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Research Article

In vitro and *In vivo* Neutralizing Activity of Uvaria Chamae Fractions on the Venoms of Naja Nigricollis in Albino Rat and Bovine Blood

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Abstract: This study was aimed at evaluating the effect of Uvaria chamae extract in protecting against Naja nigricollis envenomations. Uvaria chamae is a well-known medicinal plant in Nigeria traditional medicine for the management of snakebite. Freshly collected Uvaria chamae leaves and stems were air-dried, powdered and extracted in methanol. The median lethal dose of the extract was determined and further fractionated with n-hexane, n-butanol and ethyl acetate. Each fraction was tested for neutralizing effect against venom-induced haemolytic, fibrinolytic, hemorrhagic, and cytotoxic activities. The result obtained showed significant ($p<0.05$) antihaemolytic activity of Uvaria chamae in n-butanol 31.40%, aqueous residue 39.60% and ethyl acetate 40.70% fractions when compared to positive controls, but in n-hexane, the activity 33% was not significantly ($p>0.05$) increased. Antifibrinolytic activity was significantly ($p<0.05$) increase in n-hexane 73.88%, n-butanol 72.22% and aqueous residue 72.22% fractions. Antihaemorrhagic significant ($p<0.05$) increase in all the concentrations of each fraction except for n-butanol and aqueous residues at 400 mg/kg; antihaemorrhagic effect was more at 100 and 200 mg/mL concentration following experimental envenomation. Uvaria chamae fractions were found to possess a high level of protection against Naja nigricollis venoms-induced lethality, compared to positive controls. The results apparently provide the pharmacological rationale for the use of the leaves of Uvaria chamae in the management of snakebite envenomation.

Keywords: Albino rat and venom, fractions, naja nigricollis, snakebite, uvaria chamae

INTRODUCTION

Snakebite envenoming is a major public health problem among rural communities of West Africa, notably Benin, Burkina-Faso, Cameroun, Ghana and Nigeria (Habib, 2013). It is a global priority and neglected tropical disease (Potet *et al*., 2019). Despite this global impact, snakebite has received little attention from the global health community, the pharmaceutical industries, governments and public health advocacy groups and has a disappointingly low priority in the global health research agenda (Gutiérrez *et al*., 2013). Thse situation remains a serious medical and socio-economic problem in many parts of the world, especially in tropical and sub-tropical countries (Chippaux, 1998). The record of snakebites the obtained from West Africa sub region, notably in Benin, Burkina-Faso, Cameroon, Ghana and Nigeria, is skyrocketing yearly (Habib, 2013). It is an environmental and occupational disease that affects mostly agricultural workers and their children in rural settings of third world countries of Africa and parts of Asia, Latin America and Oceania (Potet *et al*., 2019). A recent global appraisal estimates that there were at least 1.8 million cases of snakebite with 81,000 deaths annually throughout the world (Gutiérrez *et al*., 2017). Over 5 million people are bitten by venomous snakes annually with about 100,000 mortality, mostly in Asia and Africa although the reverse is the case in the United States of America, with 6000-8000 envenomation, resulting in 15-50 mortality (Ruha *et al*., 2017).

Nigeria is reported to account for one fifth of all West African region snakebite cases (Arya, 2004). Snakebite in Africa causes thousands of deaths annually and considerable permanent physical disability. The incidence of snakebite in rural West Africa is estimated to be as high as 174 per 100,000 populations, with an 11-17% mortality rate (Habib *et al*., 2001; Visser *et al*., 2008; Habib, 2013). This number does not account for unreported incidences and those who survive envenomation will often deal with secondary effects such as myonecrosis or limb amputation (Gutiérrez *et al*., 2006). Snakebite envenoming and associated mortality are under-reported because many victims (20-70% in most cases) do not seek medical treatment in government dispensaries or hospitals and hence, are not

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recorded. This occurs because medical posts in regions of high incidence are unable to keep accurate records and because death certification of snakebite is often imprecise (Broadley, 1990). A more recent study estimated that over 314,000 bites, 7300 deaths and nearly 6000 amputations occur from snakebites annually in sub-Saharan Africa (Chippaux, 2011). It has been estimated that there are about 5 million snakebites, resulting in 2.5 million envenoming and 125,000 deaths and 375,000 cases of permanent physical disabilities worldwide annually (Finney, 2010; Leonard, 2010).

The most effective and acceptable therapy for snakebite victims is the immediate administration of anti-venom following envenomation (Mahanta and Mukherjee, 2001).

In developing countries, Nigeria inclusive, plant natural products or parts are used in the management of snakebites (Nwanguma *et al*., 1999; Ogbulie *et al*., 2004). Research to develop a treatment for local snake envenoming is, therefore, of clinical priority and is focused on the application of natural or synthetic inhibitors of snake venom protein molecules (Gomes *et al*., 2007).

