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Acute and Subchronic (28-day) Oral Toxicity Studies of the Aqueous Root Extract of *Securidaca longepedunculata* Fresen (Polygalaceae) in Mice

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Abstract: The acute and subchronic (repeat dose) oral toxicity of aqueous root extract of *Securidaca longepedunculata* was evaluated in mice. In the acute toxicity test, oral administration of limit dose of 5 g kg^{-1} of the extract produced neither mortality nor any acute signs of toxicity throughout the 14 days period of observation. In the subchronic toxicity study, there was no mortality recorded among the animals when varying doses of the extract (300, 900 and 2700 mg kg^{-1} body weight) were administered orally for 28 days consecutively. The weekly body and organ weights of the mice showed no significant differences between the control and the mice treated with the extract except on the last week (day 28) where there was significant increase ($p<0.05$) in the body weight of mice treated with 2,700 mg kg^{-1} of the extract. In the haematological analysis, there were significant ($p<0.05$) decrease in the Red Blood Cell (RBC) count at 2700 mg kg^{-1} and in Packed Cell Volume (PCV) of mice treated with 300 and 2,700 mg kg^{-1} of the extract, while the differential leucocytes count showed no differences in any of the parameter examined. Also, there was no significant changes in the biochemical parameters tested. There was neither any gross lesion nor histopathological changes observed in the organs (liver, kidney and brain) examined. These findings suggest that the aqueous root extract of *S. longepedunculata* could be relatively safe when administered orally in mice.

Key words: *Securidaca longepedunculata*, acute toxicity, subchronic (repeat dose) toxicity

INTRODUCTION

Securidaca Longepedunculata fresen commonly known as violet tree (family: polygalaceae) is a shrub or a small 2-10 m high flowering savannah tree which is widely distributed in tropical Africa and occurs naturally in the north western and western part of Nigeria and northern provinces of South Africa (Ojewole *et al*., 2000). It is know to possess a broad spectrum of medicinal, pharmacological and therapeutic properties. It is reported that almost all part of the plant (leaves, twigs, stem bark, roots and seeds) are used by man for different purposes such as medicine, source of fibers, water purifying agents, an ornament, fish poison, molluscicides, snake repellent or as an insect repellent (Neuwinger *et al*., 1964) For example, decoction of the roots are used for cough and other chest complaint; hot water poultice of the roots is said to give symptomatic relief of rheumatism; powdered roots are rubbed into scarification marks on the forehead to treat headache and the roots are chewed to relieve toothache (Watt and Breyer-Brandwijk, 1962). The root bark is said to contain flavonoids, alkaloids, saponnins, triter periods and volatile oils (Ekpendu *et al*., 2000). The plant has also been claimed to produce several other secondary metabolites, the major ones being sapogennins including, presenegenin, indole alkaloid securinine and some ergot alkaloids (Van Wyk *et al*., 1997). Methyl salicylate present in the volatile oil is said to be responsible for the plant biocide effect against stored grain insects (George *et al*., 2000) while Atawodi *et al*. (2003) reported that the methanol extract of *Securidaca longepedunculata* has some *in vitro* trypanocidal activity on both *T.brucei* and *T.congolense*.

Despite the varied uses of this plants in treating a plethora of human and animal ailments, its toxicological profile have not been reported to our knowledge. This study is therefore aimed at investigating the acute and subchronic (repeat dose) oral toxicity of the plant using the recommended Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals for safety evaluation.

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MATERIALS AND METHODS

Preparation of plants materials and extracts: The fresh roots of *S. longepedunculata* were collected in the month of October 2004 from its natural habitat at Mekujera village in Rabah local government, Sokoto, Nigeria. The plant was authenticated by Dr. B.L. Aliero of biological science Department, Usman Danfodiyo University (UDUS), Sokoto. A voucher specimen (no: D-01SL-4) was deposited at the Herbarium, Department of Biological Science, Botany unit, UDUS.

