Pakistan J. Zool., vol. 47(1), pp. 97-101, 2015.

Effect of *Odontobuthus odonturus* (Scorpiones: Buthidae) Venom on Potassium and Sodium Ions in Albino Mice

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Abstract.- Scorpion venom contains various neurotoxic and haemato-pathological peptides with versatile binding affinity of ion channels and transmembrane receptors. Therefore, scorpion toxins have been vital to investigate for researchers. *Odontobuthus odonturus* is one of the common scorpions in sandy areas of Punjab, Pakistan. In the present study, we evaluated the toxic effect of *O. odonturus* on sodium (Na^+) and potassium (K^+) ions by introducing venom in *Mus musculus*. Animals were treated with different doses (*i.e.,* 120, 170, 200 and 250µL) of scorpion venom orally and intraperitoneally. Results showed marked decline in potassium ions of treated groups in both treatment types. However, non-significant effect on sodium ions was recorded. The venom proved to be toxic because noticeable arrythmia, paralysis, congestion and frequent fecal discharge was noticed in treated groups and at 250 µL dose all the animals were found dead after 18th hour of intraperitoneal treatment. We concluded that venom of *O. odonturus* contains peptides which are selective against K+ channels which may exhibit fetal effects on nerve impulse transmission and homeostatic balance.

Keywords: *Odontobuthus odonturus,* Na^+ ions, K^+ ions, blood plasma and neurotoxins.

INTRODUCTION

Scorpion venom comprises a wide array of molecules constituting immense naturally occurring peptide library with diverse biological activities (Possani *et al.,* 1999; Shirmardi *et al.,* 2010). These peptides bind specifically on pharmacological targets especially on ion channels and mediates their effects variably in multiple ways (Martin-Eauclaire and Couraud, 1995). Toxins modulate their effect on Na^+, K^+, Cl^- and Ca^{++} ion channels selectively on nerve membranes and muscles transmembranes (Possani *et al.,* 2000). More than 400 of these toxic peptides (Shirmardi *et al.,* 2010) ranging from 1-9 KD have been identified so far (Possani *et al.,* 1999; Rates *et al.,* 2008). These toxic peptides inhibit or activate vast number of ion channels, acetylcholine receptors, acetylcholine esterase, membrane coagulation and anticoagulation pathways (Jalali *et al.,* 2012; Bogin, 2005).

Scorpion toxins can be divided into two groups based on their molecular weights. The first group contains 60-75 amino acids cross linked by four disulfide bridges and referred as "Na^+ channel long-chain toxin" (Goldin, 2001; Gordon *et al.,* 2003). Due to their pharmacological effect on sodium channels these toxins are further classified as α and β toxins. However, second group imparts serious lethal effect by blocking potassium channels, so called as "K^+ channel toxins" (Tytgat *et al.,* 1999. It contains 30-40 amino acids cross linked by three (Miller, 1995; Gracia *et al.,* 1991) or four disulfide bridges (Kharrat *et al.,* 1997). They are shorter than Na^+ channel toxins but structurally closely related to them (Bontems *et al.,* 1991). K^+ channel toxins share a common dense scaffold typically formed by an *α*-helix and a *β*-sheet stabilized by disulfide bridges (Bontems *et al.,* 1991) and K^+ channel blockers toxins are highly lethal to humans (Savio-Galimberti *et al.,* 2012). Several components from scorpion venom, those affect on Cl^- and Ca^{++} have also been described. However, they have very little or no influence on venom toxicity for mammals (Shirmardi *et al.,* 2010).

Scorpion toxin directly affects the sympathetic and parasympathetic nervous system (Vatanpour *et al.,* 2013). Presynaptic neurotoxins

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(β-neurotoxins) inhibit the release of neurotransmitters, acetylcholine and noradrenalin, while post synaptic neurotoxins (α-neurotoxins) block acetylcholine receptors (Sabatier *et al.*, 1996; Whitehead, 1995) resulting in central and peripheral neurotoxicity, cardiotoxicity and metabolic alterations (Ismail, 2003).

Odontobuthus odonturus (Pocock, 1897) is a buthid scorpion found in undisturbed sandy areas with patches of vegetation cover. In the present study we investigated the toxic effects of this scorpion species on the physiology of Na^+ and K^+ ions channels using *Mus musculus* as a model organism. We also compared the toxicity of venom, administered by two different methods *i.e.*, oral and intraperitoneal. This study is the first repot of toxicity of *O. odonturus* venom on mammals.

