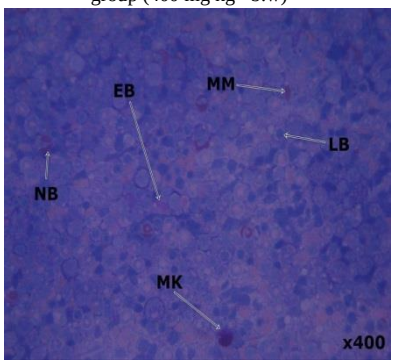


Figure 4f. Photomicrograph of the bone marrow of $CdCl_2$-challenged rats co-treated with 400 mg kg^{-1} b.w of *B. pinnatum* extract

ACKNOWLEDGEMENTS

The authors appreciate the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine for providing the laboratory space.

Conflict of interests

The authors declare that they have no conflicts of interest with respect to this work.

REFERENCES

1. Rahimzadeh M.R., Rahimzadeh M.R., Kazemi S., Moghadamnia A., 2017. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 8(3), 135-145.

2. Demir H., Kanter M., Coskun O., Uz Y.H., Koc A., Yildiz A., 2006. Effect of black cumin (*Nigella sativa*) on heart rate, some hematological values, and pancreatic β-cell damage in cadmium-treated rats. Biol Trace Elem Res. 110(2), 151-162.

3. Singh P., Chaudhary S., Patni A., 2007. Effect of cadmium chloride induced genotoxicity in bone marrow chromosomes of swiss albino mice and subsequent protective effects of *Emblica officinalis* and vitamin C. J Herb Med Toxicol. 1(2), 67-71.

4. Ercal N., Gurer-Orhan H., Aykin-Burns N., 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. 1(6), 529-539.

5. El-Boshy M.E., Risha E.F., Abdelhamid F.M., Mubarak M.S., Hadda T.B., 2015. Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. J Trace Elem Med Bio. 29, 104-110.

6. Kocak M., 2004. Kronik kadmiyum toksisites in hemostatik sisteme etkileri. Ankara Sağ Bil Enst (Doctoral dissertation).

7. Bubujuk S.P., Bojat N.C., Đjelić N., S., 2013. The effect of different acute concentrations of cadmium chloride on the frequency of micronuclei in AO rats. Genetika. 45(3), 727-736.

8. Horiguchi H., Oguma E., Kayama F., 2011. Cadmium induces anemia through interdependent progress of hemolysis, body iron accumulation, and insufficient erythropoietin production in rats. Toxicol Sci. 122(1), 198–210.

9. Aprioku J.S., Igbe I., 2017. Effects of aqueous Bryophyllum pinnatum leaf extract on hematological, renal and sperm indices in Wistar rats. Indian J Pharm Sci. 79(4), 521-526.

10. Ghasi S., Egwuibe C., Achukwu P.U., 2009. Assessment of the medical benefit in the folkloric use of BryophyllumPinnatum leaf among the Igbos of Nigeria for the treatment of hypertension. Afr J Pharmacy Pharmacol. 5(1), 83-92.

11. Afzal M., Gupta G., Kazmi I., Rahman M., Afzal O., Alam J., Hakeem K.R., Pravez M., Gupta R., Anwar F., 2012. Antiinflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. Fitoterapia. 83, 853-858.

12. Ojewole J.A.O. 2002. Antihypertensive properties of *Bryophyllum pinnatum* (Lam.) Oken leaf extracts. Am J Hypertens. 15(4), A34-A39.

13. Almeida A.P., Da Silva S.A.G., Souza M.L., Lima L.M., Rossi-Bergmann B., de Moraes V.L., Costa S.S., 2000. Isolation and chemical analysis of a fatty acid fraction of Kalanchoe pinnata with a potent lymphocyte suppressive activity. Planta Med. 66(2), 134-137.

14. Supratman U., Fujita T., Akiyama K., Hayashi H., Murakami A., Sakai H., Koshimizu K., Ohigashi H., 2001. Anti-tumor Promoting Activity of Bufadienolides from Kalanchoe pinnata and K. daigremontiana× butiflora. Biosci Biotechnol Biochem. 65(4), 947-949.

15. Asiedu-Gyekye I.J., Antwi D.A., Bugyei K.A., 2012. Comparative study of two Kalanchoe species: Total flavonoid and phenolic contents and antioxidant properties. AJPAC. 6(5), 65-73.

16. Okwu D.E., Josiah C., 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr J Biotech. 5, 357-361.

17. Cao H., Xia J., Xu D., Lu B., Chen G., 2005. The separation and identification of the flavonoids from the leaves of Bryophyllum pinnatum. J Chin Med Mater. 28(11), 988-990.

18. Schalm O.W., Jain N.C., Carol E.J., Veterinary Hematology 3rd ed., Lea and Febiger: Philadelphia, USA, 1975. pp. 1-13.

19. Ochei J.O., Kolhatkar A.A., 2000. Medical laboratory science: theory and practice. McGraw Hill Education.

20. Brar R.S., Sandhu H.S., Singh A., Veterinary Clinical Diagnosis by Laboratory Methods. 1st ed. India: Kaylani Publishers, 2000. pp. 29-150

21. Bancroft J.D., Stevens A. 1990. Theory and Practice of Histological Techniques. Edinburgh, London: Churchill Livingstone, pg. 86.