Uvaria chamae (finger root or wild banana in English), is a climbing large shrub or small tree native to tropical West and Central Africa, where it grows in wet and dry forests and scrublands (Iwu, 1993). There is a folkloric claim that Uvaria chamae, is used for the management of abdominal pain, diarrhoea, haemorhoid, snakebite, wounds and sore throat (Emordi *et al*., 2015). This study was therefore, designed to evaluate the antivenom potential of Uvaria chamae fractions.

MATERIALS AND METHODS

Location of experiment: The experiment was conducted at the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterunary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Collection of plant material: Plant specimen consisting of the leaves and stem of Uvaria chamae were collected in the month of February 2017 from Agatu Local Government of Benue State Nigeria. The sample was identified and authenticated at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria, where a voucher (V/N: 817) was deposited for future reference.

Plant extraction: The leaves and stems of Uvaria chamea were collected, cleaned and cured under shade and size reduced into powder. About 500 g was macerated in 95% methanol at room temperature for 48 h. The entire process was repeated twice for a period of 24 h each. The methanol extracts were pooled together and filtered using filter paper (Whatmann size No.1). The filtrate was concentrated on a water bath at 40°C. The dried crude extract obtained was weighed and kept in air-tight bottle in a refrigerator at 4°C until required for use.

Fractionation of extract: The crude methanol extract of leaf and stem of Uvaria chamae was dissolved in 500 mL of water and then poured into a separating funnel (1 L). An equal volume of n-hexane was gently poured into the funnel, closed and shaken carefully. The pressure that built within the flask was released by inverted and opening the tap at the base of the funnel. The process was repeated five times. The resulting suspension was allowed to stand for about 10 min and the lower aqueous layer was collected in a container. The n-hexane fraction was concentrated to dryness under a fast-moving stream of air. The aqueous fraction was extracted with n-hexane twice and allowed to stand for about 2 h under a fast-moving stream of air to remove traces of n-hexane. Furthermore, the aqueous portion of the extract was added to an equal volume of ethyl acetate and the mixture shaken gently. The separating funnel containing the mixture was inverted and the tap opened to allow the escape of built-in pressure. The resulting suspension was allowed to stand for about 15 min. The lower aqueous layer was decanted and collected. The upper ethyl-acetate fraction was collected separately. The whole process was repeated and the resulting fractions were similarly concentrated to dryness under a fast stream of air.

Experimental animals: Thirty five (35) Wistar rats of both male and female weighing between 110-129 g were used and they were obtained from the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. The rats were maintained on standard rodent feed and water ad libitum. The animals were kept in cages at room temperature (25-26°C) in the laboratory throughout the study.

Ethical clearance: Ethical approval number (ABUCAU/2018/061) for the experiment was obtained from the Ahmadu Bello University Commmittee on Animal Use and Care, Ahmadu Bello University, Zaria Venom Collection and Preparation.

The venoms were obtained from locally caught black necked spiting cobra (Naja nigricollis) using the milking method of Macfarlane (1967). The snake was properly restrained at the position of the joint between the last cervical vertebrate and the skull using the thumb and fore finger, care being taken not to strangulate the snake. A beaker covered with a piece of cellophane membrane maintained in place by a rubber band and it was placed around the buccal cavity of the snake it immediately grabbed and held on to, with the fangs through the membrane, venom was injected into the beaker. The beaker containing the venom was

immediately placed in a desiccator containing activated silica. This was allowed to dry at room temperature. The crystallized venom was subsequently transferred into a refrigerator and stored at 4°C on air tight container for subsequent use.

Determination of median lethal dose: The median lethal dose of crude extract of Uvaria chamae and Naja nigricollis venom was conducted using the method of Lorke (1983):

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality

Evaluation of the modulatory activity of Uvaria chamae methanol extract on Naja nigricollis Envenomation in albino rat:

***In vivo* neutralization assay:** Eighteen albino rats (18) were randomly divided into 6 groups of each consisting of three rats for N. nigricollis venom. The test tubes were also divided into six groups on the rag according to the groups. Neutralization of the venoms activity were estimated by mixing a fixed amount of plant extract with three times (3× LD_{50}) of the venoms inside the test tubes, the entire test tubes were incubated at 37°C for 60 min. The preincubated mixtures were administered intraperitoneally into the rats. The rats were observed at interval of 30 minutes, for toxic signs and death for over a period of 24 h. They were sacrificed by cervical dislocation and the heart, brain, kidneys, liver, spleen and lungs were harvested for subjected to histopathological analysis:

Group A: Control groups that received venom concentration at 10 mg/mL of crude venom.
Group B: Control group that received 0.2 mL of normal saline.
Group C: Administered with n-hexane extract and 3×LD_{50} of the crude venom.
Group D: Administered with n-butanol extract and 3×LD_{50} of the crude venom.
Group E: Administered with ethyl acetate extract and 3×LD_{50} of the crude venom.
Group F: Administered with aqueous residue extract and 3×LD_{50} of the crude venom.

