Archives of Razi Institute, Vol. 66, No. 2, December (2011) 133-138



*Full Article*

# Biochemical and Histopathological study of *Mesobuthus eupeus* scorpion venom in the experimental rabbits

**Zayerzadeh[1], E., Zare Mirakabadi[*2], A., Koohi[3], M.K.**

*1. Department of Anaerobic bacterial vaccine research & production, Razi vaccine and serum research institute, Karaj, Iran*
*2. Department of Venomous Animals and antivenom Production, Razi vaccine and serum research institute, Karaj, Iran*
*3. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Received 02 May 2011; accepted 20 Aug 2011

**ABSTRACT**

In tropical and subtropical countries, envenomation by scorpions (so-called scorpionism) represents a serious public health problem. In the present study, the toxic effects of mice LD50 injections of *Mesobuthus eupeus* (*Me*) venom on the kidney and liver of anesthetized rabbits were investigated. Six rabbits were selected and ALT, AST, BUN and creatinine were measured at 0, 1 and 3 hours after envenomation and histopathological studies were carried out postmortem. All the animals showed signs and symptoms of envenomation within 30-40 minutes and died 3 to 3.5 hours after venom injection. Histopathological examinations revealed glumerolar congestion, dilated vessels of interstitium and focal interstitial congestion in the kidney and focal hemorrhage, central vein congestion, congested vessels in portal areas and dilated sinusoids in the liver at 3 to 3.5 hrs following venom injection. In addition, biochemical analyses indicated significant rise in the levels of ALT and creatinine following *Mesobuthus eupeus* envenomation in animals at 3 hrs. However no significant changes were observed at 1 hr. In conclusion, scorpion (*Mesobuthus eupeus*) venom leads to damage in vital organs such as liver and kidney.

***Keywords:*** *Mesobuthus eupeus*, rabbit, envenomation, histopathological changes, ALT, creatinine.

## INTRODUCTION*

Scorpion stings represent an important and serious public health problem in tropical and subtropical countries (Gueron *et al* 2000). Although *Hemiscorpious lepturus* is one of the highly distributed medically important scorpion in south west part of Iran, but the majority of stings that occur in through our country are attributed to the Buthidae family, which includes *Mesobuthus eupeus, Androctonus crassicauda, Odontobuthus odonturus* and *Apistobuthus pertigus* (Radmanesh 1990). Scorpion *(Me)* is one of the major scorpions in Iran and its stings constitute a public health problem In addition, *Mesobuthus eupeus* shows high geographic distribution in Turkey, Iraq, Turkistan, Afghanistan and Pakistan. Therefore, *Me* is known as a central Asian scorpion

*Author for correspondence.Email: A.zare@rvsri.ir

(Ozkan *et al* 2008). Radmanesh demonstrated that 45% of all cases of scorpion stings in Iran were due to *Me* (Radmanesh 1990). Investigations showed that following a *Me sting*, victims have presented severe pain, hyperemia, edema, thirst, dry mouth, hypotension, nausea, hypertension, elevated bronchial secretion, difficulty in breathing, tachycardia and cyanosis (Ozcan *et al* 2005). In addition, there are some reports of the incidence of acute renal failure and hepatic complications following some scorpions of Buthidae and Scorpionidae family (D'Suze *et al* 2004, Ismail *et al* 1998, Pipelzadeh *et al.*, 2006, Zare *et al.*, 2007, Zare *et al.*, 2006). To the best of our knowledge, no report has been published regarding the hepatic and renal histopathological as well as biochemical effects of Iranian *Me* scorpion venom. Therefore, the present study was undertaken to investigate hepatic and renal effects in anesthetized rabbits following *Me* envenomation.

## MATERIALS AND METHODS

**Venom.** *Me* venom was obtained using electric shock at the scorpion telson in the Department of Venomous Animals and Antivenom Production, Razi Vaccine and Serum Research Institute of Iran. It was lyophilized and stored at 4°C until use.

