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EFFECT OF THE CRUDE VENOM OF HEMISCORPIUS LEPTURUS ON MORTALITY RATE OF WHITE MICE

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ABSTRACT

Introduction:
Hemiscorpius lepturus (H. lepturus) is the most venomous scorpion in Iran Its venom can damage vital body organs and cause fatal consequences. This study aimed to evaluate the effects of this scorpion venom on the mortality rates of mice.
Materials and Methods:
This experimental study was conducted on two groups of mice (treatment and control) weighing between 27 and 30 grams of the same age and race.. In the treatment group, 1 micro liter of diluted crude venom of H. lepturus scorpion in 0.1 ml of normal saline was injected with suitable syringe. The control group received 0.1 ml of sterile normal saline. Data obtained from the groups were recorded and analyzed with Chi square and Fisher's exact tests.
Results:
The results showed that H.lepturus venom could cause death by as much as 82 percent over 6 to 240 hours. The initial major symptoms of poisoning in mice after injection of venom included humping, lack of nutrition, immobilization, and piloerection. In final stages of life, contractions, severe shaking of legs and occasional moanings were observed, which continued until an hour before death.
Conclusion:
Not all injections of H.lepturus venom are fatal, in cases where mice had local wounds due to venom injection, they survived. It can be concluded that the injection-induced ulcers in mice are appropriate immune responses against this venom.

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Introduction

Poisonous, biting and stinging animals are a large group of fauna of Iran. Of arthropods scorpions are important. Hemiscorpius species are very dangerous and deadly, especially for children [1-4]. More than 2,000 species of scorpions have been identified in the world; diversity and abundance of these species are more prominent in tropical and semitropical regions [5,6] Scorpions have long been of importance because of their poisonous and painful sting [7-9]. While 50 medically important species have been reported in the world, some authors have noted 25 species of medical importance [10-14]. A review of epidemiology of scorpion sting in the world pointed out that nearly 30 potentially dangerous species for human

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exist. The authors also reported more than 3,000 deaths due to scorpion sting worldwide [15]. Scorpion sting is of great importance in the Middle East [16,17] . Hemiscorpius lepturus (H.lepturus) is considered as a dangerous scorpion in Khuzestan province of Iran, and is called "Gadim" by native people. Venom of this scorpion can affect vital organs leading to deadly outcome [18]. The mortality rate due to this species, especially among children, has reached 8 percent in recent years [19]. Severe hemolysis, deep necrotic ulcers, coagulopathy, and death can be expected due to hematotoxic and cytotoxic properties of this venom. [20-22]. Compared with medically important species, H.lepturus envenomation presents with specific manifestations. In many cases, patients complain of only mild itching on the sting area shortly after scorpion sting. Such itching might be even ignored by the patient. Some patients may seek medical help a few days later, when the sting site is swollen and painful. As time passes, the venom penetrates the dermis and causes severe inflammation and cellulites, and patient feels severe pain in the stung area. Local pain is initially mild and comparable with the insertion of a pinhead length into the skin. The scorpion venom itself does not stimulate pain receptors [23]. The frequency of of H.lepturus sting in Khuzestan province was reported 10 to 15 % by Radmanesh and colleagues, and the authors mentioned it as the most dangerous scorpion sting in the province [18] . In a study on 418 scorpion sting cases, Dehghani and colleagues reported that 28.7% of the cases were stung by Androctonus crassicauda, 24.9% by H.lepturus, 21.7% by Mesobuthus eupeus, 20.6% by Compsobuthus mattiessenis, 3.35% by Buthotus saulcyi, 0.5% by Orthochirus scrobiculosus, and 0.25% by Buthotus schach [24,25]. Given that H.lepturus (Gadim) is the deadliest and most dangerous scorpion in Khuzestan province of Iran, this study was conducted to investigate the effect of Gadim venom on mortality rate of mice.