Anti-haemorrhagic assay: Thirty (30) adult rats were used for antihaemorrhagic assay. The method previously described by Theakston and Reid (1983) was adopted. The minimum haemorrhagic dose was defined as the least amount of venom which when injected intradermally into the rat resulted in a haemorrhage lesion of 10 mm diameter in 24 h. Stock solutions of crude venom samples of Naja nigricollis of minimum lethal dose were prepared (i.e., 10 mg/mL) in two separate test tubes. Five test tubes each containing 1 mL of normal saline was arranged in a test tube racks and labelled for the rack used for Bitis venom. Individual test tubes were labelled (treatment groups) A (n-hexane), B (ethyl acetate), C (aqueous residues), D (butanol) and (control) E, for the venom. Two-fold serial dilution was made to get concentrations of crude plant extract of 400 mg/mL, 200 mg/mL and 100 mg/mL each individual test tube was incubated at 37°C for 60 min. 0.2 mL of each of these dilutions were injected subcutaneously into the backs of rat. The rats were sacrificed by cervical fracture after 60 min and then skinned and the haemorrhagic foci was measured in cm (Omori-Satoh *et al*., 1972; Furtado *et al*., 2003). The area of the haemorrhagic foci was determined using the formula πr^2 for each of the venom concentrations.

Anti-myotoxic assay: Thirty (30) rats were divided into six groups and three per group for Naja nigricollis venoms with a positive control for N. nigricollis venoms and one negative control. All the test tubes where labelled viz; Group A (n-butanol), B (ethyl acetate), C (n-hexane and D (aqueous residues) were given 100 mg/mL of crude extract diluted with 10 mg of venoms, while groups E, were given 10 mg/mL of plain venom and F where given distilled water. The test tubes were incubated for 37°C for 60 min and the mixture was administered into the thigh muscle of Wister rat. The rats were given feed and water ad libitun and they were subsequently sacrificed by cervical dislocation after 6 h. The thigh muscle was severed and sent to laboratory for histopathology.

Anti-haemolytic assay: The method described by Gomes and De (1999) was adopted in the test used to determine haemolytic activity. Briefly, 20 mL of bovine blood were collected from an abattoir using sodium citrate as anticoagulant. The blood sample was centrifuged at 489 g for 10 min and the plasma discarded. 5 mL of normal saline was mixed with the packed cell layer and centrifuged again at 489 g for 10 min and the supernatant was discarded. This procedure was repeated 10 times to obtain plasma-free packed cells. A total of 10 mg/mL of crude venoms of N. nigricollis were mixed with 100 mg/mL of crude extract fractionst in a 15 test tubes each for venom. The mixtures were diluted with 10 mL of 1% cell suspension in saline and incubated at 37°C + 1 for 60 min. The reaction was stopped by adding 3 mL of chilled phosphate buffer saline. The tubes were centrifuged at 313 g for 10 min and absorbance of the supernatant were measured at 540 nm. The supernatant of the experimental tube was treated with 3 mL of chilled water and it was taken as 100% lysis;

haemolytic activity was determined using a spectrophotometer (Spectrolap 23A) using the formula:

$$\frac{\text{Transference (\%) -Blank (cm}^2\text{)}}{\text{Wave length}}$$ (Theakston and Reid, 1983)

Anti-fibrinolytic effect: Blood sample was collected from bovine in abattoir into 3.8% sodium citrate (9:1v/v), centrifuged at 489 g and stored at 4°C for 15 min to obtain platelet-poor plasma. The different dilutions of 10 mg/mL of crude venoms for N. nigricollis were prepared using solution of 100 mg/mL of crude extract fractions. Fibrinolytic activity was measured by the method of Theakston and Reid (1983) using bovine citrated plasma (2.0 mg/mL). Breifly, 10 mg/mL of venoms was added to 0.2 mL of citrated plasma solution and clotting times were recorded at 37°C, exactly 50 µL, $CaCl_2$ (25 Mm) was added to the mixture immediately before the venoms solution. All antifibrinolytic activities were expressed as the inverse of the clotting times recorded in minutes. In control test tubes, plasma was incubated with venom alone, without extract.

Histopathological studies of some organs of envenomated albino rats: Histological samples of harvested organs were trimmed. Portions from one half were fixed in 10% formalin and analysed by adapting the routing paraffin and resin embedding method (Slaoui *et al*., 2017). Sections were viewed under microscope (H and E ×200).

