

## Haematological Effects of *Odontobuthus odonturus* (Arachnida: Buthidae) Venom in Albino Mice

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**Abstract.-** Scorpion envenomation represents a significant cause of morbidity and mortality in many countries. *Odontobuthus odonturus* is a medically important scorpion that inhabits sandy areas. In the present study we evaluated the in-vivo toxic effects of *O. odonturus* venom on male albino mice. Scorpions were collected from undisturbed sandy areas of district Sargodha (Punjab), Pakistan. We extracted the venom and injected intraperitoneally into healthy albino mice. Haemato-pathological effects of the venom were studied on different blood parameters *i.e.*, total leucocyte count (TLC), neutrophil count, RBC's count and platelet count, lymphocytes, eosinophils and monocytes. The venom of *O. odonturus* caused drastic change in blood physiology of treated animals. We observed a significant decrease in RBC's, platelets and lymphocytes of treated animals. However, treated animals showed marked increase in neutrophils, hemoglobin and TLC count. There was no difference in the count of monocytes and eosinophils between treated and control animals. It is concluded from the study that venom of *O. odonturus* is potentially harmful to mammals as it badly affects the blood physiology which may ultimately lead towards many disorders in the body.

**Keywords:** *Odontobuthus odonturus*, venom, haematological studies, toxicity, blood physiology.

### INTRODUCTION

Scorpions are venomous arachnids with highly conserved morphology (Murthy and Krishina 2002; Ruming *et al.*, 2010). They occupied tropical and subtropical areas and found everywhere except Antarctica (Isbester *et al.*, 2003; Petricevich, 2010). They have potential to bear very drastic environmental conditions; therefore, they are very successful invertebrates (Zlotkin *et al.*, 2001). Till now 16 scorpion families and approximately 1500 scorpion species and sub-species have been discovered (Bawaskar and Bawaskar, 2012). Amongst all families Buthidae is considered as the most poisonous and medically important (Chowell *et al.*, 2006; Michael and Victor, 2003).

Scorpion venom is a rich source of biochemically activated enzymes, amines and low molecular weight peptides (Gomez *et al.*, 2010). Till now 400 peptides have been isolated from scorpion venom (Shirmardi *et al.*, 2010). These peptides range in mass from 1,000 to 9,000 Da (Rates *et al.*, 2008; Schwartz *et al.*, 2007) and mainly target

sodium, potassium, calcium (Choung *et al.*, 1998) and chloride channels (Possani *et al.*, 2000). Scorpion venom also has tremendous ability to inhibit or activate the ion channels, acetylcholine receptors, acetylcholine esterase, membranes coagulation and anticoagulation pathways with highly selective affinity (Bogin, 2005; Jalali *et al.*, 2012).

On the basis of molecular weight scorpion toxins are divided into two classes. The first class contains 60-70 amino acids cross linked by four disulfide bridges (Gordon *et al.*, 2003). They are exclusive for sodium channels of excitable cells (Rochet *et al.*, 1979). The second class contains 30-40 amino acids cross linked by three or four disulfide bridges (Kharrat *et al.*, 1997). These are single chained toxins and due to their binding activities these have been described as  $\alpha$  or  $\beta$  toxins. Their particular target site is K<sup>+</sup> channels and calcium activated K<sup>+</sup> (Rodriguez and Possani, 2005).

Envenomation by the scorpions is very common in rural areas (Ozkan *et al.*, 2011). Toxic peptides of scorpion venom may cause death of their victims (Mirakabadi *et al.*, 2006). Scorpion venom also exhibits various other effects like, hemolytic (Cournet *et al.*, 1994), muscular effect (Jalali *et al.*, 2007; Vatanpour *et al.*, 2012), renal failure (De

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Sousa *et al.*, 2005), cardiovascular failure (Bakir *et al.*, 2012), pulmonary oedema and respiratory arrest (Ozkan and Carhan, 2008). Scorpion's bites may induce myocarditis and left ventricular hypertrophy due to long standing hyper tension (Sarakar *et al.*, 2008). More than 70% people suffer from gastrointestinal manifestation after scorpion stung (Deshpande *et al.*, 2005). Scorpion venom also causes pulmonary (Amaral and Rezende, 1997; Coelho and Pessini, 2007) and renal oedema (De Sousa *et al.*, 2005; Severino *et al.*, 2009).

Present study was aimed at investigating the *in-vivo* hematological effects of *Odontobuthus odonturus* venom on male albino mice. Outcome of the study will be helpful in devising better curative strategies against bites of venous scorpions of the area.

## MATERIALS AND METHODS

### Scorpion collection

The study was conducted at the Department of Zoology, University of Sargodha, Pakistan from May 2012 to April 2013. Healthy and adult scorpions were collected from undisturbed sandy areas of district Sargodha (32.083N; 72.671E), using portable ultraviolet (UV) lamps at night. Scorpion collection was done during no moon cycle period of month.

### Venom extraction and sample preparation

The venom was extracted by electrical stimulation (20 MV). Platinum electrode was used for electric stimulation to extract the venom from the swollen telsons of scorpions. The milking process continued as long as was necessary, usually between 10-15 seconds. The venom was collected in Eppendorf tubes (1.5 ml) and stored at -20°C for later use.

