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Studies on Antivenom Activities of *Ficus Iteophyla MIQ* and *Borassus Aethiopum* Plant Extracts against *Naja Mossandica* Snake Venom

Sarkiyayi S.[1], Sherif B.H.[2] and Godwin A.A.[1]
[1]Biochemistry/Chemistry section, Department of Applied Science, Kaduna Polytechnic, Kaduna, NIGERIA
[2]Biology/Microbiology Department of Applied Science, Kaduna Polytechnic, Kaduna, NIGERIA

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Abstract

The antivenom properties of methanolic extract of Ficus iteophylla miq and Borassus aethiopum plants were investigated against the venom of Naja mossandica snake. Both plant extracts effectively reduced the Naja mossandica venom induced hemolytic activity. The result reveals that hemolysis due to venom was 81.5% without extract but the heomolytic activity was drastically reduced by Ficus iteophylla miq and Borassus aethiopum plant extracts to 41.9% and 61.7% respectively. Pharmacological activities like coagulant, packed cell volume (PCV) and phospholipase A_2 were significantly neutralized by both plants extracts. The activity of phospholipase A_2 decreases from 600μmol/min to 267μmol/min and 400μmol/min by the Ficus iteophylla miq and Borassus aethiopum plant extracts respectively. The investigations revealed that Ficus iteophylla miq and Borassus aethiopum plant extracts posses potent snake venom neutralizing activity. This finding will provide an alternative ways to inhibit venom toxins in snakebite victims and may provide supplemental treatments to serum therapy.

Keywords: Plants, snake venom, *Ficus iteophylla miq*, *Borassus aethiopum, Naja mossandica.*

Introduction

Snake bite is a major socio-medical problem of tropical countries. It has been reported that globally over one million humans are bitten annually by numerous snakes resulting in 70,000 deaths[1]. India has about 216 different species of snake but 53 species are reported to be poisonous, among which most deaths are attributed to the commonest species like viper, cobra and krait. The major pathophysiological problems associated with snake bites generally starts with the systemic side effects like nausea, vomiting, headache, diarrhea, abdominal pain, fall of blood pressure etc, followed by late systemic effects, like neuromuscular blockage, respiratory, hemorrhage etc. sometimes, snake evenomation is associated with local side effects like pain, swelling and necrosis[2].

Snake bite treatment is as variable as the bite and its symptoms. The one and only medical treatment available is the usage of anti sera, since 1854, but the anti sera have its own limitations. Due to its high cost and lack of availability, it is difficult for the rural patients to access anti sera. Most of these symptoms may be due to the action of high concentration of non immunoglobulin proteins present in commercially available hyper immune anti venom[3]. Apart from these mentioned limitations, antiserum produces insufficient protection against snake bite, it fails to provide protective venom induced necrosis, hemorrhage, renal failure and its production is time consuming. Therefore, due to the problems related to Anti venom serum (AVS) therapy, people are always in search for alternative medicines, of which the anti sera treatment have gained much less importance due to their individual limitations and drawbacks[4]. The usage of medicinal plants, in the treatment of various ailments is known to be associated with various medicinal plants which have been used against snake envenomation in folk and traditional medicines. Apart from Indian traditional medicines, Chinese, Greeks and Egyptian are also motioned in the usage of folk and traditional medicinal plants in snake bite treatments. There are already 250,000 – 500,000 varieties of plants found worldwide, out of which 25% are of medicinal value[4].

Over the years, many attempts have been made for the development of snake venom antagonists especially from plants sources since there are limitations in the development of anti sera. Extracts from plants have been used among traditional healers, especially in tropical areas where there are plentiful sources as therapy for snake bite for a long time Therefore, there is a need to have a scientific validation of the folk and traditional herbal medicines, as an alternative therapy in the field of snake bite spheres. Various plants have been worked out in the laboratory as an antidote for snake envenomation. Some of which possesses strong neutralizing activity whereas others possess moderate snake bite antidote. Plants like *Pluchea indicia*, *Hemidesmus indicus, Strychnous nux vomica, Emblica Officinalis, Vitex negundo,* posses strong neutralizing capacities, where as *Aristolochia indicia*, *Andrographis Paniculata, Dollehondrous sp* posses moderate snake venom neutralizing capacity[5,6]. Protective activities of many of these plants against the lethal action of snake venom have been confirmed by biological assays.