Data analysis: Values were expressed as Mean ± SEM and analysed by One-Way analysis of Variance (ANOVA), followed by Tukey's post-hoc test for multiple comparism using GraphPad Prism 5. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

***In vivo* antihaemorrhagic potential of 100 mg/mL, 200 mg/mL and 400 mg/mL of Uvaria chamae leaf and stem fractions against Naja nigricollis venom:** The result (Fig. 1) shows the *In vivo* antihaemorrhagic effect of Uvaria chamae crude extract fractions on albino rat. There was significant ($p<0.05$) increase in antihaemorrhagic activity in all the fractions, the venom at the lower concetration exhibited greater effect compared to control group. The crude fractions at 100 and 200 mg/mL had more antihaemorrhagic effect on the N. nigricollis venom than 400 mg/mL. The antihaemorrhagic activities for the fractions were: n-butanol at concentration of 100, 200 and 400 mg/mL (34.80, 48.74 and 40.88%) ethyl acetate 100, 200 and 400 mg/mL (30.74, 40. 88 and 32.06%), aqueous residues 100, 200 and 400 mg/mL (24.27, 39.41 and 46.76%) and n-hexane 100, 200 and 400 mg/mL (100.00, 100.00 and 32.27%) compared to control (0.00%).

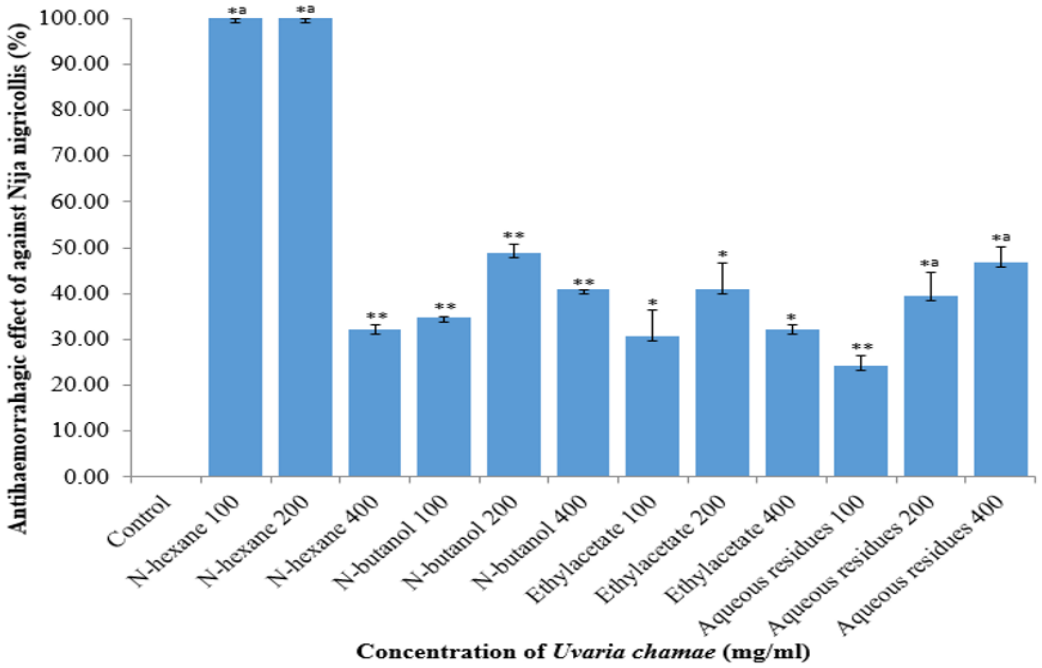

Fig. 1: Antihaemorrhagic percentage effect of extract various concentration of *Uvaria chamae* fractions incubated with *Naja nigricollis* venom administrated to albino rats;* = significantly ($p<0.05$) different from control; ** = significantly ($p<0.05$) different from each other; [a] = the same with each other

