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DETERMINATION OF MEDIAN LETHAL DOSE (LD50) OF *BITIS ARIETANS* VENOM AND ANTILETHALITY OF ADANSONIA DIGITATA AND *SECURIDACA LONGEPEDUNCULATA* LEAVES EXTRACTS

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ABSTRACT

Traditional medicine had become the oldest form of health care system in many countries of the world including Nigeria. The present study aimed at documenting ethnomedicinal plants for the treatment of snakebites in Katsina State, Nigeria. The target group of the population includes herbalists and snakebite victims; ethnobotanical information was obtained through an oral interview using a semi-structured questionnaire. A total of 300 respondents were interviewed in this study. The respondents exposed the use of 25 medicinal plants for the treatment of snakebites in the study area. The most commonly used plant family includes *Adansonia digitata* and *Securidaca longepedunculata*. Leaves were the most frequently reported plant parts by the respondents. The methods of preparation were usually by decoction or pounding the plants part into powder. Administration of the preparations is usually via topical (51%), topical/oral (26%) and oral (23%) routes. Preference ranking in this study revealed that *Adansonia digitata* and *Securidaca longepedunculata* were effective in neutralizing the venom of *Bitis arientans* this validates the claim of the traditional medical practictioners.

Keywords: Medicinal plants, Snakebites, Anti snake venom, *Bitis arietans, Adansonia digitata, Securidaca longepedunculata*

INTRODUCTION

Traditional medicine also known as alternative, complementary or ethnic medicine is the oldest form of healthcare system in the world and is used in the prevention and treatment of physical and mental illnesses (Yuan and Piao, 2016) within local and regional healing practices. Indeed, it has been well studied and documented in many countries of the world including Nigeria. Traditional medicine encompasses the utilization of substances (plants, animals and mineral elements), dosages and practices based on social cultural norms and religious beliefs as well as experiences and observation of specific group mainly traditional healers and herbalists. The knowledge of traditional medicine is handed down from generation to generation in oral or written form (Inngjerdinge *et al.,* 2004).

Snakebites cause considerable morbidity and mortality worldwide with the highest burden found in South Asia and Sub-Saharan Africa (Aguiyi *et al.,* 2018). The annual incidence of snakebites worldwide is about 5 million with about 100, 000 to 200, 000 deaths (Aguiyi, 2018; Musa *et al*., 2017). The annual snakebite incidence in savannah region of northern Nigeria has been estimated to be 497 per 100,000 populations, with 12.2% Mortality due mainly to the carpet viper, *Naja nigricollis* (Ameh *et al*., 2019). In the recent past, studies indicate that the situation has not improved as the incidence of snakebite worldwide has been reported to be in excess of 3,000,000 per year with more than 150,000 deaths (Mustapha, 2003). In another report the incidence of snakebite is still high; with an estimated 10,000 deaths occurring every year (Mustapha, 2003; Musa *et al*., 2019).

Taraba State alone, has an annual incidence of 40.4 bites per 100,000, which is one of the highest in the country (Ameh *et al*.,2019). In addition to the deaths, there are an estimated 400, 000 snakebite-related amputations each year around the world (Abubakar *et al.,* 2010) with associated tetanus cases. Analysis of mortality data suggests that snakebite contributes to 35% of all child deaths, globally (Snow *et al.,* 2014). This study investigated the herbal therapy used by the inhabitants of Katsina state for the treatment of snakebites envenomation.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Katsina state northern Nigeria. Majority of the people living in the study area belong to the Hausa ethnic group. Six local governments (Bakori, Kafur, Dutsin-Ma, Safana, Jibia and Sandamu) were selected two from each senatorial zone out of the 34 Local Governments that make up Katsina State.

