

**Full Article****Antivenom injection time related effects of *Hemiscorpius lepturus* scorpion envenomation in rabbits****Zare Mirakabadi^{*1}, A., Mahmoodi Khatoonabadi², S., Teimoorzadeh¹, S.***1. Venomous Animals and antivenom Production, Razi vaccine and serum research institute, Karaj, Iran**2. Department of Biochemistry, Mashhad Payam Noor University, Mashhad, Iran*

Received 01 Jun 2011; accepted 15 Sep 2011

ABSTRACT

Hemiscorpius lepturus is the most dangerous scorpion species, endemic in Khuzestan province and other southwestern areas of Iran, causing morbidity and mortality in children and adults. The efficacy of the antivenom for reversal of manifestation caused by venom of this scorpion is a controversial issue. In the present work, *H. lepturus* venom (1500 µg/kg) was subcutaneously injected into two separate groups of rabbits. Groups 1 and 2 of rabbits received antivenom through i.v route, at 1 and 3 hours after venom injection respectively. Electrocardiograms of all the rabbits recorded during the experiment. In group 1 rabbits blood collection carried out, before and 1 hr after venom injection as well as 3 and 24 hours after antivenom injection. In group 2 animals, blood collection was carried out before and 3 hours after venom injection as well as 3 and 24 hours after antivenom injection. Separated serum used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), creatine kinase isoenzyme, MB (CK-MB), urea, creatinine and Blood Urea Nitrogen (BUN). In group 1 rabbits highly significant ($P < 0.01$) increase in level of CPK, CK-MB and significant ($P < 0.05$) increase in level of urea, creatinine and BUN within 3 and 24 hours after antivenom injection was observed. In group 2, highly significant ($P < 0.01$) elevated level of CPK, CK-MB and significant ($P < 0.05$) elevated level of urea and creatinine within 3 hours after antivenom injection was observed. Moreover, highly significant ($P < 0.01$) elevated level of CPK, CK-MB and AST and significant ($P < 0.05$) elevated level of rest of the biochemical parameters within 24 hours after antivenom injection was observed. On the other hand, in these groups, electrocardiogram abnormalities such as ST elevation and sinus bradycardia in limb lead II, were indicator of a mild cardiac injury. In conclusion, results obtained in this study indicate that the efficacy of antivenom for reversal of cytotoxic manifestations occurring after envenomation is time limited. Therefore, it is recommended to use antivenom as early as possible before the occurrence of serious cytotoxic effect on various organs.

Keywords: *Hemiscorpius lepturus*, scorpion's venom, antivenom, biochemical parameters, Electrocardiogram

INTRODUCTION*

Scorpions are second only to snakes in causing human fatalities from envenomation (Valavi &

Alemzadeh 2008, Radmanesh 1998). Scorpion envenomation is a major problem in south and southwestern areas of Iran (Ghafourian & Ziba Mohebbi 2008). The venom comprises a complex mixture of proteins of low molecular mass, with toxic

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

or enzymatic effects (Barbaro et al 2005, DE Roodt et al 2003). This usually affecting the nervous, cardiovascular, respiratory, skeletal, and gastrointestinal systems especially in children, with death usually credited to cardiovascular and respiratory failure (Hammoudi et al 2004, Ismail 1995, Radhakrishnamurthy 2000). 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 indicated the manifestations caused by the venom of the *hemiscorpionidae* family where the most common scorpion genus is *Hemiscorpius lepturus* (Radhakrishnamurthy 2000, Zare Mirakabadi et al 2007, Jalali et al 2010, Pipelzadeh et al 2007). 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 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 serum of scorpion (Pipelzadeh et al 2007). Serotherapy of the scorpion-envenoming syndrome was a subject of controversy (Hammoudi et al 2004, Gueron et al 1993). The effectiveness of the antivenom influenced by; time of administration, route, dosage, availability, potency and the use of adjuvant or alternative therapy (Ismail Abd-Elsalam 1998). The ideal antivenom must adequately reach the different tissues in which venom produces its toxic effect and, once bound to toxin, the complex must be rapidly eliminated (Boyer et al 1999, Seifert & Boyer 2001, Va'zquez et al 2005). The aim of present study was to assess the efficacy of antivenom administration in reversing or normalization of the parameters following scorpion envenomation at the different time.

