

**DETERMINATION OF POTENCY AND PARASPECIFIC EFFECTS OF
Androctonus crassicauda (OLIVIER, 1807) ANTIVENOM AGAINST *Mesobuthus*
gibbosus (BRULLÉ, 1832) VENOM (SCORPIONES: BUTHIDAE)**

**OZKAN O. (1, 2), ADIGUZEL S. (1), KAR S. (2), YAKISTIRAN S. (1), CESARETLI
Y. (1), KARAER K. Z. (2)**

(1) Refik Saydam Hygiene Center, Ankara, Turkey; (2) Department of Entomology and Protozoology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey.

ABSTRACT: Scorpion envenomation remains a real health problem in many countries. In scorpionism cases, it is often recommended that patients be treated with species-specific antivenom. *Androctonus crassicauda* venom has been used as antigen for antivenom production in Turkey, where this antivenom, called Turkish antivenom, has also been effective in the treatment of envenomation caused by species other than *A. crassicauda*. The present study aimed at determining the paraspecific effects and potency of the Turkish antivenom against *Mesobuthus gibbosus* (Brullé, 1832) venom. To assess the venom toxicity and the antivenom efficacy, we determined the Minimum Lethal Dose (MLD) and the Minimum Effective Dose (MED) instead of LD₅₀ and ED₅₀, respectively. *Androctonus crassicauda* antivenom was capable of neutralizing *M. gibbosus* venom (20 MLD). This was the first study indicating that *A. crassicauda* antivenom can be used for the treatment of *Mesobuthus gibbosus* stings, especially in Aegean Region, Turkey.

KEY WORDS: scorpion, antivenom, *Androctonus crassicauda*, *Mesobuthus gibbosus*, paraspecific activity.

CONFLICTS OF INTEREST: There is no conflict.

CORRESPONDENCE TO:

OZCAN OZKAN, Refik Saydam Hygiene Center, Ankara, Turkey. Phone: +90 312 458 2372. Fax: +90 312 458 2395. Email: ozcanozkan_62@hotmail.com.

INTRODUCTION

Envenomation by arachnids causes significant injuries all over the world. Scorpion sting is the most important type of arachnid envenomation resulting in adult morbidity and pediatric mortality. Scorpionism remains a real health problem in developing countries, especially in tropical and subtropical regions including urban areas (3, 5, 11, 13).

Scorpion venom contains short polypeptide neurotoxins consisting of simple, low-molecular-weight proteins of lethal and paralytic effects (4, 14, 20). Lethal scorpions mostly belong to the family Buthidae (9, 12, 14), among which, species of the genera *Androctonus*, *Leiurus*, and *Mesobuthus* are the main responsible for envenomation in Turkey (10, 14).

It is recommended that patients envenomed by scorpion stings be treated with species-specific antivenom (1). The scorpion-antivenom treatment introduced in 1909 is still the only method of therapy (3, 16). In Turkey, scorpion antivenom has been produced by the Refik Saydam Hygiene Center (RSHC) since 1942 (4, 14, 16, 17). Venom obtained through maceration of telsons of *A. crassicauda* scorpions has been used as antigen for antivenom. This antivenom, called Turkish antivenom, has also been used against other scorpion species (1, 10, 17, 19).

The present study aimed at determining the paraspecific effects and potency of *A. crassicauda* antivenom against *Mesobuthus gibbosus* venom.

MATERIALS AND METHODS

Animals

Scorpions: *Androctonus crassicauda* specimens were collected from Southeastern Anatolia region (Sanliurfa) and *M. gibbosus* (Figure1) specimens were collected from Aegean region (Mugla), Turkey.

Experimental animals: Healthy female Swiss albino mice, weighed 25 ± 1 g, were used for determination of the Minimum Lethal Dose (MLD) and Minimum Effective Dose (MED). They were bred at the Animal Facility Center, Faculty of Medicine, Ankara University, Ankara, Turkey. Animals were kept in an experimental room under room temperature ($22\pm 2^\circ\text{C}$) and $60\pm 10\%$ humidity and were fed with commercial rodent pellets *ad libitum* throughout the experiment.

Venom

Venom was obtained from mature *A. crassicauda* and *M. gibbosus* scorpions through electrical stimulation of their telson. The venom was mixed with sterile double distilled water and centrifuged at 15,000rpm for 15min at 4°C. The supernatant was immediately lyophilized and stored at -20°C until use.

Antivenom

Antivenom was prepared from a pool of hyperimmune sera obtained from horses immunized with *A. crassicauda* venom.

