Archives of Razi Institute, Vol. 65, No. 2, December (2010) 83-89

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Serum Enzymes Studies in Scorpion (*Hemiscorpius lepturus)* Dose Related Envenomation in Rabbits

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Received 01 Jan 2010; accepted 10 Sep 2010

ABSTRACT

Hemiscorpius lepturus is medically important scorpion species present in the south and southwest part of Iran, causing morbidity and mortality in children and adults. Unlike other scorpions studied so far, the venom of H. lepturus is highly cytotoxic, that can be a reason for its complexity in clinical manifestations in patients stung by this scorpion. Scarce studies showed the mechanism involved in envenomation by *H. lepturus*. In the present study, *H. lepturus* venom in three doses of 50, 500 and 1500 µg/kg were subcutaneously injected into three separate groups of rabbits. Electrocardiograms of all the rabbits were recorded during the experiment. Blood collection was carried out before, one and three hours after venom injection. Serum was used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, creatinine, BUN (Blood Urea Nitrogen), creatine phosphokinase (CPK) and creatine kinase isoenzyme MB (CK-MB). In group 1 rabbits although some change were observed in serum biochemical parameters following venom injection but statistically the changes were not significant. In group 2 rabbits significant ($P<0.05$) increase in LDH, BUN, urea and creatinine was observed 3 hrs after venom (500 µg/kg) injection. In group 3, the venom injection caused highly significant ($P<0.01$) elevated level of BUN, creatinine, CPK and CK-MB and significant ($P<0.05$) elevated level of rest of the biochemical parameters. On the other hand, in this group, electrocardiogram abnormalities such as ST elevation and sinus bradycardia in limb lead II, were indicator of a mild cardiac injury. In conclusion, it seems that the venom of *H. lepturus* has the cytotoxic components that severely affecting the organs kidney and liver directly and its mechanism of action is quite different from scorpions in *buthidea* family.

Keywords: *H. lepturus*, Scorpion venom, Electrocardiogram, Biochemical disorders, Serum enzymes

INTRODUCTION

Scorpion envenomation is a public health problem in many countries. *Hemiscorpius lepturus* is medically important scorpion species, endemic in south and southwest part of Iran causing morbidity and mortality in children and adults (Radmanesh 1998, Afzali & Pezeshki 1998). Many studies have been published about the clinical and biochemical manifestations produced by the venom of scorpions from Buthidae family, but very few reports have

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indicated the manifestations caused by the venom of the hemiscorpionidae family where the most common scorpion genus is *Hemiscorpius lepturus* (Deroodtr *et al* 2003, Ismail 1995, Zare *et al* 2007, Ghafourian & Mohebby 2008). The clinical manifestations observed from *H. lepturus* envenomation include hemolysis, dermonecrotic reactions, renal failure, cardiovascular disease, and central nervous system disorders (Afzali & Pezashki 1998, Radmanesh 1990). The most important and prevalent sign of *H. lepturus* envenomation is severe hemoglobinuria, hematuria and hemolytic anemia that may lead to renal failure, and eventually death in the envenomed patients; especially in infants (Pipelzadeh *et al* 2004). Unlike other scorpions studied so far, the venom of *H. lepturus* is highly cytotoxic, that can be a reason for its complexity in clinical manifestations in patients stung by this scorpion (Pipelzadeh *et al* 2007). Systemic disturbances, such as renal failure, hemolysis and other clinical manifestations, in the envenomed patients by this scorpion may be attributable to the enzymatic components in the venom of this scorpion (Jalali *et al* 2010).However the dose dependent toxicity of *H. lepturus* venom on serum biochemical parameters as well as ECG was unknown. Hence the present study was undertaken to see the effect of sub-acute doses of scorpion (*H. lepturus*) venom on electrocardiogram as well as serum enzymes in experimental rabbits.

MATERIALS AND METHODS

Venom. For the present study, Scorpion (*H. lepturus*) Venom was milked by electric shock in department of venomous animals and antivenom production, Razi Vaccine and Serum Research Institute of Iran, then, lyophilized and preserved at -20 ˚C until used. On the day of experiment, the venom was dissolved in normal saline and the concentration was adjusted for 50, 500, 1500µg/ml and used for injection.

