

## Aqueous root extract of *Securidaca longepedunculata* linn alters haematological indices in rats

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### Abstract

The plant *Securidaca longepedunculata* is used, in combination with other plants, traditionally in the treatment of snakebites in some communities in Nigeria. A lot of biochemical and physiological alterations, like haematological or enzyme changes are commonly known to accompany snakebites. It is, therefore, necessary to evaluate the effects of this plant extract on haematological indices. The aqueous extract of the root bark was found to produce a considerable alteration in red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) and clotting time. Similarly, there was a dose dependent decrease in serum electrolytes: Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup> analysed. These changes may suggest toxicity. The investigation further revealed significant alterations (P < 0.05) in differential counts of the leucocytes. The plant could possess important biological activities but caution should be employed as these haematological alterations can be potentially toxic.

Keywords: *Securidaca longepedunculata*, haematological indices, toxicity.

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### Introduction

*Securidaca longepedunculata* Linn, belonging to the family Polygalaceae is a shrub that is widely distributed in the savannah. It is about 2 m high and has leaves that are lanceolate to oblate. The plant is commonly called "violet" plant. It is called "uwar magunguna" in Hausa, literally meaning: "The mother of medicines". The root bark of the plant is used for the treatment of snakebite. It is used in combination with other plants. The use of herbs especially in the treatment of snakebite is of interest, since the existing orthodox remedy (the anti-snake venom serum) is barely available and difficult to store. Moreover, there is a very strong belief on its therapeutic efficacy, in that the people prefer it to orthodox medicine. An oral therapy, of such will be a novel achievement. In folkloric medicine, it is believed that the root bark must be added in all traditional medicinal preparations. This is reflected in the literal meaning of the plant: "mother of all medicines" (Gunji, Personal communication 2004). The World Health Organisation (WHO) defined medicinal plants as plants, which contain substances that can be used for therapeutic purposes or precursors of useful drugs (Sofowora, 1982). Holetz *et al.* (2002) demonstrated that the presence of secondary metabolites such as alkaloids, essential oils, phenolic substances etc are responsible for therapeutic activity of plants. Previous work by Wannang *et al.* (2005), demonstrated the presence of tannins and saponins in large amount, anthraquinone, triterpenoids, sugar, flavonoids, amino acids and proteins, steroids in the root bark of *S. Longepedunculata*

One of the features of snake envenomation is the alteration of blood parameters. If this plant material is useful in the treatment of snakebites, it is expected that the extract should reverse these alterations. Similarly, the activity of the plant could result in toxicity, which can be reflected in these alterations. The claim of the herbalists inspired us in this study, to determine the possible toxic effects with regards to changes in haematological indices induced by the aqueous extract of the plant and to provide scientific proof for its use in folk medicine.

## Materials and methods

### *Plant material*

*Securidaca longepedunculata* were obtained from the fields in Vel-Pankshin, Plateau State, Nigeria and botanical authentication was confirmed by M. Musa of the Herbarium Department, Ahmadu Bello University, Zaria, Nigeria, where voucher specimens were deposited.

### *Extraction*

The bark of *Securidaca longepedunculata* were cut into pieces and air-dried for 28 days. They were reduced into powdered forms. They were subsequently reduced to coarse powder and 600 g of the powdered roots were extracted in 1000 ml of distilled water for 24 hours at room temperature. The extract was filtered and a percentage yield of 38% w/v was obtained.

### *Animals*

White wister rats (140-180 g) of either sexes, obtained from the Animal house of University of Jos, Nigeria, were kept at the laboratory animal house of the Department of Pharmacology, Faculty of Medicine, Bayero University, Kano, Nigeria were used. Animal had free access to food and water ad libitum.

### *Acute toxicity*

After a pilot study, rats were administered graded doses of extract (60-120 mg/kg,ip), mortality in each group within 24 hours was recorded. LD<sub>50</sub> was estimated using the method of Lorke (1983). 3 rats were used per group. Animals were administered 60, 80, 100, 120 mg/kg,ip. and mortality was recorded within 24 hours in the first investigation. In the second investigation, 1 rat per group of 3, was used. LD<sub>50</sub> was calculated as the geometric mean for which no death and death were found. The animals were observed for a further 7 days for any signs of delayed toxicity.

### *Haematological parameters*

The methods of Cheersbrough (2000) were used. Animals were treated for 7 days and sacrificed on the 8<sup>th</sup> day. Blood was collected in EDTA anticoagulant bottles. The PCV measurement, blood samples were collected via plain microcapillary tubes and sealed at both ends using flame, this was put in the centrifuge and spinned for 5 minutes and read in the micro haemacrit reader. For the WBC, 20 ul of blood was collected in 0.4 ml of turks fluid. This was placed on the new-improved counter chamber and read. Hb was estimated by collecting blood samples and diluting in drabkin solution in a ratio 1:250, it was read colorimetrically against a blank (drabkin). For the RBC, blood samples were collected and

Table 1: LD<sub>50</sub> Determination of Extract in Rats

Group	Dose (mg/kg)	No. Used/No. death
Control	-	3/0
1	60	3/0
2	80	3/1
3	100	3/3
4	120	3/3

Second investigation: 80 mg/kg body weight 0/1  
 100 mg/kg body weight 1/1 120 mg/kg body weight 1/1  
 LD<sub>50</sub> is V80xIOO=89.44 mg/kg.ip.