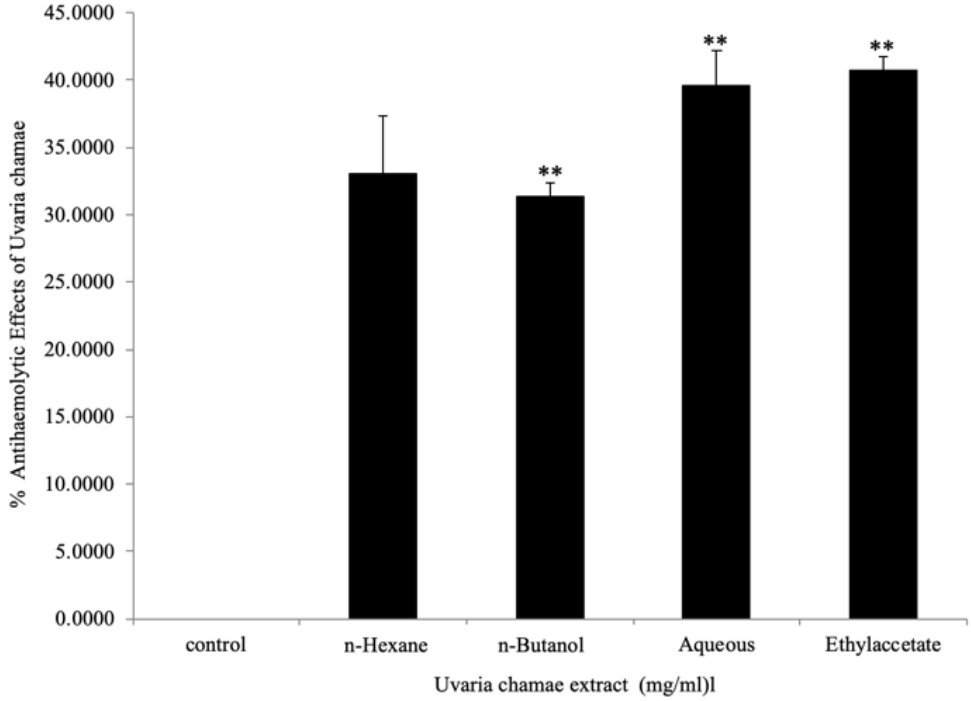

Fig. 2: Antihaemolytic effect of 100 mg/mL of crude fractions of Uvaria chamae leaves and stem extract against 10 mg/mL of venom of Naja nigricollis on 10 mL of 1% bovine erythrocytes. The values with double asterisk shows highly significant different ($p<0.05$)

***In vitro* antihaemolytic potential of crude extract fractions of Uvaria chamae leaves and stem against Naja nigricollis venom on bovine erythrocytes:** The results of the in vitro antihaemolytic effect of Uvaria chamae crude extract fractions on bovine erythrocyte are shown in Fig. 1. The minimun lethal dose of U. chamae fractions and Naja nigricollis venom plus 10 mL of 1% RBC were mixed and the mxture was pre-incubated for 60 min at the 37°C is shown in Fig. 1. There were significant ($p<0.05$) increase antihaemolytic activity in all the fractions compared to control group. The crude fractions at 100 mg/mL inhibited haemolytic effect of n. nigricollis at ethyl acetate (40.70%), aqueous residue (39. 60%), n-hexane (33. 40%), n-butanol (31. 40%), respectively as compared with control group (0. 00%) within 60 min.

***In vitro* antifibrinolytic potential of Uvaria chamae leaf and stem fractions against Naja nigricollis venom:** The result showing the *in vitro* antifibrinolytic effect of Uvaria chamae crude extract fractions on bovine citrated plasma. The minimun lethal dose of U. chamae fractions and N. nigricollis venom plus 0.2 mL of citrated plasma solution was mixed and the mxture was pre-incubated for 60 min at the 37°C and clotting time was recorded shown in Fig. 2. There was a highly significant ($p<0.05$) increase in antifibrinolytic activity in all the fractions compared to control group. The crude fractions at 100 mg/mL action of N. nigricollis to n-hexane (73.88%), n-butanol (72.21%), aqueous residue (74.64%) and ethyl acetate (72.22%) respectively as compared with control group (0.00%) within 60 min (Fig. 3).

DISCUSSION

Phytochemical screening of the Uvaria chamae leaf and stem crude extract, revealed the presence of secondary metabolites such as flavonoids, tannins, phenols, alkaloids, triterpene and steroid. Donatus and Friday (2009) reported similar phytochemical constituents from Uvaria chamae, except steroid which was not reported in his finding, these differences could be due to Soxhlet apparatus used for his extraction. It could also be due to stage of plant growth and the edaphic factors. Furthermore, Evans and Gaiere (2017) had reported the same phytochemical constituents similar to those reported in the present study.

The results of this study showed that the median lethal dose (LD_{50}) of the leaf and stem extract of Uvaria chamae was determined to be 2154.07 mg/kg (2.2 g/kg) using the method of Lorke (1983). Olumese *et al*. (2016) reported an LD_{50} for the same plant extract using oral route of administration to be 5000 mg/kg body weight in Sprague Dawley rats. The differences in the LD_{50} values might be related to the extracting solvents used in each case and the route of administration. Methanol was used in the present study, while cold maceration was used by Olumese *et al*. (2016). In addition, other factors such as difference

