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Antivenom Potentials of Some Local Medicinal Plants Against *Naja nigricollis* Associated Envenoming in North-Western Nigeria

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ABSTRACT

Background and Objective: Snakebite and its envenomation is still a vital cause of mortality especially among rural dwellers in underdeveloped countries, this phenomenon is categorized under the first class of neglected public health problem by WHO. Black-necked spitting cobra (*Naja nigricollis*), is among the important species associated with snakebite cases in Northern-Western part of Nigeria. This research work was aimed at evaluating the intraperitoneal lethal doses (50 and 100%) of *Naja nigricollis* venom and its neutralization potentials by some medicinal plants used in Kebbi State, Nigeria. **Materials and Methods:** The snake species was captured with the help of snake charmers and was duly authenticated by a zoologist. The venoms were milked and their lethal doses were determined using Probit analysis, all the plants used in this study were extracted with methanol. while the Antivenom effect of the medicinal plants was screened against venom-induced lethal effects in albino rats using standard methods. **Results:** The lethal doses, 50% (LD_{50}) and 100% (LD_{100}) of *Naja nigricollis* venom were determined to be 0.380 and 4.270 mg/kg b.wt., respectively. All the selected medicinal plant extracts presented antivenom activities at different degrees of efficacy against the venom of the *Naja nigricollis* with no significant difference ($p>0.05$) compared to the normal and positive controls. *Faidherbia albida* (Delile) A. Chev root extract revealed a significant ($p<0.05$) decrease in the mean survival time of the animals compared to normal and positive controls. **Conclusion:** The findings of this study suggest that these medicinal plants have potent antivenom potentials and thus can serve as alternatives for the treatment of snakebite envenoming involving *Naja nigricollis*.

KEYWORDS

Naja nigricollis, snakebite, medicinal plants, antivenom, envenoming

INTRODUCTION

Snakebite and its envenomation is still a vital cause of mortality especially among rural dwellers in underdeveloped countries, this prodigy is classified under class one of abandoned global health issues by the World Health Organization (WHO)[1]. Although, the data available in epidemiology statistics for

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occurrence and death as a result of snakebite incidence are said to be underestimated, this is due to inadequate computerized based and instant research software and tools needed in Asia and Sub-Saharan Africa where snakebite incident is supreme[2]. Another reason for the perceived low data estimation record of snakebite injuries, especially in Africa, is limited attendance to health facilities by most of the snakebite victims and this practice is against the WHO guidelines on first aid treatment of snakebite for Africa[3].

In Nigeria, especially in rural areas where even if there are available health care facilities provision of standard antivenins is limited[4]. Sani *et al*.[5] reported an annual incidence of snakebite of 497/100,000 and a mortality of 12.2% in Savannah Region of Northern Nigeria. A greater percentage of snakebite victims do not attend hospital. In Northern Nigeria, it was estimated that only 8.5% of snakebite victims attend hospitals, the low patronage of hospital by snakebite victims is due to the believe that it is not a hospitalized condition, some have a superstition believe of death if they go to the hospital, amputation fear and cost antevenoms[6].

The cobras originated from Africa and belong to the family Elapidae, this snake species is the most regular snake associated with snakebite cases in hospitals[7]. Generally, these spitting cobras have active venom that causes ulceration and necrosis within the bite site, accompanied by systemic neurotoxic effects. Hospital records revealed that the black-necked spitting cobra (*Naja nigricollis*) is the most urbanized and clinically essential snake in Northern Nigeria[8].

Medicinal plants have been used in traditional medicine for the treatment of snake bites many years back. Rural dwellers rely on medicinal plants to cure diseases primarily due to their safety, effectiveness, cultural preferences, inexpensive nature and accessibility. The indigenous systems of medicine use medicinal plants for the treatment of snake bites. There is a huge repository of evidence from herbalists to cure fatal snake injuries with the use of indigenous medicinal plants. Hence this study is designed to investigate the antivenom efficacies of some medicinal plants used in treating snakebite injuries against *Naja nigricollis* Reinhardt in Kebbi State, Nigeria.

