Acute cardiovascular effects of *Naja oxiana* venom in anesthetized rats

Abstract

Cobra bite is common in northwest province of Iran located in the Middle East Area. *Naja naja oxiana* envenomation is presented with neurologic manifestations like ptosis, drooling and so on. The aim of this preliminary study was to examine the hemodynamic abnormalities induced with intravascular injection of this venom in rats. Additionally, the neutralizing effects of different premedications were investigated.

Twenty male wistar rats weighting between 200-250 grams were randomly divided in to four groups (n=5). Group one was selected as control while the others were envenomed intravenously with crude venom (300µg/kg, 600 µg/kg and 1500 µg/kg) dissolved in normal saline (200µl) in two minutes. Atropine, dexamethasone, heparin and aminoguanidine were injected intraperitoneally ten minutes before envenomations to counteract its deleterious effects. All animals were sacrificed with cervical dislocation and their abdominal area were visualized for bleeding. Different organs (lung, heart and kidney) were removed and prepared for Hematoxylin and Eosin staining to reveal the pathological events.

N.oxiana venom (1500 µg/kg) induced significant ionotropic changes following intravenous infusion and all animals expired eight minutes later due to hypotension. There was no arrhythmia but heart rate was decreased statistically ($p<0.001$) in this group. Pretreatment with aminoguanidine (29±2.1%) and heparin (21±1.2%) prevented hypotension at 8 minutes but all animals eventually died at 20 minutes. Disruption of the alveolar walls of the lung with presence of the red blood cells and inflammatory components were observed while there were no pathological abnormalities with light microscope in other organs. It should be noted that according to our ionotropic and chronotropic results the last group was selected to continue our examinations. In this preliminary study, it was observed that in large doses it could produce significant negative ionotropic effects in rats. According to our results, it seems that systemic vasodilation has a major role since pretreatment of heparin and aminoguanidine diminished this effect profoundly while there were no pathological abnormalities in other organs except lungs. It seems that increasing the doses of the heparin and aminoguanidine could prolong the survival of the envenomed rats in short time since all animals were died 20 minutes later.

Keywords: *Naja oxiana*, venom, snake, hemodynamic, aminoguanidine

1. Introduction

Snake envenomation is a big problem in tropical areas of Iran located in the Middle East Area. It is of interest that 69 snake species with 9 semi-venomous and 25 venomous are living in this country and Persian gulf water(1, 2). Caspian cobra (*Naja oxiana* belonging to the elapidae family) exists in the northwest areas of Iran specially Khorasan province (3). Since the toxicity of the crude venom via intracerebroventricular injection (0.005 mg/Kg) shows the highest lethality compared with other cobras (low LD_{50}), it is regarded as one of the most dangerous snakes for humans in Iran (4). Its venom contains generally neurotoxic components causing ptosis, drooling and so on in envenomed human beings but there are additional findings representing hemodynamic effects of this poisonous snake (5, 6). Statistical electrocardiogram recordings such as tachycardia and bradycardia are seen following envenomation while its importance is usually less than neurotoxic properties (7). Additionally, in previous experiments, there was no increase in troponin-I as a sensitive enzyme one day later following envenomation in neurotoxic venoms like cobra snakes in envenomed humans determining cardiovascular effects questionable (8). According to the scarce animal studies in this issue especially in Iran, the purpose of this experiment was to evaluate the cardiovascular changes provoked with intravenous injection of the crude venom in parallel with the effective prophylactic remedies to counteract the probable deteriorating effects. In this regard, complex of drugs including dexamethasone (an anti-inflammatory medicine), atropine (an anticholinergic substance), heparin (an antihistamine drug) and finally aminoguanidine as an iNOS inhibitor will be evaluated. Additionally, pathological evidences will reveal the macro and microvascular changes in lungs and other organs leading to death.

2. Materials and Methods

Crude venom was acquired from the serpentarium of the Razi Institute of Iran. Following lyophilization, it was transported and stored at -20°C until used. Solutions were prepared with 0.9% normal saline before each experiment. Drugs and reagents used were dexamethasone (Santa cruz Biotechnology Company), heparin (Caspian Pharmaceutical Company, Iran), atropine (Santa Cruz Biotechnology Company) and aminoguanidine (Sigma Aldrich Company). All experiments were performed according to appropriate guidelines.

