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EFFECTS OF *Androctonus crassicauda* (OLIVIER, 1807) (SCORPIONES: BUTHIDAE) VENOM ON RATS: CORRELATION AMONG ACETYLCHOLINESTERASE ACTIVITIES AND ELECTROLYTES LEVELS

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ABSTRACT: Scorpions can be considered living fossils because they have changed so little during the last 400 million years. They are venomous arthropods of the Arachnida class and regarded as relatives of spiders, ticks and mites. The aim of the present study was to evaluate the toxicity of *Androctonus crassicauda* (Olivier, 1807) venom and its effects on the acetylcholinesterase (AchE) activity and on electrolytes levels in rats. Animals were divided into seven groups of five rats each. Test groups received 250µg/kg of venom solution while control group was treated with 200µl of physiological saline solution (PSS). Blood samples were collected from the animals on the 1^{st}, 2^{nd} 4^{th}, 8^{th}, 12^{th}, and 24^{th} hours after subcutaneous injection of venom. Animals were monitored for 24 hours. *Androctonus crassicauda* venom significantly reduced AchE activity on the 12^{th} hour when compared with control group. A statistically negative correlation between Na^+ and K^+ ($p<0.05$) and a positive correlation between Na^+ and CL^- ($p<0.001$) ions levels were observed after the administration of *A. crassiccauda* venom to rats. We can conclude that the differences in the electrolytes levels are due to acute renal failure, since elimination of toxin occurs primarily via the kidney.

KEY WORDS: scorpion, *Androctonus crassicauda*, venom, acetylcholinesterase, electrolytes.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Within the phylum Arthropoda, scorpions (subphylum Chelicerata, order Scorpionida) are the oldest terrestrial species known (33, 35, 36, 39). Scorpion envenomation remains a real health problem in many countries in the world, especially in tropical and subtropical regions (4, 6-8, 10, 17, 24, 25, 30-32, 37, 41, 50). Scorpions that are dangerous to humans belong to the family Buthidae, comprising approximately 1,500 species. From these, the genera *Androctonus*, *Leiurus*, *Buthus*, *Buthotus*, *Mesobuthus* and *Heterometrus* can be found in the Old World. Over 450 million years, they have developed an efficient system to subdue their preys using potent neurotoxic compounds produced in their venom glands (11, 32, 42, 46), such as a variety of biologically active components: enzymes, peptides, nucleotides, lipids, mucoproteins, biogenic amines, and other unknown substances (5, 22, 23, 26, 27, 29, 32, 38, 48, 49).

These toxins can alter neuronal action potentials and cause neurotransmitters release from cholinergic and adrenergic neurons (13, 18, 22, 23, 24, 29, 33, 38, 40, 43, 48). Neurotoxins also disrupt synaptic vesicles, leading to the cessation of acetylcholine (ACh) release and to the blockage of neuromuscular transmission (3, 24, 38, 42, 46). In vertebrates, ACh is the major transmitter at neuromuscular junctions, autonomic ganglia, parasympathetic effector junctions, sympathetic effector junctions, and at many sites in the central nervous system. It is released by motor nerve cells and once bound to its receptors on muscle fibers, it stimulates the fibers to contract. The glands that receive impulses from the parasympathetic part of the autonomic nervous system are stimulated in the same way (44, 47).

The aim of the present study was to evaluate the toxicity of *Androctonus crassicauda* (Olivier, 1807) venom as well as its effects on cholinesterase activity and on electrolytes levels in rats.

MATERIALS AND METHODS

Venom

Venom was obtained from mature *A. crasssicauda* scorpions by electrical stimulation of their telsons. The venom was mixed with sterile double distilled water and centrifuged at 15,000 rpm for 15min at 4°C. Supernatant was immediately lyophilized

and stored at -20°C until use. Dry venom was dissolved in physiologic saline solution (PSS; 0.9% chloride solution) to 200µg/ml.

Animals

Healthy male albino Wistar rats, weighed 170±10g, aged three months old, were used for quantitative determination of cholinesterase. They were bred at the Medical Faculty of Ankara University Animal Facilities. Throughout the experiment, animals were kept in the experiment room under room temperature (22±2°C) and 60±10% humidity. They were divided into seven groups of five rats each.

