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Effects of Crude Stem Bark Extract of Kigelia Africana on Local Tissue Damage in Rat Treated With Naja Nigricollis Venom

*Inya-Agha O. R.,[1] Ibiam U. A.,[1] Orji O.U.,[1] Aja P. M.,[1] Ezeani N[1]. and Ebenyi N. L.[2]
[1]*Biochemistry Department, Ebonyi State University, Abakaliki, Nigeria.*
[2]*Biotechechnology Department, Ebonyi State University, Abakaliki, Nigeria.*

Abstract: *This study investigated the effects of crude stem bark extract of Kigelia africana on local tissue damage in albino rats treated with Naja nigricollis venom. Lethal, myotoxic and edema- inducing effects were examined in rats using experiments involving co-injection and separate injection of venom and extract. The venom exhibited a mean lethal dose (LD_{50}) of 1.634mg/kg when administered intraperitoneally (i.p) in rats. At a venom challenge dose of $3\times LD_{50}$,the extract was effective in neutralizing these lethal effects with a median effective dose (ED_{50})of 83.18 mg/kg when the venom and extract were injected together. Separate injection of the extract, 5 and 10 min after the venom challenge, showed ED_{50} of 141.25 and 511.76 mg/kg respectively. In addition, the extract reduced significantly ($\rho<0.05$) myotoxic and edema-inducing activities of the venom evident by decreases in serum creatine kinase levels and footpad weight of rats respectively. The extents of neutralization of venom activities by extract were higher when venom and extract were co-injected than when injected separately. These results suggest that administration of K. africana extract may be useful in the management of N. nigricollis envenomation.*

Keywords: *Snake venom; Naja nigricollis; Kigelia africana; Lethality; Myotoxicity and Edema*

I. Introduction

The black-necked spitting cobra (Naja nigricollis), whose venom triggers local and systemic effects on its victim (WHO, 2010), is a species responsible for most accidents in Nigeria (Paramonte, 2007). There are up to 1.8 million incidences of snakebite worldwide per year leading to up to 94,000 deaths, the majority of which occur in Sub-Saharan Africa, South and Southeast Asia and Latin America (Kasturiratne et al., 2008).

Most snake bite incidences in Nigeria are caused by Echis ocellatus, Echis carinatus, Naja nigricollis and Bitis arietans (Paramonte, 2007). Envenomings by most elapids, such as Naja nigricollis are known to cause serious necrosis of tissues at the bite site, local swellings and blistering (Habib et al., 2001 and Mendez et al., 2011). Proteomic studies of the composition of this venom revealed a high content of cytotoxin/cardiotoxin protein family (Petras et al., 2011).

Plant extracts possess numerous pharmacologically active compounds that are potent in the management of snake envenoming (Soares et al., 2005). Kigelia africana, commonly known as African sausage tree, extracts have reputations as anti-inflammatory (Picerno et al., 2005, Carey et al., 2008), antibacterial (Grace et al., 2002) and anti-cancer activities. It has been mentioned by traditionally healers in Ekoli Edda, Afikpo South L. G. A of Ebonyi State that K. africana is used for treatment of snakebites. In light with the above, the aim of the present study was to investigate the effect of crude stem bark extract of Kigelia africana on the local tissue damage caused by Najanigricollis venom.

II. Materials And Methods

Plant Material

Fresh stem bark of Kigelia africana was collected from Ekoli Edda, Afikpo South Local Government Area, Ebonyi State. The plant was authenticated by Professor S. C. Onyekwelu of Applied Biology Department, Ebonyi State University Abakaliki, Nigeria.

Snake Venom

Lyophilized Naja nigricollis venom was purchased from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria Nigeria.

Animals

Albino rats (100-120 g) were procured from the Animal House of Department of Animal Sciences, University of Nigeria, Nsukka.

