American Journal of Pharmacology and Toxicology, 8 (1): 1-8, 2013
ISSN: 1557-4962

doi:10.3844/ajptsp.2013.1.8 Published Online 8 (1) 2013 (http://www.thescipub.com/ajpt.toc)

Anti-Venom Studies on *Olax viridis* and *Syzygium guineense* Extracts

Omale James, Ebiloma Unekwuojo Godwin and Ogohi Dorathy Agah

Department of Biochemistry, Faculty of Natural Sciences,
Kogi State University, PMB 1008, Anyigba, Kogi State, Nigeria

Received 2012-04-27, Revised 2013-01-16; Accepted 2013-04-12

ABSTRACT

Olax viridis (*Olacaceae*) and *Syzygium guineense* (*Myrtaceae*) are shrubs commonly found in the tropics. They are traditional folkloric medicine for a great number of sicknesses. *Olax viridis* has a wide range of applications in ethnomedicine which include treatment for ulcers, veneral diseases, ringworm, sleeping sickness, diarrhea, fever. *Syzygium guineense* has been reported as antidiarrheal agent. Liquid from the bark and roots have been reported to act as a purgative when mixed with water. Both plants have been claimed to have antivenom properties. However, there are no scientific reports on snake venom neutralizing activities of these plants. The plant samples were collected from Olowa in Dekina Local Government Area in Kogi State, Nigeria. The chemicals and reagents used were of analytical grade. Wistar albino rats (male) weighing between 180-200 g were randomly divided into seven groups of three (3). Groups 1-7 received water, normal saline, venom, venom and *Olax viridis*, venom and *Syzygium guineense*, *Olax viridis* and *Syzygium guineense* respectively. The extracts were administered orally at the dose of 400 mg kg^{-1} b.w of rats and 1 h later, the venom (0.08 mk kg^{-1}) was administered. Pulse rate, blood glucose, rectal temperature, plasma cholesterol, triacylglycerol, creatine kinase activity and edema were measured. Significant neutralization of the effects of *Naja katiensis* venom was observed in the groups of rats that received the extracts. Blood glucose, pulse rate, rectal temperature and creatine kinase activity were elevated in the untreated envenomated groups. These results suggest that oral administration of *Olax viridis* and *Syzygium guineense* extracts possess antivenom property, thus, providing the rationale for their use in treatment of sake envenomation.

Keywords: *Olax Viridis*, *Syzygium Guineense*, *Naja Katiensis*, Venom and Plant Extract

1. INTRODUCTION

Snake bites pose a major health risk in many countries, with the global incidence of snake bites exceeding 5,000,000 per year (Williams *et al*., 2010). This problem is more profound in the developing countries, particularly in areas where the access to medical service and to the antiophidic treatment is challenging (Mendes *et al*., 2008). Although, the majority of snake species are non-venomous and typically kill their prey with constriction, venomous snakes can be found on every continent except Antarctica (Kasturiratne *et al*., 2008). 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 also 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.

In many parts of the world, regular treatment for snake venom accident is serum therapy, which involves the parentheral administration of antiophidian serum (antivenoms). This therapy efficiently neutralizes the systemic toxic effects, preventing death of victims. However, antivenoms have some disadvantages, thus limiting their efficient use (Chippaux and Goyffon, 1998;

Corresponding Author: Omale James, Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, PMB 1008, Anyigba, Kogi State, Nigeria Tel: 234-08068291727

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 long been in use traditionally to treat envenomation (Mors *et al*., 2000). 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; Aung *et al*., 2010), quercetin (Nishijima *et al*., 2009) and glycyrrhizin (Assafim *et al*., 2006) can inhibit biological activities of some snake venoms *in vivo* and *in vitro*.

Olax viridis (*O. viridis*) has a wide range of application in ethnomedicine. In West Africa, the pulverized bark and root are used as dressing for ulcers and treatment of veneral diseases, ring worm (Watt and Bregar-Brandiwik, 1962). In the northern part of Nigeria, the root is used in the treatment of sleeping sickness, as anti-diarrheal agent and the treatment of febrile headache. The leaves are also used as remedy for cough, fever and wound (Ajali and Okoye, 2009).

