Antivenom activities of methanolic leaf extract of Solanum dasyphyllum *Schum & Thonn against Naja nigricollis venom* mice-induced envenomation

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARF designed and conducted the study under the supervision of authors GA and HZ. Author AR wrote the protocol, the first draft of the manuscript and managed the literature searches. All authors were involved in proofreading and approving the manuscript.

Abstract

Solanum dasyphyllum is a plant spicy often employed in ethnomedicine for the treatment of tooth pain, poisons and snakebite. The aim of this study is to evacuate the effect of methanolic leaf extract of *S. dasyphyllum* on toxicity induced by *Naja nigricollis* snake venom. There was a reduction in the mortality of albino mice treated with *S. dasyphyllum* after intra-peritoneal (i.p) administration of reconstituted venom when compared to those challenged with the venom only. Maximum protection was observed in animals exposed to the venom incubated with the different concentrations of the extract. The results suggest that the methanolic leaf extract of *S. dasyphyllum* contains bioactive constituents with significant antivenom activity both *in-vitro* and *in-vivo* and lends credence to traditional use of the plant in the management of snakebite.

Keywords: Snakebite, envenomation, *Naja nigricollis*, *Solanum dasyphyllum*, detoxification, induction, toxicity.

Introduction

Snakebite envenomation is a common neglected global health problem with frequently devastating consequences. It is an environmental and occupational disease that affects mostly people in rural communities of developing countries, especially the tropics, such as Africa, Asia and Latin America [9]. Snakebite in Africa causes thousands of deaths annually and considerable permanent physical disability. The incidence of snake bite in rural West Africa was estimated to be as high as 174 per 100,000 population, with an 11–17% mortality rate [5]. Nigeria is reported to have one fifth of all West African region snake bite cases, with 174 cases in every 100,000 hospital admissions [13]. The spitting cobra (*Naja nigricollis*) is the commonest and most widely distributed African cobra and is indeed a familiar snake in Nigerian states within the savannah terrain, where it is usually colored black, dark brown, or steel gray with pink or reddish throat bars [2]. The average length of spitting cobra (*Naja nigricollis*) is 117 cm [16].

Anti-venom immunotherapy is the only treatment available against snake envenomations. However, it is associated with many side effects which include; anaphylactic shock, pyrogen reaction and serum sickness [10]. In addition to the side effects, sub-Saharan Africa is deficient in the quality, quantity, specificity, access, and distribution of antivenoms, which significantly increases morbidity and mortality burden [7].

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times [15]. In recent years, the subject of plants used to treat snakebite has attracted the attention of several researchers. Plants and their extracts have been used for the treatment of snake bite in most areas where venomous species are endemic [1]. *Solanum dasyphyllum* belong

to the family of Solanaceae. It is an erect, perennial herb with stems that are often woody, growing 50 - 100cm tall. The plant is branched at the base, the branches heavily armed with prickles 2 - 7mm long. *S. dasyphyllum* is used ethnomedically to treat poisons. The plant is reported to possess anticonvulsant and neuromuscular properties [11]. In the South Western part of Nigeria, the fruit of S. dasyphyllum mixed with local black soap is usually applied to incisions at sites of snakebites, presumably to remove venom from bite site and reduce its absorption into the systemic circulation [1]. However, the potentials of *Solanum dasyphyllum* to treat snakebites have not been subjected to scientific evaluation; therefore, the aim of this study therefore is to carry out an in vivo assessment of the anti-snake venom neutralizing potentials of *Solanum dasyphyllum* methanolic leaf extract.

MATERIALS AND METHODS

Collection, Identification and Extraction of the Plant Sample

Fresh leaves of *S. dasyphyllum* were collected from Odeomu town in Ayedaade Local Government Area, Osun state, Nigeria. The plant was authenticated at the herbarium of Department of Botany, Obafemi Awolowo University, Ile-ife, Osun state, where a voucher specimen number IFE-17489 was deposited. The leaves were cleaned, dried and pulverized into powder. The powdered leaf (500 g) was soaked in 250mls of 95% methanol for 72hrs with constant stirring. The extract was filtered successively using muslin cloth and Whatman No1 filter paper. The filtrate was concentrated using a rotary evaporator, and lyophilizing to dryness.