2.1 Experimental protocol

Male Wistar rats (weighing 200-250 grams) were maintained in plastic cages (three in each) and kept at 12 hours light-dark cycles for 10 days. Ketamine (100 mg/kg)/xylazine (10 mg/kg) cocktail was used for muscle relaxation and anesthesia (9). Rats were placed supine on the table and their body temperature was preserved at 37 ± 1°C with a super head lamp supervised with a rectal tube (Physitemp BAT-12, Texas Scientific Instruments, San Antonio, Texas, USA). A cannula was inserted into the left femoral vein for administration of venom and drugs and the right femoral artery was used to monitor blood pressure parameters with pressure transducer (MLT844, AD instruments, Australia) for documentation of the hemodynamic changes with power lab acquisition system (AD instruments). Finally, the animals were sacrificed with cervical dislocation and their lungs, heart and kidneys were removed and maintained in formalin solution (10%) for pathological analysis. Venom (300 µg/kg, 600 µg/kg and 1,500 µg/kg) dissolved in normal saline (200 µl) were injected intravenously in two minutes via the left femoral vein followed by flushing with normal saline. The following drugs were instilled intraperitoneally ten minutes before venom injection (1500 µg/kg); atropine (1 mg/kg); dexamethasone (1 mg/kg); heparin (300 IU/kg) and aminoguanidine (1.5 mg/kg) and their counteracting effects on the cardiovascular changes were documented (10-12).

2.2 Data analysis

Data are represented as mean ± SD and analyzed using t-test or one-way analysis of variance (ANOVA). Significant differences between experiments were defined as $P<0.001$.

3. Results

3.1 Hemodynamic changes following venom injection

Crude venom injection (1,500 µg/kg) caused a significant drop in blood pressure leading to death after 8 minutes following a transient increase initially compared with normal saline as control (Fig 1).

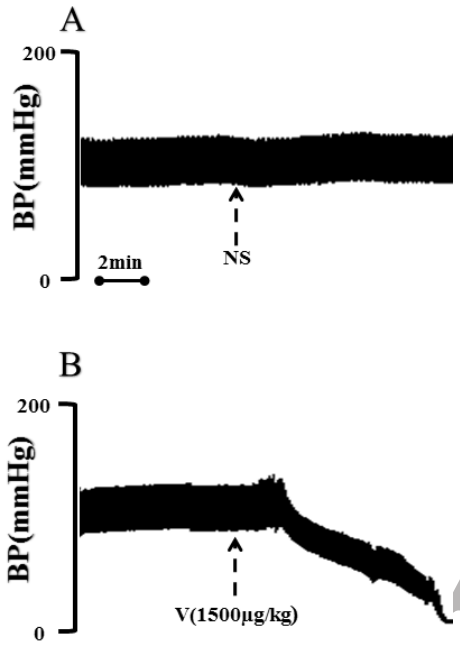

Figure 1. Individuals traces of blood pressure changes to the intravenous administration of normal saline (A) and *N. naja oxiana* venom (B) in rats. Each trace represents the mean of five experiments.

Moreover, there was a significant difference in hypotensive properties compared with lower doses (Fig 2).

