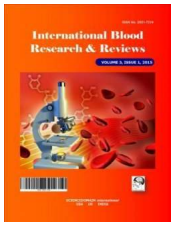

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Hematological Changes Following Oral Administration of Aqueous Root Bark Extract of *Salacia lehmbachii* in Albino Rats

G. A. Essiet[1*], A. D. Essien[1], L. P. Takem[1], G. C. Akuodor[2] and F. V. Udoh[1]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Cross-River State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author GAE designed the study, wrote the protocol and the first draft of the manuscript. Author ADE managed the literature searches. Author LPT carried out the statistical analysis. Author GCA managed the experimental process and author FVU identified the species of plant and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This study investigated the effects of oral administration of aqueous root bark extract of *Salacia lehmbachii* on some haematological indices in albino rats.
Methodology: Twenty-four male rats weighing 180-200 g were randomly divided into four groups, labeled 1-4 and each group contained six rats. Group 1 (control) received 2 mL of distilled water while groups 2, 3 and 4 had 250, 500 and 750 mg/kg body weight of aqueous root bark extract of *Salacia lehmbachii* respectively. The extract was prepared from Soxhlet extraction of petroleum ether defatted plant residue using water. Administration was through an oral gavage for 28 days. At the end of experimentation period, animals were anaesthetized by placing each in an air tight

**Corresponding author: E-mail: geessiet@yahoo.com;*

desiccator containing cotton wool soaked with chloroform and blood samples collected by cardiac puncture using sterile needles attached to 5 mL syringes into ethylene-diamine-tetra-acetic acid (EDTA) bottles for haematological studies using automated haemanalyzer.
Results: There was a significant ($p<0.05$) increase in RBC count in groups 3 ($8.72\pm0.08 \times 10^6$) and 4 ($8.97\pm0.07 \times 10^6$), when compared to the control ($6.67\pm0.24 \times 10^6$). Haemoglobin level (g/dl) in treated groups were significantly ($p<0.05$) raised to 13.59±0.11, 15.07±0.21 and 16.55±0.31 respectively compared to control (12.81±0.11). PCV was increased from 40.07±1.51 (control) to 43.07±0.71 (group 2), 45.96±1.12 (group 3) and 49.93 ±1.69 (group 4). RBC indices in treated rats were not statistically different from control. WBC count was significantly ($p<0.05$) increased in groups 2 ($10.55\pm0.41 \times 10^3$), 3 ($13.43\pm1.02 \times 10^3$) and 4 ($18.38\pm1.46 \times 10^3$) compared to control ($8.55\pm0.99 \times 10^3$). Platelet count ($\times 10^3$ cells/µL) was significantly ($p<0.05$) raised in groups 2 (840.67±0.04), 3(925.44±0.07) and 4 (962.33±0.05) compared to control (703.83±0.04).
Conclusion: Findings in this study have shown that the aqueous root bark extract of *Salacia lehmbachii increases* haematopoietic parameters.

Keywords: Salacia lehmbachii; aqueous extract; hematological indices; anemia.

1. INTRODUCTION

Herbs and other medicinal plants have been used by man for the management of many health conditions [1]. *Salacia lehmbachii* is a common herb found in uncultivated farmlands in the southern and eastern states of Nigeria. Its vernacular names include 'eba-enang-enang' (Efiks and Ibibios) and 'ara-mmanu' (Igbos). The plant is 1.5-3 meters tall and one of the species of the genus *Salacia* in the family *Celastraceae* [2,3]. Parts of this plant especially the leaves and roots have been used in folk medicine to treat many ailments especially malaria and other febrile illnesses. The pharmacological actions of the plant have been documented as antioxidant activity [4], antiabortificient effect [5], nephroprotection [6] and analgesic and anti inflammatory [7]. The median lethal dose (LD_{50}) of the aqueous root bark extract of the plant was found to be above 5000 mg/kg in albino rats and to contain alkaloids, glycosides, flavonoids, tannins, saponins and polyphenols [7].

The pharmacological actions of *S. Lehmbachii* are linked to its phytoconstituents. Its anti-inflammatory property is attributed to flavonoids, a group of phytoconstituents that specifically affect the functions of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases [8,9,10]. Flavonoids competitively bind with ATP at catalytic sites on these enzymes which are involved in signal transduction and cell activation processes involving cells of the immune system. The antioxidant property of the plant is due to phenolic compounds especially flavonoids present in the plant. These compounds can trap chain-initiating radicals at the interface of the membrane, thus, preventing the progression of the radical chain reaction. They adopt most of the mechanisms of antioxidant action including suppression of reactive oxygen species (ROS) formation (either by enzymes inhibition or chelation of trace elements involved in free radical generation); scavenging of ROS and up regulation of antioxidant defenses [11,12].