Experimental protocol

Each rat of the test groups received 250µg/kg of venom solution. Control group (Group 0) received 200µl PSS. All groups were subcutaneously injected. Blood samples were collected from each animal on the 1^{st} (Group 1), 2^{nd} (Group 2), 4^{th} (Group 3), 8^{th} (Group 4), 12^{th} (Group 5), and 24^{th} (Group 6) hours after venom injection. Groups were monitored for 24 hours. Animals were anesthetized with ether; blood samples (2.0-4.0ml) were collected through cardiac puncture (1), placed into centrifuge tubes, allowed to clot for 1h at room temperature (23±1°C) and centrifuged for serum collection. Serum samples were stored at 4°C until use. Biochemical analyses were carried out at the Biochemistry Department of the Veterinary Faculty of Ankara University. The levels of Na^+, K^+, Cl^-, and Ca^{2+} ions were evaluated as biochemical parameters; phosphorus (P) levels and AchE activity were analyzed by using cholinesterase kit (Model, Spinreact). Cholinesterase activity was expressed as unit per liter (U/l) of sample; Na^+, K^+, Cl^-, Ca^{2+} and P levels, as mmol/l. Data were presented as means ± standard error (S.E.) and were statistically analyzed using ANOVA.

RESULTS

Table 1 shows the effects of *A. crassicauda* venom on AchE activity, Na^+, K^+, Cl^- and Ca^{2+} levels in the serum, and P levels on the $1^{st,}$ 2^{nd} 4^{th}, 8^{th}, 12^{th} and 24^{th} hours after administration. Statistically significant differences (p<0.05) were found among Na^+, Cl^-, and AchE levels in the serum. *Androctonus crassicauda* venom significantly reduced AchE activity (p=0.04) only after 12 hours (Figure 1). A significant decrease

was observed in Na^+ and Cl^- levels after 1, 2, 4, 8, and 12 hours, compared with control (p<0.05).

A positive correlation between Na^+ and Cl^- levels was observed and was statistically significant (p<0.001), whereas a negative correlation was noticed (p<0.05) between Na^+ and K^+ levels (Figures 2 and 3).

Table 1: Effects of *Androctonus crassicauda* venom on acetylcholinesterase (AchE) activity and on serum Na^+, K^+, Cl^-, Ca^{2+} and P levels in rats on the 1st, 2nd, 4th, 8th, 12th, and 24th hours after administration.

	Measured parameters					
Groups	**AchE (U/l)**	**Na^+ (mmol/l)**	**K^+ (mmol/l)**	**Cl^- (mmol/l)**	**Ca^{2+} (mmol/l)**	**P (mmol/l)**
G0 (Control, n=5)	1148.00 ± 34.25	142.60 ± 0.67	4.52 ± 0.05	106.40 ± 1.16	10.34 ± 0.08	4.54 ± 0.48
G1 (1h, n=5)	1185.25 ± 85.56	137.75 ± 0.62*	4.40 ± 0.24	98.00 ± 1.08*	10.25 ± 0.21	4.15 ± 1.26
G2 (2h, n=5)	1114.00 ± 47.42	136.25 ± 1.31*	4.57 ± 0.10	99.00 ± 1.47*	10.35 ± 0.11	2.87 ± 0.20
G3 (4h, n=5)	1184.50 ± 131.21	136.25 ± 1.03*	4.77 ± 0,16	99.75 ± 1.31*	10.15 ± 0.17	5.12 ± 0.76
G4 (8h, n=5)	1146.20 ± 22.50	137.60 ± 1.02*	4.60 ± 0.26	100.40 ± 1.02*	10.20 ± 0.20	5.60 ± 0.29
G5 (12h, n=5)	832.00 ± 26.90*	136.80 ± 1.28*	4.80 ± 0.13	100.60 ± 1.40*	10.76 ± 0.15	4.41 ± 0.53
G6 (24h, n=5)	1361.25 ± 43.00	139.00 ± 0.00	4.60 ± 0.00	102.50 ± 0.28	10.42 ± 0.19	5.86 ± 0.76

Results are presented as mean ± standard error
n: Number of animals per group
* p<0.05, compared with control rats

