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In Vivo Neutralization of *Naja nigricollis* Venom by *Uvaria chamae*

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ABSTRACT

Uvaria chamae is a well known medicinal plant in Nigerian traditional medicine for the management of many diseases, but investigations concerning its pharmacological characteristics are rare. In this study, we evaluate its venom neutralizing properties against *Naja nigricollis* venom in rats. Freshly collected *Uvaria chamae* leaves were air dried, powdered and extracted in methanol. To study the antivenom properties, albino rats were orally administered with a dose of 400 mg kg^{-1} body weight and one hour later, the venom was administered intraperitoneally at a dose of 0.08 mg kg^{-1} body weight of rats. Albino rats (male) weighing between 180-200g were randomly divided into five (5) groups of three (3). Groups 1-5 received water, normal saline, venom, *Uvaria chamae* and venom, *Uvaria chamae* respectively. Blood clothing time, bleeding time, antipyretic activity, haemoglobin, RBC, WBC, creatine kinase, AST, ALP and ALT activities total protein antioxidant activity and some blood electrolytes, plasma urea and uric acid were measured. Our results showed that *Uvaria chamae* methanol extract neutralized some biological effects of *Naja nigricollis* venom. The venom increased the rectal temperature, enzyme activities, bleeding time and other blood parameters. The plant extract was able to reduce these parameters in the extract treated groups. Details of the results are discussed. From this study, it is clear that *U. chamae* leaf extract had antivenom activity in animal models. The above results indicate that the plant extract possess potent snake venom neutralizing capacity and could potentially be used for therapeutic purpose in case of snake bite envenomation.

Keywords: Packed Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC), *U. chamae* Methanol Extract Neutralized, Antipyretic Activity, Packed Cell Volume (PCV)

1. INTRODUCTION

Snake venom is a complex mixture of many substances, such as toxins, enzymes, growth factors, activators and inhibitors with a wide spectrum of biological activities (Mady, 2002; Badr *et al*., 2012). They are also known to cause different metabolic disorders by altering the cellular inclusions and enzymatic activities of different organs.

Snake bite is an important cause of mortality and morbidity and it is one of the major health problems in Nigeria. Snake bite often results in puncture wounds inflicted by the animals. Although, the majority of snake species are non-venomous rather than venomous, snakebite remains an important medical problem in both developing and developed countries (Williams *et al*., 2010). Snake bite pose a major health risk in many countries, with the global snake bites exceeding 5,000,000 per year (Mariane *et al*., 2011) Snake bite envenomations are frequently treated with parenteral administration of horse or sheep-derived antivenoms aiming at the neutralization of toxins. But despite the

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success of serum therapy, it is important to search for different venom inhibitors, either synthetic or natural, which would complement the action of antivenoms, particularly in relation to the neutralization of local tissue damage (Cardoso *et al*., 2003). Plant extracts constitute an extremely rich source of pharmacologically active compounds and a number of extracts has been shown to act against snake venom (Martz, 1992). The medicinal value associated with a plant can be confirmed by the successful use of its extract on snake bite wounds (Mors *et al*., 2000; Otero *et al*., 2000a; Soares *et al*., 2004). Application of medicinal plants with anti-snake venom activities might be useful as first aid treatment for victims of snake bites, which is particularly important in local areas where antivenoms are not readily available (Otero *et al*., 2000b; 2000c; Nunez *et al*., 2004; Sanchez and Rodriguez-Acosta, 2008). More so, antivenoms have some disadvantages, thus limiting their efficient use (Chippaux and Goyffon, 1998; Heard *et al*., 1999; Silva *et al*., 2007). For example they can induce adverse reactions ranging from mild symptoms to serious (anaphylaxis) and in addition, they do not neutralize the local tissue damage (Gutierrez *et al*., 2009).

Thus, complementary therapeutics needs to be investigated, with plants being considered as a major source (Soares *et al*., 2005). In many countries, plant extracts have been used traditionally in the treatment of snake bite envonomations. Thus, vegetal extracts have been found to constitute an excellent alternative with a range of anti snake venom properties. However, in most cases, scientific evidence of their antiophidian activity is still needed. The exact mechanisms of action of ;the plant extracts remain largely illusive, however, a number of previous reports indicate that plant- derived compounds, such as rosmarinic acid (Ticli *et al*., 2005; Aang *et al*., 2010) quercetin (Nishijima *et al*., 2009) and glycyrrhizin (Assafim *et al*., 2006) can inhibit biological activities of some snake venoms invivo and invitro.

