

TOXIC EFFECTS OF SCORPION, *HEMISCORPIUS LEPTURUS* (HEMISCORPIIDAE) VENOM ON MICE

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ABSTRACT

Scorpion stings are common in Iran, particularly in the southern and southwest regions of the country, and they pose a serious health problem. *Hemiscorpius lepturus* is the most venomous scorpion in the region. The present study was conducted to investigate the *in vivo* effects of scorpion venom in experimental mice. Haematological parameters (white and red blood cells numbers and haematocrit levels) and histological changes (skin, stomach, spleen, heart, liver, kidney, lung, pancreas, small intestine, anal, adrenal, brain, and bladder) venom were measured in mice. The results revealed that *H. lepturus* species has negative effects on both local and systemic tissues in mice, which may include damage to the skin and internal organs, respectively.

Key words: *Hemiscorpius lepturus*, venom, mice, *in vivo*, haematological studies, histological studies.

INTRODUCTION

Scorpion stings are a significant and common health problem in areas to the south and southwest (SW) of Iran. It has been reported that from 2001 to 2005, 37666, 37535, 38259, 36806, and 42085 cases of scorpion stings were happened per year in Iran, and 24, 14, 23, 29, and 14 of those respective cases resulted in death (Dehghani, 2003, 2005; Dehghani *et al.*, 2004; Azhang and Moghisi, 2006). The majority of the reported cases were from Khuzestan, a SW province of Iran (Azhang and Moghisi, 2006). *Hemiscorpius lepturus*, *Androctonus crassicauda*, and *Mesobuthus eupeus* species are thought to be responsible for envenoming this area (Chitnis *et al.*, 1993; Afzali and Pezeshki, 1998; Pipelzadeh *et al.*, 2007). However, *H. lepturus* is the most venomous scorpion and is responsible for 95% of all scorpion-sting related mortalities.

This scorpion species has been found throughout Iran, Iraq, Pakistan, and Yemen (Lorenço, 2001). However, Lowe (2010) has recently reported two new species of the *Hemiscorpius* genus in Oman, *H. falcifer* and *H. flagelliraptor*. *H. lepturus* is well known for its potent cytotoxic venom that can cause cutaneous necrosis and severe systemic pathology that may lead to death (Radmanesh, 1998; Pipelzadeh *et al.*, 2007; Jalali *et al.*, 2010; Lowe, 2010). Venom from *H. lepturus* causes severe and fatal haemolysis, secondary renal failure, deep and necrotic ulcers, ankylosis of the joints, psychological problems, and death. Venom from this particular scorpion interrupts the function of vital organs in the body and

may result in fatalities (Afzali and Pezeshki, 1998; Pipelzadeh *et al.*, 2006). In an attempt to evaluate the role of *H. lepturus* venom and its effect on organs and blood parameters, this study aimed to investigate the *in vivo* effects of venom in experimental mice.

MATERIALS AND METHODS

This study was performed during the years 2007 to 2009 at Department of Environmental Health, Kashan University of Medical Sciences, Iran.

Venom from *H. lepturus* scorpions were milked by electric shock, lyophilised, and preserved until use. The backs of fifty-healthy male mice weighing 25 to 30 grams were shaved. Each mouse received a subcutaneous injection with 0.1 ml normal saline containing 0.05mg of *H. lepturus* venom. Haematological and histological experiments were also conducted.

Blood samples were collected before injection and 3 days post-injection. Mice tails were cut and blood was collected with a 100-lambda pipette. Blood was used to measure the changes in haematological parameters including: white and red blood cells numbers and haematocrit levels. Haematocrit was centrifuged for five minutes, and levels were measured and recorded before and 3 days after the experiment, using haematocrit measuring equipment. White and red Melangure were thinned down with Marcanu and Heim solutions, respectively, in equal amounts and completely mixed using a shaker. After mixing, white and red blood cells were counted using a photomicroscope and Neobar lam.

Preparation of Tissue Samples: Three days after venom injections, the mice were sacrificed and skin, stomach, spleen, heart, liver, kidney, lung, pancreas, small intestine, anal, adrenal, brain, and bladder were removed for histological studies. Because tissue processing may cause damage, the backs of fifty-healthy male mice weighing 25 to 30 grams injected with saline as a control for comparison of tissue damage caused by venom.

