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Original Article

Pharmacoligical characterization of the iranian *Cerastes cerastes gasperettii* (Reptilia: Ophidia: Viperidae) venom

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Abstract

Objective: Snake envenomation is common in tropical and subtropical countries of the Middle East areas including Iran. *Cerastes cerastes gasperettii* is a dangerous snake living in southwestern provinces of Iran. It causes massive edema at the bite site and coagulopathy leading to death if untreated.

Methods: The purpose of this preliminary animal study was to evaluate the toxicity and proteomic of this venom for the first time in Iran. Moreover, the hemodynamic changes with intravenous injection of the venom were assessed and inotropic in addition to arrhythmogenic properties of this venom were investigated.

Results: The estimated amount of the LD50 with intraperitoneal injection was slightly less than the similar experiment in Saudi Arabia (1.32 mg/kg versus 978 µg/kg body weight). There were 8 distinct protein bands between 12 and 66 kDa in SDS-PAGE analysis that were different with Moroccan experiment due to inter and intra species variation. Inotropic potencies were not significant since the lethal dose with intravenous injection was much lower than the Arabian experiment in guinea pigs (2.4 mg/kg versus 0.8 mg/kg).

Conclusion: According to the low hemodynamic changes induced with the venom, it seems that coagulopathy and edema are the most dangerous effects of this rare snake in Iran.

Keywords: Snake, Hemodynamic, Proteomic, *Cerastes gasperettii*

Introduction

It is estimated that among 3000 known species of snakes all over the world, 27 are venomous and 11 are semi-venomous spreading in Iran (1,2). *Cerastes cerastes gasperettii* (C.cg), known as horned viper, belongs to the Viperidae family and is one of the most dangerous snakes of the sandy deserts like southwestern areas especially Khuzestan province (3). This snake is the most common species in the Saudi Arabia and lives in Jordan, Egypt and Iraq as well (4,5). There are some reports of life-threatening envenomation of this nocturnal snake including microangiopathic hemolytic edema and renal failure, while there is little knowledge about the toxicological aspects of its venom in Iran (6,7).

Clinically, hemorrhagic wounds at the bite site are the common manifestations accompanied with coagulopathy, which probably stem from the snake venom metalloproteinases like *Cerastes cerastes* matrix metalloproteinase II (CCMP-II) (8,9).

It is noted that cardiac complications are not considered primarily in all snake envenomation, but they usually provoke detrimental effects in human being (10).

Few studies have addressed the cardiovascular problems induced with C.c.gasperetti in experimental animals to clarify the hemodynamic manifestations of envenomation with this snake (11,12). Although the conducted studies seem interesting, they failed to take into account the clinical implications probably due to the limited envenomation with this snake in Iran. This study aimed at evaluating the physiological changes in the inotropic, chronotropic and arrhythmogenic parameters in anesthetized rats with Iranian C.cg venom and to find its lethal potency (LD50) with intraperitoneal injections in mice. Additionally, the protein profile of the venom was extracted with SDS-PAGE analysis.

Methods

In this interventional animal study, the crude venom was prepared by milking of the C.cg snakes captured in Khuzestan province in the southwestern part of Iran (Figure 1).

The crude venom was lyophilized and stored at -20˚C until use. During each experiment, the venom was dissolved in normal saline solution and maintained at 4˚C. Mice and rats were acclimatized for 10 days in well ventilated free cages at room temperature (20-30˚C) in the animal house of the Bushehr University of Medical Science before the initiation of the study. All experiments were performed according to the Helsinki protocol.

To determine the lethality of the C.cg venom, it was intraperitoneally injected with different concentrations dissolved in normal saline to six groups (n=5 in each) of 20-25 g male Swiss albino mice. The median lethal dose (LD50) was estimated 24 hours later by the probit analysis (13,14).

The protein content was determined based on the Bradford method, using bovine serum albumin as standard (15).

The protein ingredients of the C.cg venom (5 and 10 µg) in the non-reducing condition were resolved and electrophoresed at 90 V using 12.5% acrylamide gel as previously described (16). The protein bands were revealed after electrophoresis by staining with Coomassie Blue G-250 solution (Bio-Rad Laboratories) and were destained with distilled water. The protein ladder (CSL-BBL, Cleaver Company) was electrophoresed in the gel to determine the molecular weights of the ingredients (11-245 kDa).

Male Wistar rats weighting 250 g-300 g were anesthetized with ketamine (100 mg/kg; i.p) and xylazine (10 mg/kg; i.p). They were also placed in supine position on the operating table. A left femoral incision was made and cannulas were inserted into the femoral vein and femoral artery for administration of venom and measurement of blood pressure, respectively. Hemodynamic changes (inotropic, chronotropic and arrhythmogenicity) were obtained with a PowerLab acquisition system (AD instrument, Australia). All animals were killed at the end of the study with cervical dislocation. Animals were administered (five in each group) intravenously with *Cearstes cerastes gasperettii* venoms (300, 1200 and 2400 µg/kg) dissolved in normal saline in two minutes. Control rats were injected with the same volume of the normal saline. Cardiovascular effects (e.g., bradycardia, hypo and /- or hypertension and arrhythmogenicity) were determined during the study.

