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RESEARCH ARTICLE

Electrophoretical Comparison of Proteins of *Mesobuthus eupeus* and *Mesobuthus gibbosus* Scorpion Venoms

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Summary

Scorpion envenomation still remains a major health problem in many tropical and subtropical countries. Antivenom is still widely administered in the treatment of envenomation. Scorpion venoms is used as source of antigen to the production of antivenom. Therefore, quality control and homogeneity of venom are a crucial point. In this study, adult *Mesobuthus gibbosus* and *Mesobuthus eupeus* scorpions were collected from Osmaniye and Nigde provinces in Turkey. After extraction, the venom composition was analyzed using one-dimensional gel electrophoresis (1-DE). Only one common protein (70 kDa) band was found in all venom samples. Three protein bands (70, 87 and 100 kDa) were also detected in the venom of female scorpions in Osmaniye and Nigde provinces.

Keywords: *Scorpion, Mesobuthus gibbosus, Mesobuthus eupeus, Venom, Protein, Electrophoresis*

Mesobuthus eupeus ve *Mesobuthus gibbosus* Akrep Venom Proteinlerinin Elektroforetik Karşılaştırılması

Özet

Akrep zehirlenmesi hala birçok tropikal ve subtropikal ülkelerde önemli bir sağlık sorunu olmaya devam etmektedir. Akrep sokmalarının tedavisinde, antivenom hala yaygın olarak uygulanmaktadır. Antivenom üretimi için akrep venomları antijen kaynağı olarak kullanılmaktadır. Bu nedenle, venomun kalite kontrolü ve homojenliği çok önemli bir noktadır. Bu çalışmada, Türkiye'de Osmaniye ve Niğde illerinde yetişkin *Mesobuthus gibbosus* ve *Mesobuthus eupeus* akrepleri toplanıldı. Ekstraksiyon sonra, venom kompozisyonu tek boyutlu jel elektroforezi (1-DE) kullanılarak analiz edilmiştir. Bütün venom örneklerinde ortak tek protein bandı (70 kDa) bulundu. Ayrıca, Osmaniye ve Niğde illerindeki dişi akreplerin venomunda üç protein bandı (70, 87 ve 100 kDa) tespit edilmiştir.

Anahtar sözcükler: *Akrep, Mesobuthus gibbosus, Mesobuthus eupeus, Venom, Protein, Elektroforezis*

INTRODUCTION

The scorpion venom containing short neurotoxin polypeptides consist of low molecular weight simple proteins which have lethal and paralytic effects [1]. It has been estimated that 100.000 distinct peptides exist in scorpion venom but only limited number of these peptides have been described [2].

In many tropical and subtropical countries, venoms from medically important scorpion species are still used in the production of antivenoms to the treatment of envenomation [3]. The reason of this is the absence of vaccines or other effective agents against envenomations [4]. On the other hand, source of variation in animal venoms can be age, sex, physiological state, geographical origin, interval of venom extraction time and method and differences in the genetic makeup of population in one species [3,5]. Thence, quality control and homogeneity of venom to production antivenom are a crucial point. *Mesobuthus* is one of the most

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widely distributed genera of the family Buthidae, with species present throughout Turkey. Therefore stings by *Mesobuthus* species are more frequent than those by other scorpion species [6-10]. In many studies, gel electrophoresis, electrofocusing or liquid chromatography have already been used to detection of protein pattern of animal venoms, even if the relatively low resolution of the analytical methods [3,5].

In the present study, protein pattern of venom samples obtained from *Mesobuthus eupeus* and *Mesobuthus gibbosus* scorpions compared and analyzed by one-dimensional gel electrophoresis (1-DE).

MATERIALS and METHODS

Origin of Scorpions

During summer period of 2008, adult *M. eupeus and M. gibbosus* scorpions were collected from Nigde and Osmaniye provinces in Turkey respectively *(Fig. 1)*. They were kept in the different plastic boxes according to their geographical origins and gender at the Department of Protozoology and Entomology, Faculty of Veterinary Medicine, Ankara University. The scorpions were fed with crickets or cockroaches and receiving water daily.

