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Original Article

Hemodynamic Changes Provoked through 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 associated with this dangerous venomous snake in Iran, the current study purposed to evaluate the venom-induced hemodynamic manifestations in rats. Venom (120 µg/kg) was administered intravenously within one minute through the left femoral vein, and the hemodynamic parameters were continuously recorded using a pressure transducer (MLT844, ADInstruments, Australia). The venom caused prominent hypotension leading to death a few minutes after a transient uprise in blood pressure. It also induced a decrease in heart and pulmonary rates, yet it had no arrhythmogenic properties. Additionally, pre-treatment with the pepsin-derived Iranian polyvalent antivenom (30 µl/Kg) completely neutralized the hemodynamic responses but had no effect when instilled two minutes after venom injection. Heparin (300 IU/kg) and epinephrine (1.5 µg/kg) prevented dramatic hypotension when used 10 minutes before venom instillation; however, atropine (1 mg/kg), dexamethasone (1 mg/kg), and ketorolac (10 mg/ml) had no effects. All treated rats were killed post-injection. Histologically, the lung was the most vulnerable organ with mononuclear infiltration, microcystic formation, and significant capillary congestion. Prominent renal pathological deterioration also occurred, including mesangial cell infiltration and diffuse bleeding, leading to 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* caused hypotension leading to bradycardia, a decrease in pulmonary rate, and death without significant histological changes to the heart.

Keywords: *Echis carinatus*, venom, snake, hemodynamic, antivenom

Modifications Hémodynamiques Provoquées par l'Injection Intravasculaire de Venin d'*Echis carinatus* Chez les Rats

Résumé: *Echis carinatus* (*E. carinatus*) est connu pour ses propriétés hématologiques et néphrotoxiques chez les patients envenimés. Sur la base des données limitées concernant les changements cardiovasculaires associés à ce dangereux serpent venimeux en Iran, la présente étude visait à évaluer les manifestations hémodynamiques induites par le venin chez les rats. Du venin (120 µg/kg) a été administré par voie intraveineuse en une minute dans la veine fémorale gauche, et les paramètres hémodynamiques ont été enregistrés en continu à l'aide d'un

transducteur de pression (MLT844, ADInstruments, Australie). Le venin a provoqué une hypotension importante entraînant la mort quelques minutes après une augmentation transitoire de la pression artérielle. Il a également induit une diminution des fréquences cardiaques et pulmonaires, mais il n'avait pas de propriétés arythmogènes. De plus, le prétraitement avec l'antivenin polyvalent iranien dérivé de la pepsine (30 µl/kg) a complètement neutralisé les réponses hémodynamiques mais n'a eu aucun effet lorsqu'il a été instillé deux minutes après l'injection de venin. L'héparine (300 IU/kg) et l'épinéphrine (1.5 µg/kg) ont empêché une hypotension dramatique lorsqu'elles ont été utilisées 10 minutes avant l'instillation de venin; cependant, l'atropine (1 mg/kg), la dexaméthasone (1 mg/kg) et le kétorolac (10 mg/ml) n'ont eu aucun effet. Tous les rats traités ont été tués après l'injection. Histologiquement, le poumon était l'organe le plus vulnérable avec une infiltration mononucléaire, une formation microkystique et une congestion capillaire importante. Une détérioration pathologique rénale importante s'est également produite, y compris une infiltration de cellules mésangiales et un saignement diffus, conduisant à une nécrose tubulaire aiguë. Une inflammation portale modeste et une congestion vasculaire ont été observées dans le tissu hépatique des rats envenimés. Le venin brut d'*Echis carinatus* iranien a provoqué une hypotension entraînant une bradycardie, une diminution de la fréquence pulmonaire et la mort sans modifications histologiques significatives du cœur.
Mots-clés: *Echis carinatus,* venin, serpent, hémodynamique, antivenin