**Experimental Protocol.** Six male New Zealand white rabbits with an average weight of 2 ± 0.3 kg were used in this study. All animals were housed under conditions of controlled light (12L: 12D cycle), temperature (24 ± 1°C) and humidity (55 ± 5%), with standard diet and water available *ad libitum*. All animals were injected with 50 mg/kg ketamine and 5 mg/kg xylazine (intramuscular to induce anesthesia). All animals were kept in compliance with the recommendations of the Animal Care Committee of the Tehran University based on the Guide for Care and Use of Laboratory Animals (US NIH publication 86-23, revised 1985). All animals were subcutaneously injected with a lethal dose (4.5 mg/kg of body weight) considering LD50 of *Me* venom in mice.

**Histopathological Analyses.** After the animals died the liver and kidneys were removed carefully and immersed in 10% buffered formalin at room temperature and then sectioned transversely into 3-4 µm slices. Specimens were dehydrated in a graded series of alcohol and xylene and embedded in paraffin. Multiple slices were made and stained by hematoxilin and eosin stains. Sections were viewed and photographed using a Nikon E200® light microscope (Japan).

**Biochemical analyses.** Blood samples were collected at 0, 1 and 3 hours following venom injection by cephalic vein puncture, in tubes without anticoagulant. Serum was separated and used for determination of Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine were assayed in the sera using the photometry technology (COBAS INTEGRA® 400 plus system and ALT, AST, BUN, creatinine kits, Roche, Germany) according to the manufacturer's instructions. The enzyme values were expressed in international units (U/L).

**Statistical Analyses.** All results were expressed as mean ± SEM. The statistical significance of differences among groups was analyzed by the Student's *t*-test. Data were considered statistically significant if *p*-values were $< 0.05$.

## RESULTS

**Histopathological analyses.** Post-mortem examination revealed central vein congestion (figure 1A), congested vessels in portal areas, dilated sinusoids (figure 1B) and steatohepatitis (figure 1C) in liver of animals treated with *Me* scorpion venom. In addition, histopathological evaluation of the kidneys tissues demonstrated glumerolar congestion characterized by presence of erythrocytes in the glumeroles and interstitial space (figures 1 D, E), dilated vessels of interstitium and focal interstitial congestion (figure 1D).

**Metabolic changes.** ALT level was significantly increased at 3 h following *Me* venom injection but there were no differences between control and envenomed animals regarding ALT serum level at 0 h and 1 h after venom injections. In addition, there were no alterations in AST levels in envenomed animals at 0 h, 1 h and 3 h following *Me* venom injections (Table 1).

