Physiochemical And Biological Study Of And Hemiscorpius Persicus Scorpion Venom (Birula, 1903)

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Abstract: In this study 500 specimen of Hemiscorpius persicus scorpions were hunted from South-eastern parts of Iran after morphological diagnosis of samples under stereomicroscope using diagnosis key. The scorpions were milked by electrical stimulation and the venoms were freeze dried. The average of 0.25 mg dry venom was obtained from each scorpion. The amount of soluble protein in the venom after removal of mucus was 96.4 ±2.3 % of crude venom. The toxicity(LD_{50}) of crude venom was 135.33±6.25 µg for each 18-20 gr Albino mice. The SDS-PAGE of the venom revealed 12 proteinic bands with molecular weight ranging from 3.5 to 160 kilo dalton. Phospholipase activity of the venom was determined by using egg yolk as a substrate, found to be 122 unit/mg. the neutralization test was carried out by using Razi polyvalent scorpion antivenom which revealed that 2 mg/ml of antiserum was able to neutralize the Hemiscorpius Persicus venom equivalent to 15 LD50/ml. the immunodiffusion test of antiserum versus H.P venom showed some precipitation lines which confirms the effectiveness of Razi polyvalent scorpion antivenom against the venom of H.P scorpion.

Key words: Hemiscorpius persicus, Hemiscorpius lepturus, venom, antiserum, SDS-PAGE, Phospholipase.

1-Introduction:

From the genus Hemiscorpius, tow species Hemiscorpius lepturus and Hemiscorpius persicus are present in Iran (Farzanpay, 1988). Other researchers reported three species belonging to Hemiscorpionidae family from Iran ie Hemiscorpius lepturus, Hemiscorpius persicus and Hemiscorpius gailardy (Monod and Lourenco, 2005). Hemiscorpius lepturus has a wide distribution in 11 provinces from south-western (Bakhtaran province) to south-eastern Iran(Balochestan province) (Akbari, 2006). Due to wide distribution of this species in southern warmer areas of Iran it accounts for 15-25% of reported cases of scorpion envenomation in Khuzestan, southwest province of Iran (Taghavi et al, 2009). The venom of this species is used in the production of scorpion polyvalent antiserum (Latifi, 1991). The species H.P was reported from the provinces like Fars, Hormozgan, Balochestan and south Kerman in a scorpion fauna study of Iran by (Akbari, 2009). The population of H.P was higher than HL scorpion in so called 4 provinces. The morphologic similarities between these 2 species are so close that their recognition is so difficult by non specialist personal and it is only possible by observing the number of decoration of trichobothria in segments of pedipalps chela under stereomicroscope (Monod and Lourenco, 2005). In Iran the cases of scorpion envenomation with cutaneous and subcutaneous reactions are usually attributed to species H.L. most of the information present are about H.L and little is known about H.P (Akbari, 2007).

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The species H.P was collected from south-western to south-eastern provinces like Fars, Hormozgan and Cistan and Balochestan. Because to high distribution of this species in above provinces, it is believed that some of scorpion envenomation cases in such locations are due to H.P rather than H.L sting. The Hemiscorpius scorpions have hemolytic venoms and sever complications are known from their stings like sever and fatal hemolysis, secondary renal failure, deep and necrotic ulcers, ankylosis of joints, psychological problems and death are reported. A clinical study from Khuzestan province showed that H.L was responsible for 12% of reported stings but it responsible for 95% of mortalities (Radmanesh M, 1998).

2- Material and methods:

The H.P scorpions were collected from their habitats in Fars, Hormozgan, Sistan and Balochestan and south of Kerman provinces in temperate months of the year like Feb, March and April which is the best time for hunting scorpion as they move from deep cracks between stones to surface under stone space. The collected scorpions were kept in plastic containers and transferred to Razi Serum and Vaccine Research Institute, after approval of recognition by skilled personal they were milked by 30-50 volt electrical stimulation. The extracted liquid crud venom was instantly frozen at -70$^{\circ c}$ and freeze dried. The weight of dry venom was determined and dry venom was stored in cold, dark and dry place.

