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Evaluation of acute complication of *Naja naja oxiana* venom on some biochemical parameters in Dutch rabbit

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Abstract

The aim of this study was to evaluate the effect of *Naja naja oxiana* venom on some biochemical parameters of rabbits. The serum samples from seven rabbits were collected before venom injection (control) and then 120 µg/kg venom (*Naja naja oxiana*) was injected intramuscularly to the rabbits and their plasma at the times of 1, 3 and 24 h were collected. Electrocardiogram strips (ECG) were taken from their hearts before and after venom injection. The enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine Kinas-MB (CK-MB) and creatine phosphokinase (CPK) were determined. The result showed significant rise in the amounts of these enzymes and the irregular activity of heart after the venom injection at 1, 3 and 24 h. The results indicated that *Naja naja oxiana* venom causes time-dependent alternations in serum enzymes.

Keywords: Snake venom; heart activity; biochemical factors

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Introduction

Each year about 1.2 to 5.5 million cases of snake bites are recorded particularly in tropical countries (Langley, 2010). Snakes are vertebrates and some of its species possess dangerous venoms. *Naja naja Oxiana* is one of the types of snakes in northeast of Iran which belong to the Elapidae family. It is responsible for a large number of snake bite mortality (Akbari et al., 2010). Venom is the toxic substance produced by an animal in a highly developed secretory organ or group of cells, which is delivered during the act of biting or stinging (Meier and Theakston, 1986). *Naja naja oxiana* 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 (Ponnappa et al., 2008; Binh et al., 2010). Elapidae envenoming is known to induce multiple-organ failure, leading to death in severe cases (Cher et al., 2005). Liver and heart are the vital organs which are targeted by the venom (Satyaswaroop and Gowda, 1997). 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) (Chwetzoff et al., 1989). The enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phophatase (ALP) are considered as markers of hepatic dysfunction, while lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and creatine phosphokinase (CPK) as markers of heart failure. CK-MB test may be used as a follow-up test for an elevated CPK in order to determine whether the increase is due to heart or skeletal muscle damages. In fact, CK-MB is more specific than CPK (Dufour, 2000). It is estimated that the analyses of serum enzymes can help to detect some abnormalities in the liver and heart.

Therefore, the current study was designed to find the changes in serum biochemical parameters in rabbit.

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Materials and Methods

Venom: Seven snakes *Naja naja oxiana* were collected which were identified on the basis of their morphology from northern of Iran. Expert of Zoology helped to identify the snakes. Their venom were collected manually (by pressing the venom glands located behind the eyes) and stored at 4°C.

Toxicity assay: Toxicity of the crude venom was assayed by intravenous injection in groups of mice (five groups of male mice and four mice were used at each dose level). The mortality rate was recorded 24 h after the injection and LD_{50} values were evaluated (Meier and Theakston, 1986; Akbari et al., 2010).

Blood sample collection: Seven rabbits were anaesthetized with intra muscular injection of 10% ketamine and 2% xylazine. ECGs were taken (ECG Machine Model 8/30 AD Instruments, Australia) from their hearts before venom injection (as control). Blood samples mixed with EDTA (1.5 mg EDTA per ml of blood). Stored venoms were dissolved in 10 ml of normal saline solution and were injected intramuscularly to the rabbits (120 µg/ per kg of rabbit) and their sera at 1, 3 and 24 h were collected. Blood samples were centrifuged (Sigma centrifuge, Model 6k15) for 10 min at 2500 rpm and ECGs were taken from their hearts before and after injection of venom. Quantitative detection of ALT, AST, ALP, LDH, CK-MB and CPK were determined with the help of commercially available kits (Pars Azmon Company, Iran)

Statistical analysis

The data were analyzed using analysis of variance (ANOVA). Means were compared by Duncan multiple range test. P value less than 0.05 was considered as significant.

Results

The heart activity after the venom injection changed at 15 min, 1 and 3 h and returned to normal after 24 h (Fig. 1). These results suggested that the cause of rabbit death (two died) can be heart failure. The effect of *Naja naja oxiana* snake venom on ALT, AST, ALP, LDH, CK-MB and CPK activity before (control) and after injection of venom are showed in Table 1. These parameters showed significant ($P<0.05$) elevation compared with their control. The concentration of ALT increased drastically at the times of 1, 3 and 24 h relative to control (augmentation of ALT compared to the control after 1, 3 and 24 h was 29.5, 33.5 and 62% respectively). AST increased significantly ($P<0.05$) at 1, 3 and 24 h compared to control (growth of AST compared to the control after 1, 3 and 24 h was 97, 140 and 205% respectively) but there was no significant difference in AST at 3 and 24 h. ALP after 1 h increased non significantly 1.3 times (increase in ALP compared to the control after 1, 3 and 24 h was 36.5, 71 and 139.5% respectively). ALP increased significantly at 3 and 24 h compared to control. The concentration of LDH at 3 h increased significantly ($P<0.05$) and then decreased at 24 h. However, it showed significant rise compared with the control (elevation of LDH compared to the control after 1, 3 and 24 h was 103, 142 and 86% respectively). The level of CK-MB at 1 h reached the highest point ($P<0.05$) and then declined significantly (fall in CK-MB compared to the control after 1, 3 and 24 h was 87, 53 and 17% respectively). Similarly, CPK increased significantly ($P<0.05$) at 1 and 3 h and then declined.

