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# Preliminary studies of antidote to snake venom from plant based materials

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**ABSTRACT:** The ability of the plant extract "Danya" (*Sclerocarya birrea*) to neutralize the necrotic and haemorrhagic effect of *Naja naja* and *Naja nigricollis* venom was investigated. The plant extract of various doses were pre-incubated with the venoms for 30 minutes at room temperature prior to intramuscular administration to rats. Cold extract of this plant completely neutralized the necrotic and haemorrhagic effect of the two venoms. Cold extract of this plant also exhibited a heat stable anticoagulant property. This anticoagulant property was found to reside in the chloroform fraction of the extract which could be responsible for the neutralization of necrotic and haemorrhagic effect of the venoms. The identity of this neutralization factor and/or the anticoagulant substance(s) is currently under investigation.

## INTRODUCTION

In many parts of the world, especially in the tropical countries of Africa, S. America and S.E. Asia, snake bites are serious medical, social and economic problems, particularly because the antidotes are very expensive. In the past, this problem has been neglected, primarily because statistics on snake bite had been based on hospital figures which are often grossly misrepresentative. Most individuals bitten by snake(s) do not feature in those figures, either because they prefer traditional treatment or recover without treatment (1).

Snake venom is composed of several constituents such as neurotoxin, cardiotoxin, haemolysin, coagulating factors and many enzymes such as phospholipases. Toxicity of crude venom is either dependent on any one of the components. Any mode of treatment of snake bite should be able to inhibit one or more of the enzymatic actions or neutralize one or more of the toxic components.

In Northern Nigeria, the commonest mode of treatment of snake bite is by administering aqueous plant extracts to the envenomated subjects. It is possible that the plant extract contains substance(s) that detoxify or inhibit one or more of the toxic or enzymatic components. In this study, the effect of "Danya" (*Sclerocarya birrea*), a commonly used plant for the treatment of snake bite in Northern Nigeria on the haemorrhagic and

necrotic effect of *Naja naja* and *Naja nigricollis* venoms is investigated.

## MATERIALS AND METHODS

### Animals

Male rats were obtained from the Veterinary Research Institute, Vom, Jos. All the animals used were healthy, fed on standard pellets and had free access to clean water.

### Venoms

*Naja naja* venom was milked from the snake by local medicine men and stored at -20°C until used. *Naja nigricollis* venom was purchased from Sigma Chemical Company, U.K. The venoms were dissolved in physiological saline to give a stock solution of 0.1 mg/ml.

### Plant Extract

The extract was obtained by cold extract of the stem bark of "Danya" (*Sclerocarya birrea*) (50g/500 ml of distilled water).

### Experimental Design

The rats were divided into five groups of 4 rats each. 100 µl of the *Naja naja* venom containing 30 µg of the venom was incubated with 200 µl of the plant extract for 30 minutes at room temperature.

The mixture was intramuscularly injected to the hind leg of the rats in group 1. Similar mixture was prepared with *Naja nigricollis* venom and injected to the rats in group 2. 300 µl of the plant extract was injected to the rats in group 3. 30 µg of the *Naja naja* venom was injected to the rats in group 5. After 4 hours the rats were sacrificed and the extent of haemorrhage and necrosis in the muscle was evaluated according to the method described by Mebs (4).

## RESULTS AND DISCUSSION

The summary of the results is shown in Table 1. When the venoms injected rats were dissected (Groups 4 & 5) an extensive haemorrhage and necrosis was observed with congestion of blood in the hearts, liver and the lungs. But no haemorrhage was observed in the rats in groups 1 and 2 and hearts, livers and lungs appeared normal. Necrosis appeared only in one of the eight rats in these groups. The absence of haemorrhage in groups 1 and 2 suggests complete neutralization of the haemorrhagic activity of the venoms. Haemorrhagic activity of snake venom is attributed to the venom haemorrhagin or venom protease (4,5). The plant extract in this case could probably be neutralizing the haemorrhagin or inhibiting the protease. Further work is necessary to ascertain which, if any, of these mechanisms is the mode of action.

The absence of any haemorrhage in the plant extract injected rats (group 3) indicates the

haemorrhagic safety of the extract at the levels administered as well as its obvious benefit of obliterating the haemorrhagic actions of the venoms. In the groups of the rats whom the plant extract is compromised (groups 4 & 5) necrosis was observed at the injection sites of either of the venoms. This necrosis was not observed in the group injected with venom-plant extract mixture (with the exception of one rat). This finding therefore suggests necrosis neutralizing ability inherent in the plant extract. It has been reported that local tissue necrosis in snake bites might be caused by phospholipase A<sub>2</sub> (6). If this is the case, it is possible that the plant extract contains inhibitor of this enzyme. The possibility of the presence of this inhibitor in the plant extract is currently under investigation.

The tradition of the use of plant based material for the treatment of snake bite has been practised since prehistoric time and this has recently been validated. Aqueous extract of persimmon fruits (*Diospyros kaka* Thumb) used as a folk remedy for mamushi (*Agkistrodon halys*) snake bite in Japan is shown to have strong detoxifying activity on various poisonous venoms from many kinds of sea snakes, land snakes, scorpions etc. (2). *Schumanniphyton magnifian* extract was also reported to have protective activity against cobra venom. A fraction of this plant extract exhibited an in vitro protective effect against the cobra venom cardiotoxin (3). This work validates the rationale of using this plant, *Sclerocarya birrea* by the local community against snake bites.

Table 1: The effect of "Danya" extract on the haemorrhagic and necrotic effect of *Naja naja* and *Naja nigricollis* venoms.

Group	H*	N	Heart	Liver	Lungs
1. Plant extract-venom mixture (NN)	-ve	-ve	Normal	Normal	Normal
2. Plant extract-venom mixture (NNG)	-ve	-ve	Normal	Normal	Normal
3. Plant extract only.	-ve	-ve	Normal	Normal	Normal
4. <i>Naja naja</i> venom only	+ve	+ve	Congestion of blood	Congestion of blood	Congestion of blood
5. <i>Naja nigricollis</i> venom only.	+ve	+ve	Congestion of blood	Congestion of blood	Congestion of blood

Key: NN = *Naja naja*; NNG = *Naja nigricollis*  
H\* = Haemorrhage; N = Necrosis

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