Specimen Examined and Diagnosis

The scorpions were identified to the species and gender level under stereomicroscope using taxonomic keys as described by Kovarik [11].

Venom

Venoms were obtained from scorpion by electrical stimulation of telson as described previously [12]. The venoms were dissolved with sterile double-distilled water and centrifuged at 14.000 rpm for 15 min at 4ºC. The supernatant was taken and immediately lyophilized and stored at -20ºC until use according to their geographical origins and gender. After milking venom, the animals were housed in individual boxes for another milking process. Protein content of venoms was determined by the absorbance at 280 nm [13,14].

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoretic (SDS-PAGE) Analysis of the Venom

SDS-PAGE (7.5%) analyses of the venom and their proteins bands determined according to Laemmli [15]. Proteins were stained with 0.1% Coomassie Blue R-250 Silver [16]. Molecular mass standard (Sigma, S8445) were run in parallel in order to calculate the molecular weights of proteins. Then, the photograph of the gels was taken and molecular weights of the proteins were calculated with Molecular Imaging Software (Kodak).

RESULTS

Scorpions and Protein Content

The stereomicroscopic identification of the scorpions was confirmed as *M. eupeus* and *M. gibbosus (Fig 1)*. The average amounts of the venoms gained from the scorpions are 38.19±6.14 mg/ml (Min: 30 mg/ml - Max: 49 mg/ml) and 37.47±4.28 mg/ml from Osmaniye and Nigde provinces respectively.

SDS-PAGE Analysis of the Venom

The protein profiles of *M. eupeus and M.gibbosus* scorpion venoms were analyzed by SDS-PAGE followed by Coomassie silver blue staining. In these analyses, 17 different protein bands *(Table 1)*, each with a different molecular mass, were detected. The number of the protein bands for the investigated scorpions ranged from 3 to 9. Out of all protein bands, only the band of 70 kDa consistently appeared in all venom samples *(Fig. 2)*.