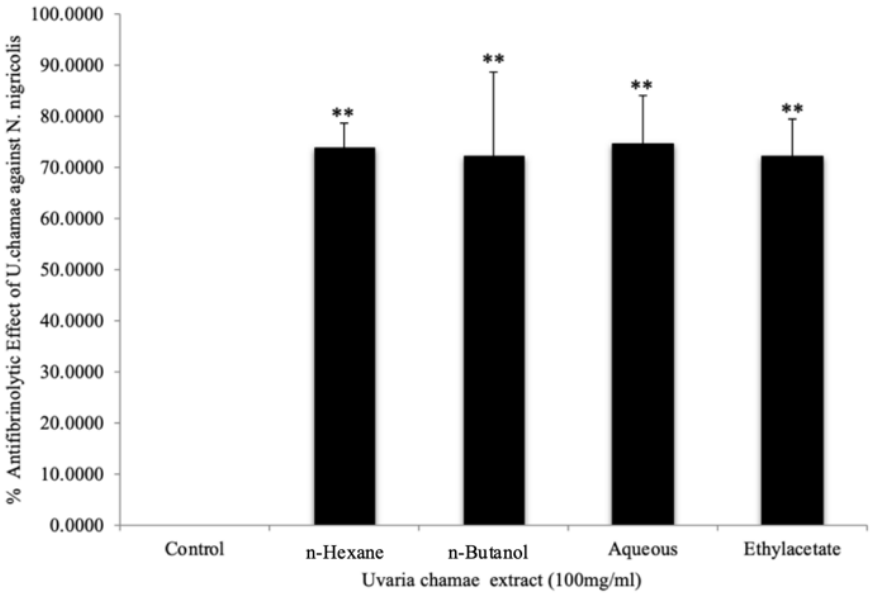

Fig. 3: Effect of crude extract of *Uvaria chamae* fractions against fibrinolytic activity of *Naja nigricollis* venom. The values with double asterisk shows highly significant ($p<0.05$), compared to control

species of rat, age, sex, body weight, percentage of active components in the plant might have also been responsible (Evans and Gaiere, 2017).

The observed antihaemorrhagic finding from this study significantly ($p<0.05$) increased at varying degrees. The abundant presence of saponins, steroids and tannins in the leaf of U. chamae may be responsible for the haemostatic activity of these plants, where they arrest haemorrhage from damaged blood vessels or capillaries by precipitating proteins to form vascular plugs (Donatus and Friday, 2009). Antihaemorrhagic activity of Uvaria chamae fractions were found in the following order of percentage against the Naja nigricollis venom: n-butanol at concentration of 100, 200 and 400 mg/mL (34.80, 48.74 and 40.88%) ethyl acetate 100, 200 and 400 mg/mL (30.74, 40. 88 and 32.06%), aqueous residues 100, 200 and 400 mg/mL (24.27, 39.41 and 46.76%) and n-hexane 100, 200 and 400 mg/mL (100.00, 100.00 and 32.27%) compared to control (0.00).

The fractions at lower concentration of 100 mg/mL and 200 mg/mL for n- butanol, ethyl acetate, aqueous residues and n-hexane showed marked antihaemorragic potential against the venom of Naja nigricollis. Donatus and Friday (2009) had earlier demonstrated Uvaria chamae crude extract to exhibit smooth muscle contraction in guinea pig. This indicated that it might possess anti-inflammatory and antihaemorragic activities in microcapillaries to control haemorrhage. Snakebite venom is composed mainly of zinc-dependent metalloproteinases that is responsible for the pathologic characteristic (Preciado *et al*., 2018). For efficient catalysis of this enzyme its require association with divalent cations such as Zn^{2+} (Bjarnason and Tu, 1978). While Zn^{2+} is required for catalytic activity of SVMPs, Ca^{2+} is involved in the structural stabilization (White *et al*., 1990). It might be possible that flavonoids in the plant inhibited these key enzymes, hence preventing progression of haemorrhage by chellating the Zn^{2+} or Ca^{2+} which is very importance in enzymes activities (Preciado *et al*., 2018).

Antihaemolytic potential of Uvaria chamae fractions might be due to presence of flavonoids which can inactivate the lysosomal enzymes secretion and arachidonic acid release from cell membrane and also inhibiting lipoxygenase, cyclooxygenase and phospholipase A_2 (Del-rio *et al*., 1977). The highest observed antihaemolytic effect showed by ethyl acetate fraction of Uvaria chamae is in agreement with the work of Ghasemzadeh and Ghasemzadeh (2011), who suggested that ethyl acetate is the best solvent for flavonoid extraction which have been demonstrated to protect biological membranes against free radical-induced oxidative damage on the red blood cell phosphplipid (Kitagawa *et al*., 1992).

The observed fibrinolytic features induced by N. nigricollis subsequently inhibited by U. chamae extract is in agreement with the work of Sanchez *et al*. (2017), although they associated the fibrinolysis to Viparine family. The presence of phenols in U. chamae could account for its anti-fibrinolytic effects. Phenols have the ability to block specific enzymes that cause inflammatory disorders. They also modify the prostaglandin pathways, and there by protect platelets

