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CROSS NEUTRALIZATION OF SOME KINDS OF SCORPION VENOMS FROM AFRICA AND SOUTH EAST USING VACSERA POLYVALENT SCORPION ANTISERA

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Abstract

Preparation of scorpion antivenin includes administration of the venom to a suitable animal - mostly horses- and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal. During such procedure the recipient animal may suffer different types of ill-health signs including, generalized asthenia, pallor, skin rashes, muscular pain, hemorrhages, cardiovascular, respiratory problems, nervous signs as paresis and paralysis, break down of tissues, and finally collapse and death, The severity and duration of the observed clinical signs depend on the nature, amount and site of the injected venoms Genus scorpion is widespread throughout Western and Central Asia. It is a genus in constant revision and recognizes a number of subspecies. An extensive study was conducted of neutralization of lethality of four species of scorpions from Egypt including *Leurus quinquestriatus, Androctonus crassicauda, Androctonus amorexi,* and *Androctonus australis* and three species of scorpions from Yemen including *Leurus quinquestriatus, Bothus* spp. and *Androctonus australis* by VACSERA equine scorpion antisera**.** The results revealed that LD_{50} venoms was 6.9, 8.3, 12.5, & 6.94 LD_{50}/mouse consequently for Egyptian venoms, while LD_{50} of the previous mentioned venoms from Yamen was 6.4±6, 20.7 & 7.0 LD_{50}/mouse consequently. Polyvalent scorpion venom antisera by injection of horses by *L. quinquestriatus, A. crassicauda* proved effective in neutralizing specifically Egyptian *L. quinquistriatus*, and *A. crassicauda,* by 70, & 35 LD50/mouse, and paraspecifically other scorpion species including Egyptian *A. amorexi*, and *A. australis* by 45, and 60 LD_{50}/mouse. Polyvalent scorpion venom antisera neutralized Yameni species as follow 70 LD_{50}/mouse for *Leurus,* 60 LD_{50}/mouse for *A. australis,* and 20 LD50/mouse for *Bothus* spp.

Key words: African scorpion venoms, VACSERA polyvalent scorpion antisera

Introduction

Scorpion venom, which has lethal and paralytic effects, is a secretion composed of water, salts and simple, low molecular-weight proteins (Ozkan *et al*, 2006a). Scorpion envenomation remains a major health problem in many tropical and subtropical countries (Ozkan *et al,* 2006b,c). Preparation of scorpion antivenom includes administration of the *Leurus quinquistriatus*, and *Androctonus crassicauda* venom to the suitable animal - mostly horses- and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal.

During such procedure the recipient animal may suffer different types of ill-health signs including, the generalized asthenia, pallor, skin rashes, muscular pain, hemorrhages, cardiovascular, respiratory problems, and nervous signs as paresis and paralysis, break down of tissues, and finally collapse and death (Ghalim *et al*, 2000). The severity and duration of the observed clinical signs depend on the nature, amount and site of the injected venoms. Genus scorpion is widespread throughout Africa, Western and Central Asia. It is a genus in constant revision and recognizes a number of subspecies. Scorpion hemocyanins induce triple enzymatic function pseudo-catalasic, peroxydasic and superoxide-dismutasic. Buthidae venom hemo-cyanins was resistant to several environmental stressors such as (dehydration-microbial infections- ionizing radiations). Oukkache *et al.* (2013) reported a high hemocyanin contamination in scorpion venom

obtained by the manual stimulation method. Indeed, the corresponding antivenom produced from the venom obtained manually presents a high percentage of specific antibodies that neutralize the hymolymph molecules and few specific antibodies that neutralize scorpion toxins.

The venoms obtained by electrical stimulation may contribute to the production of better antivenom with the higher neutralization potency. As to its advantages in relation to the manual method, electrical stimulation not only enables the collection of nearly 100% of the venom, but also yields more venom than manual stimulation with a higher content of toxins.

Material and Methods:

Venoms*:* Pure *Leurius quinquistriatus* (Egypt and Yemen), *Androctonus crassicauda* (Egypt), *Androctonus amorexi* (Egypt), *Androctonus australis* (Egypt and Yemen), and *Bothus* species (Egypt).