Analysis of Mesobuthus eupeus and Mesobuthus gibbosus Venom

Fig. 2 shows that the venoms of the scorpion differ in

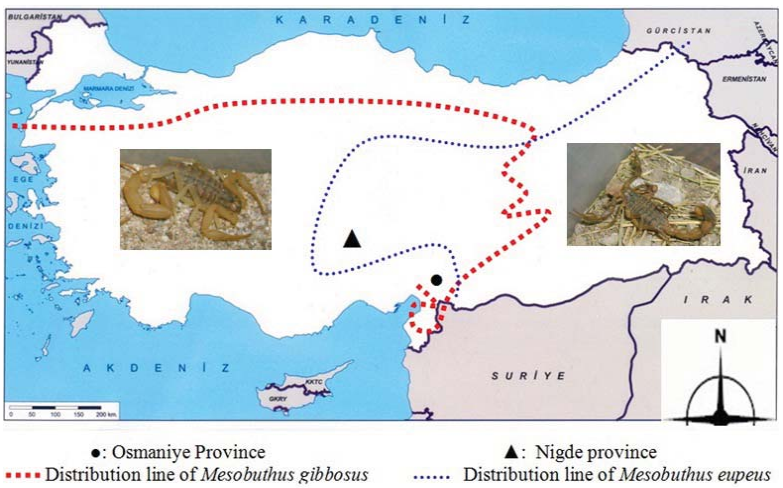

Fig 1. Map of distribution of *Mesobuthus* species in Turkey

Şekil 1. *Mesobuthus* türlerinin Türkiye'deki dağılım haritası

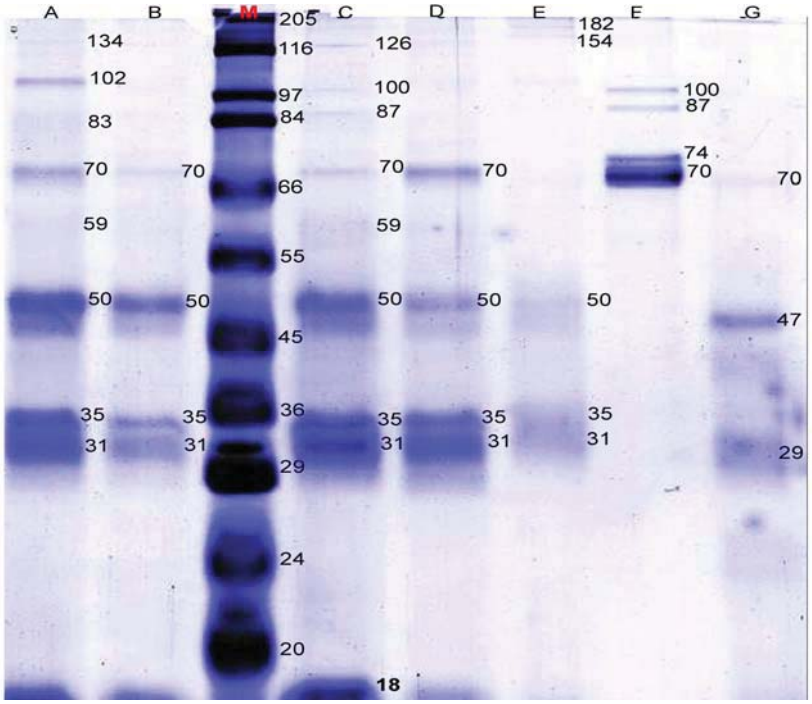

Fig 2. The proteins of venom of *Mesobuthus* species were separated by using 7.5% polyacrylamide gel electrophoresis. Venom extracts (20-30µg); *Mesobuthus gibbosus,* **(A** [Osmaniye province; ♂]**; B** [Osmaniye province; ♀ (pregnant]; **C** [Osmaniye province; ♀]**; D** [Osmaniye province; ♀ (pregnant)]**; E** [Osmaniye province; ♀ (pregnant)]**,** and *Mesobuthus eupeus***; F** and **G** [Nigde province; ♀]**),** molecular weight markers **(M)**; 205 kDa-Myosin, 116 kDa- β-Galactosidase, 66 kDa- Albumin, 45 kDa-Ovalbumin and 14.5 kDa- α-Lactalbumin

Şekil 2. *Mesobuthus* türelerinin venom proteinleri kullanılan %7.5 poliakrilamid jel elektroforezisle ayrıldı. Venom ekstrakları (20-30 µg); *Mesobuthus gibbosus,* **(A** [Osmaniye ili; ♂]**; B** [Osmaniye ili; ♀ (gebe)]**; C** [Osmaniye ili; ♀]**; D** [Osmaniye ili; ♀ (gebe)]**; E** [Osmaniye ili; ♀ (gebe)]**,** ve *Mesobuthus eupeus***; F** ve **G** [Nigde ili; ♀]**),** moleküler markır **(M)**; 205 kDa-Myosin, 116 kDa- β-Galactosidase, 66 kDa- Albumin, 45 kDa-Ovalbumin and 14.5 kDa- α-Lactalbumin

Table 1. The variations of protein in *Mesobuthus* scorpion species venom samples collected from Osmaniye and Nigde provinces

Tablo 1. Osmaniye ve Niğde illerinde toplanan *Mesobuthus* akrep türlerin venom örneklerindeki protein varyasyonları