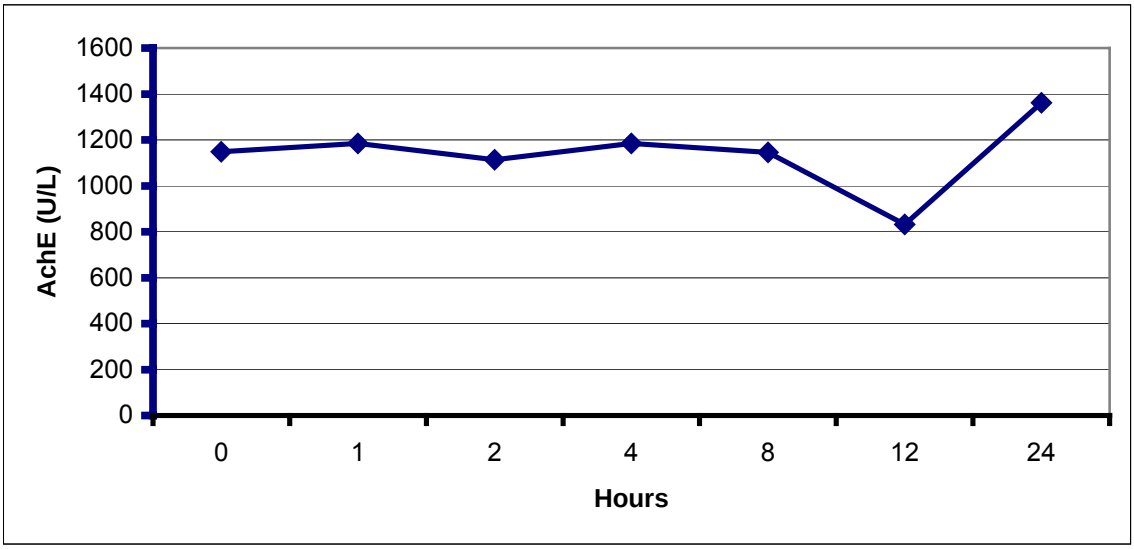

Figure 1: Acetylcholinesterase (AchE) activity throughout 24 hours after *Androctonus crassicauda* venom injection. There was a significant reduction on the 12th hour.

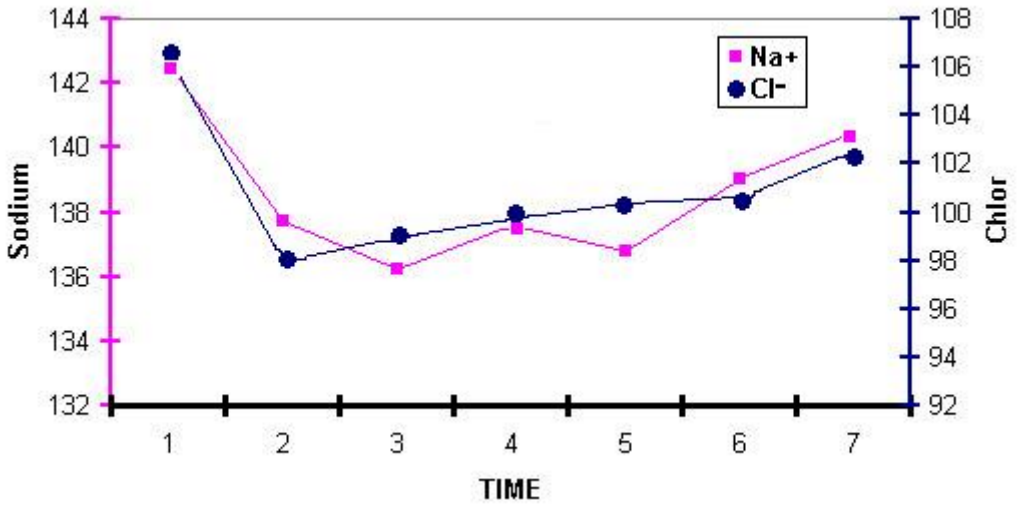

Figure 2: Comparison between Na^+ (Sodium) and Cl^- (Chlor) ions levels after administration of *Androctonus crassicauda* venom to rats. A statistically positive correlation (p<0.001) was observed.