Uvaria chamae is a Nigerian medicinal plant that belongs to the family, Annonaceae. It is commonly called by the Igala people of Kogi State as ayiloko, kaskaifi by the Hausas, oko oja by the yorubas in Nigeria as well as akotompo by the Fula-fainte of Ghana. It is a medicinal plant used in the treatment of fever and injuries (Kola *et al*., 2008). These are other oral claims that the plant can cure abdominal pain, used as treatment for piles, wounds, sore throat diarrhea.

The aim of the present study was to evaluate the ability of *Uvaria chamae* extract to neutralize some biological effects of *Naja nigricollis* venom in rats.

2. MATERIALS AND METHODS

2.1. Chemicals, Solutions and Equipment

All chemicals used in the present study were of analytical grade and purchased from reputable company (BDH, UK). Kits of triglycerides, total cholesterol, creatine kinase, AST, ALT, ALP were from Randox laboratories (UK). UV/visible spectrophotometer (Shimadzu) centrifuge (Heraeus Christ GMBH Estrode), Analytical balance, measuring cylinder, micropipette, mortar, pestle Digital thermometer and deep freezer.

2.2. Plant Material Collection and Extract Preparation

Fresh leaves of *Uvaria chamae* were collected from farm located in Odogomo in Ankpa Local Government area of Kogi State, Nigeria. The plant was identified taxonomically and authenticated by Mr. Patrick Ekwuno, a botanist in the Department of Biological sciences, Kogi State University, Anyigba, Nigeria. The fresh leaves were air-dried for four weeks, powdered using mortar and pestle and stored in an airtight container. *Uvaria chamae* leaf powder (200g) was extracted in 500 mL of methanol using cold maceration for 48 h. After that, sample solution was filtered through 0.45 mm filter to remove the insoluble materials. The filtrate was concentrated by removing the solvent completely using a water bath. For oral administration, extract was dissolved in 10 mL^{-1} Phosphate Buffer Saline (PBS). To make the extract soluble in PBS, 1% tween 80 was used.

2.3. Animal Model

Wistar albino rats (male) weighing between 180-200g was obtained from Mr. Emmanuel Titus Friday, Department of Biochemistry, Kogi State University, Anyigba, Nigeria. This study was approved by the Department of Biochemistry according to the institutional ethics. These animals were used as approved in the study of snake venom toxicity. Rats were allowed to acclimatize for two weeks with access to clean water and animal feeds (supplied by Top feeds, Anyigba, Nigeria) in the experimental site. They were maintained in standard conditions at room temperature, 60±5% relative humidity and 12 h light/dark cycle.

2.4. Experimental Design

Wistar albino rats were randomly divided into five groups of three rats:

Group 1: Control group that received only water (2 mL)
Group 2: Control group that received normal saline (2mL)
Group 3: Envenomed rats that did not receive any drug treatment.
Group 4: Envenomed rats treated with *U. chamae* extract
Group 5: Control group that received *U. chamae*

The extract was administered orally at a dose of 400 mg kg^{-1} body weight of rats and one hour later, the venom was administered intraperitoneally at a dose of 0.08 mg kg^{-1} body weight of rats.

Before and after envenomation, the rectal temperature was measured. After envenomation, different parameters such as bleeding time, clotting time, enzymes activities, (creative kinase, AST, ALP and ALT), electrolytes, plasma cholesterol and triglycerides were measured. Collected blood samples (2 mL) were centrifuged at 400 r. p. m for 10 m to separate the plasma.

Determination of activity of *U. chamae* on blood coagulation system (clothing and bleeding time) in rats.

Bleeding time-for the determination of the bleeding time, modified procedure of Mohamed *et al*. (1969) was used. 4 h after the treatment of the animals, the tail of each rat was gently pieced with lancet. A piece of white filter paper was used to blot the blood gently from the punctured surface of the body. The readings were taken every 15 sec. The end result occurs when the study was no longer stained with blood.

