

STUDY OF THE RELATIONSHIP BETWEEN *Androctonus crassicauda* (OLIVIER, 1807; SCORPIONES, BUTHIDAE) VENOM TOXICITY AND TELSON SIZE, WEIGHT AND STORING CONDITION

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ABSTRACT: Scorpions are included in the order Scorpiones; class Arachnida. Lethal scorpions are mostly of the Buthidae family. Among these, species belonging to *Androctonus*, *Leiurus* and *Mesobuthus* genera cause most scorpion envenomations in Turkey. This study was performed aiming the production of antivenom by using *Androctonus crassicauda* telsons. Venom toxicity is related to telson weight, size, and storing condition (open or closed). Telsons of *A. crassicauda* were collected in Southeastern Anatolia (especially in Harran town, Sanliurfa), Turkey. They were separated according to weight, size, and storing condition – open (a) and closed (b). Venom solution was prepared by maceration of telsons. Swiss albino mice were used to determine the lethal dose 50% (LD₅₀), which was as follows: Group 1a – 2.31mg; Group 1b – 2.66mg; Group 2a – 2.32mg; Group 2b – 2.66mg; Group 3a – 6.66mg; Group 3b – 6.88mg. Among the groups of telsons, the first and the second groups showed different characteristics. However, there were no differences between their toxicity. In the third group, a fourfold amount of telsons was used for toxicity. In other words, telsons weighting from 19.99 to 20mg (first group) and from 29.99 to 30mg (second group) presented similar LD₅₀ values, and telsons weighting from 10 to 19.99mg (third group) showed a fourfold higher LD₅₀ value. This difference was caused by the maturity of scorpions and venom toxicity was related to their size. The first and second groups were considered to be mature and the third group, not adult. Therefore, we can conclude that obtaining open telsons due to environmental factors was not effective for venom toxicity.

KEY WORDS: scorpion, *Androctonus crassicauda* (Oliver, 1807), telson size and weight, LD₅₀, acute toxicity.

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INTRODUCTION

Scorpion sting is the most important arachnidan envenomation that causes adult morbidity and child mortality. It remains a real health problem in many countries from tropical and subtropical regions (4, 13, 14, 15, 17, 20, 27).

Scorpion venom contains small neurotoxin polypeptides consisting of low-molecular-weight simple proteins with lethal and paralytic effects (1, 3, 15, 28). Several studies on scorpion stings emphasized that clinical pictures ranged from local to severe autonomic and central nervous system symptoms and also death due to cardio and respiratory failure, especially in children (5, 7, 10, 11, 12, 17, 18, 21, 22). Lethal scorpions are mostly of the *Buthidae* family (16, 18, 27).

In Turkey, scorpion envenomations are mostly caused by the genera *Androctonus*, *Leiurus* and *Mesobuthus* (18, 19). In the same country, *A. crassicauda* venom, obtained by maceration of telsons, is utilized as antigen for antivenom. This antigen has also been used against other scorpion species (23, 24). Therefore, the aim of this study was to verify the relationship between *A. crassicauda* venom toxicity and its telson size, weight, and storing condition.

MATERIALS AND METHODS

Telsons

Androctonus crassicauda scorpions were collected in Southeastern Anatolia (especially in Harran town, Sanliurfa), Turkey. Telsons were separated according to their weight, size and storing condition (Figures 1, 2 and 3).

All telsons were weighted by using an electronic scale with sensitivity of 0.001mg. Telsons were randomly divided into three base groups. They were measured by using metal capillars, sensitivity of 0.1mm, considering the morphometric parameters (length, width, height) described by Vachon (25, 26).

Mice

Four hundred and sixty-four healthy female *Swiss albino* mice, weighing 23 ± 1 g, and 8 weeks old, were used to determine LD₅₀. Throughout the experiment, the animals were kept in a laboratory under room temperature of $22\pm 2^{\circ}\text{C}$ and $60\pm 10\%$ humidity. They were fed with commercial mouse pellets *ad libitum*.

Preparation of Venom Solution

Venom solution was prepared by maceration (2, 14) of telsons from the six groups used (base groups – 1st, 2nd, and 3rd groups – and their subgroups – open and closed telsons). Telsons were ground to a fine powder, which was dissolved in physiologic saline solution (PSS; chloride solution of 0.9%).