Laboratory Animals

Albino rats (100-150g) and mice (20-40g) of both sexes were purchased from the Animal House of the Faculty of veterinary medicine, University of Maiduguri, Maiduguri, Borno state, Nigeria

and were kept at the department of Biochemistry Animal House. The animals were allowed to acclimatize for two weeks in the Animal House prior to commencement of the experiment. The animals were housed in wired cages and fed on standard rat pellets while water was provided ad libitum. Animals were handled in adherence to standard animal ethical protocols.

Venom Sample

Lyophilized *N. nigricollis* (Cobra) venom was procured from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria, and was preserved at 4°C.

Preparation of stock venom of *N. nigricollis*

25mg of *Naja nigricollis* venom was dissolved in 10ml of normal saline solution. 4.35ml was measured out into 10ml flask and used to prepare 1.00mg/ml stock solution.

Determination of the acute toxicity of methanolic leaf extract of *Solanum dasyphyllum*

The acute toxicity of methanolic leaf extract of *Solanum dasyphyllum* was carried out using Wistar strain albino rats by the method described by Lorke (1983). In the initial phase, 9 rats were randomly divided into three groups of three rat each. Groups I, II and III were given 10, 100 and 1000 mg/kg of the extract orally. In the second phase, three rats were divided at random into three groups of one rat each. Rats I, II and III were given 1600, 2900 and 5000 mg/kg of the extract, respectively. The rats were observed over a period of 24 hours for signs of toxicity and mortality.

Determination of venom minimum lethal dose (LD_{99})

The LD_{99} of *N. nigricollis* venom was determined in mice according to Theakson and Reid method (1983). Thirty mice were randomly allocated into 5 groups of 6 mice each (n = 6). Group I serve as the control where mice were injected with normal saline (0.2 ml each i.p). Mice in

groups II, III, IV and V were given the reconstituted venom at 5, 6, 8 and 10 mg/kg, respectively through the intraperitoneal route. The time of death was recorded over a period of 24 hours from venom administration. The LD_{99} value (minimum lethal dose, MLD) was determined by probit analysis.

Neutralization effect of methanolic leaf extract of *S. dasyphyllum* against *N. nigricollis* venom in induced mice

Age-matched albino mice of both sexes weighing (25-30g) were used and distributed into 5 groups (n=6). Group I and II served as the positive (normal saline only) and negative (venom only) control respectively. Group III-V were administered 100, 200 and 400mg/kg doses of the methanolic leaf extract of *S, dasyphyllum* via oral route prior to administration of 0.2mls of venom intraperitoneally. All animals were observed for mortality for 24 hrs.

In-vivo* snake venom detoxifying effect of methanolic leaf extract of *Solanum dasyphyllum

Age-matched albino mice of both sexes weighing (25-30) were used and distributed into 5 groups (n=6). Group I served as normal (normal saline only) control. Group II was administered with 0.2ml of the venom intraperitoneally, followed by treatment with polyvalent antivenom after 5minutes. Group III-V were administered with 0.2ml of venom intraperitoneally, followed by administration of 100, 200 and 400mg/kg doses of the methanolic leaf extract of *S, dasyphyllum* via I.M route. All animals were observed for mortality for 24 hrs.

***S*nake venom detoxifying effect of *S. dasyphyllum* methanolic extract**

Age-match albino mice of both sexes weighing (25-30)g were used and distributed into 4 groups (n=6). The different groups were assigned as below:

Group I: Control (Normal saline)

Group II: Venom + 100mg/kg *Solanum dasyphyllum* extract

Group III: Venom +200mg/kg *Solanum dasyphyllum* extract

Group IV: Venom + 400mg/kg *Solanum dasyphyllum* extract

Groups 2, 3 and 4 were given an equivalent of the MLD of the venom containing 100, 200, and 400 mg /kg extract, respectively. The venom and the extract were incubated at 37°C for 10 min and 0.2 ml of the incubated mixture was injected (i.p) into each animal. All animals were observed for mortality for 24 hrs.

Results

The results of phase I and II of Acute toxicity of *S. dasyphyllum* in Table 1a and 1b revealed that at high dosage, the plant extract can induce toxicity signs such as jacking and restlessness. However, the animals recover within 24hours.