Clotting time-clotting time is the time required for a firm clot to be formed in fresh blood on glass slides. The blood sample was collected from, the rats via tail bleeding and a drop was placed on a clean plain slide and every 15 sec, a tip of office pin was passed through the blood until a thread-like structure was observed between the drop of blood and tip of the pin. The thread-like structure was an indication of a fibrin clot. The time taken for this to form was recorded.

2.5. Determination of Antipyretic Activity

The method of (Sahu *et al*., 2012) was used to evaluate the antipyretic activity of the extract. The rats were fostered overnight and rectal temperature was recorded using digital thermometer with a rectal probe. The rectal temperature was recorded before and after envenomation.

2.6. Blood Sample Collection and Measurement of some Haematological Parameters

At the end of the experimental period, the animals were made inactive by chloroform anaesthization. Blood samples were collected via cardiac puncture into EDTA bottles to prevent coagulation. The blood samples were centrifuged for 5 m, results were read on the hematocrit reader for Packed Cell Volume (PCV), White Blood Cell (WBC) Red Blood Cell (RBC) and hemoglobin level, platelet as described by (Baker and Silverton, 1985).

2.7. Enzyme Activity Assays

Creative kinase activity assay: the activity of creatine kinase was determined according to the method described by (Mohammad *et al*., 2008). Randox CK110 kit was used for the quantitative invitro determination of the enzyme activity. The creatine kinase activity was calculated using the formular: U/L = 8095 X ΔA at 340 nm/m. Where ΔA = change in absorbance.

2.8. Alkaline Phosphatese (ALP) Activity Assay

The activity of this enzyme was measured as described by Schmidt and Schmidt, 1963. A portion (0.5 ml) of ALP substrate was dispensed into labeled test tubes and equilibrated to 37°C for three m. At an interval of 2 m, 0.05 mL^{-1} of each of standard, control and sample were added to respective test tubes and gently mixed. Deionized water was used as reagent blank. The tubes and their content were then incubated for 10 m. Following the same sequence as given above, alkaline phosphatease color developer was dispensed into the tubes and thoroughly mixed. The absorbance of each sample was read at 590 nm and recorded using spectrophotometer. The activity of the enzyme was calculated thus:

$$\text{Enzyme activity } (U/L) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X value of stand}$$

2.9. Alanine Aminotransferase (ALT) Activity Assay

The measurement of ALT activity was performed following standard methods, using Randox reagent kits.

2.10. A Spartate Animo Transferase (AST) Activity Assay

AST activity determination was as described by (Reitman and Frankel, 1957).

A portion (0.5 mL^{-1}) of buffer was dispensed into all test tubes and 0.1 mL^{-1} of distilled water, standard, control and sample were dispensed into respective tube, mixed and incubated for 30 m at 37°C. After incubation, 0.5 ml of 2, 4-dinitrophenyl hydrazine was dispensed into respective test tubes, mixed and allowed to stand for 20 m at 25°C. A portion (5.0 mL^{-1}) of 1.0 m sodium hydroxide was then dispensed into the tubes, mixed thoroughly and the absorbance read at 540 nm after 5 m.

2.11. Determination of Plasma Triglyceride

The plasma triglyceride level was measured according to the method described by (Tietz and Finley, 1990). Randox TR 210 assay kit was used for the quantitative invito determination of triglyceride in plasma. Triglyceride concentration was calculated using the formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X concentration of standard (MMol / L)}$$

2.12. Determination of Plasma Cholesterol

The plasma cholesterol was measured according to the method described by (Richmmd, 1973) Randox CH 200 kits was used for the quantitative invitro determination of cholesterol in plasma. Using a standard

The concentration of cholesterol in the sample was calculated by the formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X concentration of standard } (\text{mmol / L})$$

2.13. Estimation of Plasma Total Protein and Albumin

The blood plasma obtained from centrifuging was used for the estimation of total protein and albumin following the method described by (Gornal *et al*., 1949; McPherson and Everad, 1972) respectively.

2.14. Estimation of Plasma Electrolytes

The estimation of plasma electrolytes such as sodium, potassium and chloride ion was done using standard method (Nurun and Nargis, 2010).

2.15. Determination of Plasma Urea and Uric Acid

These were determined following standard methods. Carl *et al*. (2011).

2.16. Plasma Creatine Level Estimation

Blood plasma creatine was determined as described by Jaffe (1957).

