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Lethal Doses of *Bitis arietans* Venom and Antivenom Potentials of Some Medicinal Plants Found in Gwandu Emirate, Kebbi State, Nigeria

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ABSTRACT

Background and Objective: Venomous snakes pose severe health problems, especially for the rural dwellers in the tropical regions of developing countries, in Nigeria alone, snakebite injuries lead to 10,000 deaths annually and over 65% of the mortality cases result from the venomous viper species. This study aimed to assess the intraperitoneal lethal doses (50 and 100%) of *Bitis arietans* venom and investigate the antivenom potential of various medicinal plants commonly used in Gwandu Emirate, Kebbi State, Nigeria. **Materials and Methods:** The snake species was captured with the assistance of local snake charmers and authenticated by a zoologist. Venom was extracted and lethal doses were determined through Probit analysis. All plant materials were extracted using methanol. The antivenom activity of the plants was tested against venom-induced lethality in albino rats following standard procedures. Data are presented as Mean±SEM and analyzed using one-way ANOVA, with mean differences separated by New Duncan's Multiple Range Test at $p<0.05$. **Results:** The 50% lethal dose (LD50) and 100% lethal dose (LD100) of *B. arietans* venom were found to be 0.33 and 5.25 mg/kg body weight, respectively. The antivenom activity of *Mitragyna inermis* (Wild.) Kuntze root, *Sclerocarya birrea* (A. Rich.) Hochst root, *Ficus platyphylla* Delile stembark and *Faidherbia albida* (Delile) A. Chev root methanol extracts on the *Bitis arietans* venom revealed non-significant ($p>0.05$) differences in the animals mean survival time compared to both normal and positive (standard) controls. The activities of the *Sclerocarya birrea* (A. Rich.) Hochst leaves, *Catunaregam nilotica* (Stapf) Tirveng root and *Catunaregam nilotica* (Stapf) Tirveng bud extracts caused significant ($p<0.05$) decrease in the animals mean survival time compared to both normal and positive controls. **Conclusion:** Hence, the findings of this study documented the lethality of *Bitis arietans* venom and also disclosed the antivenom potentials of the tested medicinal plant extracts.

KEYWORDS

Lethal doses, *Bitis arietans*, venom, antivenom, medicinal plants, Gwandu Emirate, Kebbi State

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INTRODUCTION

Venomous snakes present serious health risks, particularly for rural populations in the tropical regions of developing countries. Despite considerable efforts to manage snake envenoming effectively, the World Health Organization (WHO) still classifies snakebite as a neglected tropical disease. This highlights the need for more comprehensive and improved strategies to address the life-threatening challenges posed by this issue. Snakebites remain one of the major health hazards in the Savanna Region of West Africa[1-3].

In Nigeria, the Viper family, notably *Bitis arietans* (Puff adder) is among the snakes with the highest incidence of morbidity and mortality and is classified as the most dangerous venomous snake species[4]. The exact fatality index resulting from snakebite by these vipers remains unknown due to limited epidemiological data, as most of the victims are rural dwellers and have limited access to healthcare facilities and therefore depend on herbal medicinal alternatives for treatments. Reports from community-based surveys suggest a higher envenomation incidence than the hospital-based estimates[5]. According to the World Health Organization (WHO), approximately 5.4 million snakebite incidents occur globally each year, resulting in 2.7 million envenomings, 138,000 deaths and 400,000 cases of disability. In Nigeria, snakebite injuries cause an estimated 10,000 deaths annually, with venomous viper species accounting for over 66% of these fatalities[6].

Many years ago, medicinal plants were used in traditional medicine for the treatment of snake bite injuries[7]. Indigenous medicinal plant species have been incorporated into several recent conventional drug formulations and preparations for fundamental human diseases[8]. Preliminary investigations on the potentials of some plants as antidotes against snake bites have been promising; this is achieved due to the presence of several pharmacologically active phytochemicals[9]. However, the present study seeks to document the lethal profile and validate the antivenom potentials of some medicinal plants used in treating snakebites incidence resulting from *Bitis arietans* in Kebbi State, Nigeria.

MATERIALS AND METHODS

Study area: The research was conducted within Aliero town, Nigeria, between January to October, 2024. 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 was used for the experiments. They were purchased from the 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 per the guidelines for the use and care of experimental animals[10].

Ethical consideration: This research was conducted per guidelines governing the conduct of research involving animals in Kebbi State University of Science and Technology, Aliero, Nigeria, after a Research Ethics Clearance Certificate was obtained from the University Research Ethics Committee with reference number; KSUSTA/DVC-R&I/RECC/003.

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).

***Bitis arietans*:** The snake species (*Bitis arietans*) used was captured and housed in a wooden cage with the help of a snake charmer. After collection, it was duly identified by a Zoologist in the Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero, Nigeria. Its venom was milked and used for the experiments.

