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RESEARCH ARTICLE

The Effects of Bezoar on the *Echis carinatus* Snake Venom Poisoning in Mice

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ABSTRACT

The bezoar, a dense material found in the stomach of wild goat, is widely used against various diseases including snakebite in traditional medicine among the southeast tribes of Iran. This study was performed to evaluate the bezoar effect on experimental mice receiving 2% and 10% concentrations of natural crude of *Echis carinatus* snake venom. Various doses of bezoar (6, 50, 100 & 200 mg/kg, i.p.) were injected. The clinical signs, mean survival time and autopsy findings were recorded 20 minute after intraperitoneal (i.p.) administration of 2% snake venom and the data were compared with control group which received saline. When 10% snake venom was administered, the mice received the most effective dose of bezoar (100 mg/kg, i.p.). Bezoar administration (50 & 100 mg/kg) increased the survival time and significantly attenuated the pathologic signs (such as bleeding in inretroperitoneal space and thoracic cavity , CNS and lung vascular congestion) caused by 2% and 10% *Echis carinatus* snake venom as compared to controls (p<0.05). The exact mechanism(s) by which the bezoar prolongs the survival time and attenuates the pathologic consequences of *Echis carinatus* snake venom in mice is not known completely and needs further investigations to elucidate the underlying mechanism(s).

Keywords: Bezoar, *Echis carinatus*, Snake venom, Survival time, Pathologic signs

Snake venom poisoning is a serious type of poisoning which not only effect the bite site, but also may affect multiple organ systems either primarily or secondary [1]. *Echis carinatus* (saw-scaled viper) snake is one of the most famous toxic snakes in Middle East, including Iran. It's widely distributed in south and southeastern part of Iran [2]. *Echis carinatus* snake bite will cause persistent pain, progressive edema and necrosis at the bite site and adjacent tissues. There may be signs of lymphangitis, with tender regional lymph nodes. Other features include local blistering, failure in blood coagulation, and spontaneous systemic bleeding. Coagulopathies are frequently seen following bites and may result in disseminated intravascular coagulation (DIC)-like manifestations [1].

Envenomation by *Echis carinatus* is associated with a 10-20 % mortality rate, if an effective treatment does not initiate early. The major cause of mortality is due to increased bleeding tendency by the venom [1-5]. Administration of antivenin is the only effective treatment for moderate to severe envenomations, along with aggressive life support in an intensive care setting. Moderate cases require the use of 10 vials administration while severe cases require the use of 15 vials or more [6]. Early reactions to antivenin are common and dose-related anaphylactic reactions mostly is induced by too-rapid infusion of antivenin [1, 6]. A skin test for hypersensitivity to horse serum should be performed prior to antivenin administration [1]. Considering these dangerous side effects of antivenin, the need for alternative or supplementary treatment of snake venom poisoning is crucial and valuable.

Bezoar is a sort of dense material rarely found in the intestine or stomach of ruminants. It was believed that bezoar has the power of universal antidote against any poison including snake bite. There is several type of bezoar; some have organic constituents and others inorganic, depending on the type of normal plant flora and vegetation of goats. However, the bezoar constituents have not been determined yet. The word "bezoar" comes from the Persian word "padzahr", which means "protection from poison" 7]. Bezoar is widely reputed antidote in traditional medicine among Iranian tribes, and is mostly used orally. People believe that varieties of bezoar may affect its therapeutic properties, depending on the type of normal plant flora and vegetation of goats. Kerman bezoar is the most wildly used bezoar among the tribes of south and southeast of Iran. Bezoar is ob-

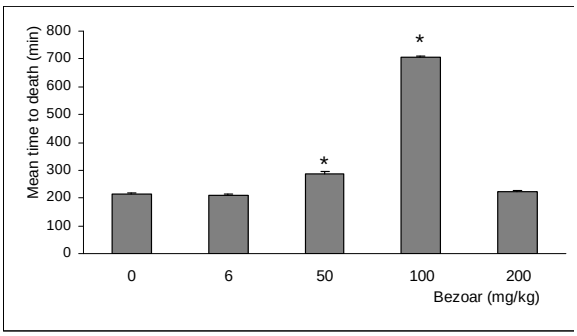

Fig 1. The effects of Bezoar administration (6, 50, 100 and 200mg/kg) on the mean survival time after 2% *Echis carinatus* snake venom administration (0.1ml/10 g body weight) in mice. Control mice received saline (0.1ml/10g body weight). Data are the Mean ± SEM of at least 6 mice in each group. $p<0.05$ as compared to control.