2.17. Measurement of DPPH Free Radical Scavenging Activity of *U. Chamae*

The free radical scavenging activity of the plant extract was measured employing the modified method of Blois (1985). A portion (1 mL) each of the different concentrations (1.0, 0.5, 0.25, 0.625 mg mL^{-1}) of extracts or standard (quercetin) in a test tube was added 1ml of 0.3mM DPPH in methanol.

The mixture was vortexed and then incubated in a dark chamber for 30 m after which the absorbance was measured at 517 nm against a DPPH control containing only 1ml of methanol in place of the plant extract:

$$\text{Percentage scavenging activity} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.18. Statistical Analysis

The mean value+S.E.M was calculated for each parameter. Results were statistically analyzed by one-way-Analysis of Variance (ANOVA) followed by Benferonis multiple comparison. $p<0.05$ was considered as significant.

3. RESULTS

3.1. Clotting Time

The result of the effects of *U. chamae* against *Naja nigricollis* venom on blood clotting time is as presented in **Table 1**. The clotting of the control (group 3) is lower when compared to the extract treated groups 4 and 5. The plant offered protection against clotting following envenomation.

Values in the same column with the same superscript are considered significant ($p<0.05$) when compared with group 3 (control).

3.2. Bleeding Time

Bleeding time is the time taken for bleeding to stop. As presented in **Table 2**, the bleeding time of the group 3 (control) which was not treated with any drug was higher (140.00±2.774) indicating a deleterious effect of the snake venom. The plant extract treated group (group 4) had a reduced bleeding time when compared with group 3.

Table 1. Effect of *U. chamae* extract on clotting time after envenomation

Treatment groups	Clotting time (sec)
Group 1 administered water	213.00 ± 2.082^{xcb}
Group 2 administered normal saline	213.33 ± 1.453^{xcb}
Group 3 administered venom	160.00 ± 2.774^{ybc}
Group 4 administered *U. chamae* and venom	212.33 ± 1.856^{xcb}
Group 5 administered *U. chamae* only	213.00 ± 1.4433^{xcb}

Values are mean ± S.E.M (η = 3)

Table 2. Effect of *U. chamae* extract on bleeding time after envenomation in rats

Treatment groups	Clotting time (sec)
Group 1 administered water	95.60 ± 2.887^{xbc}
Group 2 administered normal saline	95.00 ± 2.629^{xbc}
Group 3 administered venom only	140.00 ± 5.774^{ybc}
Group 4 administered *U. chamae* and venom	91.66 ± 4.410^{xbc}
Group 5 administered *U. chamae* only	86.66 ± 3.333^{xbc}

Values are mean ± S.E.M (n = 3)

Table 3. Antipyretic activity of *U. chamae* extract in *Naja nigricollis* envenomation

Treatment groups	Rectal temperature (°C) before administration	After administration
Group 1 administered water	33.933 ± 0.784^{xbc}	33.800 ± 0.462^{ax}
Group 2 administered normal saline	34.933 ± 1.202^{xbc}	34.467 ± 0.666^{ab}
Group 3 administered venom only	34.167 ± 0.348^{xbc}	38.933 ± 0.353^{bcd}
Group 4 administered *U. chamae* and venom	32.800 ± 0.513^{xbc}	34.567 ± 0.233^{ba}
Group 5 received *U. chamae* only	33.600 ± 0.964^{xbc}	33.800 ± 0.153^{bd}

Values in the same column with the same superscript are considered not significant ($p>0.05$). Values in the same column with different superscript are considered significant ($p<0.05$) when compared with venom control (group 3).

Values in the same column with the same superscript are considered statistically significant ($p<0.05$) when compared with the control.

3.3. Antipyretic Activity

As shown on **Table 3**, the rectal temperature of group 3 (control) which was not treated but envenomed is higher than group 4 treated with the plant drug. This is an indication of antipyretic activity of the plant.

Values in the same column with different superscript are considered significant ($p<0.05$) when compared with the control (group 3).

3.4. Hematological Parameters

Hematological parameters were significantly ($p<0.05$) reduced in group 3 (envenomed rats) when compared with the extract treated group 4 (**Table 4**). The WBC was most reduced when compared with other hematological parameters. This therefore means that the extract neutralizefd the biological effect induced by the venom in the extract treated group that had increased HGB, WBC, RBC and PCV.

