DOI: http://dx.doi.org/10.22034/ari.2016.107493

Archives of Razi Institute, Vol. 72, No. 1 (2017) 23-31

Original Article

Effects of experimental *Mesobuthus eupeus* scorpion envenomation on chicken

Khosravi * [1], M., Mayahi [2], M., Jalali [2], S.M., Rezaie [1], A., Taghavi Moghadam [3], A., Hosseini [4], Z., Barzegar [4], S.K., Azadmanesh [4],S.

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Khuzestan, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Khuzestan, Iran
3. Razi Reference Laboratory of Scorpion Research, Razi Vaccine and Serum Research Institute, Ahvaz, Iran
4. Student of Veterinary Medicine, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Khuzestan, Iran

Received 13 January 2016; accepted 24 April 2016
Corresponding Author: m.khosravi@scu.ac.ir

ABSTRACT

This study aimed to evaluate the clinical, histopathological and hematological effects of *Mesobuthus eupeus* venom on chicken organs. Adult chickens were subcutaneously injected with five doses of *M. eupeus* venom (0.5, 2, 5, 10 and 20 mg/kg; four chickens per each dose). Symptoms were recorded during the experiment and blood samples were collected for hematological analysis. Moreover, a complete necropsy was performed. After macroscopic examination, tissue samples were obtained from the liver, kidneys, heart, lungs, intestines and brain of the chickens three days after venom administration. In intravenous injection, lethal dose of the venom was determined at 15 mg/kg. The first clinical, pathological and hematological symptoms in envenomated chickens were observed at *M. eupeus* doses of 2, 5 and 0.5 mg/kg, respectively. Hematological examination revealed a reduction in lymphocyte count following experimental envenomation, which returned to the pre-experiment level in almost all the cases. On the other hand, heterophil count was found to increase during the experimental period. In addition, erythrocyte count and hematocrit level were stable at all the intervals. Pathological examination was indicative of severe pulmonary hemorrhage, pulmonary and cerebral edema, tubular necrosis of the kidneys, hemorrhage in kidneys and heart, hyaline thrombus and congestion of the liver. According to the results of this study, poultry are resistant to the toxic effects of *M. eupeus* venom.

Keywords: *Mesobuthus eupeus*, Venom, Chicken, Pathology, Hematology

Les effets d'une envenimation expérimentale au scorpion *Mesobuthuseupeus* chez le poulet

Résumé: Cette étude avait pour but d'évaluer les effets cliniques, histopathologiques et hématologiques du venin de *Mesobuthus eupeus* sur les organes du poulet.Cinq doses de venin de *M. eupeus* (0,5, 2, 5, 10 and 20 mg/kg) ont été injectées par voie sous-cutanée aux poulets (chaque dose injectée à un groupe de 4 poulets). Les symptômes ont été enregistrés au cours de chaque expérience et des prélèvements sanguins ont été soumis à des analyses hématologiques. De plus, une nécropsie complète a été menée. Après examen macroscopique, des prélèvements du foie, des reins, du cœur, des poumons, des intestins et cerveau ont été effectués trois jours après l'administration du venin. La dose létale de venin par injection intraveineuse a été déterminée à 15 mg/kg. Les premiers symptômes cliniques, pathologiques et hématologiques d'envenimation au *M. eupeus* ont été respectivement observés à des doses de venins de 2, 5 et 0,5 mg/kg. Les examens histopathologiques ont révélés

une réduction des lymphocytes juste après l'envenimation avec un retour aux taux pré-expérimentaux dans presque tous les cas analysés. D'une autre part, une augmentation dans la numération d'hétérophile a été observée alors que les taux d'érythrocytes et d'hématocrites restaient stables aux différents intervalles analysés. L'examen pathologique indiquait de sévères hémorragies pulmonaires, des œdèmes pulmonaires et cérébraux, une nécrose tubulaire des reins, des hémorragies rénales et cardiaques, des thromboses hyalines et une congestion du foie. Selon nos résultats, les volailles montrent une résistance aux effets toxiques du venin de *M. eupeus.*