Milking of venom: The venom was collected between the hours of 17.30-18.00, in a low light condition at an ambient temperature according to the method of Goswami *et al.*[11] by using short-acting general anesthesia; halothane (Piramal Healthcare Limited, UK). The glands below the eyes of the snake were compressed to release the stored venom into a clean and sterilized container.

Preparation of venom: After milking, the venom was lyophilized using a freeze-dryer (Millrock Technology, USA) and kept inside a refrigerator (HR135A, Haier-Thermocool, Lagos, Nigeria) in a light-resistant and air-tight container. Before use, the lyophilized venom was reconstituted in 0.9% saline (regarded as the venom) and kept at 4°C. The venom concentration was expressed in terms of dry weight (mg/mL)[12].

Determination of venom lethal doses, 50% (LD_{50}) and 100% (LD_{100}): Twenty rats were randomly distributed into five groups of four 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:** Injected (i.p.) with the venom at the dose of 1.0 mg/kg b.wt.
- **Group 3:** Injected (i.p.) with the venom at the dose of 2.0 mg/kg b.wt.
- **Group 4:** Injected (i.p.) with the venom at the dose of 3.0 mg/kg b.wt.
- **Group 5:** 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[13].

Collection and authentication of the plant materials: *Mitragyna inermis* (Wild) Kuntze Root, *Sclerocarya birrea* (A. Rich.) Hochst leaves, *Sclerocarya birrea* (A. Rich) Hochst Root, *F. 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 *M. inermis* (Wild) Kuntze Root [KSUSTA/PSB/H/VOUCHER No: S.N], *Sclerocarya birrea* (A. Rich.) [KSUSTA/PSB/H/VOUCHER No: 114A], *F. 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] was deposited in the herbarium.

Preparation of plants methanol extracts: The plant extracts were prepared according to a modified method of Dupont *et al.*[14]. The collected plant parts were washed with clean water, air-dried under shade and pulverized using a pestle and mortar. One hundred grams of each of the powdered plants' parts 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 a rotary evaporator.

Antivenom activity screening of the plant's methanol extracts: Forty albino rats were randomly distributed into 10 groups of 4 rats each. Venom inducement and extract treatment were conducted as follows:

Groups	Treatments
Group 1	Received orally with only distilled water and served as normal control
Group 2	Injected intraperitoneally (i.p.) only with LD_{100} of the snake venom and served as venom control.
Group 3	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/0.45 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 and died were recorded for 24 hrs[15,16].

For:

$$0\%\ \text{death} = 100\left(\frac{0.25}{n}\right)$$

$$100\%\ \text{death} = \left(\frac{n-0.25}{n}\right)$$

$$n = \text{Number}$$

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 Test at $p<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20).

RESULTS

The lethality data of the *Bitis arietans* venom is presented in Table 1. The lethality of *Bitis arietans* venom increased with dosage, with no deaths observed at 1 mg/kg (0% mortality) but reaching 100% mortality at 4 mg/kg. The corrected probit mortality ranged from 4.33 (1/4 deaths) to 6.55 (4/4 deaths), highlighting a dose-dependent increase in venom lethality.

And the LD_{50} (Median Lethal Dose) and LD_{100} (Cabsolute Lethal Dose) of the venom were calculated using a probit curve (Fig. 1) and were evaluated to be 0.33 and 5.25 mg/kg b.wt., respectively.

