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FRACTIONATION OF THE VENOM OF THE NIGERIAN PUFF ADDER(*BITIS ARIENTANS)*

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Two fractions A and B were obtained from the gel filteration on Sephadex G-75 of the crude puff adder *(Bitis orientans)* venom. When purified on ion-exchange chromatograph, fraction A gave subfractions A_1, A_2, and A_3 while fraction B gave subfraction B_1, B_2. Fractions A and B produced haemorrhagic effect although the individual subfractions A_1, A_2, A_3, B_1 and B_2 did not.

Key words: Nigerian puff adder, Venom, Fractionation.

Introduction

Toxicologically important snakes have fangs at the front of their mouths which enable them to inject venom. These poisonous snakes belong to three families namely, elapids (neurotoxic), vipers (vasculotoxic) and seasnakes (myotoxic).The vipers have long, erectile fangs, triangular heads and usually short fat bodies. Throughout the tropics viper bites are much more common than elapid bites.

The existence of twelve species of venomous snakes in Nigerian savannah has been confirmed. Only three of these are of toxicological importance *Echis carinatus, Naja nigricollis* and *Bitis arientans.*

Snake venoms have been fractionated and their constituent toxins isolated by various techniques [1-8]. The ideal method should allow the initial separation of the venom into as many discrete fractions as possible. The presence of more than one toxin in a snake venom means that fractional precipitation techniques are of limited value. Gel filtration and cation exchange chromatography appear to be the most promising techniques [9].

This paper presents the results of a preliminary investigation on the use of Sephadex gel filtration and a CM-Sephadex ion exchanger for the separation of the toxins from the venom of the Nigerian puff adder *(Bitis arientans).* Toxicity test on the separated toxins in mice was also undertaken.

Materials and Methods

Male in-bred mice, obtained from the animal breeding units of the Biological Sciences Department, University of Benin, Nigeria, weighing 25-30gm were maintained in an air-conditioned (22°±2) animal house. They were fed with a commercial diet (Livestock Feed Products Nigeria Ltd.) for three days for the purpose of acclimatization.

Ten mice were randomly chosen to constitute the control group, while a second group of ten mice represented the experimental group. Both groups were given the commercial diet and water *ad libitum.*

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An adult puff adder (*Bitis arientans*) was milked by a local snake catcher and the venom was dessicated and stored in the dark at 5°.

Gel filtration on Sephadex G-75. A column (1.5 x 93 cm) was packed with Sephadex G-75 fine (Pharmacia) and equilibrated with 0.2 M ammonium acetate buffer pH 7.3 at a flow rate of 30 ml/h. The void volume was then determined employing blue dextran 2000.

0.5 ml (100mg) of the crude venom was dissolved in 2 ml 0.2 M ammonium acetate, centrifuged at 18,000gm for 30 mins and the clear supernatant applied to the column at 22° 0.2 M ammonium acetate was used for the elution and 5 ml aliquots of the eluate was collected. The tube contents were monitored by measuring their absorbance at 280 nm using a unicam SP6-500 spectrophotometer. The optical density (O.D.) values were plotted against tube number and the fractions pooled all appropriate (Fig. 1).

Ion-exchange chromatography on CM-Sephadex. A column (2.5 x 35 cm) of CM-Sephadex, was prepared in and equilibrated with 0.05M ammonium acetate buffer pH 7.3 at a flow rate of 30 ml/h. Fraction A previously obtained from the G-75 column (Fig.1) was applied to the column. The column was eluted and 5 ml fractions of the eluate collected. These were monitored at 280 nm and the O.D. values at 280 nm plotted against tube number. The tube contents were pooled as

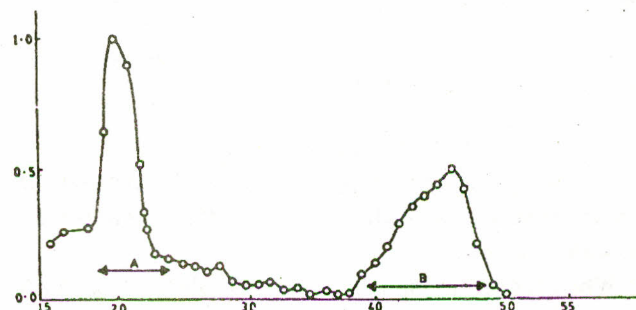

Fig.1. Gel filtration of the crude venom on 1.5 x 93cm column of Sephadex G-75 in 0.2 M ammonium acetate buffer pH 7.3; flow rate 30ml/h.

appropriate (Fig.2), dialysed against distilled water and the protein content determined by the method of lowry *et al.* [10]. Fraction B from G-75 column was similarly treated (Fig. 3). The crude venom (0.025 mg/20 gm body weight of mice) and each of the fractions A_1, A_2, A_3, B_1 and B_2 (in amount corresponding to the amount of crude venom given) were administered intravenously to groups of ten mice.