Table 1: Determination of Acute Toxicity of Methanolic leaf Extract of *Solanum dasyphyllum*

Table 1a: Phase 1

Dose (mg/kg)	Volume	No. of death/no of rat	Survival %	Sign of toxicity
10	0.2	0/3	100	-
100	0.2	0/3	100	-
1000	0.2	1/3	66.7	+

Table 1b: Phase II

Dose (mg/kg)	Volume	No. of death/no of rat	Survival %	Sign of toxicity
1500	0.2	0/1	100	++
2600	0.2	0/1	100	++
5000	0.2	0/1	100	++

Key: + =Degree of toxicity

The LD_{99} of the *Naja nigricollis* venom was estimated to be 7.57mg/kg as summarized in table 2, while Figure 1 represents the probit graph.

Table 2: Minimum Lethal Dose (LD_{99}) of venoms of *Naja nigricollis* venom in mice

Group	dose (mg/kg)	Ln dose	No of animals	No of dead animals	% mortality	Probit
1	Control	0	0	0	-	-
2	5	0.698	8	6	75	5.670
3	6	0.778	8	7	87.5	6.155
4	8	0.903	8	8	100	8.1214
5	10	1	8	8	100	8.1214

Minimum Lethal Dose (LD_{99}) = 7.57mg/kg

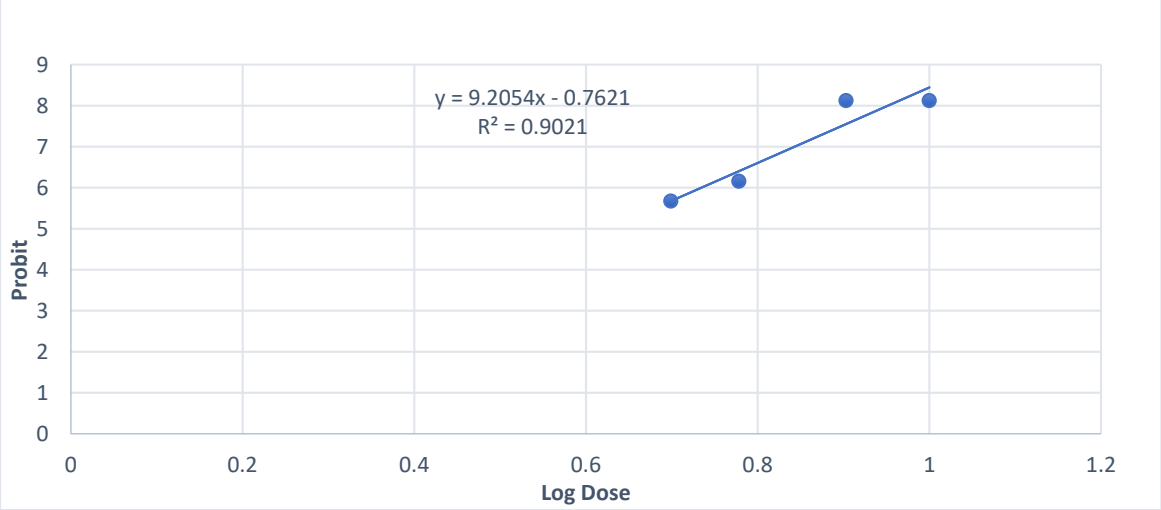

Fig 1: Graph of probit against Log dose

Tables 3, 4 and 5 summarized the results of the neutralization effect of treatment of mice with different doses of extract of plant 1 hour before inoculation of snake venom, *In-vivo* snake venom detoxifying effect of *S. dasyphyllum* methanolic extract and Snake venom detoxifying effect of *S. dasyphyllum* methanolic extract. Different doses of the extract alleviated the toxic signs and improve survival rate.

Table 3: Neutralization effect of treatment of mice with different concentrations of extract of plant 1 hour before inoculation of snake venom

Group	Dose	Number of survival/ total number of animals	% mortality	toxicity sign
Group 1	Normal saline only	6/6	0	-
Group 2:	Venom only	0/6	100	+++
Group 2	MLD venom+ 100mg/kg of extract	1/6	83.3	++
Group 3	MLD venom+ 200mg/kg of extract	1/6	`83.3	++
Group 4	MLD venom+ 400mg/kg of extract	0/6	100	+++

Key: MLD= Minimum Lethal Dose

Table 4: *In-vivo* snake venom detoxifying effect of *S. dasyphyllum* methanolic extract