Tissue processing and staining were performed using conventional methods that had been previously described (Bancroft and Gamble, 2007). Briefly, organ samples from venom-injected rats were immersed in 10% formalin for fixation. After dehydration with ethanol and clarification with xylene, tissue sections of the organs were embedded in paraffin. 4-8 μm sections were prepared from embedded tissues. Paraffin sections were placed on glass slides, de-waxed in xylene and rehydrated in distilled water. After rehydration, sections were dried and stained with eosin and haematoxylin. At least five well-prepared slides from each sample were analysed using a Leica light microscope. A t-test was used to determine statistical significance in paired samples that were collected before and after injection with *H. lepturus* venom. The histology data were interpreted as a percentage of staining performance.

RESULTS AND DISCUSSION

The haematological results from mice showed changes in the range of white and red blood cells numbers as well as the haematocrit after *H. lepturus* venom injections, however, only changes in haematocrit levels showed a significant reduction ($p=0.02$). The average number of white blood cells increased from $1.24 \times 10^4 \pm 1.4 \times 10^3$ (mm^3) pre-injection to $1.30 \times 10^4 \pm 4.8 \times 10^3$ (mm^3) post-injection. No significant change in white blood cell numbers was observed.

The average number of red blood cells decreased from $6.99 \times 10^6 \pm 1.2 \times 10^6$ (mm^3) before injection to $6.93 \times 10^6 \pm 1.95 \times 10^6$ (mm^3) after injection. The change in the red blood cell numbers was not statistically significant.

The average number of haematocrit decreased from 35.5 ± 2.48 (mm^3) before injection to 34.25 ± 2.98 (mm^3) after injection and this decrease was statistically significant.

The RBC reduction results obtained in this study were similar to the results found by others (Afzali and Pezeshki, 1998; Salimian *et al.*, 2002; Dehghani *et al.*, 2004; Pipelzadeh *et al.*, 2006, 2007; Zare Mirakabadi *et al.*, 2007; Jalali *et al.*, 2010).

These similar studies, however, used rabbits and rats as animal models instead of mice, which were used in this experiment, haemolytic activity caused by *H. lepturus* venom has also been documented in humans. Farzanpey (1994) noted that people who were stung by

H. lepturus demonstrated haemolytic symptoms from the injected venom. Pipelzadeh *et al.* (2007) reported a rapid drop in haematocrit levels and severe haemolysis in people referred to hospitals for emergencies. Emam *et al.* (2008) described similar results in another study that found a reduction in RBC numbers and haematocrit levels in people stung by *H. lepturus* in the Khuzestan province.

Our results reveal that venom from this particular scorpion causes an increase in the number of WBC. The findings from this study are in accordance with results found by Chitnis *et al.* (1993) who reported elevated numbers of WBC in the majority of patients who died following a scorpion sting.

The histology results showed that *H. lepturus* venom caused pathological changes in the kidney, liver, skin, stomach, heart, spleen, lung, small intestine, pancreas, bladder, and adrenal. According to the presented results, maximum pathological changes occurred in the liver. The changes in the liver included the following: mild congestion (15%), severe congestion (20%), severe and slight bleeding (16%), and necrosis (25%). In the lungs, the pathological changes mostly included the following: slight haemorrhage (11%), severe haemorrhage (15%), slight congestion (5%), severe congestion (8%), alveolar oedema (15%), and swelling (12%). Pathological changes in the skin were manifested in the form of mild necrosis (12%), severe necrosis (6%), swelling (22%), oedema (10%), oedema and necrosis (2%), and swelling and severe necrosis (2%). In the stomach, pathological changes were observed in the form of slight bleeding (8%), severe bleeding (2%), mucosal necrosis (18%), and swelling and inflammation (16%). The pathological changes found in the spleen consisted of the following: severe congestion (4%), slight congestion (8%), slight haemorrhage (20%), severe haemorrhage (4%), and the presence of giant cells (4%). Changes in the heart included the following: severe bleeding (4%) and slight congestion (2%). In the small intestine, pathological changes were seen as mucosal necrosis (42%), ulcer formation and severe necrosis (8%), and severe necrosis and swelling (8%). Pathological changes in the pancreas were found in the form of slight haemorrhage (14%) and haemorrhage and congestion (15%). In the bladder, the following pathological changes were noted: slight bleeding (14%) and mucosal necrosis (15%). *H. lepturus* venom caused death in 92% of mice, as determined by necropsy. Some (8%) of the mice were anesthetized with chloroform prior to a necropsy.

Histological studies indicated that *H. lepturus* venom caused focal necrosis in skin and pathologic alterations in liver, spleen, and kidney. The liver was most severely affected by *H. lepturus* toxin, with common changes including congestion or hyperaemia. The congestion intensity ranged from mild to severe. Additional changes in the liver included sinusoidal

congestion, focal necrosis, and haemorrhage in the fat tissue (near and around intestine); however, these changes were less common. The results showed that *H. lepturus* venom could also induce variable degrees of congestion and haemorrhage in kidney tissues. The results of this study demonstrated the occurrence of pathological changes in rat spleens following envenoming by *H. lepturus* was comparatively less than in liver and kidney (Ahmadizadeh *et al.*, 2006; Khamechian *et al.*, 2009).