Mots clés: *Mesobuthus eupeus*, Venin, Poulet, Pathologie, Hématologie

INTRODUCTION

Scorpions are a major health hazard for humans and animals, especially in tropical regions (Bawaskar and Bawaskar, 2012; Warrell, 2012). Scorpions belong to the *Phylum Arthropoda*, class *Arachnida* and order *Scorpiones*. *Mesobuthus eupeus* species belong to the *Buthidae* family, which are responsible for the majority of envenomation cases in the Middle East and central Asia, particularly in Iran (Karatas, 2003; Sadeghian, 2003; Dehghani and Khamehchian, 2008). Several toxic fractions in *M. eupeus* venom may give rise to sting symptoms (Tuuri and Reynolds, 2011; Sagheb et al., 2012). As short-chain peptides, biological compounds (Adiguzel, 2010) and bioactive substances in scorpion venom (e.g., enzymes, peptides, nucleotides, lipids, mucoproteins, biogenic amines and other unknown compounds) (Boyer et al., 2009) could affect vertebrate and invertebrate organisms (Upadhyay and Ahmad, 2008). Out of an estimated amount of 100,000 distinct peptides that exist in scorpion venom, approximately 400 peptides have been shown to exert toxic effects on humans and animals (Karatas, 2003).

Massive release of catecholamines following scorpion envenomation (Gueron et al., 1993) influences various regulatory hormones, such as glucagon, cortisol and angiotensin (Radha et al., 1998). Stimulation of the sympathetic system leads to adrenergic stimulation, which is associated with cardiac, metabolic, respiratory and neuromuscular disturbance (Chippaux, 2012). Consequences of scorpion envenomation mainly depend on the species of scorpions, venom compounds and physiological response of the victim. These signs and symptoms may manifest within a few minutes or days after the sting. Evaluation of the history, symptoms, haematological and chemical factors are essential to the accurate diagnosis of envenomation and prediction of the status of victims. Previous studies in this regard have denoted various biochemical (Radmanesh, 1990; Taghavi Moghdam et al., 2009), hematological (Emam et al., 2008; Taghavi Moghdam et al., 2009) and pathological manifestations (Kumar et al., 2012; Zayerzadeh et al., 2012) to be induced by scorpion envenomation in humans, rabbits, mice and rats. Median lethal dose of different scorpion venoms ranges between 0.25-3.6 mg/kg in injections to mice. In the majority of lethal scorpions, LD50 is below 1.5 mg/kg (Karatas, 2003). Vulnerability of different animals to the toxic effects of scorpion envenomation is variable (Dehesa-Dávila and Possani, 1994; Padilla et al., 2003). Therefore, assessment of the level of resistance to various toxins in animals could result in the recognition of the action mechanisms of toxic compounds. Considering the differences between birds and mammal species and lack of sufficient data regarding the effects of scorpion venom on poultry organs, this study aimed to evaluate the clinical, histopathological and hematological effects of *Mesobuthus eupeus* venom on chicken organs.

MATERIALS AND METHODS

Venom preparation. In this study, *Mesobuthus eupeus* scorpions were collected from different regions of Khuzestan province in the southwest of Iran (31°19′-32°73′ N, 48°41′-49°4′ E) and milked by electric

stimulation at the end of the tail. Freeze-dried venom was dissolved in distilled water and dialyzed against distilled water at the temperature of 4 °C for 48 hours. After dialysis, venom solution was centrifuged at 1500 rpm for 15 minutes, and the supernatant was collected. On the day of envenomation, crude venom was diluted with distilled water to obtain the final protein concentrations of 0.5, 2, 5, 10 and 20 mg/kg per body weight of the chickens. Total protein concentration was measured using regular Bradford spectrophotometry with standard bovine serum albumin.