Antivenom*:* polyvalent scorpion venom antisera from VACSERA, Egypt is a divalent antiserum which prepared by injection of horses by *Leurus quinquistriatus*, *Androctonus crassicauda* venoms and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal. Experimental antiserum used in the study consisted of equivolumetric pools of the sera of the horses in each group.

Animals*:* For lethal potency 18-20gm and 16-18gm laboratory bred Albino Swiss male mice (VACSERA),) were used for neutralization. All mice experimentation were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 2002).

Protein concentration measuring (Stoscheck, 1990): Protein was determined by absorbance measurment at 280, & 260nm for each venom type as final concentration of 1mg/ml.

Gel electrophoresis of venom (Laemmli, 1970): Electrophoresis analysis of venoms was performed on 15% polyacrylamide gel in the presence of SDS under reducing conditions. All samples were disolved in a sample buffer (50mM Tris-Hcl, pH 6.8, 0.1 M DDT, 10% glycerol, 2% SDS, and bromophenol blue). A constant electrec current of 70 mA was applied for two hours. After migration, the gel was stained with Coomassie Brillient Blue R 250.

Determination of LD_{50}: Different doses of each venom species were injected intravenously in mice (5mice/dose) according to conventional techniques (WHO, 2010a). The number of deaths 48hrs after injection was recorded and the lethal potency calculated as LD_{50}, the dose of venom in ug/mouse that causes a statistical mortality of 50%. The plot of mortality versus venom dose was analyzed by nonlinear regression.

Neutralization of lethality: (WHO, 2010) Different doses of antivenom were incubated with five median lethal doses (LD50) of each venom species for 30min at 37^{o}C. After incubation, samples were injected intravenously (i.v.) in mice (n ¼ 5/ dose level). The number of deaths 48 h after injection was recorded and the median Effective Doses (ED50) were calculated as the antivenom dose in microliters that protected 50% of the mice. Anti-venom potency was calculated using the formula Potency ¼ [(n-1)/ED_{50}] ? LD_{50}, where n-1 represents the number of lethal doses of the challenge minus one. LD50 subtracted from the total challenge dose (n)= the theoretical dose responsible for the death of half the mice, i.e. the calculation based on the total challenge minus one represents the actual quantity of venom that would be otherwise responsible for 100%mortality and was therefore neutralized by the antivenom. As the ED_{50} = expressed in LD_{50}/mouse, the final result is LD50/mouse), indicating the LD_{50} of venom doses neutralized by 1ml polyvalent scorpion antivenom.

Ethical Approval: All the procedures involving animals were in accordance with the ethical principals in animal research adopted (WHO, 2010).

Statistical analysis: Data were processed

statistically (Snedecor and Cochran, 1982), where minimum, maximum, mean value, standard deviation, standard error, and range were presented. Comparison between groups for significance was done using t. test.

Results

Geographic distribution and measurement of protein concentration (Tabs. 1 & 2)

Table1: Geographic regions of scorpions used.

Scorpion	*Description*	*Representation and region of scorpion (habitat)*
Leurus quinquistriatus,	*Leiurus quinquestriatus* yellow, and 30-77mm (1.2-3.0 inch) long, with an average of 58 mm (2.3 in). (WRBU Scorpion Identification, 2011) .	Leiurus quinquestriatus in desert and scrubland habitats from North Africa to Middle East, covering a wide sweep of territory in Sahara, Arabian Desert, and Central Asia, from Algeria and Mali in west through Egypt, Ethiopia, Asia Minor and Arabian Peninsula, eastwards to Kazakhstan and western India in north-east & southeast. (WRBU Scorpion Identification, 2011)
Androctonus crassicauda	*A. crassicauda* a desert species, an Old World scorpion. Adults can vary in color from a light brown to reddish to blackish-brown, to black, grow to over 10cm (3.9 inch) in length. (Ait *et al*, 2018).	In Palaearctic region.as Saudi Arabia, Iran, Turkey, and in north African nations (Ait *et al*, 2018).
Androctonus Amorexi	Very similar in size, appearance, & physical characters to *A. australis* & *A. crassicauda* (Living Hazards Database, 2000)	Fairly wide-spread in northern Africa & Middle East, also in Iraq, Syria, & Libya. For general description of typical habitat for a closely-related species (Living Hazards Database, 2000)
Androctonus Australis	Large in size, pale -yellow with dark spots (Gantenbein and Largiadèr, 2003).	Wide spread in north Africa, Somali, India, Pakistan, and Middle East countries in very hot deserts (Gantenbein and Largiadèr, 2003).
Bothus species	Medium to large scorpion, up to 7.5cm long. Body all dark-brown, legs lighter, brown to yellowish-brown; post-abdomen relatively thick & wide (Steve, 2011).	Semi-arid, arid, or desert areas with limited vegetation, sometimes in margins of cultivated land & oases; from near sea level up to at least 300 m elevation, southern Europe, on several Mediterranean islands, Northern Africa & Middle East (Steve, 2011).