Protein Bands (kDa)	Scorpion Venom Samples								
	Osmaniye (n: 5)						Nigde (n: 2)		
	A	B	C	D	E	Number	F	G	Number
182	-	-	-	-	+	1	-	-	0
154	-	-	-	-	+	1	-	-	0
134	+	-	-	-	-	1	-	-	0
126	-	-	+	-	-	1	-	-	0
102	+	-	-	-	-	1	-	-	0
100	-	-	+	-	-	1	+	-	1
87	-	-	+	-	-	1	+	-	1
83	+	-	-	-	-	1	-	-	0
74	-	-	-	-	-	**0**	+	**-**	**1**
70	+	**+**	**+**	**+**	-	4	+	+	2
59	+	-	+	-	-	2	-	-	0
50	+	+	+	+	+	5	+	-	1
47	-	-	-	-	-	0	-	+	1
35	+	+	+	+	+	5	-	-	0
31	+	+	+	+	+	5	-	-	0
29	-	-	-	-	-	0	-	+	1
18	-	-	+	-	-	1	-	-	0
Total number of protein bands (n: 17)	**8**	**4**	**9**	**4**	**5**	**30**	**5**	**3**	**8**

composition, number and intensity of bands between the same gender of *M. eupeus* and *M. gibbosus*. Female *M. eupeus* scorpion venom from Nigde province was characterized by the presence of 6 protein bands (29-100 kDa). The number of bands was detected 14 protein bands (18-182 kDa) in the female *M. gibbosus* venom from Osmaniye province. Female *M. gibbosus* scorpion venom showed bands of 18, 31, 35, 50, 59, 126, 154 and 182 kDa which were absent

in the female *M. eupeus* venoms. Three protein bands (29, 47 and 74 kDa) were found in *M. eupeus* scorpion venom samples and absent in *M. gibbosus* scorpion venoms. On the other hand three protein bands (70, 87 and 100 kDa) were detected in female scorpion venom samples both Osmaniye and Nigde provinces *(Table 1)*.

Analysis of M. gibbosus Scorpion Venom According to Gender

The number of bands was detected minimum four and maximum nine bands according to SDS-PAGE analysis of venoms *(Table 1)*.

Male *M. gibbosus* scorpion venoms showed bands of 83, 102 and 134kDa which were absent in the female venoms, whereas five protein bands (18, 74, 126, 154 and 182 kDa) were found in female venom samples and absent in male venoms. Five protein bands with molecular masses of 31, 35, 50, 59, 70 kDa was detected in both female and male *M. gibbosus* venom samples *(Table 2)*. On the other hand, only one protein band with molecular masses of 70 kDa was detected in both female and male venom samples as independent from geography origin and gender of scorpions.

DISCUSSION

In Turkey, there are 23 distinct scorpion species and of those, the most venomous scorpion to humans belongs to the Buthidae family [6,17-19]. *M. eupeus* and *M. gibbosus* are members of *Mesobuthus* genus, Buthidae family [20]. These species common from west Anatolia to the east of Anatolia are considered a medically important species [6,21-23]. Ozkan et al.[9] stated that the majority of scorpion stings were observed in the Mediterranean, Aegean, Central and East Anatolia regions of Turkey.

Polymorphisms of protein contents have been also observed by El Ayeb and Rochat [24] in venom of individual *Androctonus australis hector* venom. Keskin and Koc [25] indicated that venom proteins of *Iurus dufoureius asiaticus* scorpion from the Aydin province in the Aegean region of Turkey were variations individually in the number of bands. Abdel-Rahman et al.[26] reported that intersexual variations in scorpion venom were detected within the same scorpion population. They also suspected that a combination of local environmental conditions, geographical separation and genetic separation may play a major role in the intra-specific variation of venom of *Scorpio maurus palmatus*.