1. Introduction

Snake envenomation leads to a large number of deaths each year, most of which occur in Asia (15,400-57,600 annually) and sub-Saharan Africa (3,500-32,000 patients per year) (1-3). Snake bite mortality is reported to be low in Iran located in the Middle East area, (0-10.9 deaths per year), since there is strong relationship between survival and good management of envenomed patients (1). *Echis carinatus* belongs to Viperidae family is a dangerous snake found in central and southern provinces of the provinces of Iran (4). Its venom is a potent cocktail of matrix metalloproteinases responsible for coagulation and platelet aggregation (5). Historically, 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 cardiotropic changes induced by this snake venom (6). The present study aimed to evaluate the hemodynamic and arrhythmogenic deteriorations induced by *Echis carinatus* venom injection in anesthetized rats. The neutralizing ability of pre-treatment with Iranian polyvalent antivenom was also evaluated. Additionally, the mechanism of the hemodynamic changes was 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 were investigated.

2. Material and Methods

2.1. Venom, Antivenom, Reagents

The crude venom was obtained from the adult specimens in the serpentarium of the Razi Institute of Iran (Figure 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 experiments. The Persian polyvalent antivenom (pepsin-derived equine type) against five dangerous endemic snakes (*Echis carinatus, Vipera lebetina, Vipera albicornata, Pseudocerastus persicus*, and *Agkistrodon halys*) was prepared from the same place. The neutralizing potency of the product was 50 LD50/ml (7). 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 the Caspian Pharmaceutical Company (Iran).

Figure 1. Saw scaled viper (*Echis carinatus*).

2.2. Experimental Protocol

Male Wistar rats (weighing 250-300 g, 6-8 weeks of old were housed in PVC cages (three each) maintained at 22±2 ˚C, with free access to water and hard foot pellets, and kept at a 12-hour light-dark cycle starting at 7 AM. Animals were anesthetized with ketamine (100 mg/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 ℃ monitored with a rectal tube connected to a thermometer (Physitemp BAT-12, Texas Scientific Instruments, San Antonio, Texas, USA).

The venom and drugs were administered via a cannula placed into 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 sacrificed by cervical dislocation, and their internal organs (hearts, kidneys, livers, and lungs) were preserved in formalin solution (10%) for pathological analysis.

Three groups of animals (n=6 in each) were injected intravenously via the left femoral vein with different amounts of venom (15, 60, and 120 µg/Kg) dissolved in Normal saline within one minute. The same volume of normal saline was injected in the last group as control. The hemodynamic parameters were expressed as changes in the percent of mean arterial pressure (MAP), calculated with doubling the diastolic blood pressure and adding the sum to the systolic blood pressure. The composite sum was divided with three to obtain the final amount. The chronotropic and arrhythmogenic effects derived by this procedure were investigated during the experiment (8, 9).

The following drugs were added intravenously ten minutes before the venom injection (120 µg/Kg): epinephrine 1.5 µg/kg, heparin 300 IU/kg, atropine 1 mg/kg, dexamethasone 1 mg/kg, and ketorolac 10

mg/ml, and their neutralizing effects on the hemodynamic changes were recorded (8, 10-14).

2.3. Antivenom Effects

ranian polyvalent antivenom (30 µl/Kg), was injected slowly as a pretreatment agentm,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.

2.4. Data Analysis

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

3. Results

3.1. Cardiotropic Effects of the *Echis charinatus* Venom

Venom injection (120 µg/Kg) caused a rapid decline in MAP leading to death (after ≈ 5 minutes). In initial uprising of the blood pressure (≈ 1.5 minutes) occurred prior to the hypotensive state (Figure 2A and Figure 2B). There was a statistically significant difference between the hypotension induced with this venom and other treatments ($p<0.01$).

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

Prior injection of the heparin and epinephrine notably increased MAP percentage (16.21±2.34% and10.24±1.2%, respectively) six minutes after venom injection (Figure 3). No other drugs in this study could prevent death due to the hemodynamic changes.

