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Assessment of Haematopoietic Toxicity of *Salacia lehmbachii*

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ABSTRACT

Plant extracts are generally known to treat a variety of diseases but their toxic effects in other organs are at times not assessed. The aim of this study was to assess the toxic potential of aqueous (ASL) and ethanol (ESL) extracts of *Salacia lehmbachii* on hematopoietic activity of Wistar rats to ascertain their safety owing to the plant's widespread use in traditional medicine in Southeastern Nigeria. Forty two rats weighing between 150-170 g were randomized into seven groups (n=6). Group 1 (control) received 2ml of distilled water; groups 2-4 rats received 250, 500 and 750mg/kg of ASL while groups 5-7 rats received similar doses of ESL. Administration was per oral using an orogastric cannula for 28 consecutive days. At the end of treatment period, the rats were sacrificed and blood samples were collected for hematological analysis. Data obtained were computed by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison as post hoc. The results revealed that RBC, WBC, platelet counts, hemoglobin concentration and hematocrit levels showed no significant attenuation.Therefore, treatment with *S. lehmbachii* at concentrations studied has a safety profile on hematopoietic cells and justified the plant's extract in Southeastern Nigeria folkloric usage.

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INTRODUCTION

Haematopoiesis is the formation of blood cellular components from hematopoietic stem cells [1]. In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are made daily so as to maintain steady state levels in peripheral circulation [2]. These hematopoietic stem cells (HSCs) reside in medulla of bone marrow and have single ability to produce all forms of mature blood cell types [1]. HSCs are self-generating cells and when they proliferate some of their daughter cells remain as HSCs enabling the pool of stem cells not to depleted and the process is called asymmetrical division [3]. Myeloid and lymphoid do form the other daughters of HSCs which can follow any of the other differentiation pathways resulting in production of one or more specific types of blood cell but cannot renew themselves [3].

In the last 50 years, physicians and basic researchers have known and capitalized on this HSCs processes in treating many diseases which make hematopoietic stem cell transplant the most common treatment. However, in Africa, WHO reports that approximately 70-80% of the world's population depend on nonconventional medicine from herbal sources for their primary healthcare [4,5]. The main reasons are not only increased consultation fees but economic downturn, inadequate health care facilities and trained personnel [6] amongst others. Nevertheless, some of these plants are believed to cause hematopoietic toxicity and begs for in-depth toxic research into every medicinal plant used traditionally in the treatment of diseases.

One of these plants is *Salacia lehmbachii* which is traditionally used in the treatment of malaria fever, abdominal pains and inflammatory disorders [7]. Report on scientific study of *S. lehmbachii* revealed that extracts of root back have: anti-abortifacient activity [8], analgesic and acute anti-Inflammatory activities [7], non-hepatotoxic activity [9], anticholinergic property [10]. Therefore, to assess the toxic effect of ASL and ESL in Wistar rats is plausible since there are great similarity and homology between the genomes of rats and humans.

MATERIALS AND METHODS

Collection and identification of plant material

The collection of and identification of *Salacia lehmbachii* were as described earlier [9].

Preparation of the extract

The roots were washed with distilled water and dried in an electric oven, thermostatically controlled at 40^0C, for 12 hours. The bark was removed by striking the dry roots with a hammer and the pieces obtained were pulverized into a coarse powder using a mechanical grain mill (model) and the powder stored in an airtight container. The plant powder was defatted with 99.9% petroleum ether (Sigma Chemical Limited, USA) using a Soxhlet extractor at 65^0C for twelve hours. The petroleum ether residue was dried, weighed and re-extracted with water at 100^0C and ethanol at 60^0C for 72 hours to obtain aqueous and ethanol extract solutions which were then evaporated to dryness using a rotatory evaporator at a reduced temperature of 45^0C *in vacuo*. The dried extracts were weighed, put in clean specimen bottles and preserved in a refrigerator until required for the experiments.