Lethal dose 50% (LD₅₀) assay

A different concentration of venom solution was prepared for each group of mice. Then, 0.5ml was subcutaneously injected into each mouse. Control groups were treated with 0.5ml PSS, subcutaneously. After treatment, animals were monitored for 48 hours, and the number of dead animals was recorded at the end of the experiment. The dose that killed 50% of animals (LD₅₀) was calculated by probit analysis (9).

Statistical analysis

Results were statistically evaluated by using probit analysis, Pearson's chi-square test and the probability value *p*. Telson size values were presented as mean ± standart derivation (SD). Differences were considered significant when *p*<0.005.

RESULTS

Telsons

We used 524 telsons from *A. crassicauda* scorpions. According to weight, telsons were divided into three groups in the following manner: First group, 30-39.99mg; second group, 20-29.99mg; and third group, 10-19.99mg (Tables 2A, 2B and 2C). Within these base groups, there were two subgroups according to the storing condition: open (a) and closed (b). The mean weights of telsons were: First group, 33.46mg; second group, 24.64mg; third group, 16.47mg, as shown in Table 1.

Telsons were randomly divided into base groups and the measurements of their morphometric parameters (length, width, heigth) were also shown in Table 1.

A stock venom solution at a concentration of 20mg/ml was prepared through maceration of telsons.

Animals

In the determination of LD₅₀ values, Group 1 consisted of 64 mice in G1_a, 72 mice in G1_b, and 8 mice in the control group [G1¹]; Group 2 was comprised of 72 mice in G2_a, 72 mice in G2_b, and 8 mice in the control group [G2¹]; Group 3 presented 80 mice in G3_a, 80 mice in G3_b, and 8 mice in the control group [G3¹] (Tables 2A, 2B and 2C). The LD₅₀ was found (95% confidence limit – CL) for each group and is presented in Table 3.



Figure 1: Telson of *Androctonus crassicauda* (by Ozkan O. *et al.* *J. Venom. Anim. Toxins incl. Trop. Dis.*, 2006, 12, 297-309).

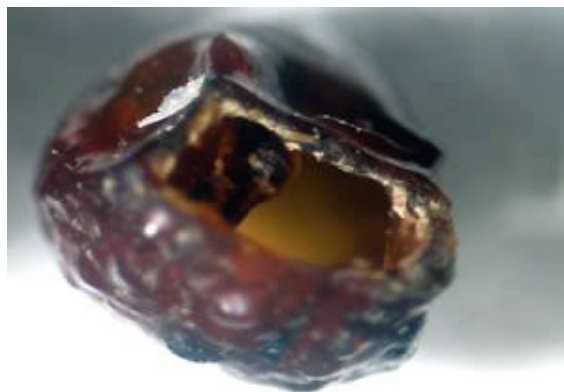


Figure 2: The view of an open telson (by Ozkan O. *et al.* *J. Venom. Anim. Toxins incl. Trop. Dis.*, 2006, 12, 297-309).



Figure 3: The view of a closed telson (by Ozkan O. *et al.* *J. Venom. Anim. Toxins incl. Trop. Dis.*, 2006, 12, 297-309).

Table 1: Groups of telsons and their morphometric characteristics. There were not statistically significant differences according to weight and morphometric parameters ($p>0.005$).

Group	Telsons (n=524)	Total weight (g)	Mean weight (mg)	Morphometric parameters (mm)		
				Width	Length	Height
				Mean \pm SD	Mean \pm SD	Mean \pm SD
1st	68	2.27	33.46	0.445 \pm 0.021*	0.104 \pm 0.039*	0.352 \pm 0.006*
2nd	241	5.93	24.64	0.397 \pm 0.020*	0.960 \pm 0.056*	0.308 \pm 0.013*
3rd	215	3.54	16.47	0.325 \pm 0.022**	0.857 \pm 0.032**	0.290 \pm 0.016**

* Mature scorpions

** Not adult scorpions

Table 2A: Number of animals in each subgroup, amount of venom used, and number of dead animals in Group 1 (injected with venom solution from telsons weighting from 30 to 39.9mg).