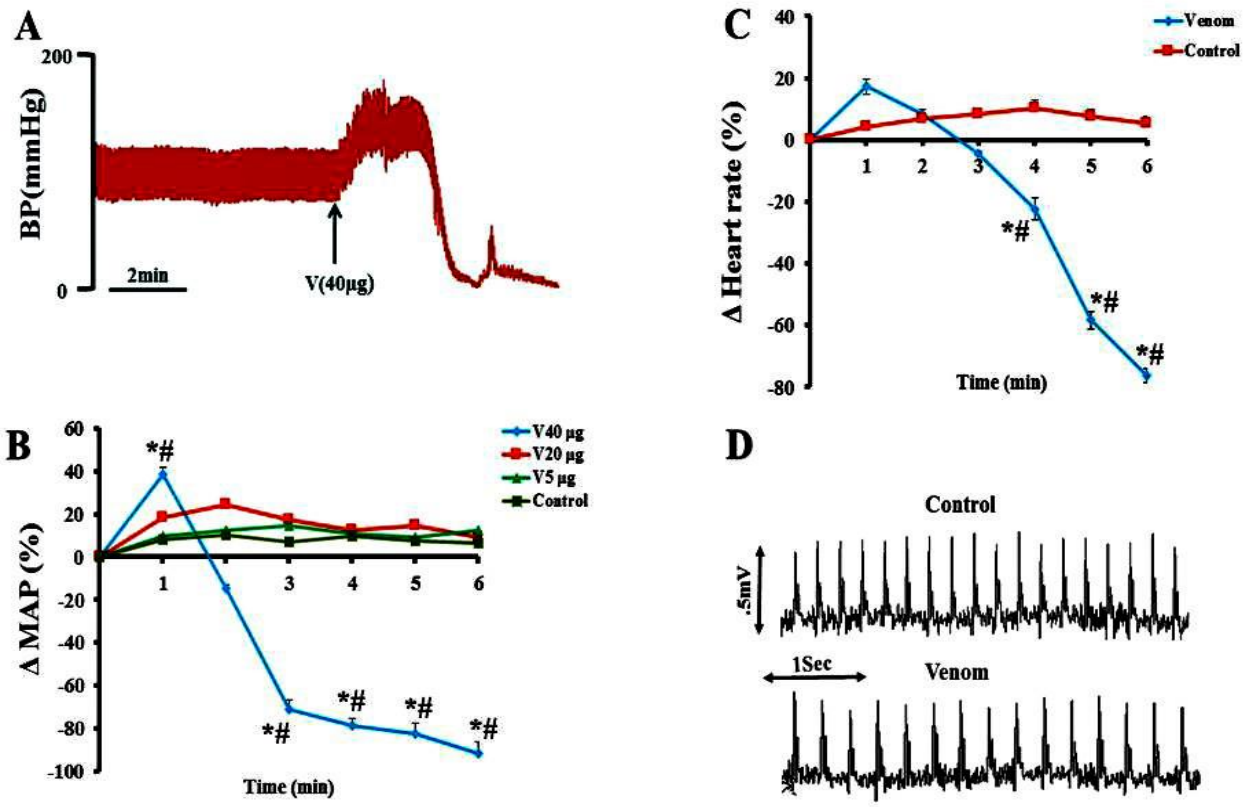

Figure 2. Individuals traces of blood pressure changes to the intravenous administration of *E. carinatus* in rats. This recording is representative of 6 experiments for 120 µg/Kg **(2A)**. Changes in mean arterial pressure, heart rate, and arrhythmogenicity after intravenous administration of the *E. 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 (#).