Venom LD50. The venom LD50 was determined in mice (18–20g) by the method of Reed and Muench (Reed & Muench 1938).

Experimental animals. Fifteen healthy New Zealand white male rabbits (1.5± 0.2 kg of body weight) were selected for this experiment. Rabbits, prior to the experiment, were maintained in quarantine for at least 3 days before the experiment The environment was climate controlled at 18–22 ˚C with food and water. No animals used in the experiment that showed any signs of ill health. They were divided in to three groups (1, 2 and 3). All the rabbits were anaesthetized with intramuscular injection of ketamine and xylazine in ratio 2:0.5 ml respectively. *Hemiscorpius lepturus* venom in three doses of 50, 500 and 1500 µg/kg of body weight was subcutaneously injected into groups 1, 2 & 3 respectively. Blood collection was carried out for all animals before, one and three hours after venom injection. Clinical signs and symptoms of all the animals were recorded during the experiment.

Biochemical parameters. Separated serum were used for analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase isoenzyme MB (CK-MB), creatine phosphokinase (CPK), urea ،reatinine and BUN levels using Pars Azmoon kits according to DGKC and IFCC method(Bergmeyer & Horder 1986).

Electrocardiography. Electrocardiogram of all the rabbits before and after the venom injection for 3 hrs was recorded continuously using electrocardiogram recorder model of ML136 Animal Bio Amp with power lab 4/30, AD Instrument, Australia. All the observations were carried out in limb lead II in various interval times.

Statistical analysis. Data were analyzed with statistical software SPSS 16.0 .Data is reported as means ± standard deviation (SD). All the results were statistically analyzed using the Student "t" test

to compare the mean values of different variables. The results were considered to be statistically significant if $P< 0.05$.

RESULTS

Clinical signs and symptoms. In group 1 animals no significant local signs were observed except a red circle of less than 0.5 cm., around the injection site without inflammation during the experiment. In groups 2 and 3 animals, the local signs appeared 15 to 20 min after venom injection by appearance of a red circle of 0.5-2 cm along with inflammation at injection site. However the inflammation which was more severe in group 3 animals did not appear in three rabbits of group 2. No significant changes appeared in breathing pattern of animals but the heart rate decreased in all the animals within 3 hours of experiment from average of 210beat/min to 80beat/min. One hour after venom injection, all animals showed muscle contraction on their feet where venom was injected.

Venom LD50. The venom LD50, determined in mice, was 126 µg/mouse or in other word it was 6.3mg/kg of body weight. However the LD50 in rabbits was not determined due to the limitation in animals scarify.

Electrocardiogram. An electrocardiogram of rabbits before and after *H. lepturus* envenomation (50 µg/kg) is shown in (figure 1A). No change in the P-wave, QRS-complexes and T-wave were observed. However, a mild bradycardia in which heart rate (HR) decreased from average of 210 beat/min to 148 beat/min was observed. When the rabbits were injected with 500 µg /kg of the venom, ECG indicated no change in the P-wave and T-wave. QRS-complexes and ST segment were not widened. However, a significant decreased in HR from average of 192 beat/min to 100 beat/min, indicative of a mild bradycardia was seen. Prolonged repolarization was also detected as a prolonged QT interval (Figure 1B).