Table 2. The variations in *Mesobuthus gibbosus* and *Mesobuthus eupeus* scorpion venom proteins samples according to gender of scorpions

Tablo 2. Akreplerin cinsiyetlerine gore; *Mesobuthus gibbosus* ve *Mesobuthus eupeus* akep venom proteinlerindeki varyasyonlar

Protein Bands (kDa)	**Scorpion Venom Samples**		
	***Mesobuthus gibbosus* (Osmaniye)**		***Mesobuthus eupeus* (Nigde)**
	Female (n: 4)	**Male (n: 1)**	**Female (n: 2)**
182	+	-	-
154	+	-	-
134	-	+	-
126	+	-	-
102	-	+	-
100	+	-	+
87	+	-	+
83	-	+	-
74	-	-	+
70	**+**	**+**	**+**
59	+	+	-
50	+	+	-
47	-	-	+
35	+	+	-
31	+	+	-
29	-	-	+
18	+	-	-
Total number of protein bands (n: 17)	**11**	**8**	**6**

Differences have been also described within venom of *M. tamulus* [27] *Androctonus mauretanicus* [28], *Tityus serrulatus* [3,29], *Leiurus quinquestriatus* [30], *M. gibbosus* [31]. Thus, the individual variability in venoms is extremely important for evaluating the venom yield and the resulting toxicity after a scorpion sting [28].

Ucar and Tas [32] showed that the crude venom of *M. gibbosus* from Manisa province consisted of 19 protein bands with molecular masses ranging between 6.5 and 210 kDa by SDS-PAGE analysis. Recently, Ozkan and Ciftci [31] indicated that protein bands with molecular masses of 28, 30, 33, 68 and 98 kDa were detected in the venom of captive male *M. gibbosus* from the same biotope during the summer period in the Mugla province. In the current study, five protein bands with molecular masses of 31, 35, 50, 59, 70 kDa was detected in both female and male scorpion venom samples according to SDS-PAGE analyses of *M. gibbosus* venom collected from Osmaniye provinces. On the other hand, only one protein band (70 kDa) was seen in *M. eupeus* scorpion venom. The reasons for this difference would be gender and hormonal status of scorpions, and geographical variation which is effective on feeding habits and consequently venom composition of scorpions.

The toxins composition, protein content and toxicity of the venoms are related to many factors [26,29,33,34]. Effectiveness of scorpion antivenoms and their neutralization capacity depends on both interspecific and intraspecific variations of venoms used for immunization of animals [35]. El Hafny et al.[28] indicated that individual variability in the venom of the same species must be considered to understand the clinical symptoms, to produce effective antivenoms and to adjust the doses of antivenom. Therefore, a critical step in the preparation of antivenoms is the selection of venoms which are used in the immunizing mixture [36].

Monovalent *A. crassicauda* antivenom was capable of neutralizing *M. eupeus* and *M. gibbosus* venom on mice [7,23] and is now widely used throughout Turkey [37]. However, the results of the current work show that the individual variation in scorpion venoms is an important point to take into account in producing effective antivenom. Therefore, it seems to be better to pool the numerous venom extractions from different scorpions of the same species and to use this mixture in the preparation of antivenoms.

In this study, we established individual variations in the number and molecular masses of the protein bands of the venoms according to geographical origins, gender and species of the scorpions both in the same and different species. It should be noted that these variations may have a significant role in venom toxicity of these scorpions from different origins or biological properties. Finally, indicated differences needs to be considered as an important factor in developing effective antivenoms. It is necessary to identify toxic and immunogenic proteins of the scorpion venom for efficient antivenom.

REFERENCES

1. Ghalim N, El-Hafny B, Sebti F, Heikel J, Lazar N, Moustanir R, Benslimane A: Scorpion envenomation and sero terherapy in Morocco. *Am J Trop Med Hyg,* 62 (2): 277-283, 2000.

2. Possani LD, Becerril B, Delepierre M, Tytgat J: Scorpion toxins specific for Na^+-channels. *Eur J Biochem*, 264, 287-300, 1999.

3. Pimenta AM, De Marco Almeida F, Delima ME, Martin Eauclaire ME, Bougis PE: Individual variability in *Tityus serrulatus* (Scorpiones, Buthidae) venom elicited by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*, 17 (5): 413-418, 2003.

