Archives of Razi Institute, Vol. 68, No. 2, December (2013) 91-99

DOI: 10.7508/ari.2013.02.001

Review Article

Hemiscorpius lepturus envenomation: Manifestations and management with specific antivenom

Zare Mirakabadi*, A.

Department of Venomous Animal & Antivenom Production, Razi Vaccine & Serum Research Institute, Karaj, Iran

Received 20 Oct 2012; accepted 05 May 2013

ABSTRACT

Scorpionism is a known significant problem of medical and social importance in many tropical and subtropical regions including the Middle East. In Iran, highest prevalence of scorpion sting about 60% of all the stings has been reported from Khuzestan province. Among the 21,000 cases of reported scorpion stung patients, 12% were caused by *H. lepturus,* but contributed to 95% of all moralities in scorpion stung patients. The sting of *H. lepturus* does not produce an immediate pain as does the sting of other scorpions, rather cause delayed swelling that may diffuse and is often accompanied by late necrosis at the sting site suggestive of less significant role of the nervous system stimulation. Since the venom from *H. lepturus* is cytotoxic in nature and the renal response and blood toxicity are normally simultaneously manifested, it is suggested that the toxin binds to kidney tissue and potentially induce acute renal failure in stung patients. Pharmacokinetic analysis revealed that Intramuscular (i.m) injection of antivenoms is ineffective in neutralizing the action of venoms. Although some reports mention the slow distribution rate of *H. lepturus* venom following sting, but since the cytotoxic effect of venom from this scorpion is irreversible by antivenom once it occurs, it is recommended to use antivenom through intravascular (i.v) route. Antivenoms of F(ab)2 fraction are the best choice of treatment for their fast extravasation, their ample distribution into the extracellular space, and their prolonged retention time.

Keywords: *Hemiscorpius lepturus,*antivenom, manifestations, management

INTRODUCTION

Scorpionism is a known significant problem of medical and social importance in many tropical and subtropical regions including the Middle East (Balozet 1971, Farghly and Ali, 1999, Silva *et al* 2000, Dehghani *et al* 2009). In Iran, highest prevalence of scorpion sting about 60% of all the stings has been reported from Khuzestan province. The highest rates of annual incidence of scorpion sting per hundred thousand populations are reported to be 1563 in Khuzestan, 1290 in Kohkiloye Boyerahmad and 826 in Ilam, provinces of Iran (Azhang & Moghisi 2006). At least 7 important scorpion species are found in Khuzestan with varied prevalence of *Androctonus crassicauda* with 28.7%, *Hemiscorpius lepturus* with

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

24.9%, *Mesobuthus eupeus* with 21.7%, *Compsobuthus matthiesseni* with 20.6%, *Hottentotta saulcyi* with 3.35%, *Orthochirus scrobiculosus* with 0.5 % and *Hottentotta schach* with 0.5% (Pipelzadeh et al 2007). Among the 21,000 cases of reported scorpion stung patients in1996, 12% were caused by *H. lepturus* and it contributes to 95% of all moralities in scorpion stung patients (Radmanesh 1990). It is interested to know that the average venom content in telson of scorpion (*H. lepturus*) is only 250µg and the LD50 of *H. lepturus* venom in mice is 126µg/mice that shows the toxicity of this venom is much less than the other scorpions venom like *O. dorea* with LD50 of 8 µg per mice (Jalali *et al* 2010), but however the mortality rate in *H. lepturus* scorpion stung patients is higher as compared to others (Jalali *et al* 2010). Adiguzel et al in the year 2007 reported that over 39% of the stings were in children aged up to 10 years, while those aged 60 and over constituted only 3.2% of the reported accidents, suggesting that the risk of suffering accidents diminishes with age. They attributed the high incidence of stings among children to the higher inquisitive nature, risk-taking behavior such as lifting up stones and putting on clothes and shoes without checking them for the presence of scorpions (Adiguzel *et al* 2007). The body extremities accounted for almost 67% of the site stings, a frequency that is not very different from other studies that invariably have shown that the afflicted body parts are mostly the extremities (hand, leg, foot, arm) (Farghly & Ali 1999, Silva *et al* 2000). These findings may be explained on the basis that the exposed limbs are usually used in most manual activities and moving makes the scorpion retreat and so stings occur in other parts of the body, such as neck and head, when resting or sleeping.