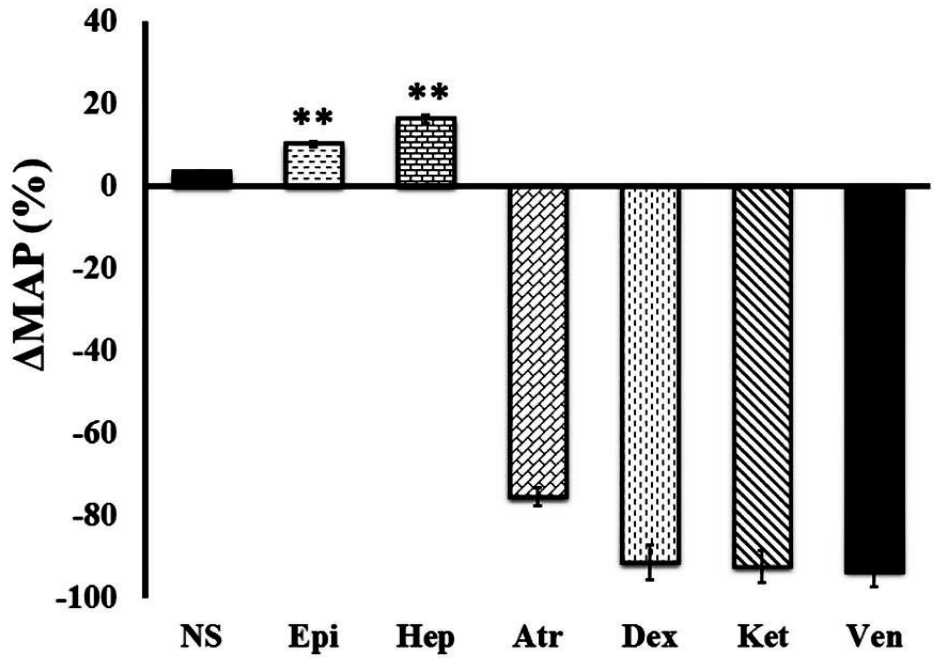

Figure 3. Changes with premedication of different drugs with the hypotensive phenomenon induced with *E. carinatus* venom in anesthetized rats. Heparin (300 IU/kg) and epinephrine (1.5µg/kg) totally neutralized the hypotensive shock, while atropine (1 mg/kg), dexamethasone (1 mg/kg), and ketorolac (10 mg/ml) had no effect. $^{**}P<0.01$ compared with the treated rats.

3.2. Antivenom Neutralizing Effects

Prior administration of the antivenom (30 µl/kg) 10 minutes before venom injection totally neutralized remarkable hypotensive effects, and there was no mortality in treated rats (Figure 4A). Notably, this remedy had no effects when it was instilled two minutes after venom injection (Figure 4B).

3.3. Pathological Analysis

Our findings suggest that the lungs were destructed damaged 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 emphysematous phenomenon were observed (Figure 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 (Figure 5B).

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

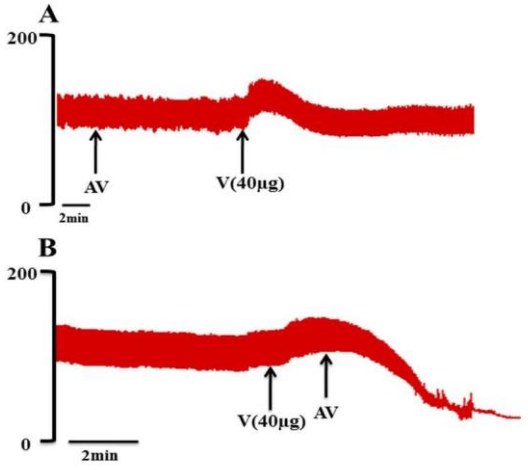

Figure 4. Effects of the Iranian polyvalent antivenom on the blood pressure changes in anesthetized rats treated with *E. carinatus* venom (120 µg/Kg). Premedication with the remedy 10 minutes before venom injection totally neutralized the hypotensive shock **(A)**, while it had no effects following venom injection **(B)**.