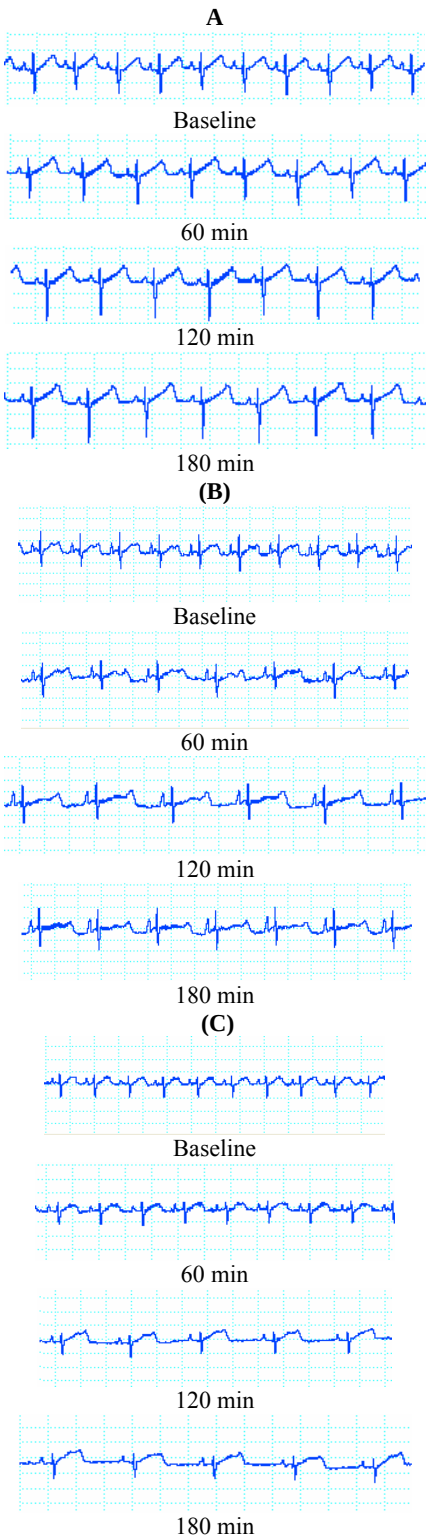

Figure 1. Electrocardiogram recordings of anesthetized rabbits treated with *HL* venom in several time intervals. (A) 50 µg/kg *HL* venom (B) 500 µg/kg *HL* venom (C) 1500 µg/kg *HL* venom.

In group 3 of rabbits (received 1500 µg/kg of venom), electrocardiogram showed mild ST elevation and sinus bradycardia in limb lead II being the prominent effect up to three hours following venom injection, the significant decreased in HR from average of 227 beat/min to 94 beat/min showed more severe bradycardia, Prolonged repolarization was also detected as a prolonged QT interval (Figure 1C).

Biochemical parameters. Injection of *H .lepturus* venom (50µg/kg) into group one animals showed non significant increase in CPK, CK-MB, LDH and AST within 1 hour. Increase in ALT level within 3 hours was not significant too. However, although the rise in CPK following venom injection at 1 and 3 hours were 3.753 and 18.912 percent respectively but statistically they were not significant in this group (table 1).

In group 2 animals which received 500µg/kg of *H. lepturus* venom although at 1 hour following venom injection a rise was seen in all the biochemical parameters but non of them were statistically significant. However the rise in all the parameters was continued till 3 hours after venom injection. The elevated levels of LDH, BUN, urea and creatinine at 3 hours following venom injection were found to be statistically significant (table 2).

In group three animals, injection of *H .lepturus* venom caused non significant increase in LDH, AST, ALT, BUN and Urea. However, the rise in CK-MB and creatinine levels was significant within 1 hour following the venom injection. Finally, the venom injection caused highly significant ($P<0.01$) increase level of CK-MB, CPK, BUN and creatinine and significant ($P<0.05$) elevated level LDH, AST, ALT and urea within 3 hours (table 3).

DISCUSSION

In the present study we observed the local signs as red circle and inflammation at the site of injection especially when the injection dose of venom increased to 500µg/kg and 1500µg/kg. The sting of *H. lepturus* does not produce an immediate pain as does the sting of other scorpions, and generally give rise to delayed swelling that may diffuse and is often accompanied by late necrosis at the sting site (Afzali & Pezashki 1998, Radmanesh 1990). The appearance of red circle and inflammation without serious pain around the injection site which appeared as local sign in the present study is likely due to the necrotizing property of the venom that may cause damage to the presynaptic sensory nerves leading to localized anesthetic effects and/or to the inhibitory effects on the release of neurotransmitters (Piplezadeh *et al* 2007, Jalali *et al* 2010). It has been reported that subcutaneous injection of the three doses (0.01, 0.1 & 1LD50) of venom produce some changes in skin structures with clear cell aggregation in epidermal and collagen precipitation in dermal layers. A marked dermal layer discontinuity, atrophy in subcutaneous