Table 2: Total protein of all venoms used

venom	TP (mg/ml)
Leurus quinquestriatus Egypt	1.523±0.47
Leurus quinquestriatus Yemen	0.91±0.4
Androctonus crassicauda Egypt	0.394±0.03
Androctonus amorexi Egypt	1.259±0.12
Androctonus australis Egypt	1.414±0.34
Androctonus australis Yamen	1.413±0.35
Bothus species Egypt.	1.3±0.12

Gel electrophoresis of scorpion venom*:* Difference between scorpions venom found differences in presence of gel bands in every species. In *Leurus* venom showed clear bands at 3.5,6,15,18, & 24 Kda and faint bands at 31,42,57,72, 83 & 240 Kda. In *Australis* venom showed clear bands at 3.5, 6, 42, & 125 kda, and faint bands at 15, 24, 31, 57, 72, & 165 kda. *Androctonus amorexi* venom showed bands appear at 3.5, 6, 15, 24, 42, and 125 Kda, and faint bands at 31, 72, & 83 kda. Bothus venom showed clear bands at 3.5, 42, 83, 165, & 240 kda, and faint bands at 6, & 125 kda (Fig. 1).

Median Lethal dose of venoms: The most potent was *L. quinquistriatus* venom was 6.9 ug/mice**,** while *Androctonus crassicauda* was (8.3ug/mice), *Androctonus amorexi* was (12.5ug/mice) *Androctonus australis* was (6.94ug/mice) as in (Tab. 3).

Table3: median lethal dose of venom (LD50 in ug/mouse, 18–20 g mice) of all venoms used

Venom	Source	LD50 (95% c.i.)
Leurus quinquestriatus	VACSERA Serpentarium	6.9 ±0.1
Leurus quinquestriatus	Elbalsam Company Yamen	*6.4±0.6*
Androctonus crassicauda	VACSERA Serpentarium	8.3±0.9
Androctonus amorexi	VACSERA Serpentarium	12.5±0.5
Androctonus australis	Elbalsam Company, Yemen	6.94±0.7
Androctonus australis	Yemen	7.0± 0.5
Bothus occitanus	Yamen	20.7 ±0.4
(-) indicates a confidence interval of <0.01 calculated with just one intermediate survival value at very close doses.		

Range of specific neutralization potency was from 35 LD_{50} *Androctonus crassicauda* venom, to 70 LD_{50} *Leurus quinquestriatus* venom from Egypt and Yamen, whereas paraspecific neutralization ranges 45 LD_{50} *Androctonus amorexi* venom, 60 LD_{50} *Androctonus australis* venom from Egypt and Yemen, and 20 LD_{50} for *Bothus* species venom (Tab. 4).