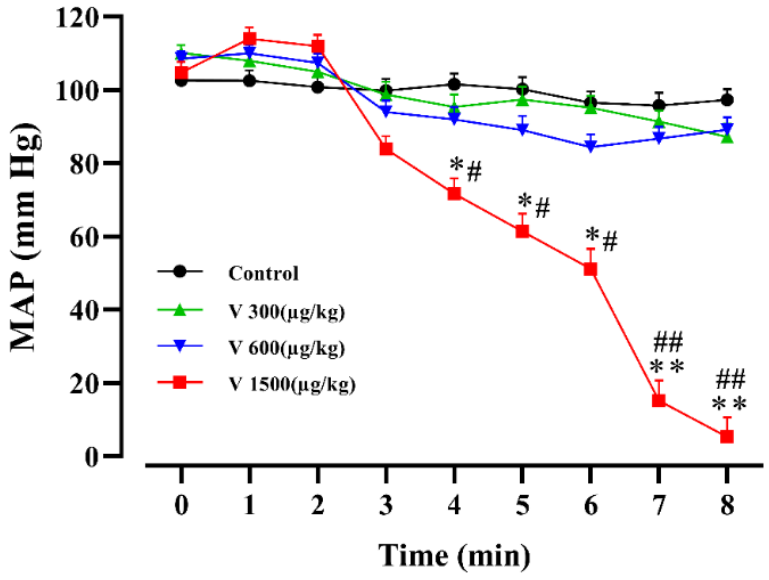

Figure 2. Mean arterial pressure values after intravenous administration of various doses of *N. naja oxiana* venom dissolved in normal saline (300, 600 and 1,500 µg/kg). The points represent mean ± SD (n= 5). *P<0.01, **P<0.001 compared with the time zero. #P<0.01, ##P<0.001 compared with the control group.

It induced transient tachycardia at the beginning and then marked bradycardia until death (Fig 3).

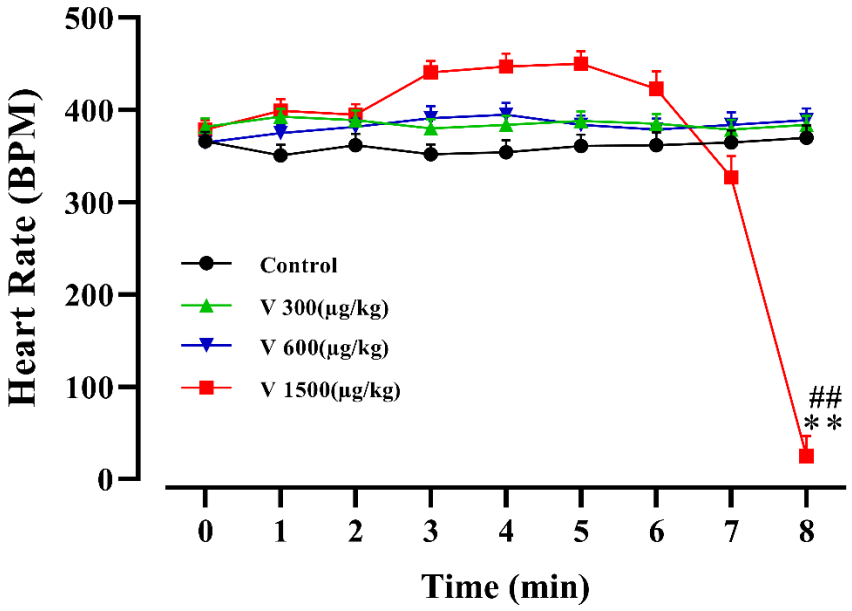

Figure 3. Heart rate values following the administration of *N. naja oxiana* venom dissolved in normal saline (300, 600, or 1,500 µg/kg). The points represent mean ± SD (n= 5). **P<0.001 compared with the time zero, ##P<0.001 compared with the control group.

while no arrhythmogenic properties were seen (data not shown). There was no internal bleeding in euthanized animals following abdominal exploration ruling out hemotoxic potential of this venom.

3.2 Effects of medication and pathological deteriorations in different organs

To examine the possibility of different pharmacological pathways, rats were pretreated with atropine, dexamethasone, heparin and aminoguanidine intraperitoneally ten minutes before venom injection. Pretreatments with atropine as an anticholinergic drug or dexamethasone as a strong corticosteroid had no effects on venom induced marked hypotension and bradycardia at 8 minutes leading to death. Heparin (21 ± 1.2%) and specially aminoguanidine (29 ± 2.1%) significantly prevented hypotension while all animals eventually died 20 minutes later possibly due to hemodynamic deteriorations (Fig 4).