MATERIALS AND METHODS

Venom. Venom from 4000 scorpions (*Hemiscorpius lepturus*) was extracted by applying a 10-12-volt

electrical shock to the telson of scorpions, avoiding contamination by digestive enzymes. Venom was lyophilized and preserved at -20°C. in the department of venomous animals and antivenom production, Razi Vaccine and Serum Research Institute, Iran. On the day of experiment, the venom was dissolved in normal saline and the concentration was adjusted for 1500 µg/ml and used for injection.

Antivenom. The polyvalent antivenom for *H. lepturus* was produced by Razi Vaccine and Serum Research Institute. It comprises a purified solution with F(ab)₂ fractions of equine immunoglobulins specifically for venoms of Six scorpion including *H. lepturus*. It was obtained from hyperimmune plasma of healthy horses that had been immunized with a mixture of venoms from six species of medically important scorpion species in Iran (*Odontobuthus doriae*, *Mesobuthus eupeus*, *Androctonus crassicauda*, *Buthotus (Hottentota) Sauleyi*, *Buthotus sach* and *H. lepturus*). Protein in the plasma mixture precipitated with ammonium sulfate, enzymatically digested with pepsin and thermodenatured. This followed by dialysis, and finally formulated for use (Latifi & Tabatabai 1979). This lot polyvalent antivenom produced in 2009 and valid until 2012. The mean protein content of the antivenom from the used batches was 3.6 mg/ml, with a neutralizing ability of 26 LD₅₀/ml. All rabbits received (one vial) single 5ml scorpion polyvalent antivenom ampoule

Experimental animals. Healthy albino male New Zealand rabbits (1.5–2 kg) were used. 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. Animals evaluated upon arrival, during quarantine and the experiment by the veterinarian. No animals used in the experiment that showed any signs of ill health. All the rabbits anaesthetized with intramuscular injection of ketamine and xylazine in ratio 2: 0.5 ml respectively. *Hemiscorpius lepturus* venom (1500 µg/kg of body weight) subcutaneously injected into two separate groups of five rabbits. In

group 1 animals, the antivenom injected 1 hour after venom injection while group 2 animals received antivenom 3 hours following venom injection. Blood sampling was carried out before venom, before antivenom (1 and 3 hour after venom injection in groups 1 & 2 respectively), 3 and 24 hours after antivenom injection. The serum separated and used for biochemical assay.

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, creatinine and BUN levels using Pars Azmoon kits According to DGKC and IFCC method (Deutsche *et al* 1972, Bergmeyer 1986).

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

Statistical analysis. Data of the biochemical parameters analyzed with statistical software SPSS 16.0. The biochemical parameters measurements expressed as means \pm SD. All the results statistically analyzed using the Student "t" test to compare the mean values obtained before the venom injection and all the values obtained after venom injection. The results considered statistically significant if P_{value} were less than 0.05.

RESULTS

Clinical signs and symptoms. 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. On the other hand, 1 hour after venom injection, all animals showed muscle contraction on their feet where venom was injected. No

further progress in the local signs observed following antivenom injection and disappeared within 24 hours. No significant changes appeared in breathing pattern of animals. However, the heart rate decreased in all the animals from average of 204 to 103 beats/min within 1hr following venom injection. The heart rate returned to normal within 24 hrs following antivenom injection.

Electrocardiogram. An electrocardiogram of rabbits before and after *H. lepturus* envenomation (1500 $\mu\text{g}/\text{kg}$) and antivenom injection at different time is shown in (figure 1 & 2). ECG In group 1 of rabbits showed no change in the P-wave, QRS-complexes and T-wave. However a mild bradycardia, decreasing heart rate (HR) from average of 204 beat/min (normal) to 103 beat/min which occurred after venom injection was recovered 24 hours after antivenom injection (figure 1). In group 2 of rabbits, ECG showed mild ST elevation and sinus bradycardia in limb lead II being the prominent effect up to 180 min following venom injection, No change in the P-wave, QRS-complexes and T-wave were observed. The significant decreased HR from average of 205 beat/min to 127 beat/min (180 min. after venom injection) showed bradycardia and prolonged repolarization as a prolonged QT interval (Figure 2). 24 hours after antivenom injection, some of the changes in ECG including heart rate of the animals recovered.

Biochemical parameters. Injection of *H. lepturus* venom (1500 $\mu\text{g}/\text{kg}$) into group one animals showed non-significant increase in levels of CPK, CK-MB, LDH, AST, ALT, urea, BUN and creatinine within 1 hour after venom injection. However, highly significant ($P<0.01$) elevated level of CPK, CK-MB and creatinine was observed at 3 and 24 hours following antivenom injection and significant ($P<0.05$) elevated level of BUN and urea within 3 hours after antivenom injection was observed. Although the rises were continued up to 24 hours following antivenom injection but none of them were significant. In addition, highly significant ($P<0.01$) elevated levels of CPK, CK-MB and significant ($P<0.05$) elevated level of creatinine within 24 hours after antivenom injection was observed.

