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Full Length Research Article

HISTOPHATOLOGICAL STUDIES ON EFFECT OF SERUM LIVER ENZYMES OF *NAJA NIGRICOLLIS* AND *BITIS ARIETAN* VENOM

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ABSTRACT
Snake envenoming is a major problem in West Africa especially Nigeria which has the highest risk to humans and animals. In this study, the histophathological effect of *Naja nigricollis* and *Bitis arietan* crude venom at a sub-lethal dose have been investigated on liver of male mice. Venom injected interpretoneally (ip) in mice and 3.5mg/kg body weight at time intervals of 3, 6 and 24h after injection. Enzymes serum aspartate transaminase (AST) showed a tendency to increase with time, the increase was recorded significant from 2, 6 and 24 h with value from 0.1699 ± 0.06841 to 0.2362 ± 0.02916 of the *Bitis Arietan* venom when compared with the *Naja nigricollis* venom of 0.1569 ± 0.0598 to 0.018297 ± 0.07783. The serum alanine aminotransferase (ALT) revealed a tendency to decrease with time and was found to significantly decrease at the 3, 6 and 24 h from the beginning of the experiment. In the present work, the elevated activity of AST might indicate liver and other vital organ damage brought about by the venoms. Knowledge of the biochemical parameters of snake venoms is very important in understanding the pathological manifestation of the envenomation and may be also fundamental in deciding on appropriate drugs to use in clinical envenomation.

Keywords: AST, ALT Creatine*, Naja Nigricollis, Bitis Arietan*.

INTRODUCTION

The most common species *Naja nigricollis* (black-necked spitting cobra) and *Bitis arietans* (puff adder) are families that are associated with envenoming in Nigeria.

Snake venom is a complex mixture of many substances, such as toxins, enzymes, growth factors, activators and inhibitors with a wide spectrum of biological activities (Theakston, 1983; Rahmy and Hemmaid, 2000). They are also known to cause different metabolic disorders by altering the cellular inclusions and enzymatic activities of different organs. The main clinical features of *B. arietans* envenoming are systemic hemorrhage, incoagulable blood, shock, local swelling, bleeding and, occasionally, necrosis. Bites may be complicated by amputation, blindness, disability, disfigurement, mutilation, tissue destruction and psychological consequences (Karaye *et al*., 2012). Antivenom remains the hallmark and mainstay of envenoming. All body systems may be affected; cardiac and hemodynamic abnormalities may result while the strongest predictor of mortality is central-nervous-system involvement with intracranial hemorrhage (Habib *et al.,* 1995 and 2011).

The liver is a multifunctional organ, responsible for vital functions and for maintenance of energy balance in the organism (Adzu *et al*., 2005) The release of intracellular enzymes into circulation is frequently used as an indicator of hepatocyte damage (Rahmy and Hemmaid 2000), although the release of some enzymes like AST and ALT is also present in tissues other than liver thus, needs to be evaluated by other injury markers to confirm any hepatic damage. Liver is considered as one of the targets for cobra venom factor (Fu *et al*., 1997). Hepatic injury due to cobra envenoming was reported by Rahmy and Hemmaid (2000) and Adzu *et al.* 2005). Therefore, in the present study, we examined the liver damage induced by *N. nigricollis and B. arietan* venom in rats through enzymatic markers of liver function and also by histological analysis.

MATERIALS AND METHODS

Venom

The Lyophilized snake venom, *Naja nigricollis* was obtained from Ahmadu Bello University, Zaria, Nigeria and was preserved at 4°C. Before use, the venom was dissolved in saline, centrifuged at 2000rpm for 10mins and the supernatant used for anti-venom studies. Venom concentration was expressed in terms of dry weight.

Animals

Twenty- five young healthy albino rats (3.0- 4.0 kg), were purchased from the Zoology Department, Faculty of Science, Bayero University, Kano. They were de-wormed and allowed to acclimatize for a week before the commencement of the experiment.

Study design

Twenty five male albino rats weighing 3.0 – 4.0 Kg were used. The rats were divided at random into three groups as follows: Group 1: 10 rats were given intraperitoneal (i.p.) injection of 0.5 mL saline (0.9 % NaCl) and served as control. Group 2: 10 rats received single i.p. injections of low dose (2 mg/kg body weight) of *B. arietans* and *N. nigricollis* crude venom. Group 3: 10 rats received single i.p. injections of medium dose (4 mg/kg body weight) of *B. arietans* crude venom. All rats in groups 1, 2 and 3 were killed by decapitation 3, 6, 24 h. after venom injection.

