HAEMATOLOGICAL EFFECTS OF MEGAPHRYNIUM MACROSTACHYUM LEAF EXTRACT IN ALBINO RATS

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ABSTRACT

Megaphrynium macrostachyum leaves are used traditionally to wrap food during cooking and serving. In this study, the effect of leaf aqueous extract of M. macrostachyum on haematological parameters (Haemoglobin, HB; Total white blood count, TWBC; Packed cell volume, PCV and Platelet, PLT) was investigated using healthy albino rats. Rats in five groups were fed for 14 days with a basal diet modified with appropriate doses of leaf extract (100 – 400mg/kg body weight, respectively for groups 1-4) while rats in group 5 served as the control and were not fed with leaf extract. Blood samples from all the rats were collected by cardiac puncture after 14 days and analyzed and the mean value for each parameter determined. The percentage increase or decrease in the parameter was calculated except for HB. The 100mg concentration (Group 1) had no effect on the animals. The HB value ranged from 12.09 to 13.34g/dl (for groups 1 – 3, respectively) and decreased to 11.7 in Group 4, whereas the control had 11.7g/dl. On the other hand, percentage increases in TWBC were 23.05, 11.8 and 12.43, respectively, for Groups 2, 3 and 4 animals. The increase in TWBC was significant (p <0.05). The PCV values also increased significantly (26.6, 17.6 and 16.5%, respectively, for Groups 2, 3 and 4). However, the PLT count was significantly lowered by 200,300 and 400mg doses. Megaphrynium macrostachyum aqueous leaf extract showed positive (haemopoietic) effects on PCV, HB and TWBC but repressive effect on PLT of the experimental rats.

KEYWORDS: Activity, extract, haematology Megaphrynium, parameters.

INTRODUCTION

Plants as medicines
Plants have been a source of medicines for man for a long time. Recently, interests in plant-based medicines have risen due to their relatively mild side effects, environment friendly nature as well as their efficacy in certain cases where orthodox medicine has failed. Different parts of the plant may be used; these include leaf, stem, root, seed, fruit and flower. These plant parts have been found to contain phytochemicals such as steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides (Wintola et al., 2010; Ajayi et al., 2011). Others are saponins, phlobatannins, cardiac glycosides, and anthraquinones (Okungbowa, 2012).

Megaphrynium macrostachyum
The plant Megaphrynium macrostachyum is a perennial rhizome-bearing herb, growing to a height of about 4m (Fig. 1). It belongs to the Family Marantaceae with synonyms such as
Sarcophrynium macrostachyum (Benth.) K. Schum, and Sarcophrynium arnoldianum De Wild. It bears many leaves directly from the rhizome, with a sheath at the basal end. Each leaf has a long petiole (about 5m long). Megaphrynium macrostachyum is believed to have originated from Guinea and Sierra Leone and spread to Democratic Republic of Congo, Angola, Sudan and Uganda and Nigeria. In Nigeria, it is commonly called ‘Yoruba Soft Cane’ with local names such as ‘Ebieba’ (Bini), ‘Ekparanga’ (Ijaw) and ‘Akwukwo –mma’ (Delta – Ibo). The plant exhibits rapid vegetative reproduction by rhizome and seed. Originally, it grows in the wild but is sometimes also cultivated.

Traditionally, the leaves find use in food wrapping (before or after cooking), packing of agricultural produce (fruits, vegetables, seeds, kolanuts, tomato, pepper, garden egg, okra etc.). Elsewhere, as well as in Nigeria, the leaves and petioles are also used for thatching, and for weaving several household materials like mats and baskets.

A previous report showed that M. macrostachyum leaf extract had some antifungal effects (Adebayo et al., 2010). Many plants have medicinal values but there is dearth of information on their mode of action. As a result, there is need for thorough scientific investigations of these plants for both efficacy and potential toxicity (Ashafa et al., 2010). Also, Okungbowa et al. (2013) reported the effect of M. macrostachyum leaf extract on eleven haematological parameters (including those examined in the current study).

Haematological Parameters
Haematological parameters such as packed cell volume (PCV), haemoglobin (HB), platelet (PLT) and total white blood cell counts (TWBC) among others are important diagnostic indices in several human diseases. Examples of such diseases are malaria, anaemia, HIV, cancer, and parasitic infections. Reduction in red blood cells can lead to anaemia, and eventually, death. The PCV and HB values are used to quantify the red blood cells and haemoglobin, respectively. White blood cells help the body to combat invading pathogens. Platelets play a major role in blood clotting and any malfunction of these cells can result in prolonged bleeding, severe blood loss and death. Platelet count is usually lowered during infection, such as malaria as reported by Chandra and Chandra, 2013 who concluded that PLT was significantly reduced in acute malarial infection caused by various species of Plasmodium (with 87.2% sensitivity as an indicator). The haematological profile of a pregnant woman has an impact on the outcome of the pregnancy (James et al., 2008). Haematological abnormalities are also common manifestations of HIV infection (Munyazesa et al., 2012) who reported that prevalence of anaemia (haemoglobin <12.0 g/dl) was higher in the HIV+ than the control group (20.5% versus 6.3%; p<0.001), and increased with lower CD4 counts.