Venom of *Hemiscorpius lepturus*. The electrophoretic profiles, with 15% acrylamide gel, of *H. lepturus* venom showed at least 10 different protein components, which were widely distributed in the range of molecular mass between 3.5 kDa and 260 kDa. There were several major bands, being located at the approximate molecular weights of 4 kDa, 30 kDa and 50 kDa (Ramin *et al* 2010). The venom of *H. lepturus* showed significant amounts of enzymatic activities against casein; gelatin, or hyaluronic acid by zympgraphy method. This venom presented a weak caseinolytic band around 30 kDa, which appears to be a metalloproteinase. Gelatinolytic activity was similarly observed in this venom; with one weak band placed around 30 kDa, and a stronger band near 50 kDa. In addition, hyaluronidase activity was detected at around 40 kDa (Ramin *et al* 2010). In fact, identification of a novel compound named Hemicalcin, a neurotoxin which opens ryanodine-sensitive Ca2+ channels similar but significantly more toxic than maurocalcine in the venom of *H. lepturus* has been reported previously (Shahbazzadeh *et al* 2007). In a study by our group of research workers we found that one ml of polyvalent snake antivenom, produced by Razi Research Institute is able to neutralize *H. lepturus* venom equivalent to 5 LD_{50} in mice (Unpublished data). It was very interesting, since almost all research workers are sure that snake antivenom is unable to neutralize the venom of scorpion. We believe that this is due to the similarity in enzymatic constitute of venom of scorpion *H. lepturus* and snake venom. However Ramin et al 2010 believe that, these enzymatic entities are quite similar by their molecular weights to those of Loxosceles desserta.

Local signs and symptoms. The local signs produced in rabbits by the venom of *H. lepturus* included red circle and inflammation at the site of injection especially when the injection dose of venom increased to above 500µg/kg (Zare *et al* 2007). 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 layers with severe hemorrhage observed in envenomed rats (Ajj 2003). The appearance of red circle and inflammation without serious pain around the injection site which appeared as local sign 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 is also suspected that the proteases of *H. lepturus* venom may play an indirect role in the activation of complement system, which participates in dermonecrosis of the envenomed patients as observed in Loxocelism (Espino-Solis et al, 2009). 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 suggestive of less significant role of the nervous system stimulation or to be due to the damaging action on the nerve fibers that transmit the pain signals (Afzali & Pezashki 1998, Radmanesh 1990). The local necrosis and various skin responses that develop following *H. lepturus* sting may last from a few days to several months. This phenomenon was attributed to the small size of the sting, which is approximately 1mm in length, and to the possibility of delay in absorption from the sting site (Radmanesh 1998). When a patient stung by *H. lepturus,* the type of cutaneous reactions, at early stages of presentation, usually correlated with the severity of intoxication. However, this classification is not absolute, since systemic effects can develop without extensive local reactions. When the patients are stung at high local blood flow areas such as face, trunk, neck or proximal extremities, systemic effects can develop without extensive local reactions. Therefore, it is likely that the direct correlation of cutaneous manifestations with eventual development of systemic is not absolute (Radmanesh 1998).

Systemic signs and symptoms. Clinical picture observed among the stung patients by *H. lepturus* suggests both local and systemic effects with the presence of several clinical syndromes and varying intensities that dominate the clinical presentation and so varied and complicated compared with other scorpion stings manifestations. Complications that were reported for human victims stung by *H. lepturus* scorpion include severe fatal haemolysis, renal failure, ankylosis of the joints, psychological problems and cardiovascular complications (Radmanesh 1990). Blood toxicity and renal failure have higher probability in children younger than 10 years old. The reason underlying the severity of symptoms of envenoming in children could be related to their smaller body mass and decreased physiological reserves compared to adults, making them vulnerable to developing the most common signs and symptoms of systemic symptoms, particularly renal failure. Since the venom from *H. lepturus* is cytotoxic in nature (Radmanesh 1998, Pipelzadeh *et al* 2006) and the renal response and blood toxicity are normally simultaneously manifested, it is reasonable to suggest that the toxin binding to kidney tissue may potentially induce acute renal failure in patients following severe scorpion accidents presented, such as decreased urinary volume, hemoglobinuria, proteinuria and lower creatinine excretion (Pipelzadeh *et al* 2006). However, a contribution from the release of endogenous neurotransmitters and other inflammatory mediators cannot be excluded. The high incidence of blood toxicity and renal involvement observed in patients stung by *H. Lepturus* had not been reported in the clinical picture following other scorpion stings. The data from percentage of incidences of various clinical signs and symptoms in relation to the presence or absence of renal toxicity, and hence degree of toxicity, showed that most of these symptoms were observed when there was a concurrent presence of renal involvement. Although most of scorpions from *buthidae* family cause significant myocardial infarction in heart (Hering *et al* 1993, Kilger *et al* 2000), in experimental animals studies on rabbits *H. lepturus* venom did not effect the heart seriously and it seems that the appearance of signs and symptoms related to the neurotransmitters release is not observable and the symptoms related to cytotoxic nature of this venom is more prominent (Zare Mirakabadi *et al* 2010). However, high dose (3000μg/kg) of H. *lepturus* venom in rabbits caused mild ST elevation and sinus bradycardia in limb lead II. The subcutaneous injection