The lyophilized dried venom (60mg) was dissolved in 900µl double distilled water and kept for 48 h at 4°C (Masihpour, 2005). After dialysis, venom was centrifuged at 14,000 rpm for 10 min at 4°C. Mucous material and un-dissolved residues were separated apart and the supernatant was collected in Eppendorf tubes (1.5ml).

### Experimental protocol

The toxicity of the *O. odonturus* venom was

determined on Stock Webster strain of albino mice, *Mus musculus*.

Twenty five healthy adult male albino mice, Webster strain, *Mus musculus* with an average weight between 25-30 g were used in the study. All the animals were housed in different cages under controlled light (325 lux), humidity (40-50%) and temperature (26-32°C). They were fed with standard diet.

Four doses *i.e.*, 120, 170, 200 and 250µl of stock solution (60mg/900µl double distilled water) were used to test the toxicity in this experiment. Five groups each of 5 albino mice were used. The first group was kept as control and given normal diet (unmarked), whereas the remaining four groups (each of 5 albino mice) were administered with venom intraperitoneally at 120, 170, 200 and 250 µl using insulin syringes. All groups were placed separately under observation for 24 h.

After 24 h, mice were anesthetized with chloroform, sacrificed and blood was drawn directly from heart using 1 ml syringe. The blood was stored in EDTA coated tubes and used for total leukocyte count (TLC), RBC's count, neutrophils, eosinophils, monocytes, lymphocytes and platelet count and determination of haemoglobin level. The departmental ethical committee granted permission to conduct the experiment.

### Statistical analyses

One way analysis of variance (ANOVA), followed by Tukey's test, was applied by using SPSS (13) to compare the blood parameters of different groups. Differences were considered significant when P-values were less than 0.05.

## RESULTS

The inflammation at the injection site, restlessness, increased urination and fecal discharge were noticed only in the treated groups. All the members of fifth group, which was injected with highest dose (*i.e.*, 250µl) were paralyzed after 12 h and died after 18 h.

Table I shows the various haematological parameter of mice after treatment with different doses of venom. The total number of RBCs decreased significantly in the treated groups ( $F_{3,16}=7.99$ ;  $P=0.002$ ), whereas, TLC increased with the

**Table I.-** Effect of crude venom of *Odontobuthus odonturus* on different blood parameters of *Mus musculus*.

Dose	Total RBCs (1x10 <sup>6</sup> /μl)	Blood parameters						Platelet count (1x10 <sup>8</sup> /μl)	Haemoglobin (g/dl)
		White blood cells (WBCs)							
		TLC (1x10 <sup>3</sup> /μl)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)			
Control	8.99±0.40 <sup>b</sup>	2.26±0.10 <sup>a</sup>	12.0 ±1. 70 <sup>a</sup>	87.4±2.73 <sup>b</sup>	1.0±0.00 <sup>a</sup>	2.0±0.00 <sup>a</sup>	1103.60±31.66 <sup>d</sup>	8.94±0.40 <sup>a</sup>	
120 μl	6.60±0.69 <sup>a</sup>	2.72±0.25 <sup>ab</sup>	27.0±3.83 <sup>b</sup>	53.0±5.35 <sup>a</sup>	1.0±0.00 <sup>a</sup>	1.6±0.20 <sup>a</sup>	880.80 ±70.07 <sup>c</sup>	9.64±0.38 <sup>b</sup>	
170 μl	6.60±0.50 <sup>a</sup>	3.54±0.23 <sup>b</sup>	36.2±2.51 <sup>b</sup>	52.8±6.46 <sup>a</sup>	1.2±0.20 <sup>a</sup>	1.8±0.24 <sup>a</sup>	788.20±91.89 <sup>ab</sup>	10.06±0.39 <sup>b</sup>	
200 μl	5.68±0.34 <sup>a</sup>	3.50±0.13 <sup>b</sup>	34.6±3.93 <sup>b</sup>	49.6±4.20 <sup>a</sup>	1.2±0.20 <sup>a</sup>	1.6±0.24 <sup>a</sup>	704.60±47.09 <sup>a</sup>	12.38±0.36 <sup>c</sup>	

**Note:** Values in a column having different superscripts are significantly different.

increase in venom concentration ( $F_{3,16} = 7.06$ ;  $P = 0.003$ ). The amount of neutrophils were considerably higher in the treated groups compared to the control ( $F_{3,16} = 13.35$ ;  $P = 0.001$ ). The percentage of lymphocytes and platelets and hemoglobin level decreased significantly among treated animals ( $F_{3,16} = 13.35$ ;  $P = 0.004$  for lymphocytes,  $F_{3,16} = 19.01$ ;  $P = 0.001$  for platelets and  $F_{3,16} = 14.67$ ;  $P = 0.011$  for hemoglobin). Non-significant differences were recorded for monocytes and eosinophils ( $F_{3,16} = 0.97$ ;  $P = 0.445$  and  $F_{3,16} = 0.667$ ;  $P = 0.58$  respectively).

