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Optimization of the Conditions for Maximum Recovery of Venom from Scorpions by Electrical Stimulation

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ABSTRACT

Aim of the present study was to extract venom from common buthid scorpions of eastern Punjab, Pakistan and to optimize the conditions to get maximum yield of venom via electric stimulation. We also recorded surveillance of scorpions without food in laboratory. We found that current intensity of 25V is the best suitable for venom extraction. When current was applied at the base of telson we obtained 5.214±0.36, 7.17±1.20 and 4.90±0.31 (µl) venom from *Odontobuthus odonturus, Androctonus finitimus* and *Mesobuthus tamulus*, respectively. There was significant difference in the quantity of venom yielded from studied species. *A. finitimus* produced maximum quantity of venom followed by *O. odonturus* and *M. tamulus.* It is also evident from study that *M. tamulus* is more resistant to hunger as compared to *O. odonturus* and *A. finitimus. M. tamulus* can survive for more than 3 months without food while *O. odonturus* and *A. finitimus* are less tolerant to starvation. It is concluded from the study that extracting venom from living scorpion by electric stimulation of telson is a convenient method as it is least harmful for scorpion and most efficient in venom recovery. Furthermore, keeping of scorpions in the laboratory for investigatory studies is very easy and need less maintenance care and efforts.

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HMT and MMA collected the samples from field. RY and SN executed the experiment work. HMT analyzed the data. MA wrote the article.

Key words
Scorpion venom, venom extraction, electric stimulation.

INTRODUCTION

Scorpion's venom has developed much attraction for researchers because of its toxic properties, which help to enhance our understanding about various biological phenomenon. It is a mixture of various bioactive compounds, which are being used in investigatory study of pharmaceutical research (Oukkache *et al*., 2008). Therefore, venom extraction or venom milking has been the crucial and primary step of such studies. Previously various methods have been applied for extraction of scorpion's venom (Wee *et al.*, 1993; Plessis, 2005), such as mechanical stimulation (Louis, 1976; Nisani *et al*., 2007), maceration of whole telson (Ozkan *et al.*, 2006) or puncturing of venom gland and electric stimulation of venom gland (Candido and Lucas, 2004; Gopalakrishnakone *et al*., 1995). However, every method has its own limitations. The composition, quantity and activity of venom greatly varies with method of extraction (Oukkache *et al.*, 2013).

Being stinging in nature scorpions remained unfamiliar and forbidden creatures as a research object. In fact scorpions are vastly adaptable creature that can survive in extreme conditions (Rochet *et al.*, 1979; Zlotkin *et al.*, 1978; Huber *et al.*, 2005). Due to their low metabolic rate, scorpions do not require food frequently (Neubauer, 2013). Likewise, due to nocturnal feeding habit and preference of living in burrows or under litter layer, scorpions can withstand long dark periods (Hadley, 1974). These properties made them model organisms to keep in laboratory for various studies.

Scorpion venom is chiefly composed of protenaceous molecules (Nisani *et al*., 2007) and protein biosynthesis is mainly associated with the high metabolic costs (Morgensten and King, 2013). So venom production is an expensive phenomenon in terms of metabolic cost. Furthermore, venom regenerated shortly after envenomation is biochemically less efficient (Boeve *et al*., 1995). Therefore, scorpions limit their physical activity and seek for shelter to reduce the chance to encounter a predator or cannibalism, and enhance their metabolic activity after envenomation or venom milking (Nisani *et al*., 2007).

Scorpions of genus *Androctnous, Mesobuthus* and *Odontobuthus* are commonly found in eastern Punjab, Pakistan. Venom of these scorpions could be used for antivenom production, diagnostic and pharmacological research. To get considerable quantities of venom it is a convenient way to keep and rear them in the laboratory. However, to maintain their populations in an artificial habitat for venom, one should be aware of some basic principles of their life history such as their food preferences, life expectancies, tolerance to hunger,

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drought and heat etc. Conditions and methods must be drawn which are suitable to achieve maximum venom recovery and putting minimal stress on animal.

Keeping in view above facts, present study was designed. The aim of the study was to determine efficiency of venom milking by electrically stimulating the venom gland and optimize conditions such as current intensity and site of current application to get maximum yield of scorpion venom. We also tried to observe injurious effects (if any) of electric stimulation on general activity of scorpion. Furthermore their ability to resist hunger was also recorded.