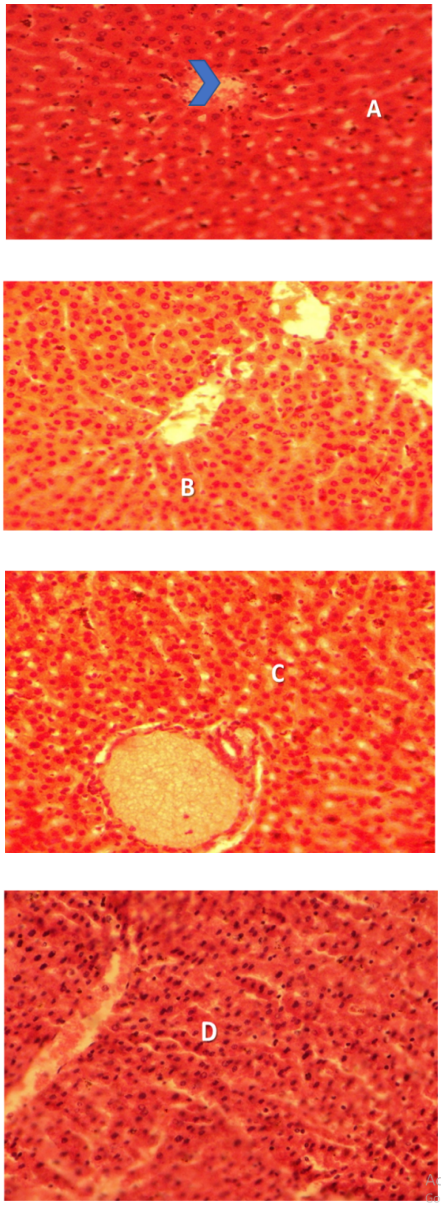

Fig. 4: Photomicrograph of panoramic view (H & E x 200) of section of liver from albino rat treated with Uvaria chamae fractions against the N.nigricollis venom, (A) Model control, arrow shows the area of congestion, (B, C and D) are treated groups with ethyl acetate, n-hexane and n-butanol fractions plus against Naja nigricollis venom with the liver showing normal tissue architecture

from clumping (Okwu, 2005). The anti-fibrinolytic effects of U. chamae against Naja nigricollis venoms could also be due to the presence of bioactive compounds that are able to arrest Zn^{2+} modulating activity, the Zn^{2+} is required for activation of metalloproteinase, when Zn^{2+} is removed from the enzyme the catalytic activity is lost (Preciado *et al*., 2018). It was observed that fractions exerted higher significant ($p<0.05$) antifibrinolytic effect in Naja nigricollis snake venom to a varying degree which might be due to phenolic compound in that extract. Antifibrinolytic activity of the fractions were found in the following decreasing order: Aqueous residues (74.64%), n-hexane (73.88%), Ethyl acetate (72.22%), n-butanol (72.2167%) and control (0.00%) for Naja nigricollis. These results are in contrast with results obtained by previous studies (Claude *et al*., 2014), but similar to those reported by Omale *et al*. (2013). It is well known that these divalent ions play an important role in fibrinolysis by increasing the activity of several fibrinolytic toxins (Celedón *et al*., 2009), which probably explain the inhibition of fibrinolysis due to the Zn^{2+} chellating ability of the crude extract in the venoms. Amakoha *et al*. (2002), who observed that saponins showed modulation in zinc transport in isolated inflamed cell and thereby showed reduction in fibrinolysis. Calcium is also required in fibrinolytic activation cascade by Kini *et al*. (2001), who showed that the snake venom, elapine, contains pro-coagulant proteins, either serine proteinases or metalloproteinases, which are either Zn^{2+} or Ca^{2+} dependent enzymes.

The results showed that the Uvaria chamae crude extract neutralizing the lethality induced by the venom of Naja nigricollis on the tested animals and bovine blood by administration of 100 mg/mL of Uvaria chamae fractions agaist 10 mg/mL of snake venoms when compared with the control groups. Denson *et al*. (1992) reported similar result on Uvaria chamae methanolic extract. The Neutralization could be as a result of effects of phytochemical constituent which exert antioxidant and chaleting properties on the snake venoms enzymes (Nasidi, 2007). Furthermore, the present study demonstrated antihaemolytic effect across the fractions against Naja nigricollis venom. It was observed that Uvaria chamae posses neutralizing activity against the venom of Naja nigricollis, ethyl acetate fraction possesses strongest activity (40.70%) when compared to other fractions (aqueous residues (39.60%), n-butanol (31.40%), n-hexane 33.000%).

However, histopathological studies, it was observed that the plain venom administered to rats caused serious tissue damage to liver and thigh muscels in Fig. 4A and 5A 24 h after and these damage was completely neutralized by Uvaria chamae fractions in Fig. 4B to 4D and 5B to 5D groups. This observed histopathology neutralization capacity by Uvaria chamae fractions is in agreement with the work of Olumese *et al*. (2016). The anticytotoxin and neutralization by the extract could be due to ability of the fractions to inhibit cell membrane pores of the skeletal muscle cells induced by the snake venoms and also to prevent the breaking down of phospholipid bilayers in the cell membrane. In vitro experiments using model membranes (Gasanov *et al*., 1990; Dubovskii *et al*., 2014), have shown that the hydrophobic core of cytotoxins represents the principal membrane-binding motif and upon binding, cytotoxins