Groups	Subgroups	Number of animals	Amount of venom (mg/mouse)	Number of dead animals
G1 ¹	-	8	-	0
	G1 _{a-1}	8	1.50	0
	G1 _{a-2}	8	1.75	1
G1 _a	G1 _{a-3}	8	2.0	3
	G1 _{a-4}	8	2.25	5
	G1 _{a-5}	8	2.50	6
	G1 _{a-6}	8	2.75	6
	G1 _{a-7}	8	3.0	6
	G1 _{a-8}	8	3.25	7
	G1 _{a-9}	8	3.50	8
G1 _b	G1 _{b-1}	8	1.50	0
	G1 _{b-2}	8	1.75	0
	G1 _{b-3}	8	2.0	1
	G1 _{b-4}	8	2.25	2
	G1 _{b-5}	8	2.50	3
	G1 _{b-6}	8	2.75	4
	G1 _{b-7}	8	3.0	6
	G1 _{b-8}	8	3.25	7
	G1 _{b-9}	8	3.50	8

G1¹: Control group

G1_a: Groups injected with venom solution from closed telsons

G1_b: Groups injected with venom solution from open telsons

Table 2B: Number of animals in each subgroup, amount of venom used, and number of dead animals in Group 2 (injected with venom solution from telsons weighting from 20 to 29.9mg).

Groups	Subgroups	Number of animals	Amount of venom (mg/mouse)	Number of dead animals
G2 ¹	-	8		0
	G2 _{a-1}	8	1.50	0
	G2 _{a-2}	8	1.75	0
G2 _a	G2 _{a-3}	8	2.0	4
	G2 _{a-4}	8	2.25	5
	G2 _{a-5}	8	2.50	6
	G2 _{a-6}	8	2.75	6
	G2 _{a-7}	8	3.0	7
	G2 _{a-8}	8	3.25	7
	G2 _{a-9}	8	3.50	8
G2 _b	G2 _{b-1}	8	1.50	0
	G2 _{b-2}	8	1.75	1
	G2 _{b-3}	8	2.0	3
	G2 _{b-4}	8	2.25	5
	G2 _{b-5}	8	2.50	6
	G2 _{b-6}	8	2.75	6
	G2 _{b-7}	8	3.0	7
	G2 _{b-8}	8	3.25	7
	G2 _{b-9}	8	3.50	8

G2¹: Control group

G2_a: Groups injected with venom solution from closed telsons

G2_b: Groups injected with venom solution from open telsons

Table 2C: Number of animals in each subgroup, amount of venom used, and number of dead animals in Group 3 (injected with venom solution from telsons weighting from 10 to 19.9mg).

Groups	Subgroups	Number of animals	Amount of venom (mg/mouse)	Number of dead animals
G3 ¹	-	8		0
G3 _a	G3 _{a-1}	8	5.75	0
	G3 _{a-2}	8	6.0	1
	G3 _{a-3}	8	6.25	2
	G3 _{a-4}	8	6.50	5
	G3 _{a-5}	8	6.75	5
	G3 _{a-6}	8	7.0	6
	G3 _{a-7}	8	7.25	6
	G3 _{a-8}	8	7.50	7
	G3 _{a-9}	8	7.75	7
	G3 _{a-10}	8	8.0	8
G3 _b	G3 _{b-1}	8	5.75	0
	G3 _{b-2}	8	6.0	0
	G3 _{b-3}	8	6.25	1
	G3 _{b-4}	8	6.50	4
	G3 _{b-5}	8	6.75	4
	G3 _{b-6}	8	7.0	5
	G3 _{b-7}	8	7.25	5
	G3 _{b-8}	8	7.50	6
	G3 _{b-9}	8	7.75	7
	G3 _{b-10}	8	8.0	8

G3¹: Control group

G3_a: Groups injected with venom solution from closed telsons

G3_b: Groups injected with venom solution from open telsons

Table 3: Lethal dose 50% (LD₅₀) and confidence limit determined for each group of mice. LD₅₀ were not significantly different between groups ($p>0.005$).

Groups	LD ₅₀ (mg*)	95% Confidence Limit
G1 _a	2.31	2.05-2.53
G1 _b	2.66	2.47-2.86
G2 _a	2.32	2.11-2.52
G2 _b	2.66	2.46-2.87
G3 _a	6.66	6.42-6.88
G3 _b	6.88	6.66-7.11

* Dry telsons

G1_a: Mice injected with venom solution from closed telsons weighting from 30 to 39.9mg

G1_b: Mice injected with venom solution from open telsons weighting from 30 to 39.9mg

G2_a: Mice injected with venom solution from closed telsons weighting from 20 to 29.9mg

G2_b: Mice injected with venom solution from open telsons weighting from 20 to 29.9mg

G3_a: Mice injected with venom solution from closed telsons weighting from 10 to 19.9mg

G3_b: Mice injected with venom solution from open telsons weighting from 10 to 19.9mg

DISCUSSION

The aim of this study was to determine the structure of *A. crassicauda* telsons, the LD₅₀ of its venom, and the relationship between its telson weight, size and storing condition.

