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## EFFICACY OF *SECURIDACA LONGEPEDUNCULATA* ON THE PARAMETERS OF BLOOD GLUCOSE LEVEL AND PULSE RATE OF ENVENOMED ALBINO RATS

J. Sanusi<sup>1</sup>, J.A. Bawa<sup>\*2</sup>, I.S. Aghemwenhio<sup>3</sup>, Z.S. Rabi'u<sup>4</sup>, M.G. Sani<sup>5</sup> and S. Liadi<sup>6</sup>

Biology Department<sup>1,6</sup>, Isa Kaita College of Education Dutsin-ma, Katsina State Nigeria.

Department of Biological Sciences<sup>2,3</sup>, Federal University Dutsinma, P.M.B. 5001, Dutsin-ma Katsina State Nigeria.

Department of Biological Sciences<sup>4</sup>, Federal University Gusau, Zamfara State Nigeria.

Biology Department<sup>5</sup>, Zamfara State College of Education Maru, Zamfara Nigeria.

### Keywords:

Aqueous extract, Root bark, *Securidaca longepedunculata*, Snake venom, Blood glucose, Pulse rate

### Correspondence to Author:

**J. A. Bawa**


(Lecturer) Department of Biological Sciences, Federal University Dutsin-Ma, P.M.B. 5001, Dutsin-Ma Katsina State, Nigeria.

**E-mail:** bawa51@yahoo.com

**ABSTRACT:** The study was undertaken to investigate the in vivo activity of aqueous and ethanol extracts of Violet plant, *Securidaca longepedunculata* leaves and root bark against snake venom of *Naja nigricollis* and blood glucose level along with pulse rate in experimental albino rats. Healthy adult albino rats weighing 250-300g were used and randomly divided into five groups for in vivo anti-snake venom activity. Extracts were prepared using two hundred grams (200g) from the dried plant material in 1000 ml of ethanol and water (solvent). The pulse rate was determined using Blood Pressure (B.P.) machine before and after administering the glucose. Statistical significance was determined by one-way analysis of variance ANOVA with SPSS 16.0 Version, followed by Duncan's Multiple Range. The results indicated that organic extract has the highest percentage (20.20%) yield for the leaves and 11.86% for the root bark extract. The percentage yield of ethanol extract is significantly higher (M+SD 2.5+1.48) than the aqueous extraction. Potentials activity of the plant extract revealed that combined root bark with leaf extracts are able to neutralize snake venom at 200 and 300mg/kg body weight with 100% survival. The plant extracts showed significant effect (P<0.05) on blood glucose level, on leaf (1.79 and 1.69%), root bark (1.91 and 64.33%), combined root bark and leaves extracts (1.82 and 1.25%) at all the concentrations of the PR1 and a decreased of the blood glucose level at PR2 respectively. The blood glucose level increase with a mean of 77.66 and 73.00% before administration, and 69.66 and 65.66% after administering glucose. But, in combined leaves and root bark extracts showed no significant. Snake venom had significant effect on the blood glucose level of the treated rats. The extracts used in this research indicated a potential activity at 200 and 300 mg/kg concentrations of the glucose administered, with reduced blood glucose level and pulse rate in the laboratory animals. Further study is needed to determine the mechanism of action for the active component and phytochemical metabolites responsible for anti snake venom and anti diabetic properties of the plant.

**INTRODUCTION:** Snake bite is a major public health problem with a large number of lives in the African continent and the world at large. The outcome of snake bite depends on numerous factors including species of snake, the area of the body bitten, the amount of venom injected and the condition of the victim.

Bites from non-venomous snakes can cause injury, often due to lacerations caused by the teeth or from a resulting infection. A bite may also trigger an anaphylactic reaction, which is potentially fatal. It is a common belief that snakes are generally venomous. However, of the 2,700 known species of snakes, only about 300 are venomous<sup>6</sup>. The most common symptoms of poisonous snake bites include 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<sup>7</sup>.