Syzygium guineense (*S. guineense*) fruits are used as remedy for dysentery. In traditional medicine, liquid from the pounded bark and roots has been reported to act as purgative when mixed with water. The present study was carried out to determine the venom neutralizing effects of *O. viridis* and *S. guineense* in rats.

2. MATERIALS AND METHODS

2.1. Plant Material

Fresh leaves of *Olax viridis* and *Syzygium guineense* were collected from farms located in Olowa in Dekina Local Government Area of Kogi State, Nigeria. The fresh leaves were rinsed with clean water to remove dirt and were air-dried in the laboratory for three weeks and pulverized into fine powders using mortar and pestle. Prior to air-drying, the plant samples were identified in the Department of Biological Sciences (Botany unit), Kogi State University, Anyigba, Nigeria. A voucher specimen has been kept.

2.2. Preparation of Plant Extracts

Powdered samples (200 g) each were extracted using cold maceration for 48 h in 1000 mL^{-1} of methanol. The mixtures were there after filtered. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency. The percentage yields of the extracts were 14.5 and 3.55% for *O. viridis* and *S. guineense* respectively.

2.3. Animals

Wistar albino rats (male) weighing between 180-300 g was obtained from Mr. Friday Emmanuel, 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) 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

2.4.1. Animal Grouping and Treatment

The wistar albino rats were randomly divided into seven groups of three rats.

Group: Control group that received only water (2 mL)
Group 2: Control group that received normal saline (2 mL)
Group 3: Envenomed rats that did not receive any treatment
Group 4: Envenomed rat treated with *Olax viridis*
Group 5: Envenomed rats treated with *Syzygium guineense*
Group: Control group that received only *Olax viridis*
Group: Control group that received only *Syzygium guineense*

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

2.5. Antiedematogenic Activity Evaluation Design

Antiedematogenic property of the extracts was measured in the right hind paw edema model (Bispo *et al*., 2001). The rats were divided into four groups of three rats each.

Group 1: Received *O. viridis* extract and venom
Group 2: Received *S. guineense* extract and venom
Group 3: Received venom only (control)
Group 4: Received indomethacin (positive control)

The extracts were administered orally at the dose of 400 mg kg^{-1} body weight of rats. 1 h later, the animals

were injected subcutaneously in the right hind paw with venom (0.08 mg kg^{-1} body weight). The paw volumes were measured 1, 2, 4, 6 and 24 h after venom injection. Group 4 rats were treated with indomethacin (100 mg kg^{-1} body weight, I.P) as control for anti inflammatory activity. Group 3, negative control was injected with venom only in the right hind paw and with normal saline in the left hind paw. Edema was expressed as percentage of the difference between the left and right paw volumes and compared with venom control.

2.6. Biological Assays

2.6.1. Determination of Pulse Rate

The pulse rate was determined using the femoral artery in the groin of the femur of the hind leg. The rats were restrained and once settled, the pulse rate was taken by placing finger over the femoral artery. The pulse was counted for one min using a stop watch.

2.7. Blood Glucose Determination

The blood glucose level was determined according to the method described by (Nelson *et al*., 2012). ACCU Check glucose test meter was used for the determination of the blood glucose in the experimental rats before and after envenomation. A drop of blood from 2 mL collected via tail bleeding of rats was applied to the strip area containing the chemical leading to glucose dye oxidoreductase reaction, causing colour change to occur. The strip was inserted into the meter and the blood glucose concentration was displayed. Before the determination the rats were fasted overnight.

2.8. Antipyretic Activity Determination

The method of (Laura and Dorian, 2008) was used to evaluate the antipyretic activity of the extracts. The rats were fasted overnight and their rectal temperature was recorded using digital thermometer with a rectal probe. The rectal temperature was recorded before and after envenomation.

