EFFECT OF SUBLETHAL DOSE OF *Najanaja* SNAKE VENOM ON LEVELS OF SOME LIVER ENZYMES IN ALBINO MALE RATS

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ABSTRACT

The effects of (*Najanaja*) snake cobra venom on some liver enzymes in albino male rats have been investigated. The effects of a single sublethal dose of *Najanaja* snake venom (0.04µg/g) body weight on the activities of certain serum enzymes levels: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were studied. Samples from the serum were collected 3 and 24 h following venom dose intraperitonealy injected in male albino rats. The activities of these enzymes showed significant elevation compared to the control. *Najanaja* snake venom caused damage and hepatic dysfunction in enevomated male rats.

Keywords: *Najanaja* venom, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP)**.**

تأثير الجرعة تحت المميتة لسم ألأفعى Najanaja على بعض إنزيمات الكبد في ذكور الجرذان البيض

المستخلص

تم في الدراسة الحالية التعرف على تأثير سم أفعى الكوبرا (*Najanaja*) في بعض أنزيمات الكبد في ذكور الجرذان البيض. تمت دراسة تأثير الجرعة المنفردة تحت المميتة (0.04 µg/g) لسم ألافعى المحقونة داخل الغشاء البريتوني لبعض الإنزيمات (ALT.AST & ALP). أخذت العينات من مصل الحيوانات بعد 3 و24 ساعة من الحقن. أظهر نشاط هذه ألانزيمات أرتفاعا معنويا عند مقارنتها بمجموعة السيطرة. سبب سم ألافعى تلف واختلال وظيفي للكبد في الحيوانات المحقونة بالسم.

Introduction

Snakes are cold-blooded vertebrates, and some species possess dangerous venoms. Cobras, which are widely distributed over the world, belong to the Elapidae family. *Najanaja*is one of the most dangerous snake species in the world , where it provokes a high number of human deaths due to envenomations [1]. *Najanaja*cobra venom contains a mixture of many different proteins, including a variety of enzymes (proteases and phospholipases), non-enzymatic polypeptide toxins (neurotoxins and cardiotoxins), and other substances [2,3]. Cobra envenoming is known to induce multiple-organ failure, leading to death in case of severe envenoming [4]. Liver is considered as one of the targets for cobra venom factor [5]. Moreover, the toxicity of the venoms of *Naja*species has been attributed to the presence of cardiotoxins or other cytotoxins (cytotoxin P4) and nigexine (basic phospholipase A2) [6]. There are reports showing the effects of various snake venomson ALT ,AST and ALP in rat that venom inceasing the level s of these enzyme and damage of the hepatocyes of the liver [7,8,9,]

The liver is a key organ actively involved in numerous metabolic and detoxifying functions. The objective of this study is to determine some biochemical changes in the liver of rats following snake cobra (*Najanaja*) envenomation in an attempt to improve our understanding of snake envenomation in rats .

Materials and Methods

Venom:

Lyomphilized*Najanaja*venom was obtained from India (Sigma loeate Ltd).Lyophilizedvenom was dissolved in phosphate buffered saline(PBS), pH 7.2.

Toxicological studies:

The determination of the median lethal dose LD_{50} of the snakevenom by intraperitoneally (i.p.) injection was carried on 40 adult healthy albino rats .The LD_{50} was determined in rats according to the method of Meier and Theakston[10], (Table 1).

Animals and Experimental design:

A total number of 24 adult healthy male albino rats weighing (180-200 g) obtained from the Institute of Embryo Researches and Infertility Treatment , AL-Nahrain University and used throughout this study. . All animals were given free access to standard laboratory chow and tap water. The animals were divided randomly into two main groups:

Group I- normal control (NC):

Eight normal healthy rats, each received a single i.p injection of 0.25 ml saline and remained intact serving as normal control.

Group II

This group includs 16 normal healthy rats, each received a single i.psublethal dose 0.04 µg/g body weight of snake venom in 0.25 ml phosphate buffered saline. These animals were divided into two subgroups (A and B). Each consisted of 8 rats, and was sacrificed by decapitation after 3 and 24 hours of the injection.