To obtain venom, we used maceration, manual techniques, and electrical stimulation (18). Maceration is one of the oldest and simplest methods based on the principle of keeping telsons of dead scorpions in physiologic saline solution (1, 18, 23, 24).

We prepared three different venom solutions containing 20mg of grounded telson/ml. These solutions were subcutaneously injected into mice when the average of telsons used was evaluated as one telson; mean LD₅₀ was considered as 7% of the first group, 9.4% of the second group, and 40.4% of the third group. Although average LD₅₀ values of the first and second groups were different, there were no significant differences between their toxicity; in the third group, it was observed that an amount of venom fourfold higher, compared with the other groups, was used because it presented lower toxicity, which was probably due to the fact that the scorpions were not mature yet. Also, toxicity changed because scorpions may have used some of

their venom for defense against being killed or caught or because they did not receive enough food during the experiment. In this study, similar results were seen in the first and second groups of telsons (20-40mg). Differently, Balozet declared the relationship between empty telson and full telson was 1:4 in order to kill mice (1). Although Balozet mentioned that factors such as transportation, drying method, storage conditions and usage period adopted could affect measurements (1), in this study, there were no differences between LD₅₀ of telsons (open/closed), which became open due to environmental conditions.

In addition to Turkey, *A. crassicauda* has high prevalence in Azerbaijan, Iran, Iraq, Syria, Jordan, and Saudi Arabia (12, 18, 21, 27); and venom toxicity also changes according to regions and scorpion species (2). Total length of mature *A. crassicauda* varies from 90 to 100mm (6, 20). Measurements of telsons from the first and second groups (considered mature scorpions), and from the third group (considered not adult scorpions) are shown in Table 1.

According to lethality tests, different LD₅₀ values were found for the same species. Ismail *et al.* found that LD₅₀ of *A. crassicauda* venom was 0.64mg/kg, whereas venom obtained from Latoxan laboratory had an LD₅₀ of 0.87mg/kg (12). Altinkurt and Altan (1) reported that LD₅₀ of venom of *A. crassicauda* from Sanliurfa region was 11.5mg/kg. Therefore, envenomation due to scorpion stings as well as local or systemic clinical effects could be related to the scorpion species, its feeding state, the telson structure, the amount of venom injected, the number of stings, the patient's sensitivity, age and weight, and the climate of the region (1, 7, 8, 21).

In this study, the average amount of venom obtained by maceration and subcutaneously injected into mice was 20mg/ml. The quantity that would kill a mouse (weighing approximately 23g) was calculated as 2.31mg [closed telsons] and 2.66mg [open telsons] (Table 3). Since a telson is about 20±1mg, 7%-8% of it corresponds to the LD₅₀ of a mouse. This conclusion was similar to that of Ozkan and Filazi (18), who used the same species of scorpions. They stated that 3.6mg of grounded dry telson corresponds to the LD₅₀ of a mouse (18). In our study, there were no differences between the subgroups and the base groups but the first and second groups of telsons were more toxic than the third group. However, the weight and size of telsons were not statistically significant ($p>0.005$) and did not correlate with

toxicity. Tulga, using the same species of scorpions but different methodology, showed that 3.3%-5% of a telson corresponded to the LD₅₀ of a mouse (23, 24).

Another study reported that differences between toxicities of scorpion venoms could be explained by the collection of scorpions as well as changes in their physiologic statuses and feeding patterns. Furthermore, during capturing and killing, the scorpions could have used some quantity of their venom to protect themselves. In addition, as already stated, factors such as transporting conditions, drying method, storage, and period of use of telsons could have influenced these differences (18).

Consequently, in our study, telsons weighting from 19.99 to 20mg (first group) and from 29.99 to 30mg (second group) had LD₅₀ values similar to each other and telsons weighting from 10 to 19.99mg (third group) presented fourfold higher values. This difference was caused by the maturity of scorpions (biological status) and venom toxicity was related to their size. Therefore, it was seen that obtaining open telsons due to environmental factors was not effective for venom toxicity.

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