Preparation of Plant Extract

The plant extract was prepared according to the methods of Magalhaes et al. (2011). Fresh stem barks (500 g) of Kigelia africana were washed, ground with manual grinder and pressed to get a liquid suspension. This choice of extract was made so as to reflect the way the plant is used by traditional healers, i.e., without solvents, like water or ethanol. Then, the suspension was filtered through Whatman filter paper (No. 1) and the filtrate was evaporated to dryness at room temperature.

Experimental Design

The experiment was designed according to the method of Yingprasertchai et al. (2003). A total of 128 rats were used for this study. The rats were grouped as shown below:
Group I: rats were injected with normal saline alone
Group II: rats were injected with venom alone
Group III – V: rats were injected with venom and varying extract doses.
Groups III – V were further divided into three subgroups as described below:
Zero delay: venom and extract were incubated together at 37°C for 30mins before injection.
5 mins delay: venom and extract were injected separately. Here, the extract was injected 5 minutes after venom
10 mins delay: venom and extract were injected separately. Here, the extract was injected 10 minutes after venom

For neutralization of PLA_2 activity, cytotoxicity and superoxide anions production, zero delay subgroup only was used while for lethality, myotoxicity and edema; the three subgroups were utilized.

Neutralization of Lethality of N. nigricollis Venom

The median lethal dose (LD_{50}) of N. nigricollis venom was determined according to the method of Theakston and Reid (1983). For lethality studies of the venom, five groups of five rats were administered with different doses of venom (1, 2, 4 and 8 mg/kg, i.p) in 500 µl of normal saline. Control groups received 0.5 ml of normal saline only. The rats were monitored for symptoms of venom toxicity and death occurring within 48 hours recorded. LD_{50} of venom was estimated by probit analysis according to the method of Finney (1971). For neutralization studies; the experimental groups were injected intraperitoneally with 500 µl of saline, venom (3 x LD_{50}, 5mg/kg) and extract doses (50, 100, and 200 mg/kg) as may be described in the experimental design. In all cases, the rats were observed for symptoms/signs of toxicity and death occurring with 48 hours recorded.

Neutralization of Myotoxic Activity of N. nigricollis Venom

Myotoxic activity of N. nigricollis venom was determined according to the method of Evans and Ownby (1999) by measuring serum levels of creatine kinase (CK) activities using a reagent kit (Auto span diagnostics). For neutralization studies; the experimental groups were injected intradermally with 50 µl of saline, venom (1/2 x LD_{50}) and extract doses (40, 80 and 160 mg/kg) as may be described in the experimental design. In all cases, the serums of the experimental rats were assayed for CK activities. Activity was expressed as IU/L.

Neutralization of Edema-Inducing Activity of N. nigricollis Venom

Edema-inducing activity of N. nigricollis venom was estimated based on the method of Soares et al. (1998). For neutralization studies; the experimental groups were injected subcutaneously with 50 µl of saline, venom (0.08 mg/kg) and extract doses (4, 8 and 16 mg/kg) as may be described in the experimental design.

Statistical Analysis

Results were presented as mean ± SD and the significance of the differences among experimental groups was evaluated using one-way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

III. Results

Neutralization of Lethality

Results of the lethality of N. nigriollis in albino rats showed that smaller venom doses of 0.5 mg/kg did not induce mortality, and doses of 1.0, 2.0, 4.0 and 8.0 mg/kg produced 40, 60 80 and 100% mortality respectively (Table 1). Local symptoms like local swelling, impaired movement, blistering/bruising and eventual coma were also observed. Using, probit analysis, the LD_{50} value of the venom obtained was 1.634 mg/kg. In the neutralization studies, rats treated with venom challenge dose of $3xLD_{50}$ only were unable to survive within 48 hours (Table 2). Co-injection of the venom and extract (200 mg/kg) offered 100% protection on the rats. However, separate injections of venom, followed by the extract after 5 and 10 mins, provided 40 and 20% protection in the rats, respectively. Venom toxicity symptoms were abrogated in all the survived animals.