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Snake bites may trigger the occurrence of diabetics with specific reference to high cholesterol accumulate heart and excessive glucose in the body of many vertebrates<sup>6</sup>.

Therefore, uncontrolled blood pressure and pulse rates may lead to several micro-vascular and macro-vascular (atheroma) complications that affect many organs of the body of vertebrates<sup>8,9</sup>. In many parts of the world, regular treatment for snake venom accident is serum therapy, which involves the parenteral administration of antiophidian serum (antivenoms). This therapy efficiently neutralizes the systemic toxic effects, preventing death of victims.

Plants used traditionally as medicines constitute potentially useful resources of new drugs for treatment and control of diseases<sup>10-13</sup>.

Violet plant (*Securidaca longepedunculata*) is a medicinal plant used in parts of Africa. The plant is a savanna shrub with twisted bole or slender erect branches and grows up to 30 ft high. It grows in various parts of Western, Northern and Eastern Nigeria<sup>14</sup>, and in Malaysia, Guinea, Cuba and several Asian countries<sup>15</sup>. The active constituents of *S. longepedunculata* are used as folk medicine in traditional therapies of about 80% of the world's population and over 50% of all modern clinical drugs are of natural product origin<sup>16, 17</sup>. In Tanzania, the dried bark and root are used as a laxative for snake bites and nervous system disorders, with one cup of the mixture being taken daily for two weeks. In East Africa, dried leaves from the plant are used in the treatment of wounds and sores, coughs, venereal diseases, and snakebites. In Malawi, the leaves are also used for wounds, coughs, venereal diseases, and snakebites, as well as bilharzia, and the dried leaves are used to cure headaches. In other parts of the continent, parts of the plant are used to cure skin diseases, malaria, impotence, epilepsy, and are also used as an aphrodisiac<sup>18</sup>.

According to World Health Organization<sup>19</sup> the use of a variety of plant extracts and phytochemicals, both with known anti snake venom and anti diabetic properties, can be of great significance in therapeutic treatments.

This research was carried out to determine the in vivo efficacy of violet plant (*Securidaca longepedunculata*) on parameters of blood glucose level and pulse rate of envenomed Albino rat

## MATERIALS AND METHODS:

**Collection, Identification and Processing of Plant Material:** Fresh roots and leaves of *Securidaca longepedunculata* were collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State, Nigeria.

The plant was identified by Dr. Auwal Umar and preserved in the Herbarium with Voucher Specimen No.: D-01SL-7, in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The 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 polyethene bag at room temperature for use.

**Preparation of Extract:** Two hundred grams (200g), each of dried plant material was extracted by soaking in 1000 ml of ethanol and water (solvent) in 1000 ml of conical flask, and covered with aluminum foil and allowed for 24 hours. The extracts were filtered and the solvents removed by warming in oven at 40°C for 3 days. The evaporated extract was stored for 48-hours in sterile universal bottles at room temperature, this methods is adopted by<sup>20, 21</sup>.

**Percentage yield of the Crude Plant Extract:** The percentage yield of the extracts was obtained using the following formula in equation:

$$\% \text{ yield} = \frac{s_2}{s_1} \times 100$$

Where  $s_1$  - weight of powdered sample (g)

$s_2$  - Weight of the extract obtained (g)

**Collection and Preservation of Snake Venom:** 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 a sheet of polyethene. The snake was pressured to

strike the polyethene and penetrate it with it fans until it releases the venom in to the container<sup>22</sup>. The venom was transferred in a sterilized sample bottle and preserved at 4°C.

**Laboratory Animals:** Healthy adult male Albino rats weighing in 250-300g were used for the study. They were housed in polypropylene cages, maintained under standard conditions (12h light and 12h dark). The Albino Rats were allowed to acclimatize to the environmental conditions for five days with access to clean water and animal feed (supplied by vital feed Sokoto) at the Botanical garden, Usmanu Danfodiyo University, Sokoto Nigeria. Ethical clearance was obtained and duly followed from the Nigerian Laboratory animal welfare commission and Veterinary Council of Nigeria (VCN).