Serum analysis

Blood was collected from each rat into plain centrifuge tubes, left for 1hr at room temperature (25°C ± 2) and serum was separated by centrifugation at 600g for 15 min and analyzed, without delay. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) were determined using Kits purchased from Sera-Pack (Ames division, Miles Ltd. England)

Serum Enzyme Activities and Bilirubin Levels

Serum alanine aminotransferase (ALT), alkaline phosphatase

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(AP), aspartate aminotransferase (AST) and ã-glutamyl transferase (GGT) activities were determined spectrophotometrically, as described by Reitman and Frankel (1957), Bilirubin tests were made by a chosen diazo-coupling reagent and by a spectrophotometric method, according to Winsten and Cehelyk (33)

Plasma creatine level estimation: Blood plasma creatine was determined as described by Jaffe (1957).

Histological Analysis
Animals were sacrificed by chloroform inhalation and the following organs were removed: spleen, skeletal muscle, kidneys, liver and lungs, which were then fixed in 10% formalin, dehydrated and included in paraffin wax for 5µm sections that were stained with high emulsion and observed under light microscope

RESULTS

Table 1: Serum Liver Enzymes Levels of Albino Rats Injected (i.p.) with the venom *Bitis Arientans* and *Naja nigricollis* Venoms

Parameters	Normal	*Bitis arietans*	*Naja nigricollis*
AST(U/L)			
3hrs	0.014±0.003	0.1699± 0.068	0.2058±0.139
6hrs	0.07±0.007	0.1820± 0.071	0.2312± 0.142
24hrs	0.090±0.006	0.2362±0.022*	0.289±0.321*
ALT (U/L)			
3hrs	0.016±0.008	0.157± 0.057	0.275 ± 0.184
6hrs	0.015±0.008	0.166± 0.82	0.353± 0.259
24hrs	0.073±0.009	0.183± 1.34	2.06± 0.253*
CK (U/L)			
3hrs	0.0125±0.011	0.039± 0.037	0.23± 0.016
6hrs	0.26±0.015	0.029± 0.020	0.33± 0.019
24hrs	0.03±0.002	0.056± 0.034	0.036± 0.016

Values are Presented as Mean ± S. D. (n=5)
*: Significant Difference with the Normal Group (P<0.05)

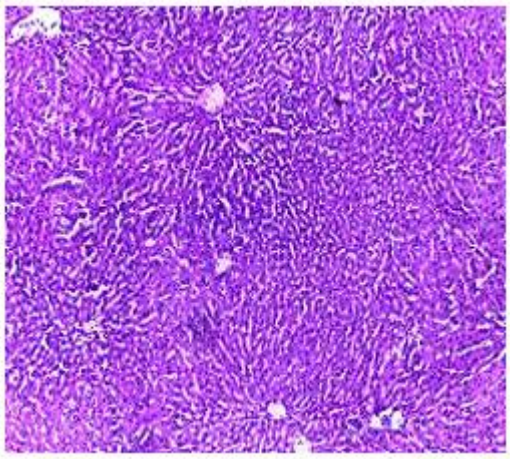
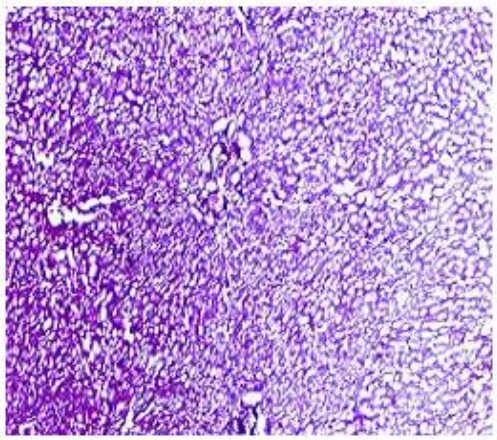


Plate A



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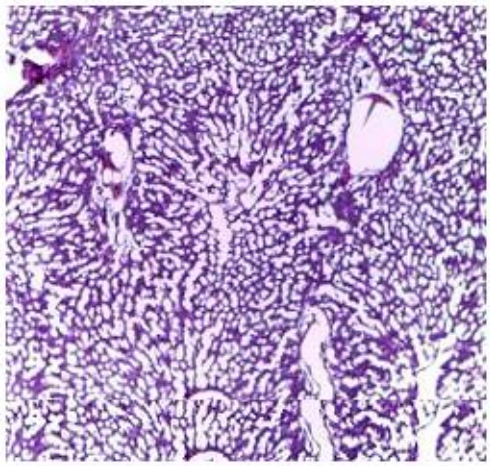

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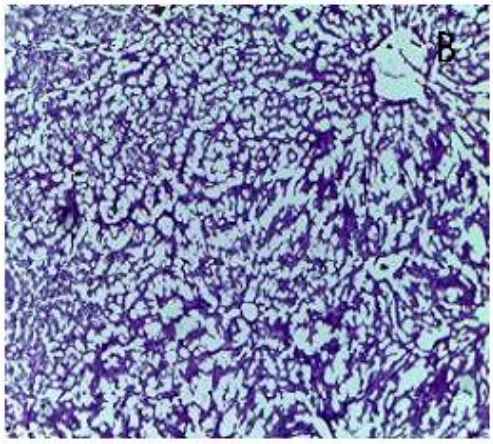

Plate D

Fig 1: Histogical changes induced by treatment with 3.5mls of crude venom *Naja nigricollis* at 3hr envenomination. **Plate A**: A Control group injected with normal saline shows normal hepatocytes arranged as radiating cord. **Plate B**: shows moderate cytosis preserved connective tissue architecture. **Plate C&D**: shows a severe cytosis and the lost of connective tissue architecture.