3.5. Lipid Profile

The triglyceride and cholesterol were reduced by the snake venom in rats (group 3) as shown in **Table 5**. This reduction for cholesterol was statistically significant ($p<0.05$) when compared with the extract treated group 4. The extract (*U. chamae*) had some measure of protection against lipolysis induced by the snake venom.

3.6. Enzyme Activity Assay

The result of the effect of *U. chamae* extract on the activities of the enzymes assayed is as presented in **Table 6**.

The snake venom induced increased activity (group 3). The extract treated group i.e., 4 and 5 had reduced enzyme activity in the entire enzyme assayed. These reductions were statistically significant ($p<0.05$) when compared with the control (group 3).

Values in the same column with the same superscript are considered not statistically significant ($p>0.05$). Values in the same column with different superscript are statistically significant ($p<0.05$) when compared with control (group 3).

Values are mean ± S.E.M (η = 3).

Values in the same column with the same superscript are considered significant ($p<0.05$), l (group 3):

CK = Creatine kinase
AST = Aspartate amino trasferase
ALP = Alkaline phosphatase

ALT = Alanine amino transferase

Values in the same column with the same superscript are considered not statistically significant ($p>0.05$). Values in the same column with different superscript are statistically significant ($p<0.05$), when compared with control (group 3):

Table 4. Effect of *U. chamae* extract on some hematological parameters in rats

Treatment groups	HGB (g/dI)	WBC($X10^9$/L)	Platelet ($X10^9$/L)	RBC ($x10^{12}/_2$)	PCV (%)
Group 1 administered water	16.967 ± 1.391^{xcb}	9.906 ± 6.872^{xbc}	$9.887\pm3,896^{xbc}$	9.887 ± 3.896^{xbc}	43.497 ± 0.673^{xbc}
Group 2 Received normal saline	14.400 ± 1.274^{xcb}	8.033 ± 3.246^{xbc}	8.910 ± 3.517^{xbc}	8.910 ± 3.577^{xbc}	40.917 ± 0.597^{xbc}
Group 3 administered venom	5.833 ± 0.933^{bbc}	3.567 ± 3.661^{ybc}	4.287 ± 3.428^{ybc}	4.287 ± 3.428^{ybc}	25.807 ± 0.582^{ybc}
Group 4 administered venom nd *U. chamae*	13.200 ± 1.002^{xbc}	8.067 ± 0.994^{xbc}	8.513 ± 6.193^{xbc}	8.513 ± 6.193^{xbc}	39.333 ± 0.634^{xbc}
Group 5 Received *U. chamae* only	13.147 ± 0.876^{xbc}	8.967 ± 5.798^{xbc}	9.483 ± 2.375^{xbc}	9.483 ± 2.375^{xbc}	

Values are mean ± S.E.M (η = 3)

Table 5. Effect of *U. chamae* extract on two plasma lipid profiles in rats after *Naja nigricollis envenomation*

Treatment groups	Rectal temperature (°C) before administration	After administration
Group 1 administered water	1.426 ± 0.0821^{xbc}	4.920 ± 0.019^{xbc}
Group 2 received normal saline	1.493 ± 0.098^{xbc}	4.820 ± 0.0139^{xbb}
Group 3 received venom	1.337 ± 0.158^{ybc}	1.227 ± 0.113^{ybc}
Group 4 received *U. chamae* and venom Group 5 received *U. chamae*	1.816 ± 0.069^{xbc}	4.329 ± 0.436^{xbd}
	1.426 ± 0.121^{xbc}	4.721 ± 0.324^{xbc}

Values are mean ± S.E.M (η = 3)

Table 6. Effect of *U. chamae* extract on enzyme activities after *Naja nigricollis* envenomation

	Enzyme activities (U/L)			
Treatment groups	CK	ALP	AST	ALT
Group 1 received water	50.663 ± 4.674^{b}	19.440 ± 1.350^{a}	18.160 ± 3.750^{c}	110.470 ± 8.751^{d}
Group 2 received normal saline	48.567 ± 4.690^{b}	19.310 ± 1.551^{a}	18.270 ± 3.250^{c}	110.330 ± 10.720^{d}
Group 3 received venom only	110.630 ± 2.700^{cd}	29.790 ± 1.450^{bc}	281.360 ± 4.360^{d}	131.021 ± 9.411^{e}
Group 4 received *U. chamae* and venom	50.745 ± 0.041^{b}	22.330 ± 0.060^{a}	132.390 ± 1.320^{c}	120.880 ± 0.341^{d}
Group 5 received *U. chamae* only	50.140 ± 4.239^{b}	20.920 ± 0.160^{a}	2018.270 ± 3.250^{c}	111.461 ± 6.390^{d}