Animals. In this study, 24 adult broiler chickens were equally divided into six groups (n=4), categorized as A-F. Subjects in the control group (A) received 500 µL of ultra-pure water via subcutaneous injection into the breast region using a disposable 1-mL hypodermic syringe. Via an identical route, animals in the experimental groups received 500 µL of a solution containing 500 µg (group B), 2 mg (group C), 5 mg (group D), 10 mg (group E), and 20 mg (group F) of the scorpion venom dissolved in ultra-pure water. At 48 hours after venom injection, symptoms were recorded in all the study groups.

Toxicity verification. All the experiments in this study were performed in accordance with the ethical guidelines of the National Ethics Advisory Committee (2006). To determine toxicity, higher concentrations of the scorpion venom (50-200 µg) were administered via subcutaneous (SC) and intraperitoneal injection (IP) to albino mice (weight: 20±2 g). Moreover, increased concentrations of the venom (5-40 mg/kg) were administered via intravenous (IV) and SC injection to adult chickens. Following the treatment, animals were monitored for 24 hours, and the number of dead animals was recorded at the end of the experiment. In addition, lethal dose of the scorpion venom was calculated at this stage.

Hematological analysis. At intervals of 0.5, 1, 2, 4, 6 and 24 hours after venom administration, venous blood samples were collected from study groups (A, B, C, E and F) using 10% ethylenediamine tetra-acetic acid anticoagulant, and hematological evaluation was performed immediately afterwards. Blood smears were prepared on glass slides. After fixation, prepared samples were stained with Giemsa solution for differential leukocyte counting. In the next step, we determined total leukocytes, lymphocytes, heterophils, erythrocytes and hematocrit level. Furthermore, total counts of erythrocytes and leukocytes were recorded through manual haemocytometer chamber counting. In total, 100 lymphocytes, neutrophils, monocytes, eosinophils, and basophils were counted in order to determine the relative microscopic differential counts. Moreover, hematocrit level was verified through blood centrifugation in a capillary tube.

Pathological analysis. A complete necropsy was carried out on two randomly selected animals from each experimental group, including those that died or had to be euthanized three days after venom administration. Macroscopic examination was performed, and tissue samples were obtained from the liver, kidneys, heart, lungs, intestines and brain of the animals. Tissue processing and staining were conducted using conventional methods, as previously described (Bancroft and Gamble, 2007). In brief, organ samples of the chickens with venom injection were immersed in 10% formalin for fixation. After dehydration with ethanol and clarification with xylene, tissue sections of the organs were embedded in paraffin. In the next stage, 5-µm sections were prepared from the embedded tissues. Paraffin sections were placed on glass slides, dewaxed with xylene and rehydrated with distilled water. Afterwards, sections were dried and stained with haematoxylin and eosin. Finally, a minimum of two well-prepared slides containing each tissue sample were further studied using a light microscope.

Statistical analysis. In this study, statistical significance of the differences between the study groups was assessed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. In all statistical analyses, P value of less than 0.05 was considered significant.

RESULTS

Clinical presentation. In this study, no specific clinical symptoms were observed in groups A (control) and B (0.5 mg/kg venom injection) at different time intervals. On the other hand, shortness of breath and lethargy were observed in the chickens administered with 2 mg/kg of the scorpion venom (group C) after one hour. It is noteworthy that the clinical signs diminished after two hours, and complete symptom remission occurred after four hours. Clinical symptoms of envenomation manifested 20 minutes after the SC administration of 5 and 10 mg/kg of scorpion venom in groups D and E, respectively. These symptoms included increased respiration, increased oral secretion, and bloody stool. Complete symptom remission in these groups occurred after 24 hours. However, in study group F, additional symptoms of envenomation prevailed until the end of the experimental period (e.g., breathing problems, excessive mucus secretion, diarrhea, and leg paralysis). Furthermore, SC administration of 30 mg/kg of scorpion venom immediately caused mucous diarrhea, lameness, increased respiration and drooping wings in the animals. After one hour, clinical signs of envenomation were recorded as increased heart rate and respiratory rate, breathing disorders, excessive mucus secretion, diarrhea, leg paralysis, neck rotation, seizures, and death (after two hours). However, experimental mice died following the SC injection of 11.5 mg/kg of the venom. According to our findings, lethal doses of *M. eupeus* venom in IV administration were 15 and 4.5 mg/kg in chickens and mice, respectively.