#### **In vivo Anti snake Venom Activity:**

**Animal Grouping and Treatment:** Wister albino rats male and female were randomly divided into five groups of six rats.

**Group 1-** Group were injected with venom only

**Group 2-** Group were injected with Snake Venom and treated with leaves extract

**Group 3-** Group were injected with Snake Venom and treated with root bark extract

**Group 4-** Group were injected with snake venom and treated with root bark extract and leaves Extracts

**Group 5-** 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<sup>7</sup>. The snake venom was on survival and mortality of model rats was calculated using the formula:

$$\chi^2 = \frac{((ad-bc)-n/2)2n}{(a+b)(c+d)(a+c)(b+d)}$$

**Determination of in vivo Effect of *S. longepedunculata* on Blood Glucose level on Albino Rats: Animal Grouping and Treatment:** Wister albino rats male and female were randomly divided into four groups of three rats each.

**Group 1-** administered Leaf extract at 200mg/kg and 300mg/kg

**Group 2-** administered root bark extract at 200mg/kg and 300mg/kg

**Group 3-** administered leaf and root bark extracts at 200mg/kg and 300mg/kg

**Group 4-** administered snake venom at 200mg/kg and 300mg/kg

The blood glucose level was determined according to the method described by<sup>23</sup>. ACCU check glucose meter was used for the determination of the blood glucose level using test strip in the experimental rat before and after plant extract and venom administration. A drop of blood (2ml) were collected via tail bleeding of rats and applied to the strip area containing the chemical leading to glucose dye oxidoreductase reaction coursing color change to occur. The strips were inserted in to the meter and the blood glucose concentration was displayed.

**Determination of Pulse Rate:** The pulse rate was determined using Blood Pressure (B.P.) machine before and after administration. Five rats were inserted into the B.P machine and allowed to restrain and once settled for 20 minutes. The pulse rates were observed using machine meter and recorded.

**Statistical Data Analysis:** The parameters analyzed in all the phases of the study were subjected to statistical analysis. Statistical significance was determined by one-way analysis of variance with SPSS 16.0 Version. P < 0.05 considered as significant followed by Duncan's Multiple Range Test to detect significant differences among the means as well as the interactions between the variable.

#### **RESULTS:**

**Percentage Yield of Plant Extract: Table 1** shows result of weight of solute extracted from 400g of powdered leaves and root, percentage yield of the plant crude extract using ethanol and distilled water extract as solvents of extraction, the result indicate that organic extract has the highest percentage yield with 20.20% for the leaves and 11.86% for the root bark extract. The percentage yield of ethanol extract is significantly higher than the aqueous extraction P < 0.05.

**TABLE 1: CRUDE YIELD OF EXTRACTION OF *S. LONGEPEDUNCULATA* LEAVES AND ROOT BARK**

Solvent	% Yielded		M+SD
	Leaves	Root bark	
Aqueous	18.18	8.40	1.79+17.78
Ethanol	20.20	11.86	2.5+1.48

**In vivo Anti snake Venom Activity:** Following injection of the snake venom at 0.2 and 0.3ml and application of the plant extracts at a dose of 200mg/kg and 300mg/kg respectively; a total

number of survivals and mortality of the rat models treated with untreated ones of extracts is indicated in **Table 2**.

**TABLE 2: 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

The value of calculated  $\lambda^2$  is 0.280 which is less than the critical level, at an allowable error ( $\alpha$ ) of 5% and degree of freedom of 1 given as 3.84.

