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Anti Snake Venom Potential of Securidaca longepedunculata Leaf and Root Bark on Spitting Cobra (Naja nigricollis Hallowel) in Envenomed Wister Rats

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Abstract: *This study was undertaken to investigate the anti snake venom potential of aqueous extracts of Securidaca longepedunculata leaves and root bark extracts against Snake venom of Spitting cobra (Naja nigricollis). The venom of Naja nigricollis snake was used to test the anti snake venom efficacy of the extracts, using experimental rats. The anti snake venom activity revealed that root bark extract are able to neutralize snake venom at 300mg/kg body weight with 100% survival. And the leaf extract recorded lower anti venom activity of 0% at 200mg/ml survival and 33.33% of mortality at 300mg/ml. But in the combined root bark and leaves extract it shows higher activity of 66.67% survival at 300mg/ml and 33.33% mortality at 200mg/ml. The result indicate that phytochemical constituents of S. longepedunculata leaves and root bark extracts could also be used to enhance the treatment of the snake venom or bites as it shows a significant anti venom effect ($p<0.05$) against Naja nigricollis venom. Sub-acute toxicity and determination of LD_{50} of the plant extracts should be carried out and to enlighten pharmacists on the use of such plant extract for the treatment of various snake bites.*

Key words: *Aqueous extract, Envenomation, Mortality, Phytochemical, Securidaca longepedunculata, Snake venom.*

I. Introduction

Snake bites were considered emergency threats for human life. Perhaps, venomous bites show as double teeth marks than ordinary bites. Snake venom is one of the most amazing and unique adoptions of snakes in animal planet. Venoms are mainly toxic modified saliva consisting of a complex mixture of chemicals called enzymes found in snake poisons throughout the world known to man. Broadly there are two types of toxins namely neurotoxins, which attack the central nervous system and haemotoxins which target the circulatory system. Snakes with neurotoxic venom include cobras, mambas, sea snakes, kraits and coral snakes. Snakes with haemotoxic venom include rattlesnakes, copper head and cottonmouths (Blanchard, 2001).

It is a common belief that snakes bite is devastating and of the 2,700 known species of snakes, only about 300 are venomous (Wang *et al.*, 1997; Alabi, 2005). The most common symptoms of poisonous snake bites likely bloody wound discharge, fang marks in the skin and swelling at the site of the bite, severe localized pain, diarrhea, burning, convulsions, fainting, dizziness, weakness, blurred vision, excessive sweating fever, increased thirst, nausea, vomiting, numbness, tingling and rapid pulse (Wannang, 2005). Mainly the venom is made in special glands located on the head of the animal and that are delivered by transferring method from gland to prey (Wang *et al.*, 1997; Alabi, 2005).

It was estimated worldwide that about 30,000 to 40,000 people die annually due to snake bites. Of these, about 25,000 people die in India, mostly in rural areas, about 10,000 people in United States and rest of in other countries. Under the Wild Life Protection Act, 1972, all snakes are protected (with the venomous once being at the top of the list of the protected species) and there was a ban on the selling of snake skins since 1976. Snake venom is badly needed to produce anti venom required to treat potentially fatal snakebites (Dravidamani *et al.*, 2008).

Recent scientific investigations have confirmed the efficacy of many plants with constituents, to be effective in controlling snake poisons. The use of plant remedies to treat snake bite victims in rural areas and poor communities in developing countries is a common practice (Kuntal, 2009).

The antivenins taken from the horses are used to treat humans suffering from snake envenomation. When injected into the human blood stream, the antibodies attack the venom, neutralizing its effect. But the usage of snake venoms antiserum has its own limitations due to its high cost and lack of availability. It is, therefore, difficult for the rural patients to access antiserum. The natives whose majority are rural farmers come in contact with snakes during their farming engagements.

Due to high cost of hospital treatment and unavailability of antivenins, most often, the rural people find it more convenient to consult native doctors who are acclaimed for curing snake bite patients. There are also many anecdotal evidences which indicate that plant remedies used by native doctors are effective in curing snake bites, and there appears to be a high rate of survival among snake bite patients in advanced clinical stages of venom toxicity (Moses, 2005). In Nigeria, nearly all plants are associated with some medicinal value. The use of plants especially in traditional medicine is currently well acknowledged and accepted in Nigerian health care practice (Hassan and Kamba, 2010). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity and reduced the risk of using them. It facilitates pharmacological studies and leads to the synthesis of pure and potent compounds with decreased toxicity (Hassan and Kamba, 2010).

This research work attempted to determine the anti-snake venom potentials of *S. longepedunculata* on the venom of *Naja nigricollis* using aqueous induced rats as experimental models.

1.1 Research Question

Does the plant; *S. longepedunculata* have anti snake venom potential against the venom of the Spitting Cobra (*Naja nigricollis*)?