Using probit analysis, the median effective doses (ED_{50})of K. afriana extract in zero, 5 and 10 mins delays obtained were 79.94, 141.25 and 221.76 mg/kg respectively. Experiments involving zero delay in venom and extract injection were more effective than 5 or 10 mins delays.

Neutralization of Myotoxic Activity

N. nigricollis venom exerted pronounced myotoxic effects in exposed rats evident by a rise in the level of serum CK activities. In the zero delay group, the extract reduced significantly ($p< 0.05$) the venom- induced myotoxicity in a dose dependent manner (Figure 1). The extract was also effective in neutralizing the myotoxic activities of the venom, if injected within 5min after the injection of venom. Injection of extract dose of 40mg/kg, 10mins after envenomation, did not reduce significantly ($p> 0.05$) the levels of serum CK activities.

Neutralization of Edema

N. nigricollis venom exhibited pronounced edematogenic activity in the footpads of experimental rats, evident by increases in weight of footpads. Co-injection of venom and extract (zero delay groups) reduced significantly ($p< 0.05$), in a dose dependent manner, the weight of the right footpad of experimental rats compared to that of the left footpad (Fig. 2). Separate injection of extract, at 5 and 10 mins delays after venom injection, also reduced significantly ($p< 0.05$) edema in all extract doses except at extract dose of 4 mg/kg, when given 10min after venom injection.

IV. Discussion

Our results have clearly showed that the stem bark of K. africana possesses active components which are capable of inhibiting the enzymatic and toxic activities of N. nigricollis venom.

The results of the lethality studies of N. nigricollis venom revealed a cytotoxic pattern of envenoming. This was characterized by progressive local swelling, impaired movement and blistering/bruising of the injection site. Our results also revealed that these symptoms followed a venom dose dependent manner, leading to death at high doses. Behavioural changes leading to eventual death are physiological indicators used in snake venom toxicity studies (WHO, 2010).

The strategies employed in the neutralization of the lethal effects of N. nigricollis venom include preincubation of venom with K. africana extract prior to injection or separate injection of venom and then the extract, 5 and 10 mins later. In all the strategies employed, our results showed that the extract was effective in abrogating the cytotoxic symptoms, as well as promoting percentage survival of the rats injected with thrice the LD_{50} dose of the venom in a dose dependent manner. However, co-injection of venom preincubated with the extract offered better protection than separate injection of venom, followed by the extract.

The results of the present study revealed that the myotoxic activities of the N. nigricollis venom evident by increases in level of serum creatine kinase. These myotoxicity was associated with symptoms like muscle paralysis and resultant local necrosis. Administration of K. africana extract reduced significantly ($P < 0.05$) the myotoxic activities of the venom in a dose dependent manner. Our results also revealed that delaying the time of extract administration in envenomed rats, lowered the extent of neutralization of the myotoxic activity of the venom.

Local inflammation is, an important characteristic feature of Elapid envenoming, evoked by the action of myotoxic PLA_2 enzymes to release of arachidonic acid metabolites (Teixeira et al., 2003, Ketelhut et al., 2003 and WHO, 2010). In a rat footpad test, our results evidenced the edema-inducing capability of N. nigricollis venom. Incubation of the venom and K. africana stem bark extract prior to injection revealed that the extract was potent in neutralizing edema induced by of N. nigricollis venom in a dose dependent manner. In an alternative approach, the venom was injected separately before the extract at 5 and 10 mins delays. In the latter technique, our results showed poor neutralization of edema in a time dependent manner. In conclusion, the crude stem bark extract of K. africana is effective in the neutralization of the enzymatic and toxic actions of N. nigricollis venom, validating its ethnomedical use in the treatment of snakebites.

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Table 1: Effect of administration (i.p) of N. nigricollis in albino rats.