Table 2: Effect of extract on haematological indices in rats

Treatment (mg/kg)	PCV (C/o)	Hb (G/dL)	RBC (x10 <sup>12</sup> /L)	WBC (x10 <sup>9</sup> /L)	Clotting time(min)
Control	2.0	11.4	1.37	0.7	0.1
5	1.0	11.9	0.4	0.1	0.2
10	1.2	10.3	1.7	0.1	*2.3d LO.I
15	2.1	12.0	0.1	4.1 + 1.1	2

\*P<0.05 C/o-percentage, G/dL-grams per deciliter, L-liter, min-minutes

Table 3: Effect of Extract on differential count in rats

Treatment (mg/kg)	C/oN	C/oL	C/oM	C/oE	C/oB
Control			00	00	00
5			00	00	00
10				00	00
15			00	00	0.01

N-Neutrophilis, L-Lymphocyte, M-Mesophilis, E-Eosinophilis, B-Basophilis

Table 4: Effect of extract on serum electrolyte in rats

Treatment mg/kg	Na <sup>+</sup> mmol/L	K <sup>+</sup> mmol/L	Cl <sup>-</sup> mmol/L	HCO <sub>3</sub> <sup>-</sup> mmol/L
Control				
5				
10				
15	12			

n= 3

Na<sup>+</sup>-Sodium ion, K<sup>+</sup>-Pottasium ion, Cl<sup>-</sup>-Chloride ion, HCO<sub>3</sub><sup>-</sup>-Bicarbonate ion, mmol/L -Millimole per liter.

placed on an auto analyser, for the count. Clotting time was determined by a cut on the distal part of each rat's tail using a sterile scissors. The blood was placed on a grease-free glass slide. A stop clock was started immediately. A needle was passed through the blood on the glass slide every 15 seconds until a thread-like structure was seen. This is taken as the clotting time.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  were analyzed titrimetrically.

### Statistics

All the parameters measured were expressed as means  $\pm$  standard deviation. The difference between means values was analysed by students t-test at 5% significant level.  $P < 0.05$  was considered to be significant

## Results

From the result (Table 1) of the  $\text{LD}_{50}$  estimation, the extract is toxic, as it produced an  $\text{LD}_{50}$  of 89.44 mg/kg using the intraperitoneal route. Table 2 revealed that there were alterations in haematological indices in rats administered the aqueous extract (5-15 mg/kg). There was no significant difference between PCV, Hb and RBC counts for the test and control groups. On the RBC count, the control samples showed the normal RBC picture while the test samples (5-15 mg/kg of extract) showed microcytosis with evidence of mild haemolysis, as it revealed schistocytes. However, there was considerable low count (leucopaenia) in total WBC count ( $P < 0.05$ ). There was dose-dependent decrease in clotting time.

In the differential count on the WBC (Table 3), relative neutrophilia was noticed on the test samples administered the extract (5-15 mg/kg) when compared to the control (administered with distilled water), which showed higher lymphocyte count. The study shows that the changes in RBC, Hb, PCV were not significant but evidence abound on lysis of RBC which were manifested as precipitates in the test tubes (not shown in results).

Various concentrations of crude extract of *S. longepedunculata* produced a significant reduction in serum electrolytes in rats (Table 4). Similarly, there was a dose-dependent decrease in the anions ( $\text{CP}$  and  $\text{HCO}_3^-$ ) as increase in concentration of extract showed a significant ( $P < 0.05$ ) decrease in both anions analyzed.

## Discussion

The data obtained from this work, show an alteration in haematological indices in rats administered the aqueous extract of *Securidaca longepedunculata*. There was a biphasic change in PCV and Hb concentration and a corresponding decrease in total leucocyte. Elevated PCV and Hb values are commonly associated with pathologies like polycythaemia (Schafer, 1984). An elevation in PCV is indicative of a decrease in fatty acid components, while the decrease in leucocyte is an indication of an infection, thus a decrease immunology. The decrease in Hb and RBC could be as a result of direct hemolytic activity of the RBC and Hb or could be a suppression of the production site of these cells. Similarly, saponins are haemolytic components, thus, their presence in the root bark could responsible for the breakdown of the cells. The decrease in clotting time is a direct effect of the platelets. Jackson and Nemerson (1980) showed that platelets are the blood cells involved in coagulation; increasing platelet population and/or function will result in decreasing clotting time, resulting in coagulation (Burstein et al., 1981). These authors further demonstrated that

the bone marrow has a narrow reserve of platelets. Thus decreasing clotting time, is suggestive that the extract produced a general increase in platelet function. The results on differential counts contradict the use in snakebite as there was a recorded decrease in lymphocytes, which depicts a vulnerability to immunity. The lymphocytes undergo transformation to T-lymphocytes (cell mediated immunity) and B-lymphocytes (humoral immunity). The effect of increasing concentration of the two operational ions  $\text{Na}^+$  and  $\text{K}^+$  on the ion motive Na-K-ATPase is quite significant in mechanisms. These ions exert stimulatory effect on enzymes (Rossier et al., 1987). Thus, variation of concentration of either will influence binding and enzyme activities. The decreased levels of  $\text{Na}^+$  and  $\text{K}^+$  will increase activity of Na-K-ATP to compensate ATP hydrolysis resulting in hypoglycaemia and weakness. Eveloff and Warnock, (1985) demonstrated that coordinated action of the  $\text{Na}^+/\text{K}^+$  exchanger and the  $\text{Cl}^-/\text{HCO}_3^-$  exchanges would lead to net uptake of NaCl to initiate the restoration of cellular volume (Gala et al., 1986). Boron and Knalkal (1992) demonstrated that the reduction in  $\text{Na}^+$ -dependent  $\text{Cl}^-/\text{HCCV}$  exchanger will reduce alkalinisation.

This report revealed some haematological changes in rats administered *Securidaca longepedunculata* and further studies to elucidate the involvement/implications in therapeutics are recommended.

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