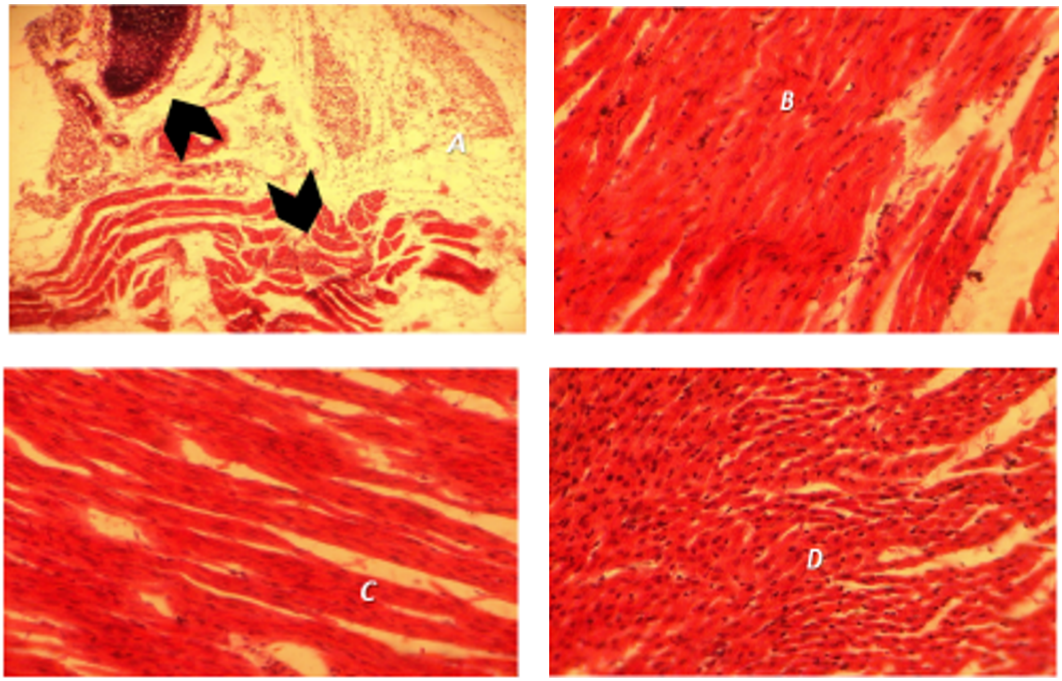

Fig. 5: Photomicrograph showing the panoramic view (H & E x 200) of section of thigh muscle of albino rat (A) Model control, blacks arrows showing areas of muscle fibers degeneration and (B C and D) Rat treated with ethyl acetate, n-butanol and n-hexane fractions against the Naja nigricollis venom and observed for 24 h

produce structural defects in lipid bilayers. The histopathological study established that the venoms caused serious damage to connective tissues in rats 24 h after injection of plain venoms to positive control group. Iddon *et al*. (1987) had earlier described Naja nigricollis venoms to cause necrosis. The venoms of Naja nigricollis could induce both haemorrhage and myonecrosis in muscular tissues. The finding from our histopathological study is in agreement with the work of Gürkan and Hayretdağ (2012), who observed haemorrhage and necrosis in muscular tissues at the point of injection. Koh *et al*. (2006) pin piont the presence of phospholipase A_2 and myotoxin in elapids and viparids. The observed necrosis and damage to tissues was apparently neutralized in those treated groups in which venoms and fractions were co-administered compared to the positive control.

All fractions of Uvaria chamae significantly ($p<0.05$) neutralized the pathological lesions seen in the vital organs after treatment. It was observed that the present experimental results indicate that U. chamae extract was effective in ameliorating the toxic effects of Naja nigricollis venoms and may provide alternative or complementary treatment strategy of envenomation in Naja nigricollis victims. Further studies should isolate and characterize U. chamae fractions in order to identify the bioactive compounds responsible for the observed efficacy, establish safety and the antiophidian mechanism of action which could possibly lead to the development of pharmaceutical formulations for treating snakebite envenomation.

CONCLUSION AND RECOMMENDATIONS

Conclusions: In this study, we were able to identify some phytochemical compounds such as alkaloids, steroids/triterpenes, saponins, phenols, tannins and cardiac glycosides in Uvaria chamae, that maybe responsible for the observed antivenom activity.

Interaction of such compounds with the toxins/enzymes from snake venoms especially N. nigricollis leads to the neutralization/inhibition of their activities like Lethality, Haemorrhagic and Myocytic activities.

Recommendations:
Based on the findings, the following recommended:

- Uvaria chamae's potentials should be further investigated to identify candidates for development of tools for treatment and managment of Naja nigricollis and other venomous snake species envenomations.
- More studies should be conducted to identify the exact mechanism of action of the constituents of Uvaria chamae with a view to improve their antivenom potency.
- A convenient non-rodent animal model should be used to evaluate the effect of Uvaria chamae extract on N. nigricollis envenomation.
- Investigations on the geographical and seasonal variations of the bioactive constituents of the plant stem and leaves

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