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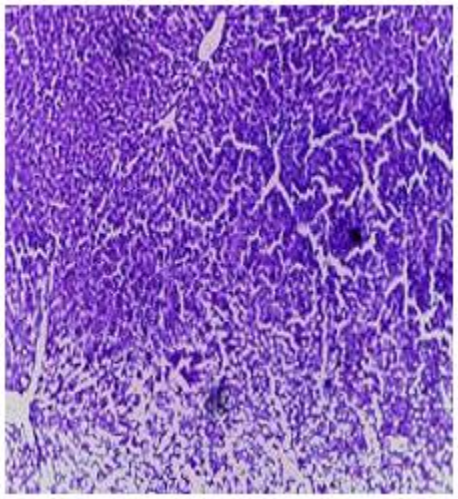
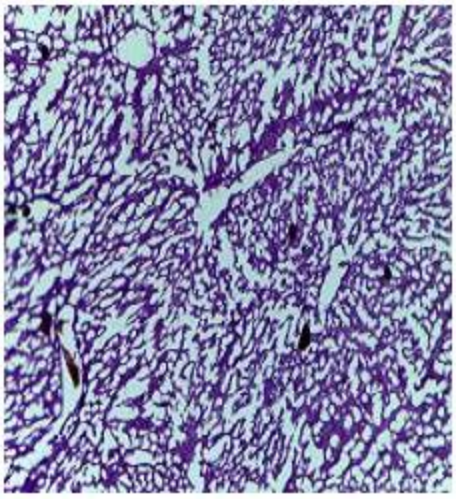


Plate A



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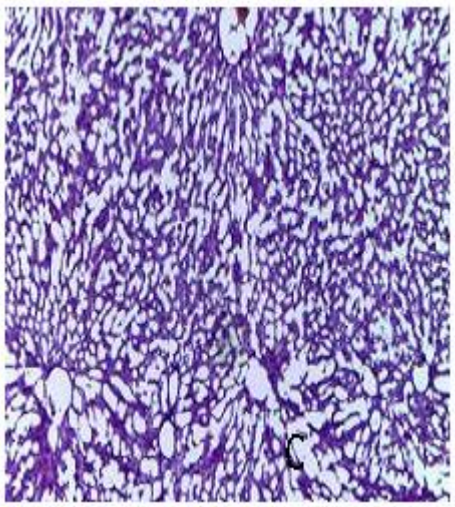

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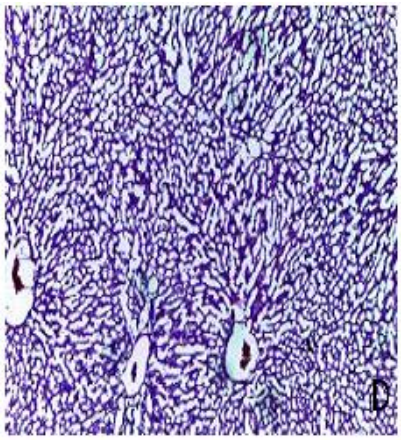

Plate D

Fig 2: Histogical changes induced by treatment with 3.5mls of crude venom *Bitis arientans* at 3, 6 and 24hr envenomination. **Plate A**: A Control group injected with normal saline shows normal hepatocytes arranged as radiating cord. **Plate B**: shows moderate cytosis preserved connective tissue architecture. **Plate C&D**: shows a severe cytosis and the loss of connective tissue architecture.