Effect of Plant Extracts on Haematological Parameters
Extracts of some plants such as Telfaria occidentalis (Onu et al., 2012) and Magnifera indica (Madunagu et al., 1990) have been reported to have effects on haematological parameters. When the leaves of M. macrostachyum are used to wrap food (especially before cooking) there is the tendency of some of the extracts diffusing into the food during cooking. This is attested to by the appealing flavor usually detectable in any food wrapped with the leaves of M. macrostachyum. It is therefore desirable to know if the extract has positive effects on the blood and at what concentrations the plant extract might be toxic. This informed the current study with the aim
of determining the effects of *M. macrostachyum* leaf extract on some haematological parameters of healthy albino rats and make recommendations on the safety of its use for wrapping food consumed by humans.

**MATERIALS AND METHOD**

*Megaphrynium macrostachyum* leaves

*Megaphrynium macrostachyum* leaves (local name, ‘Ebieba’, Fig. 1) were purchased from a local market (Edaiken Market) in Benin City, Edo State, in southern Nigeria and taken to the final year students research laboratory, Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

**Preparation of sample**

The leaves were then sliced transversely across the petiole into small pieces (8 mm diameter wide) and air-dried under shade for seven days, after which they were ground into fine powder with a kitchen blender.

**Extraction**

The leaf powder was then weighed (weight of ground plant sample was 40370g) and 900ml of sterile distilled water was added to it in one litre conical flask. The flask was stoppered with cotton wool ( ) wrapped with aluminium foil (Alufoil Products, India) and left to stand for 24 hrs according to Idu and Timothy (2012) but modified. This was followed by filtration of the mixture using Whataman Number 1 filter paper (Sigma-Aldrich). The filtrate was transferred to an evaporating dish (SEOH Model 3760-65LC, Scientific Equipment of Houston, USA) on a water bath (Model TW20, JULABO GmbH) so as to allow excess water to gradually evaporate. The resultant paste was weighed (Analytical weighing balance,Model PBF03-S/FACT Maximum C, Spectro Lab Equipment, Pvt Ltd, New Delhi, India) and stored in a sterile universal bottle (ESSCO Glass, India) and kept in the refrigerator (Haier Thermocool, Model HR-170T) at 4°C. The concentrated plant extract was reconstituted in sterile distilled water (from Chemistry Dept, University of Benin, Nigeria) to get a concentration of 100mg/ml stock.

**Source of experimental animals**

Twenty-five Sprague – Dawley albino rats weight 148 - 215g, obtained from the Department of Pharmacology of the University of Benin were randomly divided into five groups of five rats (that is, five replicates) of each group (giving rise to groups 1, 2, 3, 4 and 5, respectively). The study conformed to the provisions of the Ethics Committee on Experimentation with Animals, University of Benin, which also granted permission for the study.

**Care of rats**

The test animals were kept in five plastic cages with lids. To ensure good hygiene, the cages were cleaned at regular intervals (two times a week) and the floor of the cages was covered with a little quantity of saw dust so as to maintain warmth and dryness while the animals were observed daily for social behaviour.

**Feeding of rats**

All the animals were fed with a basal diet of known composition (Tab. 1) and clean water for 14 days. In addition to the basal diet, Group 1 animals were given 100mg/kg body weight of the leaf extract daily while Groups 2, 3 and 4 animals were given 200,300 and 400mg/kg of extract, respectively, with the aid of an orogastric tube (HOSHIN China. Model FET01).
Group 5 animals were also fed with the same animal feed pellets and clean water but without administration of plant extract; this group served as control.

**Bleeding of the rats and analysis of blood samples**
Anaesthetization with 95% w/v chloroform (Merck) was done on all the rats. Then 2.5ml of blood samples were collected via cardiac puncture into potassium Ethylene Diaminetetra-acetic acid (EDTA) bottles (Sigma-Aldrich). The PCV, TWBC and PLT count were done following the method of Cheesbrough (2000) while the HB value was determined by the Cyanmethaemoglobin method.

**Determination of total white blood cells**
The whole blood was diluted 1:20 in an acid reagent (EDTA) which haemolyzes the red cells (but not the nucleus of the nucleated red cells) leaving the white cells to be counted. The white cells were counted microscopically using an improved Neubauer ruled counting chamber and the number of WBCs per litre of blood calculated.

