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In vitro Neutralization of *Naja haje* Venom with Methanol Extract of Bulb of *Urginea altissima* linn

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Abstract

The use of medicinal plants to treat snake envenomation is a common practice among the rural dwellers especially in Africa. This could be attributed to the scarcity of anti-venom readily available or its high cost. It has been claimed that the extract of the plant Urginea altissima Linn if taken orally alleviates the pain and hence neutralizes the venom effect in envenomed human. This study was therefore, aimed to determine the neutralizing effect of Urginea altissima Linn. On a key enzyme (phospholipase A_2) found in Naja haje venom. Result of the phytochemical analysis of the crude methanolic extract of Urginea altissima Linn. Showed the presence of saponins, flavonoids, anthraquinones, and alkaloids which are potential anti-venom compounds in plants. The LD_{50} of the venom of N. haje was found to be 0.183 mg/kg. The venom-induced hemolytic activity was significantly reduced by 1mg/mL of Urginea altissima extract on the venom. The PLA_2 activity of crude venom was reduced by extracts of Urginea altissima Linn. Significantly at ($p < 0.05$). The extracts of Urginea altissima have some beneficial effects on some toxic effects of venom. Thus, the use of this plant in ethno-medicine has some scientific basis.

Keywords: Phospholipase, *Urginea altissima*, anti-venom, *Naja haje*

Introduction

Snake envenomation is a public health problem well known in Nigeria (Theakston and Warrell, 2000; Lallo et al., 1995). However, it has been estimated that mortality rate ranges from 2 to 16 per 100,000 per year in Nigeria, Kenya, Senegal and west Bengal (Theakston et al., 2003). The Kaltungo snake envenomation treatment center recorded an average of 6 snakebites per day (EchiTab, 2009). At certain periods of the year about 74% of hospital beds in this region are occupied by snake bite victims (Revault, 1996). The major species responsible for bites in North-Eastern Nigeria is the saw scaled or carpet viper i.e. *Echis ocellatus* (Habib et al., 2008; Abubakar et al., 2010). Others include *Naja nigricollis*, *Bitis arietans* and to a lesser extent *Causus maculatus, Naja katiensis, Naja haje, Atractaspis microlepidota, Telescopus variegatus* and several species of rattle snakes, sand snakes and *Lycophidion semicinctum* (Abubakar, 2010; EchiTab, 2010).

Snake venoms are also known to cause various physiological changes in organs of different animals. The composition of snake venom determines the physiological effects on their target and it varies with snake type, age and environment where they are found. The components of snake venom are mostly

enzymes which include; L- amino acid oxidase, Alanine amino transferase, Phospholipase A_2, 51 Nucleotidase, Phosphodiesterase, Deoxyribonuclease, Ribonuclease I, Adenosine triphosphatase, Amylase, Hyaluronidase, NAD-Nucleotidase, Glusamine ammonium lyase and Kininogenase which are found in all snake types and Lactate dehydrogenase, Lysophospholipase, Acetylcholinesterase, Alkaline Phosphatase, Acid phosphatase, Factor-X activator, Heparinase, α-fibrinogenase, β-fibrinogenase, α-β-fibrinogenase, Fibrinolytic enzyme, Prothrombin activator, Collagenase and Elastase which are found in some species (Bauchot, 1994). There are reports by locals that the extract of the plant *Urginea altissima* has effects on snake envenomation. This study was therefore, aimed at establishing scientific bases for the use of this plant.

Materials and Methods

Plant Preparation

The plant *Urginea altissima* Linn was collected at Jere, Borno State and authenticated at the Department of Botany, University of Maiduguri. The bulbs were then removed, cleaned, air-dried, pulverized into powder and stored in an airtight container.

Experimental Animals

Adult male and female albino rats weighing 150-200g were obtained from animal house of the Department of Biochemistry, University of Maiduguri,Borno State. The rats were housed in plastic cages and maintained under standard laboratory conditions with free access to rat's pellets and tap water *ad libitum*.

Methanol Extraction

Exactly 300g of the powdered plant sample was weighed. The powder was extracted by cold maceration method (Sofowara, 1984) and stored.

Phytochemical Screening of the Plant Extracts of *Urginea altissima* Linn.

The crude extract was partitioned with Methanol. The residue left was dissolved in water and the various fractions obtained were all subjected to phytochemical screening employing the standard screening test. The tests conducted includes: test for saponin glycosides, tannins, flavonoids and alkaloids.