Determination of mucus to soluble protein percentage:

A specific weight of dry venom was weighted and suspended in known amount of distilled water and centrifuged at 8000 rpm for 20 minutes. The supernatant was separated and freeze dried and soluble dry material was used for determination of the ratio of venom soluble material to insoluble mucus.

Determination of protein concentration:

The amount of protein in the venom was determined by micro kjeldahl method (John. D. Bauer, 1990).

Lethality assay:
For determination of LD50, a dilution of 1 mg dry venom suspended in distilled water was made and used for preparation of further dilutions. Each dilution was injected to four 18-20 gram albino mice through tail vein and the mortality of mice were recorded after 48 hours (Spearman-karber, 1975).

Determination of Phospholipase A_2 activity:
This was performed according to Marinetti method by using egg yolk as substrate. The procedure includes suspension of an egg yolk in saline and making the final volume to 100 ml (first dilution). 10 ml of first dilution was mixed with 90 ml of saline to make 10% solution (second dilution). Four test tubes were chosen and 2 ml of second dilution was added to each test tube. First test tube was kept as control without addition of venom then to tube 2, 3 and 4 was added 0.01, 0.02 and 0.05 mg of Hemiscorpius persicus venom respectively. All 4 test tubes were incubated in a 37$^{\circ c}$ water bath for 20 minutes. After this time the test tubes were removed from water bath 3 ml of cold saline was added to each test tube to stop enzymatic reaction. Then the optical density (absorbance) of each test tube was measured against saline at 925 nm wavelength (Biochrom Libra S_{22} Spectrophotometer). The specific phospholipase A_2 activity was expressed as unit of phospholipase per mg of venom which is determined from following formula. (Absorbance of sample - absorbance of control) = 100 units of phospholipase A_2 Unit of phospholipase A_2 is defined as the amount of phospholipase A_2 that cause 0.01 degree decrease in the absorption of egg yolk suspension at 925 nanometer.

Determination of PH:
A solution containing 2 mg dry venom per ml distilled water was made and its PH was determined with PH meter (Metler Tolledo MP 120).

Determination of protein nitrogen in venom:
In this test a solution containing 1 mg dry venom in 1 ml distilled water was made and the amount of protein nitrogen was determined by micro kjeldahl method.

Protein nitrogen × 6.25 = protein

SDS-PAGE of venom:
With the use of 13×18 cm glass plates of Biorad gel electrophoresis first 15% separating gel and then5% condensing gel was applied to the plates then the venoms from 3 species of scorpion HL and HP belonging to Hemiscorpionidae family and Androctonus crassicauda from Buthidae family and molecular weight lather were applied to the gel. The glycine tris buffer at 150 voltage was carried out for 5 hours then it was stained with Comassi blue and destained with methanol and acetic acid.

Neutralization of capacity HL antivenom against HP scorpion venom:
In this experiment 1.5, 2, 2.5 and 3 mg dry venom from HL scorpion were weighted and each weight was dissolved in 1ml saline then to each dilution was added 1ml of polyvalent scorpion antisera, mixed and incubated at 37$^{\circ c}$ for 30 minutes. From each dilution 0.5 ml was injected to 3 albino mice (18-20 grams) and kept under observation for 24 hours.The neutralization of HP venom by antiserum produced against HL scorpion venom was determined and rate of mortality was reported as LD_{50} and neutralization was reported as mg of venom neutralized by 1ml of antisera.

In vitro Gel-immunodiffusion test:
This test was done on a 9×13 cm glass plate covered with agarose (1.5 g%). In this test, the venoms of HP and HL scorpions were studied against monovalent and polyvalent antiserums. The gel plate was incubated at 37 $^{\circ c}$ for 72 hours then the precipitation lines produced were stained with Fushine stain (Duchterlony, 1962).