2.9. Creatine Kinase Activity Assay

The activity of serum creatine kinase was determined according to the method described by Szasz *et al*. (1976). Randox CK 110 kit was used for the quantitative *in vitro* determination of the enzyme activity. The creatine activity was calculated using the formula: U/I = 8095 X ΔA at 340nm/min where ΔA = Change in absorbance.

2.10. Plasma Triglyceride Level Measurement

The plasma triglyceride level was determined according to the method described by Tietz *et al*. (1990). Randox TR 210 kit was used for the quantitative *in vitro* determination of triglyceride in plasma.

Triglyceride concentration was calculated using this formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mmol / L)}$$

2.11. Determination of Plasma Cholesterol

The plasma cholesterol was measured by the method of Richmond, 1973. Randox CH 200 kit was used for the quantitative i*n vitro* determination of cholesterol in plasma. Using a standard, the concentration of cholesterol in the sample was calculated by the formula:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mmol / L)}$$

2.12. Statistical Analysis

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

3. RESULTS

3.1. Pulse Rate

In the pulse rate study, there was a significant ($P<0.05$) increase in the pulse rate of the group (3) administered *Naja katiensis* venom compared with the control groups (**Table 1**). Reduction in the pulse rate of the extract treated groups following envenomation was observed, thus, indicating hypotensive effect of the extracts and this reductions were statistically significant ($P<0.05$) when compared with group 3.

3.2. Blood Glucose

The effects of the two plant extracts on the blood glucose level of rats following envenomation is presented in **Table 2**. The plant extracts significantly ($P<0.005$) reduced hyperglycemia induced by the snake venom (Group 3). As seen in **Table 2** for group 6 and 7, the extracts reduced blood glucose level indicative of hypoglycemic properties of the plants.

3.3. Antipyretic Activity

In the antipyretic study, the rectal temperature of group 3 animals after envenomation is 40.00±0.404°C and 34.06±0.493°C before envenomation. The plant extract treated groups showed significant ($P<0.05$)

reduction in rectal temperature (**Table 3**). *S. guineense* showed more antipyretic activity than *O. viridis*.

3.4. Creatine Kinase Activity

There was elevated creatine kinase activity in group 3 rats (**Table 4**). The extract treated groups 4 and 5 showed reduced activities of the enzyme significantly ($P<0.05$). *S. guineense* had more protective effect than *O. viridis*.

3.5. Lipid Profile

The lipid profiles were reduced by the venom, 1.157±0.078 and 1.217±0.110 mmol/L for triglyceride and cholesterol respectively in group 3. The extract treated groups (**Table 5**) offered some protection for both lipids even though this is not statistically significant when compared with the control (group 3) for triglyceride but significant for cholesterol. Values in the same column with the same superscripts are considered not significant ($P>0.05$). Values in the same column with different superscripts are statistically significant ($P<0.05$), when compared with control (group 3).

3.6. Antiedematogenic Effect of the Plant Extracts

The snake venom (*Naja katiensis*) produced a rapid on set in paw edema in group 3 but not in group 1, 2 and 4 (**Table 6**). The plant extracts reduced edema formation in the treated groups and the reduction is comparable to the standard drug used (indomethacin).

Table 1. Effect of the extracts on pulse rate (per minute) of rats after envenomation

Treatment groups	Pulse rate before administration	Pulse rate after administration
Group 1 administered Water	60±1.202^{a}	61±1.528^{ab}
Group 2 administered Normal Saline	56±1.856^{a}	60±1.764^{ac}
Group 3 administered Venom only	57±0.088^{a}	75±1.732^{ad}
Group 4 administered *O. viridis* and venom	56±0.88^{a}	61±2.646^{ae}
Group 5 administered *S. guineense* and venom	57±1.453^{a}	58±1.764^{af}
Group 6 administered *O. viridis* only	58±1.155^{a}	63±3.930^{ag}
Group 7 administered S. gunieense only	57±1.453^{a}	58±1.764^{ah}

Values are mean ± SEM (n = 3)
Values in the same column with the same superscripts are considered not significant ($P>0.05$). Values in the same column with different superscripts are considered significant ($P<0.05$) when compared with group 3 (control).