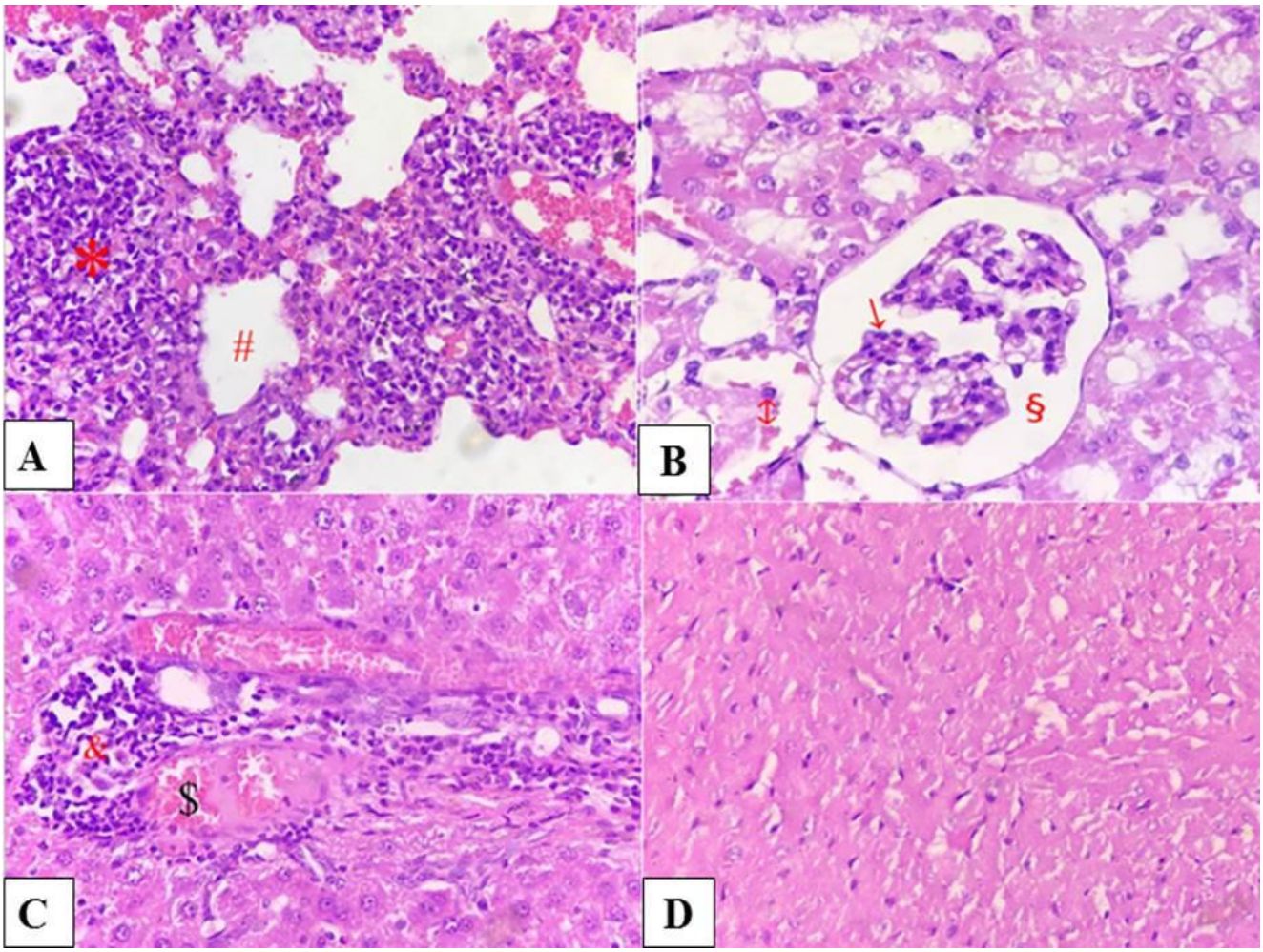

Figure 5. Light micrograph showing the pathological changes in different organs (lung, kidney, liver, and heart) induced by 40 µg/rat of *E. carinatus* venom after its intravenous injection stained with hematoxylin and eosin (H&E). Lung **(A)**: Notice massive hemorrhage, leukocyte infiltration (*), 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)**.

