A Study on Specific and Nonspecific Effects of Antivenoms of *Androctonus crassicauda*, *Buthotus saulcyi* and *Odontobuthus doriae* Against Venoms of Important Species of Scorpion in Iran

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Summary

The venoms of Androctonus crassicauda, Buthotus saulcyi *and* Odontobuthus doriae *were obtained by means of electrical stimulation and telson maceration. Three specific antisera (monovalants) were prepared, in the horse, against the venoms of the afore mentioned species. The immunoglobulin was precipitated with ammonium sulphate and cleaved with pepsin to produce* $F(ab)'_2$ *fragments. The specific antisera were more effective against their respective venoms (more than 10* LD_{50}*). However, sera with more than 10* LD_{50} *gave some protection against the venoms of other Buthid scorpions, whereas these antisera didn't show any protective effects against the venom of scorpionid scorpions.*

Immunological tests indicated that there is a strong cross-reactivity between the venoms of Androctonus crassicauda, Odontobuthus doriae and Mesobuthus eupeus.

Introduction

Scorpion stings are a major health problem in the world. Injuries and deaths due to scorpion stings occur in most parts of the world, especially in the tropics and the subtropics. Despite the great number of species (more than 1000 species), only the venom of a few species of scorpion (about 20 species in the world) are capable of causing human death .

The most dangerous scorpions in Iran are *Androctonus crassicauda*,

Buthotus saulcyi and *Odontobuthus doriae*, belonging to the *Buthidae* family (1). In Iran, approximately several thousand people are annually stung by scorpions. The only specific medical treatment for envenomation by dangerous scorpions is the use of antisera, which is prepared in hourses. In this study 3 specific antisera (monovalent) were prepared, against the venoms of *Androctonus crassicadua*, *Buthotus saulcyi* and *Odontobuthus doriae*, in the horse .

The specific and non-specific effects of these antisera were evaluated in vivo and in virto with venoms of 6 different species of scorpion from Iran.

Materials and methods

Venoms: The venom from 3 species of scorpion, from different parts of Iran, collected during spring and summer, were obtained by 2 methods as follows:

1. Venoms from *A. crassicauda, B. Saulcyi and O. doriae* were obtained in the laboratory by electric stimulation, centrifuged (12000 rpm) and pooled. The precipitate (mucoprotains) was discarded and the supernatant was freeze-dried and kept until used(1).

2. Telsons macerates, from 3 of the above mentioned species, were prepared by grinding and homogenisation in distilled water, dialysed against water for 24-48 h. These procedures eliminated about 50% of mucoprotenis, without reducing the toxicity (Table 1). After centrifugation, the supernatant was frozen at -20°C until use.

Table 1. Preparation of antigens

Venoms	Electrical stimulation venom (pooled) (mg)	Gland macerates venom (mg)	Concentration mg/ml	Toxicity (LD50)ug
Androctonus	240	150	10	10
Buthotus	480	200	10	30
Odontobuthus	400	200	10	10

Toxicity, in vivo, was tested in mice of 18-20 g body weight by intravenous (iv) injection. The lethal dose killing 50% of the animals (LD_{50}) was determined according to the method of Spearman-Karber (2).
Immunization: Horses were immunised by subcutaneous injections, 9-11 times, at intervals of one week, with separate venoms, increasing the amount of the injected venom in 3 periods. Blood-clots were kept for 24 h at 4°C. Crude antivenom was aspirated into containers, stored at 4°C prior to purrification.
Purification of the Antivenoms: The amount of crude antivenoms taken from immunized horses were 68 l. The immunoglobulin was precipitated with ammonium sulphate, then cleaved with pepsin(3). The antibody molecule was split by pepsin to yield 2 identical fragments ($F(ab)'_2$ and Fc). The Fc fragment was discarded.