of 6500µg/kg (LD 50) of *H. lepturus* venom in rabbits caused the signs and symptoms 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). It seems the signs and symptoms appears in case of acute envenomation by *H. lepturus* is due to the neurotoxin in the venom of this scorpion species (zare Mirakabadi *et al* 2010). Identification of Hemicalcin, a neurotoxin which opens ryanodine-sensitive Ca2+ channels similar but significantly more toxic than maurocalcine in the venom of *H. lepturus* has been reported which can explain the neurological disturbances in patients specially children stung by this scorpion (Shahbazzadeh *et al* 2007). In the same study, a rise in the CK-MB and CPK in animals following *H. lepturus* venom injection at 3 hours following venom injection my be indicators for the delayed type of damage to the heart. It is reported that 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). A marked rise in BUN, urea, creatinine, ALT and AST were observed especially at 3 hours after venom injection (Zare *et al* 2006). The elevated levels in these parameters are indicative of damage to the kidney and liver. When RBCs were exposed *in vitro* to various concentrations of *H. lepturus* venom, there was a highly significant ($p<0.001$) increase in osmotic fragility. This is an indicator of the venom direct action on RBCs. *In vivo* studies also confirm these results which may be due to the presence of an enzyme, like phospholipase A2, in the venom of *H. lepturus.* This phenomena may be the cause of hematuria in patients stung by this scorpion species(Zare *et al* 2006). The hyaluronidase of this scorpion venom may affect the stability of blood vessel walls (Veiga *et al* 2001) and increase the spreading of venom toxins. 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 (Ramin *et al* 2010).

Pharmacokinetics of scorpion venom. pharmacokinetical studies of scorpion venom were performed either by measuring the plasma level of 125I-labeled venom (Ismail *et al* 1974, 1983, 1380, Calderon- Aranda *et al* 1999) or by following toxin concentrations by ELISA (Revelo *et al* 1996, Santana *et al*, 1996, Krifi *et al* 2001). The result of the pharmacokinetic analysis, performed on the venom of scorpions from buthidea family revealed that the time to reach the maximal venom concentration in the blood, Tmax, was virtually brief (about 2 hr) and the apparent terminal half-life was 496 min. The pharmacokinetics of the venom showed that after a rapid ascending phase, which means a fast absorption of scorpion toxins, the toxin concentrations in plasma reached a maximal value (C max) after T max of 120 min. Then the curve followed a rather slow bi-phasic decline, followed by a slow and a more slowly declining phase (Ismail *et al* 1998). In another study biodistribution studies were carried out in Wistar rats at different time intervals after IV administration of the labeled venom from scorpion of buthidea family. Within 5 min of administration, the labeled venom was found in the blood (27%), muscle (30%), bone (13%), kidney (12%), liver (10%), and other organs. The level of venom in the kidneys was higher than in the liver. The labeled venom was excreted through renal and hepatobiliary pathways (Murugrnsan *et al* 1999). Hence it is clear that the venom distribution in scorpion stung patients in extravascular compartment is fast, explaining the early appearance of the symptoms. After subcutaneous injection in rabbit, 70% of the venom is detected in the blood circulation in less than 15 min, and the maximum serum concentration is reached in less than 2 h (Devaux *et al* 2004). The accumulation of toxins in some internal organs is associated with direct tissue effects. The concentration of venom in the kidneys not only depends on the renal excretion but also on a specific phenomenon of concentration (Abdel-Haleem *et al* 2006), which induces functional effects due to the reduction of local flow of the blood (De Sousa *et al* 2005). When the radiolabelled *H. Lepturus* venom was

injected subcutaneously to rats, the time to reach the maximal venom concentration in the blood, Tmax, was about 2hr and the apparent terminal half-life was 103min. This report showed slow absorption and gradual distribution from blood into tissue and slow removing of *H. Lepturus* venom. So it is likely that the release of venom from the site of injection was gradual (Jalali *et al* 2012). The main reason was attributed to large molecular size of active proteins in the venom of *H. lepturus.* Recently our group examined the biodistribution of the venom of *H. Lepturus* injected by intravascular rout in rats and found that at 4 hours following venom injection more than 80% of the venom is accumulated in the kidney and bladder (unpublished data).

