Hemodynamic Changes Provoked with Intravascular Injection of the *Echis carinatus* Venom in Rats

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Abstract

*Echis carinatus* (*E.carinatus*) is known for its hematological and nephrotoxic properties in the envenomed patients. Based on the limited data upon the cardiovascular changes with this dangerous venomous snake in Iran, the objective of the current study was to evaluate the induced hemodynamic manifestations in rats. Venom (120 µg/kg) was administered intravenously within one minute via the left femoral vein and the continuous recording of the hemodynamic parameters was performed by means of a pressure transducer (MLT844, ADInstruments, Australia). It caused a prominent hypotension leading to the death in few minutes after a transient up rise in the blood pressure. Meanwhile, it induced a decrease in the heart and the pulmonary rate while it had no arrhythmogenic properties. Additionally, pre-treatment with the pepsin derived Iranian polyvalent antivenom (30µl/Kg) neutralized the hemodynamic responses totally while this remedy had no effect when instilled two minutes after venom injection. Heparin (300 IU/kg) and epinephrine (1.5µg/kg) prevented the dramatic hypotension when used 10 minutes before venom instillation while atropine (1 mg/kg), dexamethasone (1 mg/kg) and ketorolac (10mg/ml) had no effects and all treated rats were killed post injection.
Histologically, the lung was the most vulnerable organ with mononuclear infiltration, micro cystic formation and significant capillary congestion. There were prominent renal pathological deteriorations including mesangial cell infiltration and diffuse bleeding leading to the acute tubular necrosis. Modest portal inflammation and vascular congestion were observed in the hepatic tissue of the envenomed rats. The crude venom of Iranian *Echis carinatus* causes hypotension leading to death, bradycardia and decrease in pulmonary rate without significant histological changes in the heart.

**Key words:** *Echis carinatus*, Venom, Snake, Hemodynamic, Antivenom

**INTRODUCTION**

Snake envenomation leads to death mostly in Asia (15,400-57,600 patients annually) and sub-Saharan Africa (3,500-32,000 patients per year) (Harrison et al., 2009, Kasturiratne et al., 2008, White, 2000). Snake bite mortality is reported to be low in Iran locating in the Middle East area (0-10.9 deaths patients per year) since there is a strong relationship between survival and good management of envenomed patients (Harrison et al., 2009). *Echis carinatus* belongs to Viperidae family, is a dangerous snake found in central and southern provinces of provinces of Iran (Fathi et al., 2011). Its venom as a cocktail including potent matrix metalloproteinases is responsible for coagulation and platelet aggregation (Kornalik and Blombäck, 1975; Gan et al., 1988). Historically, the envenomation has been recognized as the leading cause of blood disorders and renal malformations in envenomed patients. However, little attention has been paid to the cardio toxic changes induced by this snake venom with intravenous injection in rats (Sifrija, 2008). The aim of the present study was to evaluate the hemodynamic and arrhythmogenic deteriorations induced by *Echis carinatus* venom injection in anesthetized rats. Furthermore, the neutralizing ability of the pre-treatment of Iranian polyvalent antivenom was evaluated. Additionally, the mechanism of the hemodynamic changes were investigated using pre-treatment with intravenous injection of different drugs along with pathological changes induced in critical organs including kidney, lung, heart and liver in the killed animals.
MATERIALS AND METHODS

Venom, antivenom, reagents. The crude venom was obtained from the adult specimens in the serpentarium of the Razi Institute of Iran (Fig 1).

It was lyophilized and stored at -20°C until use. Fresh solutions were prepared in 0.9% normal saline and maintained on ice during experiment. The Persian polyvalent antivenom (pepsin derived equine type) against five dangerous endemic snakes (Echis carinatus, Vipera lebetina, Vipera albicornata, Pseudocerastes persicus, and Agkistrodon halys) was prepared from the same place. The neutralizing potency of the product was 50 LD50/ml (Dehghani et al., 2014). Atropine sulfate, dexamethasone, and ketorolac were purchased from the Santa Cruz Biotechnology company. Epinephrine (as HCl) was supplied from Iranian Daru Pakhsh pharmaceutical company. Heparin Sodium (25000 IU/5ml) ampoules were purchased from Caspian pharmaceutical company (Iran).