Our findings show that *H. lepturus* venom caused pathological changes in mouse liver, kidney, and lung tissues in 76%, 68%, and 66%, of mice respectively. These changes also included heavy or light haemorrhage, congestion, and necrotic foci. Results from this study are in accordance with those found by others. Dehghani *et al.* (2004) indicated pathological changes in 70.6%, 35.3%, and 27.4% of rat liver, kidney, and spleen, respectively. The pathological damages included the following: haemorrhage, congestion, and heavy or light necrosis (Dehghani *et al.*, 2004; Khamechian *et al.*, 2009). It seems that the mechanism for the pathological changes is the same in rats as it is in mice. Dehghani *et al.* (2004) reported some damage to human veins due to *H. lepturus* venom. It may be possible that haemorrhage in liver, spleen, and kidney tissues is attributed to vascular damage, and these results conform to our findings. Farzanpey (1994) also reported kidney failure in people stung by *H. lepturus*. These results correlate with our studies and show that venom from this scorpion leads to kidney failure in both animals and humans.

Cheng (2002) described the effects of *H. lepturus* venom on necrosis in humans. This study also agrees with our findings, which revealed skin damage in 54% of mice injected with *H. lepturus* venom. Radmanesh (1998) reported the rate of the skin manifestations from scorpion stings is 95.5% in humans. He also pointed out that most of the skin manifestations lead to necrosis and sore formations, which demands a long period of recovery. Afzali and Pezeshki (1998) based on an earlier study reported the occurrence of kidney failure in people stung by scorpions. In fact, some of them needed to be dialysed and others have died.

The results of our study revealed that *H. lepturus* venom has a destructive effect on mouse lung tissue, which led to pathological changes in 66% of the mice. Heavy or light bleeding, congestion, oedema, alveolar oedema, and swelling were all reported as main pathological changes in mice caused by scorpion venom. According to this recent study, pathological changes caused by *H. lepturus* venom are a concern not only in the aforementioned organs but also in the heart, stomach, brain, pancreas, adrenal glands, and bladder. This is supported with a study using *Odontobuthus doriae* venom in the experimental rabbits (Zare Mirakabbadi *et al.*, 2007). They injected the venom of *O. doriae* in the

experimental rabbits (using IV route and dosage of 0.5 mg/kg). They have concluded that the venom of *O. doriae* has damaged the liver tissue organ. However, in the another study the venom of *H. lepturus* was injected in the experimental rabbits, applying 6.3 mg/kg of body weight and LD₅₀ of 126 µg/mouse and the main effects have been observed in the kidney organ (Zare Mirakabbadi, 2007). Study of Shahbazzadeh *et al.* (2007) also confirms indirectly the results of the present study regarded to the neurotoxic symptoms observed in the mice after injecting the venom of *H. lepturus* due to hemicalcin activity against the CNS. This is similar to the effects of Buthidae scorpion family as a neurotoxic venom. However, they suggested that venom of *H. lepturus* has cytotoxic effect, because hemicalcin is resistant to proteolysis which helps the venom of *H. lepturus* in cell penetration and doing tissue damages in the different organs (Shahbazzadeh *et al.*, 2007).

Results of the present study revealed that the venom of *H. lepturus* have targeted liver, kidney, lung and adrenal organs more than the other organs, however the most sever damages have regarded to the liver. It is not similar to the results of the other authors who reported the kidney as the main organ target, using *H. lepturus*. This disagreement is due to the using of different experimental animals and dosage of the toxins. The applied dosage in this study was 0.05 mg/experimental mouse as a lethal dose. Our results show that the venom of *H. lepturus* acts as scorpions of Buthidae family venoms by applying at high dosage (lethal dosage) and targets the liver organ.

The *in vivo* effects of *H. lepturus* venom have previously been investigated using rats and rabbits as alternative animal models (Ahmadizadeh *et al.*, 2006; Zare Mirakabbadi *et al.*, 2007; Khamechian *et al.*, 2009; Lowe, 2010). However, in the present study mice were chosen as the preferred animal model. Therefore, the obtained results in the present study are new and innovative. The *in vivo* effects of *H. lepturus* have been recorded based on haematological and histological data collected during the current study.