MATERIALS AND METHODS

Study area: The research was conducted from December, 2023 to July, 2024 within Aliero Town, Nigeria. It was performed in Biochemistry Research Laboratory, Department of Biochemistry, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.

Experimental animals: Adult Wister albino rats of both sexes aged 4-5 months and weighing between 120-150 g were used for the experiments. They were purchased from National Veterinary Research Institute, Vom, Nigeria and kept under standard laboratory conditions (22-24°C; 12:12 hrs dark/light cycle). The animals were allowed free access to both food (commercial rodent pellets) and water ad libitum; they were allowed to acclimatize for 2 weeks. The weight of each rat was taken before the commencement of the experiment. All animal experiments were conducted in accordance with the guidelines for the use and care of experimental animals[9].

Ethical consideration: This research was conducted in accordance with guidelines governing the conduct of research involving animals in Kebbi State University of Science and Technology, Aliero, Nigeria, after ethical approval was obtained from the University Research Ethics Committee.

Standard snake venom antiserum (antivenin): The lyophilized polyvalent snake venom antiserum (Batch No.: 8904012480039, Manufacture Date: November, 2022, Expiry. Date: October, 2026) was used as a standard to compare with the efficacy of the plant extract. It was produced by a standard pharmaceutical company (Bharat serums and Vaccines Limited, India).

***Naja nigricollis* reinhardt:** After capturing the snake species (*Naja nigricollis* Reinhardt), it was housed in a wooden cage with the help of a snake charmer. Furthermore, it was identified accordingly by a zoologist in the Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero, Nigeria. The snake venom was milked and used for the experiments.

Milking of venom: The snake venom was milked in a low light condition at a temperature of 25°C according to the method of Goswami *et al.*[10] Using a halothane (a short acting general anesthesia); (Piramal Healthcare Limited, U.K.). The venom was collected by pressing the glands below the eyes of the snake into a clean and sterilized container.

Preparation of venom: Immediately after milking, a freeze-dryer (Millrock Technology, USA) was used to lyophilize the venom and further kept inside a refrigerator (HR135A, Haier-Thermocool, Lagos, Nigeria) in a light resistant and air-tight container. Prior to use, the lyophilized venom was reconstituted in 0.9% normal saline (regarded as the venom) and kept at a temperature of 4°C. The venom concentration was expressed as dry weight in (mg/mL)[11].

Determination of venom lethal doses, 50% (LD_{50}) and 100% (LD_{100}): The lethal doses of the venom were determined using a modified method of Theakston and Reid[12]. The 20 rats were randomly distributed into 5 groups of 4 rats each. The venom was reconstituted in normal saline and was administered intraperitoneally (IP) as follows:

Group 1	Served as normal control and were administered with normal saline (i.p.)
Group 2	Were injected (i.p.) with the venom at the dose of 1.0 mg/kg b.wt.,
Group 3	Were injected (i.p.) with the venom at the dose of 2.0 mg/kg b.wt.,
Group 4	Were injected (i.p.) with the venom at the dose of 3.0 mg/kg b.wt.,
Group 5	Were injected (i.p.) with the venom at the dose of 4.0 mg/kg b.wt.,

Mortality was recorded within 24 hrs of venom administration and the lethal doses (LD_{50} and LD_{100}) were estimated using probit analysis[12-14]

Collection and authentication of the plants material: *Mitragyna inermis* (Wild) Kuntze root, *Sclerocarya birrea* (A. Rich.) Hochst leaves, *Sclerocarya birrea* (A. Rich) Hochst root, *Ficus platyphylla* delile stembark, *Faidherbia albida* (Delile) A. Chev root, *Catunaregam nilotica* (Stapf) Tirveng root and *Crinum ornatum* (Aiton) Herb. bud was collected within Aliero town, Kebbi State, Nigeria. The plants were then authenticated at the herbarium of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero and voucher specimen for *Mitragyna inermis* (Wild) Kuntze Root [KSUSTA/PSB/H/VOUCHER NO: S.N], *Sclerocarya birrea* (A. Rich.) [KSUSTA/PSB/H/VOUCHER No: 114A], *Ficus Platyphylla* Delile Stembark [KSUSTA/PSB/H/VOUCHER NO: SN], *Faidherbia albida* (Delile) A.Chev [KSUSTA/PSB/H/VOUCHER NO: 319], *Catunaregam nilotica* (Stapf) tirveng [KSUSTA/PSB/H/VOUCHER NO: SN] and *Crinum ornatum* (Aiton) Herb. [KSUSTA/PSB/H/VOUCHER NO: SN] were deposited in the herbarium.

