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Evaluation of anti-snake venom activity of the aqueous root extract of *Securidaca longipedunculata* in rats

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Abstract

The anti-snake venom properties of *Securidaca longipedunculata* Frens root extract have been evaluated in rats by monitoring the levels of the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CPK), lactate dehydrogenase (LDH), and amylase. The extract produced a significant ($P < 0.05$) dose-dependent alteration in the serum enzymes and urea analyzed. The alterations in these parameters may be responsible for pharmacologic activity of the plant extract.

Keywords: *Securidaca longipedunculata,* serum enzymes, anti-snake venom activity.

Introduction

Securidaca longipedunculata called "violet plant" in English, and "uwar magunguna" (mother of all drugs) in *Hausa*, is a shrub, which grows widely in savannah and temperate climates. The plant is reputed to possess over one hundred medicinal uses which probably explain the literal meaning of its name in Hausa. In many parts of Africa, the plant is employed in traditional medicine principally for its psychotropic properties. In Guinea Bissau, Malawi and South Africa, aqueous extracts of the roots are used as psychopharmacological agents, a property believed to be as a result of its ergot alkaloid content in the dry season (Winkelman and Dobkin De Rios, 1989). *Securidaca longipedunculata* is a common plant in the Pankshin area of Plateau State, Nigeria where it is used extensively in the treatment of snakebites.

Serum chemistry parameters are important in the assessment of vital organ functions. Changes in blood chemistry, particularly with respect to serum enzymes, are indicative of some form of vital tissue damage (Wilkinson, 1976). Snake venom is known to be a complex mixture of various chemicals including about twenty enzyme sub types that are responsible for the toxic nature of the venom (Moore 2004). A consistent feature of a victim of snake envenomation is an altered hematological, haemostatic and clinical chemistry of the individual (Lepow *et al.*, 1969). Ibrahim and Al-Jammoz (2001) observed that crude venom of the snake

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Walterinnesia aegypties gave variable results on the serum enzyme activity of rats. There was a significant increase in the serum level of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and amylases. Aspartate aminotransferase (AST) level initially increased but, with time, the level dropped significantly below that of control rats. Lactate dehydrogenase (LDH), on the other hand, was initially lower than control, but rose with time. In another study, (De sousa-e-silva *et al.*, 2003) found that the venom of *Crotalus terrificus* caused an increase in serum levels of alkaline phosphatase, alanine aminotransferase, myoglobin, creatinine kinase and aspartate aminotransferase in dogs. Mukherje *et al.*, (2001) found similar patterns of enzyme alteration following envenomation with *Daboia russeli* venom.

The most reliable treatment for snake envenomation is the use of antivenom prepared by generating antibodies against the toxic venom in horse serum. The claimed efficacy of many plants in the treatment of snakebites presents an alternate means of venom detoxification. Abubakar *et al.*, (2000) reported on the ability of the leaf extract of *Guirera senegalensis* to detoxify, *in-vitro*, venom of two snake species: *Echis carrinatus* and *Naja nigrocollis*. Similarly, Aguiyi *et al.*, (2001), investigated the effect of the aqueous extract of the seed of *Mucuna pruriens* in rats and found that pretreatment with the extract inhibited the venom, induced increase in the level of serum enzymes and altered coagulation parameters.

The aim of this study, is to investigate the effect of the aqueous root extract of *Securidaca longipedunculata* on some enzyme profiles and blood chemistry of rats, with a view to rationalizing the use of the plant in the treatment of snake bite by the natives of Pankshin.

Experimental

Collection of Plant Materials. The plant was collected from Vel-Pankshin, Plateau State, Nigeria, in September 2004. Identification was performed by David Wonang, Department of Botany, University of Jos, Nigeria, and confirmed by M. Musa, Herbarium Department, Ahmadu Bello University (A.B.U) Zaria, Nigeria. A Voucher specimen has been deposited at the Herbarium (A.B.U) Zaria. The roots were washed in clean water, cut into pieces and air dried for 28 days. They were subsequently reduced to coarse powder.

Extraction procedure. 600g of the root powder 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. This filtrate referred hereafter as the *Securidaca longipedunculata* extract was used for this study.

Rats. Adult white albino rats of both sexes (200-250 g) were purchased from the National Veterinary Research Institute Vom, Nigeria. They were kept in clean cages under a 12/12 hours normal light/dark cycle and allowed to adjust to the laboratory environment for a period of three (3) weeks before the commencement of the experiment. Food (Pfizer products Lagos, Nigeria) and water were provided *ad libitum* during the stabilization period.
The rats were randomly selected from the colony of female and male. Each rat was used for one experiment/treatment only.

Snake. The black spitting cobra (*Naja nigrocollis*) was obtained through the help of traditional snake charmers. Animal was kept in the animal house for 2 weeks before extraction of venom. The venom was extracted and a pilot study was conducted on the venom for 1 week. This consistently killed the rats within 2-3 minutes. The venom was stored in the refrigerator until used.