Table 2. Effect of the plant extracts on blood glucose after *Naja katiensis* envenomation in rats

Treatment groups	Glucose (mg/dl) before administration	Glucose (mg/dl) after administration
Group 1 administered Water	89.67±1.553^{b}	80.66±3.180^{bb}
Group 2 administered Normal Saline	107.33±0.133^{b}	97.33±1.881^{bc}
Group 3 administered Venom	104.67±9.330^{b}	137.00±6.028^{bcd}
Group 4 administered *O. viridis* and venom	101.00±2.082^{b}	106.00±2.000^{bd}
Group 5 administered *S. guineense* and venom	133.00±2.868^{bd}	103.33±2.848^{be}
Group 6 administered *O. viridis* only	102.00±2.517^{b}	90.33±4.333^{bf}
Group 7 administered *S. gunieense* only	99.00±2.082^{b}	88.33±2.333^{bg}

Values are mean ± SEM (n = 3)
Values in the same column with the same superscripts are considered not significant ($P>0.05$). Values in the same column with different superscripts are considered significant ($P<0.05$) when compared with control (group 3).

Table 3. Antipyretic activities of the plant extracts in *Naja katiensis* envenomation

Treatment groups	Rectal temperature (°C) before administration	Rectal temperature (°C) after administration
Group 1 administered Water	33.200±0.723^{b}	33.133±0.491^{ba}
Group 2 administered Normal Saline	34.99±0.603^{b}	32.500±0.929^{bb}
Group 3 administered Venom only	34.067±0.493^{b}	40.000±0.404^{bcd}
Group 4 administered *O. viridis* and venom	33.000±0.854^{b}	34.333±3.486^{bd}
Group 5 administered *S. guineense* and venom	32.800±0.513^{b}	33.667±0.218^{bd}
Group 6 administered *O. viridis* only	33.500±1.127^{b}	33.133±0.712^{be}
Group 7 administered *S. gunieense* only	34.300±0.251^{b}	33.267±0.693^{bf}

Values are mean ± SEM (n = 3)
Values in the same column with the same superscripts are considered not significant ($P>0.05$). Values in the same column with different superscripts are considered significant ($P<0.05$) when compared with the control group 3.

Table 4. Effects of the plant extracts on creatine kinase activity

Treatment groups	Creatine kinase activity (U/I)
Group 1 administered Water	50.663±4.674[a]
Group 2 administered Normal Saline	48.567±4.679[b]
Group 3 administered Venom only	110.630±2.698[cdc]
Group 4 administered *O. viridis* and venom	80.953±4.674[d]
Group 5 administered *S. guineense* and venom	51.268±0.793[c]
Group 6 administered *O. viridis* only	80.347±0.372[f]
Group 7 administered *S. gunieense* only	51.000±7.139[g]

Values are mean ± SEM (n = 3)
Values in the same column with different superscripts are considered significant (P<0.05), when compared with the group 3 (control).

Table 5. Effects of the plant extracts on plasma lipid profiles in rats after *Naja katiensis* envenomation activity

Treatment groups	Triglyceride (mmol/l)	Cholesterol (mmol/l)
Group 1 administered Water	1.426±0.082[a]	4.921±0.18[aa]
Group 2 administered Normal Saline	1.493±0.098[a]	4.820±0.139[bb]
Group 3 administered Venom	1.157±0.078[a]	1.217±0.110[ccd]
Group 4 administered *O. viridis* and venom	1.494±0.287[a]	4.620±0.359[dd]
Group 5 administered *S. guineense* and venom	1.479±0.218[a]	4.651±0.819[ee]
Group 6 administered *O. viridis* only	1.270±0.085[a]	4.823±0.195[ff]
Group 7 administered *S. gunieense* only	1.416±0.086[a]	4.944±0.073[gg]