4. World Health Organization: WHO Expert Groups on Requirements for Biological Substances. Requirements for immune sera of animal origin. Geneva: *WHO Tech Report Series*, 413, 45-59 [Appendix 2], 1969.

5. Escoubas P, Corzo G, Whiteley BJ, Célérier ML, Nakajima T: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and high-performance liquid chromatography study of quantitative and qualitative variation in tarantula spider venoms. *Rapid Commun Mass Spectrom,* 16 (5): 403-13, 2002.

6. Ozkan O, Kat I: *Mesobuthus eupeus* scorpionism in Sanliurfa region of Turkey. *J Venom Anim Toxins Incl Trop Dis,* 11 (4): 479-491, 2005.

7. Ozkan O, Adiguzel S, Kar S, Yakistiran S, Cesaretli Y, Karaer KZ: Determination of potency and paraspecific effects of *Androctonus crassicauda* (Olivier, 1807) antivenom against *Mesobuthus gibbosus* (Brulle, 1832) venom (Scorpiones: Buthidae). *J Venom Anim Toxins Incl Trop Dis,* 13 (2): 500-508, 2007.

8. Ozkan O, Kar S, Güven E, Ergün G: Comparison of proteins, lethality and immunogenic compounds of *Androctonus crassicauda* (Olivier, 1807) (Scorpiones: Buthidae) venom obtained by different methods. *J Venom Anim Toxins Incl Trop Dis,* 13 (4): 844-856, 2007.

9. Ozkan O, Uzun R, Adıguzel S, Cesaretli Y, Ertek M: Evaluation of scorpion sting incidence in Turkey. *J Venom Anim Toxins Incl Trop Dis*, 14 (1): 128-140, 2008.

10. Adiguzel S: *In vivo* and *in vitro* effects of scorpion venoms in Turkey: A mini-review. *J Venom Anim Toxins incl Trop Dis,* 16 (2): 198-211, 2010.

11. Kovarik F: Review of European scorpions, with a key to species. Sekret, 6, 38-44, 1999.

12. Ozkan O, Filazi A: The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different methods from scorpions, *Androctonus crassicauda* (Olivier 1807). *Acta Parasitol Turcica*, 28 (1): 50-53, 2004.

13. Layne E: Spectrophotometric and Turbidimetric Methods for Measuring Proteins. *Meth Enzymol,* 3, 447-455, 1957.

14. Stoscheck CM: Quantitation of protein. *Meth Enzymol,* 182, 50-69, 1990.

15. Laemmli K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227, 680-685, 1970.

16. Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG: Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis,* 25, 1327-1333, 2004.

17. Kovařík F, Fet V, Soleglad ME, Yağmur EA: Etudes on iurids, III. Revision of the genus *Iurus* Thorell, 1876 (Scorpiones: Iuridae), with a description of two new species from Turkey. *Euscorpius,* 95, 1-212, 2010.

18. Yagmur EA, Koc H, Kunt KB: Description of a new species of *Leiurus* Ehrenberg, 1828 (Scorpiones: Buthidae) from Southeastern Turkey. *Euscorpius.* 85, 1-20, 2009.

19. Yagmur EA: First record of Orthochirus Karsch, 1891 (Scorpiones, Buthidae) from Turkey. *Journal of Anatolian Natural Sciences,* 1 (1): 15-19, 2010.

20. Fet V, Braunwalder ME: The scorpions (Arachnida: scorpiones) of the aegen area: Current problems in taxonomy and biogeography. *Belg J Zool,* 130 (1): 15-20, 2000.

21. Karatas A, Karatas A: First record of *Mesobuthus eupeus* (C.L. Koch, 1839) from central Anatolia (Scorpiones: Buthidae). **In**, Fet V, Selden PA (Eds): Scorpions 2001: In Memoriam Gray A, Polis. pp. 297-299, Burnham Beeches, Bucks, British Arachnological Society, 2001.