The $F(ab)_2$ fragment with 2 combining sites for the antigen, was very pure and more effective than the non-purified $F(ab)'_2$. The protein contents of specific antisera are shown in Table 2.

Table 2. Protein content of the sera

Antisera	Amount of plasma (l)	Amount of serum (ml)	Amount of protein (g%)
Androctonus	19.5	800	5.1
Buthotus	17	800	5.1
Odontobuthus	32	1250	4.7

Neutralisation tests: Neutralisation capacities of the specific antisera were determined as follows: One ml of serial dilutions of each venom was mixed with the same amount of antisera and the mixtures were incubated at 37°C for 1 h. Then 0.5 ml of each mixture was injected intravenouly into 3 mice. Survival or death of the mice were recorded 24 h after the injection(4).
Immunological test: Ouchterlony's immuno-diffusion technique was performed in plates of agar. Venom solutions in saline (6 different species of Iranian scorpions) were used at a concentration of 5 mg/ml against the 3

specific antisera and one kind of polyvalent antisera, Lot 57,(5).

The central well was filled with 0.25 ml of antisera and venoms were added into peripheral wells. The plates were incubated at 37°C. The precipitate bands were observed after 48 h as shown in Table 3 and Figs. 1-5.

Results

Total amount of the venoms used for immunization of horses were 300, 470 and 820 mg from venoms of *Androctonus, Buthotus* and *Odonto buthus*, respectively.

The amount of crude antivenoms taken from immunized horses were 19.5 (*And.*), 17 (*Buth.*) and 32 (*Od.*) litre, and those of purified antisera were 800 (*And.*), 800 (*Buth.*) and 1250 (*Od.*) ml containing 5.1%, 5.1% and 4.4% protein, respectively (Table 2).

One ml of purified antisera to *Androctonus*, *Buthotus* and *Odontobuthus* neutralised 0.06 mg, 0.5 mg and 0.1 mg of the respective venoms.

In Ouchterlony double diffusion, *Androctonus* antisera showed precipitation lines against venoms of *Odonotobuthus* and *Mesobuthus* but not against the venoms of other scorpions. *Odonotobuthus* antisera produced precipitation lines against the venoms of *Androctonus* and *Mesobuthus* but not against other venoms (Table 3).

Table 3. Immunological tests

Venom / antisera	Buthidae family				Scorpionidae family	
	And.	Buth.	Od.	Mes.	Scorp.	Hemis.
Androctonus	5	0	1	2	0	0
Buthotus	0	3	0	0	0	0
Odontobuthus	1	0	2	1	0	0
And.+Buth.+Od.	2	2	1	2	0	0
Polyantisera (Lot 57)	1	2	2	3	1	1

Cent: Mon. And.

1-And. Venom

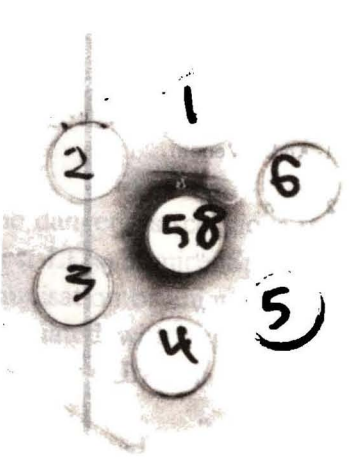
2-Buth. "

3-Odont. "

4-Mes. "

5-Scorp. "

6-Hem.



Cent. Mon. Buth.

1-And. Venom

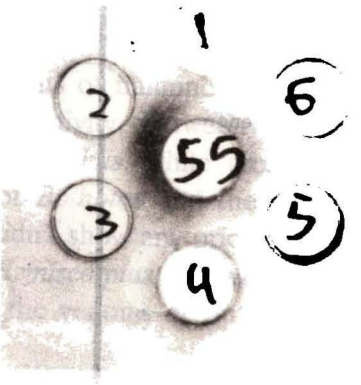
2-Buth. "

3-Odont. "

4-Mes. "

5-Scorp. "

6-Hem.