Experimental animals

Mature male and female Wistar rats weighing between 150 and 170 g obtained from the Animal House Unit of the Department of Pharmacology, University of Calabar, Calabar were used for the study. 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 %, température 28 ± 2^0C and 12h 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 were given standard rat chow (Agro Feeds, Calabar) and water (Water board, Calabar) *ad libitum.* The guidelines on Care and Use of Laboratory animals [11] strictly adhered.

Experimental procedure

Forty-two rats were randomly divided into seven groups (n=6) and labeled 1 to 7. Group 1 rats (Control) had 2 ml of distilled water; groups 2, 3 and 4 received 250, 500 and 750 mg/kg of ASL while groups 5, 6 and 7 were treated with 250, 500 and 750 mg/kg of ESL respectively. Administration was per oral via a gastric cannula and administered daily for 28 consecutive days. In the morning of the 29^{th} day following an overnight fast, the rats were sacrificed by a sharp blow to the back of the head. The thorax was surgically open and blood was drawn by cardiac puncture.

Haematological analysis

Blood from the EDTA-treated bottles was used for hematological studies using an automated haem analyzer (Sysmex KX- 21N, Japan).

Processing of data and statistical analysis

SPSS software version 20.0 was used for data processing and values obtained from descriptive statistics expressed as means ± standard error of mean (SEM). Data was analyzed using ANOVA with Turkey's multiple comparison post hoc tests. Level of significance was predetermined as $P< 0.05$

RESULTS

The toxic effect of ASL and ESL on red blood cell parameters of Wistar rats are shown in Table 1. The total red blood cell count, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were not significantly attenuated.

The toxic effect of ASL and ESL on platelet count of Wistar rats are shown in Fig. 1. Again both extracts showed no toxic effect and instead potential platelet production in a dose-dependent manner.

The toxic effect of ASL and ESL on white blood cell count in Wistar rats are shown in Table 2. Total white blood cell count, lymphocytes, neutrophils and eosinophils were not significantly attenuated.

Table 1: Toxic effect of the ASL and ESL one rythrocyte parameters of Wistar rats

Study groups	tRBC ($x10^6$cells/µL)	Hb (g/dl)	PCV (%)	MCV (f)	MCH (pg)	MCHC (g/dl)
Control	7.67±0.24	15.07±0.23	45.07±1.51	54.87±1.33	19.14±0.45	33.0±0.09
ASL:						
250mg/kg	8.00±0.13	14.76±0.24	44.25 ±0.71	56.25 ±1.18	18.97 ±1.15	33.36±0.11
500mg/kg	8.72±0.08	15.76±0.15	44.96 ±1.12	53.14 ±1.64	18.76 ±0.43	33.36±0.04
750mg/kg	8.97±0.07	15.43±0.25	45.93 ±1.69	55.15 ±1.74	19.02±0.49	33.59±0.12
ESL:						
250mg/kg	8.67±0.20	15.02±0.37	45.07±1.14	55.15 ±2.69	18.68 ±0.58	33.32±0.03
500mg/kg	8.00±0.16	14.80±0.35	44.07±2.85	52.99 ±2.60	18.79 ±0.26	33.58±0.11
750mg/kg	7.85±0.10	14.51±0.14	43.51±1.61	53.13 ±2.85	18.67 ±0.35	33.35±0.15