Materials and Methods

a. Venom Extraction:

Extraction of 1-2 micro liter of crude and fresh venom was applied using an electroshock device after the two electrodes were stuck to the Telson (the posterior- most division of the tail) under 4-5 voltage over 0.5-1 second. The venom came out of the stinger in close contact with 100 and 200 µLs pipettes, due to capillary effect; therefore, the exact volume of the extracted venom could be measured. With such method, venom was sometimes accompanied by a milky mucosal liquid. The volume of venom obtained from 10 scorpions was 15 µLs in the first time.

b. Amount of the injected venom using standard methods:

According to a study by Mirakabbadi et al [26], LD50 of H.lepturus dried venom was 126 micrograms per mouse or 6.3 mg/kg for intravenous administration in mice. This value was not applicable to our study due to the administration of fresh liquid venom via cutaneous injection. In a pilot study on mice weighing 27-30 grams, 0.6 µLs of fresh H.lepturus venom was dissolved in 1.0 ml of 50% normal saline solution, and 0.9 µLs of fresh venom resulted in 100 deaths. Since the purpose of our injections was induction of death over time, a volume of 1.0 of fresh H.lepturus venom in 0.1 ml of normal saline(0.9%) was considered as the lethal dose in a mouse weighing between 27 and 30 grams.

Groups allocation: In this experimental study, 56 mice, each weighing 27-30 grams, were randomly and equally divided into treatment and control groups. The control group received 1mm3 of saline while treatment group was treated with 1 µL of fresh H.lepturus venom. Mortality rate and clinical manifestations were recorded over 20 days. The injection sites in the case and control groups were examined every other day. Each animal was then placed in individual cages with food and water access ad libitum and mortality due to venom injection was monitored.

Results

Our results showed that 23 mice (82%) in the experimental group were dead while 5 (18%) survived following injection of 1µL of venom. All 28 animals survived in the control group. The mortality rates between the case and control groups were significant using Fisher's exact tests ($P<0.0001$). Among total of 23 deaths in the treatment group, time of death was variable from 6 to 240 hours after venom injection. The majority of deaths occurred 11-20 hours after injection (24 animals; 37%), and the lowest incidence of death occurred after 41-240 hours (8 animals; 12%) (Fig 1).The major early signs of toxicity in mice after injection of venom were humping, lack of nutrition, immobilization, and piloerection. In the final stages of life, contractions sever shaking of legs and occasional moaning was observed which continued up to an hour before death. In cases where death did not occur in the experimental group, most animals gradually showed an increase in mobility after 3-4 days and were heading towards began a full recovery. Among the experimental group mice that were injected with the venom, a wound was observed at the site of the injection after 4- 6 days ,which started healing after nearly14 days but left behind a scar. All of the animals in which the venom injection caused a wound, survived in the study (Image 1). The dead mice were then underwent a laparatomic incision to open the abdomen, where darkness of skin at the injection site was detected. Other observations among the dead animals in terms of frequency included excessive edema and inflation of stomach that was mostly filled with fluids. Intestinal congestion and edema as well as bleeding were also clearly observed in most animals (Image 2).

Discussion

Results showed that a volume of 1 µL of H.lepturus venom caused 82% deaths in the treatment group.Time of death ranged from 5 to 240 hours. The most frequent time of death was 11 to 24 hours after injection, for 24 cases (37%), while the

fewest number of deaths occurred after 41-240 hours, for 8 cases (12%). The mechanism of effect of this scorpion venom is different from that of Buthidae scorpions, and has a lower rate of distribution within the victims' body [26]. Unlike the H.lepturus venom, Buthidae venom has a short half-life; and should the victim survives, complete remission will be achieved after a few days [27]. Thus, due to the nature of this scorpion venom in the mice, different and prolonged time of death can be considered normal.

The results also showed that H.lepturus venom caused severe poisoning in mice with major signs of toxicity including humping, lack of nutrition, sheltering, immobilization, and piloerection. In the final stages of life, spastic movements sever tremor in legs and moaning occurred that sometimes lasted up to an hour before death. In further research on laboratory animals, some of the effects of the venom were reported as edema at the injection site, changes in the heart rate, and increased salivation. Death occurred due to an increase in the heart rate, respiratory disorders and complete paraplegia, similar to that in cyanide toxicity [26,28].

