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Erythropoietic Effects of *Eremomastax polysperma* Leaf Extracts on Female Prepubertal and Pubertal Wistar Rats

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Authors' contributions

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

Original Research Article

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ABSTRACT

This study assessed the erythropoietic effect of *Eremomastax polysperma* leaf extracts in female albino Wistar rats. Method: Twenty eight (28) female rats were divided into two major groups based on their weight and age. The duration of administration of *E. polysperma* extracts lasted for twenty one (21) days. This study was carried out in the Department of Biochemistry University of Calabar, between February and March 2013. A significant increase ($P<0.05$) in red blood cell count (RBC) (8.17±0.48, 6.46±0.37) and Haemoglobin (Hb) count (15.13±1.03, 13.27±0.7) was observed in the prepubertal group compared to the control, while packed cell volume (PCV) was significantly increased ($P<0.05$) in the pubertal group compared to the control (55.40±4.40, 48.63±2.33 respectively). This suggests that *E. polysperma* leaf extract can be used as a haematinic and a therapy for anaemic conditions.

Keywords: Eremomastax polysperma; erythropoietic; packed cell volume; haemoglobin; red blood cell; wistar rats.

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1. INTRODUCTION

Anaemic conditions are characterised by an abnormal decrease in red blood cell count or haemoglobin deficiency resulting in inadequate supply of oxygen to the tissues. Women of reproductive age and pregnant women are at a risk of anaemia [1]. Reports have shown that women are increasingly using medicinal herbs for various therapeutic applications [2]. Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used for therapeutic purposes [3]. Many of these phytochemicals have beneficial effects on long term health when consumed by humans. Chemical compounds in plants mediate their effect on the human body through processesidentical to those in conventional drugs [4,5]. Also, modern medicine now tends to use the active ingredients from plants as a precursor for the synthesis of useful drugs [3]. Previous reports have shown that *Eremomastax polysperma* leaf contains some bioactive principles [6].

1.1 Medicinal Uses of *Eremomastax polysperma*

Eremomastax polysperma belongs to the Acanthaceae family. It is commonly found in the tropics and subtropical forest [7]. It is known as Pindula in Swahili [8] and Edemididuot in Ibibio [9]. The part of the plant commonly used for medicinal purposes is the leaf. Petiole anatomy of the leaves show a succulent leaf with a purple underside at maturity [10]. Oral administration of *Eremomastax polysperma* leaf mixed with egg helps in the treatment of anaemia and internal heat [11]. It is also used to treat penfigures in children[12].

2. OBJECTIVE OF RESEARCH

This study is set out to investigate the therapeutic efficacy of *Eremomastax polysperma* leaf extracts in possible anaemic conditions in females.

3. MATERIALS AND METHODS

3.1 Plant Materials and Authentication

Fresh, mature *Eremomastax polysperma* leaves were obtained from Akai-Effa, Calabar municipality, Cross River state. The plant was identified and authenticated in the herbarium in botany department, Faculty of Sciences, University of Calabar.

3.2 Preparation of Plant Extract

The mature leaves were rinsed in clean water to remove dirt and properly dried under shade for seven (7) days. The dried leaves were blended to fine powder using a manual blender. About 1kg of the blended leaves was weighed out using an electronic weighing balance and soaked in 3000mL of 95% ethanol at the ratio of 4:1 powder to ethanol respectively for 72 hours. It was later sieved with sieve cloth to get the liquid portion. The liquid portion was filtered with a filter paper in order to get a clear filtrate. The filtrate was concentrated using a steam bath at a regulated temperature of 40ºC. This gave rise to a crude ethanolic extract.

3.3 Experimental Animals

Twenty eight (28) albino rats of Wistar strain weighing between 110-125g and 180-210g were used for this study. The animals were obtained from the animal house of Zoology

Department, Faculty of Sciences, University of Calabar. The animals were housed at the Department of Biochemistry, University of Calabar animal house, in wooden cages with wire-mesh tops. The animals were acclimatized for a period of seven7 days under controlled environmental conditions of temperature 27±2ºC and a 12 hour light/dark cycle. The animal room was adequately ventilated and the animals were fed on normal rat chow and drinking water*ad libitum.*

3.4 Experimental Design

The twenty eight albino Wistar rats were assigned into two major groups prepubertal and pubertal rats of 7 animals each. The control group was orally administered with 0.3ml and 0.6ml of normal saline. Also, the experimental group was administered with 150mg/kg and 300mg/kg body weight of *E. polysperma* extract daily for 21 days (3 weeks), through the same route Table 1. The concentrations administered were used after the determination of the medial lethal dose (LD_{50}) of 1414, using the method of [13]. "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

3.5 Collection of Blood Samples for Analysis

Twelve hours (12 hours) after last feeding and administration of plant extract. The animals were anaesthesized under chloroform vapour, and sacrificed. Whole blood samples were collected from the heart by cardiac puncture into ethylenediaminetetracetate (EDTA) tubes, and then taken to the laboratory for analysis.