Blood is the medium through which therapeutic agents reach their sites of action. It is composed of plasma and formed elements namely, erythrocytes (red blood cells), leucocytes (white blood cells) and thrombocytes (platelets). While some therapeutic agents are carried by the plasma, others may be bound to the cellular component. Another key function of blood is transportation and delivery of nutrients and oxygen to cells and removal of waste products of metabolism and carbondioxide from them. The red blood cells (RBC) contain the oxygen carrying protein, hemoglobin (Hb) and have an average lifespan of about 120 days. Its total count and other indices which include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are useful in the diagnosis of the severity and type of anaemia. These indices may be determined by mathematical calculation or use of a haemanalyzer [13,14]. Red blood cell count is different for males and females, being higher in males than females [15]. Normal Hb concentration is also different for the two sexes and varies with age [16]. Haematocrit or packed cell volume (PCV) is the percentage of RBC in a person's volume of whole blood.

White blood cells (WBC) are concerned with immune response in the organism. The normal count varies with age, sex and is affected by certain disease states [17]. They migrate to the tissues sites to perform their immunological functions and die predominantly by apoptosis.

Platelets are non-nucleated disk like cell fragments of 2-4 µm in diameter and originate from the fragmentation of giant polypoid megakaryocytes in the bone marrow. They have a lifespan of about 10 days and are involved in blood clotting [18].

The formed elements of blood and even the bone marrow, the site of haematopoiesis have been shown to be affected by circulating xenobiotics [19,20,21]. Physical characteristics of these cellular components of blood are useful in the diagnosis of certain disease states and may be affected by therapeutic agents including herbal preparations. Notably, these medications affect blood counts. *Salacia lehmbachii* is widely used by the local dwellers for therapy of ailments. This study was carried out to determine the effect of aqueous root bark extract of the plant on blood parameters in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

The roots of *Salacia lehmbachii* were bought from Watt market, Calabar, Cross river state, Nigeria in September, 2014. Authentication of the plant was carried out by a Botanist in Botany department, University of Calabar where a Voucher Specimen was deposited. The root of the plant was authenticated by Professor Ani Nkang of the Botany department, University of Calabar and the specimen with herbarium number 688 was deposited in the herbarium of that department. The roots were washed with water to remove dirt and dried in an electric oven, thermostatically controlled at 40ºC, for 12 hours. The bark was obtained by striking the dry roots on a hard surface and the pieces obtained were pulverized into a coarse powder using a mechanical grain mill (Corona®, Columbia) yielding 1000 g of powder which was stored in an airtight container. The plant powder was defatted with 99.9% petroleum ether (Sigma Chemical Limited, USA) using a Soxhlet extractor at 65ºC over twelve hours. The petroleum ether residue was dried, weighed and re-extracted with water at 100ºC for 72 hours to obtain aqueous extract solution which was then evaporated to dryness using a rotatory evaporator at a reduced temperature of 45ºC in vacuo. The solid extract was weighed, put in a clean specimen bottle and preserved in a refrigerator, until required for the experiment.

2.2 Experimental Animals and Extract Administration

Twenty four mature male albino rats weighing between 180 and 200 g were obtained from the animal house of the department of Pharmacology, University of Calabar, Calabar.

They were housed in wire gauzed topped plastic cages, each cage accommodating six rats appropriately branded for identification. The animals were allowed to acclimatize to normal laboratory conditions (relative humidity of 50±5 %, temperature 28±2ºC, good ventilation and 12 hours of light: dark cycle) over a seven day period before the start of the experiment and maintained in the same conditions for the duration of the experiment. They had standard rat chow (Agro Feeds, Calabar) and water (Water board, Calabar) *ad libitum.* The animals were randomly divided into four (n=6) and labeled 1 to 4. Group 1 rats (Control) had 2 mL of distilled water, the solvent (vehicle) for the extract; groups 2, 3 and 4 received 250, 500 and 750 mg/kg body weight of aqueous extract of *Salacia lehmbachii* root bark respectively. Administration was orally via a gastric cannula and carried out daily for twenty eight days. All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals [22]. The Research and Ethical Committee of the Faculty of Basic Medical Sciences of University of Calabar approved the study protocol.

2.3 Determination of Hematological Indices

At the end of the experimentation period (morning of the 29th day), the rats were anaesthetized by placing each of them in an air tight desiccator containing cotton wool soaked with chloroform. Blood samples were drawn from them by cardiac puncture using sterile needles which were attached to 5 mls syringes. The collected blood was put into EDTA-treated sample bottles. These blood samples were used for haematological studies using an automated haemanalyzer (Sysmex, England) as earlier used

by [23]. Some of the WBC differentials that were not picked by the machine were counted using the improved Neubauer counting chamber [24].