Values are mean ± SEM (n = 3)

4. DISCUSSION

Snake bites being a major public health problem claim a large number of lives in the African continent and the world at large. Anti-snake venom remains the specific (antidote) for snake venom poisoning. This anti-snake venom are usually derived from horse sera. They contain horse immunoglobulins, which frequently causes complement medicated side effects and other proteins that cause serum sickness and occasionally, anaphylactic shock. Although, the use of plants against the effects of snake bites has been long recognized, more scientific attention has been given to since last 20 years (Alam and Gomes, 2003).

In this investigation, venom neutralizing potential of *O. viridis* and *S. guineense* plant extracts were studied against *Naja katiensis* venom rats. Many biochemical parameters such as blood glucose, lipid profile, creatine kinase activity, pulse rate were measured. The measurement of these parameters in plasma is of importance in the assessment of the pathophysiological state of snake bite victims. The results suggest that *Naja katiensis* venom can disturb rat metabolism and the plant extracts were capable of neutralizing the lethality induced by the venom.

The result of the effect of the plant extracts on pulse rate of rats after envenomation is presented in **Table 1**. There was a significant (P<0.05) increase in the pulse rate of group 3 rats that were administered the snake venom only. This increase in pulse rate might be due to increased metabolic activity or the heart disease (Pangana and Pangana, 2010). In other groups and groups treated with the plant extracts pulse rate was reduced. This reduction in the extract treated groups revealed the hypotensive effect of the plant extracts. In blood glucose level measurement (**Table 2**) there was significant increase in blood glucose in group 3 envenomed rats. Many snake venoms are known to cause hyperglycemia in rats and mice (Al-jammaz *et al*., 1999; Pung *et al*., 2005; Sleat *et al*., 2006). A few venoms induced hypoglycemia.

In the present study, the levels of blood glucose were significantly increased in the envenomated animals in group 3 that were not treated with the extracts. This increase in blood glucose level could be attributed to the effects of venom in glycogen metabolism in the hepatocytes, muscle fibres and medullary catecholamines that stimulate glycogenolysis and gluconeogensis in the tissues (Ohhira *et al*., 1991; Marsh *et al*., 1997). In group 4 and 5 animals that received extracts of *Olax viridis* and *Syzygium guineense*, there was no significant increase in their blood glucose before and after envenomation. Furthermore; there was no significant change in blood glucose level in group 6 and 7 animals which only received the extracts of *O. viridis* and *S. guineense* without envenomation. These results indicate hypoglycemic activity of the plants. This might be due to insulin-like mechanism most probably through the peripheral glucose consumption.

Table 3 presents the result obtained from the evaluation of the antipyretic activity of the plant extracts. There was a significant increase in the rectal temperature of group 3 rats that were injected intraperitoneally with *Naja katiensis* venom but were not treated with extracts when compared with the values obtained before envenomation. The drastic reduction in rectal temperature in extract treated groups is indicative of antipyretic activity of the two plant extracts.

Table 6. Antiedematogenic effects of the plant extracts

	Paw volume (mm) after envenomation				
Treatment groups	1	2	4	6	24 h later
Group 1 received *O. viridis* and venom	56.197±3.081^{xab}	52.357±3.460^{xyz}	45.720±4.535^{xyz}	40.897±2.353^{xyz}	37.683±1.534^{xyz}
Group 2 received *S. guineense* and venom	60.787±3.081^{xab}	54.013±2.953^{xbb}	45.410±4.712^{xac}	40.153±4.152^{xad}	36.097±4.965^{xab}
Group 3 received Venom only	78.67±1.414^{xaa}	76.610±237^{xab}	75.877±1.237^{xab}	75.210±1.792^{xac}	75.337±0.999^{xad}
Group 4 received Indomethacin (100mg kg^{-1})	35.920±0.184^{xcb}	33.670±0.099^{xcd}	33.580±0.123^{xcc}	33.580±0.123^{xcc}	33.257±0.0633^{xcb}

Values are mean ± SEM (n = 4)
Values in the same column and row with different superscripts are considered significant (P<0.05).

