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Solanum anguivi saponins inhibit basal erythropoiesis in *Rattus novergicus*

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ABSTRACT

The effect of saponin purified from *Solanium anguivi* Lam. fruits on haematology parameters of rats (*Rattus novergicus*) was investigated. Thirty six (36) rats (*Rattus novergicus*) of average weight (125± 12g) were divided into six (6) groups of six animals each designated as A (n=6, 2.0 ml distilled water), B (n=6, 20mg/kg saponin), C (n=6, 40mg/kg), D (n=6, 60mg/kg), E (n=6, 80mg/kg), and F (n=6, 100mg/kg). Saponin was administered orally once daily to groups B, C, D, E and F for (21) days while group A served as control. The animals were then sacrificed and blood was obtained for estimation of the data herein presented. *Solanum anguivi* fruit saponin had a significant reduction ($P<0.05$) in red blood cells (RBC), white blood cells (WBC) and Haemoglobin (Hb) concentration and the decrease was dose dependent. There was no significant difference in packed cell volume (PCV), basophil, lymphocyte and eosinophil. There was significant increase in neutrophil at 100mg/kg ($P<0.05$). The observed significant reduction in RBC and Hb concentration were indication of defective haematopoiesis and anaemia respectively. Therefore, saponins from S. anguivi fruits inhibit erythropoiesis.

INTRODUCTION

Solanum anguivi Lam. a native of Africa, is a medical plant that has improved health both in the ancient times and the modern age of today. The green fruits of *Solanum anguivi* Lam. are collected and consumed as a vegetable. In Ghana they are used as an appetizer [1]. In Nigeria, it is consumed as vegetable, and in south western part of Nigeria where it is called "Igba yirin". It is consumed mainly with the believe that it cures hypertension. Recently, there have been a tremendous commercially driven promotion of saponins as dietary supplement and nutriceutricals, and there is evidence of the presence of saponins in traditional medicine preparation [2]. Saponin from different plant sources vary widely in their toxicity [3]. Despite the high toxicity of many saponins when given intravenously to higher animals, their toxic effect are greatly reduced when administered orally [4]. Ability of saponin to form pores in membranes have been reported [5,6,7]. Cholesterol lowering properties of saponin from S. anguivi has also been reported [8]. The fruits of *Solanum anguivi* Lam is the richest source of edible saponin from south western part of Nigeria. The effect of saponin from fruit of *S. anguivi* Lam. on haematological parameters have not been documented. The present study was therefore aimed at evaluating the effects of saponin from *Solanum anguivi* Lam fruits on haematological parameters of rats.

MATERIALS AND METHOD

Plant materials

The fruits of *Solanum anguivi* were collected from Adekunle Ajasin University, Akungba Akoko horticultural garden. They were identified and authenticated at the herbarium of plant science and Forestry department, University of Ado Ekiti, Nigeria. The fruits were air dried and grounded into a powdery fine texture and stored at room temperature in air tight polythene bag prior to use.

Preparation Saponin extract

100g of ground sample was extracted with 500ml of petroleum ether (40-60^{0}C) in a soxhlet extractor for 12 hours. The air-dried, deffated sample was extracted with methanol (500ml) for 12 hours. The methanolic extract was partitioned between mixture of n-butanol and water (1:1.v/v). After a thorough shaking and allowing to stand overnight, the n-butanol layer was separated. The aqueous layer was washed five times with aliquots of n-butanol until it became colourless. The pooled butanolic layer was evaporated in vacuo to give a residue which was dissolved in 100ml methanol and precipitated by adding a large

amount of diethyl ether to obtain a solid crystalline dark brown compound [3]

Qualitative thin layer chromatography

The crude saponin fraction was spotted onto pre-coated silica gel TLC plate (Merck, Kleselgel 60F-254). The plates were developed with n-butanol: acetic acid: water (60:10:30 v/v/v). The spots on the chromatograms which were due to saponins were identified by spraying with Lieberman-Burchard reagent (methanol: sulphuric acid: acetic acid (50:5:5 v/v/v). Solanum anguivi saponin extract was spotted alongside a standard solution (5g/litre) of saponin white as a reference [3].

Saponin extract purification

Concentrated crude saponin extract was applied to a silica gel column of (60-120 mesh). The impurities were washed with n-hexane through a 2.4x50cm bed of silica gel. The column was eluted with n-butanol: acetic acid: water (1:1:1 v/v/v). The fractions were collected and aliquots applied as a series of spots to aa trip of TLC plate, dried, sprayed with Lieberman-Burchard reagent and heated. Positive fractions were pooled together and used for the experiment.

Animal grouping

Twenty four albino rats of average weight 125±12g were obtained from Animal unit of Federal university of Technology, Akure Ondo state. They were divided into six groups of four animals each and allowed to acclimatize to experimental condition for two weeks. They were housed in clean cages and maintained under standard laboratory conditions (temperature 25±2^{0}C with dark/light cycle 12/12h). They were fed *ad libitum* on rat pellets by (Top Feeds, Nigeria) and water. Groups A (control) was given distilled, B, C, D, E and F were given daily oral dose of 20, 40, 60, 80, 100mg/kg body weight of saponin respectively as previously reported by [3] for 21 days.

Haematological estimations

The blood samples were obtained via cardiac puncture, collected into heparinized tubes and were immediately used for determination of haematological parameters. Total red blood cell and white blood cell counts were estimated according to the visual method of [9]. The percentage packed cell volume was determined according to the hematocrit method of [10] while the blood haemoglobin concentration in all samples were estimated according to the cyanomethaemoglobin method of [10].