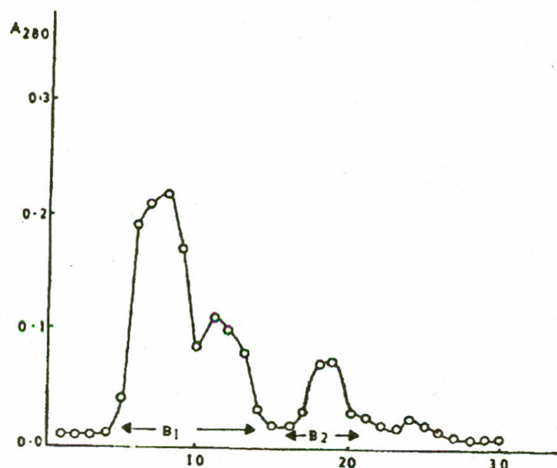

Fig.2. Ion exchange chromatography of Gel filtration fraction A on a 2.5 x35 cm column of CM-Sephadex equilibrated with 0.05 M ammonium acetate buffer pH 7.3; flow rate 30 ml/h.

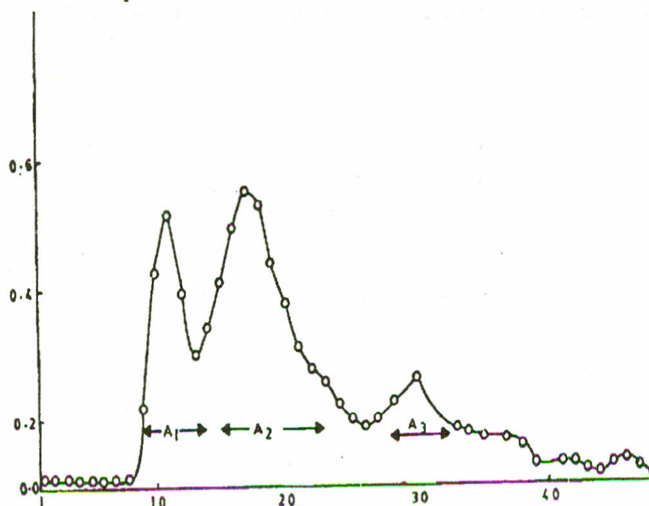

Fig. 3. Ion exchange chromatography of Gel filtration fraction B under the same conditions as used for fraction A.

Results and Discussion

Two peaks A and B were obtained from the elution profile of the crude venom on Sephadex G-75. Lethality was associated with each peak.

When applied to the CM-Sephadex column, fraction A gave three sub-fractions designated (A_1, A_2 and A_3) while fraction B gave two subfractions (B_1 and B_2). Although the individual fractions obtained by the latter treatment showed no lethal effect, when they were combined, lethality was observed as with fractions A and B. Administration of the crude venom and fractions A and B from G-75 column resulted in severe haemorrhage. However, the crude venom produced more severe effects. Subfractions A_1, A_2, A_3, B_1 and B_2 showed no lethal effect. The failure of these subfractions to produce this effect may mean that the combined action of either some or all of these various components is necessary if the haemorrhagic effect is to be produced. However, combination of subfraction A_1, A_2, and A_3 was almost as lethal as fraction A. A combination of subfractions B_1 and B_2 was also lethal but not as toxic as the untreated fraction B.

The separation and the demonstration of the presence of discrate haemorrhagic fractions in the Nigerian puff adder (*Bitis arientans*) has been effected.

The haemorrhagic and tissue sloughing effect of the venom of puff adder (*Bitis arientans*) from some African countries has been observed [11]. The result of our present investigation is the first recorded demonstration of the presence of discrete haemorrhagic fractions in Nigerian puff adder (*Bitis arientans*). The non-clotting blood is a useful sign of systemic poisoning in many viper envenomings. The non-clotting blood, a factor causing spontaneous bleeding, is caused by the consumption of fibrinogen. Spontaneous bleeding has also been found to be due to a vasculotoxic factor acting directly on the blood vassel [12].

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