Original Article

Oral Absorption of *Mesobuthus eupeus* Scorpion Venom in Mice

Zohreh Hosseini [1], Mohammad Khosravi[2], Masoud Ghorbanpoor [2], Mansour Mayahi [3]*

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ABSTRACT

Background: To explore the oral absorption of scorpion venom an ELISA were designed in this study. Scorpions and their venom were been used for centuries as medical treatments in traditional medicine. The oral administration of drug referred as the convenient way, as there was not any publication about gastro-intestinal absorption of scorpion venom; this experiment checked oral absorption of *Mesobuthus eupeus* scorpion venom in mice.

Methods: Six groups of mice orally received 0, 0.2, 0.5, 1, 2 and 5 mg/kg *of M. eupeus* venom and their blood samples were tacked after 15, 30, 60 min and 2, 4, 6, 24, 48 h after that. The presence of venom the blood samples were detected with a house- antigen capture ELISA.

Results: The venom was absorbed after its feeding to mice. The animals expressed no signs of envenomation and, the venom was detectable by AC-ELISA as soon as 15 min after its feed. M*aximum* serum *levels* were *2 h after* its meal.

Conclusion: The orally administrated venom was absorbed to the blood circulation without any clinically symptoms.

Keywords: *Mesobuthu Eupeus*, Oral Absorption, Scorpion, Venom.

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INTRODUCTION

Scorpions have been living on earth since silurian period; they are the oldest group of terrestrial arthropods [1, 2]. *Mesobuthus eupeus* is a species of Buthidae family; it is populous in southern and central Asia and the most stings occur in these areas pertained to these species [3, 4]. They use potent neurotoxic compounds made in their venom glands to subdue their praise and for defense [5, 6].

Venom comprises a mixture of a variety of biologically active components: enzymes, peptides, nucleotides, lipids, mucoproteins and biogenic amines that most of which have not been investigated yet [7-11]. Scorpion sting can cause a broad range of condition from sever local skin pain to multisystem failure followed by death [12]; despite that, scorpions and their venom has been used for centuries as medical treatments in traditional medicine in India, China, Africa and Cuba [13]. Mammalian and chicken expressed no clinical signs of envenomation following scorpion venom feeding [14]. Therapeutic effects of orally administration of scorpion venom have been found without serious noxious consequences [15, 16]. Besides, anti- nociceptive and anti-inflammatory effects of oral intoxication of cardiotoxin of snake venom are reported before [17]. Goirgi et al. [18] refute the general view that digestive enzyme or chemical degradation will make venom biologically inactive in gastrointestinal tract.

As there was no scientific publication on oral absorption of scorpion venom; this experiments checked its oral absorption in mice.

MATERIALS AND METHODS

Preparation of Venom Solution

Electroshock lyophilized *M. eupeus* scorpion's venom was provided from the Razi Institute (Ahwaz, Iran). The freeze-dried venom was dissolved and was diluted with distilled water to obtain a final protein concentration of 0.2, 0.5, 1, 2 and 5 mg/kg of the mice's body weight. The total protein concentration was measured using the usual Bradford spectrophotometric method with Bovine Serum Albumin (BSA) as standard.

Toxicity Determination

All experiments were performed according to the guidelines of the Ethical Committee (National Ethics Advisory Committee) [19].

1. Student of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.
2. Department of Pathobiology, Shahid Chamran University of Ahvaz, Iran.
3. Department of Clinical Sciences, Shahid Chamran University of Ahvaz, Iran.
*Corresponding Author: E-mail: m.khosravi@scu.ac.ir

Toxicity determinations were performed by subcutaneously (SC) injection of increasing concentrations of the venom to albino mice. Following treatment, animals were monitored for 24 h, and the number of dead animals was recorded at the end of the experiment, then, LD100 was calculated.