The activities of anti venom against snake bites are lacking in the rural areas and as such, many people died of snake bites in the region. Anti serum, being the only therapeutic agent, its development from animal source is time consuming, expensive and has many limitations, in view of this, there is the need to look for local medicinal plants for the treatment of snake bites and drug development. The objectives of this research is to investigate the effects of *Ficus iteophylla miq* and *Borassus aethiopum* plant extracts against venom of Naja mossandica snake and determine the efficacy of the *Ficus iteophylla miq* and *Borassus aethiopum plant* extracts on *Naja mossandica* enveno mation.

Material and Methods

Materials: *Ficus iteophylla miq* and *Borassus aethiopum* plants were obtained from Dr.Bello's farm at kawo Kaduna,brought to Biological science department and identified by officer in charge of habarium with voucher numbers 1894 and 1708 for *Ficus iteophylla miq* and *Borassus aethiopum* respectively.

The venom of *Naja mossandica* was obtained from biological dept Ahmadu Bello Universisy(A B U), Zaria and experimental animals (mice) from pharm dept A B U Zaria

Reagents: All reagents used were of analytical grades

Methodology: Sample collection: The leaves of *Ficus iteophylla miq* and fruit of *Borassus aethiopum* were collected from Dr.Bello's farm at kawo Kaduna, Kaduna state, Nigeria popularly known as cow slaughter. The leaves of *Ficus iteophylla miq* and fruits of *Borassus aethiopum* were dried at room temperature for five weeks and crushed into powdery form and kept. The soxhlet extraction method was used and methanol was used as the extracting solvent

Test for anti-snake venom properties: The following parameters were investigated: Red blood cell fragility test: The red blood cell fragility test was determined by method described by Doughterty[7]. Briefly,200ul of cow's blood were collected in heparinised capillary tubes and transferred to 5ml of sodium chloride buffer solution and centrifuged for 5 minutes. After centrifugation the cells were suspended in 5ml of the same buffer solution and one milliliter of the cell suspension was added in duplicate to test tubes containing 4ml of distilled water. This was repeated for the test samples. All samples were incubated at 37^{o}C for six hours, then mixed and centrifuged. Percentage heamolysis was determined by comparing A_{54O} of the supernatant of the control or test samples with A_{540} of completely heamolysed sample (distilled Water sample).

Calculation :

$$\% \text{ Heamolysis } = \frac{\text{A540 of } of\ Test\ sample}{\text{A540 } of\ completely\ heamolysed\ in\ water} \ x \frac{100}{1}$$

Effect of the extract on crude venom phospholipase A_2: Phospholipase A_2 neutralization has been determined using the method described by Simuzu[8].Thus 100ul of the crude venom was incubated with 100ul of egg folk at 37^{o}c for 30minutes. At the end of the incubation, the reaction was stopped by immersing in boiling water for five minutes. The reaction mixture was titrated using 20mM sodium hydroxide and phenolphthalein as indicator. The same procedure was repeated using extracts incubated with the venom. The volume of sodium hydroxide used to neutralize free fatty acids was recorded and the activity of phospholipase A_2 calculated.

Plasma recalcification time determination: This was done by determining the effect of the venom on recalcification time of citrated plasma using the method described by Theakston and Reich[9]. 200µl of citrated cow's plasma was incubated in water bath at 37^{o}C for 5 minutes. To each sample, 10µl of crude venom was added; the mixture was then diluted with 100ul sodium chloride buffer solution pH 7.0. Finally, 10µl of 25mM $CaCl_2$ was added and the coagulation activity was recorded. The same procedure was repeated for extracts incubated with venom.