There was a significant(P<0.05) increase in the activity of Creatine kinase enzyme assayed for in group 3 rats when compared with group 4 and 5 that received oral doses of the plant extracts (**Table 4**). The plant extracts showed protective effects, the activity of the enzyme was reduced in the extract treated groups. The increase in activity obtained in group 3 might be due to muscle necrosis causing the enzyme to leak out of the muscle into the plasma; however, the plant extracts were able to render protection against this.

The results of the effects of the plant extracts for the plasma lipid profiles in rats after *Naja katiensis* envenomation is as presented in **Table 5**. There are few reports on the effects of snake venom on the rate of lipid metabolism. Decreased plasma 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 splited tissue lipid with the liberation of free fatty acids (Dev and Papasani, 2006). It has also been reported that increased total plasma lipid levels caused by administration of snake venom and the disturbance of lipid metabolism, could be attributed to liver damage and destruction of cell membranes of animal tissues (Al-Sadoon *et al*., 2011). However, plasma cholesterol and triglycerides have been shown to decrease following some other venoms injection in rats (Salman, 2011). In this study, the plant extracts offered some protection against the lipolytic activity of the venom. Cholesterol is more in the extract treated groups than the control (group 3).

In this study, the antiedematogenic effects of the two plant extracts were demonstrated (**Table 6**). The extracts of the plants were able to neutralize the edema induced by *Naja katiensis* venom. One of the consequences of snake bite is local inflammation. The snake venom induces a striking dose-dependednt edema. This snake bite may lead to shock, because of loss of fluid and tissue compression (Garfin *et al*., 1985) which could contribute to the development cardiovascular disturbances. There are many inflammatory mediators which participate in the production of edema in a variety of inflammatory conditions. Among others, histamine, prostaglandins, kinins and leukotrienes could be implicated in the resulting edema in the case of snake venoms. Edema seems to be clearly related with prostaglandin production, because an important reduction of the inflammatory effects is induced by indomethacin, a known inhibitor of cyclooxygenase.

Olax viridis and *Syzygium guineense* extracts significantly (P<0.05) reduced venom induced edema. *Olax viridis* has already been reported as a plant that inhibits inflammation (Ajali and Okoye, 2009). This study therefore confirms the anti-inflammatory property of this plant. Although, this study was not designed to investigate the mechanism of inhibition, it might be said that the two plant extracts are capable of inhibiting the production of mediators involved in the inflammation induced by *Naja katiensis* venom, effect that has been found in studies made with plant extracts (Kiuchi *et al*., 1983). Plant extracts constitute a rich source of novel compounds of potential therapeutic interest in the inhibition of venom toxins. This result suggests that the plant extracts investigated contain anti- inflammatory agents that reduced the *Naja katiensis* venom-induced edema.

5. CONCLUSION

In, the results of the present study indicate the potent snake venom neutralizing capacity of these plant extracts against *Naja katiensis* venom and have the potential of an alternative or complementary treatment strategy of envenomation by *Naja katiensis*. However, further specific studies need to be conducted to discover the exact compounds responsible for these observations,

their efficacy, safety and the antiophidian mechanism of action which could possibly lead to the development of a new chemical antidote for snake envenoming.

6. ACKNOWLEDGEMENT

The researcher are grateful to Mr. Friday T. Emmanuel for his technical assistance on this study.

7. REFERENCES

Ajali, U. and F.B.C. Okoye, 2009. Antimicrobial and anti-inflammatory activities of *olax viridis* root bark extracts and fractions. Int. J. Applied Res. Natural Prod., 2: 27-32.