Specific Antivenom. According to (Touloun *et al* 2001), about 40% of the scorpion stings were treated exclusively with traditional medicine in Morocco, 27% by both the traditional and modern medicines, 28% by modern medicine alone and 7% remained without treatment. The role of antivenom in the treatment of scorpion stings remains controversial and the effectiveness of antivenom treatment depends on the potency of the antivenom (Hisham, 1997, Isbister *et al* 2003, Hammoudi-Triki *et al* 2004). Different approaches to the treatment of scorpion envenoming have been advocated by different investigators. Some investigators recommend treatment of mild cases of envenoming with symptomatic measures and/or antivenin and severe cases with symptomatic measures, support of vital functions and i.v. injection of antivenin [Freire-Maia and Campos 1987, Freire-Maia and Campos 1989]. Others recommend close monitoring in ICU for pulmonary or CNS complications, especially for children (Gueron *et al* 1992). The crucial factors in the success of serotherapy can be the potency of the antivenom and its dose and route of administration. The common remedy of the patients envenomed by *H. lepturus* is the intramuscular or intraventricular injection antivenom to neutralize the undesirable venom effects and other symptomatic treatments (Jalali *et al* 2010). It is important to note that it results in a delayed neutralization of toxins that is 10-fold lower efficacy compared to the intravenous route (Ismail and Abd-Elsalam 1998). Ismail and Abd-Elsalam concluded the i.m injection of antivenoms is certainly ineffective in neutralizing the action of venoms, as the venoms and antivenom have no opportunity to meet quickly enough in the central or tissue compartments. Ismail and his co-investigators showed that low doses of antivenom are unable to neutralize completely the electrocardiographic effects of the venom in experimental animals and the ineffectiveness of antivenom in preventing or abolishing cardiovascular manifestations of scorpion envenoming had been ascribed to the low titers of commercial antivenoms used (Ismail 1993, Ismail 1995, Ismail and Abd-Elsalam 1988, Ismail *et al* 1993). The i.m injection of antivenom is also reported to be useless in the treatment of scorpion envenoming because of the slow absorption and distribution of the immunoglobulins compared with the rapid absorption and distribution of scorpion venom. (Mohammad 2003). In contrast to all the reports indicating inefficacy of intramuscular injection of antivenom in neutralizing the scorpion venom in central compartment of stung patients, a report by Jalali et al 2012 considering Tmax value of *H. lepturus* venom and polyvalent F (ab) 2 antivenom in rats and the results indicate that venom absorption is comparatively slower than antivenom and the maximal concentration of venom reached 1.5hr after antivenom. Hence the research workers mentioned that intramuscular injection route of antivenom would be useful, only if administered in referrals under 2hr following *H. Lepturus* sting (Jalali *et al* 2012). It is needed to clarify that unlike the scorpions from *buthidae* family which the cause of manifestation in human is due to the neurotoxins with molecular weight about 7KD (Ismail *et al* 1974, 1980, 1983), most of the manifestations caused by scorpion, *H. lepturus* is due to the cytotoxic components of molecular weight about 30 KD in the venom (Ramin *et al* 2010). The medium molecular size of cytotoxins in the venom of H.Lepturus can be the reason for efficacy of antivenom

against *H.Lepturus* when injected intramuscularly within two hours following scorpion sting (Jalali *et al* 2012). However since the cytotoxic effect of venom is proved to be irreversible by antivenom if once it occurs (Zare *et al* 2011, Hering *et al* 1993), it is recommended to inject the antivenom by i.v rout if any systemic signs and symptoms are seen in stung patients. The time of antivenom injection has important role in effectiveness of antivenom. When antivenom is injected at early time following scorpion sting the chance to prevent the appearance and progress in systemic signs and symptoms increases significantly. Antivenom injection time related effects of *Hemiscorpius lepturus* in rabbits studied by our group. It was found that although the antivenom at 1 hrs following venom injection was unable to reverses the biochemical changes occurred following venom injection but, the acute rise in the various parameters stopped following antivenom administration (Zare *et al* 2011). This indicated that the antivenom is able to neutralize the circulating venom and prevent further disruption of tissues by the venom. In next group of animals which received the antivenom 3 hours following venom injection the rise continued even after 24 hours in most of parameters which indicate that the disruption of tissues by the venom is not able to be reversed, once it occurs (Hering *et al* 1993, Zare *et al* 2011). Antivenom binds to and neutralizes the venom, halting further damage, but do not reverse damage already done. (Gueron *et al* 1993). Some scorpion stings which were previously inevitably fatal have become only rarely fatal provided that the antivenom is administered soon enough (Pipelzadeha *et al* 2006).

