

Research Paper: Investigation of the Behavioral and Clinical Effects of Black Scorpion Venom in Albino Rats



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ABSTRACT

Background: Aggressive behaviors in human and experimental animals have previously been described following induced pain. Aggression in rodents has been attributed to genetic and environmental factors, such as pain. A major complication of scorpion envenomation is severe pain in animals and humans. Considering that envenomation by black scorpion (*Androctonus crassicauda*) induces severe pain, the present study was conducted to investigate the effects of the venom on inducing aggressive behaviors in rats due to the pain from stinging.

Methods: Rats in the control (n=20) and experimental groups (n=20) were injected with 0.5ml physiological serum or 1µg/ml of black scorpion venom dissolved in 0.5ml physiological serum, respectively, in the dorsal vein near the tail. Changes in behaviors were monitored photographically among the rats in both groups.

Results: Following the injection of the scorpion venom, considerable agitation and fights occurred among the experimental rats, presumably due to the severe pain induced by the venom. However, there was no such abnormal behavior observed in the control rats and in the experimental rats before the venom injection.

Conclusion: The induced pain post envenomation in rats caused violent changes in their behaviors, which were highly likely associated with the venom injection.

Keywords: *Androctonus crassicauda*; Scorpion venom; Clinical manifestations; Behavioral changes; Albino Wistar rats

Introduction

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tudies in laboratory animals and humans have revealed that pain can induce aggression toward a target organism [1, 2]. Various forms of aggression have been observed

in animals including primates [3, 4], mammals, arthropods, reptiles, fish and rodents due to genetic changes or environmental factors [5-7]. Among the factors causing aggression in animals, particularly in social organisms, such as mice and rats, pain is capable of inducing aggressive reactions, such as threat, attack, defense and fight for survival [8].

Venomous animals, such as snakes and scorpions apply their venoms for defense against their enemies and threats. A major complication of scorpion envenomation is severe pain, which repulses humans and animals that are threats to them [9-12]. Scorpion envenomation has been reported as an important public health concern in some tropical and sub-tropical nations [11]. Black scorpion, *Androctonus crassicauda*, is one of the most venomous scorpion species with neurotoxic effects in the Middle East and Iran, and generally lives in hot and dry areas. In some arid regions of Iran, scorpion sting and envenomation have frequently led to severe pain or even fatal consequences [13, 14]. The lethality of scorpion envenomation varies with age of the victim, scorpion species, dose of injected venom, and access to medical treatment and supportive care [14]. Regardless of the eventual consequences, in many cases scorpion sting evokes severe pain, swelling and tissue edema [15, 16].

The investigation of aggression in laboratory animals can be used as a valuable experimental model to elucidate the causative agents of violent behaviors in human [17]. It is believed that various environmental and behavioral factors may induce aggression in animals and humans such as threat, attack, and defense [17]. The experience of pain that often occurs in many animals is an aversive sensory reaction due to a wide variety of injurious stimuli subsequent to tissue damages. Also, humans often experience emotional pain due to social crisis and lack of fulfillment [18, 19]. Indeed, there is evidence to suggest that pain is one of the factors causing aggressive behavior and violence. This study was conducted to investigate the effect of black scorpion envenomation on inducing pain and the resultant aggressive behavior in rats.

Materials and Methods

Forty albino rats of Wistar strain (*Ratus norvegicus*) of both sexes with an average weight of 200-250g and 2-3 months old were procured from animal breeding house of the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. They were kept in standard cages under controlled conditions, and were freely provided with food and water. The experimental protocol was approved by the Ethics Committee, Kashan University of Medical Sciences, Kashan, Iran. The animal welfare law of Britain were observed for the care and use of experimental animals [20].

