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ANTIVENOM REVERSAL OF BIOCHEMICAL ALTERATIONS INDUCED BY BLACK SCORPION *Heterometrus fastigiousus* COUZIJN VENOM IN MICE

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ABSTRACT: In the present study, *Heterometrus fastigiousus* venom (HFV) was employed as antigen to produce species-specific scorpion antivenom (SAV) in albino mice (NIH) strain. To determine SAV efficacy, it was pre-incubated with 10 LD_{50} of HFV and then injected subcutaneously into mice. Subsequently, mortality was observed after 24 hours. Minimum effective dose (MED) was 12.5 LD_{50} of HFV/mL of SAV. SAV effectiveness to reverse HFV-induced biochemical alterations in mice was analyzed by challenge method. Simultaneously, mice received subcutaneously 40% of 24-hour-LD_{50} of HFV and intravenously SAV. After four hours, changes in serum glucose, free amino acids, uric acids, pyruvic acid, cholesterol, total protein, alkaline phosphatase, acid phosphatase, lactic dehydrogenase and glutamate-pyruvate transaminase enzyme level were determined. Treatment with species-specific SAV resulted in the reversal of HFV-induced biochemical alterations.

KEY WORDS: venom, antivenom, envenomation, *Heterometrus fastigiousus*, serotherapy.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Scorpion stings cause several clinical manifestations in the victim and are common in tropical and subtropical countries (1). There are several drugs that are employed in the treatment of scorpion envenomations; however, the relief of venom induced hypertension, these substances may cause side effects. Therefore, scientists have turned their attention towards serotherapy as a safer treatment for scorpion stings. Nevertheless, there are contradictory opinions about the effectiveness of the scorpion antivenoms either in experimental animals or in scorpion sting victims. Some researchers consider antivenom the only specific treatment against scorpion stings, while others have questioned its usefulness in eliminating scorpion sting complications (2-5). However, for quick neutralization of scorpion venom toxic effects, serotherapy is a well-tested pharmaceutical method safely used in patients around the globe (6-8).

In India, the efficacy of species-specific antivenoms in neutralizing lethal factors of red scorpion venom has been tested by different scientific groups (9, 10). Despite the several attempts to investigate the consequences of scorpionism and to produce species-specific antivenom against scorpion venom, Asian black scorpions have attracted little attention. Still, several clinical manifestations of black scorpion stings have been reported (11-14). Chaubey (11) reported reduction in erythrocyte and increase in total leucocyte counts, blood hemoglobin, mean corpuscular hemoglobin, packed cell volume and plasma hemoglobin in mice envenomated by *Heterometrus fastigiousus* venom (HFV). Chaubey and Upadhyay (12) observed that HFV increases levels of glucose, free amino acids, uric acid, pyruvic acid and total protein while decreases cholesterol level in sera of experimentally envenomated albino mice. The same treatment also augmented enzyme activity levels of alkaline phosphatase, acid phosphatase, lactic dehydrogenase and glutamate-pyruvate transaminase in albino mice sera. The present study was carried out to determine the efficacy of species-specific SAV produced against HFV -induced abnormalities in albino mice.

MATERIALS AND METHODS

Venom

Living black scorpions *H. fastigiousus* were purchased from the animal supplying agency Zoological Animal UP, India. Venom was obtained by electric stimulation of

scorpion telson. Both toxicity and LD_{50} of HFV has already been determined in albino mice by Chaubey and Upadhyay (12).

Production of SAV

Species-specific SAV against HFV was produced in albino mice (NIH strain, 25±5 g) using Freund's complete and incomplete adjuvants (respectively, FCA and FICA). Immunogen for primary immunization was prepared by emulsifying equal volumes of venom and FCA, while boosting immunogen was prepared by emulsifying equal volumes of venom and FICA. Primary immunization was performed by injecting intramuscularly 100 µL of immunogen containing 100 µg HFV. The interval between the first two doses was two weeks and then boosting was done regularly in one-week intervals with gradually increasing HFV doses. During this period animals were bled randomly to check detectable amount of antibodies by Ouchterlony method (15). After four months, mice were killed and collected plasma was processed using ammonium sulphate precipitation and pepsin treatment according to Kankonkar *et al*. (9)

Neutralizing Ability of Species-specific SAV

The neutralizing ability of the species-specific SAV was tested in albino mice (25 ± 5 g) that were injected subcutaneously with 10 LD_{50} of HFV (in 100 µL solution) pre-incubated with 100 µL, 200 µL, 400 µL, 800 µL and 1000 µL of SAV for two hours at 37°C. Each pre-incubated mixture was subsequently adjusted with physiological buffer saline (PBS) to a final volume of 2 mL. The injection volume was kept constant to 400 µL/mouse. Control group received PBS only. Animal mortality was registered 24 hours after treatment.