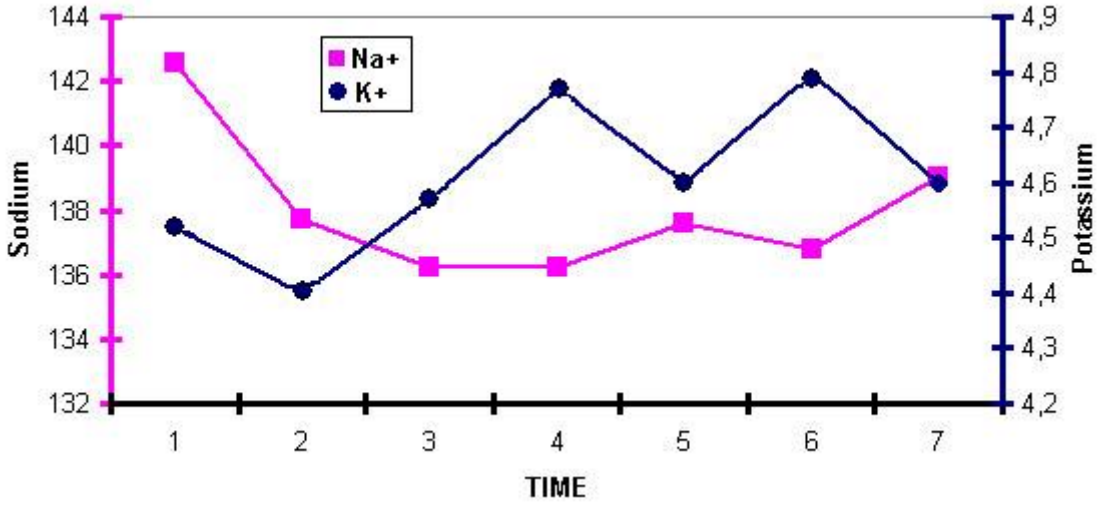

Figure 3: Comparison between Na^+ (Sodium) and K^+ (Potassium) ions levels after administration of *Androctonus crassicauda* venom to rats. A statistically negative correlation (p<0.05) was observed.

DISCUSSION

Scorpions use their venom for both prey capture and defense. They become active at night and sting for their own protection against humans (37). Several studies have reported that severe scorpion envenomation can cause an autonomic storm resulting in a massive release of catecholamine, angiotensin II, glucagon and cortisol, and can change insulin secretion in the human body (9, 34). These changes in the hormonal milieu lead to a syndrome of energy deficit and to an incapability of vital organs to utilize the existing metabolic substrates, resulting in myocardial damage, cardiovascular disturbances, peripheral circulatory failure, pulmonary edema, and many other clinical manifestations alone or in combination, producing multi-system organ failure and death (34).

Scorpion venom stimulates neural sodium channels, thus resulting in autonomic storm. Both branches of the autonomic system are stimulated leading to sweating, salivation, vomiting, fasciculation, hypotension, hypertension, bradycardia or tachycardia, ventricular premature contraction, cool extremities, pulmonary edema, shock, and priapism in men. The majority of the envenomation symptoms are due to a massive release of catecholamines, which play an important role in the pathogenesis of scorpion sting; they are released from the adrenal glands and postganglionic nerve endings and act either on α-adrenergic receptors, increasing the peripheral resistance, or on β-adrenergic receptors, increasing the cardiac contractility or causing release of renin from the kidneys. Pulmonary edema, as a result of scorpion envenomation, is due to myocardial dysfunction. Bradykinin-induced secretory pulmonary edema is secondary and occurs because of kallikrein stimulation due to tissue damage caused by anoxia and accumulation of oxygen free radicals if cardiogenic manifestations are not managed early enough with prazosin, which enhances insulin secretion by blocking α-receptors on β cells in the pancreas. Hyperkalemia and hyperglicemia may also occur in the victim due to autonomic storm (16, 18-20, 27, 28, 31, 33, 34, 47).

Scorpion venoms are composed of different concentrations of neurotoxins, cardiotoxins, nephrotoxins, phosphodiesterases, hyaluronidases, glycoaminoglycans, histamines, serotonins, tryptophans, and cytokine releasers (14, 48).

The venom can act either at the pre-synaptic or at the post-synaptic region. The long-chain neurotoxin causes stabilization of voltage-dependent sodium channels in the

open position, leading to prolonged, repetitive firing of somatic, sympathetic, and parasympathetic neurons. This repetitive firing results in autonomic and neuromuscular rather than excitatory symptoms and prevents normal nerve impulse transmission. It also causes an excessive release of neurotransmitters such as epinephrine, norepinephrine, glutamate, aspartate and acetylcholine (14).