Table 4: Neutralization of lethality by polyvalent scorpion antivenom

Venom	*LD50 doses neutralized by 1ml polyvalent scorpion antivenom
Leurus quinquestriatus Egypt	70±4.0
Leurus quinquestriatus Yemen	70±*6.6*
Androctonus crassicauda	35±*3.6*
Androctonus amorexi	45±*4.2*
Androctonus australis Egypt	60±*6.1*
Androctonus australis Yemen	60±*6.3*
Bothus species	20±*2.6*
(*units = LD50/mouse)	

Discussion

Scorpion bites are a common problem in human and veterinary medicine. Scorpion venom contains a short sequence of neurotoxin polypeptides consisting of simple, low-molecular weight proteins that have lethal and paralytic effects. Venom toxicity varies according to several factors such as genus, species, age, physiology, feeding state and region of the scorpion. Then, major difficulties are related to standardizing venom quality (WHO, 2010b). To develop an antivenom that neutralizes the toxic effects of venoms as much as possible, we must have a high quality of venom with a high toxic activity (LD_{50}) containing a large amount of toxins. Scorpion antivenom immunoglobulins are the only specific treatment against envenomation from stings. That can prevent or reverse most effects of stings, and play crucial roles in minimizing mortality and morbidity as toxicity widely differs among species. There was an urgent need to ensure the availability of safe, effective and affordable antivenoms, particularly for developing countries, and to improve regulatory control over the manufacture, import, and sale of antivenoms (WHO, 2010a). Concerning gel electopheresis pattern we found that both *Leurus* venom from Egypt and Yemen gave the same bands, also *Androctonus australis* venom from Egypt and Yamen showed same bands. There were difference between other species of scorpions due to differences in components of their proteins and enzymes detected. These results agreed with Ismail *et al.* (1993) and Fet *et al.* (2000) as there are more than 1500 different species of scorpions worldwide of which 50 species proteins or peptides were characterized. Concerning venom toxicity, *Leiurus* venom from Egypt was nearly of the same toxicity as that of Yemen (6.9 for Egypt and 6.4 for Ymen). Also *Androctonus Australis* venom was 6.9 (Egypt) and 7.0 (Yemen). This result represented almost the same toxicity of the same species, and more or less agreed with Saganuwan (2018) who found that LD50 of *A. Australis* venom was 0.25mg/kg. As to toxicity of *A. crassicauda*

venom was 8.3 (Egypt), which more or less agreed with Saganuwan (2018) who found LD_{50} of A**.** crassicauda venom was 35ug/kg. In the present study, toxicity of *A. amorexi* was 12.5, which agreed with Ozkan *et al.* (2007). LD_{50} of scorpion venom varied even if the venom was extracted by using a single method. Ismail *et al.* (1994) found that of *A. crassicauda* venom LD50was 0.64mg/kg, (12.8ug/mouse), but Latoxan Laboratory reported the LD_{50} of 0.87mg/kg (17ug/ mouse) for the same venom by the same method. Toxicity of *Bothus* venom was 20ug/mouse, which was more or less similar to Oukkache *et al.* (2014) who found it 15.2ug/mouse, but disagreed with Ait *et al.* (2018) who found 0.52 mg/kg due to geographical variations. The VACSERA polyvalent scorpion antisera were specifically neutralized by venom *Leurus quinquestriatus* from Egypt and Yemen by 70 LD_{50} for both and also neutralize Egyptian *Androctonus crassicauda* by 35 LD_{50}. *Ad hoc*, it was neutralized par specifically by Egyptian *Androctonus amorexi* by 45 LD_{50} and *A. australis* from both Egypt and Yemen by 60 LD_{50}.

Bothus species venom was neutralized by VACSERA scorpion antivenom by 20 LD_{50}. Theses results agreed with Mohamed *et al.* (1974) who found weak neutralization by the Egyptian serum (Agouza Laboratories) of *Leiurus quinquestriatus* against the two Libyan scorpion venoms *Androctonus aeneas* and *Buthus occitanes* as 1ml neutralized 6& 14 LD_{50}, respectively (Garrigues *et al*, 2005). This reflected the antigenic difference between the specific venoms used in immunization (Michael, 2007).