Efficacy Determination of Species-specific SAV in the Reversal of HFV-induced Biochemical Abnormalities

Experimental albino mice were divided into the following three groups:

- Group A: received physiological buffer only (Control group);
- Group B: received 40% of 24-hour-LD_{50} of HFV (150 µg);
- Group C: received 40% of 24-hour-LD_{50} of HFV (150 µg) + simultaneous intravenously administration of SAV.

After four hours of treatment, serum levels of glucose, uric acid, pyruvic acid, free amino acids, total protein and cholesterol levels were calculated according to the method of Mendel *et al.* (16), Folin (17), Friedeman and Haugen (18), Spies (19), Lowry *et al.* (20) and Abell *et al.* (21). Additionally, serum levels of alkaline phosphatase (ALP), acid phosphatase (ACP), lactic dehydrogenase (LDH) and glutamate-pyruvate transaminase (GPT) enzyme activities were determined by Bergmayer (22), Annon (23) and Reitman and Frankel (24) methods respectively.

Statistical Analysis

Results were expressed as mean ± SE of six replicates and were analyzed using Student's t-test to detect significant changes with controls and between treated groups (25).

RESULTS

To determine antivenom efficacy, mice were injected subcutaneously with 10 LD_{50} of HFV pre-incubated with species-specific SAV (100 µL, 200 µL, 400 µL, 800 µL and 1000 µL). After 24 hours, animal survival was recorded and found to rise with increased amount of SAV (Table 1). Species-specific SAV neutralizes 12.5 LD_{50} of HFV/mL.

In another experiment, the efficacy of species-specific SAV to revert augmented levels of serum glucose, free amino acids, pyruvic acid, uric acid as well as to diminish cholesterol and to improve levels of alkaline phosphatase, acid phosphatase, lactic dehydrogenase and glutamate-pyruvate transaminase enzyme activity was determined by challenge method.

SAV significantly reversed biochemical changes induced by HFV (Tables 2 and 3). Glucose, free amino acid, uric acid, pyruvic acid and total protein levels in group B mice were 140.48%, 138.39%, 127.27%, 144% and 122.09% of the control group; and decreased to 106.04%, 104.89%, 107.27%, 108% and 104.53% in animals of group C after treatment with SAV (Table 2). Cholesterol level in group B was 71.48% of the control group; and increased to 96.41% in group C mice (Table 2). Activities of ALP, ACP, LDH and GPT in mice of group B were 135.64%, 120.78%, 131.37% and 156.16% of control group; and after treatment with SAV the values diminished to 103.96%, 101.30%, 104.29% and 105.60% in group C (Table 3).

Table 1. Determination of neutralizing capacity of scorpion antivenom (SAV) against *H. fastigiousus* venom (HFV) in albino mice (NIH strain)

Amount of HFV (µg)	Amount of SAV (µL)	Number of living mice / Number of total mice
1,500	0	0/10
1,500	100	1/10
1,500	200	3/10
1,500	400	7/10
1,500	800*	10/10
1,500	1,000	10/10

*Minimum effective dose (MED).

Table 2. SAV effect on the reversal of HFV-induced alterations in serum levels of glucose, free amino acids, uric acids, pyruvic acid, cholesterol and total protein in albino mice (NIH strain)

Parameters	Group A	Group B	Group C
Glucose[1]	64.15 ± 0.89 (100)	90.12* ± 0.93 (140.48)	70.03 ± 1.35 (106.04)
Free amino acids[1]	6.33 ± 0.09 (100)	8.76* ± 0.08 (138.39)	6.64 ± 0.14 (104.89)
Uric acid[1]	2.20 ± 0.04 (100)	2.80* ± 0.07 (127.27)	2.36 ± 0.08 (107.27)
Pyruvic acid[1]	0.25 ± 0.002 (100)	0.36* ± 0.002 (144.00)	0.29 ± 0.006 (108)
Cholesterol[1]	167.00 ± 2.20 (100)	119.38* ± 1.53 (71.48)	178.00 ± 2.89 (96.41)
Total protein[2]	3.53 ± 0.04 (100)	4.31* ± 0.07 (122.09)	3.69 ± 0.09 (104.53)

[1]Values represent mg/100 mL serum; [2]values represent g/100 mL serum. Values in parentheses indicate the percent of change with respect to the control group, considered as 100%.
*Significance: $p < 0.05$ (Student's t-test).