Acetylcholine is synthesized in certain neurons by the enzyme choline acetyltransferase from the compounds choline and acetyl-CoA. Normally, acetylcholine is quickly removed after having performed its action; this is done by the enzyme acetylcholinesterase which converts acetylcholine into choline and acetate. The devastating effects of nerve agents are due to the inhibition of acetylcholinesterase, leading to continuous stimulation of the muscles, glands and central nervous system. Certain insecticides are effective because they act as cholinesterase inhibitors in insects and cause a build-up of ACh in the synaptic junction, resulting in muscle fasciculation (9, 12, 44).

According to several studies, cardiovascular manifestations are due to the direct effects of excessive catecholamines circulating in the blood after autonomic hyperstimulation of the cholinergic system. The sympathetic branch of the autonomic nervous system usually predominates (3, 5, 12, 19, 24). However, early parasympathetic effects caused by scorpion venom toxins may respond to atropine, which reduces the muscarinic effects of ACh when there is blockage of AchE. Thus, the use of atropine is supported in animal models that have shown an increase in survival time following envenomation (3, 42, 44).

Venoms from species of the *Androctonus* genus are very toxic (37). According to lethality tests, the same species presented several LD_{50} values. Signs and symptoms depend on various factors including the genus, species, age, weight, feeding state and structure of the scorpion as well as the amount of venom injected, the number of stings, the sting site, the susceptibility of the patient, the period elapsed between the sting and the first medical aid, and the climate of the region (3, 15, 19, 37).

Androctonus crassicauda venom can specifically stimulate acetylcholine receptors throughout the body and therefore it can be considered as a neurotoxic venom (27, 37). Similarly to those of other scorpion species from different parts of the World, *A. crassicauda* venom can directly increase ACh concentration (27, 37). Radmanesh (40) emphasized that adrenergic signs, pain and no clinical symptoms occur with low

concentrations of the venom, because it cannot reach a threshold to stimulate ACh receptors. On the other hand, cholinergic signs only occur with high venom concentrations (27, 37). Most of the symptoms are caused either by the release of catecholamines from the adrenal glands (sympathetic nerves) or by the release of acetylcholine from postganglionic parasympathetic neurons (3, 5, 18, 19).

In the current study, a significant decrease in AchE activity occurred only 12 hours after venom administration, compared with control. Probably because the venom dose used was lower than the dose needed to stimulate acetylcholine receptors.

Several studies have reported that the electrolytes levels in the serum change during scorpion envenomation (2, 7, 12, 16, 19-21, 24, 29, 34, 36, 40, 45). Similarly, hyponatremia and hypocloremia were observed in the rats of the present study following *A. crassicauda* venom injection. There was a positive correlation between Na^+ and Cl^- ions levels. Potassium levels increased slightly, but they were not statistically significant on the 2^{nd}, 4^{th}, 8^{th}, 12^{th}, and 24^{th} hours after venom administration, compared with control. There was a statistically significant negative correlation between potassium and sodium levels. We have found results similar to other studies in electrolyte disturbances like hyponatremia and relative hyperkalemia (2, 28, 29, 42, 47).

In animal models, elimination of toxins is primarily via the kidney. Following venom injection into mice, the highest toxin concentration was found in the kidney, heart and lungs (12). Isolated cases of acute renal failure have been reported following stings by scorpions (39, 44). After scorpion envenomation, cardiovascular failure could be observed as a complication of pulmonary edema as well as respiratory arrest (35, 40). These cardiac effects result from sympathetic and parasympathetic stimulations, direct myocardial toxicity and possible electrolyte disturbances (40). In the present study, autopsy was not performed on all animals; however, we can consider differences in the electrolytes levels are due to acute renal failure, since elimination of toxin occurs primarily via the kidney.

Rats developed hyponatremia, hypocloremia and relative hyperkalemia, which corroborate several previous studies (12, 16, 19, 20, 27). There was a statistically positive correlation between Na^+ and Cl^- ions levels, although a statistically negative correlation was observed between Na^+ and K^+ ions levels. *Androctonus crassicauda* venom showed its toxicity by altering the electrolytes balance, especially sodium and

chlorine ions levels in the serum, rather than by inhibiting AchE activity. We considered that all changes in the measured parameters are due to acute renal failure, as the toxin is eliminated primarily via the kidneys.

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