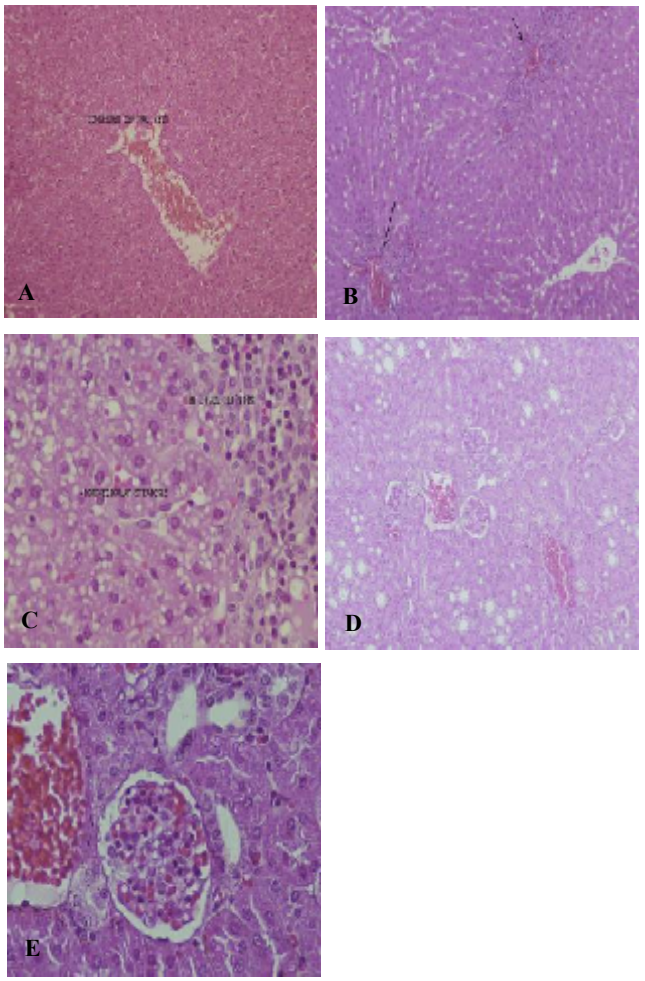


**Figure 1.** Histopathological changes of liver (A, B, C) and kidney (D, E) tissue induced by *Mesobuthus eupeus* venom (90 µg/20 g body weight). From 3 to 3.5 hours after evenomation.
(**A**) central vein congestion, (B) congested vessels in portal areas and dilated sinusoids, (c) steatohepatitis in liver are present. Three hours after envenomation, (**D**) glumerolar congestion, dilated vessels of interstitium and focal interstitial congestion (10x) (E) glumerolar congestion (40x) in kidney were present.
Sections were viewed and photographed using a Nikon E200® light microscope (Japan).

Significant creatinine serum elevation was observed at 3 h after *Me* venom injection. However, there were no significant differences between control and envenomed rabbits regarding creatinine serum level at 0 h and 1 h after venom inoculations. Furthermore, there were no significant changes in BUN serum levels in envenomed animals at different time following *Me* venom inoculations (Table 2).

## DISCUSSION

Stings of medically important scorpions from buthidae family scorpions with medical importance produce some disturbances in major organs. In the present study the dose of venom was selected based on LD50 of the venom in mice to observe the acute toxic effects of the *Me* venom on vital organs. In this study, injection of *Me* scorpion venom in the rabbits caused histopathological complications such as central vein congestion, congested vessels in portal areas, dilated sinusoids and steatohepatitis. D´ Suze et al reported aggregation of leukocytes, mainly neutrophils, in the portal vein and hepatocytes with Mallory's body near to sinusoids in liver of envenomed rams with *Tityus discrepans* scorpion venom (D'Suze *et al* 2004). In addition, they have shown that *Tityus discrepans* venom caused macrophages, neutrophils and lymphocytes infiltration in liver. Another observations were hepatocytes necrosis in zones surrounding portal venules through which leukocyte were infiltrating (D'Suze *et al* 2004). On the other hand, in our study, although the rise at 1hr following venom injection was non significant as compared with control group , but at 3 hrs it was found to be significant. ALT is the most important hepatic enzyme that increaseses during hepatic injury or inflammation. These change was similar to those observed in rabbits receiving venom of scorpions of the Buthidae family (Zare *et al* 1994b, Zare *et al* 1994a). In addition, Zare et al showed ALT increment in envenomed rabbits with *Hemiscorpius lepturus* scorpion venom (Zare *et al* 2007). Moreover, Hepatic toxicity has reported in *Hemiscorpious lepturus* scorpion victims (Pipelzadeh *et al* 2006). Pharmacokinetic studies of the distribution of scorpion venom in rats have shown that the concentration of

**Table 1.** Serum ALT and AST levels in anesthetized rabbits at 1 and 3hrs following *M.e* venom injection.

| | ALT(U/L) | | | AST(U/L) | | |
|---|---|---|---|---|---|---|
| **Time(h)** | **control** | **envenomed** | ***P* value** | **control** | **envenomed** | ***P* value** |
| 0 | 26.2 ± 2.6 | 23.4 ± 3.8 | NS | 17.4 ± 2.1 | 19.1 ± 3.5 | NS |
| 1 | 28.4 ± 3.1 | 35.5 ± 4.1 | NS | 22.2 ± 4.1 | 25.1 ± 5.4 | NS |
| 3 | 25.3 ± 4.4 | 57.2 ± 3.7 | S | 20.1 ± 5.4 | 29.5 ± 4.9 | NS |

Mean values (standard error) are shown for the six animals in each group. NS: Not significant, S: significant