Table 3. SAV effect on the reversal of HFV-induced alterations in serum levels of alkaline phosphatase, acid phosphatase, lactic dehydrogenase and glutamate-pyruvate transaminase enzyme activity in albino mice (NIH strain)

Parameters	Group A	Group B	Group C
ALP	1.01 ± 0.03 (100)	1.37* ± 0.04 (135.64)	1.05 ± 0.07 (103.96)
ACP	0.688 ± 0.008 (100)	0.831* ± 0.008 (120.78)	0.697 ± 0.010 (101.30)
LDH	103.87 ± 1.16 (100)	136.46* ± 1.66 (131.37)	108.33 ± 1.16 (104.29)
GPT	15.17 ± 0.52 (100)	23.69* ± 0.51 (156.16)	16.02 ± 0.52 (105.60)

ALP and ACP: µmoles of p-nitrophenol formed per 30 minutes per mg protein. LDH: µmoles of pyruvate reduced per 45 minutes per mg protein. GPT: units of glutamate-pyruvate transaminase activity per hour per mg protein. Values in parentheses indicate the percent of change in enzymatic activity compared to the control group, considered as 100%.
*Significance: $p < 0.05$ (Student's t-test).

DISCUSSION

Lethality due to scorpion stings is a common health problem in developing countries. Of the 1,500 scorpion species distributed throughout the world, 50 have proven lethal to humans and most of them belong to Buthidae family (26). However, some species within the family Scorpionidae have also been reported as poisonous including *H. fastigiousus* (11-14). Still, no species-specific antivenom has been produced to treat black scorpion envenomations up to now. Despite contradictory opinions regarding scorpion antivenom effectiveness against scorpionism, research on scorpion antivenom production is advancing.

In the present study, species-specific SAV against HFV was produced and its efficacy in the reversal of biochemical changes induced by HFV was determined by challenge method in mice. Species-specific SAV could significantly revert biochemical alterations induced by black scorpion venom. Increase in levels of serum glucose, free amino acid, uric acid, pyruvic acid and total protein as well as decrease in cholesterol after HFV envenomation were notably recovered after SAV treatment. Augmented serum levels of ALP, ACP, LDH and GPT enzyme activity following venom inoculation returned to normal values after treatment with scorpion antivenom. Similar results were reported by several other scientific groups. Freire-

Maia and Campos (27) suggested the intravenous administration of antivenom as a better manner to neutralize the circulating venom and the venom that is absorbed into the sting site. Kankonkar *et al*. (9) prepared a potent antiserum against *Buthus tamulus* scorpion venom capable of neutralizing toxic lethal substances. Reversal of ECG, cardiovascular, hemodynamic, metabolic and hormonal changes in experimental dogs caused by scorpion *Mesobuthus tamulus* venom were registered after utilization of species-specific antivenom (10, 28, 29). These previous results on the usefulness of serotherapy against scorpion envenomation support the current study.

According to Ismail (3), scorpion venom presents an overall elimination half-life of 33.7 hours from the body and 26 hours from the peripheral compartments. This long half-life of the venom in the body increases the risk of toxicity and provides an opportunity of treatment with antivenom even few hours after scorpion envenomation. Since scorpions generally inoculate their venom into interstitial space and not directly into circulation, venom must be absorbed and reach circulation to cause toxicity. To reach maximum blood venom concentration approximately 101 minutes are required whereas the venom complete absorption from the sting site takes 7 to 8 hours (3). Based on these data, it may also be concluded that successfulness of serotherapy depends upon specificity, dose, time and route of antivenom administration.

Despite usefulness of serotherapy against scorpionism, a universal anti-scorpion venom has not yet been created to neutralize venoms of different scorpion species. However, in this regard, Devaux *et al.* (30) produced antibodies against a synthetic polypeptide that represents a conserved region in a set of 25 scorpion toxin sequences. These antibodies have proven to cross-react with several scorpion toxins of different serotypes and to neutralize pharmacological effects and biological activities. However, differences in venom composition and variation in amino acid sequence in active site regions among numerous scorpion species, widely distributed over the globe, limits the possibility of a universal antivenom production. Therefore, it is quite necessary to develop species-specific scorpion antivenoms.

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