The clean roots cut into pieces were air dried to constant weight and pulverized to a dry powder. About 250g of the powder were macerated with 3 liters of distilled water for 24 h. The liquid obtained by filtration was oven-dried at a temperature of 55°C and the percentage yield of the extract was calculated to be 15.04% (w/w). Appropriate concentrations of the extracts were made in distilled water and used in the experiments.

Experimental animals: Swiss Albino mice of either sex (bred in animal facility unit, University of Jos, Jos, Nigeria) weighing 20-25 g were used. The animals were fed with standard mouse diet (Vital Feeds, Jos), had free access to water under well ventilated condition of 12 h light cycle. They were kept in metal cages with wood shavings as bedding and were adapted to laboratory conditions for 7 days prior to the experiments. This study was carried out according to the Organization for Economic Cooperation and Development (OECD) principles on Good Laboratory Practice (GLP) (OECD, 2001).

The extract was administered orally to all animals using a suitable intubation cannula, after each aliquot extract constitution. The animals were fasted prior to dosing according to OECD guidelines, food but not water was withheld for 3-4 h. Following period of fasting, the animals were weighed to determine the appropriate weight (mg kg^{-1}) of the extract residue for making various concentrations for each of the experiments. In all cases, the maximum volume of the aliquot portion of the extract used did not exceed 1 mL/100 g of the animal body weight as recommended by OECD guideline (OECD, 2000). The animals were randomly assigned to cages for grouping and individual animal was fur marked with picric acid for easy identification.

Acute toxicity test: The limit test dose of 5,000 mg kg^{-1} was used as described by Organization for Economic Cooperation Development (OECD) guideline and Interagency Research Animal Committee (IRAC, 2004) recommendation. Five female mice each sequentially dosed at interval of 48 h (short term observation period) were used for the test. Animals were observed individually for any sign of acute toxicity, morbidity or mortality during the first 24 h daily thereafter for a total of 14 days (long term observation period).

Sub-Chronic toxicity test: the repeat dose 28 day oral toxicity study was carried out according to OECD guideline, 407 (OECD, 1995). A total of twenty apparently healthy mice of either sex selected randomly into four groups were placed into four different cages.

Group 1 received 10 mL kg^{-1} of 0.9% saline water and served as control. Group 2, 3 and 4 received test doses of 300, 900 and 2700 mg kg^{-1}, respectively of aqueous extract of *S. longepedunculata.* The animals were dosed daily for a period of 28 days and the doses were given at similar time each day. Adjustment was made as necessary to maintain a constant dose level in term of animal body weight.

Animals were observed at least twice daily for morbidity and mortality. All animals were weighed weekly. Animals that survive after 28 days were anaesthetized on the 29th day with chloroform and blood sample for haematological and biochemical analysis were collected by cardiac puncture into coated EDTA container and plain vials respectively. The Packed Cell Volume (PCV) was determined by the microhaemocrit method. The Haemoglobin concentration (Hb) was evaluated by cytomethamoglobin method using Beckman Model of Spectrophotometer. White Blood Count (WBC) and Red Blood Count (RBC) were determined using improved Neubauer Haemocytometer. Plasma concentrations of glucose, total protein, albumen, globulin and urea were analysed using humalyzer 2000 (Human, Germany). Necroscopy of all animals was carried and the organ weights (heart, lung, liver, stomach, kidney, brain and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each mouse. Subsequently, the animals organs harvested (the brain, liver and kidney) were fixed in 10% formalin saline solution for histopathological examination. Thin cryostat sections were stained with haematoxylin and eosin, periodic and schiff reagent with and without diatase, respectively. The sections were examined under light microscope at high (x 400 objective) power magnification.

Statistical analysis: All data were expressed as the mean±standard error of the mean (SEM). One way analysis of variance (ANOVA) with subsequent Dunnett's post hoc analysis was used to detect further differences between groups. Values of $p<0.05$ were considered significant. All statistical analysis were carried out using the Instat Statistical package (Graph pad software, Inc. USA).