The scorpions were collected from Kashan area (Isfahan province, Iran) and were transferred to the laboratories of the Department of Environmental Health of Kashan University of Medical Sciences. They were fed live German

cockroaches, and the venom was obtained by an electric shock apparatus (constructed at Kermanshah University, Kermanshah, Iran). The collected venom was mixed with physiological serum at the concentration of 1 µg/ml and used over the next 24 hours. The rats were divided randomly into control or experimental group of 20 rats each. The control and experimental groups were injected subcutaneously with 1ml of pure physiological serum (control) or 1ml of the serum containing 0.44 µg of the venom (experimental) in the dorsal vein of the rats near the tail, using a mouse holder. Thereafter, the rats were placed in standard cages, had free access to water, food and optimum physical parameters, and were observed throughout the study with respect to their behaviors, activities and clinical manifestations. All of the data were tabulated in tables for statistical analyses and observations were recorded by photographs until the study ended. At the completion of the study, all live rats from both control and experimental groups were returned to their normal environment in the animal housing of the university.

Results

The results provided evidence that the injection of the black scorpion's venom into the rats caused extensive and abnormal behaviors and clinical manifestations. Initially, once the first injected rat was placed in the cage with others, it started licking the injection site. When all of the rats had been injected, aggressive behaviors and fighting broke up amongst them. The fights were characterized in upright posture, using their front paws for hitting the head and face of the opposing rats. Such behaviors were observed only in the experimental group. Other clinical manifestations, which occurred only in this group, included abnormal pulse (80%), spontaneous muscle contractions (60%), frothing from the nose (80%), bleeding from the eyes (70%), nose bleed (50%) and severe salivation (40%) (Figures 1 & 2). Additionally, the venom injection caused death in 90% of the experimental rats (Table 1).

Further, the envenomation caused paraplegia in 50% of the experimental rats. The other 50% developed paralysis in one leg only. The leg paralysis in 50% of the rats started out in a single leg initially but advanced to both legs later on. In addition to the above mentioned clinical manifestations, other symptoms also appeared, such as asymmetrical limb movements, urination, priapism (persistent & painful erection), sever muscle spasm and shivering of the back. The rats' mortality rate was approximately 90% (Table 1), which on average occurred 51 minutes post injection in the experimental rats (minimum: 10 min.; max: 150 min.). The recovery in 10% of

Table 1. Frequency distribution of the behaviors and clinical manifestations found in the albino rats following the injection of black scorpion venom

Clinical Situation	No. (%)							
	Clinical Manifestation							
	Violence	Irregular Pulse	Twitching	Frothing From the Nose	Bleeding From the Eyes	Nosebleed	Salivation	Mortality
Asymptomatic	0 (0)	4 (20)	8 (40)	4 (20)	6 (30)	10 (50)	12 (50)	2 (10)
Symptomatic	20 (100)	16 (80)	12 (60)	16 (80)	14 (70)	10 (50)	8 (50)	18 (90)
Total	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

the experimental rats following intoxication symptoms occurred up to 28 hours post injection. Thereafter, the rats started eating food, drinking water, grooming activities, and they survived similarly to those in the control group. In the control group, no intoxication symptoms were observed following the injection of sterile physiological serum. These rats demonstrated normal activities without fights and aggressive behaviors being evident.

Discussion

The results demonstrated that the subcutaneous injection of black scorpion venom in rats caused extensive behavioral and clinical manifestations. Aggressive behaviors and fighting by pairs of rats were observed in 100% of the injected animals. Other behaviors and clinical manifestations, including paralysis, irregular pulses, spontaneous muscle contractions, frothing of the nose, sever salivation, bleeding from the eyes and nose were also observed. The scorpion venom contains several low molecular weight proteins and amino acids that are that are toxic to the rat

nervous system, kidneys, blood and heart [11]. Further, there may be multiple toxins in just one scorpion species [15]. According to one study [14], the venom from black scorpion is known to be neurotoxic, affecting the acetylcholine receptors in the central nervous system, resulting in severe pain and paralysis plus local and generalized muscle contractions. The development of excruciating pain due to neurotoxins arising from the bites of venomous animals has been frequently reported, which cause the human victims to seek medical care [15, 21, 22].