Determination of Crude Venom LD_{50}

An approximate LD_{50} of the crude Venom was determined by grouping the animals into four groups (0.1mg/kg, 0.2mg/kg, 0.3mg/kg and 0.4mg/kg) and the venom was administered intraperitoneal into Wistar-strain albino rats. After twenty-four (24) hours the number of death animals was recorded and the LD_{50} was estimated (Karber's method modified by Aliu and Nwude, 1982).

Determination of *Urginea altissima* Extract LD_{50}

An approximate LD_{50} of *Urginea altissima* extract was determined using Lork's method, 1983.

Determination of Hemolytic Activity of the crude venom

Bovine erythrocyte was used to determine the hemolytic activity. Erythrocyte was washed with saline (0.9%) by centrifugation (2000 RPM) for 10 minutes. After repeated washings with saline, a 1% cell suspension was prepared. Tubes with the diluted venom (equivalent to 10µL) are mixed with 10ml of 1% cell suspension in saline which was

incubated at 37^{o}C ±1 for 60 minutes. The reaction was stopped by adding 3ml of chilled PBS, then the mixtures was centrifuge at 2000RPM for 10 minute and absorbance of the supernatant was measured at 540nm. (Abubakar, et al., 2006).

Neutralization of Phospholipase A2 activity of the Crude venom

The phospholipase A_2 activity of the venom was determined by a modified egg yolk coagulation method as described by Habermann and Neumann, (1954). Fresh egg yolk was homogenized in distilled water to yield a concentration of 100mg/ml. The venom (10µL) and 10µL of 50mM Tris HCl buffer (pH 8.0) was incubated with 1000µL substrate at 37±1^{o}C for 10 minutes. The phospholipase A_2 activity was given as the number of moles of acid (average of five determinant ±SEM) liberated by 1mg of venom in 1 hour.

Statistical Analysis

Median lethal dose, LD_{50} of the venoms was expressed as mean with 95% confidence intervals (CI) and was reported as the mean ± S.D.

Results

Table 1 shows the possible secondary metabolites found in the methanolic bulb extract of *Urginea altissima* Linn. Alkaloids, saponins, anthraquinone and flavonoids were detected in the extract, while tannin was not detected.

Table 1. Phytochemical screenining of *Urginea altissima* Linn

Metabolites	**Phytochemicals**
Alkaloids	+
Tannins	-
Flavonoids	+
Saponins	+
Anthraquinones	+

Key: + = Positive - = Negative

Table 2 shows the LD_{50} of the methanolic bulb extract of *Urginea altissima.* In the first phase of the experiment, the first three groups of mice that received a dose range of 10, 100, and 1000 mg/kg of the extract had no mortality. The other group in the second phase of the test that received a higher dose of 1600, 2500, and 5000 mg/kg of the extract had no mortality as well.

Table 2. Results of the LD_{50} of Methanolic Bulb extract of *Urginea altissima* Linn.

Group	Dose (mg/kg)	No of death	Behavior
Phase I	10	0/3	Normal
	100	0/3	Normal
	1000	0/3	Normal
Phase II	1600	0/1	Normal
	2500	0/1	Normal
	5000	0/1	Normal

LD_{50} value is > 5000 mg/kg since there was no mortality.

Table 3 shows the LD_{50} value of *Naja haje* venom. The first group that received a dose of 0.1mg/kg had no mortality, while the other group that received 0.2, 0.3 and 0.4mg/kg had mortality at 66.6% and 100% respectively. According to Karber's method, the LD50 of the crude venom is 0.183mg/kg which is close to the reference value 0.29mg/kg.

Table 3. Results of the LD_{50} experiment of *Naja haje* venom

Group (µg/ml)	Dose difference	Dose Death	No of death	Mean	DDxMD
A	0.1		0/3		
B-A=	0.1		1.0	0.10	
B	0.2		2/3		
C-B=	0.1		2.5	0.25	
C	0.3		3/3		
D-C=	0.1		3.0	0.30	
D	0.4		3/3		
Total				0.65	

$LD_{50} = LD_{100} - \frac{MD \times DD}{N}$ = 0.183mg/kg/

Key:
N= number of replicates

Table 4 shows anti-phospholipase A_2 activity of methanolic bulb extract of *Urginea altissima* Linn on *Naja haje* venom. Phospholipase A_2 activity was reduced in the presence of the extract of *Urginea altissima* Linn significantly at ($p > 0.05$) 26.04%.