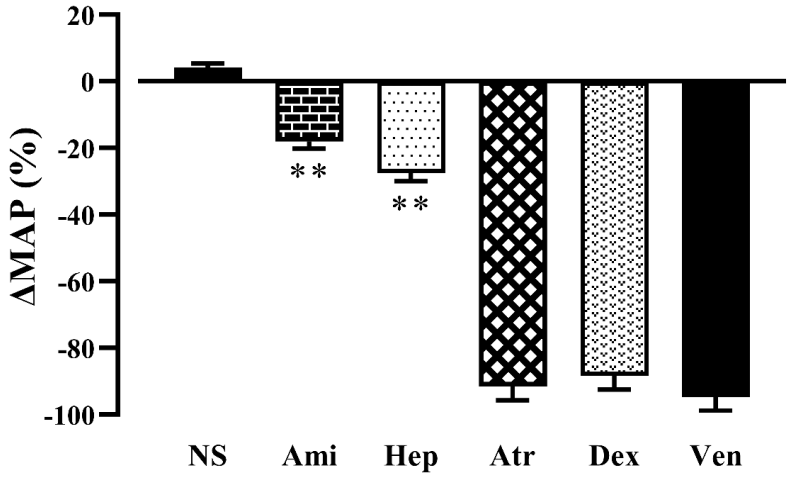

Figure 4. Impacts of premedication using various therapeutic drugs upon hypotensive properties induced by *N. naja oxiana* injection in rats (1,500 µg/kg). Aminoguanidine (1.5 mg/kg) and heparin (300 IU/kg) had significant ameliorating effects on this property. The points represent mean ± SD (n= 5). **P<0.001 compared with the venom alone.

Bradypnea induced with neurotoxic properties was not persuaded during our experiment. According to Fig 5, structural architecture of the lungs was significantly disrupted with rupture of the alveolar walls and presence of the red blood cells and mononuclear cell infiltration causing emphysematous positions. There were no significant changes in heart and other internal organs following this experiment (data not shown).

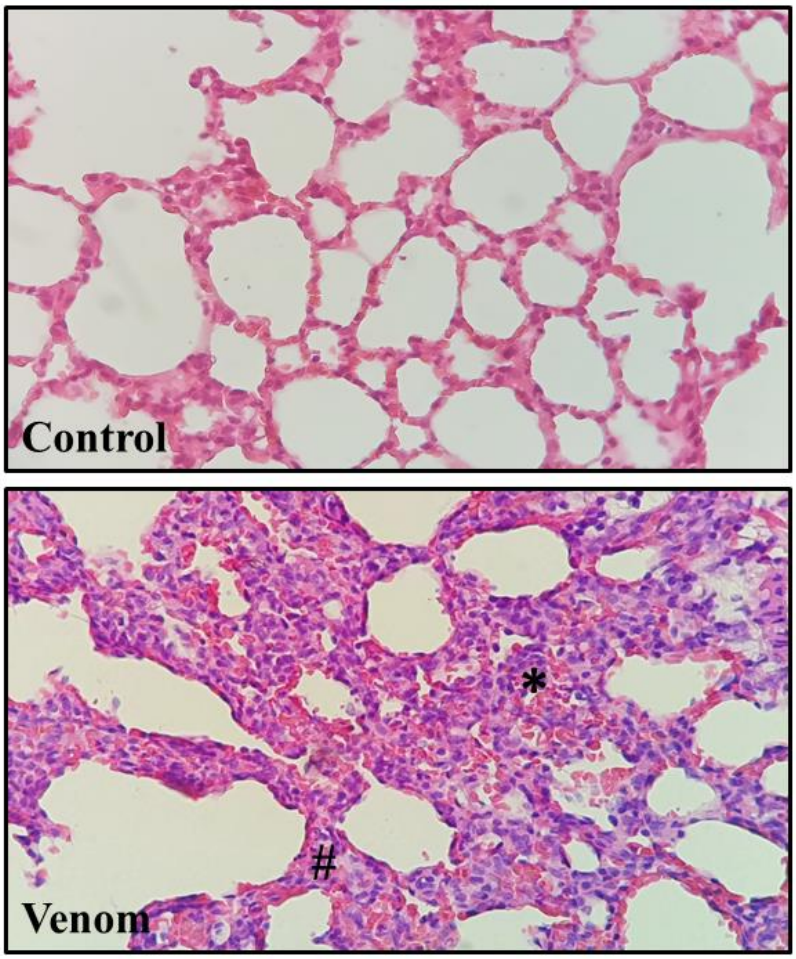

Figure 5. Light microscopic views show the pathological changes in lung stained with hematoxylin and eosin (H&E). Massive hemorrhage and leukocyte infiltration (*) with destruction of the lung structure (#) are seen in envenomed rats compared to control.