Preparation of plants methanol extracts: The plant extracts was prepared according to a modified method of Dupont *et al.*[15]. The collected plant part was washed with clean water and air-dried under shade, pulverized using pestle and mortar. The 100 g each of the powdered plant part was measured and soaked in 100 mL of 99% methanol. The mixture was then kept at room temperature for 24 hrs and filtered twice; initially with a muslin cloth and later with a Whatman filter paper No.1. The filtrate was evaporated to dryness at 45°C using rotary evaporator (NYC USA R-205D).

Antivenom activity screening of the plants methanol extracts: The 35 albino rats were randomly distributed into 7 groups of 5 rats each and venom inducement and extract treatment were conducted as follows:

Groups	Treatment
Group 1	Received orally with only distilled water and served as normal control
Group 2	Were injected intraperitoneally (i.p.) only with LD_{100} of the snake venom and served as venom control
Group 3	Were injected (i.p.) with the LD_{100} of the snake venom, then after 30 min, they were administered intravenously (i.v.) with the standard conventional serum antivenin at the dose of 1 ml/0.6 mg venom and served as standard control
Group 4	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Mitragyna inermis* (Wild.) Kuntze root 300 mg/kg b.wt.
Group 5	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Sclerocarya birrea* (A.Rich.) Hochst leaf 300 mg/kg b.wt.
Group 6	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Sclerocarya birrea* (A.Rich.) Hochst root 300 mg/kg b.wt.
Group 7	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Ficus platyphylla* Delile Stembark 300 mg/kg b.wt.
Group 8	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Faidherbia albida* (Delile) A.Chev root 300 mg/kg b.wt.
Group 9	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Catunaregam nilotica* (Stapf) Tirveng root 300 mg/kg b.wt.
Group 10	Injected (i.p.) with the LD_{100} of the snake venom, then after 30 min treated with *Crinum ornatum* (Aiton) Herb. bud 300 mg/kg b.wt.

All the groups received the same volume of preparations. In all the groups, the duration of survival and the number of rats that survived were recorded for 24 hrs[14]

Statistical analysis: The data generated from the study are presented as Mean±SEM and subjected to One-way Analysis of Variance (ANOVA) and statistical differences between the means were separated using new Duncan's multiple range t-test at $p<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20).

RESULTS

Lethal profile of *Naja nigricollis* venom: The lethality data of the *Naja nigricollis* venom was presented in Table 1. The LD_{50} (median lethal dose) and LD_{100} of the venom were calculated using a probit curve (Fig. 1) and were determined to be 0.380 and 4.27 mg/kg b.wt., respectively.