Null hypothesis (Ho): The plant *S. longepedunculata* has anti snake venom potential against the venom of the Spitting Cobra (*Naja nigricollis*)

Alternate hypothesis (Ha): The plant *S. longepedunculata* have no anti snake venom potential against the venom of the Spitting Cobra (*Naja nigricollis*)

Rejection rule: Ho is rejected if calculated λ^2 is $\geq \lambda^2$, α (0.05) and DF (1)

Chi square (λ^2) will be calculated using the formula, $\lambda^2 = \frac{(\{ad-bc\}-n/2)^2 n}{(a+b)\,(c+d)\,(a+c)\,(b+d)}$

II. Materials And Methods

2.1 Collection and Identification of Plant

Fresh root bark of *Securidaca longepedunculata* was collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State Nigeria, located on latitude $7^0$29'08"N and longitude $12^0$31'68"E.

The climate is characterized as having a cool dry (harmattan) season from December to February; a hot dry season from March to May; a warm wet season from June to September; a less marked season after rains during the months of October to November, characterized by decreasing rainfall and a gradual lowering of temperature.

The plant was identified by the officer in charge of the Herbarium where the Voucher specimen was preserved in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Samples deposited at the Herbarium have a Voucher No. D-01SL-7.

2.2 Processing of Plant Material

Fresh plant materials were properly washed under tap water, rinsed with distilled water, dried under shade and pulverized with a pestle and mortar and kept in a transparent sterile polythene bag at room temperature for use. Forty gram (40g) of root bark was soaked in 500ml of distilled water in 1000ml conical flask for 24 hours (Lorke, 1983); the extracts were filtered with muslin cloth in to a beaker of 1000ml.

2.3 Testing for Toxicity of the Plant Extract

Ten gram (10g) of leaves and root were soaked in 100ml of distilled water and 5g of each leaves and roots were also soaked for 24 hours (Lorke, 1983), the extracts were filtered with muslin cloth in to conical flask. Four groups of six rats each were used to test for toxicity of the plant. Twenty four (24) hours later numbers of rat that died and survived was recorded. The extracts were administered orally at the dose of, 200mg/kg, and 300mg/kg body weight (Lakshmi and Vadivu, 2010).

2.4 Collection of Snake Venom

The lyophilized snake venom was obtained from traditional snake catcher Baba Mai Maciji of the Department of Biological Sciences Zoology Unit, Usmanu Danfodiyo University Sokoto. The venom of *Naja nigricollis* was milked by holding the head of the snake over a snake chilled beaker cover with the sheet of polythene. The snake was pressured to strike the polythene and penetrate it with it fans until it releases the venom in to the container (Haruna and Choudhury, 1995). The venom was preserved in a sterilized sample bottle at 4^oC.

2.5 Experimental Animals

Wister albino rats male and female were divided into five groups of six rats each in a Complete Randomized Design (CRD).

Group 1- Group were injected with venom only

Group2- Group were injected with Snake Venom and treated with leaves extract

Group3- Group were injected with Snake Venom and treated with root bark extract

Group4- Group were injected with snake venom and treated with root bark extract and leaves extracts

Group5- Group were injected with snake venom and treated with anti-snake venom

Rats were injected subcutaneously in the right hind paw with venom (0.2ml body weight), thirty minutes (30) later the rats was treated with plant extracts. The extract was administered orally using canola syringe at the dose of 200mg/kg, and 300mg/kg respectively of the body weight of the rats as shown by Wannang *et al*. (2005).

The extract was administered orally using canola syringe at the dose of 200 mg/kg distilled and 300mg/kg dissolved in 10mls of distilled water respectively of the body weight of the rat, thirty minutes (30) later the animal were injected subcutaneously in the right hind paw with venom (0.2ml body weight) as described by Tanko *et al*. (2011).

III. Statistical Data Analysis

The parameters derived in the study were subjected to statistical analysis. Statistical significance was determined using Chi square for testing association in 2 x 2 contingency table at 95% CI and allowable error of 0.05.

IV. Results

4.1 Toxicity Test of Plant

Table 1 shows the toxicity test of the plant extract at two different concentrations of 200 and 300 mg/ml body weight. All the rats given the crude extracts of Leaves and Roots bark of *S. longepedunculata* at different concentrations were able to survive without any sign of weakness or illness within 24 hours (Table 1).

Table 1. Toxicity Test of the Plant Extracts

Plant part	Doses mg/kg	No. Died	No. Survived	% Died	% Survived
Leaves	0.2	0	3	0	100
	0.3	0	3	0	100
Root bark	0.2	0	3	0	100
	0.3	0	3	0	100
Root+Leaves	0.2	0	3	0	100
	0.3	0	3	0	100
	0.2	0	3	0	100
Total		0	24	0	100

4.2 In vivo Antisnake Venom activity of *S. longepedunculata*

4.2.1 Anti venom activity of *S. longepedunculata*

Table 1 had indicated the effect of plant extracts on *Naja nigricollis* Venom at 2 different concentrations of leaf and root bark extract, the result showed that the root bark extract has higher anti venom activity of 100% survival at 300mg/ml and the leaf extract recorded lower anti venom activity of 0 % at 200mg/ml survival and 33.33% of mortality at 300mg/ml. In the combined root bark and leaves extract it shows higher activity of 66.67% survival at 300mg/ml and 33.33% mortality at 200mg/ml (Table 2)