3-Result and Discussion:
Our Results revealed that 0.25 mg dry venom was obtained from each Hemiscorpius persicus scorpion. The estimated LD50 of Hemiscorpius persicus venom was 135.33 ±6.25 micro gram for 18-20 gram albino mice. According to Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Hemiscorpius persicus venom, 12 proteinic and polypeptide fractions with molecular weight ranging from 3.5-160 kilo Dalton were detected. The phospholipase activity of the venom was estimated by using egg yolk as a substrate and found to be 122 unit/ mg. The neutralization test was performed against polyvalent scorpion antivenom produced by Razi Research Vaccine and Serum Institute which was immunized against Hemiscorpius lepturus belonging to Hemiscorpionidae family and 5 other species belonging to Buthidae family showed potency against Hemiscorpius persicus venom, the result showed that 2mg/ml of antivenom was able to neutralize Hemiscorpius persicus venom equivalent to 15 LD50/ml. this shows great similarity among the venoms of these 2 species. classical immune diffusion test of polyvalent scorpion antiserum and monovalent scorpion antiserum against the venoms of Hemiscorpius persicus and Hemiscorpius lepturus and Androctonus crassicauda showed 3 strong and sharp precipitating lines for persicus and 5 weak precipitating lines for lepturus. This shows that the venoms of Hemiscorpius persicus and Hemiscorpius lepturus are closely similar in antigenicity.

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Fig1: male hemiscorpius persicus scorpion

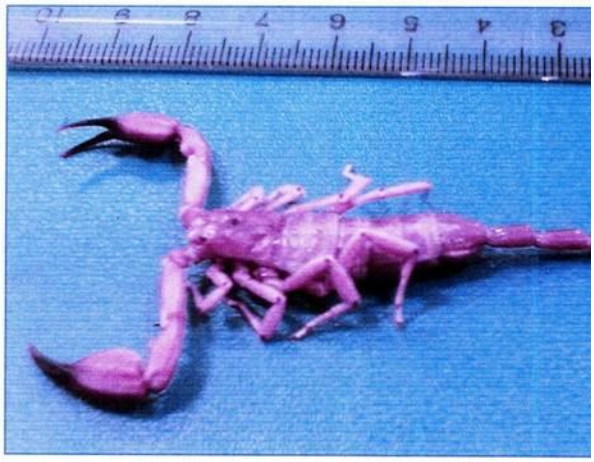

Fig2: female hemiscorpius persicus scorpion

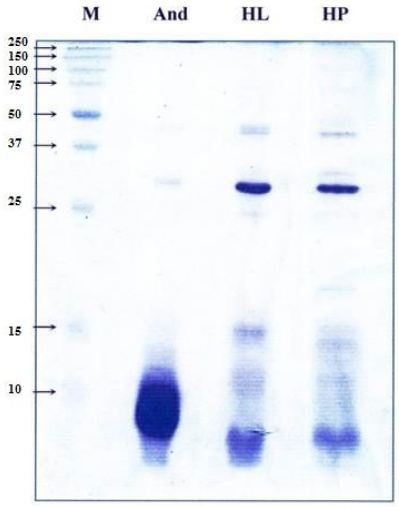

Fig3: *SDS-PAGE of venoms*
HP. hemiscorpius persicus venom
HL. hemiscorpius lepturus venom
AC. Androctonus crassicauda venom
M. marker

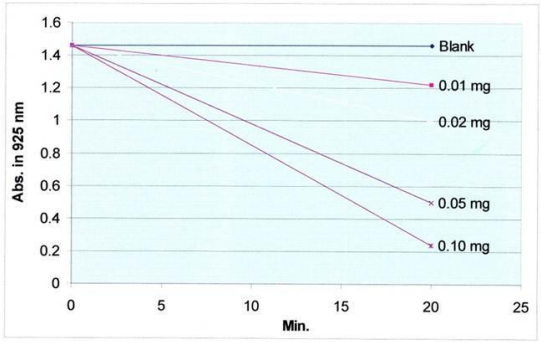

Fig4: *clarification of egg yolk emulsion by phospholipase A2 in different concentrations of H.P. venom*