## DISCUSSION

Most of the scorpions of family buthidae are medically important as their venom is highly toxic (Gwee *et al.*, 2002; Mebs, 2002). Peptides of scorpions are more poisonous and dangerous than that of the snake toxins (Sofer and Gueron, 1990). Scorpion's toxins act particularly at the voltage gated sodium and potassium ion channels in the excitable nerve cells. Therefore, they can easily kill their pray and predator (Adiguzel, 2010). Envenomation by the scorpions of buthidae family causes respiratory distress, tachycardia, tachypnea, sialorrhoea and myocarditis. Scorpion venom also causes hemorrhage, severe and mild necrosis and congestion (Deghani *et al.*, 2004).

Deghani *et al.* (2012) and Abdoon and Fatani (2009) reported considerable decrease in hematocrit and RBC's count after envenomation by scorpion venom. Their finding is in accordance with our results, that all the envenomed groups showed significant decrease in total RBC's count compared to the control group. It suggests that envenomation

induced hemolysis of RBC's. The venom of *O. odonturus* was abstemiously poisonous because at 250 μl dose all the intraperitoneally treated mice were died. Emam *et al.* (2008) recorded about 48.73% erythrocytes hemolysis in a scorpion envenomed persons. The venom of genus *Odontobuthus* is highly toxic for the blood vessels (Klassen and Watkins, 2003). According to Farzanpey (1994) scorpion stung modulates hemolytic symptoms of their victim's body. Pipelzadeh *et al.* (2007) and Jalali *et al.* (2012) also observed a rapid drop in hematocrit and preferential increased hemolysis in the victim of scorpions. Similarly, Emam *et al.* (2008) also reported the decreased hematocrit and considerable reduction in the RBC's number in the scorpion stung patients. Histopathological changes recoded by Zayerzadeh *et al.* (2010) after injecting venom were myocytolysis (lysis of myocytes), coagulation necrosis, focal hemorrhage, thrombus formation both in myocardium and on endocardial surfaces.

In the present study significant increase in average TLC count after intraperitoneal treatment was observed. Results are in accordance with Deghani *et al.* (2012) and Chitnis *et al.* (1993), they also found elevated number of WBC's in the envenomed people. This leads towards the leukocytosis after envenomation. Significant diminution in lymphocytes was observed in intraperitoneally treated groups. However, non-significant difference in the count of eosinophils and monocytes was recorded.

The level of neutrophils increased more than twice in treated animals compared to the control. Altenburg *et al.* (1997) elucidated that injection of scorpion venom cause mobilization of neutrophils

from the bone marrow to the blood circulation in rats that caused neutrophilia which explained the release of norepinephrine and its action on  $\alpha_1$ -adrenoceptors. Borges *et al.* (2000) reported that number of neutrophils circulating in the blood stream after envenomation by scorpions increased, while matured neutrophils simultaneously decreased. These increased neutrophils may participate in the propagation of lung injury in children and older age people. Neutrophilic leukocytosis attributed by scorpion envenomation has previously been studied in humans (Guéron *et al.*, 1993; Bucarechi *et al.*, 1995) and animals (Cordeiro *et al.*, 2006).

The platelet count in the present study decreased markedly which is in concurrence with Pinto *et al.* (2010). However, in controversy to our findings Emam *et al.* (2008) reported 61.4% increase of platelet count due to action of scorpion poison. According to Heemskerk *et al.* (2002) blood platelets impart promising role in the regulation of adhesive and coagulation properties and confers a primary physical barrier necessary to arrest the bleeding. Moreover, they provide a catalytic surface by assembling the enzymes complexes and catalytic cascades and also accelerate the fibrin formation. This alteration is consequent for the occurrence of pulmonary hemorrhage and thrombocytopenia which is reported by Longenecker and Longenecker, (1981). Corrêa *et al.* (1997) observed hemorrhagic lesions in the heart, lungs, and kidneys of rats after poisoning with *Tityus serrulatus* venom.

The level of hemoglobin in the intraperitoneally treated animals was also increased with venom dose. This increase in hemoglobin is probably due to excessive hemolysis of erythrocytes (Shahbazzadeh *et al.*, 2007). Furthermore, increase in hemolysis of erythrocytes may increase the amount of bilirubin which may leads towards hemoglobinuria. Emen *et al.* (2008) confirmed the presence of hematuria in the envenomed animals and negative effect of scorpion poison on the kidney and damage to the capillary system of glomeruli. On the other hand, Nunan *et al.* (2003) and Nunan *et al.* (2004) have showed in histo-pathological studies that venom caused glomerular congestion, dilated vessels of interstitium and focal interstitial congestion in kidneys of all animals.

## CONCLUSIONS

It is concluded that scorpion venom cause drastic changes in the blood physiology which may leads to towards renal failure, hemolytic anemia by erythrocytes fragmentation, vasoconstriction in blood vessels, haemoglobinuria, pulmonary oedema, myocarditis and thrombocytopenia. In the present study we first time reported the toxicity of venom of *O. odonturus*. More research is needed so that effective and improved treatments for scorpion venom manifestations can be designed.

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