2.4 Statistical Analysis

SPSS software version 20.0 was used for data processing and values obtained from descriptive statistics were expressed as Mean ± standard error of mean (SEM). Inter group comparison was done using one way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests. P values of P< 0.05 was considered significant.

3. RESULTS

The RBC count, haemoglobin concentration and packed cell volume level in the treated rats were raised significantly ($p<0.05$) at high doses (500 and 750 mg/kg) compared to control as shown in Table 1. At low dose of the extract (250 mg/kg), the above parameters were not significantly ($P>0.05$) different from control. The RBC indices (MCV, MCH and MCHC) in treated rats were not statistically ($P>0.05$) different from control (Table 1). There was a dose dependent increase in total WBC count that was significant ($p<0.05$) at all doses of the extract compared to control as shown in Table 2. White blood cells differentials were equally increased without distortion of the normal proportion. Platelet count (x 10^3 cells/µL) was significantly ($p<0.05$) increased in a dose dependent manner in all treated rats compared to control (Fig. 1).

4. DISCUSSION

Blood, the major constituent of the hematopoietic system transports nutrients, oxygen and other substances including medicinal agents to different cells of the body and removes from them, the waste products of metabolism [25]. The formed elements of blood originate from hematopoietic stem cells, the only bone marrow cells with the capability to differentiate into all blood cell lineages [26]. Hematopoietic stem cell formation requires inputs from multiple cell signaling pathways during embryonic and sometimes postnatal developments. The signaling pathways include bone morphogenetic protein (BMP) pathway [27], Hh (Hedgehog) pathway [28], Vegf pathway [29], Notch pathway [30], Wnt signaling [31] and protein-tyrosine kinase pathway [32].

Table 1. Effects of the aqueous extracts of roots of *Salacia lehmbachii* on erythrocyte parameters of albino rats

Study groups	tRBC (x10^6 cells/µL)	Hb (g/dl)	PCV (%)	MCV (f)	MCH (Pg)	MCHC (g/dl)
Control	7.67±0.24	12.81±0.11	40.07±1.51	54.87±1.33	19.14±0.45	33.00±0.09
ASL:						
250 mg/kg	8.00±0.13	13.59±0.11	41.07±0.71	56.25±1.18	18.97±1.15	33.36±0.11
500 mg/kg	8.72±0.08*	15.07±0.21*	45.96±1.12*	53.14±1.64	18.76±0.43	33.36±0.04
750 mg/kg	8.97±0.07*	16.55±0.31*	49.93±1.69*	55.15 ±1.74	19.02±0.49	33.59±0.12

*Values are expressed as mean ± SEM. n = 6, *significantly different from control (p<0.05), tRBC =Total red blood cells, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean concentration haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, ASL = Aqueous extract S. lehmbachii root bark*

Table 2. Effect of the aqueous root bark extracts of *Salacia lehmbachii* on albino rat WBC total count and differentials

Study group (n=6)	tWBC (x 10^3 cells/µL)	Lymphocytes (%)	Neutrophils (%)	Esinophils (%)
Control	8.55±0.99	82.07±1.57	16.17±1.33	1.76±0.45
		(7.34±1.57)	(1.45±1.33)	(0.16±0.45)
250 mg/kg of ASL	10.55±0.41*	82.00±0.71	16.20±1.18	1.77±1.15
		(8.65±0.71)	(1.71±1.18)	(0.19±1.15)
500 mg/kg of ASL	13.43±1.02*	82.06 ±1.52	16.14 ±1.64	1.80±0.43
		(11.02±1.52)	(2.17±1.64)	(0.24±0.43)
750 mg/kg of ASL	18.38±1.46*	82.03 ±1.69	16.15 ±1.74	1.82±0.49
		(15.08±1.69)	(2.97±1.74)	(0.33±0.49)

*Values are expressed as mean SEM. n = 6, *significantly different compared to control (p<0.05), tWBC = total WBC count, WBC differentials count (10^3 /µL) is in parenthesis; ASL = Aqueous extract of root bark of Salacia lehmbachii*