Enzyme analysis. One group of rats, (200-250 g), were administered graded doses of the extract (15- 20 mg/kg ip) for 2 days. Another group was administered the venom (1 mg/kg) and after 30 minutes the animals were sacrificed. Blood samples were collected and centrifuged at a speed of 240 rpm for 10 minutes. The serum was analyzed for ALT, ALP, CPK, LDH, and amylase using the automated enzyme analyses at room temperature.

Statistical Analysis. Values (mean $\pm$ S.E.M) were analyzed using one-way ANOVA and all the statistical comparison (comparing paired and unpaired samples) were by student- t test.

Results

The results from this experiment showed a biphasic alteration in serum enzyme activity of ALT, ALP, CPK, and amylase. Low dose (5 mg/kg) produced an increase in ALT, ALP, CPK and amylase while 20 mg/kg dose of the extract produced a decrease in the concentration of ALP, CPK and LDH when compared to 5 mg/kg, but results were higher than control. In the case of ALP and CPK 20 mg/kg dose of the extract caused an increase in ALP and amylase compared to 5-10 mg/kg dose and control. The kidney, liver, pancreatic and cardiac enzymes tests showed statistically significant ($P<0.05$) dose-dependent increases between the control and all treated groups for ALT, AST, ALP, CPK, and amylase (Table 1). However, there was a significant decrease in the concentration of LDH in a dose-related fashion.

Discussion

This experiment showed a biphasic dose related alteration in serum levels of ALT, ALP, CPK, amylase and urea. Alternation in the serum level of urea is synonymous to renal damage, while LDH, CPK are cardiac enzymes. Hepatic and renal dysfunctions will produce increased levels of serum, AST, ALT and ALP. Generally an increase in the level of serum enzymes such as aspartate transaminase, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase is indicative of cell or tissue damage (Mukherje *et al.*, 2000). Criteria for the retrospective diagnosis of myocardial infarction rely heavily on increase in LDH. Low doses (5-10 mg/kg) produced a dose-dependent decrease, while 20 mg/kg dose produced an increase greater than (5-10 mg/kg) but insignificantly lower than control. The reasons for the discrepancies remain unclear.

One of the principal characteristics of snake envenomations is alteration in enzyme level, which renders the victims vulnerable. The extract was found to produce statistically significant bi-phasic dose-dependent alterations in serum enzyme activities. These alterations are at variance with variations observed during envenomation with snake venom. Muller-Eberhand and Fjellstrom (1971) have shown that snake venoms are highly complex mixtures of enzymes which act together to produce toxic effects. This implies that there might not be a single pathway for toxicity. Thus, these non-uniform alterations could be unpredictable, as it may compound or enhance the toxic actions of the venom. However, investigations have revealed that some other mechanisms, apart from enzyme activity, are involved in toxicity of venoms Lepow *et al.*, (1969) demonstrated that venoms could be cardiotoxic, proteolytic, haemorrhaginic, cytolytic or neurotoxic. Hemorrhagic and/or necrotic activities appear to play a major role in lethality of snake venom (de Roodt *et al.*, 2000). Moore (2005) suggested that the presence of venom components like carbohydrates, lipids and some metals, play an active role in the lethality of venoms. It may be possible that the extract may possess activity not mediated via the enzyme alterations.

Table 1: Effect of extract on mean values of serum ALT, AST, ALP, amylase, LDH, CPK in rats

Dose (mg/kg)	AST µ/L	ALT µ/L	ALP µ/L	CPK µ/L	Amylase µ/L	LDH µ/L
Control	380.5 ± 20.2	113.5 ± 20.1	181.5 ± 50	1306 ± 105	1168 ± 105	1264 ± 96.5
5	524 ± 260	244.5 ± 105	213 ± 20	3751 ± 205	1688 ± 230	846.5 ± 4.5
10	734 ± 40	245 ± 60	192 ± 30	232.5 ± 135	36.5 ± 8.0	808 ± 10
20	791 ± 57	167 ± 42	276 ± 40	2256 ± 487	3910 ± 767	1111 ± 108
Venom(1)	420 ± 37	288 ± 21.5	301 ± 60	1870 ±98	2850 ± 210	1010 ± 47

Our previous experiment (Wannang *et al.*, 2005) showed that the extract possessed hypotensive properties. Thus, the increased blood pressure induced by some venom may be countered by the hypotensive activity of this extract. However, in cases where there is a rapid drop in blood pressure (observed with venom containing phosphodiesterases), it may aggravate the condition with a potential risk of a circulatory collapse.

It is obvious that there are many mechanisms of action in a single venom. It is therefore, possible that the extract has different actions from enzyme alternations. Based on the data obtained in this work, the extract does not counter the increased enzyme profile induced by the venom of *Naja nigrocollis*.

The results show that there is contradiction, thus, no justification in the use of *Securidaca longipedunculata* in the overall treatment of snake bites. However, the pharmacological activities of individual phytochemical constituents may be elucidated to further elaborate on the acclaimed anti snake activity of this plant.

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