Statistical analysis was performed by using IBM SPSS software version 19. Responses were expressed as mean ± SD. Multiple comparisons were made using a one-way ANOVA followed by Tukey's test. A *P* value <0.01 was considered as statistically significant.

Results

The results showed that the estimated amount of the LD50 of the venom with intraperitoneal injection was 1.32 mg/kg body weight in mice.

Proteomic analysis of the C.cg venom revealed the presence of the components of at least 8 distinct bands between 12 and 66 kDa. There were 7 bands located at 12, 16, 19, 24, 32, 37 and 66 kDa and one fair band at 56 kDa (Figure 2).

Contrary to the lower doses of the *Cerastes cerastes gasperettii* venom (300, 1200 µg/kg), the higher dose (2400 µg/rat) caused a marked hypotensive effect in anesthetized animals leading to death in 8 minutes after injection (Figure 3).

Discussion

Cesrastes cerastes gasperettii is one of the most abundant snakes of the Middle Eastern areas and it is found in Khuzestan province in Iran. Hemorrhagic wound at the bite site and coagulopathy are the common clinical manifestations in the Saudi Arabia. Due to the rare reports of the snakebites in Iran, it is not possible to firmly express the envenomation effects and the best way of its management (8,17).

Based on the results, the approximate lethality (LD50)

Figure 1. *Cerastes cerastes gasperettii* captured in the southwestern province of Iran.

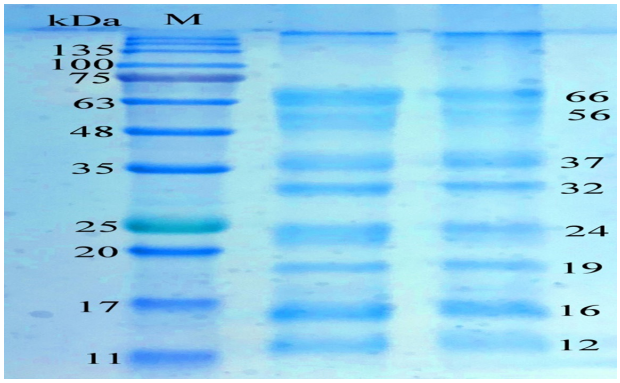

Figure 2. The protein constituents of the *Cerastes cerastes gasperettii* venom (lane 1:10 and lane 2:5 µg) were separated with the SDS-PAGE stained with the Coomassie blue. Numbers show the molecular weights of the components.

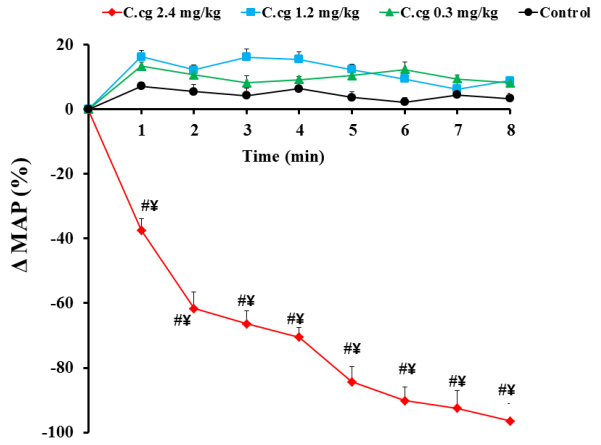

Figure 3. Changes in mean arterial pressure after intravenous administration of *Cerastes cerastes gasperettii* venom (300, 1200 and 2400 µg/kg). Control rats (n=5) received normal saline (400 µL, i.v). The points represent the mean ± SEM. $^{\#,¥}P<0.01$ compared to the elementary value (time zero) of the same group and (#) to the control group (¥).

of the Iranian snake was less than the previous reports performed in the Saudi Arabia (1.32 mg kg versus 978 µg/kg body weight) probably due to the species variation (18).

The protein profile showed two bands around 32 kDa and 38 kDa similar to the previous reports likely due to the snake matrix metalloproteinases (9,19,20). Furthermore, it had 7 various extra bands different from the other experiment performed on the Moroccan snake. In our further examination, venom separation must be carried out with the sephadex column and HPLC to extract the molecular weight of the different enzymes such as hemorrhagic, fibrinogenolytic, and proteolytic.

As shown in Figure 3, our results demonstrate that the venom is weak to induce hemodynamic effects in rats compared to the previous experiment in guinea pigs (2.4 mg/kg versus 0.8 mg/kg). This can be due to inter or intraspecies variation (12). There was bradycardia in the lethal dose as observed also in the Arabian study but no arrhythmia was recorded before death in rats. It seems that cardiovascular injuries have little role in envenomed patients.

Conclusion

According to our results, it is evident that cardiovascular changes have a minor role in the envenomed patients compared to other snakes. This preliminary experiment paves the way for further examination to separate different metalloenzymes causing pathological manifestations in stung patients.

Authors' contribution

This study was designed and performed by RS and SZ. Venom extraction and toxicological experiments were performed by AS and ZA, while the hemodynamic interpretation was analyzed by NRM. All authors accepted the final revision.

Ethical Issues

This animal study was conducted by the local research ethics committee of the Bushehr University with code number IR.BPUMS.REC.1398.133.

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