Alam, M.I. and A. Gomes, 2003. Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. J. Ethnopharmacol., 86: 75-80. PMID: 12686445

Al-Jammaz, I., M.K. Al-Sadoon and A. Fahim, 1999. Effect of LD_{50} dose of Echis coloratus venom on serum and tissue metabolites and some enzyme of male albino rats. J. King Saud Univ. Sci., 11: 61-68.

Al-Sadoon, M.K., A. Fahim, S.F. Salama and G. Badr 2011. The effects of LD50 of Walterinnesia aegyptia crude venom on blood parameters of male rats. Afr. J. Microbiol. Res., 6: 653-659. DOI: 10.5897/AJMR11.395

Assafim, M., M.S. Ferreira, F.S. Frattani, J.A. Guimaraes and R.Q. Monteiro *et al*., 2006. Counteracting effect of glycyrrhizin on the hemostatic abnormalities induced by Bothrops jararaca snake venom. Br. J. Pharmacol., 148: 807-813. DOI: 10.1038/sj.bjp.0706786

Aung, H.T., T. Nikai, M. Niwa and Y. Takaya, 2010. Rosmarinic acid in *Argusia argentea* inhibits snake venom-induced hemorrhage. J. Natural Med., 64: 482-486. DOI: 10.1007/s11418-010-0428-3

Bispo, M.D., R.H.V. Mourao, E.M. Franzotti, K.B.R. Bomfim and M.D.F. Arrigoni-Blank *et al*., 2001. Antinociceptive and antiedematogenic effects of the aqueous extract of Hyptis pectinata leaves in experimental animals. J. Ethnopharmacol., 76: 81-86. DOI: 10.1016/S0378-8741(01)00172-6

Chippaux, J.P. and M. Goyffon, 1998. Venoms, antivenoms and immunotherapy. Toxicon, 36: 823-846. DOI: 10.1016/S0041-0101(97)00160-8

Dev, K.S. and V.S. Papasani, 2006. Modulation of the activity and arachidonic acid selectivity of group X secretory phospholipase A2 by sphingolipids. J. Lipid Res., 48: 683-692. DOI: 10.1194/jlr.M600421-JLR200

Garfin, S.R., R.R. Castilonia, S.J. Mubarak, A.R. Hargens and W.H. Akeson *et al*., 1985. The effect of antivenin on intramuscular pressure elevations induced by rattlesnake venom. Toxicon, 23: 677-680. PMID: 4060178

Gutierrez, J.M, H.W. Fan, C.L.M. Silva and Y. Angulo, 2009. Stability, distribution and use of antivenoms for snakebite envenomation in Latin America: Report of a workshop. Toxicon, 53: 625-630. DOI: 10.1016/j.toxicon.2009.01.020

Heard, K., G.F. O'Malley and R.C. Dart, 1999. Antivenom therapy in the Americas. Drugs, 58: 5-15. PMID: 10439926

Kasturiratne, A, A.R. Wickremasinghe, N.D. Silva, N.K. Gunawardena and A. Pathmewaran, 2008. The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. Lancet, 5: e218-e218. DOI: 10.1371/journal.pmed.0050218

Kiuchi, F., M. Shibuya, T. Konoshita and U. Samkawa, 1983. Inhibition of prostaglandin biosynthesis by the constituents of medicinal plants. Toxicon, 31: 3391-3396. PMID: 6671219

Laura, C.Y. and S.H. Dorian, 2008. Thermal tolerance in bottlenose dolphins (*Tursiops truncatus*). J. Exp. Biol., 211: 3249-3257. DOI: 10.1242/jeb.020610

Marsh, N., D. Gattullo, P. Pagliaro and G. Losano, 1997. The gaboon viper, bitis gabonica: Hemorrhagic, metabolic, cardiovascular and clinical effects of the venom. Life Sci., 61: 763-769. PMID: 9275005

Mendes, M.M., C.F. Oliveira, D.S. Lopes, L.H.F. Vale and T.M. Alcantara *et al*., 2008. Anti-snake venom properties of *Schizolobium parahyba* (Caesalpinoideae) aqueous leaves extract. Phytother. Res., 22: 859-866. DOI: 10.1002/ptr.2371