Animals

Eighteen N-mary mail mice were divided randomly in to 6 groups (A, B, C, D, E and F) consisting of three mice each. Groups A to E respectively received orally, 500 µL of a solution containing 0.2 mg, 0.5 mg, 1mg, 2 mg and 5 mg of scorpion venom in ultra-pure water. The controls (group A), received 500 µL of ultra-pure water. Blood samples were taken from tail vein at 15, 30, 60 min and 2, 4, 6, 24, 48 h following scorpion venom meal and their sera were kept frozen at -20 ºC until use.

Development of an Antigen Capture ELISA

Production of Polyclonal Antibody

Three Ross 308 broiler chickens and three New Zealand white male rabbits were acclimatized to room temperature at 25 °C for two weeks former to immunization. Preimmune sera were attained throughout this period. In initial immunization, animals were each injected subcutaneously with 250 µg of venom in 0.5 of PBS emulsified with 0.5 ml of complete Freund's adjuvant by a multiple injection method (10 sites/rabbit) [8]. These first injections were pursued by three sets of booster injection. Booster injections were made at 2 nd, 4 th and 6 th weeks with 130 µg of immunogen in 0.5 mL of PBS and 0.5 ml of incomplete Freund's adjuvant at two sites in both thighs intramuscularly. The existence of antibodies in serum was determined by immunodiffusion and Dot ELISA tests. Finally, 10 days after last immunization, blood was directly collected into sterilized glass tubes without any anti-coagulants and allowed to clot. Serum was pipette out and centrifuged at 1500 rpm for 10 min and then isolated in a sterilized vial and stored for bioassay tests.

Purification of Polyclonal Antibody against Venom

Polyclonal antibody against venom was first purified by ammonium sulfate precipitation (50% saturation for the final solution) and dialyzed in PBS and then subjected to an affinity column conjugated with venom. The column was prepared by conjugating 20 mg of venom with 7 ml of activated CH-Sepharose 4B (Sigma-Aldrich, Product Number: 4B200). Cyanogen bromide activation was performed by the method of March et al [20]. Antibody was eluted from the column with 0.1M glycine (pH=2.5) and fractions were collected and neutralized immediately by adding an appropriate amount of 1 M tris (pH= 9) to each fraction.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Dot Enzyme-Linked Immunosorbent Assay (Dot-Elisa) Analysis

Purity of polyclonal antibody against venom was analyzed by SDS-PAGE in an 11% polyacrylamide gel according to the Laemmli method [21]. Proteins were stained with 1% Coomassie blue R.250 and their affinity against venom, were confirmed by DOT-ELISA before being used in the next steps.

Detection of Mesobuthus Eupeus Scorpion Venom by Antigen Capture-ELISA (AC-ELISA)

An in-house AC-ELISA technique was used for scorpion venom detection in mice sera. For this purpose, after optimization of reagents with checker board titration, the developed antigen-capture ELISA technique briefly consist of the following steps: the microtiter plates sensitized overnight with 100 µL of a solution containing 33 µg/ml of chickens' specific antibody in coating buffer (carbonate bicarbonate, pH= 9.6) at 4 ºC. The subsequent washing cycles were done three times with PBST (phosphate buffered saline containing 0.05% tween 20] and one time with PBS (pH=7.2). Blocking of the unoccupied sites of microtitration wells was attained by overnight incubation at 4 ºC with 200 µL per well of PBST containing 4% skimmed milk. After washing as above, 50 µl of each samples were added to the plates and incubated 45 minutes at room temperature. Thereafter, washing was done and 100 µl of rabbit's anti-*M. eupeus* scorpions venom IgG (20 µg/mL) in PBS containing 1% skimmed milk was added to each wells and incubated 45 min at room temperature, the ELISA plate was washed again as previously described, and goat anti-rabbit IgG-HRP conjugate (Immuno

Chemistry Technologies company, USA, HRP AffiPure Goat anti-Rabbit IgG Fc, CatalogNumber: 6293), freshly diluted 1:20000 and 100 µL was added to the wells. After 45 min incubation at room temperature the plate was washed again and 75 µL of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was added to the wells and incubated for 10 min. The reactions were then stopped by addition of 0.1M H_2SO_4 and finally, optical density (OD) values were measured at 450 nm with an ELISA reader.