**Table 2.** Serum Creatinine and BUN levels in anesthetized rabbits at 1 and 3 hrs following *M.e* venom injection**.**

| | Creatinine(mg/dL) | | | BUN(mg/dL) | | |
|---|---|---|---|---|---|---|
| | **control** | **envenomed** | ***P* value** | **control** | **envenomed** | ***P* value** |
| 0 | 0.87 ± 0.23 | 0.79 ± 0.36 | NS | 24.3 ± 6.1 | 21.1 ± 9.4 | NS |
| 1 | 1.02 ± 0.34 | 0.9 ± 0.44 | NS | 23.7 ± 3.3 | 24.4 ± 10.8 | NS |
| 3 | 1.07 ± 0.2 | 1.93 ± 0.31 | S | 25.4 ± 4.6 | 32.8 ± 4.2 | NS |

Mean values (standard error) are shown for the six animals in each group. NS: Not significant, S: significant

toxins in the liver reached their highest level 15 min after inoculation (Nunan *et al* 2003, Nunan *et al* 2004). Hence, ALT increament and liver complications in the present work confirmed that lethal dose of *Me* venom caused alterations in the liver function of rabbits. In addition, although the rise in serum was observed AST at 1 and 3hrs following venom injection, but the rises were non significant .The non significant rise in AST can be due to limitation of number and variation in physiological response of animals. In our study, injection of *Me* scorpion venom in rabbits caused histopathological complications such as glumerolar congestion, dilated vessels of interstitium and focal interstitial congestion in kidneys of all animals. Pharmacokinetic studies in rats have previously shown that scorpion venom spreads rapidly from blood to tissues, and especially the kidneys, reaching a maximum concentration around 15 min after inoculation (Ismail *et al* 1998, Nunan *et al* 2003, Nunan *et al* 2004). Martins et al have demonstrated that Snake (*Crotalus durissus cascavella)* venom induced proximal tubular damage in perfused isolated kidney within two hours (Martins *et al* 1998).

With respect to creatinine level (table 1), the significant statistical difference ($p<0.05$) was observed between groups at 3 h but there was no alteration in BUN level, which done to evaluated renal lesions. Creatinine and BUN are the final products of protein metabolism and their levels will be increased in renal failure. There are some reports of the incidence of acute renal failure, with increased urea and uric acid concentrations, reduced urinary volume and diminished creatinine secretion, in victims that had been stung by *Androctonus*, *Leiurus* and *Buthus* scorpions (Ismail *et al* 1998). Zare et al demonstrated creatinine and BUN increment following *Odonthobuthus doriae* scorpion venom injection in experimentally rabbits (Zare *et al* 2006). However, based on the creatinine elevation and kidney complications observed in this study, it is clear that *Me* venom in the doses used caused alterations in the renal function rabbits. Clinical manifestations of scorpion envenomation are usually complex in nature and can be attributed mainly to noticeable overactivity of the autonomic nervous system similar to pheochromocytoma. Many toxins in scorpion venoms can interfere selective actions on voltage-gated Na+

channels and/or on voltage-gated and other K+ channels that result in the massive release of autonomic neurotransmitters (described as 'an autonomic storm') as a major donor to the pathophysiolgy of scorpion envenomation (Gwee *et al* 2002). Therefore, hepatic and renal injury can be due to massive release of catecholamines and resultant vasoconstriction and hypertention in liver and kidney. However, these lesions may also be caused by action of other substances related to scorpion envenomation such as cytokines and inflammatory mediators (D'Suze *et al* 2003, Fukahara *et al* 2003). Furthermore, *Me* venom may be toxic to the vessel wall. Endothelial cells are the first cellular barrier to blood-borne toxins. These cells are susceptible to toxic effects. Toxins that reach the subendothelial space may cause injury to medial smooth muscle cells and/or adventitial fibroblasts (Klassen & Watkins 2003). Therefore, the direct effect of *Me* venom on endothelial cells of liver and kidney vessels leading to hepatic and renal injury are to be considered too.

In conclusion, *M. eupeus* venom administrated at dose of 4.5 mg/kg was able to induce alterations in liver and kidney organs of rabbits. Our results suggest that these complications induced are multi-factorial occurrences induced by the toxins present in the venom.