RESULTS

The limit dose of 5 g kg^{-1} did not cause any mortality or any signs of acute toxicity in any of the five mice tested in the short term (i.e., 48 h) and long term (i.e., 14 days) observatory period. LD_{50} of the extract according to OECD guidelines is therefore greater than the limit dose tested. No mortality was also observed when varying doses of 300, 900 and 2700 mg kg^{-1} of aqueous root extract of *S. longepedunculata* were administered orally daily for a period of 28 days.

There were no significant differences in the mean organ weight between the control and the animals treated with the extract (Table 1). Similarly, there were also no significant differences in the weekly mean body weight between control and the animals treated with the extract except on day 28 where the group treated with the highest dose (2,700 mg kg^{-1}) of the extract produced significant higher weight ($p<0.05$) compared to control and other treated group (Table 2). Haematological analysis showed significant ($p<0.05$) decrease in the Red Blood Cell (RBC) count at the highest dose of 2700 mg kg^{-1} and also in the Packed Cell Volume (PCV) at low dose (300 mg kg^{-1}) and high dose (2700 mg kg^{-1}), (Table 3). The leucocytes differential count however showed no significant differences between the control and the animal treated with the extract (Table 4). The result of the biochemical study also showed no significant difference between the control and the animals treated with the extract in all the biochemical parameters tested (Table 5). Observation for gross pathological lesions of the organs examined immediately after dissection show no visible lesion. Histopathological examination of the liver, kidney and the brain in the control and the animals treated with the

Table 1: Mean organ weight of mice[a] after 28 days treatment with aqueous root extract of *S. longepedunculata*

Treatment and dose (mg kg^{-1})	Mean organ weight (g)						
	Heart	Lung	Liver	Kidney	Stomach	Brain	Spleen
Control	0.11±0.01	0.06±0.01	1.39±0.06	0.17±0.01	0.48±0.11	0.41±0.02	0.18±0.01
300	0.10±0.01	0.05±0.01	1.17±0.20	0.14±0.02	0.35±0.02	0.40±0.01	0.15±0.03
900	0.10±0.01	0.06±0.02	1.06±0.10	0.15±0.03	0.35±0.04	0.42±0.03	0.15±0.03
2700	0.11±0.01	0.06±0.00	1.11±0.09	0.12±0.01	0.36±0.03	0.39±0.01	0.14±0.03

[a]Data are means±SEM; n = 5

Table 2: Mean body weight of mice[a] after 28 days treatment with aqueous root extract of *S. longepedunculata*

Treatment and dose (mg kg^{-1})	Weekly mean body weight (g)					Mean weight gain (g)
	Day 1	Day 7	Day 14	Day 21	Day 28	
Control	21.09±1.14	21.58±0.81	22.91±0.69	23.43±0.81	24.13±0.33	3.04
300	20.56±0.52	21.58±0.97	22.75±1.29	23.09±1.09	23.17±1.76	2.61
900	21.13±0.71	21.75±0.91	22.04±1.65	22.89±1.58	23.15±1.64	2.02
2700	21.00±0.73	22.21±1.10	24.40±0.84	25.18±0.59	*28.48±1.38	7.04

[a]Data are means±SEM; n = 5. ANOVA, * $p<0.05$

Table 3: Haematological parameters of mice[a] after 28 days treatment with aqueous root extract of *S. longepedunculata*

Treatment and dose (mg kg^{-1})	RBC (×10^6 L^{-1})	Haemoglobin (g δL^{-1})	PCV (%)	WBC (×10^3 L^{-1})
Control	4.18±0.38	7.70±0.60	22.00±4.00	4.85±1.25
300	3.05±0.29	5.73±0.62	*12.33±0.67	3.67±0.35
900	3.20±0.42	6.73±1.80	15.75±2.50	4.40±0.55
2700	*2.48±0.34	4.93±0.37	*11.60±0.98	3.70±0.68