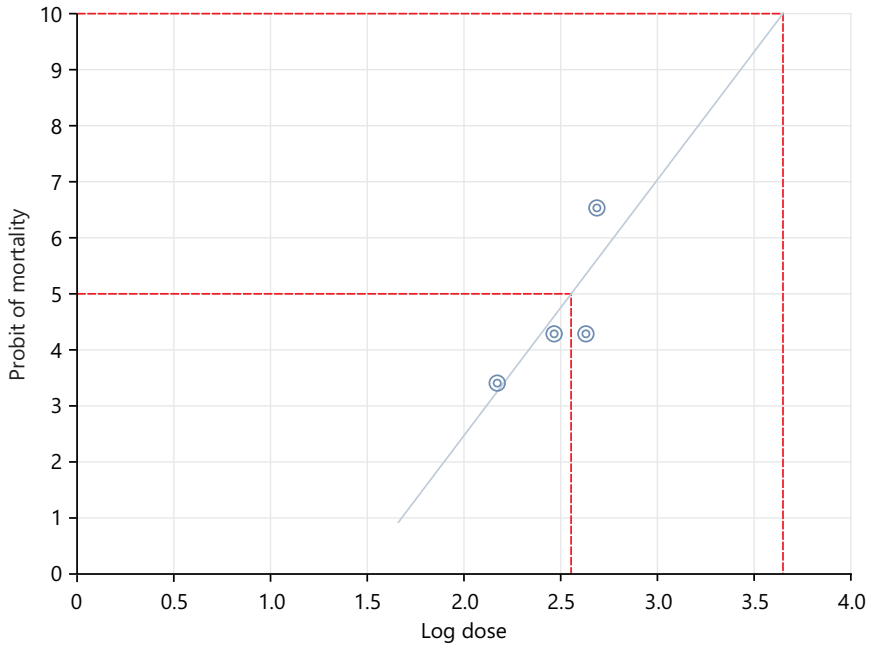

Fig. 1: Probit curve for *Naja nigricollis* venom lethal doses (LD_{50} and LD_{100})

LD_{50}: Antilog of 2.58 = 380.19 µg/kg b.wt., = 0.380 mg/kg b.wt. and LD_{100}: Antilog of 3.63 = 4265.795 µg/kg b.wt., = 4.27 mg/kg b.wt.

Table 1: Lethal profile of *Naja nigricollis* venom

Average animal weight (g)	Venom dose (mg/kg b.wt.)	Average dose of venom Administered (µg/kg b.wt.)	Log dose	Number of death/ rats used	Death (%)	*Corrected (%)	Probit of mortality
153.53	-	-	-	0/4	0	0	-
148.43	1.00	148.4	2.17	0/4	0	*6.25	3.45
150.18	2.00	300	2.47	1/4	25	25	4.33
139.38	3.00	418.1	2.62	1/4	25	25	4.33
152.3	4.00	479.5	2.68	4/4	100	*93.75	6.55

*Corrected formula: 0% death = 100 (0.25/n), 100% death = (n-0.25/n)

Table 2: Anti-venom effect of the selected medicinal plant extracts on *Naja nigricollis* venom LD_{100}

Treatment	Treatment (dose)	Survival/ number of animal used	Survival (%)	Mean survival time
Normal control	Normal saline (0.5 cmlIP)	4/4	100	24.00 ± 0.00^{c}
Negative control	-	0/4	0	2.73 ± 0.93^{a}
Positive control (standard polyvalent anti-venom)	1 ml/0.6 mg venom	4/4	100	24.00 ± 0.00^{c}
Mitragyna inermis (Wild.) Kuntze root methanol extract	300 (mg/kg b.wt.)	2/4	50	15.33 ± 5.46^{abc}
Sclerocarya birrea (A.Rich.) Hochst leaves methanol extract	300 (mg/kg b.wt.)	3/4	75	18.31 ± 5.69^{bc}
Sclerocarya birrea (A.Rich.) Hochst root methanol extract	300 (mg/kg b.wt.)	4/4	100	24.00 ± 0.00^{c}
Ficus platyphylla Delile stembark methanol extract	300 (mg/kg b.wt.)	2/4	50	13.85 ± 5.95^{abc}
Faidherbia albida (Delile) A.Chev root methanol extract	300 (mg/kg b.wt.)	1/4	25	9.47 ± 5.02^{ab}
Catunaregam nilotica (Stapf) Tirveng root methanol extract	300 (mg/kg b.wt.)	2/4	50	15.30 ± 5.51^{abc}
Crinum ornatum (Aiton) Herb. Bud methanol extract	300 (mg/kg b.wt.)	2/4	50	16.42 ± 4.51^{bc}

Values are presented as Mean±SEM (n = 4), Value having similar alphabetical superscripts are not significantly different at (p>0.05) analyzed using One-way Analysis of Variance (ANOVA), followed by Duncan's multiple comparison t-test with SPSS version 20.0

Neutralization effect of the selected medicinal plants against the *Naja nigricollis* venom: The neutralization activity of some medicinal plants against *Naja nigricollis* is presented in Table 2. The result revealed non-significant ($p>0.05$) differences in the mean survival time of *Mitragyna inermis* (Wild.) Kuntze root, *Sclerocarya birrea* (A.Rich.) Hochst leaves, *Sclerocarya birrea* (A.Rich.) Hochst root, *Catunaregam nilotica* (Stapf) Tirveng root and *Crinum ornatum* (Aiton) Herb. bud methanol extracts compared to both normal and positive control. While *F. albida* root revealed a significant ($p<0.05$) decrease in mean survival time compared to both normal and positive control.