**Effect of *S. longepedunculata* on Blood Glucose level on Albino Rats:** The effect of the plant extracts in the blood glucose level of the rats

following the envenomation is presented in **Table 3**. The plant extracts showed significant effect ( $P < 0.05$ ) on blood glucose level, except that of combine leaves and root bark extracts which showed no significant effect ( $P > 0.05$ ). Snake venom also has significant effect on the blood glucose level ( $P < 0.05$ ) of treated rats.

**TABLE 3: EFFECT OF *SECURIDACA LONGEPEDUNCULATA* ON BLOOD GLUCOSE LEVEL N=3**

Plant part	Conc. mg/kg	Glucose 1 (mg/dl)	Glucose 2 (mg/dl)	P-value compared to the snake venom group
Leaf extract	200	80.33+ 11.50	68.00+ 16.82	0.08
	300	86.00+ 9.53	65.33+ 8.32	0.18
Root bark	200	88.66+23.96	76.66+20.03	0.18
	300	67.33+9.86	66.33+4.93	0.84
Root + leaf	200	77.66 +14.50	69.66+13.65	0.02
	300	73.00+3.60	65.66+3.05	0.16
Snake venom	200	63.00+5.65	74.50 +3.53	0.02
	300	72.00 +18.68	94.66 +14.84	0.04

Values are means + standard error of three replications

Glucose 1= Glucose level before administration

Glucose 2= Glucose level after administration

**Effect of *S. longepedunculata* on Pulse Rate:** The effects of the plant extracts on pulse rate on the albino rats following the envenomation are presented in **Table 4**. The results showed

significant effect ( $P > 0.05$ ) on root bark, leaves combined root bark and leaves extracts at all the concentrations and snake venom at 0.2 ml.

**TABLE 4: EFFECTS OF *SECURIDACA LONGEPEDUNCULATA* ON PULSE RATE IN ALBINO RATS N=3**

Plant part	Conc. Mg/kg	PR 1(BPM)	PR 2(BPM)	P-value compared to the snake venom group
Leaf extract	200	1.75 +13.45	2.36 + 17.32	0.66
	300	1.69 + 47.37	3.88 + 106.09	0.02
Root bark extract	200	1.91 + 23.43	74.33 + 20.55	0.06
	300	64.33 + 19.29	3.146 + 126.76	0.01
Root + leaf extract	200	1.82 + 33.28	2.43 + 29.00	0.03
	300	1.25 + 30.02	1.80 + 33.56	0.01
Snake venom only	200	1.64 + 7.50	3.01 + 60.06	0.06
	300	83.66 + 22.05	2.63 + 12.58	0.03

Values are means + standard error of three replications

PR 1= pulse rate before administration

PR 2= pulse rate after administration



**DISCUSSION:** It has been established in this study that the percentage yield of the plant crude extract using ethanol and distilled water as solvents of extraction has been reported. The result indicated that organic extract has the highest (20.20%) percentage yield, for the leaves and 11.86% for the root bark extract. The percentage yield of ethanol extract is significantly higher ( $P < 0.05$ ) than the aqueous extraction.

The presence of chemical ingredients extracted from *Securidaca longepedunculata* plant might be used in diseases management. The chemical components incorporated in the plant extracts might possess some medicinal anti venom activity on the experimental animals used in this research.

These findings are in conformity with those reported by<sup>24-25</sup> on phytochemical composition and acute toxicity of root bark extracts of *S. longepedunculata*. The presence of these bioactive compounds extracted from *S. longepedunculata* plant might have one or more therapeutic usage. This report is in accordance with the work of<sup>26</sup> who conducted phytochemical screening of leaves of twenty eight woody species from different plant families in Nigeria and discovered the presence of secondary metabolites in all samples, thus, the secondary metabolites seems cosmopolitan in plant but varying degrees and types.