[a]Data are means±SEM; n = 5; ANOVA, with post hoc Dunnett test, * $p<0.05$

Table 4: Leucocytes differential counts of mice[a] after 28 days treatment with aqueous root extract of *S. longepedunculata*

Treatment and dose (mg kg^{-1})	Neutrophil (%)	Eosinophil (%)	Monocyte (%)	Lymphocyte (%)	Basophil (%)
Control	33.00±5.00	2.0±1.00	6.00±1.00	59.00±7.00	0.00
300	32.67±2.33	2.0±0.58	6.61±0.35	58.33±2.60	0.00
900	37.33±2.33	2.3±0.67	7.00±0.58	53.33±3.18	0.00
2700	32.00±0.00	2.0±0.00	6.00±0.00	59.50±0.50	0.00

[a]Data are means±SEM; n=5

Table 5: Biochemical parameters of mice[a] after 28 days treatment with aqueous root extract of *S. longepedunculata*

Treatment and dose (mg kg^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Urea (mmol L^{-1})	Serum glucose (mmol L^{-1})
Control	5.82±0.29	4.17±0.17	1.65±0.12	13.67±0.73	1.45±0.22
300	7.63±1.19	4.44±0.11	3.19±1.08	9.11±0.71	3.17±0.23
900	8.00±0.45	4.37±0.04	3.63±0.41	10.17±0.72	3.66±0.88
2700	6.94±097	3.92±0.20	3.02±0.77	10.16±1.67	5.37±1.40

[a]Data are means±SEM; n=5

extract showed no lesion that could be attributed to the effect of oral administration of the aqueous root extract of *S. Longepeduculata* on the mice for 28 days. The lowest observed adverse effects level of the aqueous root extract range between 300 to 2700 mg kg^{-1} following oral administration.

DISCUSSION

The limit test is a modification of acute systemic test design (Gad and Chengelis, 1988) using principles of reduction of animals and changes to study design and techniques in order to make the testing more humane as well as scientifically more valid.

The results of the acute toxicity study indicated that the LD_{50} of the aqueous root extract of *S. longepedunculata* is greater than 5,000 mg kg^{-1}/oral which is above the 2,000 mg kg^{-1}, limit dose recommended by the IRAC and in line with the 2000 or 5000 mg kg^{-1} limit dose stipulated by Organization for Economic Cooperation and Development (OECD). The limit test is primarily use in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity (OCED, 2000). The result of the acute oral toxicity study therefore suggest that the aqueous root extract of *S. longepedunculata* at the limit dose tested is essentially non-toxic and safe in oral formulation.

A 28 day study considered as subchronic, is well accepted for eliciting any toxicity on long term by dosing repeatedly for 28 days with specific endpoint in mind. The extract has no effect on organ weight and weekly body weight except at the dose of 2700 mg kg^{-1} on day 28 where there was a significant increase in the body weight of the mice. The extract produced significant decrease in red blood cell count at the highest dose and also in the Packed Cell Volume (PCV) at low and high dose but not at moderate doses. This could be an indication of anaemia that may have occurred as a result of inhibition of RBC production by bone marrow or haemolysis of the blood cells by the active component of the extract. The reason for low PCV not occurring at moderate dose is not known, but one possibility could be that different active principles present in the extract are acting at different doses tested. The result of the biochemical studies indicates that the extract have no significant adverse effect on the parameters studied. The histopathological examination of the liver, kidney and the brain in the control and the treated groups showed no differences, suggesting that the extract at those doses tested did not result in any adverse toxicological effect on these organs. The sub-chronic toxicity findings therefore indicated that the extract appear to have low toxicity and could be as well tolerated for the 28 days study period.

The findings of both acute oral and 28 days sub-chronic toxicity could be an indication that the aqueous root extract of *S. longependunculata* has some high level of safety margin in oral formulation, thus justifying its wide application in various communities coupled with lack of any reported serious side effect with the traditional use of the plant.

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