Conclusion

The neutralization of four species of scorpions from Egypt and three from Yemen by VACSERA equine scorpion antisera prepared by injection of horses by *Leiurus quinquestriatus, Androctonus crassicauda* was highly effective in neutralizing specifically Egyptian *Leurus quinquestriatus*, and *Androctonus crassicauda*, by 70 & 35 LD_{50}/ mouse, and par-specifically other scorpions species including Egyptian *A. amorexi,* and *A. australis* by 45, & 60 LD_{50}/mouse. On the other hand, polyvalent scorpion venom antisera from vacsera neutralize Yemen species were 70 LD_{50}/mouse for *Leurus* 60 LD_{50}/ mouse for *A. australis,* & 20 LD_{50}/ mouse for *Bothus* spp.

Recommendations

More scorpion venoms from other countries could be tested for verification of their neutralization effect to wide range the use of VACSERA scorpion anti-venom for different countries and decrease incidence of toxicity due to scorpion envenomation

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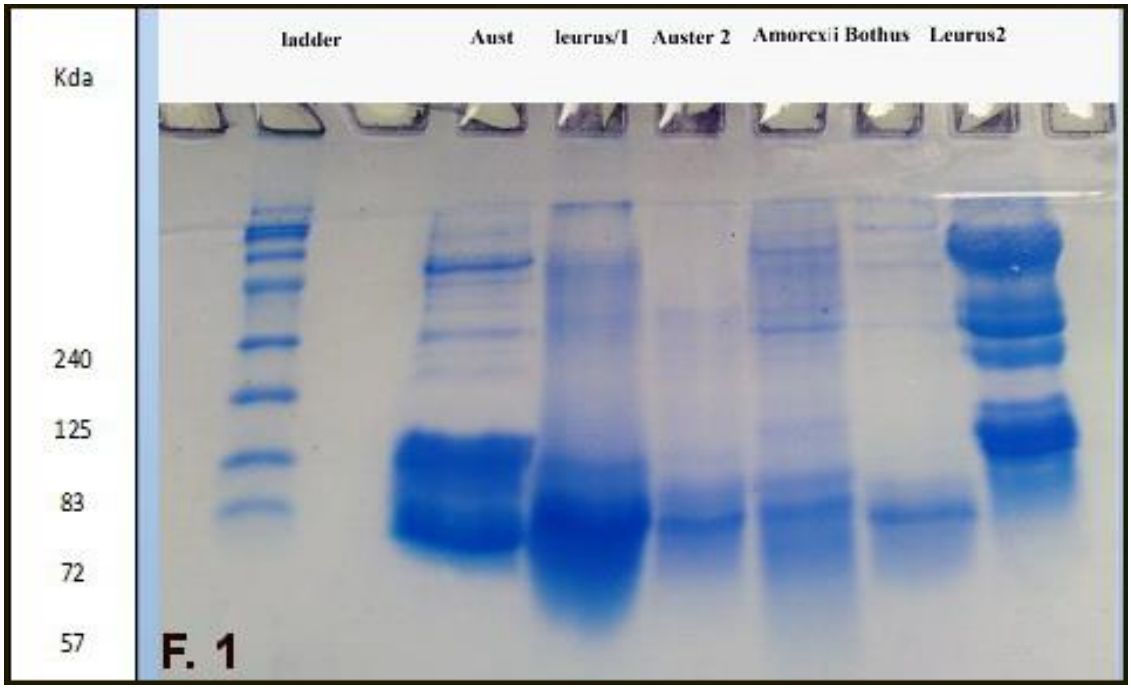
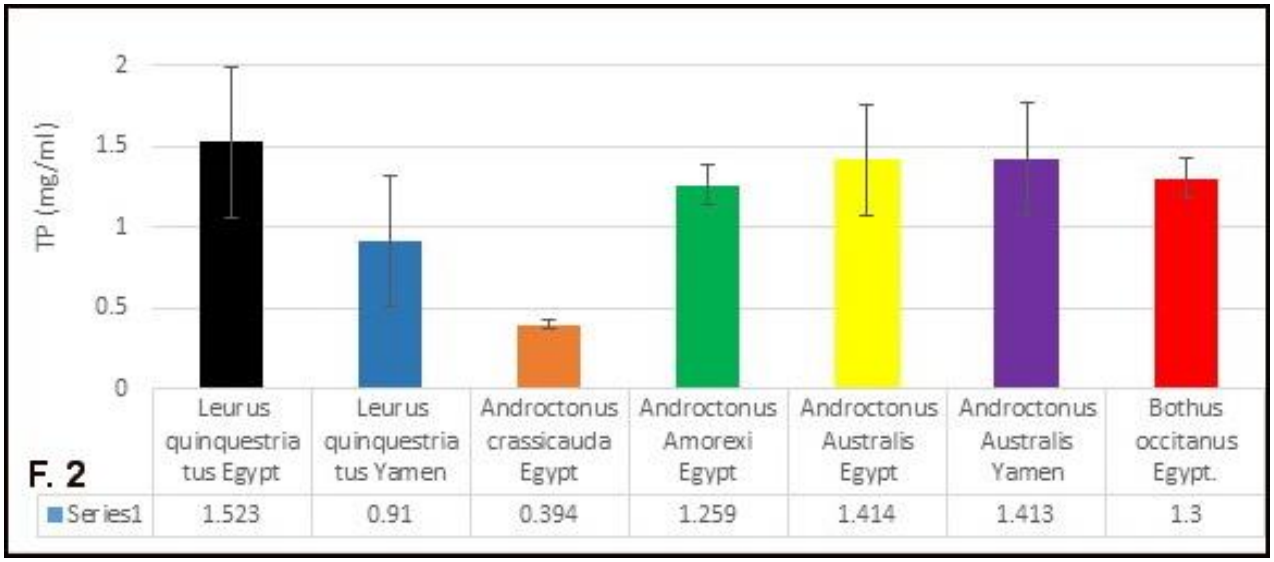
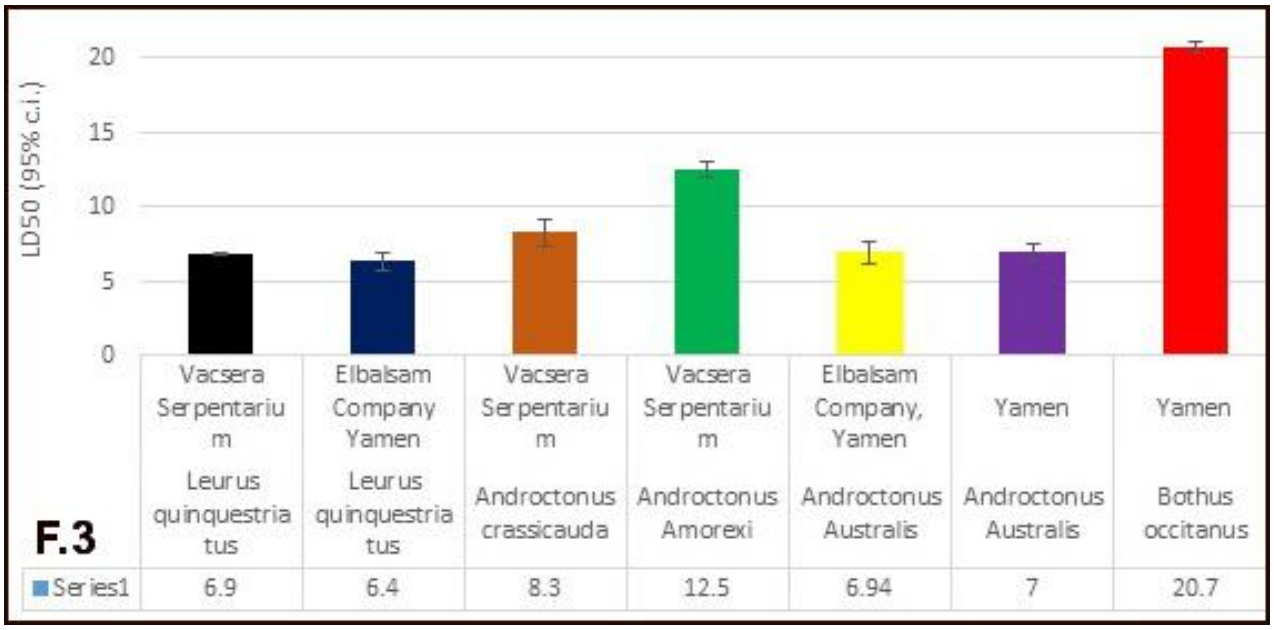
Explanation figures

Fig. 1: Gel electropheresis of scorpion venoms Leu1 Egypt, Leu 2 Yemen---Aust1 Egypt, Austr 2 Yemen, Bothus, Amorexi
Fig. 2: Total proteins of venoms
Fig. 3: Median lethal dose of venom
Fig. 4: Neutralization of lethality by polyvalent scorpion antivenom produced by VACSERA, Egypt.