Values are mean ± S.E.M (η = 3)

Table 7. Changes in plasma constituents of rats following envenomations and treatment with *U. chamae* extract

Treatment groups	TP (mg/dI)	Alb (mg/dI)	Uric acid (mg/dI)	Urea (mg/d)	Creatinine (mg/dI)
Group 1 received water	5.50 ± 0.85^{b}	1.83 ± 0.16^{c}	3.49 ± 0.60^{d}	16.09 ± 0.55^{aa}	0.08 ± 0.03^{f}
Group 2 received normal saline	5.50 ± 0.35^{b}	1.77 ± 0.06^{c}	5.64 ± 2.17^{dd}	16.09 ± 0.55^{ab}	0.08 ± 0.03^{f}
Group 3 received venom	4.42 ± 0.62^{b}	1.55 ± 0.90^{c}	93.97 ± 5.36^{dc}	9.56 ± 1.74^{dc}	0.33 ± 0.02^{f}
Group 4 venom and extract	5.06 ± 0.30^{b}	2.00 ± 0.29^{c}	52.26 ± 1.56^{da}	10.87 ± 1.90^{ac}	1.94 ± 1.05^{f}
Group 5 received *U. chamae*	5.60 ± 0.60^{b}	2.27 ± 0.19^{c}	5.64 ± 2.16^{df}	14.68 ± 0.36^{ad}	1.19 ± 0.62^{f}

Values are mean ± S.E.M (n = 3)

Table 8. Effect of *U. chamae* extract on plasma electrolytes after snake envemomation

Treatment groups	Na^+(mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)
Group 1 administered water	115.90 ± 8.72^{a}	4.99 ± 1.07	102.91 ± 4.38^{bc}
Group 2 administered normal saline	113.85 ± 3.21^{ad}	5.93 ± 1.54^{f}	102.53 ± 5.51^{bd}
Group 3 administered venom	145.88 ± 1.89^{abc}	4.82 ± 92^{f}	157.16 ± 2.80^{abc}
Group 4 administered venom and extract	133.85 ± 1.32^{aa}	5.85 ± 3.18^{f}	84.15 ± 4.22^{cc}
Group 5 administered extract only	113.85 ± 3.21^{ab}	6.58 ± 1.03^{f}	$85.15.\pm3.23^{dd}$

Values are mean ± S.E.M (η = 3)

HGB = Haemoglobin
WBC = White blood cell
RBC = Red blood cell
PCV = Packed cell volume
TP = Total protein
Alb = Albumin

Table 9. DPPH radical scavenging activity of *U. chamae*

Plant extract/ standard	Concentration (mg/mL)	Percentage of scavenging activity	IC_{50} (mg/mL)
U. chamae	1.000	91.55	
	0.500	72.18	
	0.250	59.68	0.355[a]
	0.125	40.18	
	0.625	19.22	
Quercetin	1.000	90.68	
	0.500	78.60	
	0.250	56.10	0.296[b]
	0.125	41.62	
	0.625	18.58	

Linear equation: y = 199. 0558 X-20.5602 linear equation: y = 69. 25 X + 29.48

3.7. Changes in Protein and some Blood Constituents

As presented in **Table 7**, 18 total protein, albumin, creatinine and urea were reduced by the snake venom in rats. Uric acid concentration was increased and this was reduced by the plant extract in the extract treated group 4. The electrolytes also increased in the envenomated group 3 except for potassium.

Values in the same column with the same superscript are not statistically significant ($p>0.05$). Values in the same column with different superscript are statistically significant ($p<0.05$), when compared with control (group 3).

3.8. Antioxidant Activity of Extract

The result of the antioxidant activity of the plant extract is as presented in **Table 9**.

The free radical scavenging activity of the plant extract (0.355 mg mL^{-1}) is comparable with the standard quercetin used (0.296 mg mL^{-1}).