Efforts have been made to investigate the pharmacological characteristics of pain responses induced by scorpion envenomation. Bai et al. [23] demonstrated that the venom from scorpion *Buthus martensi* induced spontaneous pain and hypersensitivity responses following its injection into the hind paws of rats. These authors reported other spontaneous responses in rats, such as flinching, licking and lifting the injected paws following their envenomation [23]. Jiang et al. [24] identified the molecular mechanisms underlying the pain-related behaviors in rats induced by the



Figure 1. Bleeding from the eyes in the albino rat following the injection of black scorpion venom



Figure 2. Frothing from the nose in the albino rat following the injection of black scorpion venom

scorpion *Buthus martensi* venom. They attributed the pain-related behaviors in rats to the activation of spinal astrocytes and microglia [24].

A previous study [14] reported that the black scorpion venom caused increased acetylcholine levels in the nervous system, accounting for the observed pathological disorders in the stung victims. The venom's toxins stimulated the muscarinic and nicotinic receptors in the stung animals, causing the subsequent neurological manifestations [14]. The initial target of the venoms is the voltage gated ion channels that leads to alterations in the nervous system followed by prolonged and repeated stimulation of the sympathetic system, increased sodium and calcium ions and accumulation of neurotransmitters at receptor sites [25, 26]. Other studies have attributed clinical complications, such as muscle spasm, paralysis, excessive salivation, numbness, priapism in male rats, weeping and twitching due to scorpion envenomation [26, 27]. Further, studies have reported priapism occurring in rats due to scorpion envenomation and the authors suggested that the stimulation of nitrenergic nerves could explain the priapism [15, 28]. These nerves use nitric oxide as the neurotransmitter. Our results are consistent with the above studies, which have investigated the pain subsequent to the injection of *Androctonus crassicauda* venom that led to the associated behaviors and clinical manifestations in animal models.

Various aggressive behaviors have been studied in laboratory animals. For instance, Patki et al. [29] have suggested that aggressive behaviors in rats were largely associated with stress and enhanced plasma levels of corticosterones as evident by their biochemical data. Much research has been carried out in laboratory animals to examine the causes of anger and aggression. Aggressive behaviors, such as threat, attack and defensive reactions in different species of animals could be related to competitions, fights for survival and mating opportunity [1, 2]. It is, therefore, evident that aggressive behaviors and violence in animals happen due to environmental and biological stimuli. The investigation of factors associated with aggression in laboratory animals can provide valuable experimental data and models to elucidate the causative factors and may ultimately help the investigation of stimuli involved in violence among humans [16, 30].

Conclusions

Based on the findings of this study, it can be concluded that severe pain in rats due to injection of black scorpion venom may be associated with putative neurotoxins present in the venom. Also, the aggressive behaviors

and fighting among the rats were secondary to the severe pain following the venom injection. This assumption is supported by our observations in the control group where no fighting broke up among the rats at all while no aggression happened before the venom injection in the experimental group. Thus, it may be postulated that the aggression in the experimental animals happened largely due to the interactions of the components of the venom with specific sites in the nervous system.

Ethical Considerations

Compliance with ethical guidelines

Ethical guidelines against plagiarism, misconduct, data fabrication or falsification, double publication or submission and redundancy have been fully observed by the authors throughout the conduction of this study. The ethical approval was obtained from Kashan University of Medical Sciences' Ethics Committee (Registration code No.: 655).

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Author's contributions

Investigation, concept: Rouhollah Dehghani; Methodology, Validation: Tahereh Khamechian; Experiments: Azar Dehghani; Writing, review & editing: Mojtaba Limoe; Experiments: Mazdak Limoe; Writing, review & editing: Hooshyar Hossini.

Conflict of interest

The authors declared no conflict of interest.

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