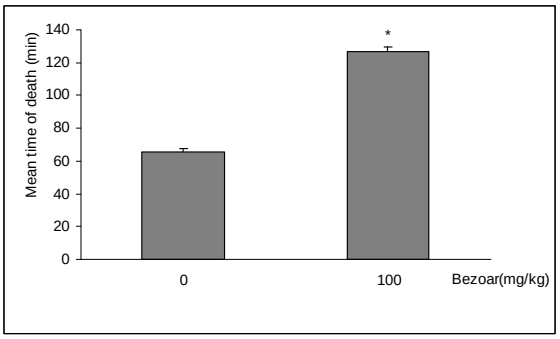

Fig 2. The effects of Bezoar administration (100 mg/kg) on the mean survival time after 10% *Echis carinatus* snake venom administration (0.1ml/10g body weight) in mice. Control mice received saline (0.1ml/10g body weight). Data are the Mean ± SEM of at least 6 mice in each group. $p<0.05$ as compared to controls.

tained as a by-product from some, but not all, wild goats, and it is purchased deliberately to be used as an antidote.

Since the Bezoar efficacy as snake antidote has not been reported yet, this study was performed to evaluate the bezoar effect on clinical signs, survival time and autopsy findings of experimental mice receiving crude *Echis carinatus* snake venom.

MATERIALS AND METHODS

Animals

Albino mice, weighing 25-30 g, were supplied from Kerman Neuroscience Research Center (Kerman, Iran). Animals were housed in standard plastic cages with wood chip bedding and were kept in controlled temperature (22±2°C), and 12-hour-light/dark-cycle- controlled rooms, with food and water available ad libitum, unless otherwise indicated. All testing procedures were carried out in accordance with Ethical Issues committee Recommendations of Neuroscience Research Council (Kerman, Iran).

Drugs

Bezoar was purchased from the native tribes of Sirjan City (160 km far away from Kerman, the center of Kerman province, Iran). The bezoar which was used in this study was grounded and macerated for 24 hours and then an aqueous solution was extracted for experimental use.

Echis carinatus venom was collected from the live snakes in Kazeroon City (150 km far away from Shiraz, the center of Fars province, Iran) and was immediately placed in an ice bag chamber, and then transferred to toxicology laboratory in Kerman Pharmacy School (Kerman, Iran).

The crude *Echis carinarus* snake venom were serially diluted and then injected to mice to determine the concentration of the venom which caused animals death in 1 and 3 hours. The 10% and 2% concentrations of crude snake venom caused animals death in 1 and 3 hours, respectively. These venom concentrations were used for further investigations. The bezoar was grounded and macerated for 24 hours and then an aqueous solution was extracted. Various concentrations (0.6, 5, 10 & 20 mg/ml) of the bezoar were prepared for intraperitoneal (i.p.) injections.

Experimental protocols

The 10% and 2% crude snake venom solutions were injected (0.1 ml/ 10 g mice, i.p.) to control group and then the clinical signs and survival time (minute) were recorded up to 3 hours following snake venom administration. The autopsy findings were recorded immediately following the death of the mice. Also, the effect of the 2% crude snake venom on the recorded parameters in mice pretreated with bezoar solution (6, 50, 100 & 200 mg/kg, i.p.), 20 minutes before i.p. crude venom injection (0.1 ml/l0 g mice) was investigated.

In 10% crude *Echis carinarus* snake venom administration, mice were received only 100 mg/kg, i.p. bezoar, 20 minutes prior to venom injection.

Statistical analysis

The mean survival times are the mean ± SEM of at least 6 mice in each group. Each mouse was only used for one treatment. Pathologic findings were expressed as severe, moderate and mild. The mean survival time were analyzed by one-way analysis of variance (ANOVA) followed by Turkey t-test. The value of $p<0.05$ was considered statistically significant.

RESULTS

Effects of bezoar on survival time after snake venom poisoning

Fig 1 shows that administration of 6 mg/kg, i.p. bezoar had no significant effect on 2% *Echis carinatus* snake venom poisoning compared with controls. However, 50 & 100 mg/kg, i.p. bezoar, 20 minute prior to *Echis carinatus* poisoning, increased the survival time significantly, while 200 mg/kg bezoar had no significant effect on survival time, compared with controls ($p>0.05$).

Table 1: Autopsy findings in saline and Bezoar treated mice after 2% and 10% *Echis carinatus* snake venom i.p administration.