Cent: Mon. Odont.

1-And. Venom

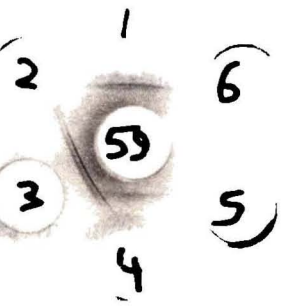
2-Buth. "

3-Odont. "

4-Mes. "

5-Scorp. "

6-Hem.



Cent: Poly. Mix
(A + B + O)

1-And. Venom

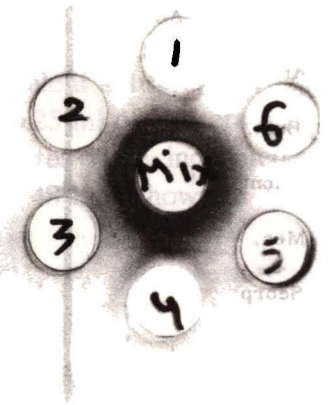
2-Buth. "

3-Odont. "

4-Mes. "

5-Scorp. "

6-Hem. "



Cent: Poly. (57)

1-And. Venom

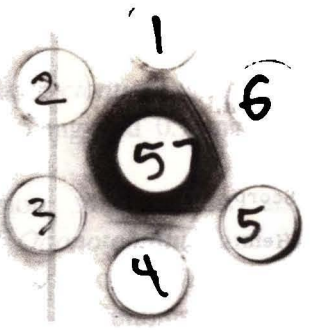
2-Buth. "

3-Odont. "

4-Mes. "

5-Scorp. "

6-Hem. "



Discussion

In this study we chose 3 species of the dangerous scorpions from *Buthidae* family, because they showed the highest LD_{50} in mice. Specific antigens, as shown in Table 1, were used for immunisation of animals.

Three specific antisera were evaluated with regard to specific and non-specific effects against the few selected Iranian scorpion venoms(In vivo and In vitro).

The results of neutralisation tests indicated that the specific antisera were more effective against their respective venoms (more than 10 LD_{50}) but, at the same time, showed some degree of protection against other Buthid scorpions. However, these antisera did not show any protection against scorpionid scorpions. The results of immunological tests indicated that there was a strong cross-reactivity between the venoms of *Androctonus, Odontobuthus* and *Mesobuthus*, while cross-reactions were not observed between the venom of *Bothotus* and the venoms of other Buthid family. All specific antisera didn't show any pricipitate band against the venom of *Scorpio maurus* and *Hemiscorpius lepturus*. This means that there is no cross-reactions between the venoms of Buthid and scorpionid scorpions.

Therefore, according to neutralisation and immunological results, on polyvalent antisera against scorpion venom, it is necessary to use the venoms of 4 important scorpions, namely, *Androctonus crassicauda, Odontobuthus doriae, Buthotus* and *Hemiscorpius lepturus*.

Although, the lethality of *Hemiscorpius* venom is not high in mice and the yield of its venom in each milking is very low (0.1 mg), the sting of this scorpion is a medical problem in south of Iran. For this reason, it is suggested that the venom of this scorpion be further studied.

References:

1. *Latifi, M. (1978). Immunological studies on antiscorpion serum and antivenin. In: Toxins, Animal and Microbial. P. 619.*
2. *Finney, D. J. (1964). The Spearman-Karber method. In: statistical method in Biological Assay. P. 524, London.*
3. *Latifi, M. (1978). Commercial production of antisnakebite serum. In:*

Biology of the Reptilia. Vol. 8, P. 561, London.

4. *Latifi, M. (1979). Immunological studies on Iranian scorpion venom and antiserum. Toxicon. 17: 61.*

5. *Ouchterlony, (1962). Diffusion-in-gel method for immunological analysis, prg. Allergy. 6: 30.*