Venom dose (mg/kg)	Number of rats dead within 48 hrs (n = 5)	Percentage mortality (%)	Local symptoms/signs
0.5	0	0	+
1.0	2	40	+
2.0	3	60	++
4.0	4	80	++
8.0	5	100	+++

+ indicates mild local swelling; ++ indicates prominent local swelling and impaired movement; +++ indicates prominent local swelling, impaired movement, blistering/bruising and coma.

Table 2: Neutralization of lethality of N. nigricollis venom by crude stem bark extract of K. africana.

Groups (n = 5)	No of rats dead within 48hr (n = 5)					
	0 min delay	Survival (%)	5 mins delaydelay	Survival (%)	10 mins	Survival (%)
I	0	100	0	100	0	100
II	5	0	5	0	5	0
III	4	20	5	0	5	0
IV	2	60	4	20	4	20
V	0	100	3	40	4	20

Group I = saline only, group II = venom only, group III - V = Venom + extract (50, 100 and 200 mg/kg) respectively.

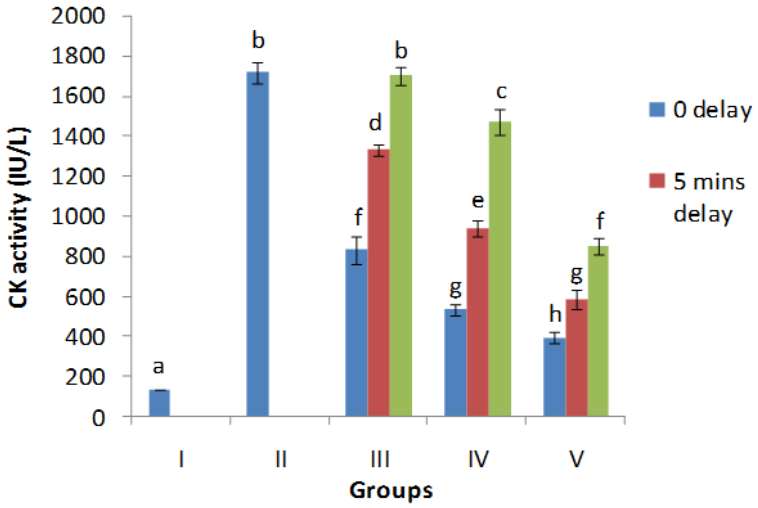

Figure 1: Neutralization of myotoxic activity of N. nigricollis venom by crude stem bark extract of K. africana. Columns with different letters (a, b, c, d, e, f, g, h) differ significantly ($p<0.05$) with each other.Group I = saline only, group II = venom only, group III - V = Venom + extract (40, 80 and 160 mg/kg) respectively.

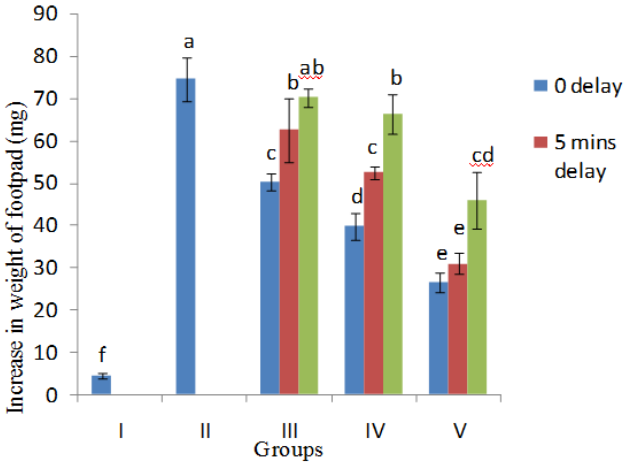

Figure 2: Neutralization of edema-inducing activity of N. nigricollis venom by crude stem bark extract of K. africana. Columns with different letters (a, b, c, d, e, f) differ significantly ($\rho<0.05$)with each other.Group I = saline only, group II = venom only, group III -V = Venom + extract (4, 8 and 16 mg/kg) respectively.