Experimental protocol. Male Wistar rats (250-300g, 6-8 weeks old) were housed in PVC cages (three each) and maintained at 22±2°C with free access to water and hard foot pellets and kept at 12 hours light-dark cycles starting at 7AM. Animals were anesthetized with ketamine (100mg/kg; i.p) and xylazine (10 mg/kg; i.p) and placed supine on the surgical table where their body temperature was preserved at 37±1 °C monitored with a rectal tube connected to a thermometer (Physitemp BAT-12, Texas Scientific In-struments, San Antonio, Texas, USA). Administration of the venom and drugs were performed via a cannula that was placed in to the left femoral vein. Another one was inserted in the left femoral artery to monitor cardiotropic changes. It was connected to a pressure transducer (MLT844, AD instruments, Australia) for continuous recording of the hemodynamic changes by means of a power lab acquisition system (AD instruments). At the end of the study, the animals were killed by cervical dislocation and their internal organs (hearts, kidneys, livers, and the lungs) were preserved for pathological analysis in formalin solution (10%).
Three groups of animals (n=6 in each) were injected intravenously via the left femoral vein, with different amounts of the venom (15, 60, and 120µg/Kg) dissolved in normal saline within one minute. Normal saline with the same volume was injected in the last group as control. The hemodynamic parameters were expressed as the changes in the percent of the mean arterial pressure (MAP), calculated with doubling the diastolic blood pressure and added to the systolic blood pressure. The composite sum was divided by three to obtain the final amount. The chronotropic and arrhythmogenic effects derived by this procedure were investigated during the experiment (Ziaee et al., 2015, Chaisakul et al., 2014).

The following drugs were added intravenously ten minutes before the venom injection (120µg/Kg): epinephrine 1.5µg/kg, heparin 300 IU/kg, atropine 1 mg/kg, dexamethasone 1 mg/kg, and ketorolac 10mg/ml and their neutralizing effects on the hemodynamic changes were recorded (Chaisakul et al., 2013, Chaisakul et al., 2014, Costa et al., 1996, Dias et al., 2016a, Smits et al., 1987, Rochette et al., 1988).

**Antivenom effects.** Iranian polyvalent antivenom (30µl/Kg) was injected slowly, as a pretreatment agent, 10 minutes before the venom injection and the cardiac contractility changes were evaluated. In parallel experiments, this remedy was injected 2 minutes post venom instillation and its neutralizing effects were assessed.

**Data analysis.** Statistical data were analyzed using IBM SPSS 19. Responses were demonstrated as mean ±SD. Multiple comparisons were made using ANOVA, followed by Tukey test. The significant value was considered to be as $p<0.01$

**RESULTS**

**Cardiotropic effects of the *Echis charinatus* venom.** Venom injection (120µg/Kg) caused a rapid decline in MAP leading to death (after $\approx$ 5 minutes). Initial uprising of the blood pressure
(≈ 1.5 minutes) was occurred prior to the hypotensive state (Fig 2A and 2B). There was a statistically significant differences between the hypotension induced with this venom against other treatments \((p<0.01)\).

There was a prominent bradycardia following envenomation while there were no arrhythmogenic properties in envenomed rats (Fig 2C and 2D).

Prior injection of the heparin and epinephrine notably increased MAP percentage \((16.21±2.34\% \text{ and } 10.24±1.2\% \text{ respectively})\) six minutes after venom injection (Fig 3). No other drugs in this study could prevent death due to the hemodynamic changes.