We observed an extensive internal bleeding in mice due to H.lepturus venom. Radmanesh et al reported that abnormal neuronal behavior, occult or severe hemolysis, fever, sweatings, chills, shivering, tachycardia, asthenia, lethargy, lackof appetite, petechiae, skin rashes, hypotension and pallor as the major symptoms in patients with H.lepturus poisoning [29, 18], which is consistent with our study.Venom of the above mentioned scorpion shows hematotoxic and cytotoxic properties, and widespread internal multi organ damage is expected [30,31]. Movements turned back to normal after 3-4 days in the survived mice , when they gradually started to recover. Similarly, an ulcer formed in the injection site after 4-6 days, which was repaired after 14 days with a remaining scar. All mice that had ulcer formation due to venom injection survived. Borchani and colleagues isolated dermonecrotic toxin from the venom of this scorpion [32]. Severe hemolysis and even the need for blood transfusion have been reported following H.lepturus sting. In these cases, if another H.lepturus sting accidentally occurred within the next year, no local or systemic symptoms were found except for a small erythema at the site of the sting,. This indicates that H.lepturus venom could possibly stimulate immune system, especially in non- allergic cases and induce immunity in the injected cases [18,23]. Therefore, formation of a necrotic ulcer after venom injection and survival of the victim is anticipated. Autopsy of dead mice due to venom injection showed darkening of the injection site, swelling of stomach mucosa and gastric distention due to fluid retention, congestion and swelling of the intestines, and rectal bleeding. These findings were consistent with previous reports [33-35].

Conclusion

Not all injections of H.lepturus venom are fatal; in cases where mice had local wounds due to venom injection, they survived. H.lepturus scorpion envenomation has a wide variety of clinical manifestations, both local and systemic. The use of the animal model of Hemiscorpius lepturus venom effect has given an accurate explanation of the pathology mechanism. The venom of this scorpion involves the vital organs of the victims. Due to the formation of ulcers caused by the injection of Hemiscorpius lepturus venom, in live mice, it can be concluded that the injection-induced ulcers in mice are appropriate immune responses against this venom.

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Conflict of Interest:

None

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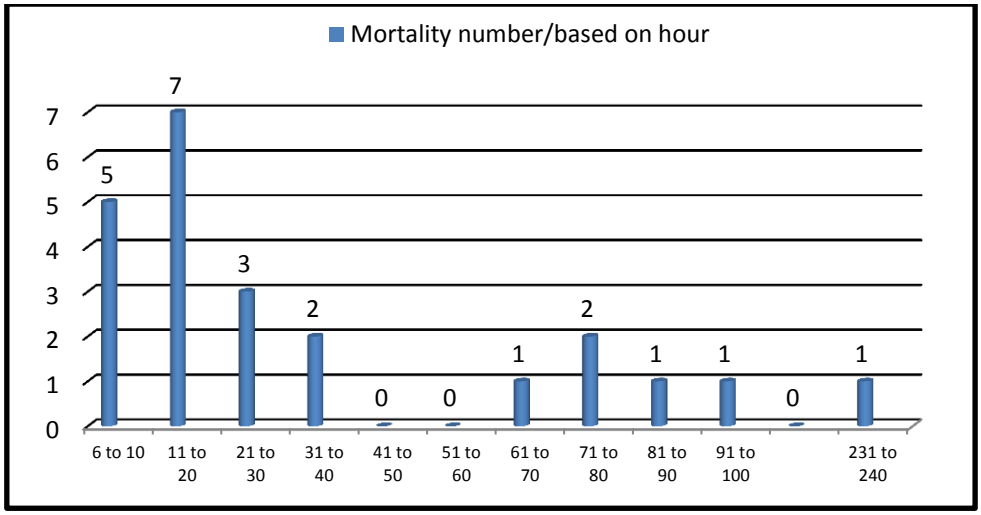

Figure 1: Frequency of death in mice injected with the venom of scorpion, based on time of death

Image 1: Wound caused by injection of H.lepturus venom at the abdominal site of mice

Image 2: Post mortem effects of H.lepturus venom in dissected mice