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Histopathological changes induced by *Hemiscorpius lepturus* scorpion venom in mice

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ABSTRACT

Envenomation by *Hemiscorpius lepturus* (*H. lepturus*) is associated with local necrosis, followed by systemic manifestations. In this work the LD_{50} of *H. lepturus* venom were determined by subcutaneous (SC) injection in white Balb/c mice (5 mg/kg). Histopathological alterations in organs such as kidney, heart, liver, lungs, stomach and intestine were determined in 3, 6, 12 and 24 h following experimental (SC) envenoming injection of one LD_{50} of the venom in Balb/c mice. Histological studies showed degenerative changes in the kidney with disorganized glomeruli and necrotic tubular in 3 h and reached to its climax in 6 h. Myocardium showed massive myocytolysis with interstitial necrosis in 3 h and reached to its peak after 6 h past envenoming. Bowels showed edema of lamina propria and slight villous necrosis. The enzymatic activities of creatine kinase (CK) and lactate dehydrogenase (LDH) were significantly increased in the serum in 9 h. No necrotic lesion observed in lungs and liver. The results indicate that the venom of *H. lepturus* is a highly cytotoxic, and induces massive tissue damages in specific organs, starting from the heart and kidney as the first target in 3 h and ends to the bowels in 6 h post envenomation.

1. Introduction

There are at least twenty-three scorpion species in Iran from which twenty species belong to Buthidae family; two belong to Liochelidae and one to Scorpionidae (Prendini, 1802; Vachon, 1966; Farzanpay, 1987; Fet et al., 2000). The latter, *Hemiscorpius lepturus* is spread from West to East in the Southern country, as well as South of Iraq (Pringle, 1960). This scorpion is endemic in Khuzestan, Southwestern Iran with hot and humid climate and is considered the most dangerous species in the region. Ten to 15% of total scorpion stings during the hot season and almost all cases of scorpion stings during winter are due to *H. lepturus*. Envenomation by *H. lepturus* is a major public health problem in Khuzestan, particularly in children (Mirdehghan and Motlagh, 2001) and contributes to 95% of all mortalities in scorpion-stung individuals in the province (Radmanesh, 1998).

Envenomation by *H. lepturus* is characterized by various symptoms such as pain, sweating, fever and hypertension and neurological disorders, and even with cutaneous reaction (Radmanesh, 1998). In the more severe cases, hematuria due to hematolytic effect of the venom may occur (Radmanesh, 1990).

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Less information about the pharmacokinetics and pharmacodynamic characteristics of the venom of *H. lepturus* scorpion is available. In one study some toxic effects of *H. lepturus* venom were studied in rat (Pipelzadeh et al., 2006). The results, however, suggest that the venom from *H. lepturus* is primarily a cytotoxic agent over cultured fibroblasts and possesses hemolytic, nephrotoxic and to some extent hepatotoxic activities (Pipelzadeh et al., 2006).

The present study was undertaken in order to study the toxicity effects of *H. lepturus* venom in the experimental Balb/c mice. Histological damages to the heart, kidney, liver, intestine and stomach of the mice were assessed after subcutaneous (S.C.) injection of one lethal LD_{50} of the venom. Alterations in LDH and CK enzymes in the mice serum were also determined.

2. Materials and methods

2.1. Scorpion venom

Venom of *H. lepturus* scorpion from Khuzestan (Iran) were collected by the veterinarian service of the RAZI Vaccine Development and Serum Research Institute of Iran and kept frozen at −20 °C in their crude forms until use.

2.2. Determination of toxicity in vivo

To determine LD_{50}, the Spearman-Karber's method was employed (World Health Organization: WHO, 1981). Briefly, appropriate venom concentrations were prepared in PBS containing 1% BSA in order to obtain at least four points within the linear portion of the dose–response curve and to cover the full range between zero and 100% of induced animal mortalities with a symmetrical distribution in comparison with 50%. Toxicity in Balb/c white mice (average weight 18–20 g) was tested by subcutaneous (SC) injection of 100 μl of the solutions, and the LD_{50} was determined by a lethality test 24 h after injection. Deaths were monitored in 24 h and LD_{50} was then calculated.

2.3. Histopathological studies in mice

Four groups of six Balb/c male mice were each subcutaneous (SC) injected with one LD_{50} of crude venom for pathological study. The fifth group was each intramuscularly (IM) injected with the same amount of venom concentration in order to study the morphological changes in the site of injection. The sixth group was intradermally (ID) injected with one LD_{50} of crude venom to study the pathological alteration. One group of six mice which was assigned as control was injected with PBS by the same route of SC, ID or IM. Each mouse was injected with the volume of 100 μl of solution in all experimental groups. The mice were sacrificed by cervical dislocation in 3, 6, 12 and 18 h and the organs of lung, liver, heart, kidney, stomach and intestine along with the calf muscle from one group of mice that were injected in the muscle, were taken and fixed in 10% formalin solution. After 24–48 h the sample of organs were dehydrated in a grade alcohol series and embedded in paraffin wax. Sections of 4–5 μm thick were stained with hematoxylin–eosin (H&E) for pathological studies based as described by Kiernan (1999).

2.4. Enzyme assays

The myotoxic activity was measured by determining creatine kinase (CK) and lactate dehydrogenase (LDH) activities in the serum of the mice described in Section 2.3. The serum was obtained from the centrifugation of the blood samples collected from the orbital sinus of mice after 2 h incubation at 37 °C. The creatine kinase (CPK reagents, Bayer commercial Kit, code SFBC: LO, BIO-2407-83, USA) and lactate dehydrogenase (LDH reagents, Bayer commercial Kit, code SFBC: BO, BIO-2111-83, USA) were assayed in the sera using Bayer commercial Kit according to the manufacturer's instructions. The enzyme values were expressed in international units (IU/l).

2.5. Protein estimation

Protein concentration was determined according to the method of Bradford (1976), using BSA as standard.

2.6. Statistical analysis

The results are reported as the means ± SEM of n experiments, when appropriate. The significance of differences between means was evaluated by analysis of variance followed by Student's *t*-test when various experimental groups were compared with the control group. The confidence limit for significance was 5%.

3. Results

3.1. LD_{50} determination

The LD_{50} amounts of *H. lepturus* venom were determined 5 mg/kg by SC injection in white Balb/c mice.

3.2. Histopathology and cellular changes

The histological analysis of some organs from mice after subcutaneous exposition of one LD_{50} dose of the *H. lepturus* venom for 0, 3, 6, 12 and 24 h revealed remarkable alterations in the kidney, heart, intestine and stomach tissues. After 24 h post histopathology studies, the renal showed degeneration in the Bowman's space, glomerular and tubular epithelial cells, with no deposition of eosinophilic material inside the proximal and distal renal tubules (Fig. 1a′). Pathological effect of *H. lepturus* venom on heart muscle was studied as intravascular fibrin network deposition, thrombosis of the dermal blood vessels, and degeneration of the blood vessel wall. The muscular edema was also present with myonecrosis of myofibrils (Fig. 1b′). There was no obvious injuries observed in liver, but it seems that it is suffering from the envenomation (Fig. 1c′). There was no dermonecrotic manifestation of *H. lepturus* venom in Balb/c mice in the site of injection (Fig. 1d′). Bowels showed elective histopathological changes including edema of lamina propria and slight villous necrosis (Fig. 2a). After shivering and stretch attend