

Asian Journal of Medical Sciences 3(3): 121-124, 2011

ISSN: 2040-8773

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Received: April 06, 2011

Accepted: May 13, 2011

Published: June 20, 2011

The Effect of Crude Methanolic Leaf Extract of *Bryophyllum pinnatum* on Some Haematological Parameters in Wistar Rats

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Abstract: *Bryophyllum pinnatum* (*B. pinnatum*) is a perennial herb growing widely and used in folkloric medicine. Much research work has not been carried out on its effect on hematological parameters. We therefore decided to investigate the effect of crude methanolic leaf extract of *B. pinnatum* on some hematological parameters in Wistar rats. Twenty (20) male Wistar rats aged 2 to 3 months obtained from the Animal House of College of Medicine, University of Nigeria Enugu Campus were acclimatized for two weeks. They were divided into five groups labeled A to E. Groups A to D were orally fed with graded doses of the crude leaf extract (100, 200, 400, and 600 mg/kg body weight respectively) once daily for 28 days in lower concentrations compared to an oral LD 50 of 800 mg/kg body weight. Group E served as control without receiving the extract. On Day 29 about 2.5 mL of blood sample were collected from each rat through the median canthus into K₃-EDTA anticoagulant containers for hemoglobin (Hb), Packed Cell Volume (PCV), Total White Blood Cell (TWBC), and Platelet count. The results revealed significantly increased Hb in all the treated groups, A = 15.9 ± 1.0, B = 16.8 ± 1.0, C = 17.5 ± 1.0 and D = 18.7 ± 1.0 g/dL when compared with control E = 13.5 ± 0.5 g/dL (p < 0.05). The PCV of the treated groups were also significantly increased, A = 0.48 ± 0.01, B = 0.51 ± 0.01, C = 0.53 ± 0.01 and D = 0.56 ± 0.01 L/L when compared with control E = 0.38 ± 0.01 L/L (p < 0.05). The TWBC were significantly increased in all the treated groups (A = 6.2 ± 1.0, B = 6.5 ± 1.0, C = 7.1 ± 1.0 and D = 7.7 ± 1.0 × 10⁹/L when compared with control E = 4.0 ± 1.0 × 10⁹/L. The platelet count were decreased in all the treated groups but was significant only in group A = 135 ± 13 × 10⁹/L when compared with control E = 225 ± 20 × 10⁹/L (p < 0.05). The blood film examination revealed normocytic and normochromic red blood cells. This result pattern suggests that crude methanolic leaf extract of *B. pinnatum* may have properties that increase the Hb, PCV and TWBC, while decreasing the platelets, hence care should be taken while consuming such extracts to avoid haematological disturbances such as thrombocytopaenia. Also, constant monitoring the hematological parameters should be ensured in subjects taking such medicinal herbs for whatever purposes.

Key words: *Bryophyllum pinnatum*, hematological parameters, methanolic leaf extract

INTRODUCTION

Traditional herbal medicines are naturally-occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices (Tilburt and Kaptchuk, 2008). Government, international

agencies and corporations are increasingly investing in traditional herbal medicine research (Tilburt and Kaptchuk, 2008).

Herbal medicine usually contains an active ingredient of aerial or underground part of plant, such as their petal and/or seed material. Furthermore 80% of world population is dependent on plant based drugs (WHO,

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1996). Most developing countries of the world, rural and urban dwellers, literate or illiterate depend heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine (Nwabuisi, 2002). In Nigeria, traditional and herbal healing systems play an important role in health care delivery, and about 70-80% of the population depends on traditional healers for most of their ailments (Akah *et al.*, 1998).

Bryophyllum pinnatum (*B. pinnatum*) belongs to the family *crassulaceae*, classified as a weed, and its common names include: "African never die", "Resurrection plant", "Love plant", "life plant", "air plant" etc. It is a fleshy herb, about 60 to 120 cm tall, which branches from the base, 10 cm long and 5 to 6 cm broad. The margins are notched with irregular, blunt or rounded teeth which sometimes bear bulbils in their axes (Oliver-Bever, 1983). It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia, tropical America, and southern part of Nigeria (Gill, 1992).

Phytochemically, *B. pinnatum* contains flavonoids, glycosides, steroids, bufadienolides, and organic acid (Marriage and Wilson, 1971) and previous research revealed that the leaves of *B. pinnatum* has been used in diverse ways, such as an anti-ulcer agent, anti-fungal, anti-inflammatory, anti-hypertensive and an analgesic (Oliver-Bever, 1983). The plant is also needed for the treatment of earache and in ophthalmic preparations and also the poultice used for sprains, dysmenorrhea and cold in the head (Wong, 1976).

Hematological parameters, which include complete blood count-Hemoglobin, Packed cell volume, Leukocyte (total and differential), Platelet, Red blood cell, Reticulocyte and absolute indices, are all important in the diagnosis and classification of anemia. Anemia is the reduction in hemoglobin and hematocrit in relation to age, sex and location of individual considered (Ukaejiofo *et al.*, 1979). It is believed that during usage of this crude extract as a herbal remedy, it may be either stimulating the bone marrow to produce more blood cells or suppressing the bone marrow, leading to anemia.

The numerous medicinal properties and uses of the plant extract, coupled with the paucity of hematological information towards this crude extract in the science literature, makes it necessary to investigate the effect of crude methanolic leaf extract of *Bryophyllum pinnatum* on some hematological parameters such as Hemoglobin (Hb), Packed Cell Volume (PCV), Total White Blood Cell count (TWBC), and Platelet count in Wistar rats.

MATERIALS AND METHODS

Collection of plant materials: The plant materials were obtained and authenticated by the Department of Botany, University of Nigeria Nsukka and a voucher specimen was kept in the herbarium for future reference.