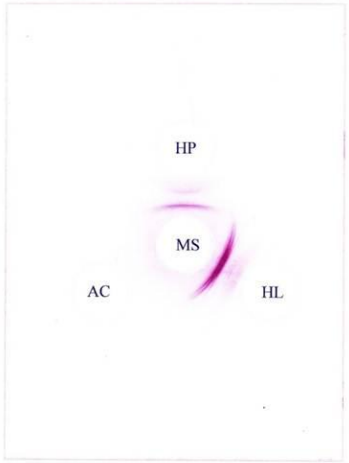

Fig5: *immunodiffusion pattern of venoms with monovalent antisera against H.Lepturus venom*
MS: hemescorpius lepturus Monovalent antisera
HP. Crude venom of hemescorpius persicus
HL. Crude venom of hemescorpius lepturus
AC. Crude venom of Androctonus crassicauda

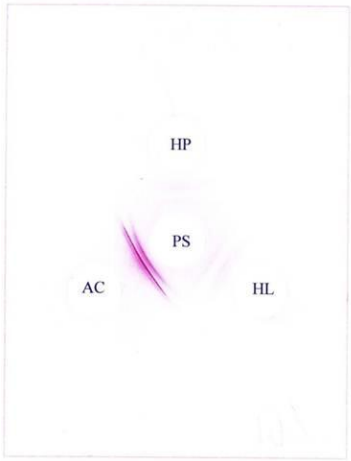

Fig6: *immunodiffusion pattern of venoms with polyvalent antisera*
PS. Polyvalent antisera
HP. Crude venom of hemescorpius persicus
HL. Crude venom of hemescorpius lepturus
AC. Crude venom of Androctonus crassicauda

x	$\bar{x}$	$x-\bar{x}$	$(x-\bar{x})^2$	$(x-\bar{x})^2/(N-1)$	s	c.l.	
15.40 %	16.23333333	-0.833333333	0.694444444	0.723333333	0.850	2.11	
16.20 %	16.23333333	-0.033333333	0.001111111				
17.10 %	16.23333333	0.866666667	0.751111111				
		0	0				
		0	0				Result
16.23333333			1.446666667				16.23 ± 2.11 %

Table1: *the amount of mucosa in hemiscorpius persicus venom*

x	$\bar{x}$	x- $\bar{x}$	(x- $\bar{x}$)^2	(x- $\bar{x}$)^2/(N-1)	s	c.l.	
6.19	6.203333333	-0.013333333	0.000177778	0.002633333	0.051	0.13	
6.16	6.203333333	-0.043333333	0.001877778				
6.26	6.203333333	0.056666667	0.003211111				
		0	0				
		0	0				Result
6.203333333			0.005266667				6.20 ± 0.13

Table2: *the PH of venom suspension (2mg/ml) of hemiscorpius persicus venom*

x	$\bar{x}$	x- $\bar{x}$	(x- $\bar{x}$)^2	(x- $\bar{x}$)^2/(N-1)	s	c.l.	
97.50 %	96.4	1.1	1.21	0.93	0.964	2.39	
96.00 %	96.4	-0.4	0.16				
95.70 %	96.4	-0.7	0.49				
		0	0				
		0	0				Result
96.4			1.86				96.40 ± 2.39 %

Table3: *the protein concentration in mucus free venom of hemiscorpius persicus*

x	$\bar{x}$	x- $\bar{x}$	(x- $\bar{x}$)^2	(x- $\bar{x}$)^2/(N-1)	s	c.l.	
133.00	135.3333333	-2.333333333	5.444444444	6.333333333	2.517	6.25	
138.00	135.3333333	2.666666667	7.111111111				
135.00	135.3333333	-0.333333333	0.111111111				
		0	0				
		0	0				Result
135.3333333			12.66666667				135.33 ± 6.25 µg/mouse

Table4: *the lethal dose (LD50) of hemiscorpius persicus venom*