Pharmacokinetics of the antivenom. It is recognized that antivenom should contain suitable pharmacokinetic parameters and be distributed rapidly to the tissues to neutralize distributed toxins. The choice of preparing specific IgG or fragments appears to depend on the size and toxicokinetics of the principal toxin(s) of the venoms. Large relative molecular mass (Mr) bivalent antibodies (IgG and F(ab)2 fragments) may be effective for the complete and prolonged neutralization of intravascular toxins (e.g. procoagulant enzymes) which have a long half-life in envenomed patients, whereas low Mr and monovalent IgG fragments such as Fab may be more appropriate against low-molecular-mass neurotoxins which are rapidly distributed to their tissue targets and are rapidly eliminated from the patient's body (Gutiérrez *et al 2003*). The time course of antivenom concentration in plasma, determined by radioactivity measurements, showed a bi-exponential decline, indicating that the antivenom was distributed into two compartments with a terminal half-life of 496.43 min. Extravasation of IgG antivenom is likely to occur via convection, i.e., movement of IgG with fluid flow from blood to tissue through paracellular routes, or following an endocytic route in endothelial cells (Lobo *et al* 2004). The elimination half-life of IgG antivenom was reported to be 82 hrs in experimental rabbits. These values, together with those of clearance and mean residence time, point to a prolonged presence of IgG antivenom in the body. A relatively prolonged elimination half-life has been also described for IgG and F (ab)2 antivenoms by other workers. (Ismail et al 1998, Pe´pin-Covata *et al* 1996, Rivie`re *et al* 1997). A wealth of literature indicates that IgG, F(ab)2 and F(ab) antibodies are equivalent in their efficacies to neutralize their antigens. Yet F (ab)2 antivenoms are the best choice treatment for their fast extravasation, their ample distribution into the extracellular space, and their prolonged mean retention time (MRT) (Va´zqueza *et al* 2005).

Antivenom Impurities. The presence of impurities in antivenom increases the possibility of anaphylactic shock characterized by several actions including increased vascular permeability, vasodilatation, bronchial and visceral smooth-muscle contraction, mucous secretion and local inflammation hypersensitivity reaction characterized by edema in several tissues and drop in blood pressure, secondary to vasodilatation (Abbas & Litchman 2003, Cruce & Lewis 2004). Sera incorrectly purified, or with excessive total protein load, can contribute to the development of this reaction. The Razi institute

antivenom appeared to have some impurities; especially below 30 kDa. However an ELISA assay showed that Razi institute polyvalent antivenin has a high affinity to *H. lepturus* venom, suggesting that the antivenom has specificity for detection and inhibition of the enzymatic activities of this venom. (Ramin *et al* 2010).

Conclusion

Based on information provided in this review article it can be concluded that the venom of scorpion *H. lepturus* is mainly cytotoxic in nature causing various tissue damage in stung patients. The specific polyvalent antivenom is capable of neutralizing the venom if it is injected at early time. Intramuscular injection of specific antivenom is useful before appearance of any systemic signs and symptoms in patients. However since the cytotoxic effect of venom is proved to be irreversible by antivenom if once it occurs, it is recommended to inject the antivenom by i.v rout if any systemic signs and symptoms are observed in stung patients.

References

Abbas, A.K., Lichtman, A.H., Pober, J.S. (2000). (editors). *Cellular and molecular immunology*. 4th ed. Philadelphia: WB Sanders Co. Pp;309-334.

Abbas, A.K., Litchman, A.H. (2003). Immediate Hypersensitivity. In: Abbas AK, Litchman AH (editors). *Cellular and molecular immunology*. 5th ed. Philadelphia: Saunders Elsevier Science Pp;323-336.

Abdel-Haleem, A.A., Meki, A.M.A., Noaman, H.A., Mohamed, Z.T. (2006). Serum levels of IL-6 and its soluble receptor, TNF- and chemokine RANTES in scorpion envenomed children: their relation to scorpion envenomation outcome. *Toxicon* 47: 437–444.

Ajj, K. (2003). Efficacy study of skin excition site of injection of *H Lepturus* venom in Rats. Pharm. D. *Thesis. School of Pharmacy University of Jundishapur, Ahvaz* p 474 (in Farsi, English summary).

Jalali, A., Bavarsad-Omidian, N., Babaeig, M., Najafzadeh, H., Rezaei, S. (2012). the pharmacokinetics of *Hemiscorpius lepturus* scorpion venom and Razi antivenom following intramuscular administration in rat. *Journal of Venom Research 3*: 1-6.

Azhang, N., Moghisi, A.R., 2006. Surveying of Scorpion Sting and Snake Bite during 2001–2005. *Report of Center of Management of Preventing and Fighting with the Diseases*, Pp. 1–29 (in persian).