**Antivenom neutralizing effects.** Prior administration of the antivenom \((30\mu\text{l/kg})\), 10 minutes before venom injection totally neutralized remarkable hypotensive effects and there was no mortality in treated rats (Fig 4A). Notably, this remedy had no effects when it was instilled two minute after venom injection (Fig 4B).

**Pathological analysis.** Our findings suggest that the lungs were destructed significantly after envenomation with *Echis carinatus* venom. Marked structural demolition was produced due to the significant mononuclear cell infiltration and excessive hemorrhage. Furthermore, vascular congestion with huge red blood cells extravasation and destruction of the alveolar spaces leading to emphysemalos phenomenon were observed (Fig 5A).

Some tubules of the kidney showed cytoplasmic vacuolization, sloughing and regenerative changes, leading to acute tubular necrosis. Additionally, some glomeruli revealed mild mesangial proliferation and marked vascular congestion (Fig 5B).

There was vascular congestion with regenerative changes and moderate portal inflammation in the liver (Fig 5C) while no significant pathological changes in the heart were observed (Fig 5D).

**DISCUSSION**
Snake envenomation is a tropical disease that affects people especially in rural areas. Due to the complex mixture of substances that make the venom cocktail, there are different therapeutic strategies to cure the injured patients. It involves the correction of the hemodynamic, neuroleptic and cytotoxic properties with different remedies including antivenom, analgesics, fluid therapy and even hemodialysis in severe cases. *Echis carinatus* is responsible for envenomation in large number of people in India and Middle Eastern countries like Iran (Patra et al., 2017). Moreover, envenomation by this dangerous snake principally causes hematological consequences including coagulation and gum bleeding due to some metalloproteinase enzymes and small proteins like echistatin as a platelet aggregation inhibitor (Gan et al., 1988, Gutiérrez et al., 2017). Up to date, the potential mechanisms behind the hemodynamic changes following snake envenomation have not been defined exclusively. Our results showed for the first time that the mean arterial pressure had a transient increase with intravascular injection of the *Echis carinatus* venom (120µg/kg) and then significant decrease in pulse pressure leading to death in 6 minutes in all rats (Fig 1A and B). This scenario is similar with other previous experiments carried out on the cardiovascular effects of the poisonous venoms (Noguchi et al., 2005, Szoló et al., 2001). Notably, the hypotensive properties of this venom was more than the previous investigation performed with Fatehi et al on this snake (120 µg versus 7 µg/rat) probably due to the season and inter or intra species variation (Fatehi-Hassanabad et al., 2004, Chippaux et al., 1991). It has been proposed that autonomic overactivity due to the adrenal release is responsible for the brief hypertensive up rise (Radha Krishna Murthy et al., 2003).

Furthermore, there was no sign of atrial fibrillation or other arrhythmic events (Fig 2D) due to myocardial ischemia in envenomed rats ruling out its direct effects on the *in vivo* electrical conduction system contrary to the other poisonous snakes (Omran and Abdel-Nabi, 1997).
It is obligatory to study the possible effects of the *Echis carinatus* venom on rat isolated heart in further experiments (Dias et al., 2016). Our results support the hypothesis that getting epinephrine and heparin protects against the hypotensive property of this venom in treated animals (Fig 3). It has been shown that heparin inhibits histamine release and the results of this study were in line with the previous experiment demonstrating the major role of this mediator in hypotension (Inase et al., 1993). Additionally, ineffectiveness of the pretreatment with atropine as an anticholinergic drug is ruling out the direct cholinergic activity of this venom while beneficial effects of the epinephrine shows that the venom causes a negative inotropic and chronotropic effects probably via blocking the adrenergic receptors (Church and Hodgson, 2002).

Polyvalent snake antivenom has been suggested in previous reports to neutralize the detrimental changes including neural and cardiovascular in envenomed animals (Tarasiuk et al., 1998, Tarasiuk et al., 2003). Pretreatment with the Razi Institute polyvalent antivenom, 10 minutes before the venom injection totally counteracted its great hypotensive property while its injection following envenomation had no effects (Fig 4). It has been shown that in cardiac manifestations of the scorpion and snake envenomation, the time interval of the antivenom administration should not exceed an hour in human patients (de Davila et al., 2002). Additionally, it is evident that this venom has the potency to produce effective neutralizing antivenom from hyperimmune horses and this product must be infused as soon as possible to envenomed patients.