Animal housing: Twenty (20) adult male Wistar rats were purchased and housed in the Animal House of the College of Medicine, University of Nigeria Enugu Campus, where they were allowed to acclimatize for two weeks. They were fed with commercially available rat feed and allowed access to water *ad libitum*.

Preparation of the plant extract: One hundred (100) gram of the powder from the grinded shade dried *Bryophyllum pinnatum* leaves were extracted exhaustively with methanol and the mixture sieved. The remaining methanol in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% Dimethylsulphoxide (DMSO) and stored in the refrigerator until needed.

Experimental design: The twenty (20) adult male Wistar rats were divided into five (5) groups of four (4) rats per group, labeled A to E. Groups A to D were orally administered with graded doses (mg/kg body weight) of the crude extract (100, 200, 400 and 600 mg/kg body weight) once daily for 28 days. Group E, however, served as control and did not receive the crude extract but was orally administered with Dimethylsulphoxide (DMSO) as vehicle since DMSO was used to dissolve the crude extract. On the 29th day, blood samples (2.5 mL) were collected from each of the 20 rats through the median canthus of the eyes into K₃-EDTA anticoagulant containers for the analysis of Hemoglobin (HB), Packed Cell Volume (PCV), Total White Blood Cell (WBC), Platelet count and Blood film using standard operative procedures as described by Dacie and Lewis (2006). The study was conducted between June-July, 2010.

Statistical analysis: The Statistical Package for Social Science (SPSS) computer software version 15 was used for data analysis. The results of the tests were analyzed using student's t-test at 95% confidence interval with $p \leq 0.05$ being considered as significant. The results were expressed as mean \pm standard deviation (\pm SD).

RESULTS

Table 1 shows the mean \pm standard deviation of the hematological parameters assayed in the treated and control rats in this study. The results of Hemoglobin (HB), Packed Cell Volume (PCV) and Total White Blood Cell (WBC) of all the treated rats were increased significantly ($p < 0.05$) when compared with the control. The platelet count were decreased in all the treated groups, but only significant in group A ($p < 0.05$) when compared with the control. The blood film report revealed normocytic and normochromic red blood cells in both the treated and control rats.

Table 1: Mean±standard deviation of the hematological parameters of both treated and control rats in the study with the test of difference

Groups	A	B	C	D	E (Control)
Extract dose mg/kg body wt	100	200	400	600	1 mg/mL of 3% DMSO
Hemoglobin g/dL	15.7±0.8 p = 0.001*	17.0±0.4 p = 0.000*	17.6±0.8 p = 0.000*	18.5±0.8 p = 0.000*	13.0±0.6
PCV L/L	0.47±0.02 p = 0.002*	0.51±0.02 p = 0.000*	0.53±0.02 p = 0.000*	0.56±0.02 p = 0.000*	0.39±0.01
TWBC×10 ⁹ /L	5.9±0.6 p = 0.002*	6.7±0.7 p = 0.001*	6.7±0.6 p = 0.000*	7.4±0.8 p = 0.000*	3.9 ±0.4
Platelet ×10 ⁹ /L	135±13 p = 0.001*	212±15 p = 0.766	215±13 p = 0.488	220±17 p = 0.408	225±20

*: Statistically significant, compared with the control

DISCUSSION

This study revealed significant increase in Hemoglobin, Packed Cell Volume, and Total White Blood Cell count in all the treated groups when compared with the control group ($p < 0.05$). A significant decrease in Platelet count was also observed in group A rats when compared with the control.

This result pattern indicates that some of the phytochemical constituents of the crude methanolic leaf extract of *Bryophyllum pinnatum* (*B. pinnatum*) may have stimulatory effect on the bone marrow for leukocyte production and hemoglobin synthesis. This observed effect may be as a result of the tannin, ascorbic acid (Okwu, 2004) and phenol content (Ofokansi *et al.*, 2005). Other phytochemical constituents of *B. pinnatum* which may have likely affected the hematological parameters in this study include flavonoid (an anti-oxidant and free radical scavenger), zinc, riboflavin, and niacin (Mckenzie *et al.*, 1985; Salahdeen and Yemitan, 2006).

From the previous research work this extract has been reported to be an anti-microbial agent, anti-fungal, anti-inflammatory, anti-hypertensive, anti-ulcer, and analgesic (Wong, 1976; Iwu, 1993). It has also been reported that *B. pinnatum* is effective in the treatment of typhoid fever, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* infections (Okwu, 2001). This could probably be due to its leucocytic activity and the blood film also revealed mild to moderate leucocytosis with lymphocyte as the predominant leukocyte. The observed significant increase in total white blood cell count in the treated groups of rats agrees with the findings of previous researchers that it has anti microbial activity (Okwu, 2001). The observed significant increase in hemoglobin concentration and packed cell volume suggests that this crude methanolic leaf extract may have properties that stimulate the bone marrow to produce more hemoglobin when orally administered and may be very useful in the treatment of anemia.

CONCLUSION

These observed changes in hematological parameters indicate that the crude methanolic leaf extract of *B. pinnatum* may be stimulating the bone marrow to produce

increased leukocyte count and increased hemoglobin concentration when orally administered. There is however need for the crude leaf extract of *Bryophyllum pinnatum* to be fractionated using HPLC in other to characterize the active constituent that is possibly causing the observed effects on the hematological parameters in the Wistar rats.

ACKNOWLEDGMENT

The authors wish to acknowledge the staff of the Botany Department of University of Nigeria Nsukka, and the Animal House of College of Medicine University of Nigeria Enugu Campus for their valuable contribution to the success of the study.

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