Adiguzel, S., Ozkan, O., Inceoglu, B. (2007). Epidemiological and clinical characteristics of scorpionism in children in Sanliurfa,Turkey. *Toxicon* 49: 875–880.

Afzali, N., Pezeshki, N. (1998). Acute renal failure evaluation in children envenomation by *Hemiscorpius lepturus*. Scientific *Journal of Ahvaz University of Medical Sciences & Health Services* 25:13-18 (in persian, English summary).

Barbaro, K.C., Knysak, I., Martins, R., Hogan, C., Winkel, K. (2005). Enzymatic characterization, antigenic cross-reactivity and neutralization of dermonecrotic activity of five Loxosceles spider venoms of medical importance in the Americas. *Toxicon* 45: 489–499.

Balozet, L., (1971). Scorpionism in the old world. In: Bucherl, W., Buckley, E. (Eds.), Venomous Animals and their Venoms. Venomous Invertebrates. *Academic Press, New York* 56: 349–371.

Calderon-Aranda, E.S., Rivie`re, G., Choumet, V., Possani, L.D., Bon, C. (1999). Pharmacokinetics of the toxic fraction of *Centruroides limpidus limpidus* venom in experimentally envenomed rabbits and effects of immunotherapy with specific Fab0 2. *Toxicon* 37: 771–782.

Cruce, J.M., Lewis, R.E. (2004). Types I, II, III, and IV hypersensitivity. In: *Atlas of immunology*. 2nd ed. Florida: CRC Press;.

Devaux, C., Jouirou, B., Krifi, M.N., Clot-Faybesse, O., El Ayeb, M., Rochat, H. (2004). Quantitative variability in the biodistribution and in toxicocinetic studies of the three main alpha toxins from the *Androctonus australis hector* scorpion venom. *Toxicon* 41: 661–669.

De Sousa Alves, R., Do Nascimento, N.R., Barbosa, P.S., Kerntopf, M.R., Lessa, L.M., De Sousa, C.M., Martins, R.D., Sousa, D.F., de Queiroz, M.G., Toyama, M.H., Fonteles, M.C., Martins, A.M., Monteiro, H.S. (2005). Renal effects and vascular reactivity induced by *Tityus serrulatus* venom. *Toxicon* 46: 271–276.

Espino-Solis, G.P., Riano-Umarila, L., Becerril, B., Possani, L.D. (2009) .Antidotes against venomous animals: state of the art and prospectives. *Journal of Proteomics* 72: 183–199.

Freire-Maia, L., Campos J.A. (1987). Response to the letter to the Editor by Gueron and Ovsyshcher on the treatment of the cardiovascular manifestations of scorpion envenomation. *Toxicon* 25:125- 30.

Freire-Maia, L., Campos, J.A. (1989). Pathophysiology and treatment of scorpion poisoning. *Proceedings of the Ninth World Congress on Animal, Plantand Microbial Toxins,* Stillwater, Oklahoma, Oxford: Pergamon Press; August 1988.

Farghly, W.M., Ali, F.A. (1999). A clinical and neurophysiological study of scorpion envenomation in Assiut, Upper Egypt. *Acta Paediatrica* 88: 290–294

Gueron, M., Ilias, R., Sofer, S. (1993). The management of scorpion envenoming syndrome. *Toxicon* 31:1071-1076.

Gutiérrez J.M, León G, Lomonte B. (2003). Pharmacokinetic-pharmacodynamic relationships of immunoglobulin therapy for envenomation. *Clinical Pharmacokinetics* 42:721–741.

Gueron, M., Ilia, R., Sofer, S. (1992). The cardiovascular system after scorpion envenomation. A review. *Journal of Toxicology - Clinical Toxicology* 30:245-58.

Green., T.R, Golper, T.A., Swenson, R.D. *et al* (1986). Diagnostic value of creatine kinase MB isoenzyme in chronic hemodialysis patients: A longitudinal study. *Clininical Nephrology* 25: 22-28.

Hisham, M.A., (1997). Scorpion sting syndrome: epidemiology, clinical presentation and management of 2240 cases. *East Mediterranean Health Journal* 3 (1): 82–99.

Hammoudi-Triki, D., Ferquelb, E., Robbe-Vincent, A., Bonb, C., Choumetb, V., Laraba-Djebaria, F. (2004). Epidemiological data, clinical admission gradation and biological quantification by ELISA of scorpion envenomations in Algeria: effect of immunotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 98 (4): 240–250.