Values are expressed as mean ± SEM. n = 6

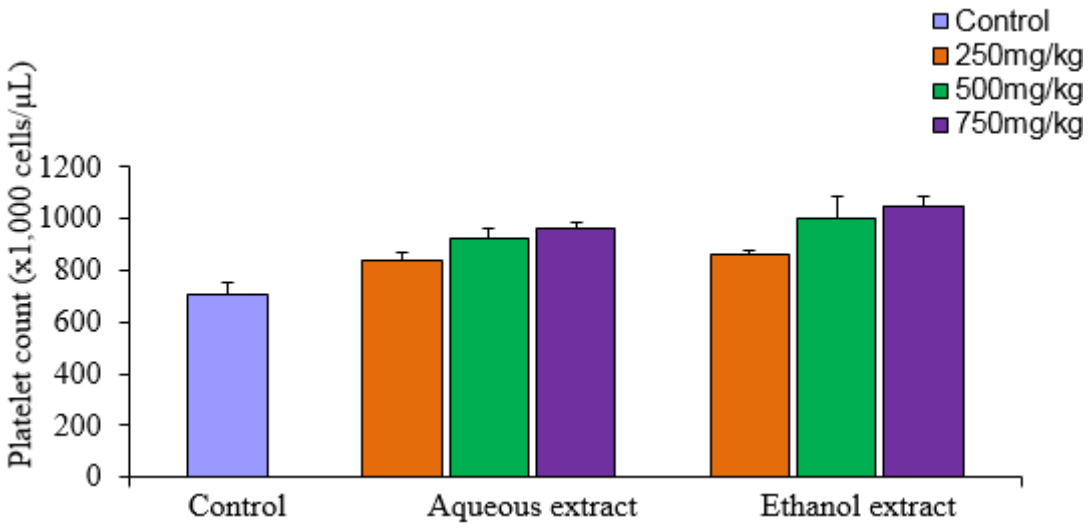

Fig.1: Toxic effect of the ASL and ESL on platelet count of Wistar rats

Table 2: Toxic effect of ASL and ESL WBC total count and differentials of Wistar rats

Study groups (n=6)	tWBC (x 10^3cells/µL)	Lymphocytes (%)	Neutrophils (%)	Eosinophil (%)
Control	8.95 ± 0.99	82.07±1.57 (7.34±1.57)	16.17±1.33 (1.45±1.33)	1.76±0.45 (0.16±0.45)
ASL:				
250mg/kg	10.55± 0.41	82.00 ±0.71 (8.65±0.71)	16.20 ±1.18 (1.71±1.18)	1.77 ±1.15 (0.19±1.15)
500mg/kg	13.43±1.02	82.06 ±1.52 (11.02±1.52)	16.14 ±1.64 (2.17±1.64)	1.80 ±0.43 (0.24±0.43)
750mg/kg	18.38 ± 1.46	82.03 ±1.69 (15.08±1.69)	16.15 ±1.74 (2.97±1.74)	1.82±0.49 (0.33±0.49)
ESL:				
250mg/kg	10.96±1.76	81.97±1.14 (8.93±1.14)	16.15 ±2.69 (1.77±2.69)	1.88 ±0.58 (0.21±0.58)
500mg/kg	13.50±1.38	82.07 ±2.85 (11.13±2.85)	16.14 ±2.60 (2.19±2.60)	1.79 ±0.26 (0.24±0.26)
750mg/kg	15.50±1.80	82.10 ±1.61 (15.23±1.61)	16.13 ±2.85 (2.99±2.85)	1.77 ±0.35 (0.33±0.35)

Values are expressed as mean ±SEM. n = 6

DISCUSSION

Toxicity is normally non-specific and specific. Non-specific acting mode results in narcosis being regarded as generalized depression in biological activity due to the presence of toxicant molecules [12]. However, the target site and mechanism of toxic action through which narcosis affects organisms are still unclear [12]. Nevertheless, hypotheses do support its occurrence through alterations in specific sites in the plasmatic membranes like lipid layers or the proteins bound to these membranes. In the present study, hematopoietic stem cells seemed not to be affected by ASL and ESL which suggests that the both extracts do not contain toxicants.

Specific acting mode of toxicant refers to toxicants acting at low concentration to modify or inhibit biological process by binding to a specific site or molecule [12]. However, at high enough concentrations, toxicants showing specific acting modes can produce narcosis that can or can not be reversible. In the present study, low concentrations instead potentiate biological process of hematopoiesis suggesting equally that no toxicants are involved in the two extracts.

CONCLUSION

Therefore, aqueous and ethanol extracts of *S. lehmbachii* are not toxic in hematopoiesis. This supports the traditional use of the plant by herbalists in Southeastern Nigeria.

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