4. DISCUSSION

Snake bite is an important cause of morbidity and mortality and is one of the major health problems in Nigeria. The most effective and acceptable therapy for snake bite victims is the immediate administration of antivenom following envenomation (Mahanta and Mukherjee, 2001). The orthodox medical treatment of snake venom poisoning so far is limited by the use of anti-venom, which is prepared from animal sera. Although, the use of plants against the effects of snake bites has been recognized, more scientific attention has been given to since last 20 years (Alam and Gomes, 2003). Like plants, snakes venom can also be considered a sophisticated laboratory of biotechnology. The search for bioactive molecules in plants used in folk medicine has been growing in the past few years. In this study we have reported that *U. chamae* neutralized some biological effects induced by *Naja nigricollis* venom including various parameters such as blood clotting time, bleeding time, some hematological parameters, lipid profile, enzyme activities which were measured. The measurement of these parameters in plasma is of importance in the assessment of the pathphysiological state of snake bite victims.

The results suggest that *Naja nigricollis* venom can disturb rat metabolism. The study showed that the extract of *U. chamae* was effective in neutralizing the lethality and the effects of *Naja nigricollis* venom in animals. Several workers have studied the ability of plants as well as their purified fractions to inhibit biological activities of snake venoms (Melo *et al*., 1994; Maiorano *et al*., 2005; Oliveira *et al*., 2005; Cavalcante *et al*., 2007; Lomonte *et al*., 2009; Depaula *et al*., 2010). However, only a few have investigated the neutralizing mechanism of their action. In some cases a direct interaction with catalytic sites of enzymes or with metal ions which are essential for enzymes activities may be involved (Borges *et al*., 2005; Nunez *et al*., 2005).

Regardless of the precise mechanism *U. chamae* appear to be a promising chemical agent for use as first and treatment, or in combination with antiserum. Many snake venoms are known to cause pathological properties associated with haematological disturbances leading to in coagulability of blood. Some local tissue necrosis always accompany envenomation from this snake species. Spontaneous bleeding and coagulation disturbances are some of the haematological effects of *Naja nigricollis* in patients (Warrell *et al*., 1976). The fundermental differences between blood clotting and bleeding determination is that bleeding is associated with integrity of blood vessels while clotting is a function of clotting factors deficiency. The decrease in clotting time level observed in **Table 1** establishes the fact that treatment of animals with extract/venom

mixture abolished the blood incoagulability. The capacity of plasma to form thrombin is also relevant in the blood coagulation system. These entire blood characteristic are affected by the toxic components of *Naja nigricollis* venom (Kini, 2006).

In the envenomated animals (group 3) that were not treated with extract there was significant ($p<0.05$) reduction in clotting time due to the presence of venom. In groups 4 and 5 treated with *U. chamae* extract, the extract neutralized this effect of the venom and the clotting time was maintained at the normal level when compared with the control groups 1 and 2.

Bleeding time is associated with integrity of blood vessels and is known to cause pathological disturbances leading to incoagulability of blood.

In this present study, the level of bleeding time increased significantly ($p<0.05$) in the envenomated animals in group 3 that were not treated with extract. The increase in bleeding time in this group established the blood incoagulability (Kini, 2006).

Pro-coagulability commonly found in cobra venom cause blood coagulation to occur due to its thrombin-like effect and also it can cause the activation of factor X to Xa. The anticoagulant prevents blood from clotting essentially due to the effect of the venom fribrinolysis or fribrinogenolysis or action of phospholipase on platelets or plasma phospholipids. The two chemical may be found in the same venom. The conflicting results obtained in the clotting and bleeding time (**Table 1 and 2**) could be as a result of the presence of pro-coagulant and anticoagulant in the same venom.

Table 3 presents the results obtained from the measurement of antipyretic activity of *U. chamae* extract. The victims of *Naja nigricollis* envenom action also present fever as one of the symptoms of event on action (Warrell *et al*., 1976). Rectal temperature increased signfically ($p<0.05$) in group 3 rats that received *Naja nigricollis* venom compared with the value obtained before envenomation. This effect was neutralized in groups 4 and 5. The result revealed the antipyretic activity of the plant.

As presented in **Table 4**, Packed Cell Volume (PCV) of the envenomed rats were reduced significantly ($p<0.05$), when compare with non-envenomed ones. This is consonance with the report of Mwangi *et al*. (1995).