Increase of ALT, AST and LDH within 3 and 24 hour after antivenom injection was not significant too.

(Bhaskara & Suvarnakumari 1972, Hering *et al* 1993, Gueron *et al* 1993).

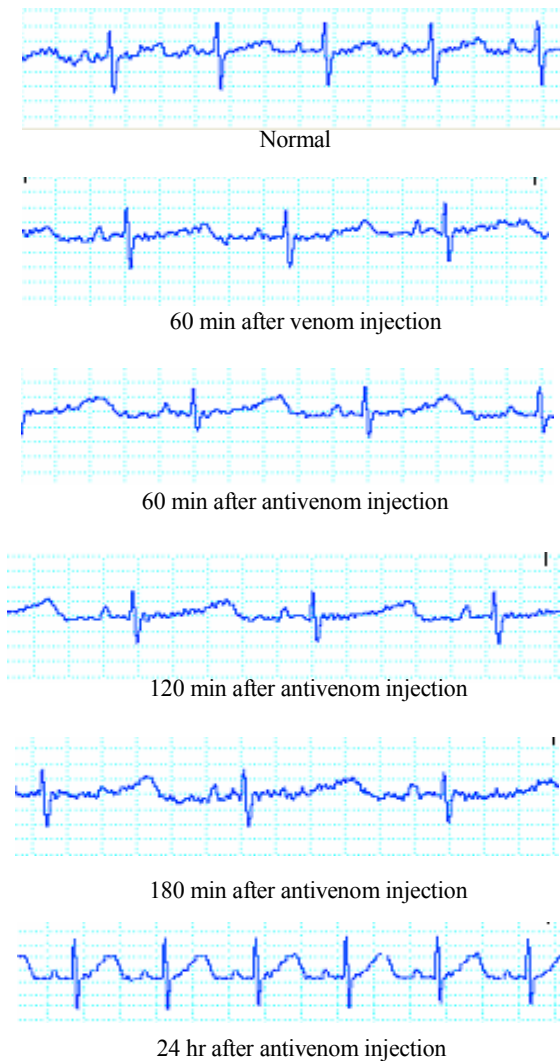


Figure1. Electrocardiogram recordings of anesthetized rabbits treated with antivenom at 60 min. after venom injection (group1).

DISCUSSION

Death due to severe scorpion envenoming syndrome is a common event in the developing tropical and subtropical countries (Bhaskara & Suvarnakumari 1972). In spite of zoological differences resulting in venoms of differing chemical structure, the signs and symptoms following stings by scorpions from *Buthidae* family in all over the world are remarkably similar