Autopsy Findings / Groups	Thoracic hemorrhage	Echymotic bleeding in diaphragm & peritoneum	Mesenteric vascular congestion	Blood clot around intestines	Brain vascular congestion
1(n=6)	+++	+++	+++	+++	++
2(n=6)	+++	++++	++++	++++	+++
3(n=6)	+++	+++	+++	+++	++
4(n=6)	++	++	++	++	+
5(n=6)	++	++	++	++	++
6 (n=6)	+++	+++	+++	+++	+++
7 (n=12)	++	++	++	++	+

Severe=++++, Moderate = +++, Mild = ++
1-Saline +2% *Echis carinatus* snake venom.
2-Saline +10% *Echis carinatus* snake venom.
3-Bezoar (6mg/kg) +2% *Echis carinatus* snake venom
4-Bezoar (50mg/kg) +2% *Echis carinatus* snake venom
5-Bezoar (100mg/kg) +2% *Echis carinatus* snake venom
6-Bezoar (200mg/kg) +2% *Echis carinatus* snake venom
7-Bezoar (100mg/kg) + 10% *Echis carinatus* snake venom

As shown in Fig 2, the Bezoar administration (100mg/kg) significantly increased the survival time after 10% snake venom injection, compared with control (p<0.05).

Effect of bezoar on pathologic signs of snake venom poisoning

Echis carinatus snake venom injection (2% and 10%) caused severe retroperitoneal & thoracic bleeding, Schematic hemorrhage in diaphragm & peritoneum, lung and CNS vascular congestion, pulmonary congestion, in a dose dependent manner. The severity of pathologic signs after 10% crude snake venom were more than 2% crude snake venom injection (Table 1) and the severity of pathologic signs of snake venom poisoning following 6 mg/kg and 200 mg/kg of bezoar were almost the same as those of controls.

The effect of Bezoar on clinical signs of snake venom poisoning

Echis carinatus snake venom injection caused persistent pain and strain tail within 2 minute after snake venom injection. Orofacial edema (lip and eyelid edema) and ptosis were observed after 25 min. Dyspnea, cyanosis, ataxia, somnolence and intermittent tremor were observed during the first 120 minute after snake venom injection. Death occurred during 120-180 minute after 2% snake venom administration.

DISCUSSION

The results of this study showed that intraperitoneal administration of crude *Echis carinatus* snake venom caused severe persistent pain, progressive orofacial edema, systemic bleeding and finally death which are in agreement with other reports [5,8]. *Echis carinatus* snake venom contains phospholipase A2, which induces edema in mice [9]. Also complement activation via classical and alternative pathways may have contributed to vascular damage [5]. Snake venom is a complex mixture containing many different biologically active proteins and peptides. A number of these proteins interact with component of the human haemostatic system, including blood coagulation pathway, endothelial cells, and platelets [10]. Both platelet aggregation inducers and inhibitors were isolated from *Echis carinatus* snake venom [11]. For example, *Echis carinatus* snake venom contains echistatin, which inhibits fibrinogen-dependent platelet aggregation and echicetin which causes platelet agglutination [12,13].

Bezoar administration, 20 minutes before *Echis carinatus* snake venom injection increased the survival time and decreased the severity of pathologic findings (retroperitoneal & thoracic hemorrhage, CNS and lung vascular congestion, etc.) in a dose dependent manner. For example, 6 mg/kg bezoar did not affect the snake venom poisoning, but 50 & 100 mg/kg, i.p. bezoar increased the survival time and attenuated the severity of pathological signs of poisoning in mice.

The precise mechanism(s) by which bezoar have neutralized the *Echis carinatus* snake venom is not determined yet. But the protective effect of bezoar may be due to possible interaction of bezoar components with protein components of the *Echis carinatus* snake venom [14-16].

Guerranti *et al.* reported the antivenin property of a water extract of *Mucuna pruriens* seeds through an immune mechanism. They mentioned that the antibodies of the mice treated with non-lethal doses of venom reacted against some proteins of *Mucuna pruriens* extract [17]. Some other studies also have reported the ability of traditionally-used plants to reduce the effects of snake venom poisoning in several experimental models [15,18,19].

Houghton *et al.* reported that aqueous extract of some traditional plant showed a dose-related ability to

prolong the time taken to clot blood treated with a standardized dose of venom of *Echis carinatus* [20]. Also others have reported remarkable mortality reduction of albino mice after i.p. administration of reconstituted venom incubated with the leave extract of Guiera senegalensis [15]. Since plants constitute the central part of bezoar, so it is probable that the anti-snake-venom activity of bezoar be due to its plant component. The beneficial effects of bezoar on snake venom toxicity may be through its interaction with haemostatic system which is altered by snake venom injection [15,18,19].

Despite the widespread use of bezoar among the native tribes of south and southeast part of Iran, the efficacy of bezoar against snake bite was not reported yet and this is the first preliminary report which shows the anti-snake venom activity of bezoar.

In conclusion, the results of this study show that bezoar increases survival time and attenuates the pathologic signs of *Echis carinatus* snake venom poisoning in mice. The precise mechanism(s) by which bezoar possess the snake venom neutralizing effects needs further investigation to be elucidated.

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