Hematological findings. In hematological examinations, maximal reduction of lymphocyte count was recorded in all the study groups six hours after the treatment (figures 1, 2 & 3). Although changes in this regard were not statistically significant, total leukocyte count was observed to elevate significantly after 24 hours in experimental group F (P=0.018). Moreover, heterophil count was observed to increase in all the study groups two hours after venom injection, and this change was

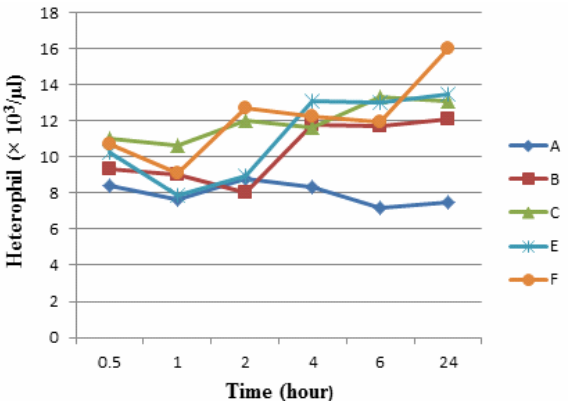

Figure 1. The effect of experimental *M. eupeus* envenomation on heterophil counts of chickens (A: control, B: 0.5, C: 2, E: 10, F: 20 mg/kg).

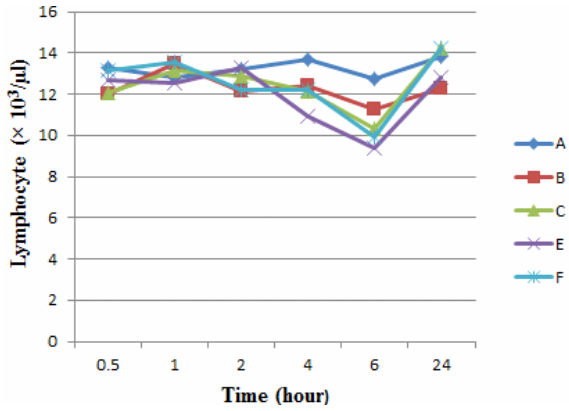

Figure 2. The effect of experimental *M. eupeus* envenomation on lymphocyte counts of chickens (A: control, B: 0.5, C: 2, E: 10, F: 20 mg/kg).

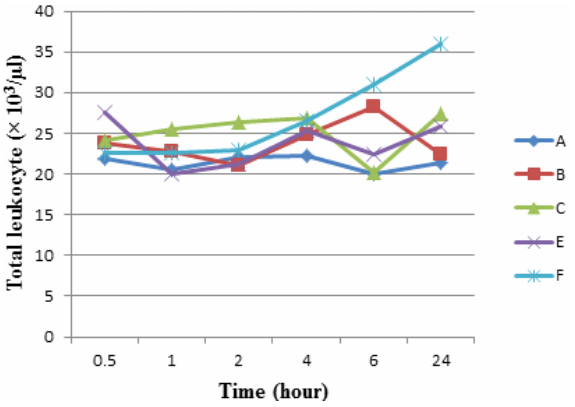

Figure 3. The effect of experimental *M. eupeus* envenomation on total leukocyte counts of chickens (A: control, B: 0.5, C: 2, E: 10, F: 20 mg/kg).

considered significant in group F after 24 hours (P=0.039). Our findings were indicative of no significant changes in the erythrocyte count and hematocrit level at different time intervals. However, a slight reduction was observed in all the study groups in this regard. Accordingly, lymphocyte count returned to pre-injection levels in all the groups after 24 hours, while the heterophil count constantly increased during the experiment.