Hering, E.S. Jurca, M., Vichi, F.L., Azevedo-Marques, M.M., Cupo, P. (1993). Reversible cardiomyopathy' in patients with severe scorpion envenoming by *Titvus serrulatus*: evolution of enzymatic. Electrocardiographic and echocardiographic alterations. *Annual tropical Pediatrics* 13: 173-182.

Isbister, G.K., Graudins, A., White, J., Warrell, D. (2003). Antivenom treatment in Arachnidism. *Journal of Toxicology - Clinical Toxicology* 41 (3): 291–300.

Ismail, M., Kertesz, G., Osman, O.H., Sidra, M.S. (1974). Distribution of 125I-labelled scorpion (*Leiurus quinquestriatus H* and *E*) venom in rat tissues. Toxicon 12: 209.

Ismail, M., Abdoulah, M.E., Morad, A.M., Ageel, A.M., (1980). Pharmacokinetics of 125I-labelled venom from the scorpion *Androctonus amoreuxi* (*Aud*. and *Sav*.). *Toxicon* 18: 301–308.

Ismail, M., Shibl, A.M., Morad, A.M., Abdullah, M.E. (1983). Pharmacokinetics of 125I-labelled antivenin to the venom from the scorpion *Androctonus amoreuxi*. *Toxicon* 21: 47–56.

Ismail, M., Abd-Elsalam, M.A. and Al-Ahaidib, M.S. (1998). Pharmacokinetics of 131I-labelled *Walterinnesia aegyptia* venom and its specific antivenins: flash absorption and distribution of the venom and its toxin versus slow absorption and distribution of IgG. Fab02 and Fab of the antivenin. *Toxicon,* 36: 93-114.

Ismail, M. (1993). Serotherapy of the scorpion envenoming syndrome is irrationally convicted without trial. *Toxicon* 31, 1077-87.

Ismail, M. (1995). The scorpion envenoming syndrome. *Toxicon* 33: 825-58

Ismail, M., Abd-Elsalam, M.A. (1988). Are the toxicological effects of scorpion envenomation related to tissue venom concentration? *Toxicon*, 26: 233-56.

Ismail, M., Fatana, J.Y., Dabeas, T.T. (1993). Experimental treatment protocols for scorpion envenomation, a review of common therapies and an effect of kallikrein-kinin inhibitors. *Toxicon* 30: 1257-79.

Jalali, A., Pipelzadeh, M., Sayedian, R., Rowan, E.G. (2010). A review of epidemiological, clinical and in vitro physiological studies of nvenomation by the scorpion *Hemiscorpius lepturus* (Hemiscorpiidae) in Iran Review Article. *Toxicon* 55: 173-179.

Kilger, E., Pichler, B., Weis, F., Goetz, A., Lamm, P., Schutz, A. (2000). Markers of myocardial ischemia after minimally invasive and conventional coronary operation. *Annual Thoracic Surgery* 70: 2023-2028.

Krifi, M.N., Miled, K., Abderrazek, M., el Ayeb, M. (2001). Effect of antivenom on *Buthus occitanus tunetanus* (Bot) scorpion venom pharmacokinetics: towards an optimization of antivenom immunotherapy in a rabbit model. *Toxicon* 39: 1317–1326.

Lobo, E.D., Hansen, R.J., Balthasar, J.P. (2004). Antibody pharmacokinetics and pharmacodynamics. *Journal of Pharmaceutical Sciences* 93: 2645–2668

McLaurin, M.D., Apple, F.S., Voss, E.M. (1997). Cardiac troponin I and troponin T, and creatine kinase MB in dialysis patients without ischemic heart disease: Evidence of cardiac troponin T expression in skeletal muscle. *Clinical Chemistry* 43: 976-981.

Murugesan, S., Radhakrishna Murthy, K., Noronha, O.P.D. (1999). Samuel AM. Tc 99m -scorpion venom: labeling, biodistribution and scintiimaging. *Journal of Venomous Animals and Toxins* 5: 46-55.

Mohammad, I. H. (2003). Treatment of the scorpion envenoming syndrome: 12-years experience with

serotherapy *International Journal of Antimicrobial Agents* 21: 170_174.

Pipelzadeh, M.H., Jalali, A., Taraz, M., Pourabbas, R., Zaremirakabadi, A. (2007) A An epidemiological and a clinical study on scorpionism by the Iranian scorpion *Hemiscorpius lepturus* .*Toxicon* 50 (7): 984–992

Pipelzadeha, M. H., Dezfulianb, A., Jalali, M. T,. Mansourian, A. (2006). In vitro and in vivo studies on some toxic effects of the venom from *Hemiscorpious lepturus* scorpion *Toxicon* 48:93–103.