MATERIALS AND METHODS

Scorpion collecting and venom extraction

Adult scorpions (n=35) were collected from undisturbed sandy areas of Sargodha district. Collection was done at night using portable Ultraviolet (UV) lamps. Venom extraction was done by electrically stimulating the base of scorpion's telson with commercial electric adapter adjusted to 15 mVolt. One electrode of the adapter was connected to steel plate while the other to a steel pin. During electric stimulation telson of scorpion was inserted into 1.5 mL plastic tube to collect the venom. Collected venom was stored at -20°C for later use.

Sample preparation

Freeze dried venom weighing 60 mg was dissolved in 1.5mL double distilled water to make stock solution and allowed to dialyze for 48 hours at 4°C (Masihipour, 2005). After dialysis, venom was centrifuged at 14,000 rpm for 15 minutes and supernatant was collected in 1.5 mL plastic tube. Four doses *i.e.*, 120µl, 170µl, 200µl and 250µl were prepared from stock solution.

Experimental protocol

A total of 45 healthy albino mice with an average weight between 25-30 g were used in this study. All the animals were housed in nine different cages under controlled light (325 lux), humidity (40-50%) and temperature (26-32°C). They were fed with standard diet.

Venom treatment

Animals were divided into nine groups *i.e.*, Group 1 to 9. Each group contained 5 animals of similar weight and age. Group 1 was kept as a control. Groups 2 to 5 were treated with 120, 170, 200 and 250 µl venom orally, whereas group 6-9 received these does intraperitoneally (*i.p.*).

All groups were kept separately and observed for 24 h. After 24 h, the mice were anesthetized with chloroform in excess and then sacrificed. By using 1 cc syringe, 300 µL blood from each mouse was collected in gel coated tube using heart puncture technique. The Na^+ and K^+ concentrations were determined with the help of flame photometer (FP 20).

Statistical analyses

One way analysis of variance (ANOVA) followed by Tukey's test was applied to compare concentrations of sodium and potassium among different groups. The Na^+ and K^+ concentrations between two differently treated groups were compared using t-test. SPSS (13) was used for statistical analyses.

RESULTS

Oral treatment

A significant decline in K^+ in all treated groups at 24 h post treatment was observed (df=3, 16; F= 30.161; P=0.001). Multiple comparison performed by using Tukey's test showed that concentration of K^+ among treated groups did not differ statistically but all treated groups have significantly lower concentration of K^+ compared to control (see Table I vertically). The difference in Na^+ of orally treated and control groups was non-significant (df=3,16; F=1.541 and P =0.242, Table II).

Intraperitoneal treatment

Significant difference in K^+ of control and *i.p.* treated mice groups at 24 h post treatment was recorded (df= 3, 16; F= 30.16; P= 0.001). The

concentrations of ions decreased by increasing the venom dose (Table I vertically). Non-significant difference for Na^+ ions in the control and *i.p.* treated groups was observed (df= 3, 16; F= 1.54; P= 0.24, Table II).

In Tables I and II, values of K^+ and Na^+ ions against the venom dose of 250 µL are not given as all animals treated *i.p.* with this dose died at 18 h of post treatment.

Table I.- Comparison of K^+ ions concentration in oral and intraperitoneally treated mice at 24 h post treatment.

Concentration (µl)	K^+ ions		T-value	P-value
	Oral treatment	Intra-peritoneal treatment		
0 (control)	8.58±0.17[b]	8.58±0.17[b]	Control was same for both treatments	
120	4.90±0.14[a]	4.68±0.40[a]	2.58	0.06
170	4.78±0.30[a]	4.36±0.18[a]	8.55	0.001*
200	4.67±0.58[a]	4.22±0.18[a]	6.14	0.009*
250	4.53±0.58[a]	all animals at this dose died	-	-
F-value	26.28	30.16		
P-value	>0.001	>0.001		

Note: P-value with * sign is indicating a significant difference. Superscripts in the table are only valid vertically.

Table II.- Comparison of Na^+ ions concentration in oral and intraperitoneally treated mice at 24 h post treatment.