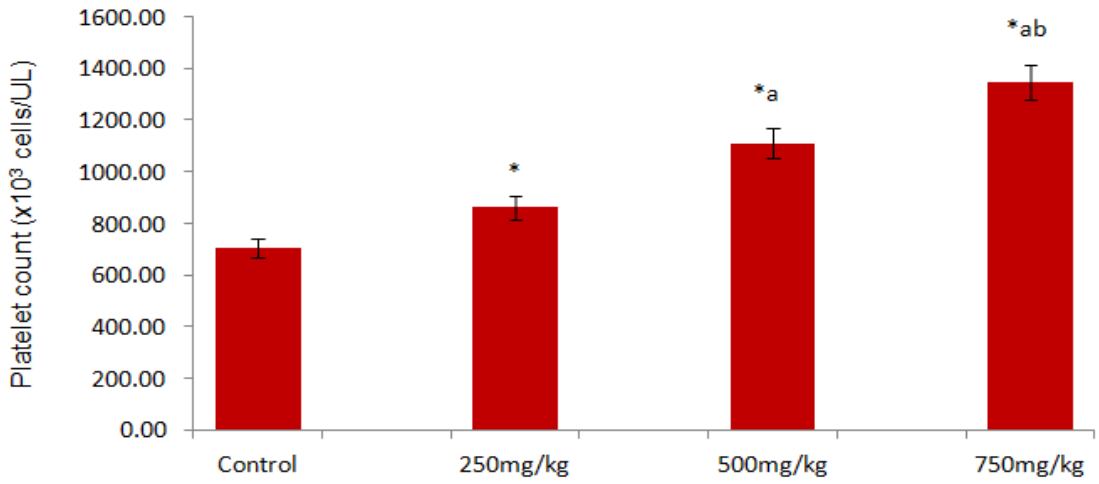

Fig. 1. Effect of aqueous root bark extract of *Salacia lehmbachii* on rat platelet count

Values expressed as mean ± SEM. n = 6
**different significantly from control at p<0.05*
a = different significantly from 250 mg/kg ASL at p<0.05
b = significantly different from 500 mg/kg ASL at p<0.05

Therapeutic agents and other xenobiotics may affect blood cells at any stage of hematopoiesis resulting in alteration of the number (count), quality, morphology and proportions (differential counts) of the different blood cells [33-36].

Hematological parameters may therefore provide useful information on the effects of xenobiotics and the basis for alterations in the levels of some biomolecules like enzymes [37,38]. Rats treated with the aqueous extract of the root bark of *Salacia lehmbachii* had significantly increased RBC count at high doses (500 and 750 mg/kg). The increase suggests that the extract may stimulate erythropoiesis probably through an increase in erythropoietin levels since this cytokine hormone is the main stimulant of erythropoiesis. The enhancement of erythropoiesis increases the RBC counts thus explaining the observed rise in haemoglobin and packed cell volume. These erythropoietic properties of the extracts may be useful in correction of the anaemia that almost always complicates malaria especially in children and for which the plant is also most commonly used as treatment. The stimulant effect of the extracts on erythropoiesis is probably caused by flavonoids found in them. This phytochemical has been reported to stimulate erythropoiesis [39].

Platelets are formed from stem cells in the bone marrow and are responsible for haemostasis. The main stimulant is thrombopoietin, a glycoprotein produced mainly in the liver [40]. The dose dependent increase in platelet counts in treated rats may have occurred because the extract caused an increase in the production of the cells in the bone marrow. It may also be speculated that the enhanced production of stem cells is skewed in favour of megakaryocytes by increased levels of thrombopoietin. Increase in platelets is also a natural response to anaemia (reactive thrombocytosis), an unlikely scenario here because erythrocytes counts were increased in the treated rats, they could therefore not have been anaemic. As increase in platelet count enhances the formation of blood clots, the risk of thrombus formation and subsequent embolization is to be borne in mind when considering the use of this extract of *Salacia lehmbachii* especially in vulnerable groups like sicklers, the elderly and people with myeloproliferative disorders.

The white blood cells are the major effectors of both innate and adaptive immunity. The granulocytes, basophils, monocytes and macrophages together with complement, natural killer cells and mast cells are the effectors of innate immunity while the B and T lymphocytes are for adaptive immunity [41]. The B lymphocytes produce antibodies and the T lymphocytes act as helper, cytolytic and suppressor cells. WBCs are thus important in normal response to infection and tumour suppression even as they can mediate transplant

rejection and autoimmunity [42]. This study revealed a dose dependent increase in total WBC with all doses of the extract. The WBC differentials were equally increased without affecting the usual distribution. The results clearly show that the aqueous extracts at the doses used in this study may stimulate the immune system thereby enhancing immunity. This may be one of the mechanisms the extracts use to destroy *plasmodia,* the causative protozoa of malaria. The immunomodulatory actions of the extract may be due to two of its major chemical constituents, saponins and stilbenes. These two phytochemicals have been documented to have immuno-stimulant actions [43,44].

5. CONCLUSION

The results obtained from this study show clearly that the aqueous root bark extract of *Salacia lehmbachii* at 250, 500 and 750 mg/kg body weight administered for 28 days have stimulant effects on the haematopoietic system leading to increase hematopoietic indices. This effect may be attributed to the chemical constituents in the extracts notably flavonoids, saponins and stilbenes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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