Determination of Minimum Lethal Doses

The Minimum Lethal Dose (MLD) was determined to assess the lethal toxicity of *A. crassicauda* and *M. gibbosus* venoms. Groups of five mice were subcutaneously injected with venom diluted in physiologic saline solution (PSS; 0.9% sodium chloride solution) at doses ranging from 5.0 to 35.0µl/mouse (*M. gibbosus* venom) and from 2.5 to 15.0µl/mouse (*A. crassicauda* venom). The volume of venom injected was kept constant at 500µl/mouse. Control group was injected with PSS only. Following treatment with venom solution, animals were monitored for 48 hours and the number of dead animals was recorded at the end of the experiment. The dose that killed 100 percent animals was considered the MLD.

Serum Neutralization Tests

A solution of *A. crassicauda* venom (40 MLD) and a solution of *M. gibbosus* venom (20 MLD), both diluted in PSS to a 2500µl volume, were prepared. Then, each solution was mixed with hyperimmune horse sera and incubated for one hour at 37°C for antigen-antibody reaction. Groups of six mice were subcutaneously injected with the solutions. The injection volume was kept constant at 500µl/mouse. Control groups were injected with PSS only. The number of living mice was recorded after 48h.

Determination of Minimum Effective Doses

Androctonus crassicauda antivenom was prepared at doses ranging from 100µl to 1000µl. The final volume was made up to 5000µl with PSS and the solutions were

incubated at 37°C for 60 min. Then, 500µl of each solution was subcutaneously injected into groups of six mice previously injected with 20 MLD of *M. gibbosus* venom or 40 MLD of *A. crassicauda* venom. Positive control groups were only injected with 20 MLD of *M. gibbosus* venom and 40 MLD of *A. crassicauda* venom diluted in PSS. After administration, animals were monitored for 48 hours and the number of living animals was recorded. The antivenom doses that prevented 100% deaths in the groups were considered the minimum effective doses (MED).

RESULTS

The MLD of *A. crassicauda* and *M. gibbosus* venoms were 10µl/mouse and 30µl/mouse, respectively (Table 1).

To assess the antivenom potency, increasing doses of hyperimmune sera were used (100, 400, 700, 1000µl) while the amounts of *A. crassicauda* and *M. gibbosus* venoms were kept constant (40 and 20 MLD, respectively). Changes in the neutralization capacity of the hyperimmune horse sera against *A. crassicauda* and *M. gibbosus* venoms were shown in Table 2. The minimum effective dose of the antivenom against both *A. crassicauda* and *M. gibbosus* venoms was 400µl. All control mice died. The antivenom dose needed to neutralize *M. gibbosus* venom was 20 times higher than the MLD of a mouse.



Figure 1. *Mesobuthus gibbosus*, Aegean Region (Mugla), Turkey (Photographed by Ozkan).

Table 1. Determination of Minimum Lethal Doses (MLD) of *Androctonus crassicauda* and *Mesobuthus gibbosus* venoms subcutaneously injected into mice.

<i>A. crassicauda</i>		<i>M. gibbosus</i>	
Venom (µl/mouse)	Mice (No. dead animals/total)	Venom (µl/mouse)	Mice (No. dead animals/total)
2.5	0/5	5.0	0/5
5.0	1/5	10.0	1/5
7.5	2/5	15.0	2/5
10.0*	5/5*	25.0	4/5
12.5	5/5	30.0*	5/5
15.0	5/5	35.0	5/5
Control Groups			
PSS (µl/mouse)	Mice (No. dead animals/total)	PSS (µl/mouse)	Mice (No. dead animals/total)
500	0/5	500	0/5

* Minimum Lethal Dose

Table 2. Determination of the antivenom neutralization capacity, expressed as Minimum Effective Dose (MED), against *Androctonus crassicauda* and *Mesobuthus gibbosus* venoms subcutaneously injected into mice.

<i>A. crassicauda</i>			<i>M. gibbosus</i>		
Venom (µl)	Antivenom (µl)	Mice (No. living animals/total)	Venom (µl)	Antivenom (µl)	Mice (No. living animals/total)
400	100	0/5	600	100	0/5
400	400*	5/5	600	400*	5/5
400	700	5/5	600	700	5/5
400	1000	5/5	600	1000	5/5
Control Groups					
Venom (µl/mouse)	Mice (No. dead animals/total)	Venom (µl/mouse)	Mice (No. dead animals/total)		
40	5/5	60	5/5		

* Minimum Effective Dose

DISCUSSION

Out of the approximately 1500 scorpion species described so far, 50 present venoms that are dangerous for humans. Most of these species belong to the genera *Buthus*, *Parabuthus*, *Mesobuthus*, *Tityus*, *Leiurus*, *Androctonus*, and *Centruroides* of the Buthidae family (5, 8, 12, 14, 16). The most common and lethal species are: *Tityus serrulatus* and *T. bahiensis* in South America, especially in Brazil; *Centruroides suffusus*, *C. limpidus*, *C. sculpturatus* in Mexico; *Leiurus quinquestriatus*, *Androctonus crassicauda*, *A. mauretanicus*, *A. australis*, *A. amoreuxi*, and *Buthus occitanus* in Middle East and North Africa; *Parabuthus granulatus* and *P.*

transvaalicus in South Africa; *Mesobuthus tamulus* and *Palamneus swammerdami* in India (3, 6, 16).