Concen. (µl)	Na^+ ions		T-value	P-value
	Oral treatment	Intra-peritoneal treatment		
0 (control)	144.0±1.86[a]	144.0±1.86[a]	Control was same for both treatments	
120	143.00±1.73[a]	144.5±1.50[a]	2.22	0.08
170	148.20±1.39[a]	142.40±2.45[a]	6.08	0.02*
200	147.80±2.83[a]	143.80±2.47[a]	5.91	0.01*
250	149.20±2.92[a]	all animals at this dose died	-	-
F-value	1.541	1.491		
P-value	0.242	0.247		

Note: P-value with * sign is indicating a significant difference. Superscripts in the table are only valid vertically.

DISCUSSION

In present study, we observed a rapid decline in concentrations of K^+ ions after venom treatment by both methods (*i.e.,* oral and *i.p.*). From this finding we can predict that venom of *O. odonturus* may act selectively against all the classes of K^+ activated channels but does not impart considerable change in Na^+ activated ion channels. Our results are in accordance with Pamela and Papponel (1987) who reported that scorpion venom from number of species blocked K^+ channels of nerve fibers. Similarly, toxicity of *Odontobuthus doriae* (Thorell, 1876) towards neuromuscular preparations and axons of nerve cells has also been reported by Jalali *et al.* (2007).

Jover *et al*. (1980) divided the scorpion toxins into two classes and each class consists of several peptides according to their pharmacological action. The long chained toxins are considered as Na^+ toxins. These toxins are primarily divided into two major subtypes (*i.e.*, α and β-toxins) and their molar mass is between 6-8 KDa. Lebrun *et al*. (1997) have demonstrated that short peptides with molar mass ranging between 3-4 KDa are specific toxins for all K^+ activated channels. Zafar *et al.* (2013) found that out of five peptides in the venom of *O. odonturus,* four were below 5KDa. The lower levels of K^+ observed during present study in both types of treatments might be due to selective action of low molecular weight peptides against K^+ channels. Low molecular scorpion toxins also exhibit devastating effects on mammals, insects and crustaceans (Zlotkin *et al.,* 2001; Loret and Hammock, 2001). This might be the reason of death of all scorpions at 250µL venom dose in the present study.

Sofer and Gueron (1990) have reported that the neurotoxins of the scorpion venom are more potent and severely lethal than the neurotoxins of snake venom. Oyama and Takahashi (2003) have further explained that this toxicity is due to selective target of scorpion venom neurotoxins on voltage gated Na^+ and K^+ channels including calcium activated K^+ channels on the excitable cells of nerves and muscles. Gwee *et al.* (2002) reported that most of the toxins in the scorpion venom can interfere selectively the voltage gated active N^+ and K^+ channels which ultimately results in the release

of autonomic neurotransmitters.

According to Becerril *et al.* (1970) the scorpion venom usually increases the membrane permeability to sodium by opening the voltage sensitive Na^+ channels. It is reversed with calcium entry and blockage of calcium-activated K^+ channels that leads towards hyperkalemia with the release of catecholamines. Mandal and Deshpande (2004) have reported the patients with extreme hyperkalemia are at the risk of potentially fatal abnormal heart rhythms (arrhythmia). Furthermore, increased K^+ influx contributes the hyperkalemia condition that causes prolonged hyperglycemia and enhanced glycolysis (Masihipour, 2005). D'Suze *et al.* (2003) and Fukahara *et al.* (2003) have explained that hepatic and renal injury may consequence due to excessive release of catecholamine, so resultant vasoconstriction and hypertension in the liver occurs. However, other substances may be attributable in the scorpion envenomation such as cytokines and inflammatory mediators. Strong *et al.* (2001) examined that iberiotoxin and tamulotoxin are the toxins isolated from *Mesobuthus tamulus* and these toxins inhibit K^+ channels selectively.

CONCLUSION

The toxins of *O. odonturus* showed modulated effects on nerve membranes of excitable tissues and therefore can cause hyperkalemic condition with excessive release of catecholamines and neurotransmitters. For this reason, these toxins are capable to exhibit fatal effects in mammals, insects and other crustaceans.

ACKNOWLEDGEMENTS

This project was supported by the University of Sargodha, Sargodha. We are thankful to Dr. Kawaja Raees Ahmed and Tahir Abbas for providing animals and for their technical support during the experiment. We are also thankful to Mr. Haroon and Muhammad Ali (laboratory staff) of Department of Biological Sciences, University of Sargodha for their assistance in the laboratory.

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(Received 18 March 2014, revised 28 March 2014)