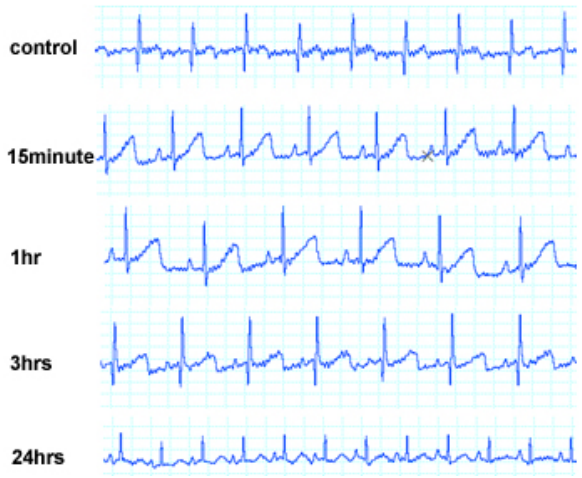

Fig. 1: ECGs taken from rabbit´s heart before and after venom injection

Discussion

Venom causes multi-organ system and failure and death. The severity of clinical signs is related to neurological and cardiovascular dysfunction (Meki et al., 2003).

The results of this study showed that the venom of *Naja naja oxiana* induced significant elevation in biochemical parameters of liver (ALT, AST and ALP) and heart (CK-MB, LDH and CPK). These results are correlated with the (Abdel-Aal, 1998; Rahmy and Hemmaid, 2000; Al-Sadoon et al., 2012; Omale et al., 2012). Venomous snakes produce their harm through alternation in enzymatic activities of serum and mammalian tissues (Al-Jammaz et al., 1999). This effect is manifested by either activating or inhibiting the enzymes activities in the cells or the liberated enzymes causes destruction of the cell organelles.

Table 1: Serum ALT, AST, ALP, LDH, CK-MB and CPK in rabbits of all groups

Parameters (U/l)	Control	Experimental group		
		1 h	3 h	24 h
ALT	67.29±5.24^{c}	87.10±10.17^{b}	89.85±12.81^{ab}	109.5±14.03^{a}
AST	52.52±10.41^{b}	103.66±14.19^{ab}	126.56±12.2^{a}	160.39±23.3^{a}
ALP	24.87±2.9^{b}	33.98±4.9^{b}	42.60±5.4^{a}	59.47±7.4^{a}
LDH	214.42±17.2^{b}	435.83±30.7^{ab}	520.36±46.7^{a}	399.00±37.3^{ab}
CK-MB	218.33±20.5^{c}	410.00±39.5^{a}	334.33±31.5^{ab}	257.00±26.4^{b}
CPK	375.85±34.5^{b}	1369.67±88.5^{a}	1366.6±94.5^{a}	828.33±81.05^{ab}

Nakada et al. (1984) reported proportional increase in serum CPK to the dose and the microscopic alterations in venom-injected mice and concluded that CPK value is a useful indicator of myonecrosis due to snake venom. Meki et al. (2003) found that CPK, CK-MB and LDH increased significantly in scorpion envenomation in children. Sofer et al. (1991) proved that CK-MB was significantly high in patients with cardiovascular disease. LDH is a glycolytic enzyme catalyzes the reversible oxidation of lactic acid to pyrouvic acid. Its increase level indicate that the metabolic pathway is disturbed Al-Jammaz et al., 1999). CPK is known as an important enzyme which is used for clinical diagnosis in tissue injury such as muscle dystrophy (Nakada et al., 1984). Al-Jammaz et al. (1999) showed that intra peritoneum envenomation with LD_{50} dose of *E. coloratus* venom caused significantly higher serum AST and LDH concentration in male albino rats and suggested that the parallel increase in AST and LDH indicate the myocardial infarction. ALP activity in the present study increased significantly after 3 h. This enzyme is mainly found in the bile duct and its rise shows obstructive liver disease. According to Al-Sadoon et al. (2012), Walterinnasia aegyptia crude venom causes fluctuation in ALT, AST and ALP activity with a tendency to normalise. These values began to rise from 1 to 3 h which agreed with our findings.

From the present study, we concluded that *Naja naja oxiana* venom can cause time-dependent disorders in the biochemical enzymes.

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