Pathological signs. No pathological manifestations were observed in study groups A, B and C (Figure 4). On the other hand, pathological examination revealed different lesions in study groups D, E and F (figures 5, 6 & 7), including severe pulmonary hemorrhage and pulmonary edema, which were characterized by the accumulation of hyaline degeneration around the vessels and bronchi. Moreover, cardiac hemorrhage, tubular necrosis, congestion in the kidneys, hyaline thrombus, and congestion of the liver were observed in the animals. The mentioned symptoms appeared to be dose-dependent, and the most severe lesions were reported in study group F.

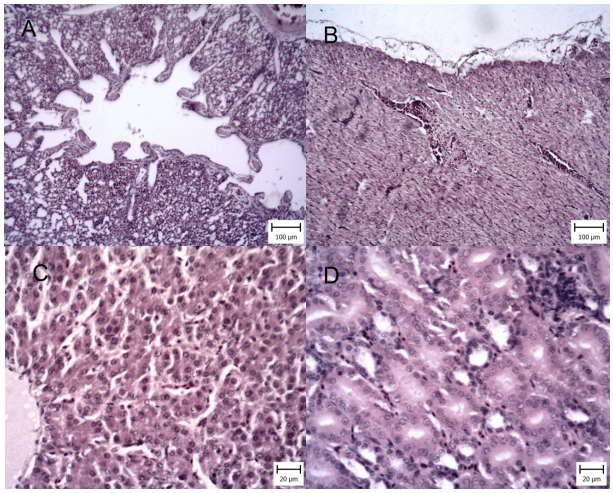

Figure 4. Photomicrograph of effects of experimental *M. eupeus* envenomation on study group A (H&E staining; note to normal structure of A. lungs, B. heart, C. liver and D. kidneys)

DISCUSSION

According to the results of the present study, approximate lethal doses of *M. eupeus* venom in chickens were 15 and 30 mg/kg in IV and SC administration, respectively; these values have been reported by previous studies conducted on mice (Khoobdel et al., 2013).

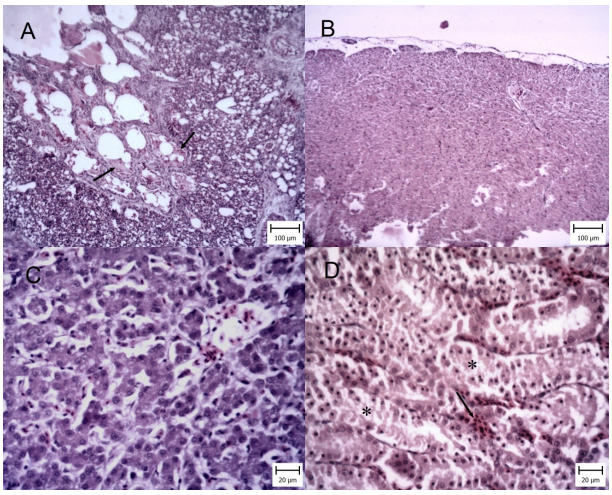

Figure 5. Photomicrograph of effects of experimental *M. eupeus* envenomation on study group D (H&E staining; A. lungs, pulmonary edema (arrows); B. heart, normal structure; C. liver, accumulation of hyaline degeneration in sinusoids; D. kidneys, congestion (arrows) and tubular necrosis (stars)