Blood Collection and handling:

At the end of the experimental period, the animals from the experimental groups together with the normal control group were decapitated, and the blood was collected by heart puncture and immediately placed into non heparinized tubes to obtain the serum for analysis of (ALT, AST,and ALP). Blood samples in the non- heparinezed tubes were allowed to clot at room temperature for 1h. Serum samples were obtained by centrifugation of non heparinized tubes at 3000 r.p.m. for 20 min. Clear serum was aspired and stored at refrigerator until used in the same day. The kinetic measurement of plasma ALT , AST and ALP by spectrophotometer using commercially available diagnostic kit (BioMareuix , France).

Statistical analysis

The results are given as mean ± standard error (X± S.E.). Significance of the differences was tested by analysis of variance (ANOVA) test. The levels of significance were taken at p <0.01.

Results and Discussion:

Venom Lethality:

The approximatei.p. LD_{50} for *Najanaja* snake was determined in rats to be equal to 0.05 µg/g body weight , as shown in table 1. the present results showed that the LD50 of *Najanaja*snake venom is approximately equal to 0.05µg/g body weight . Other investigators reported that the LD50 of the same venom is 0.066 µg/g body weight [11], 0.50 µg/g body weight [12] . These differences of LD50 could be attributed to differences in geographical distribution of *Najanaja* snake , seasonal variations in composition and potency of venoms (13,14,15].

Table 2 showed the effect of sublethal dose *Najanaja*snake venom on serum ALT, AST and ALP activity. There was significant elevation in serum ALT, AST, and ALP levels (P<0.01) in rats after 3 and 24 hrs treated with 0.04 µg/g (body weight) *Najanaja*snake venom in comparison with control group. Biochemical results showed that treatment with snake venom induced a significant increase in activity of serum ALT, AST and ALP activity. The principal marker enzymes include alanine (ALT) and aspartic (AST) aminotransferases, which catalyze the transfer of α-amino groups from alanine and aspartate to the α-keto group of ketoglutaric acid to produce pyruvic acid and oxaloacetic acid, respectively [16]. Other enzymes such as alkaline phosphatase (AP)

may also be used as markers of hepatic dysfunction [17]. Serum enzymes analysis proved to be very useful for liver diseases diagnosis . Serum alanine aminotransferase ALT), aspartate aminotrans- ferase (AST) and alkaline phosphatase (ALP) serve as markers for hepatocellular damage [18]. The result of this study is in agreement with that of [19] who reported that i.p of *Najahaje venom* to male rats induced changes in the activities of ALT , AST ALP activity . Elevated activities of ALT, ALP and AST have been reported due to envenoming with animals venom [20,21]. Rats treated with the *Najanaja*snake venom suffer from hepatocellular injury and dysfunction which are represented by significant elevations in the activities of serum ALT , AST and ALP. The present study was similar with previous studies which revealed harmful effects of venom on hepatocytes and induction of degenerative changes the liver [22]. The general rise in the activities of ALT, AST and ALP that indicate the damage of liver ,heart and other organs brought about by the venom [23,24].

Table (1). Determination of LD_{50} of *Najanaja* snake Cobra venom on rats.

Dose µg/g body weight	No. of animals	Survival (S)	Death (D)	% Mortality
0.02	8	8	0	**0%**
0.04	8	5	3	**37.5%**
0.06	8	3	5	**62.5%**
0.08	8	1	7	**%87.5**
0.1	8	0	8	**100%**

LD_{50} = 0.05 µg/g body weight rats

Table (2): Serum ALT, AST ,and ALP in rats of all groups

Parameters	No. of rats	Group 1	GroupII	
		Normal (Control)	Time after (i.p) venom injection	
			3 hours	24 hours
S ALT (U/L) Mean + S.E % change P <0.05	8	62.3±5.9	89.1±5.6 * 43.01	99.6±4.1 * 59.87
S AST (U/L) Mean + S.E % change P <0.05	8	123.7±6.3	143.8±4.8 * 16.24	167.9±3.4 * 35.73
S ALP (U/L) Mean + S.E % change P < 0.05	8	313.8±3.5	344.2±4.7 * 9.68	357.4±6.9 * 13.89

*P<0.01 (significantly different from the control)

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