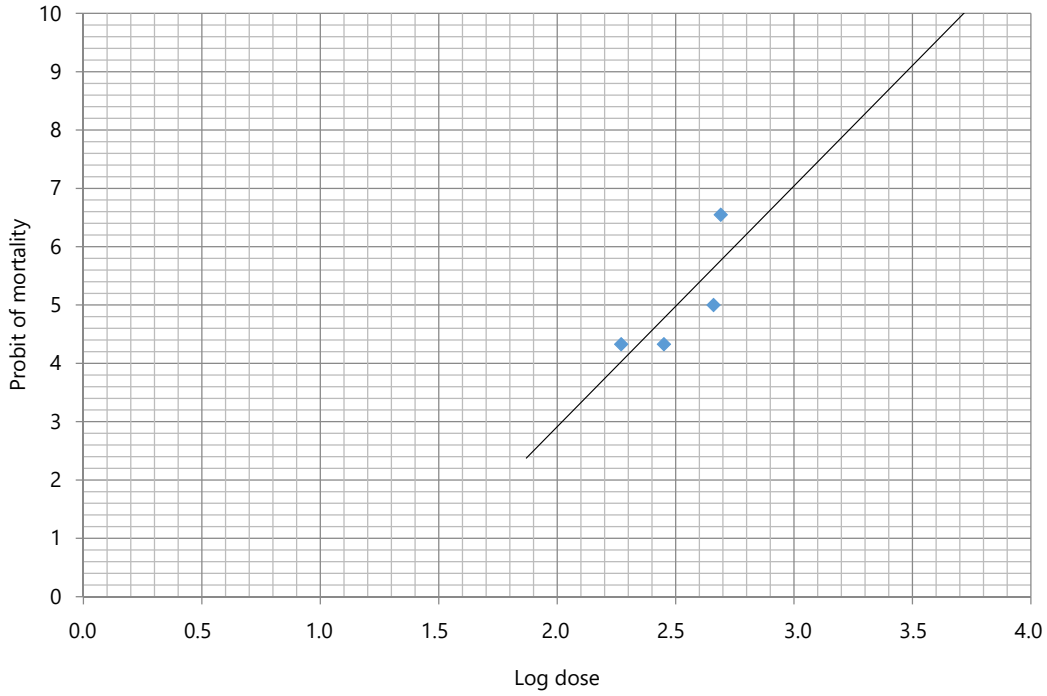

Fig. 1: Probit curve for *Bitis arietans* venom lethal doses (LD_{50} and LD_{100})

LD_{50} = Antilog of 2.52 = 331.13 µg/kg body weight = 0.33 mg/kg body weight. LD_{100} = Antilog of 3.72 =5248.07 µg/kg body weight = 5.25 mg/kg body weight

Table 1: Lethal profile of *Bitis arietans* venom

Group	Average animal weight (g)	Venom dose (mg/kg b.wt.)	Average dose of venom administered (µg/kg b.wt.)	No. of death/ No. of rats used	Log dose	Death (%)	Corrected (%)*	Probit of mortality
1	153.53	-	-	-	0/4	0	0	-
2	186.63	1.00	185.97	2.27	1/4	25	25	4.33
3	139.732	2.00	278.65	2.45	1/4	25	25	4.33
4	150.6	3.00	451.88	2.66	2/4	50	50	5.00
5	121.67	4.00	486.70	2.69	4/4	100	*93.75	6.55

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

Table 2: Antivenom activities of the plant extracts on the *Bitis arietans* venom LD_{100}

Groups	Treatment	Treatment (dose)	Survival (%)/ No. of animal used	Mean survival time
1	Normal control	Normal saline (0.5 mL IP)	100	24.00 ± 0.00^{c}
2	Negative control	-	0	2.75 ± 0.19^{a}
3	Positive control (Standard Polyvalent Anti-Venom)	1 mL/0.45 mg Venom	100	24.00 ± 0.00^{c}
4	*Mitragyna inermis* (Wild.) Kuntze root methanol extract	300 (mg/kg b.wt.)	50	15.07 ± 5.16^{bc}
5	*Sclerocarya birrea* (A.Rich.) Hochst leaves methanol extract	300 (mg/kg b.wt.)	0	5.33 ± 1.04^{ab}
6	*Sclerocarya birrea* (A.Rich.) Hochst root methanol extract	300 (mg/kg b.wt.)	50	14.90 ± 5.27^{bc}
7	*Ficus platyphylla* Delile Stembark Methanol Extract	300 (mg/kg b.wt.)	50	14.95 ± 5.24^{bc}
8	*Faidherbia albida* (Delile) A.Chev root methanol extract	300 (mg/kg b.wt.)	50	18.50 ± 4.34^{c}
9	*Catunaregam nilotica* (Stapf) Tirveng root methanol extract	300 (mg/kg b.wt.)	0	5.40 ± 1.21^{ab}
10	*Crinum ornatum* (Aiton) Herb. Bud methanol extract	300 (mg/kg b.wt.)	0	5.69 ± 1.27^{ab}

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 ANOVA and followed by Duncan multiple comparison test with SPSS version 20.0

Neutralization effect of some selected medicinal plants against *Bitis arietans* venom: The neutralization activity of some medicinal plants against *Bitis arietans* is shown 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 root, *Ficus platyphylla* Delile stembark and *Faidherbia albida* (Delile) A.Chev root methanol extracts compared to both normal and positive control. While *Sclerocarya birrea* (A.Rich.) Hochst leaves, *Catunaregam nilotica* (Stapf) Tirveng root and *Crinum ornatum* (Aiton) Herb. bud revealed a significant ($p<0.05$) decrease in mean survival time compared to both normal and positive control.