Data Analysis

In order to quantities the amount of venom present in serum samples, a titration curve was constructed by plotting the venom concentration against absorbance value for each groups. The known amounts of venom spiked with mice serum from normal controls were included with each test to obtain a titration curve. Descriptive and inferential statistical methods were applied to data analysis; the online software of ELISA analysis (http://www.elisaanalysis.com) used for calculation of venom concentration in each samples.

RESULTS

The *M. eupeus* Lethal Dose for mice was 11.5 mg/kg in S.C injection. When venom was administered orally, the animals expressed no signs of envenomation and no death was accrued at the test periods. The venom was detected within 15 min until 6 hours after treatments. The maximum level in serum occurred at 2 hours after administration (Table 1).

Table 1. The mice serum *M. eupeus* venom concentration (µg/50 µl) (mean + standard error) in different times after feeding of five doses of venom.

Time intervals	0.2mg	0.5mg	1mg	2mg	5mg
15min	0.065±0.005	0.079±0.02	0.068±0.03	0.056±0.02	0.082±0.01
30min	0.091±0.03	0.133±0.03	0.141±0.04	0.134±0.057	0.095±0.024
1h	0.118±0.006	0.189±0.02	0.168±0.005	0.203±0.01	0.453±0.05
2h	0.145±0.03	0.203±0.05	0.247±0.02	0.531±0.04	0.830±0.04
4h	0.119±0.01	0.037±0.01	0.08±0.005	0.103±0.03	0.144±0.03
6h	0.043±0.008	0.052±0.04	0.037±0.07	0.022±0.01	0.048±0.02
24h	–	–	–	–	–
48h	–	–	–	–	–

DISCUSSION

Venom may be a candidate for treatment of the many diseases and disorders in future [22, 23]. Scorpion's venom has various medicinal properties [24] that need the safe and accurate *administration* route. The oral administration of scorpion venoms had no toxic effects [14]. The main remained question was orally administrated venom enters to the systemic circulation or not? The oral administration of drug referred as the convenient way, but it is assumed that enzymatic or chemical degradation of peptides in stomach or intestines will be making them less effective. However, contrary to the general idea, oral administration of the component of *Crotalus durissus terrificus* venom could induce analgesia [18]. Also oral utilization of hannalgesin caused the analgesic effects. It is unlikely that hannalgesin can be absorbed as a protein with a long chain of 72 amino acids [25]. Since it is susceptible to the actions of proteases, peptide fragments of hannalgesin could be absorbed through the gastrointestinal tract.

In agreement to present study, Weir [26] has shown that *Crotalus* venom could be ingested by the pigeon, one of the most susceptible animals to this venom and no poisonous principle is recoverable from the excreta of the pigeon. Similarly, oral administration of denatured *Naja Naja Atra* venom (NNAV) temperate the inflammation by regulating the immune system [27]. Therefore, the denatured NNAV may be a novel therapeutic drug for rheumatoid arthritis.

Unlike to the oral administration of the scorpion venoms, Malleswari et al. [28] showed that oral administration of *Naja naja* venom was toxic as well as the S.C injection. Oral administration of *M. lemniscatus* venom formed effective anti-nociceptive property [29]. Orally ingestion of snake venom could alter the protein

profiles in the liver [30]. Also, it has been previously proved that neurotoxin of cobra venom can be quickly absorbed through rectal mucosa [31].

CONCLUSION

The oral administration of the crude venom of *M. eupeus* has no toxic effects. This may be due to the detoxification of venom by enzyme or pH of gastrointestinal tract. The *M. eupeus* scorpion venom was inactivated and absorbed through digestive tract; determination of the deactivation mechanism could facilitate the topical treatment of scorpion envenomation. In addition, the use of oral route of scorpion venom administration can facilitate the venom application in medical treatments. This study for the first time showed the oral absorption of *M. eupeus* scorpion venom. Absorption was initiated within the first 15 min; *maximum* serum levels are reached 2 h after treatment. The further investigations may be proved the medically advantage of oral administration of peptides of *M. eupeus* venom.

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