Table 2. Antisnake Venom Activity of *S. longepedunculata*

Plant Extract	Doses mg/kg	Venom (ml)	No. Died	No. Survived	% Died	% Survived
Leaf	0.2	0.2	3	0	100	0
	0.3	0.2	2	1	66.67	33.33
Root	0.2	0.2	1	2	33.33	66.67
	0.3	0.2	0	3	0	100
Root + Leaf	0.2	0.2	1	2	33.33	66.67
	0.3	0.2	1	2	33.33	66.67
Antivenom	0.2	0.2	1	2	33.33	66.67
	0.3	0.2	0	3	0	100
Snake venom	00	0.2	3	0	100	0

The value of λ^2 is 0.280 which is less than λ^2, $P < 0.05$ and DF (1); 3.84

Hence, the null hypothesis (Ho) is accepted: The plant *S. longepedunculata* has anti snake venom potential against the venom of the Spitting Cobra (*Naja nigricollis*). Number of survived and mortality of the rat models treated with untreated ones of extracts (Table 3).

Table 3. Testing statistical association of Anti-snake Venom potential of *S. longepedunculata*

Treatment	No. Survived	No. Died	Total
Treated with plant Extract	8	7	15
Not treated with plant Extract	5	4	9
Total	15	11	24

V. Discussion

The in vivo activity of the root bark extract of *Securidaca longepedunculata* at 200mg/kg increased the survival rate of the envenomed rats at $P<0.05$ which was comparable to that of standard anti-venom. This might be due to the toxicity which may be incorporated with some anticoagulant substances in the extract of the plant parts used. Nahed *et al*. (2011) reported that in a daily orally treated envenomed rats with *Ambrosia maritima* extract (100 mg/kg b.wt.) following injection with 1/3 LD_{50}, 2/3 LD_{50} or LD_{50} of the venom, rats showed normal ileal histological structures, but slight erosion of few villi after 45 days of treatment. Robaszkiewicz *et al*. (2007) proved the anti oxidative effects of quercetin flavonoid (the well-known phenolic compound widely present in compositae plants) on the cells of in vitro culture at low concentrations.

It was reported in this research that snake bite is an important cause of morbidity and mortality and is one of the devastating health problems in the study area. The results of the study however, suggested that *Naja nigricollis* venom can disturb rat metabolism. The study showed that the extract of *S. longepedunculata* was effective in neutralizing lethal effects of *Naja nigricollis* venom in rat animals. Fatani *et al*. (2006) proved the protective effects of the antioxidant *Ginkgo biloba* extract against *Leiurus quinquestriatus* venom which induced tissue damage in rats. This report is in line with the work of Goje *et al*. (2013) who noticed that aqueous extract of *Boswellia dalzielli* stem bark concentration of 400mg/kg at 0.1mg/kg for 24 hours on the parameters of hepatic and energy metabolism of *Naja nigricollis* 15 envenomed albino rats was shown to yield 100% survivals with significant ($p\leq 0.05$) anti snake venom activity.

It was also observed in this study that the survival of the rats increased (66.7-100%) progressively with the increasing dose of the extracts in a dose dependent manner. The progressive increase in the dosage (0.2-0.3 mg/kg) of the extracts and antivenom applied respectively might be responsible for the less number of mortality of the envenomed rats.

It has also been established in this study that the water extracts of *S. longepedunculata* has potent snake venom neutralizing capacity against venom of *N. nigricollis*. This could be explained by the fact that the combined root bark and leaves extract might possess a toxic potential or activity that acted against the snake venom, which yielded highest (66.67%) number of survival at 300mg/ml and 33.33% mortality at 200mg/ml.

This is in agreement with the study conducted by Ahmed *et al*. (2010) to determine anti-snake venom activity of different extracts of *S. longepedunculata* against Russel viper venom.

The results revealed that the ethanol and aqueous extracts were found to possess most significant activity. The separate leaves extract that are not in combination root bark had 100% rate mortality at 0.2 mg/kg, but at 0.3mg/kg had 66.67%. This might be due to increased metabolic action by the envenomed rats in trying to mobilize and resist energy in order to cope up with the cytotoxicity of the *N. nigriccollis* venom. This disagrees with Omale *et al*. (2013) who used methanol extracts of *Uvaria chamae* leaves at 0.08mg/kg body weight of male albino rats intraperitoneally and found up to 100% anti venom activity in the animal models.

Therefore, the root bark and leaves extract of *S. longepedunculata* can be said to possess an anti snake venom activity thereby neutralizing the toxic effects of *N. nigricollis* venom. The potent snake venom neutralizing capacity this extract could potentially be used for therapeutic purpose in case of snake bite envenomation.

VI. Conclusion

Neutralization of the *S. longepedunculata* extracts was checked for *Naja nigrocollis* venom. The in vitro and in vivo activities was shown to determine the anti venom potential of the plant extracts using two dose levels and it has indicated significant neutralization, hence root bark extracts at 300mg/ml has shown a good anti venom activity. Isolation, identification, characterization of this extract should be carried out in order to elucidate the structure of the bioactive compounds and mechanism of action of *S. longepedunculata*, with need to enlighten pharmacists can develop formulations against snake bites.

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