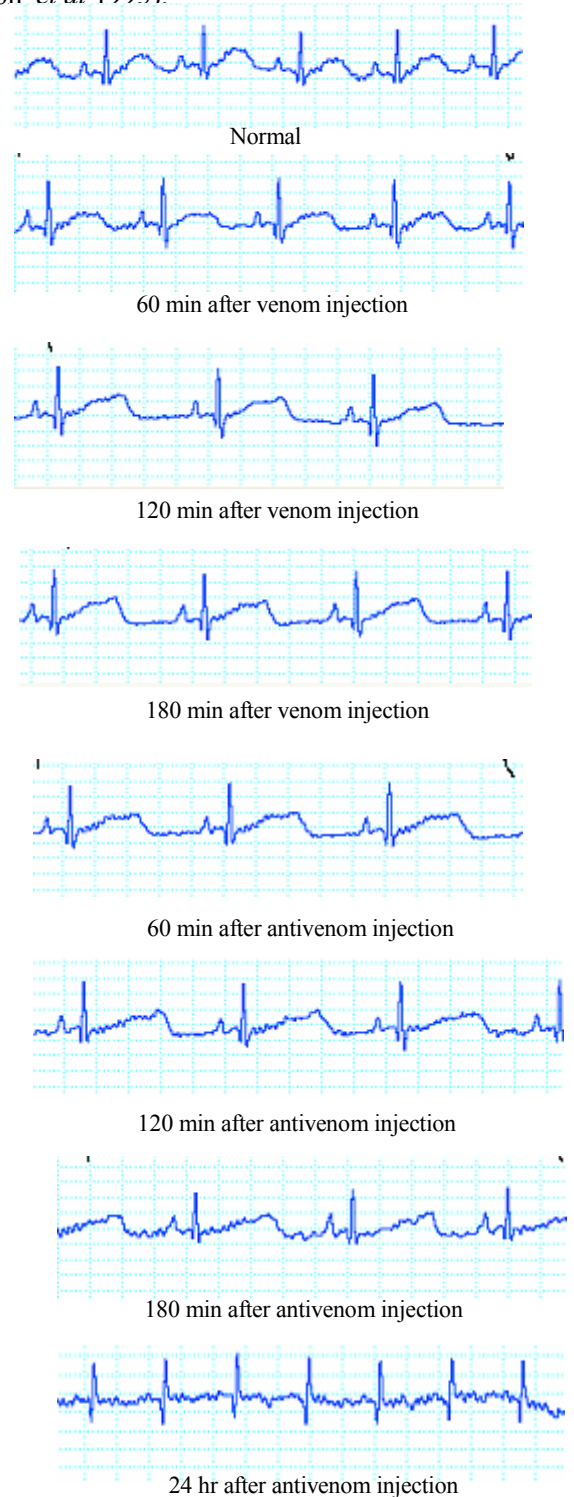


Figure2. Electrocardiogram recordings of anesthetized rabbits treated with antivenom at 180min following venom injection (Group 2).

Table 1. Serum biochemical changes during antivenom treatment at 1hr. following venom injection in group 1 experimental rabbits.

Parameters	Before venom injection, (mean ± S.D.)	1hr. after venom injection	P Value	3hrs. after antivenom injection	P Value	24hrs. after antivenom injection	P Value
LDH (U/L)	166.22±29.37	192.91± 61.18	NS	285.33 ± 167.63	NS	279.91 ±208.44	NS
AST (U/L)	9.59 ± 4.76	15.59 ± 3.41	NS	19.05 ± 9.93	NS	20.57 ± 13.06	NS
ALT (U/L)	11.91 ± 6.49	12.92 ± 3.55	NS	14.11 ± 4.45	NS	16.68 ± 15.03	NS
CK-MB (U/L)	258.25±45.96	646.93 ± 559.39	NS	1326.54±299.00	P<0.01	1338.5 ±478.77	P<0.01
CPK (U/L)	126.83±16.80	235.92± 110.50	NS	576.88 ±164.69	P<0.01	611.6 ±111.70	P<0.01
BUN (mg/dl)	10.77 ± 4.35	12.97 ± 5.05	NS	15.89 ± 6.10	P<0.05	23.15 ± 12.57	NS
Urea (mg/dl)	28.72 ± 10.06	29.04 ± 9.76	NS	42.52 ± 13.07	P<0.05	44.45 ± 18.49	NS
Creatinine (mg/dl)	1.06 ± 0.27	1.64 ± 1.05	NS	2.29 ± 0.11	P<0.01	2.16 ± 1.74	P<0.05

NS=Not Significant. (P>0.05). All the values were compared with the values of before venom injection.

Table 2. Serum biochemical changes during antivenom treatment at 3hrs. Following venom injection in group 2 experimental rabbits.

Parameters	Before venom injection	3hrs. after venom injection	P Value	3hrs. after antivenom injection	P Value	24hrs. after antivenom injection	P Value
LDH (U/L)	180.33±38.22	850.43 ± 452.46	P<0.01	972.8 ± 370.57	P<0.01	1145.97 ± 487.09	P<0.01
AST (U/L)	11.41 ± 1.90	119.880 ± 50.75	P<0.05	145.15 ± 52.07	P<0.05	188.57 ± 45.10	P<0.01
ALT (U/L)	11.42 ± 2.80	54.91 ± 12.96	P<0.05	67.85 ± 17.62	P<0.05	94.48 ± 34.33	P<0.05
CK-MB (U/L)	245.55 ± 10.38	1027.62 ± 364.82	P<0.05	1479.5 ± 144.7	P<0.01	1524.65 ± 129.51	P<0.001
CPK (U/L)	128.35 ± 13.75	728.59 ± 412.83	P<0.05	809.67 ± 45.84	P<0.01	901.90 ± 149.95	P<0.001
BUN(mg/dl)	10.01 ± 8.56	16.53 ± 2.67	P<0.01	16.90 ± 5.78	P<0.05	19.30 ± 5.47	P<0.05
Urea(mg/dl)	32.632 ± 7.36	41.76 ± 7.73	P<0.05	43.51 ± 7.39	P<0.05	82.81 ± 35.49	P<0.05
Creatinine(mg/dl)	0.995± 0.27	1.87 ± 0.45	P<0.05	2.65 ± 1.03	P<0.05	3.70 ± 1.87	P<0.05

NS=Not Significant. (P>0.05). All the values were compared with the values of before venom injection.