3.6 Full Blood Count Assays

Full blood counts including PCV, Hb, RBC, WBC, platelet count, differential WBC (lymphocytes and mixed), and red cell indices (MCHC, MCH and MCV), were estimated using the Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan. The pre-diluted (PD) sample method was used where blood was diluted manually, and then fed into the transducers. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there are electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current changes, the blood cell size is detected as electric pulses. Blood cell count is then calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data including differential whole blood count, red cell indices and derived values

3.7 Statistical Analysis

The results were expressed as mean ± standard deviation. Significant differences between control and experimental values were assessed by student's t-test. The results were considered significant at *P* values of less than .05 ($P<0.05$).

4. RESULTS

Tables 2 and 3 Show the comparison of the haematological indices between control and experimental animals in the prepubertal and pubertal group.

Table 1. Experimental design

Experimental group	Number of animals	Dosage/prepubertal	Dosage/pubertal
Control	7	0.3ml	0.6mls
E. polysperma	7	150mg/kg	300mg/kg

Table 2. Estimation of haematological indices in prepubertal control and experimental animals

Group	RBCx10^6/µl	WBCx10^3/µl	PLTx10^3/µl	PCV (%)	MCV(fl)	LYMx10^3/µl	NEUTx10^3/µl	HB (g/dl)	MCH (pg)	MCHC (g/dl)
Control	6.46±0.37	16.40±0.40	964.33 ±67.33	49.67± 2.73	77.85±8.19	8.47±1.57	1.97±0.03	13.27±.037	20.61±0.57	26.97±2.35
Experimental	8.17±0.48*	12.57±1.13*	896.33±61.33	53.70±4.30	65.57±1.33	10.00±1.40*	2.10±0.10	15.13±1.03*	18.50±0.17*	28.23±0.31

PLT: platelet count, PCV: packed cell volume, MCV: mean cell volume, LYM: lymphocyte count, NEUT: neutrophil count, RBC: red blood cell count, WBC: white blood cell count, HB: haemoglobin, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

Table 3. Estimation of haematological indices inpubertal control and experimental animals

Group	RBCx10^6/µl	WBCx10^3/µl	PLTx10^3/µl	PCV (%)	MCV(fl)	LYMx10^3/µl	NEUTx10^3/µl	HB (g/dl)	MCH (pg)	MCHC (g/dl)
Control	7.20±0.12	12.23±0.13	899.33±0.33	48.63±2.33	67.58±3.39	8.47±1.57	3.23±1.27	14.13±0.27	19.64±0.49	29.24±1.86
Experimental	7.09±0.58	11.80±2.70	939.0±59.00	55.40±4.40*	80.09±11.77	11.07±2.47	0.00±0.00	14.03±0.93	19.85±0.25	25.98±4.07

PLT: platelet count, PCV: packed cell volume, MCV: mean cell volume, LYM: lymphocyte count, NEUT: neutrophil count, RBC: red blood cell count, WBC: white blood cell count, HB: haemoglobin, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

5. DISCUSSION

Medicinal plants can alter a wide range of haematological parameters in biological systems [14]. Previous studies on other medicinal plants with erythropoietic values have been reported, the pumpkin leaf extract for instance [15] and *Argeratum cornyzoides* [16]. Haematological parameters are good indicators of the physiological status of humans and animals, significant alterations in these indices could be used to assess in vivo responses to various physiological situations [17]. The various haematological indices investigated in this study are useful indices in evaluating the potential heamatopoetic effects of the plant extract in animals. Assessment of haematological parameters cannot only be used to determine the extent of deleterious effect of extracts on the blood of animal, but it can also be used to explain blood relatedfunctions of a plant extract or its products [18]. Analysis of blood indices is relevant in risk evaluation as a change in haematological system has higher predictive value for human toxicity when the data are translated from animal studies [19]. Anaemia can also result from low PCV and RBC counts [15]. PCV values below 30%have been reported to indicate anaemic condition in rats [20]. Results from this study have shown a significant increase in the PCV values in the pubertal experimental rats when compared with the control. RBC, Hb, and MCH values were significantly increased in the prepubertal experimental rats when compared with the control. This suggests a positive effect of the extracts on the haematopoietic system of Wistar rat models.

5.1 Possible Mechanism of Action

It can be suggested that the bioactive components in *Eremomastax polysperma* leaf extract stimulates the kidney directly to cause the formation of erythropoietin to stimulate haematopoiesis. Erythropoietin is a glycosylated hormone which is synthesized primarily in the kidney. The regulation of erythropoietin production by the kidneyis central to the control of erythropoiesis [21].

6. CONCLUSION

Results from this study have shown that ethanolic extracts of *E. polysperma* leaves have an erythropoietic effect in experimental female animal models.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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