4. Discussion

Snake envenomation is a life-threatening problem in tropical and subtropical areas. Despite advances in the specific antivenom production and the therapeutic approaches, snake bite still have significant mortality rates and severe clinical complications (13). In humans, the respiratory failure and neurological problems are the leading causes of death in the early hours after envenomation with cobra bites, while there are limited data on animal studies (9, 14). In addition, the potential mechanisms responsible for hemodynamic changes as a result of snake envenomation have not

been identified exclusively. Therefore, in this preliminary study, for the first time, we investigated causative problems following intravascular injection of *Naja oxiana* venom in anesthetized rats and explored the mechanisms involved.

Based on the previous articles explaining low cardiovascular potency of the elapidae family (12, 15) and our pilot studies, three different groups were selected. The strength of *Naja oxiana* venom for cardiovascular problems was weak compared to *Naja haje* venom, as another member of the cobra family (16). For this reason, the results of the present study demonstrated that the intravenous injection (1,500µg/kg) led to a transient increase in mean blood pressure followed by a sharp drop and death within 8 minutes in all animals close to the potency of *Lachesis muta* snake venom in another study (12). It should be noted that this profile of blood pressure following envenomation have similarities to the cardiovascular effects of other snake venoms (12, 17). It has been proposed that the adrenergic storm with a great release of catecholamines induced by snake venom is likely to have contributed to transient hypertension (18). In addition, several potential mechanisms have been suggested for the venom-induced hypotension, including overactivation of cholinergic system, adrenergic system block, and the releases of nitric oxide, histamine, prostaglandins and other vasodilators by the components of the venom acting either directly or indirectly on the vascular system (12, 19, 20).

Limited studies have investigated the cardiovascular mechanism of *Naja* venom. In one of these published articles, it was shown that the cardiotoxins of *Naja oxiana* causes the decreases of contractility in the heart muscle and tonic contraction in the aorta rings as well as disturbances in calcium currents following injection (21). Systemic vasodilation has a cardinal responsibility for cardiovascular collapse in snake envenoming, and nitric oxide and histamine are important factors in this regard (9). Our findings provided evidences that aminoguanidine as an iNOS inhibitor caused delay in death due to the development of hypotension, implicating that nitric oxide probably plays an important role in this phenomenon. In addition, it has been reported that histamine-like substances in venom or the stimulation of histamine release by snake venom is one of the causes of hypotension (19, 22). It should be noted that neutralizing effects of higher doses of aminoguanidine and heparin will be investigated separately or together for these events in our further studies. On the other hand, atropine as an anticholinergic drug and dexamethasone as a phospholipase A2 inhibitor did not inhibit venom-induced hypotension that rejects the role of these

factors in this situation. According to the inhibitory effects of premedications like heparin and aminoguanidine, it is mandatory that hypotension should be evaluated as one of the main causes of the animal mortalities.

No signs of arrhythmia and histological alterations in heart and other internal organs were seen in our study following exposure to venom .Angaji et al. exhibited that the intramuscular injection of *Naja oxiana* venom (140 µg/kg) caused the bradycardia and T tall in the rabbit's ECG (23). The discrepancies between the ECG findings between our studies may be stemmed from differences in the animal used or the type of snake species. Finally, our histological analyses showed that the lung architectures were destructed with hemorrhage that may be attributed to negative inotropic effects leading to pulmonary edema. Moreover, there was no bleeding in abdominal area after venom injection ruling out hemotoxic properties of this venom (24, 25). According to our data, anaphylactic shock is possibly introduced as a leading cause of the mortality due to vasodilation in this study, making further experiments mandatory to determine the therapeutics approaches for envenomed patients.

Author's contribution

This study was designed and performed by RS and SZ. Venom extraction and toxicological experiments were performed by HFK, ZA, NMD, EK and ZD, while the hemodynamic interpretation was analyzed by RS. All authors accepted the last revision.

Conflict of interest

None

Ethical issues

This study was performed by the local research committee with code number IR.BPUMS.REC.1400.099.

Acknowledgements

All these experiments were performed based on the approved protocols of our own research department. We thank the members of our animal house for their cooperation in this study.

Funding

This study was approved by the science and research committee of the Bushehr University of Medical Sciences.

Data availability

The data are available on request from corresponding author.

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