Table 2. Biochemical Parameters at different time interval following H. Lepturus venom (500 µg/kg) injection

Parameters	Before venom injection (Mean ±SD)	1 hr. after venom injection (Mean ±SD)	P Value	3 hr. after venom injection (Mean ±SD)	P Value
LDH (U/L)	80.155 ± 40.868	173.124 ± 53.645	NS	432.812 ± 127.738	P<0.01
AST (U/L)	9.13 ± 4.98	10.32 ± 6.18	NS	25.01 ± 15.97	NS
ALT (U/L)	9.92 ± 3.71	9.13 ± 3.60	NS	12.70 ± 4.98	NS
CK-MB(U/L)	208.116± 79.219	285.156 ± 112.506	NS	365.183 ± 169.323	NS
CPK (U/L)	176.82 ± 64.226	215.915 ± 42.336	NS	205.309 ± 56.436	NS
BUN (mg/dl)	11.911 ± 2.954	14.992 ± 4.169	NS	20.347 ± 4.404	P<0.05
Urea (mg/dl)	28.251 ±7.658	30.954 ± 4.533	NS	45.227 ± 13.486	P<0.05
Creatinine(mg/dl)	1.052 ± 0.213	0.924 ± 0.431	NS	1.720 ± 0.510	P<0.05

NS=Not Significant

layers with severe hemorrhage observed in envenomed rats (Ajj 2003). While in our previous study with injection of 6500µg/kg of *H. lepturus* venom in rabbits, the signs and symptoms observed were similar to the signs and symptoms reported for the envenomation by the venom of scorpions from *buthidea* family (Zare *et al* 2006, Jalali *et al* 2010), but in the present study the signs and symptoms like increased heart beat rate, salivation, lacrimation, and altered breathing rhythm were not observed in rabbits following venom injection. It seems the signs and symptoms appears in case of acute envenomation is due to the neurotoxin in the venom of scorpion *H. lepturus*. However in sub-lethal dose (1500µg/Kg) the appearance of signs and symptoms related to the neurotransmitters release is not observable rather the signs and symptoms related to cytotoxic nature of this venom is more prominent. Although most of scorpions from *buthidae* family cause significant myocardial infarction in heart (Hering *et al* 1993, Kilger *et al* 2000), in the present study *H. lepturus* venom did not effect the heart seriously even in dose of 1500µg/kg. However, in group 3 of rabbits electrocardiogram showed mild ST elevation and sinus bradycardia in limb lead II following venom injection. In a report by Ajj in 2003 which studied the histopathological effects of *H. lepturus* venom, the results showed that the effect of *H. lepturus* is more prominent on kidney and skin (at the site of injection) rather than cardiac and lung tissues (Ajj 2003). Althogh there was a rise in the CK-MB and CPK in group 1 animals following venom injection but it was not significant. It may be due to high SD value of the results which can be attributable to variations in animal's physiological responses. The rise observed in the CK-MB as well as CPK especially with envenomation dose of 1500µg/kg at 3 hours following venom injection my be indicators for the delayed type of damage to the heart. Approximately 30 to 50 percent of dialysis patients without evidence of myocardial injury exhibit an elevation in the CK-MB fraction (McLaurin *et al* 1997, Green *et al* 1986). The percentage is even higher among

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LDH (U/L)	80.155 ± 40.868	173.124 ± 53.645	NS	432.812 ± 127.738	P<0.01
AST (U/L)	9.13 ± 4.98	10.32 ± 6.18	NS	25.01 ± 15.97	NS
ALT (U/L)	9.92 ± 3.71	9.13 ± 3.60	NS	12.70 ± 4.98	NS
CK-MB(U/L)	208.116± 79.219	285.156 ± 112.506	NS	365.183 ± 169.323	NS
CPK (U/L)	176.82 ± 64.226	215.915 ± 42.336	NS	205.309 ± 56.436	NS
BUN (mg/dl)	11.911 ± 2.954	14.992 ± 4.169	NS	20.347 ± 4.404	P<0.05
Urea (mg/dl)	28.251 ±7.658	30.954 ± 4.533	NS	45.227 ± 13.486	P<0.05
Creatinine(mg/dl)	1.052 ± 0.213	0.924 ± 0.431	NS	1.720 ± 0.510	P<0.05