In agreement with the effects of snake bite including myonecrosis, edema, dermonecrosis and hemorrhage (Cher et al., 2005), the current study showed diffuse histological malformations in the pulmonary, renal, hepatic and cardiac organs. In lung, there was generalized mononuclear infiltration with capillary congestion, probably due to the increase in the red blood cells count in the first minutes to compensate the hypoxia (Al-Sadoon and Fahim, 2012). There was a
significant decrease in respiratory rate and the rats experienced respiratory distress syndrome before the death. *Echis carinatus* venom caused a great hepatotoxic deterioration (inflammatory cells infiltration in the portal areas and vascular congestion) more pronounced than *Microviper a lebetina* injection in mice (Yücel Aquan and Hayretdağ, 2019).

Moreover, the renal destruction was occurred probably due to the significant bleeding, consumption coagulopathy and direct nephrotoxicity caused by the actions of the phospholipase A2 enzyme. It was similar to the previous experiment carried out on the *Echis pyramidum* venom (Samy et al., 2010, Warrell et al., 1976). There were no significant pathological changes in the heart, except the signs of myocyte degenerations (Al-Johany et al., 2015).

**CONCLUSION**

The results of this study show that intravenous injection of the *Echis carinatus* venom has a significant hypotensive effects leading to death in few minutes. Premedication with Iranian polyvalent antivenom could neutralize totally the lethal cardiovascular effects. Furthermore, it seems that vasodilatation due to the histamine release and blocking of the adrenergic receptors are the main reasons for hypotension. We believe that our research will serve as a base for future studies to investigate the in vitro cardiotropic effects of this dangerous venom.

**Disclosure of statements**

The authors report no conflict of interest.

**Acknowledgement**

This study was performed according to the experimental protocol approved by the research department of the Bushehr University of the Medical Sciences (IR.BPUMS.REC.1398.133).

**References**


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Figure legends:

Fig 1: Saw scaled viper (Echis carinatus).
Fig 2. Individual trace of blood pressure changes to the intravenous administration of *E. carinatus* in rats. This recording is representative of 6 experiments for the 120µg/Kg (2A). Changes in mean arterial pressure, heart rate and arrhythmogenicity after intravenous administration of the *E. carinatus* venom versus normal saline injection (2B, C, and D). The points represent the mean±SD (N=6). *#P<0.01 compared with the initial values (time zero) of the corresponding group (*) and to the control (#).
Fig 3: Changes with premedication of different drugs upon hypotensive phenomenon induced with E. carinatus venom in anesthetized rats. Heparin (300 IU/kg) and epinephrine (1.5µg/kg) totally neutralized the hypotensive shock while atropine (1mg/kg), dexamethasone (1 mg/kg) and ketorolac (10mg/ml) had no effects. **P<0.01 compared with the treated rats.
Fig 4: Effects of the Iranian polyvalent antivenom on the blood pressure changes in anesthetized rats treated with the E.carinatus venom (120μg/Kg). Premedication with remedy, 10 minutes before venom injection totally neutralized the hypotensive shock (A) while it had no effects following venom injection (B).
**Fig 5:** Light micrograph showing the pathological changes in different organs (lung, kidney, liver and heart) induced by 40µg/rat of *E.carinatus* venom after its intravenous injection stained with hematoxylin and eosin (H&E). Lung (A): Notice massive hemorrhage, leukocyte infiltration(*) and microcystic formation(#). Kidney (B): Mesangial proliferation and pyknotic glomeruli ($\$). Liver (C): Moderate portal inflammation (&) and vascular congestion ($) and finally there was no pathological lesion in the heart (D)