Aqueous extract was also used for *in vivo* anti snake venom activities. The activity of the root bark extract at 200mg/kg increased the percentage survival which was comparable to that of standard anti venom. Similar study was also conducted by<sup>27</sup> who determined anti-snake venom activity of different extracts of *Pouzolzia indica* against russel viper venom. The result revealed that the ethanol and aqueous extracts were found to possess most significant activity. This could also be explained by the fact that the survival of the rats increased progressively with the increasing dose of the extracts in a dose dependent manner. It is also confirmed that the potent snake venom had a neutralizing capacity of water extracts of *S. longepedunculata* against the snake venom. Extract of the root bark of *S. longepedunculata* exhibited neuromuscular blocking and negative inotropic and chronotropic cardiac effects<sup>28</sup> and also demonstrated spasmolytic activity on vascular and

extra vascular smooth muscles in experimental animals<sup>28</sup>.

It was reported in this research that plant extracts in the blood glucose level (200 and 300 Conc. Mg/kg) of the rats, with 80.33 and 86.00% in mg/dl; and 68.00 and 65.33% after administration. Following the envenomation it shows significant effect ( $P < 0.05$ ) on blood glucose level, except that of combine leaves and root bark extracts which showed no significant effect ( $P > 0.05$ ); with a mean 77.66 and 73.00 respectively before administration; and 69.66 and 65.66% after administering the glucose. Snake venom also had 63.00 and 72.00% before administration; 74.50 and 94.66 after glucose administration, indicating significant effect ( $P < 0.05$ ) on the blood glucose level of treated rats.

Although, the chemical compounds that are present in the plant extract used in this research some were not determined, their presence and effectiveness might be responsible in controlling high glucose levels or tolerance in the albino rats. This report is in line with work of<sup>29</sup> reported that methanolic extract of *Cochlospermum planchonii* root at 250, 500 and 1000mg/kg b. w. reduced the blood glucose levels of alloxan-induced diabetic mice in a 360 minutes experimental period. The effects of the plant extract on pulse rate and glucose level of blood pressure of the albino rats was reported in this research, after administering the snake venom at 200 and 300 concentrations of 0.2 and 0.3 ml application. The results showed significant effect ( $P > 0.05$ ) on leaf (1.79 and 1.69%), root bark (1.91 and 64.33%), combined root bark and leaves extracts (1.82 and 1.25%) at all the concentrations of the PR1 and a decreased of the blood glucose level at PR2 respectively. The blood glucose level and pulse rate in the experimental models was reduced at different concentrations as a result of regular administration of the extracts. This report is in accordance with the study of<sup>30</sup> noticed that the blood glucose of alloxanized rats after 48 hours ranged from 20.50-30.20 mmol/L and were significantly ( $P < 0.05$ ) and progressively reduced in the glibenclamide and Aqueous root extract of *Cochlospermum planchonii* treated albino rats.

The *in vivo* activity of the *S. longepedunculata* reduced blood glucose level as a result of the

increased concentration of the extracts of the root bark, leaves and combined root bark and leaves extracts. This showed that the plant could be useful as anti diabetic treatment. The increased blood pressure of the glucose level might likely be due to the observed signs and symptoms after administration of the snake venom, to include: localized edema and swelling at the site of injection, increased heart beat, paralysis, and subsequently death were confirmation of earlier findings on the researches carried out on this venom. Therefore, determination of glucose concentration in blood of the experimental rats could serve as a useful, quantitative index of diabetes.

**CONCLUSION:** Neutralization of the *S. longepedunculata* extracts was checked for *Naja nigrocollis* and the *in vivo* activities were used to determine the anti venom activity of the plant extracts with a two dose levels and have shown significant neutralization. Hence, root bark extracts at 300mg/ml also showed a good anti venom activity. The plant extract indicated potential activity at 200 and 300 mg/kg concentrations of the glucose administered, with reduced high blood glucose level and pulse rate in the experimental rats.

**Ethical Approvals:** Ethical approval was gotten from the ethical committee of Sokoto and Katsina State Ministries of Health in Nigeria, before the commencement of the research. Authors in this work were hereby, declared that all experiments have been examined and approved by the appropriate ethics committee and have been carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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