Differential White Blood Cell counts

These were estimated using the method of [11]. A dry micropipette was used to suck in blood from the blood sample bottle, a small drop of blood was applied to one end of a slide and quickly placed on the bench holding it in position, the end of the second slide was then placed in the drop and held there until the blood had spread across it. It was then drawn slowly over the whole length of the first slide being held at an angle of 45°. After the blood had spread, it was dried before staining with Leishman's stain. The film which was washed off in a gentle stream of water was dried with filter paper and examined under low and high power microscope and the different kinds of cells counted.

Statistical analysis

The data are expressed as mean± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA). Difference were considered to be statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Results in Table 1

DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal [12]. It can also be used to explain blood relating function of chemical compounds/ plant extract [13].

The changes in the mean Red blood cell count (RBC), Packed cell volume (PCV), Haemoglobin concentration (Hb), total white blood cell count and white blood differentials are presented in Table 1.*Solanum anguivi* fruit saponin had a significant reduction ($P<0.05$) in red blood cells (RBC), Haemoglobin concentration and WBC counts. The decrease was dose dependent. There was no significant difference in packed cell volume (PCV), white

Table 1. Effect of *S. anguivi* saponin on haematology parameters of rats (n = 6, X ± SEM).

Grp/Test	Control	20mg/kg	40mg/kg	60mg/kg	80mg/kg	100mg/kg
RBC (10^6/µl)	8.44±0.28	6.80±0.44^a	6.23±0.79^b	5.92±0.31^b	5.37±0.11^a	5.25±0.50^a
PCV (%)	44.00±2.04	43.5±2.02	41.25±1.88	42.25±1.03	44.00±1.41	44.00±1.82
Hb (g/dl)	18.03±1.47	13.40±0.58^a	13.05±0.69^a	12.90±0.56^a	12.45±0.50^a	12.10±0.58^a
WBC (10^6/µl)	7.65±0.67	4.25±0.25^c	3.65±1.00^c	3.07±0.66^c	2.13±0.20^b	1.50±0.12^a
Neutrophils (%)	29.00±0.91	28.00±1.96	26.25±1.49	25.00±1.91	24.75±1.18	34.00±0.91^a
Eosinophil(%)	1.75±0.47	2.50±0.50	2.25±0.48	2.00±0.00	1.75±0.25	1.75±0.48
Lymphocytes(%)	63.25±3.44	68.75±1.88	66.00±4.88	69.00±1.87	70.25±1.31	67.33±1.14
Basophil(%)	1.50±0.29	1.25±0.25	1.25±0.25	1.50±0.50	1.00±0.00	1.75±0.48

Values are expressed as mean± standard error mean (SEM). Values with different superscript are significantly different.

blood cells count (WBC), basophil, lymphocyte and eosinophil. There was significant increase in neutrophil at 100mg/kg ($P<0.05$).

The observed decrease in red blood cell count in all test groups (Table 1) may be due to the suppressive effect of saponin from *S. anguivi* on bone marrow. Saponin may have suppressed the growth and differentiation factors in the bone marrow [14]. Another probable reason for the observed decrease in RBC count may be due to haemolysis mediated via saponin from *Solanum anguivi* fruits or may be the saponin caused failure of erythropoietin production. It is a known fact that various antinutritional substances and xenobiotic chemicals like saponin and tannins cause haemolysis, nutrient malabsorption and abnormal haemopoeisis [15,16,17,18].

The decrease level of haemoglobin (Hb) concentration in test groups compared with control may be an indicator of rapid haemolysis leading to haemolytic anaemia. Hb estimation measures the amount of of Hb in grams per 1dl of whole blood and provides an estimate of oxygen- carrying capacity of the red blood cell. The reduction in the levels of haemoglobin which is a protein utilized by red blood cells for the distribution of oxygen to other tissues and cells of the body is an indication of anaemia [19]. Therefore it appears that saponin from *S. anguivi* has harmful effect on the red blood cells and heamoglobin metabolism at the dosages tested and might reduce the oxygen carrying capacity of the rat's erythrocytes, which is an indication of anaemia [20].

The significant reduction observed in WBC counts following oral administration of saponin from *Solanum anguivi* is not in line with the normal physiological response following perception of a foreign attack by body defense mechanism. The decrease observed may have resulted from suppression of leucoytosis by saponin and also from the suppression of their production in the bone marrow [21]. The effect of saponin from *Solanum anguivi* on differential count was not significant. This showed that saponin from S. anguivi did not influence WBC differential count. This means that saponin may not be able to protect much against some form of infections at the dosages under investigation.

The reduction in the white blood cells and neutrophils observed in this study also suggests selective and localized toxicity [22]. However a significant increase was observed in neutrophil in group administered 100mg/kg body weight saponin. A higher dose of saponin may have a significant effect on neutrophil. Depletion of erythrocyte number and Hb content is an indicator of defective haematopoiesis [23].

CONCLUSION

The study has shown the effect of saponin from *Solanum anguivi* fruit on haematological parameters of rats. It shows that saponin from *Solanum anguivi* Lam fruit given at dosages investigated adversely affect haemotological parameters of rats due to its significant lowering effect on RBC, WBC counts and Hb concentration. Further experimental studies to elucidate the mechanism of action involved are in progress.

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