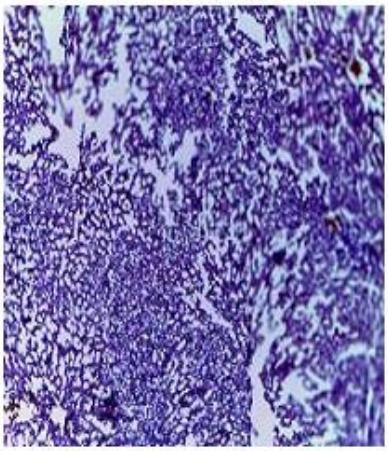

Plate A

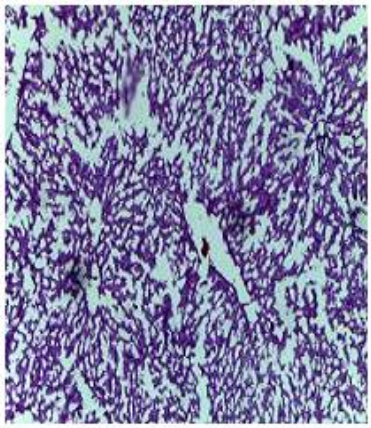

Plate B

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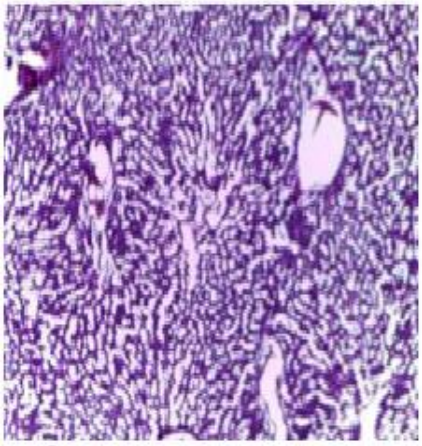

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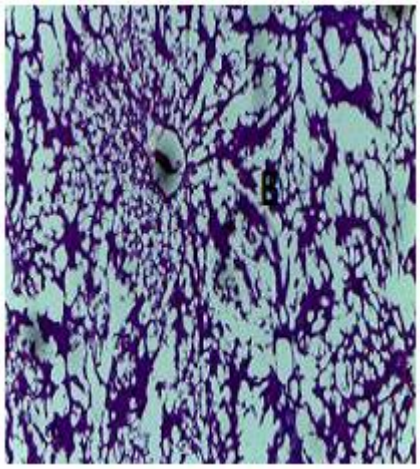

Plate D

Fig 3: Histogical changes induced by treatment with 5mls of crude venom *Bitis arientans and Naja nigricollis* at 24hr envenomination. **Plate A&B**: shows moderate cytosis preserved connective tissue architecture. **Plate C&D**: shows a severe cytosis and the loss of connective tissue architecture.

DISCUSSION

Snake bite is an important cause of morbidity and mortality and is one of the major health problems in Nigeria. The most effective and acceptable therapy for snake bite victims is the immediate administration of antivenom following envenomation (Mahanta and Mukkerjee, 2001). Many snake venoms are known to cause pathological properties associated with haematological disturbances leading to in coagulability of blood. Some local tissue necrosis always accompany envenomation from this snake species. Spontaneous bleeding and coagulation disturbances are some of the haematological effects of *Naja nigricollis* in patients (Warrell *et al*., 1976). AST and ALT enzymes are markers for cellular damage and ALT enzyme is essentially present in hepatocytes (Abdulrahman *et al*., 2015).These enzymes are of importance in assessing and monitoring liver inflammation and necrosis which result in the release of both enzymes in circulation due to increased permeability of the cell membrane or breakdown of the cells (Abdel Moneim *et al.,* 2013). Animals inoculated with copra venom showed an increase in AST and ALT activities as demonstrated by James *et al.,* 2013) for *Naja nigricollis* in rats. The changes included variable degrees of cellular swelling, cytoplasmic changes, cellular necrosis and cellular damage accompanied with loss of the common architecture of the hepatic parenchyma different stages of envenoming. The recorded cytoplasmic granulation of the hepatocytes was also observed by Rahmy and Hemmaid (2000). Moreover, liver damage was found after bitten by the family of Elapidae (Omran *et al.,* 2004). This result for liver enzymes, AST have been found to be increased with *N. nigriollis* at different hour of envenomation which agreed with Mohammed et al 1981 reported that Naja haje venom induced a significant increase in liver AST activity which may be due to destruction of hepatic cellular organelles and intracellular liberation of enzymes. Decrease in the liver ALT could be explained by the glucose-alanine cycle in which pyruvate produced from glucose is transaminated to alanine via ALT enzyme and transported to liver to be reconverted to glucose by gluconeogenesis to enhance the enhance the hyperglycemic phenomenon observed after envenomation (Felig, 1975). The live enzymes of *Bitis arietans* shows a lower level of AST and ALT against the venom of *N. nigricollis* which indicate the disturbances of vital organ. Such disturbances appeared to continue for at least 24h after envenomation regardless of the dose.

Conclusion

The snakebites have complex venomous effects may lead to various changes in hemostasis. In this experiment, snakebite-induced coagulopathy by both *N.nigricollis* and *B.arientans* resulted in hepatic necrosis and rupture with active bleeding. Therefore, further detailed studies on time and dose dependent manner and isolated purified venom toxins are essentially required in order to clarify the mechanism of action of this venom, and can be used on development of rapid diagnostic kit

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