White blood cells are effectors of the immune system,(in group 3 there was significant reduction in the WBC compared to group 4 that received venom and extract . This suggests that the plant extract must have combated the venom directly without cells of the immune system produceing effectors cells. Pathological properties of *Naja nigricollis* are mainly associated with haematological disturbances leading to hemorrhage. The platelet inhibition was not due to either serine proteases or metalloproteinase which may be present in the venom. In this study it was demonstrated that the venom effectively inhibits clot formation and platelet aggregation.

The reduction in number of platelets in blood also leads to spontaneous bruising and prolonged bleeding as observant on **Table 2**.

Haemoglobin is the principal molecule responsible for the transport of both oxygen and carbon oxide in blood in group 3 the haemoglobin level decreased due to the effect of the venom compared to group 4 and 5.

The results of the effects of *U. chamae* extract on the plasma lipid profiles in rats after *Naja nigricollis* envenomation is as presented in **Table 5**. These are few reports on the effects of snake venom on plague cholesterol and triglyceride levels were observed in group 3 rats. This result suggests that the snake venom might have mobilized lipids from adipose and other tissues. Lipolytic enzymes, which are present in many snake venoms, could have splitted tissues lipids. With the liberation of free fatty acids. Increase in total plasma lipids levels caused by administration of snake venom and the disturbance of lipids metabolism, could be attributed to liver change and distruction of cell membrane of animal tissues (Vasquez-Colon *et al*., 1966). However, plasma cholesterol and triglycerides have been shown to decrease following some other venoms injection in rats (Meier and Stocker, 1991). In this study, the plant extract offered some protection against the lipolytic activity of the venoms cholesterol is more in the extract treated group than the control (group 3).

As presented in **Table 6**, there was a significant ($p<0.05$) increase in the activity of the enzymes assayed for in group 3 rats when compared with group 4 and 5 that received oral dose of the plant extract in these group the activity of the enzymes were reduced suggesting protective effect of the plant. The increase in enzyme activities in group 3 might be due to muscle necrosis causing the enzymes to leak out of the muscle in to the plasma. The present study revealed (**Table 7**) that the injection of crude venom of *Naja nigricollis* caused reduction in total protein albumin, urea, creatinine and increase uric and concentration in envenomated rats (group 3) but these blood constituents were increased in the extract treated groups. It might be assumed that, the reduced levels of these constituents could be due to disturbances in renal functions as well as haemorrhages in some internal organs. In addition, the

increase in vascular permeability and haemorrhages in vital organs due to the toxic action of various snake venoms were described by (Marsh *et al*., 1997). The increased values of these blood constituents in the extract treated groups 4 and 5 are indication of the protective effect of *U. chamae*.

There are few investigations regarding the effect of snake venoms on serum electrolytes. An initial decrease in blood sodium and initial increase in blood potassium following W. Aegyptia and E. coloratus envenomation in rats has been reported (Al-Jammaz, 1995). In this present study snake venoms produced increased sodium and chloride levels and reduction in potassium, in the undecorated rats (**Table 8**). The disturbance in electrolyte levels might be due to acute renal failure and glomerular tubular damage (group 3).

The extract treated group 4 showed reductions in electrolyte levels implying neutralization of the venom toxicity.

The DPPH (2, 2-diphenyl-1 picrylhydrazyl) radical is considered to be a model lipophilic radical. The radical scavenging activity of *U. chamae* was determined from the reduction in absorbance at 517nm due to scavenging of stable DPPH free radicals. The scavenging effect of the leaf extract on the DPPH radical is shown in **Table 9**. This positive DPPH test suggests that the sample is a free radical scavenger. The neutralizing effect of the plant on the snake venom toxicity could as well be linked to the free radical scavenging properties of U. chance extract. The free radical scavenging activity of the plant is concentration dependent and this is a good attribute of pharmacological agents.

5. CONCLUSION

The present experimental results indicate that *U. chamae* extract was effective in neutralizing the toxic effects of *Naja nigricollis* venom and or has an alternative or complementary treatment strategy of envenomation by *Naja nigricollis*. Further experiment could address the fractioning of the *U. chamae* extract in order to identify the bioactive compounds responsible for these observations, their efficacy, safety and the antiophidian mechanism of action which could possibly lead to the development of pharmaceutical formulations for treating snake bite accidents-victims.

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