However the mechanism of manifestations caused by the venom of scorpion *H.lepturus* seems to be quite different from scorpions from *Buthidae* family (Radmanesh 1998, Pipelzadeha et al 2006). Results of this study indicate that although the changes in group 1 animals at 1 hour following venom injection were nonsignificant, and the rise stopped after antivenom injection, but in group 2 animals the elevated levels in all the parameters occur at 3 hours following venom injection were significant. This indicates a delayed type of venom action, which may be due to cytotoxic nature of venom. The venom of *H. lepturus* reported to produce nephrotoxicity, which demonstrated by proteinuria and presence of intact RBCs in the urine.

Rise in creatinine, BUN and Urea following venom injection in this study also found to be in correlation with the histological findings, showing disruptions in the general structures in the nephron and presence of widespread casts in the tubular structures. (Jalali et al 2010, Pipelzadeha et al 2006). However Jalali et al report on the effect of *H. lepturus* venom (60 µg/kg) on serum enzyme levels indicate that, the level of serum BUN, creatinine and total bilirubin and amylase were within normal range in both pre- and post-venomation, while AST, ALT and ALP were increased significantly following venom injection (Jalali et al 2010). This report is in contradiction with our findings. This may be due to the dose of venom used by them i.e 20 fold less than the dose of venom used by our group.

The significant decreased HR from average of 205 beat/min to 127 beat/min (180 min. after venom injection) showed bradycardia and prolonged repolarization as a prolonged QT interval (Figure 2). 24 hours after antivenom injection, some of the changes in ECG including heart rate of the animals recovered. The mild changes in the heart function as compared to kidney and liver following venom injection indicate that the target organ of the *H. lepturus* venom is not heart. The venom from this scorpion primarily produces cytotoxic effects, although pharmacological findings on the isolated ileum, indicate a definite

increase in the release of acetylcholine, Hence the bradycardia observed in this study may be due to the release of acetylcholine (Jalali et al 2010). In the present study although the antivenom was unable to reverse the biochemical changes occurred following venom injection but, the acute rise in the various parameters stopped following antivenom administration in group 1 animals. This indicates that the antivenom is able to neutralize the circulating venom and prevent further disruption of tissues by the venom. In group 2 animals which received the antivenom 3 hours following venom injection the rise continued even after 24 hours in most of parameters. This reveals that the disruption of tissues by the venom is not able to be reversed, once it occurs. Hence the effectiveness of the antivenom influenced by; time of administration, route, dosage, availability, potency and alternative therapy (Hering et al 1993, Gueron et al 1993). Antivenom bind to and neutralize the venom, halting further damage, but do not reverse damage already done. (Gueron et al 1993). Some bites which were previously inevitably fatal have become only rarely fatal provided that the antivenom is administered soon enough (Pipelzadeha et al 2006). Antivenom is typically the sole effective treatment for a life-threatening condition, and once the precautions for managing these reactions are in place, an anaphylactoid reaction is not grounds to refuse to give antivenom if otherwise indicated. The side effects are manageable, and antivenom should be given as rapidly as the side effects can be managed (Gueron et al 1993, Ismail 1993).

In conclusion the present study revealed that the main target of the *H.lepturus* venom is kidney and liver through direct effect and the efficacy of antivenom against the venom is time dependent. Hence, we recommend the use of antivenom in all the patients stung by *H. lepturus* as early as possible, whether the systemic signs and symptoms is present or not.