Mors, W.B., M.C. Nascimento, B.M. Pereira and N.A. Pereira, 2000. Plant natural products active against snake bite--the molecular approach. Phytochemistry, 55: 627-642. PMID: 11130675

Nelson, I.O., P.C. Chioli and I.G. Samuel, 2012. Effects of aqueous leaf extract of Ocimum gratissimum on oral glucose tolerance test in type-2 model diabetic rats. J. Pharmacy Pharmacol., 6: 630-635. DOI: 10.5897/AJPP11.811

Nishijima, C.M., C.M. Rodrigues, M.A. Silva, M. Lopes-Ferreia and W. Vilegas *et al*., 2009. Anti-hemorrhagic activity of four brazilian vegetable species against bothrops jararaca venom. Molecules, 14: 1072-1080. DOI: 10.3390/molecules14031072

Ohhira, M., S. Gasa, A. Makita, C. Sekiya and M. Namiki, 1991. Elevated carbohydrate phosphotransferase activity in human hepatoma and phosphorylation of Cathepsin D. Brazilian J. Cancer, 63: 905-908. PMID: 1648948

Pangana, K.D. and T.J. Pangana, 2010. Mosby's Manual of Diagnostic and Laboratory Tests. 4th Edn., Mosby/Elsevier, Louis, ISBN-10: 0323057470, pp: 1312.

Pung, Y.F., P.T .Wong, P.P Kumar, W.C. Hudgson and R.M. Kini, 2005. Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice. J. Biol. Chem., 280: 13137-13147. PMID: 15668253

Salman, M.M.A., 2011. The acute effects of scorpion (Leiurus quinquestriatus) venom on some clinicalpathological parameters in Guinea pigs. J. Am. Sci., 7: 794-801.

Silva, D.N.M., E.Z. Arruda, Y.L. Murakami, R.A. Moraes and C.Z. El-Kik, 2007. Evaluation of three brazilian antivenom ability to antagonize myonecrosis and hemorrhage induced by bothrops snake venoms in a mouse model. Toxicon, 50: 196-205. PMID: 17466354

Sleat, D.E., Y. Wang, I. Sohar, H. Lackland and Y. Li *et al*., 2006. Identification and validation of mannose 6-phosphate glycoproteins in human plasma reveal a wide range of lysosomal and non-lysosomal proteins. Mol. Cell Proteomics, 5: 1942-1956. PMID: 16709564

Soares, A.M., F.K. Ticli, S. Marcussi, M.V. Lourenco and A.H. Januario, 2005. Medicinal plants with inhibitory properties against snake venoms. Curr. Med. Chem., 12: 2625-2641. PMID: 16248818

Szasz, G., W. Gruber and E. Bernt, 1976. Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clin. Chem., 22: 650-656. PMID: 4240

Ticli, F.K., L.I. Hage, R.S .Cambraia, P.S. Pereira and A.J. Magro *et al*., 2005. Rosmarinic acid, a new snake venom phospholipase A2 inhibitor from Cordia verbenacea (Boraginaceae): Antiserum action potentiation and molecular interaction. Toxicon, 46: 318-327. PMID: 15992846

Tietz, N.W., P.R. Finley, E.L. Pruden and A.B. Amerson *et al*., 1990. Clinical Guide to laboratory Tests. 2nd Edn., Saunders Company, Philadelphia, ISBN-10: 0721624863, pp: 931.

Watt, J.M. and M.G. Bregar-Brandiwik, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa: Being an Account of Their Medicinal and Other Uses. 2nd Edn., E and S Livingstone, Edinburgh, pp: 1457.

Williams, D., J.M. Gutierrez, R. Harrison, D.A. Warrell and J. White, 2010. The global snake bite initiative: An antidote for snake bite. Lancet, 375: 89-91. DOI: 10.1016/S0140-6736(09)61159-4