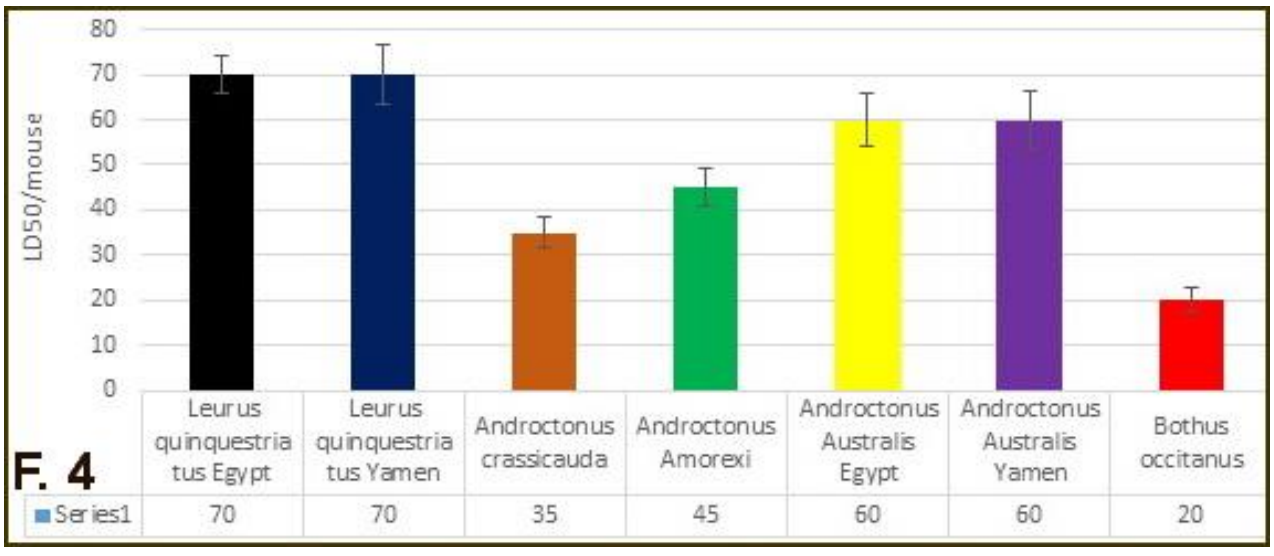



TP (mg/ml)
2
1.5
1
0.5
0
Leurus quinquestriatus Egypt
Leurus quinquestriatus Yamen
Androctonus crassicauda Egypt
Androctonus Amorexi Egypt
Androctonus Australis Egypt
Androctonus Australis Yamen
Bothus occitanus Egypt.
Series1
1.523
0.91
0.394
1.259
1.414
1.413
1.3

F. 2


LD50 (95% c.i.)
20
15
10
5
0
Vacsera Serpentarium
Leurus quinquestriatus
Elbalsam Company Yamen
Leurus quinquestriatus
Vacsera Serpentarium
Androctonus crassicauda
Vacsera Serpentarium
Androctonus Amorexi
Elbalsam Company, Yamen
Androctonus Australis
Yamen
Androctonus Australis
Yamen
Bothus occitanus
Series1
6.9
6.4
8.3
12.5
6.94
7
20.7

F.3


LD50/mouse
80
70
60
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Leurus quinquestriatus Egypt
Leurus quinquestriatus Yamen
Androctonus crassicauda
Androctonus Amorexi
Androctonus Australis Egypt
Androctonus Australis Yamen
Bothus occitanus
Series1
70
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F. 4