DISCUSSION

The present study revealed intraperitoneal (IP) LD_{50} of *N. nigricollis* venom to be 0.380 mg/kg b.wt. This is lower than the IP lethal dose concentration (LD_{50}) of *N. nigricollis* venom was 1.0 mg/kg b.wt., reported by Bala *et al.*[16] and higher than 0.341 mg/kg b.wt., as reported by Abd El-Aziz *et al.*[17]. According to Massey *et al.*[18] venom constituents differ widely between species and even within the same species. Other factors, such as environmental conditions, age, sex, or type of prey available, can also affect venom composition[19]. Therefore, the differences in the LD_{50} observed in the present study might be attributed to the aforementioned factors.

This research documented seven medicinal plants (*Mitragyna inermis*(Wild.) kuntze root, *Sclerocarya birrea* (A.Rich.) hochst leaves, *Sclerocarya birrea* (A.Rich.) hochst root, *Ficus platyphylla* delile stembark, *Faidherbia albida* (delile) A.chev, *Catunaregam nilotica* (Stapf) Tirveng root and *Crinum ornatum* (Aiton) herb. bud)

with a potent anti-venom effect against *N. nigricollis* these findings support the claims that pharmacological studies of some plants used in folkloric medicine can antagonize the activity of various venoms and neutralize their toxins[20]. The venom-neutralizing potentials observed in this study might be due to the formation of Antigen-antibody complex as suggested by Ledsgaard *et al*.[21] who reported the formation of Antigen-antibody complex as mechanism through which the toxins of snake venoms are neutralized by antivenins. Phytochemical components of *Mitragyna inermis*, *Sclerocarya birrea*, *Ficus platyphylla*, *Faidherbia albida*, *Catunaregam nilotica* and *Crinum ornatum* have been established in several researches and these include tannins, saponins, alkaloids, phenols, flavonoids, anthraquinones, cardiac glycosides and steroids[22-27].

Secondary metabolites from medicinal plants such as flavonoids, polyphenols, saponins, tannins, terpenoids, xanthenes, quinonoids, steroids and alkaloids have been reported to make a complexes with toxic proteins of snake venoms, thereby neutralizing their toxicity[28]. Similarly, plant metabolites (flavonoids, terpenoids, tannins, polyphenols, vitamins A, C, E and minerals such as selenium) inhibit oxidative stress resulting from venom phospholipase A_2 activity through active sites binding or modifying the enzyme structure hence altering its catalytic activity, this is described as antioxidant potentials[29]. The antivenin activity observed in the present study might be due to the presence of these pharmacologically active phytochemicals in the plant extracts.

CONCLUSION

The findings of this study document the lethality profile of *Naja nigricollis* venom and also disclose that the medicinal plants *Mitragyna inermis*, *Sclerocarya birrea*, *Ficus platyphylla*, *Faidherbia albida*, *Catunaregam nilotica* and *Crinum ornatum* used in this study exhibit potent antivenom potential. The present research provides valid scientific explanations on the use of medicinal plants in curing snake-bite envenoming and thus further studies towards isolation and synthesis can serve as a guide in developing plant-derived potent conventional antivenoms.

SIGNIFICANCE STATEMENT

The plant extracts are potential antidotes against *Naja nigricollis* venom. The research shows that, the plants can serve as a lead for the development of safe, readily available and affordable antivenoms. These plants if used as local first aid for victims of snakebite can lead to a significant decrease in the morbidity and mortality due to snakebite involving *Naja nigricollis*. Hence, there is need to further isolate and identify the active component(s) with the antivenom activity and find a suitable formulation of the active component that can be readily available for use by the victims of snakebite.

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