References

- 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.
- Bergmeyer, H.U., Horder, M., Rej, R. (1986). International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L - aspartate: 2- oxoglutarate aminotransferase, EC 2.6.1.1.). *Journal of Clinical Chemistry and Clinical Biochemistry* 24: 497 - 510.
- Bhaskara Reddy, D., Suvarnakumari, G. (1972). Fatal cases of scorpion sting. Autopsy study of seven cases. *Clinician* 36:94-96.
- Boyer, L.V., Seifert, S.A., Clark, R.F., McNally, J.T., Williams, S.R., Nordt, S.P., Walter, F.G., Dart, R.C., (1999). Recurrent and persistent coagulopathy following pit viper envenomation. *Archive of International Medicine* 159: 706-710.
- De Roodt, A.R., Garcia, S.I., Salomon, O.D., Segre, L., Dolab, J.A., Funes, R.F., DE Titto, E.H. (2003). Epidemiological and clinical aspects of scorpionism by *Tityus trivittatus* in Argentina. *Toxicon* 41: 971-7.
- Deutsche Gesellschaft (1972). für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC) Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. *Z Klin Chem Klinische Biochememistry* 10: 182- 92.
- Gueron, M., Ilias, R., Sofer, S. (1993). The management of scorpion envenoming syndrome. *Toxicon* 31:1071-1076.
- Hammoudi-Trikid, F., Robbe-Vincent, A., Bon, C., Choumet, V., Laraba, D. (2004). Epidemiological data, clinical admission gradation and biological quantification by ELISA of scorpion envenomation in Algeria effect of immunotherapy. *Royal Society of Tropical Medicine and Hygiene* 98: 240-50.
- Hering, S.E. Jurca, M. Vichi. F., Azevedo-marques, M.M., Cupo, P. (1993). Reversible cardiomyopathy in patients with severe scorpion envenoming by *Tityus serrulatus*: evolution of enzymatic, electrocardiographic and echocardiographic alterations. *Annual Tropical Paediatrics* 13:173-82.
- Ismail M. (1995). The scorpion envenoming syndrome. *Toxicon* 33: 825-58.
- Ismail, M., Abd-Elsalam, M.A. (1998). Pharmacokinetics of ¹²⁵Ilabelled IgG, F (ab₀)₂ and F(ab) fractions of scorpion and snake antivenins: merits and potential for therapeutic use. *Toxicon* 36: 1523-1528.
- Ismail, M. (1993). Serotherapy of scorpion envenoming syndrome is irrationally convicted without trial. *Toxicon* 31:1072-1083.
- alali, A., Pipelzadeh, M.H., Sayedian, R., Rowan, E.G.(2010). A review of epidemiological, clinical and in vitro physiological studies of envenomation by the scorpion *Hemiscorpius lepturus* (Hemiscorpiidae) in Iran. *Toxicon* 55: 173-179.
- Latifi, M., Tabatabai, M. (1979). Immunological studies on Iranian scorpion venom and antiserum. *Toxicon* 17: 617-621.
- Ghafourian Boroujerdnia, M. & Mohebbi, Z. (2008). A survey on comparison of *Hemiscorpius lepturus* and black scorpion effects on peripheral blood subpopulations of patients with scorpion bite under 15 years old referred to Ahwaz Abuzar Hospital, *Toxicology Letters* 180: 32-246.
- Pipelzadeha, M. H., Dezfulianb, A., Jalalic, M. T., Mansourian, A. (2006). In vitro and in vivo studies on some toxic effects of the venom from *Hemiscorpius lepturus* scorpion *Toxicon* 48:93-103.
- Pipelzadeh, M.H., Jalali, A., Taraz, M., Pourabba, R., Zaremirakabadi, A. (2007). An epidemiological and aclinical study on scorpionism by the Iranian scorpion *Hemiscorpius lepturus*. *Toxicon* 50: 984-992.
- Radmanesh, M. (1998). Cutaneous manifestations of the *Hemiscorpius lepturus* sting: A clinical study. *International Journal Dermatology* 37: 500-7.
- Radhakrishnamurthy K. (2000). The scorpion envenoming syndrome. *Journal of Venomous Animals and Toxins* 6: 4-51
- Seifert, S.A., Boyer, L.V. (2001). Recurrence phenomena after immunoglobulin therapy for snake envenomations: part 1. Pharmacokinetics and pharmacodynamics of immunoglobulin antivenoms and related antibodies. *Annual Emergency Medicine* 37: 189-195.
- Valavi, E, Alemzadeh Ansari, M.J. (2008). Hemolytic uremic syndrome following *Hemiscorpius lepturus* (scorpion) sting. *Indian Journal of Nephrology* 18:166-168.
- Va'zquez, H., Cha'vez-Haro, A., García, W. (2005). Pharmacokinetics of a F (ab₀)₂ scorpion antivenom in healthy human volunteers. *Toxicon* 46 : 797-805.
- Zare Mirakabadi, A., Zolfagharian, Z., Hedayat, H., Jalali, A. (2007). Clinical and biochemical manifestation produced by scorpion (*Hemiscorpius lepturus*) venom in experimental animals. *Journal of Venomous Animals and Toxins* 13: 758-765.