Effects of venom/extract on packed cell volume (PCV): Method described by Cole[10]. The mice were grouped into four groups, one mouse each, before the injections the PCV of the mice were determined. Group one, which is the control group was injected with sodium chloride solution (0.9%), group two was injected with venom (0.1mg/kg), while group three was injected with venom (0.1mg/kg) incubated with the *Ficus iteophyla miq* extract (0.1mg/kg), group four was injected with venom (0.1mg/kg) incubated with *Borassus aethiopum extract*. All injections were done intraperitoneally. The PCV of the animals were determined after 24 hours of injection respectively. Blood from the tails of all animals were used for determination of PCV by the micro heamatocrit method described by Cole[10].

Results and Discussion

The figure 1 shows the percentage hemolysis of the test samples and that of completely hemolysed in water at A_{540}.

Table-1
Effects of extracts/venom on plasma recalcification time

Group	Time recorded (minutes)
Venom + Plasma only	2 ±0.12
Venom + Plasma + *Ficus iteophylla miq* extract	3 ±2.08
Venom + Plasma + *Borassus aethiopum* extract	5 ±5.77

Values in table 1 are mean of three determinations. Delay in clothing time of plasma incubated with venom, and venom/extract were given in minutes.

Table-2
Results for the PCV of the mice taken before and after 24 hours of the injection

Group	PCV before injection %	PCV after injection %
Control	27±0.21	32±0.31
Venom only	32±0.32	35±0.22
Venom+*Ficus iteophylla miq* extract	32±0.12	36±.52
Venom + *Borassus aethiopum* extract	28±0.52	30±.43

The results in table 2 are mean ±SD of five determinations.

Snake bite is a common medical emergency encountered in the tropics. The major cause of mortality is due to increased bleeding tendency caused by the venom. Snake venom is a highly complex mixture of a variety of biological substances including 90% of water, 20-25 enzymes, a large number and low molecular weight peptides. Envenomation by *Naja Mossamdica* is associated with a mortality rate of 10-20%, if effective treatment is not initiated early. Venom of *Naja Mossamdica* is a mixture of multiple enzymes and low molecular weight peptides, some of which are responsible for the bleeding manifestations. Multiple mechanisms have been suggested for the bleeding occurring after envenomation. It is suggested that the small dose of venom, as typically injected in humans, leads to continuous activation of fibrinogen, producing a fragile fibrin more susceptible to lyses than ordinary fibrin, leading to bleeding manifestations. Vascular endothelial damaged caused by the hemorrhage present in the venom also contributes to bleeding manifestations[11]. Anti venom is the specific antidote for snake bite envenomation. Antivenom against snake bites are lacking in the rural areas of coastal region. Antiserum; being the only therapeutic agent, its development from animal source is time consuming and expensive. Although, use of plants against the effects of snake bites has been long recognized, more scientific attention has been given since last 20 years[12]. Many Indian medicinal plant as recommended for treatment of snake bites[13]. But in this present project, the anti venom potential of *Ficus iteophylla miq* and *Borassus aethiopum* were investigated. It is essential to understand the pharmacological action of snake venom in order to device a rational treatment for snake bites. Thus, various pharmacological activities like hemolytic activity, phospholipase A_2 (PLA_2), coagulant activity and packed cell volume (PCV) activity caused by *Naja mossamdica* venom were investigated. The results showed that both plant extracts were capable of neutralizing hemolytic activity from 81.5% to 41.9% and 61.7% for *Ficus iteophylla miq* and *Borassus aethiopum* respectively as shown in figure 1. Our findings also reveals that the activities of phospholipase A_2 were neutralized by the two plant extracts from 600µmol/minutes to 267µmol/minutes and 400µmol/minutes for *Ficus iteophylla miq* and *Borassus aethiopum* respectively as revealed in figure 2. The medicinal plants *Thea sinensis Linn* and *Cordia verbenacca* were effectively neutralized the phospholipase activity induced by snake venom[14]. Pro coaqulant activity induced by *Naja mossamdica* venom was studied using cow citrated plasma, *Ficus iteophylla miq* and *Borassus aethiopum* plant extracts were found to be effective in prolonging the coagulant activity as shown in table 1. Packed cell volume activity (PCV) induced by venom was studied using experimental animals.The findings revealed that the effect of venom and venom/extracts on PCV were not very significant as shown in table 2.