Conclusion: According to the results of the present study, it is concluded that *H. lepturus* is a species which can effect both local and systemic on the mice including skin tissue damages and internal organs, respectively. However, the target organs might be different regarding the applied dosage of the venom of *H. lepturus*. Finally, we propose that *H. lepturus* venom includes neurotoxic, cytotoxic and haemolytic effects on the mice and human

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REFERENCES

- Afzali N. and N. Pezeshki (1998). Surveying of kidney acute dysfunction following *Hemiscorpius lepturus* sting in children. *Med. Sci. J., Ahvaz Univ. Med. Sci.* 25: 42-48.
- Ahmadizadeh, M., M. Razi Jalali and B. Mohammadian (2006). Effects of scorpion envenomation on the different organs of rat. *Bioch. Cell Arch.* 6: 289-296.
- Azhang, N. and A.R. Moghisi (2006). Surveying of scorpion sting and snake bite during 2001-2005, Centre of Management of Preventing and Fighting with the Diseases, Iranian Ministry of Health, Tehran.
- Bancroft, J. D. and M. Gamble (2007). *Theory and Practice of Histological Techniques*. 5th ed. Churchill Livingstone, Edinburgh (UK). 800 p.
- Cheng, D. (2002). Scorpion sting. *E-med. J.* 3: 1-29.
- Chitnis, P. A., S. Maraghi and B. Vazirianzadeh (1993). Epidemiological and laboratory study on scorpion stings in Khuzestan province. *J. Med. Fac., Guilan Univ. Med. Sci.* 2: 5-12.
- Dehghani, R. (2003). The impact of thermotherapy on healing of *Hemiscorpius lepturus* scorpion sting in mice, and its clinical and haematological features in rat; determining the fractions of the venom of native scorpion *Mesobuthus eupeus* using Tricine-SDS-PAGE. PhD thesis (unpublished). Department of Medical Entomology, Tehran University of Medical Sciences, Tehran.
- Dehghani, R., T. Khamechian, H. Vatandoost, M. A. Asadi and G. A. Mosavi (2004). The effect of *Hemiscorpius lepturus* venom on pathologic changes of rat organs. *Quart. Res. J. Lorestan Univ. Med. Sci. Health Serv.* 6: 37-41.
- Dehghani, R. and N. Valaie (2005). The review of status of scorpion sting in Iran and problems from it. *Feyz.* 9: 73-92.
- Emam, S. J., A. D. Khosravi and A. Alemohammad (2008). Evaluation of haematological and urine parameters in *Hemiscorpius lepturus* (Gadim) victims referred to Razi Hospital, Ahwaz, Iran. *J. Med. Sci.* 8: 306-309.
- Farzanpey, R. (1994). Scorpion sting and the following of it. *Pajouhesh va Sazandegi.* 259: 123-125.
- Jalali, A., M. H. Pipelzadeh, R. Sayedian and E. G. Rowan (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.
- Khamechian, T., R. Dehghani and B. Vazirianzadeh (2009). Histopathological changes induced in rat organs by the venom of *Hemiscorpius lepturus* (Scorpionida: Hemiscorpiidae). *Bioch. Cell Arch.* 9: 289-296.
- Lorenço, W. R. (2001). The scorpion's families and their geographical distribution. *J. Venom. Anim. Toxins.* 7: 3-23.
- Lowe, G. (2010). Two new *Hemiscorpius* Peters, 1861 (Scorpiones: Hemiscorpiidae) from Northern Oman. *Euscorpius.* 91: 1-24.
- Pipelzadeh, M. H., A. R. Dezfulian, M. T. Jalali and A. K. Mansori (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., A. Jalali, M. Taraz, R. Pourabbas and A. Zaremirakabadi (2007). An epidemiological and a clinical study on scorpionism by the Iranian scorpion *Hemiscorpius lepturus*. *Toxicon.* 50: 984-992.
- Radmanesh, M. (1998). Cutaneous manifestation of *Hemiscorpius lepturus* sting: a clinical study. *Intern. J. Dermatol.* 37: 500-507.
- Salimian, J., J. Zargan, F. Ebrahimi, A. Farahmandnejad and A. Hajibeigi (2002). Surveying of role *Hemiscorpius lepturus* scorpion venom in red cell haemolysis. *Kowsar Med. J.* 7: 185-189.
- 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. *Biochemical Journal*, 404, 89-96.
- Zare Mirakabadi, A., H. Zolfagharian, A. Hedayat and A. Jalali (2007). Clinical and biochemical manifestation produced by scorpion (*Hemiscorpius lepturus*) venom in experimental animals. *J. Venom. Anim. Toxins incl. Trop. Dis.* 13: 758-765.