NS=Not Significant

Table 3. Biochemical Parameters at different time interval following H. Lepturus venom(1500 µg/kg) injection

Parameters	Before venom injection (Mean ±SD)	1 hr. after venom injection (Mean ±SD)	P Value	3 hr. after venom injection (Mean ±SD)	P Value
LDH (U/L)	141.064 ±59.332	234.038 ± 115.926	NS	1368.962 ± 820.557	P<0.05
AST (U/L)	9.13 ± 3.00	18.26 ± 14.54	NS	281.07 ± 162.30	P<0.05
ALT (U/L)	11.51 ± 4.73	17.36 ± 5.92	NS	72.25 ± 45.75	P<0.05
CK-MB(U/L)	195.671 ± 60.680	418.382 ± 155.652	P<0.05	812.805 ± 282.155	P<0.01
CPK (U/L)	181.466 ± 53.503	320.956 ± 125.882	NS	620.556 ± 172.953	P<0.01
BUN (mg/dl)	11.472 ± 3.646	12.176 ± 4.289	NS	19.350 ± 3.519	P<0.01
Urea (mg/dl)	28.721 ± 7.133	35.423 ± 5.677	NS	40.467 ± 4.869	P<0.05
Creatinine(mg/dl)	0.992 ± 0.278	1.408 ± 0.272	P<0.05	1.824 ± 0.167	P<0.01

NS=Not Significant

those with elevations of total CK. More than 80 percent of these individuals also had at least one increased fraction of CK-MB (Green *et al* 1986). All these reports as well as the present study can be the support for the hypothesis that the main point of *H. lepturus* venom effect is not the heart. Aspartate aminotransferase, as a transferase enzyme, mostly concentrates in the liver and the heart; therefore, the increment in its levels may be attributed to myocardial infarction or hepatic injury. It increases more and remains longer than AST during hepatic failure or inflammation (Zare *et al* 2006) .In all the groups of animals the rise in ALT is much higher that AST which can be the indicator for the hepatic injury rather than damage to the hearth, Therefore, the increase in AST and ALT levels may be due to a direct action of the venom on the liver. In the present study a marked rise in BUN, urea and creatinine were observed especially at 3 hours after venom injection. The elevated levels in these parameters are indicative of damage to the kidney. Results of this study confirms the report that showed, histopathological examination of the kidney in *H. lepturus* envenomed rats showed oedema with lymphocyte accumulation near to medulla and cortex area as well as glumerular destruction (Pipelzadeh *et al* 2007). It is interested to know that the average venom content in telson of scorpion (*H. lepturus*) is only 250µg and the LD_{50} of *H. lepturus* venom is 126µg per mice that shows the toxicity of this venom is much less than the other scorpions venom like *O. dorea* which is 8 µg per mice(Jalali *et al* 2010), but the mortality rate in *H. lepturus* scorpion stung patients is higher as compared to others(Jalali *et al* 2010). This may be due several factors including the delayed appearance of systemic signs and symptoms and absence of pain in stung patients by scorpion (*H. lepturus*) and hence delay in treatment by antivenom. Cytotoxic effect of the venom on kidney and liver which cause the direct damage to these organs and hence reversibility of these damages may not be possible when antivenom is injected late. We recently examined the pharmacokinetic of the venom and its accumulation in the rat blood circulation following intravenous injection of the venom and found that at 4 hours following venom injection more than 80% of the venom is accumulated in the kidney and bladder (unpublished data). In conclusion the results of this study reveals the direct effect of *H. lepturus* venom on kidney and liver and the cause of death in patients stung by this scorpion seems to be due to multiple organ dysfunction syndromes through direct and indirect effects of the venom on various organs. Hence, base on our studies as well as other research workers reports, unlike other scorpions which the appearance of systemic signs and symptoms is indicator for the need of antivenom in stung patients, we recommend the use of antivenom in all patients stung by *H. lepturus* scorpion at early stage with or without systemic signs and symptoms.

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