Pe´pin-Covata, S., Lutsch, C., Grandgeorge, M., Lang, J., Scherrmann, J.M. (1996). Immunoreactivity and pharmacokinetics of horse anti-scorpion venom F (ab0)2-scorpion venom interactions. *Toxicology and Applied Pharmacology* 141: 272–277.

Revelo, M.P., Bambirra, E.A., Ferreira, A.P., Diniz, C.R., Chaves- Olortegui, C. (1996). Body distribution of Tityus serrulatus scorpion venom in mice and effects of scorpion antivenom. *Toxicon* 34: 1119–1125.

Rivie`re, G., Choumet, V., Audebert, F., Sabouraud, A., Debray, M., Scherrmann, J.M., Bon, C., (1997). Effect of antivenom on venom pharmacokinetics in experimentally envenomed rabbits: toward an optimization of antivenom therapy. *Journal of Pharmacology and Experimental Therapeutics* 281: 1–8.

Ruhollah Dehghani , Navid Dinparast Djadid , Dellavar Shahbazzadeh, Shahlla Bigdelli. (2009) Introducing *Compsobuthus matthiesseni* (Birula, 1905) scorpion as one of the major stinging scorpions in Khuzestan, Iran. *Toxicon* 54: 272–275

Radmanesh, M. (1998). Cutaneous manifestations of the *Hemiscorpion lepturus*. *International Journal of Dermatology* 37: 500–507.

Santana, G.C., Freire, A.C.T., Ferreira, A.P.L., Chaves-Olortegui, C., Diniz, C.R., Freire-Maia, L. (1996). Pharmacokinetics of *Tityus serralatus* scorpion venom determined by enzyme-linked immunosorbent assay in the rat. *Toxicon* 34: 1063–1066.

Seyedian, R., Pipelzadeh, M.H., Jalali, A., Kim, E., Lee, H., Kang, C., Cha, M. et al (2010). Enzymatic analysis of *Hemiscorpius lepturus* scorpion venom using zymography and venom-specific antivenin. *Toxicon* 56: 521–525

Shahbazzadeh, D., Srairi-Abid, N., Feng, W., Ram, N,. Borchani, L., Ronjat, M., Akbari, A., Pessah, I.N., De Waard, M., El Ayeb, M. (2007). Hemicalcin, a new toxin from the Iranian scorpion *Hemiscorpius lepturus* which is active on ryanodine-sensitive Ca2+ channels. *Biochemistry Journal* 404: 89–96

Silva, R.L.M., Andrea, M., Amorim, T.K. (2000). Envenomation by *Tityus stigmurus* (Scorpiones; Buthidae) in Bahia, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 33: 239–245.

Touloun, O., Slimani, T., Boumezzough, A. (2001). Epidemiological survey of scorpion envenomations in southwestern Morocco. *Journal of Venomous Animals and Toxins* 7: 199–218.

Va´zqueza, H., A., Cha´vez-Harob, W,. Garcı´a-Ubbelohdea, R. Mancilla-Navac, J. Paniagua-Solı´sa, A. Alago´nd, C. Sevcik, d. (2005). Pharmacokinetics of a F(ab0)2 scorpion antivenom in healthy human volunteers. *Toxicon* 46:797–805.

Veiga, S.S., Zanetti, V.C., Franco, C.R.C., Trindade, E.S,. Porcionatto, M.A., Mangili, O.C., Gremski, W., Dietrich, C.P., Nader, H.B. (2001). In Vivo and in vitro cytotoxicity of Brown spider venom for blood vessel endothelial cells. *Thrombosis Research* 02: 229–237.

Zare Mirakabadi, A., Jalali, A., Jahromi, A. E. , Vatanpur, H., 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.

Zare Mirakabadi, A., Mahmoodi Khatoonabadi, S., Teimourzadeh, Sh., Sabiri, Gh.H. (2010). Serum Enzymes Studies in Scorpion (*Hemiscorpius lepturus*) Dose Related Envenomation in Rabbits. *Archives of Razi Institute* 65(2): 83-89.

Zare Mirakabadi, A., Mahmoodi Khatoonabadi, S, Teimoorzadeh, S. (2011). Antivenom injection time related effects of *Hemiscorpius lepturus* scorpion envenomation in rabbits. *Archives of Razi Institute* 66(2): 139-145.

Zare Mirakabadi, A., Zolfagharian, H., Hedayat, A., Jalali, A. (2007). Clinical and biochemical manifestations produced by scorpion (*Hemiscorpius lepturus*) venom in experimental animals. *Journal of Venomous Animals and Toxins including Tropical Diseases* 13: 759-765.