Group	Dose	Number of survival/ total number of animals	% mortality
Group 1	Normal saline only	6/6	0
Group 2	MLD +100mg/kg of extract	4/6	33.3
Group 3	MLD + 200mg/kg of extract	3/6	50
Group 4	MLD + 400mg/kg of extract	3/6	50

MLD= Minimum Lethal dose of the venom

Table 5: Snake venom detoxifying effect of *S. dasyphyllum* methanolic extract

Group	Dose	Number of survival/ total number of animals	% mortality
Group 1	Normal saline only	6/6	0
Group 2	Venom only	0/6	100
Group 3	MLD +100mg/kg of extract	6/6	0
Group 4	MLD + 200mg/kg of extract	6/6	0
Group 5	MLD + 400mg/kg of extract	5/6	16.6

MLD= Minimum Lethal dose of the venom (LD_{99}).

Discussion

The phase I and phase II of the acute toxicity study of the *Solanum dasyphyllum* leaf extract on rats is presented in table 1a and 1b. The results showed that no mortality was recorded at various doses used in phase I and phase II except 1 death at 1000mg/kg, which maybe not be due to the plant extract. The rats administered with 10mg/kg and 100mg/kg did not exhibit any toxicity symptoms. However, those that were administered 1000 and above exhibited signs of toxicity. Toxic effects evoked by the rats are restlessness and jacking. The plant is thought to be safe as suggested by lorke [8] since the LD_{50} exceed 500mg/kg b.w. Also, the absent of significant death among the rats within 72hrs seems to support this claim.

The minimum lethal dose (LD_{99}) of *N. nigricollis* venom was estimated to be 7.57mg/kg by probit analysis as presented in table 2 and fig 1 respectively. The LD_{99} obtained from is study was lower than that reported by Abubakar *et al* [3] (9.7mg/kg) and higher than the 6mg/kg reported in Nusuka, Nigeria by Ode and Asuzu [12]. The difference in minimum lethal dose might be explained by venom variability composition due to a number of factors such as geographic location, season, age and diet.

N. nigricollis envenomation is characterized by a progressive, neuromuscular paralysis, leading to respiratory failure and death [14]. The efficacy of antivenom is due to the ability of antivenom molecules to binds with toxins in the venom, and the most widely used method for antivenom efficacy testing is rodent lethality testing [4].

Many plant extracts have been reported to possess detoxifying and neutralization effects on snake venoms (Abubakar *et al*., 2000). Several investigators have reported naturally occurring

substances such as cinnamic acid derivatives; polyphenolic compounds, sitosterol, tannin, flavonoids and pentacyclic terpenes have protein- binding and enzyme-inhibiting properties [6]. The neutralization and detoxifying effect of *S. dasyphyllum* on *N. nigricollis* venom in mice is presented in tables 3, 4, and 5 respectively. The alleviation of toxic symptoms and survival of experimentally protected laboratory animals (within 24 h after being challenged with lethal venom doses) are used to infer the antivenin property.

In table 3, a total of 24 mice were used and all of them survive when exposed to the plant extract before inoculation with snake venom. Mice treated with various dose of methanolic leaf extract of *Solanum dasyphyllum* one (1) hour prior to inoculation with MLD of venom showed excitement followed by depression, paralysis and death. These are classical symptoms of neurotoxicity. However, the mortality recorded was very high, but the time of death was delayed when compared with the positive control.

Table 4 and 5 presented the *In-vivo* and snake venom detoxifying effect of *S. dasyphyllum* methanolic extract. The survival rate of envenomed mice was remarkably increased when treated with different concentrations of methanolic leaf extract of *Solanum dasyphyllum* (p.o) as compared with the control group (venom only) as shown in table 4. In the entire group apart from the control, all the animal survival percentage is up to 50%.

In table 5, the co-administration of MLD of *N. nigricollis* venom and the various concentrations of the methanolic leaf extract of *S. dasyphyllum* significantly increased the survival of the animals. All the animals survive the inoculation for a period of 24hours, except in group 5, where one death is recorded.

Conclusion

Solanum dasyphyllum possesses bioactive ingredients that have the potential to be used in the management of *Naja nigricollis* envenomation, thereby rationalizing its ethnomedicinal use in the treatment of poison and snake bites.

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