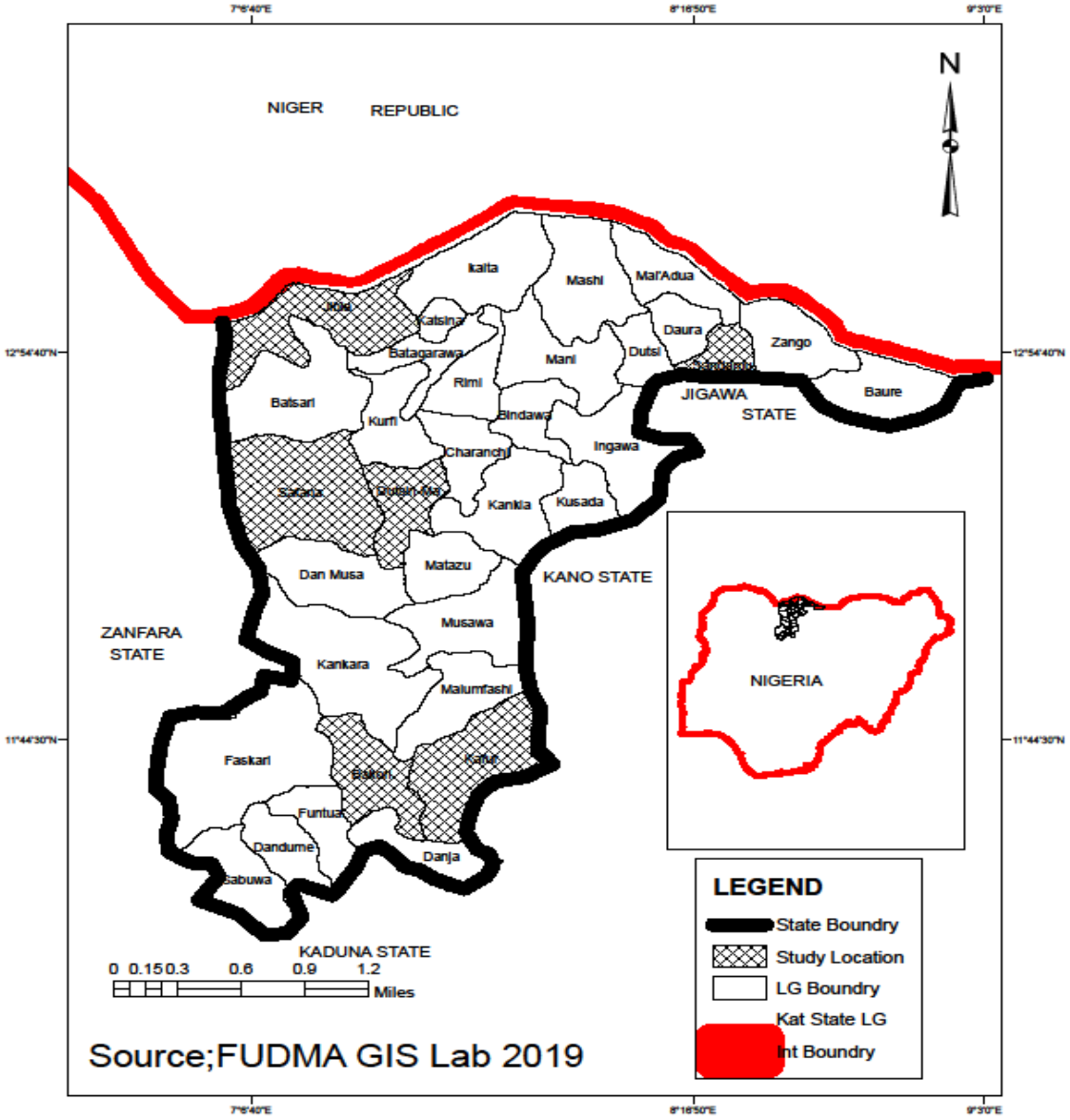

Figure 1: Map showing study area and sample locations

Study groups

Katsina state as many other northern states are known to have high number of traditional herbalists who provide health care services as their main source of income. The herbalists and victims of snakebite form the target groups of the population for the ethnobotanical survey. The study design was a cross-sectional survey and systematic random sampling was used to select the target groups.

Identification of target groups

Locations of knowledgeable herbalists from the study area were identified by local administrators

Informed consent

Respondents were contacted verbally and their consent to participate in the research was sought prior to the scheduled interview (Tugume *et al*., 2016).

Ethnobotanical survey

The survey was conducted between June and December 2019 using a semi-structured interview in Hausa language. The semi-structured questionnaire was utilized to document information given by the respondents. The questionnaire consists of demographic data, knowledge of snakebites and information of the plants used which includes local plant names, plant part use, methods of preparation and mode of administration. A total of 300 respondents were consulted with certain incentives given to some of the traditional herbalists to stimulate their participation in the research. Studies in Africa have resorted to rewarding the informants for their time and information (Gbolade, 2009).

Collection of Plant Materials

Plant samples were collected from the wild using a Soccateurs, plant parts were collected and transported to the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto for identification, and voucher specimens were prepared and deposited in the Herbarium. Collected plants parts were washed under a running tap to remove dirt, and were shade-dried in the Laboratory. Dried plant parts were pulverized into powder and kept in air-tight containers until required. The powdered materials were sieved, labeled and stored at 4°C.

Extraction of Plant Materials

The stored plant powder was weighed using an electric weighing balance out of which 20g of the plant powder was soaked in Macerating bottles containing 60mls of ethanol for 48 hours, the filtrate was collected, drained and filtered using Whatman no.1 filter paper. In the second phase, 1:2 of the solvent (40mls) was soaked for 24 hours filtered and filtrate collected and transferred into an evaporating dish and placed in an oven at 40^0C for 2-3 days (Verpoorte, 2019).

Collection of Experimental Animals

Swiss albino mice (20) were purchased from the animal house of the Pharmacology and Toxicology Department of ABU Zaria and kept in wooden cages, allowed food and water *Ad libitum* and allowed to acclimatize for two weeks before the experiment.