**Determination of platelets**
The blood sample was diluted 1:20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets were counted as described above and the number of platelets per litre of blood calculated.

**Determination of haemoglobin concentration**
Using a pipette, 0.02ml of well-mixed blood sample was transferred into a test tube and 4ml of Drabkin’s solution (Merck) was added to it and left on the bench at room temperature for 10 minutes. The test and standard were treated the same way. Drabkin’s solution was used as blank. Absorbance was read at a wavelength of 540nm (LAMBDA BIO, PerkinElmer) for both standard and test samples.

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\text{Haemoglobin concentration (g/l) = } \frac{\text{Absorbance of test} \times \text{concentration of standard} \times 200}{\text{Absorbance of standard} \times 1000}
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The concentration was then expressed in g/l (Cheesbrough, 2000).

**Determination of packed cell volume**
Anticoagulated blood in a glass capillary tube was centrifuged in a microhaematocrit centrifuge (UNICO Micro-Hematocrit Centrifuge - Model C-MH30) at RCF 12000-15000g for 3-5 minutes to obtain a constant packing of the red cells. The PCV was read using the microhaematocrit reader (UNICO) and the result expressed as a percentage.

**Data analysis**
Determination of each parameter was done thrice and the mean value was used. The data were analyzed using analysis of variance (ANOVA) for HB and Duncan’s multiple range test for the other parameters. The software used was the Statistical Package for Social Sciences, SPSS, version 20 and comparison was done at 0.05 probability level.
RESULTS AND DISCUSSION
The results indicated increases in HB, WBC and PCV values for 200 and 300mg concentration while there was no effect at 100mg/kg. Also, HB decreased at 400mg/kg. There were increases in PCV and WBC but the platelet counts for 200, 300 and 400mg/kg concentrations declined.

The HB values of Group 2 animals (fed with 200mg/kg extract) were 12.09 (Group 1), 13.34 (Group 2), 12.95 (Group 3), 11.7 (Group 4) and 12.1g/dl (control) as shown in Fig. 2. The percentage increases in TWBC were 23.05, 11.8 and 12.43, respectively, for Groups 2, 3 and 4 animals (Fig. 3). The increase in TWBC was significant. The PCV values also increased significantly (26.6, 17.6 and 16.5% increase respectively, for Groups 2, 3 and 4) as shown in Fig 4. However, the PLT count was significantly lowered by both 200,300 and 400mg doses represented by Groups 2, 3 and 4 animals, respectively (Fig. 5). The increase in HB, PCV and TWBC shows that the plant extract may have a positive haemopoietic effect (enhances production of blood cells).

Okungbowa et al. (2013) reported dose-dependent increases in these haematological parameters except that in their report, the difference in TWBC was insignificant whereas in the present report, it was significant. The reason could be due to differences in batches of rats. At 400mg/kg concentration where there was reduction in HB value, the dose may be said to be lethal. The finding agrees with that of Okungbowa et al. (2013) in which HB decreased at 400mg/kg. In the formulation of this extract for use as medicine, therefore, the recommended dose should be less than 400mg/kg body weight. Besides, since PCV is an indirect measure of haemoglobin and the former increased at 400mg, the repressive effect should be on the synthesis of haemoglobin itself, rather than on red cell production.

Osime et al. (2008) earlier suggested that potato leaf (whose extract increased HB and TWBC in their work) should be added to human daily diets. Fluted pumpkin leaf extracts have also been shown to increase haematological parameters such as PCV, HB and TWBC in chickens (Onu, 2012).

In view of the findings of the current study, the consumption of the leaves of *M. macrostachyum* as vegetable (which is already being practiced in some parts of Africa such as Democratic Republic of Congo) should be encouraged.

The drastic and significant reduction in PLT count calls for caution when the leaves are to be eaten especially in people with poor or compromised blood clotting system. The result corroborates previous report by Okungbowa et al. (2013). The oral use of the leaf decoction as an antidote for snake bite in Cote d’Ivoire (Jiofack-Tafokou, 2011) may not be unconnected (directly or indirectly) with this ability to reduce PLT since certain snake venoms cause blood clotting which can lead to death. Further work to determine safe levels of the extract for PLT is necessary.

CONCLUSION
*Megaphrynium macrostachyum* aqueous leaf extract has positive (haemopoietic) effects on PCV, HB and TWBC but repressive effect on PLT of the experimental rats. While 200 and 300 mg extract/kg enhanced HB, the 400mg/kg dose caused a decline. The use of the leaves for wrapping, cooking food, and as vegetable is beneficial. However, it is to be strongly noted that despite these benefits, the ability to reduce PLT count should not be undermined, especially, in people predisposed to or with blood clotting problems.
REFERENCES