22. Al Amery S.F., Al Garaawi N.L., 2020. Phytochemical profile and antifungal activity of stems and leaves methanol extract from the *Juncus maritimus* Linn. Juncaceae family against some dermatophytes fungi. In: AIP Conference Proceedings. 2290(1), 020-034.

23. Kamaruding N.A., Ismail N., Sokry N., 2020. Identification of antibacterial activity with bioactive compounds from selected marine sponges. Pharmacogn J. 12(3), 493-502.

24. Lotfy W.A., Hassan S.W.M., Abd El-Aal A.A., 2019. Enhanced production of di-(2-ethylhexyl) phthalate (DEHP) by Bacillus subtilis AD35 using response surface methodology (RSM). Biotechnol Biotechnol Equip. 33(1), 1085-1096.

25. El-Sayed M.H., 2012. Di-(2-ethylhexyl) Phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from the culture filtrate of newly isolated soil Streptomyces (Streptomyces mirabilis strain NSQu-25). World Appl Sci J. 20(9), 1202-1212.

26. Kumari L., Mazumder A., Pandey D. 2019. Synthesis and biological potentials of quinoline analogues: A review of literature. Mini-Rev Org Chem. 16(7), 653-688.

27. Kısadere İ., Aydın M.F., Ündağ İ., 2021. Partial protective effects of melatonin on cadmium-induced changes in hematological characteristics in rats. Biotech Histochem. 97(3), 1-7.

28. Mackova N.O., Lenikova S., Fedorocko P., Brezani P., Fedorockova A., 1996. Effects of cadmium on haemopoiesis in irradiated and non-irradiated mice: II. Relationship to the number of circulating blood cells and haemopoiesis. Physiol Res. 45, 101-106.

29. NagoorMeeran M.F., Javed H., Al Taee H., 2017. Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development. Front Pharmacol. 8, 380.

30. Wojdyło A., Oszmiański J., Czemerys R., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 105(3), 940-949.

31. Travlos G.S., 2006. Normal structure, function, and histology of the bone marrow. Toxicol Pathol. 34(5), 548-565.

32. Horiguchi H., Sato M., Konno N., 1996. Long-term cadmium exposure induces anemia in rats through hypoproduction of erythropoietin in the kidneys. Arch Toxicol. 71, 11- 19.

33. Ufelle S.A., Ukaejiofo E.O., Neboh E.E., 2011. The Effect of Crude Methanolic Leaf Extract of *Bryophyllum pinn*atum on Some Haematological Parameters in Wistar Rats. Asian J Med Sci. 3(3), 121-124.

34. Okwu D.E. 2004. Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. J Sustain Agric Environ. 6(1), 30- 37.

35. Ofokansi K.C., Esimone C.O., Anele C.R. 2005. Evaluation of the in vitro combined antibacterial effect of the leaf extracts of *Bryophyllum pinnatum* (Fam: *Crassulaceae*) and *Ocimum gratissimum* (Fam: *Labiatae*). Plant Prod Res J. 9, 23-27.

36. Mckenzie R.A., Franke F.O., Duster P.J., 1985. Flavonoids and Glycosides of *Bryophyllum pinnatum*. Antivet J. 64, 10-15.

37. Salahdeen H.M., Yemitan O.K., 2006. Neuropharmacological effects of aqueous leaf extract of Bryophyllum pinnatum in mice. Afr J Biomed Res. 9, 101-107.

38. Sani H.L., Malami I., Hassan S.W., 2015. Effects of standardized stem bark extract of *Mangifera indica* L. in wistar rats with 2, 4-dinitrophenylhydrazine-induced haemolyticanaemia. Pharmacogn J. 7(2), 89-96.

39. Obianime A.W., Aprioku J.S., Esomonu C.T., 2010. Antifertility effects of aqueous crude extract of *Ocimum gratissimum* L. leaves in male mice. J Med Plant Res. 4(9), 809-816.

40. Guyton A.C., Hall J.E., 2006. Medical Physiology (Textbook of Physiology Medical) (Jakarta: Book Medical Publishers EGC).

41. Zhang W.L., Fievez E., Cheu F. 2010. Anti-inflammatory effects of formosterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats. Eur J Pharmacol. 628, 171-178.

42. Okwu D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J Pure Appl Sci.7, 455-459.

43. Almeida A.P., Da Silva S.A.G., Souza M.L.M., 2000. Isolation and chemical analysis of a fatty acid fraction of Kalanchoepinnata with a potent lymphocyte suppressive activity. Planta Med. 66(02), 134-137.

44. Pal S., Chaudhuri A.N., 1991. Studies on the anti-ulcer activity of a *Bryophyllum pinnatum* leaf extract in experimental animals. J Ethnopharmacol. 33(1-2), 97-102.

45. Staessen J.A., Roels H.A., Emelianov D., 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Lancet. 353(9159), 1140-1144.

46. Ma Y., Ran D., Shi X. 2021. Cadmium toxicity: A role in bone cell function and teeth development. Sci Total Environ. 144646.

47. Cruz E.A., Reuter S., Martin H., 2012. Kalanchoe pinnata inhibits mast cell activation and prevents allergic airway disease. Phytomedicine, 19(2), 115-121.