DISCUSSION

The present study established intraperitoneal (IP) lethal doses LD_{50} and LD_{100} of *Bitis arietans* venom of 0.33 and 5.25 mg/kg b.wt., respectively. The LD_{50} obtained in the present study is lower compared to the reports of Ajisebiola *et al*.[17], who document 0.5 mg/kg b.wt. and Oukkache *et al*.[18], who reported LD_{50} of 0.87 mg/kg b.wt. (IP route) in their studies, respectively. It is reported that geographical inhabitation of snakes, diet type and age contribute to the variability of the venom composition[19]. *Bitis arietans*, a species of viper, possesses cytotoxic and hemotoxic venom characterized by the presence of various enzymes, predominantly Snake Venom Metalloproteinases (SVMPs). These enzymes play a crucial role in causing rapid hemorrhagic effects both locally and systemically following envenomation.

Phytochemicals, including alkaloids, flavonoids, saponins and steroids, have been widely recognized for their potential to neutralize snake venoms. For instance, atropine, an alkaloid from the *Solanaceae* family, has been shown to counteract the venoms of black and green mambas (*Dendroaspis polylepis* and *D. angusticeps*, respectively). This effect is achieved by blocking cholinergic receptors, which likely reduces the impact of neurotransmitters released at cholinergic nerve terminals by these venoms.

Additionally, PLA2 inhibitors, such as AIPLAI extracted from various plants, effectively inhibit the phospholipase A2 enzymes found in cobra and viper venoms in a dose-dependent manner. Another alkaloid, aristolochic acid (AA), identified as 8-methoxy-6-nitrophenanthro (3,4-d-1,3-dioxole-5-carboxylic acid), is found in certain plants and has been shown to suppress the enzymatic and pharmacological activities of basic PLA2 enzymes from *Vipera russelli* venom[19-22].

Steroids form complexes such as Sitosterol and stigmasterols are active against different snake venoms. These phytochemicals also show inhibitory potential against PLA2 and L-amino acid oxidases of Naja naja venom. The high affinity binding between β-sitosterol and enzymes was investigated by using molecular docking studies[23].

Flavonoids are among the key plant-based compounds known to inhibit enzymes such as PLA2 and lipoxygenase. They exhibit diverse biological activities, including anti-inflammatory, hepatoprotective, antihypertensive, antiarrhythmic, hypocholesterolemic, antiallergic, antitumor and antioxidant properties. For example, the flavonoid rutin has been shown to have a mild inhibitory effect on group I PLA2 from *Naja naja* while strongly inhibiting group II PLA2. Additionally, it has demonstrated the ability to counteract enzymatic, myotoxic, edema-inducing and anticoagulant effects caused by venom PLA2[21].

Terpenes, another significant group of plant compounds, possess antivenom properties, including anti-proteolytic and anti-hemorrhagic effects. These compounds can neutralize hemorrhagic, fibrinogenolytic and caseinolytic activities of P-I and P-III class metalloproteinases found in the venom of various *Bitis* species. Some terpenes have also been reported to reduce edema caused by crude venoms, metalloproteases and both basic and acidic PLA2s. The antivenom activity observed in this study may be attributed to the presence of these phytochemicals in the plant extracts, as supported by previous studies by researchers[24-26].

For instance, Toklo *et al*.[27] reported that extensive phytochemical analysis revealed that *M. inermis* contains polyphenols, triterpenoids, indole and oxindole and alkaloids. *Sclerocarya birrea* was reported to contain polyphenols, tannins, coumarins, flavonoids, triterpenoids and phytosterols. The phytochemical study from previous research has confirmed the presence of flavonoids, tannins and saponins in *Ficus platyphylla* stem bark[28,29]. According to Oni *et al*.[30], *Faidherbia Albida* contains flavonoids, gallic tannins, anthocyanins, leucoanthocyanins, saponins, mucilage, coumarins and the reducing compounds.

CONCLUSION

This study demonstrates the significant lethality of *Bitis arietans* venom, with LD_{50} and LD_{100} values of 0.33 and 5.25 mg/kg, respectively. While the methanol extracts of several medicinal plants, including *Mitragyna inermis*, *Sclerocarya birrea*, *Ficus platyphylla* and *Faidherbia albida*, exhibited antivenom potential, their effects on mean survival time were generally non-significant ($p>0.05$). In contrast, extracts of *Sclerocarya birrea* leaves and *Catunaregam nilotica* roots and buds significantly ($p<0.05$) decreased survival time. These findings suggest that these plants hold potential for further exploration in developing effective antivenom therapies.

SIGNIFICANCE STATEMENT

Snakebite leads to morbidities and mortalities as a neglected health problem in Nigeria, especially in rural areas where modern health facilities are very poor or not available completely. The screened plant extracts demonstrated degrees of efficacies against the venom of the *Bitis arietans*. Hence, this research proved that plants can serve as lead for the development of safe, readily available and affordable plant-based antivenoms. Additionally, these plants if used as local first aid for victims of snakebite (involving *Bitis arietans*) can lead to a significant decrease in the morbidity and mortality due to the snakebite.

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