4. Discussion

Snake envenomation is a tropical disease that affects people, especially in rural areas. Due to the complex mixture of substances that compose the venom cocktail, there are different therapeutic strategies for curing injured patients. Our cure involves 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 a large number of people in India and Middle Eastern countries like Iran (15). 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 (16). To date, the potential mechanisms behind the hemodynamic changes following snake envenomation have not been defined exclusively. The current results showed for the first time that the mean arterial pressure had a transient increase with intravascular injection of *Echis carinatus* venom (120 µg/kg) then a significant decrease in pulse pressure leading to death in 6 minutes in all rats (Fig. 1A and B). This scenario similar with other previous experiments carried out on the

cardiovascular effects of poisonous venoms (17, 18). Notably, was more the hypotensive properties of this venom was more than the current study than the previous investigation performed with by Fatehi et al. (120 µg versus 7 µg/rat), probably due to seasonal and inter- or intra-species variations (19, 20). It has been proposed that autonomic overactivity due to the adrenal release is responsible for the brief hypertensive uprise (21).

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 (22).

It is required to study the possible effects of *Echis carinatus* venom on rat isolated heart in further experiments (12). Our results support the hypothesis that epinephrine and heparin protect against the hypotensive property of this venom in treated animals (Fig. 3). It has been shown that heparin inhibits histamine release, and our results were in line with the previous experiment demonstrating the major role of this mediator in hypotension (23). Additionally, ineffectiveness of pretreatment with atropine as an anticholinergic drug is ruling out the direct cholinergic activity of this venom, while the beneficial effects of epinephrine show that the venom causes negative inotropic and chronotropic effects probably via blocking adrenergic receptors (24).

Polyvalent snake antivenom has been suggested in previous reports to neutralize detrimental changes (including neural and cardiovascular alterations) in envenomed animals (25, 26). Pretreatment with the Razi Institute polyvalent antivenom 10 minutes before injection the venom totally counteracted the its great hypotensive property, while its injection following envenomation had no effect (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 (27).

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 (28), the current study showed diffuse histological malformations in the pulmonary, renal, hepatic, and cardiac organs. There was, generalized mononuclear infiltration with capillary congestion was observed, probably due to the increase in red blood cells in the first minutes to compensate the hypoxia (29). There was a significant decrease in respiratory rate, and the rats experienced respiratory distress syndrome before death. *Echis carinatus* venom caused great hepatotoxic deteriorations (inflammatory cells infiltration in the portal areas and vascular congestion) more pronounced than those caused by *Microvipera lebetina* injection in mice (30).

Moreover, renal destruction occurred, probably due to significant bleeding, consumption coagulopathy, and direct nephrotoxicity caused by the actions of the phospholipase A2 enzyme. These results were similar to the previous experiment carried out on *Echis pyramidum* venom (31, 32). There were no significant pathological changes in the heart, except signs of myocyte degeneration (33).

5. Conclusion

The results of this study show that intravenous injection of *Echis carinatus* venom has significant hypotensive effects leading to death in a few minutes. Premedication with Iranian polyvalent antivenom could neutralize the lethal cardiovascular effects. Furthermore, it seems that vasodilatation due to the histamine release and blocking of 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.

Authors' Contribution

Study concept and design: S. Z., R. S. and E. K.
Acquisition of data: P. K. and A. S.
Analysis and interpretation of data: N. M. D. and A. E.
Drafting of the manuscript: H. F. and M. K.
Critical revision of the manuscript for important intellectual content: R. S. and E. K.
Statistical analysis: R. S.
Administrative, technical, and material support: N. M. D.

Ethics

All the procedure and animal handling were approved by the Animal Ethics Committee at the research department of the Bushehr University of Medical Sciences (IR.BPUMS.REC.1398.133).

Conflict of Interest

The authors declare that they have no conflict of interest.

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Acknowledgment

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

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