Collection and Milking of Snakes (source of Venom)

Live snakes were caught (with the help of a snake handler) from the fringes of Saran Makama forest and was taken to the Department of Pharmacology and Toxicology ABU Zaria for identification. Identified as Puff adder (*Bitis arietans*) and kept in the Herpeterium. Milking of the snake venom was done as described by Markfalane (1985). Milked venom was crystallized in a dessicator containing silica gel which absorbs all the moisture in the venom leaving behind crystallized venom which was stored at -18^0C until required

Determination of the Median Lethal Dose LD50 of the Snake Venom (*Bitis arietans*)

The median lethal dose of the snake venom was obtained using the OECD Guideline 425 using the Up and down method

Oral Acute Toxicity Study: Experimental Design

The acute oral toxicity study was conducted in compliance with OECD guideline 425, which stipulate the use of only three animals (Sankhari *et al*., 2010; Tugume, *et al*., 2016 Al Ghasham *et al.*, 2017). Three of the test animals were fasted overnight (~12 h) and weighed and administered via oral gavage at 2000 mg/kg. The animals were regularly and individually observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days (Nana *et al.*, 2010).

Data Analysis

Probit Analysis was used to determine the LD50 of the snake venom as well as the ED50 of the selected plants.

RESULTS AND DISCUSSION

Determination of Median Lethal Dose (LD50) of *Bitis arietans* Venom and Antilethality of *Securidaca longepedunculata*

The assessment of snake venom median lethal doses (LD50) is an important step for an accurate evaluation of the toxic activity of specific venom, and is also regularly used to select the relevant anti-venom batch, as well as to establish the neutralizing capacity of each vial. According to the WHO, venom lethality is expressed as median lethal dose (LD50). Result revealed 0.166mg/kg as the LD50 value that is required to kill 50% of the mice. This finding is similar to that of Fingesi et al. (2018) who reported that the LD50 of *Bitis arietans* is 0.0156mg/kg. the lethality of the snake venom varies according to the environmental conditions of the place the snake is sourced not necessarily the size, age or colour of the snakes especially of members of the same species. The determination of the LD50 is a crucial stage in the develo pment of critical antivenin in clinical trials. Albino mice were injected intraperitoneally with the 2XLD50 of *B. arietans* venom followed (after 5minutes) by varying concentrations of methanolic extract of the selected plants and observed for a period of twenty four hours (24hrs) for mortality rate as presented in table 2. Results revealed 204mg/kg, as the Median Effective Dose of the plant. For the two plants following the Venom pre-treated approach.

Results revealed 161mg/kg, and 204mg/kg for *A.digitata, and S.longipedunculata*, ED50 of the plants using the venom/extract approach as presented in Table 2 and 3. Result also revealed 180 and 270mg/kg as the as the ED50 of *A.digitata and S.longipedunculata*, plants using the extract pretreated approach as presented in Table 2 and 3. This findings is similar to that of Ameh et al. (2019) who reported that the root extracts of *Leptadenia hastata* has effectively neutralized the venom of *Echis occelatus*, they reported that 80% of the envenomed albino rats were saved from the effect of *Echis occelatus* venom. *E. occelatus* is considered the most clinically important snake in Nigeria followed by Bitis *arietans* and *Naja nigricollis*. Plants contains Secondary metabolits which are often indicted for the ability of these plants to neutralize the cocktail of protein related venom of snakes. Similarly, Musa et al. (2017). Reported that *Annona senegalensis* has the ability toneitralize the effect of snake venoms.

Table 1: Determination of Median Lethal Dose (LD50) of *Bitis arietans* venom

Venom conc. (mg/ml)	No. of mice(18-20g)	Mortality (%)
1	6	6(100)
0.5	6	6(100)
0.25	6	4(66.7)
0.125	6	3(50)
0.0625	6	1(16.7)

Table 2: Medium Effective Dose of *Adansonia digitata* Methanolic Extract Against Envenomed Albino Mice

Conc.(mg/ml)	N	Mortality (%)		
		Venom pre-treated	Venom/extract Pre-treated	Extract Pre-treated
0(control)	8	8(100)	8(100)	8(100)
50	8	6(75)	5(62.5)	7(87.5)
100	8	5(62.5)	3(37.5)	5(62.5)
200	8	4(50)	2(25)	3(37.5)
400	8	2(25)	0(0)	1(12.5)

N= Number of mice used per each group. Values in parenthesis are mortality rate

Table 3: Medium Effective Dose of *Securidaca longepedunculata* Extract against Envenomed Albino Rats

Conc.(mg/ml)	N	Mortality (%)		
		Venom pre-treated	Venom/extract Pre-treated	Extract Pre-treated
0(control)	8	8(100)	8(100)	8(100)
50	8	6(75)	5(62.5)	7(87.5)
100	8	5(62.5)	3(37.5)	5(62.5)
200	8	4(50)	1(12.5)	4(50.0)
400	8	2(25)	0(0.0)	1(12.5)

N= Number of mice used per each group. Values in parenthesis are mortality rate

CONCLUSION

This study reveals that the use of medicinal plants for the treatment of snakebites by traditional medicine practitioners is common in Katsina State. Twenty five medicinal plants were reported to be used in the treatment of snakebite envenomation. However, further pharmacological and phytochemical studies need to be carried out to prove the efficacy of the plants.

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