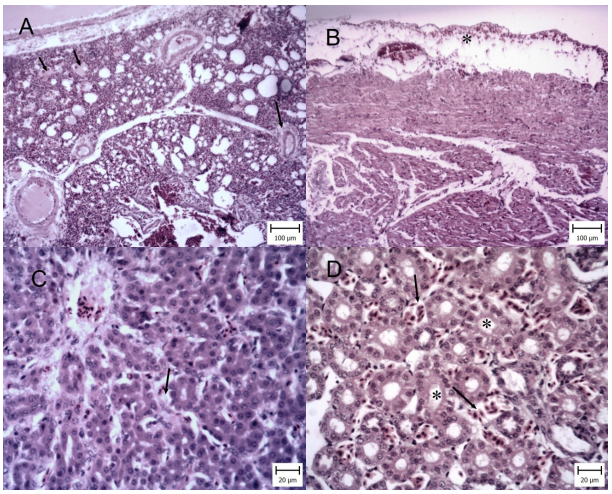

Figure 6. Photomicrograph of effects of experimental *M. eupeus* envenomation on study group E (H&E staining; A. lungs, pulmonary edema (arrows); B. heart, haemorrhage (star); C. liver, accumulation of hyaline degeneration in sinusoids (arrows); D. kidneys, congestion (arrows) and tubular degeneration (stars)

This finding is suggestive of the higher resistance of chickens to the toxic effects of *M. eupeus* scorpion venom compared to mice. In the current research, the

first clinical, pathological and hematological symptoms of envenomation in chickens were observed at doses of 2, 5 and 0.5 mg/kg, respectively.

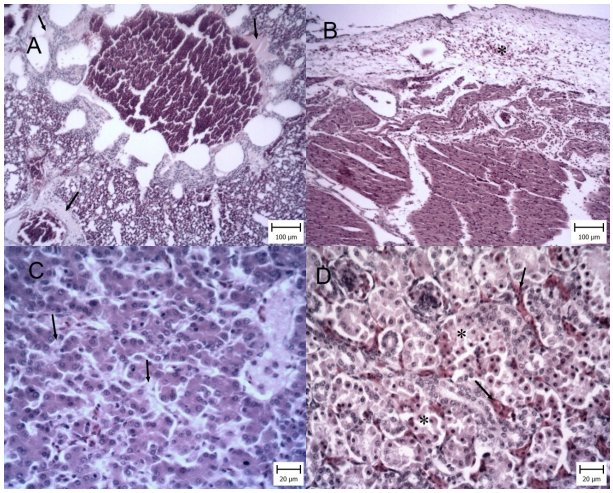

Figure 7. Photomicrograph of effects of experimental *M. eupeus* envenomation on study group F (H&E staining; A. lungs, pulmonary edema (arrows); B. heart, haemorrhage (star); C. liver, accumulation of hyaline degeneration in sinusoids (arrows); D. kidneys, congestion (arrows) and tubular necrosis (stars)

The symptoms of scorpion envenomation in humans are excessive sweating, severe pain in the site of sting, increased salivation and vomiting, generalized tingling and numbness, transient hypertension, tachycardia, tachypnoea, and pulmonary edema (Natu et al., 2006). These symptoms are similar to those of envenomated chickens and reported signs in other animal species. In humans, manifestations of the central nervous system following scorpion envenomation start with abnormal behaviors, such as altered sensorium, agitation, confusion and delirium (Das et al., 1995). In a study in this regard, cerebrovascular manifestations with neurological deficits were reported in four patients out of 50 cases with scorpion envenomation (Upadhyay and Ahmad, 2008). Similar neurological symptoms were observed in the chickens with scorpion envenomation in the present study, which is indicative of the same mechanism of toxic peptides in poultry and mammals. Normal hematological values for chickens are presented in Table 1 (Wakenell, 2010). According to the results of the present study, total count of heterophils in animals of experimental groups was higher compared to the control group, with the maximum value observed in chickens administered with the highest dose of *M. eupeus* venom (group F).