22. Karatas A: *Mesobuthus eupeus* (C.L. Koch, 1839) (Scorpiones: Buthidae) in Anatolia. *Euscorpius,* 7, 1-7, 2003.

23. Ozkan O, Carhan A: The neutralizing capacity of *Androctonus crassicauda* antivenom against *Mesobuthus eupeus* scorpion venom. *Toxicon,* 52, 375-379, 2008.

24. El Ayeb M, Rochat H: Polymorphism and quantitative variations of toxins in the venom of the scorpion *Androctonus australis hector*. *Toxicon*, 23 (5): 755-760, 1985.

25. Keskin NA, Koc H: A study on venom proteins of *Iurus dufoureius asiaticus* Birula, 1903 (Scropiones: Iuridae). *Acta Parasitol Turcica,* 30 (1): 60-62, 2006.

26. Abdel-Rahman MA, Omran MAA, Abdel-Nabi IM, Veda V, McVean A: Intraspecific variation in the Egyptian scorpion *Scorpio maurus palmatus* venom collected from different biotopes. *Toxicon,* 53, 349-359, 2009.

27. Badhe RV, Thimas AB, Harer SL, Desphande AD, Salvi N, Waghmare A: Intraspecific variation in protein of red scorpion (*Mesobuthus tamulus*, Coconsis, Pocock) venoms from Western and Southern India. *J Venom Anim Toxins Incl Trop Dis,* 12 (4): 612-619, 2006.

28. El-Hafny B, Chgoury F, Adil N, Chen N, Nassar M: Intraspecific variability and pharmacokinetics, characteristics of *Androctonus mauretanicus* scorpion venom. *Toxicon,* 40 (11): 1609-1616, 2002.

29. Kalapothakis E, Chavez-Olortegui C: Venom variability among several *Tityus serrulatus* specimens. *Toxicon,* 35 (10): 1523-1529, 1997.

30. Omran MA, Mcvean A: Intraspecific variation in scorpion *Leiurus quinquestriatus* venom collected from Egypt (Sinai and Aswan deserts). *J Toxicol Toxin Rev,* 19 (3-4): 247-264, 2000.

31. Ozkan O, Ciftci G: Individual variation in the protein profile of the venom of *Mesobuthus gibbosus* (Brullé, 1832, Scorpiones: Buthidae) from Turkey. *J Venom Anim Toxins Incl Trop Dis,* 16 (3): 505-508, 2010.

32. Ucar G, Tas C: Cholinesterase inhibitory activities of the scorpion *Mesobuthus gibbosus* (Buthidae) venom peptides. *FABAD J Pharm Sci*, 28, 61-70, 2003.

33. Bücherl, W: Classification, biology and venom extraction of scorpion **In,** Bücherl W, Buckley E (Eds): Venomous Animals and Their Venoms. Venomous Invertebrates. 3, 317-47 New York, Academic Press, 1971.

34. Krifi MN, Marrakchi N, El Ayeb M, Dellagi K: Effect of some variables on the in vivo determination of scorpion and viper venom toxicities. *Biologicals,* 26, 277-288, 1998.

35. Padilla A, Govezensky T, Possani LD, Larralde C: Mortality and antibody responses of mice to three successive episodes of experimental scorpion (*Centruroides limpidus limpidus*) envenomation and immunological recue. *Toxicon,* 46, 142-149, 2005.

36. Calvete JJ: Antivenomics and venom phenotyping: A marriage of convenience to address the performance and range of clinical use of antivenoms. *Toxicon,* 56 (7): 1284-1291, 2010.

37. Adigüzel S, Ozkan O, Inceoglu B: Epidemiological and clinical characteristic of scorpionism in children in Sanlıurfa. *Toxicon,* 49 (6): 875-880, 2007.