Scorpions as well as human envenomation cases are common in Turkey due to its geographical location, climate and socioeconomic structure (11, 14). Therefore, scorpion envenomation is an important health problem in several regions of the country, especially in South-Eastern Anatolia (2, 11, 15, 17). Important scorpions threatening public health in Turkey are *A. crassicauda*, *L. quinquestriatus*, *M. gibbosus* and *M. eupeus* (2, 10, 12, 14, 15).

The toxicity of scorpion venoms depends on several factors including the genus, species, age, structure, physiological and feeding states of the animal; the amount of venom injected; the climate of the region; and the time of animal collection (8, 11, 14).

Krifi *et al.* (7) reported that the difficulties in standardizing the venom quality and the LD₅₀ determination are in part related to: the species used; the animals' geographical origin, age and body weight; the season and procedures of venom extraction; the number of specimens milked; the breeding conditions; the venom injection route; and the LD₅₀ determination method.

The above-mentioned parameters must always be specified for the determination of any venom LD₅₀ or antivenom potency. As the subcutaneous route provides the highest LD₅₀ value and is the most frequent route of accidental scorpion envenomation, it should be used to estimate the antivenom potency (7). The venom variability must also be considered during the production of antivenom, which has to be capable of neutralizing the toxic effects of all kinds of venoms from a specific scorpion species (7, 16).

Theakston *et al.* (16) carried out a review of the potency assays used in several countries and noticed that the most acceptable test is the standard murine lethality assay (determination of LD₅₀ of venom and ED₅₀ of antivenom). However, considerable variations were found such as the way the test was carried out, the volumes injected, the routes used for injection of venom/antivenom mixtures, the LD₅₀ value, the mouse strain used and the animals' weight range.

The antivenom activities should be expressed according to national or regional standards and a toxin neutralization unit must be used.

In recent years, *in vivo* tests other than mouse lethality assay have been vastly used (16). In the present study, MLD and MED were determined instead of LD₅₀ and ED₅₀. The role of antivenom on the treatment of scorpion stings remains controversial (16). Bücherl (4) emphasized that the antivenom must be administered to the patient at an early stage since it can be much more effective if given within two hours after the sting. Alexander (1) recommended that scorpion-stung patients be treated with species-specific antivenom. In case the latter is not available, antivenom prepared against *A. crassicauda* venom could be used to treat envenomation caused by Old World scorpions and antivenom prepared against *Centruroides sculpturatus* venom could be used to treat envenomation by New World scorpion species (1).

Whittemore *et al.* (19) reported that the Turkish antivenom (antivenom produced against *A. crassicauda* venom at Refik Saydam Hygiene Center) was more effective than homologous antivenom in neutralizing *A. australis* venom in Algeria and had the same neutralization capacity as homologous antivenoms against *B. occinatus* venom in South Europe and North Africa, *T. serrulatus* and *T. bahiensis* venoms in South America, and *L. quinquestriatus* venom in Anatolia (19).

Tulga (18) observed that antivenom prepared against the Turkish *A. crassicauda* venom was capable of neutralizing *L. quinquestriatus* venom from Israel. However, Kasapoğlu (6) stated that further studies are needed about the potency and paraspecific effects of the Turkish antivenom on the treatment of envenomation by *M. gibbosus*.

Altinkaynak *et al.* (2) recommended that the Turkish antivenom be used to treat *M. gibbosus* envenomation cases since it was effective in 91.6% of the cases studied. The antivenom was not effective in only two cases (9 and 13-month-old infants) because the patients had received late first aid and hospitalization and the sting site was on the neck (2).

Soker and Haspolat (15) verified that the Turkish antivenom was effective in 90.3% of all scorpionism cases in South-Eastern Anatolia.

In the present investigation, *A. crassicauda* antivenom neutralized both *A. crassicauda* and *M. gibbosus* venoms. The Turkish antivenom was effective against *M. gibbosus* venom. Therefore, antivenom produced against *A. crassicauda* venom can be used in the treatment of envenomation by *M. gibbosus*, especially in Aegean region of Turkey.

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