Table 4. Anti-Phospholipase A_2 Activity of Methanolic Bulb Extract of *Urginea altissima* on *Naja haje* Venom

Sample	**PLA_2 Activity (µmoles mg^{-1} h^{-1})**
Crude Venom	835.8 ± 2.56^b
Urginea altissima + Venom	654.0 ± 7.04^a

Table 5 shows the effect of methanolic bulb extract of *Urginea altissima* Linn on hemolytic activity of *Naja haje* venom. Hemolytic activity of the venom was reduced by the extracts of *Urginea altissima* Linn (63.71%). The crude venom produces hemolysis of the bovine red blood cells (89.71%0) and its established fact that blood hemolysis by venoms is due to the presence of phospholipase enzymes.

Table 5. Effect of *Urginea altissima* Linn on hemolytic Activity of *Naja haje* Venom

Sample	Absorbance	100% Lysis
Crude Venom	1.526 ± 0.099^{b}	1.701 ± 0.069^{b}
Urginea altissima + Venom	0.853 ± 0.129^{a}	1.339 ± 0.131^{a}

Discussion

The results of the preliminary phytochemical screening indicate the presence of alkaloid, saponins, anthraquinones, and flavonoids, in extracts of *Urginea altissima* Linn. The antivenom activity observed with extracts of *Urginea altissima* in this study could be due to the presence of alkaloids, saponins, flavonoids, steroids and terpenoid (Rajendran et al., 2010; Yadav et al., 2014). Lans et al., (2001) reported that plant alkaloids are effective against snake bites. They also reported that phenolic compounds and tannins can bind to proteins and can directly act on venom constituents. Saponins and steroids from different plants have also demonstrated anti-snake venom activity in laboratory tests (Mors et al., 2000; Ticli et al., 2005). Similarly, flavonoids have been shown to possess the ability to bind to biological polymers and can inhibit the effects of toxins (Mors et al., 2000; Ticli et al., 2005; Batina et al., 2000). Flavonoids also have many useful properties like anti-inflammatory and enzyme inhibition effects (Prabha and Savithramma, 2014; Galati and O'Brien, 2004) which may be useful biologically in the event of snake bites.

Iwu (2014) reported that *Urginea altissima* Linn contains cardiac glycoside which can readily bind to proteins and this corroborate with the result of this study. Szent Gyorgyi suggested in 1953 that cardiac glycosides are not drugs but a substitute for an endogenous compound that is a physiological regulator of heart muscle contraction. This is useful in neutralizing proteins/enzymes present in snake venoms thus accounting for the high efficacy as anti-venom. The crude venom produces hemolysis of the bovine red blood cells (89.71%). The venom-induced hemolytic activity was significantly reduced by the extracts of *Urginea altissima* Linn (63.67%) and it is an established fact that blood hemolysis by venoms is due to the presence of phospholipase enzymes (Salach et al., 1971; Teng et al., 1986).

The PLA_2 activity of crude venom was found to be 835.8 ± 2.56 (μmoles mg^{-1} h^{-1}): this activity was reduced by the extracts of *Urginea altissima* significantly at ($p<0.05$) 26.04%. Because the venom is incubated with extracts prior to addition of substrate, the observed reduction in PLA_2 activities may be as a result of interaction of amino acid in the catalytic site with saponins, anthraquinones, alkaloids or flavonoids. The current observation, the reduction of PLA_2 activity, suggests the presence of some active ingredients in the plants that interact with the enzyme. Inhibition of PLA_2 activity by the extracts may be responsible for alleviation of these symptoms, including hemolysis of blood due to these pathogenic enzymes.

The acute toxicity studies showed that the venom of *Naja haje* had the LD_{50} of 0.183 mg/kg, The LD_{50} value obtained for the methanol extract of *Urginea altissima* Linn in this study was above 5000 mg/kg. It has been suggested that any substance with an intraperitoneal LD_{50} of above 1000 mg/kg should be regarded as safe (WHO, 2015). The intraperitoneal route was used to determine the LD_{50} in this study.

The use of the plant *Urginea altissima* by the local population in some parts of Nigeria to alleviate the pain due to snake envenomation is justifiable based on the result presented in this study. However, it can be of great advantage if the constituents can further be identified through isolation to determine their effects on the purified venom constituents.

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