Table 1. Hematological values for chickens (Wakenell, 2010)

Parameter	Interval
Leukocytes (/µl)	12,000-30,000
Heterophils	3000-6,000
Lymphocytes	7,000-17,500
Packed cell volume (%)	22-35
Erythrocytes (/µl)	2,500,000-3,500,000

This is in line with the hematological signs reported in other animal species. Neutrophilic leukocytosis has been previously reported in humans (Gueron and Ovsyshcher, 1987; Gueron et al., 1993; Bucaretchi et al., 1995) and animals with scorpion envenomation (Cordeiro et al., 2006; Ribeiro et al., 2009; Pinto et al., 2010). This condition is considered a consequence of stress and recruitment of heterophils to circulating blood compartments. According to the results of the current research, erythrocyte count and hematocrit level were stable in envenomated chickens. This finding is in congruence with the results of a study conducted on Wistar rats with envenomation (Pinto et al., 2010), while inconsistent with another investigation on envenomated dogs (Ribeiro et al., 2009). In the present study, lymphocyte values were observed to decrease in envenomated chicken, which was previously reported in envenomated dogs (Cordeiro et al., 2006; Nogueira et al., 2007). However, different results have been proposed in dogs and rats with envenomation (Ribeiro et al., 2009; Pinto et al., 2010). After scorpion envenomation, the highest concentrations of toxins are found in the kidneys, liver, heart and lungs (Petricevich, 2010). Furthermore, these organs are highly susceptible to the pathological effects of scorpion venom in chicken. Scorpion venom contains variable concentrations of neurotoxins, cardiotoxins, nephrotoxins, phosphodiesterases, hyaluronidases, glycosaminoglycans, histamine, serotonin, tryptophans,

and cytokine-releasing peptides (Mahadevan, 2000). These toxins may cause diverse pathological manifestations. Previous studies have focused on the pathological effects of scorpion envenomation in humans and experimental animals (e.g., rabbits, rats and mice). Moreover, some of the pathohistological complications caused by the inflammatory cytokine induction of the *M. eupeus* venom include severe alveolar edema and hemorrhage, thrombosis, congestion, interstitial lung inflammation, myocytolysis, coagulative necrosis, myocardial edema, and cardiac hemorrhage (Zayerzadeh et al., 2012). Consistently, these manifestations were observed in the chicken organs evaluated in the present study.In a research in this regard, cause of death in two individuals with envenomation was reported to be pulmonary edema, which is in line with the results of the current research (Das et al., 2013). Pulmonary edema may occur due to cardiac failure, hemodynamic disorders or release of chemical mediators (Kumar et al., 2012). Furthermore, cardiorespiratory failure through the release of catecholamines and myocardial ischemia are other causes of death in cases with envenomation (Karnad, 1998; Cupo and Hering, 2002; Maheshwari and Tanwar, 2012). In-vivo effects of *M. eupeus* venom have been previously investigated in mice, rats and rabbits as alternative animal models (Khamechian et al., 2009; Lowe, 2010). In the current study, we evaluated the in-vivo effects of *M. eupeus* venom on chicken organs based on hematological and histological analyses. According to the results, the main organs targeted by *M. eupeus* venom are the liver, kidneys, lungs and brain; however, the most severe damages caused by envenomation occurred in the respiratory and nervous systems. This is inconsistent with previous findings in this regard, which suggested kidneys, liver and spleen as the major susceptible organs to envenomation by *H. lepturus* or venom of other scorpions belonging to the *Buthidae* family. This discrepancy could be due to the use of different experimental animals and venom dosages.

According to the results of this study, *M. eupeus* venom affects the organs of broiler chicken similar to other animal species. Furthermore, review of the literature is indicative of the higher resistance of poultry to the toxic effects of scorpion venom compared to human.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Suport

This study was financially supported by a grant from Shahid Chamran University of Ahvaz, Iran.

Acknowledgments

Hereby, we extend our gratitude to the personnel of Razi Vaccine and Serum Research Institute for assisting us in this research project.

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