## References


D'Suze, G., Monceda, S., Gonzalez, C., Sevcik, C., Aguilar, V., and Alagon, A. (2003). Relationship between plasmatic levels of various cytokines, tumour necrosis factor, enzymes, glucose and venom concentrations following *Tityus* scorpion sting. *Toxicon* 41: 367-375.

D'Suze, G., Salazar, V., Diaz, P., Sevcik, C., Azpurua, H., and Bracho, N. (2004). Histophatological changes and inflammatory response induced by *Tityus discrepans* scorpion venom in rams. *Toxicon* 44:851-860.

Fukahara, Y.D.M., Reis, M.L., Dellalibera-Joviliani, R., Cunha, F.Q.C., and Donadi, E,A. (2003). Increased levels of IL-1b, IL-6, IL-8, IL- 10 and TNF-a in patients moderately or severely envenomed by *Tityus serrulatus* scorpion sting. *Toxicon* 41:49-55.

Gueron, M., Ilia, R., and Margulia, G. (2000). Arthropod poisons and the cardiovascular system. *The American Journal of Emergency Medicine* 1:708-714.

Gwee, M.C.E., Nirthanan, S., Khoo, H.E., Gopalakrishnakone, P., Kini, R.M, and Cheah, L.S. (2002). Autonomic effects of some csorpion venoms and toxins. *Clinical and Experimental Pharmacology and Physiology* 29: 795-801.

Ismail, M. and Abd-Elsalam, M.A. (1998). Are the toxicological effects of scorpion envenomation related to tissue venom concentration? *Toxicon* 26:256.

Klassen, C.D. and Watkins, J.B. (2003). Essentials of Toxicology. ed: 281-283.

Martins, A.M, Monteiro, H.S, and Junior E.O. (1998). Effects of *Crotalus durissus cascavella* venom in the isolated rat kidney. *Toxicon* 14410-14450.

Nunan, E.A., Moraes, M.F.D., Cardoso, V. N., and Moraes-Santos, T. (2003). Effect of age on body distribution of tityustoxin from *Tityus serrulatus* scorpion venom in rats. *Life science* 73:319-325.

Nunan, E.A., Arya,V., Hochhaus,G., Cardoso,V.N., and Moraes-Santos, T. (2004). Age effects on the pharmacokinetics of tityustoxin from *Tityus serrulatus* scorpion venom in rats. *Brazilian Journal of Medical and Biological Research* 37:385-390.

Ozcan, O. and Kat, I. (2005). *Mesobuthus eupeus* scorpionism in Saliurfa region of turkey. *Journal of Venomous Animal and Toxins Including Tropical Diseases* 11:479-491.

Ozkan, O. and Carhan, A. (2008). The neutralizing capacity of *Androctonus crassicauda* antivenom against *Mesobuthus eupeus* scorpion venom. *Toxicon* 52: 375-379.

Pipelzadeh, M.H., Dezfulian, A.R., Jalali, M.T., and Mansouri, A. K. (2006). In vitro and in vivo studies on some toxics effects of the venom from Hemiscorpious lepturus scorpion. *Toxicon* 48:93-103.

Radmanesh, M. (1990). *Androctonus crassicauda* sting and its clinical study in Iran. *J Trop Med Hyg* 93:323-326.

Zare, M.A., Murthy, K.R.K., and Haghnazari, L. (1994a). Scorpion venom poisoning in experimental animals. *Archives of Razi Institute* 44/45:67-72.

Zare, M.A, Murthy, K.R.K., and Jafar, M.B. (1994b). Insulin as an effective drug in scorpion venom poisoning. *Archives of Razi Institute* 44/45: 59-65.

Zare, M.A., Zolfagharian, H., Hedayat, A, and Jalali, A. (2007). Clinical and biochemical manifestations produced by scorpion *Hemiscorpius lepturus* venom in experimental

animals. *Journal of Venomous Animal and Toxins Including Tropical Diseases* 13:758-765.

Zare, M.A., Jalali, A., Jahromi, A.E., Vatanpur H., and Akbary, A. (2006). Biochemical changes and manifestations of envenomation produced by *Odonthobuthus doriae* venom in rabbits. *Journal of Venomous Animals and Toxins including Tropical Diseases* 12: 67-77.