Conclusion

Our findings revealed that *Ficus iteophylla mig* and *Borassus aethiopum* plants were found to be effective in neutralizing the main toxic and enzymatic effects of *Naja mossandica* venom. It can be concluded that the results from this preliminary study indicates that both plant extracts could be used for therapy in patients with snake bite envenomation.

Recommendation

The time has come that the knowledge of herbal medicinal should be applied since there are limitations associated with the development of antiserum from animal source as the only therapeutic agent. Finally, the use of herbs should be encouraged by federal government by encouraging extensive research in area and giving it all the necessary support it deserves.

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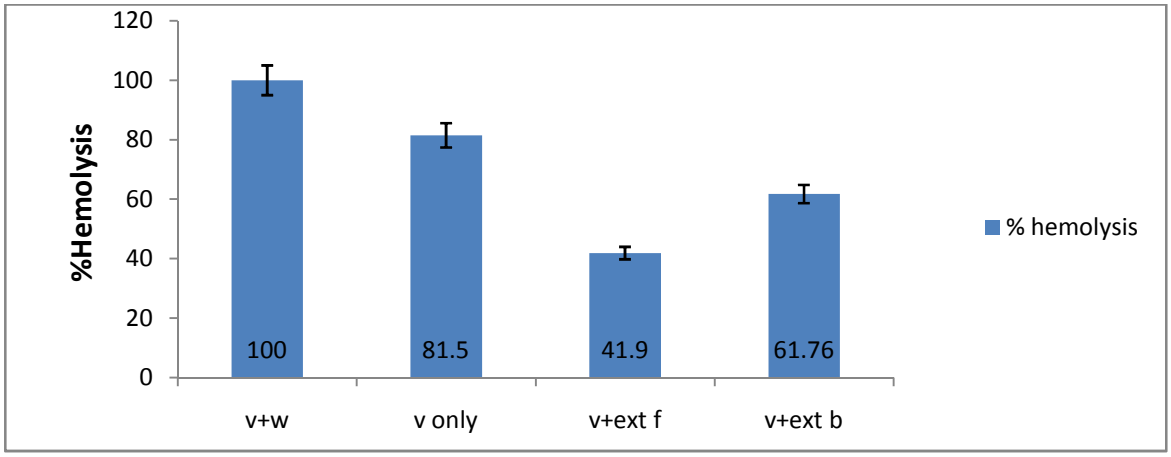

Figure-1
Results of percentage heamolysis of red blood cells due to PLA_2 Activity, Bars represents Mean ±SD for five determinations. Heamolysis was significantly different at P<0.05 for the extract groups

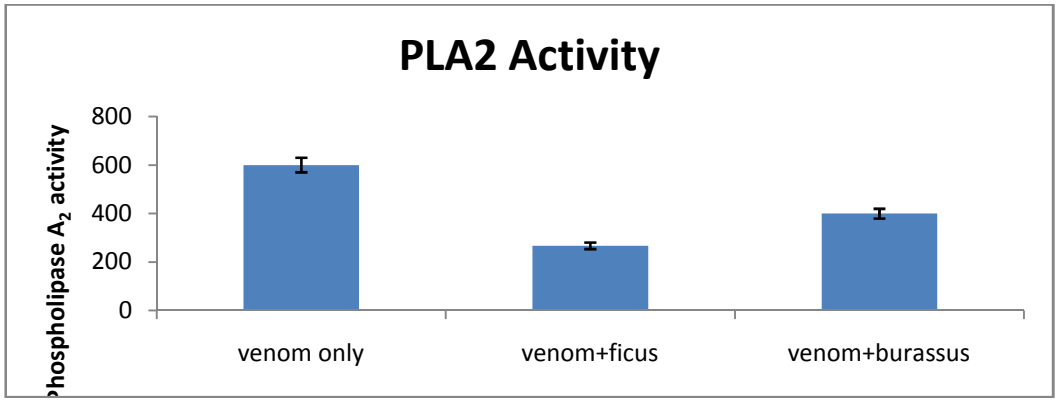

Figure-2
Results of phospholipase A_2 activity on extract/venom, Bars represents Mean ±SD for five determinations. The phospholipase A_2 activity for the extracts groups were significantly different at P<0.05