MATERIALS AND METHODS

Animal collection and maintenance

Study was conducted from May 2014 to October 2014. For the study, *Odontobuthus odonturus* and *Androctonus finitimus* were collected from sandy areas of district Sargodha, Punjab, Pakistan. Portable battery operated ultra violet (UV) lamps were used to collect scorpions during no-moon nights (at 9-12pm). *Mesobuthus tamulus* were collected from mud houses, under bricks or mud lumps and picked up with 12 forceps. Scorpions were brought to laboratory in tightly closed plastic bottles. In laboratory, ten to fifteen scorpions were kept in plastic boxes (35×16×11m) (L×W×H). Boxes were layered with 2 inch thick layer of gravel and sand. Water was sprayed periodically to maintain humidity and a shallow bottom container filled with water, was kept in corner of the box as drinking water source. Lid of boxes were designed to provide proper aeration. Scorpions were fed upon cockroaches, house flies, crickets or grasshoppers, once in a week (one large prey *i.e*. cockroach/cricket/grasshopper and 5house flies per scorpion).

Venom extraction

Scorpion venom was extracted according to method described by Ozkan and Fillazi (2004) with some modifications. Electric current of regular intensity was applied for 5 seconds, with the help of pointed electrodes. Current was applied at the base of telson to shock the animal until venom was ejected. For better conductance of current scorpion body was immersed in 10% saline solution. We used batteries of varying current intensity (*i.e.,* 5v, 10v, 15v, 20v, 25v, 30v) for scorpion milking. Extracted venom was collected in graduated capillary tubes to determine volume of venom. Once the current intensity, best suitable for maximum venom yield was achieved, current of that intensity was applied at various body parts (*i.e.,* cephalothorax, metastoma, joints between metasoma and the base of telson) of each scorpion species, to determine the best site for current application for venom extraction.

Surveillance without food

To observe the ability of scorpion that how long they can withstand hunger, individuals of each species were divided into experimental and control groups (n= 10 for each group). Scorpions of both groups were kept individually in separate wide mouth plastic bottles (3 width ×6 height). Scorpions of experimental group were kept without food for 3 months however; each scorpion of control group was offered one cockroach after a week. Mortality of scorpion in both groups was assessed weekly.

Statistical analysis

To compare the quantity of venom extracted from different species, one way analysis of variance (ANOVA) followed by Tukey's test was applied on raw data by using SPSS 13. Difference between groups was considered significant if p value was less than 0.05.

RESULTS AND DISCUSSION

In our study, we applied different current intensities at various body sites. Our result revealed that base of telson was the best site for application of current to get maximum yield of venom (Table I).

Quantity of venom obtained was also high when current was applied at joints between metastoma. Base of telson was best because this site is nearest to the venom gland and thin chitenous covering at joints made that site more sensitive. Venom recovery was poor when current below the 20V was applied for 3-5 seconds. Maximum amount of venom was recovered at 25V while venom quantity remained same at 30V (Table II). Stiffness of joints between metastoma (in all species under study) was observed when current of more than 30V was applied or repeatedly applied for 5 seconds with five minutes interval (more than 4-5 times, within an hour). Sometimes death of scorpion followed by stiffness of joints was also observed. Death rate was higher in *O. odonturus* as compared to *A. finitimus* and *M. tumulus,* respectively. However, it is not obvious that trauma caused by high intensity current was the only reason of scorpion death. Further study is required to establish this fact. These results suggest that current intensity of more than 30V may be harmful to scorpion species under study. We do not find any documented data addressing physical damages caused by extraction of venom or harmful effects of electric stimulation, however, few publications are available related to metabolic costs of envenomation in terms of induced oxygen consumption (Nisani *et al.,* 2007; McCue, 2006).

Table I.- **Amount of venom recovered (in µl) after application of electric current at different body parts of the scorpion.**

Species	Venom recovery in µl (Mean ±SE)			
	Cephalothorax	**Metastoma**	**Joints between metastoma**	**Base of telson**
Odontobuthus odonturus	1.1±0.41	1.6±0.29	3.6±0.44	5.21±0.36
Androctonus finitimus	1.3±0.57	1.8±0.33	3.7±0.18	7.17±1.20
Mesobuthus tamulus	0.6±0.32	1.4±0.67	2.8±0.19	4.90±0.31

Table II.- **Intensity of electric current best suited for venom extraction from scorpion.**

Species	Venom recovery*				
	5-10volt	**11-15 volt**	**16-25 volt**	**26-30volt**	**31 volt and above**
Odontobuthus odonturus	Poor	Good	Best	Best	Harmful to scorpion
Androctonus finitimus	Poor	Good	Best	Best	Harmful to scorpion
Mesobuthus tamulus	Poor	Poor	Best	Best	Harmful to scorpion

*Poor, 0-2 µl; Good, 2-4 µl; Best, more than 4µl.

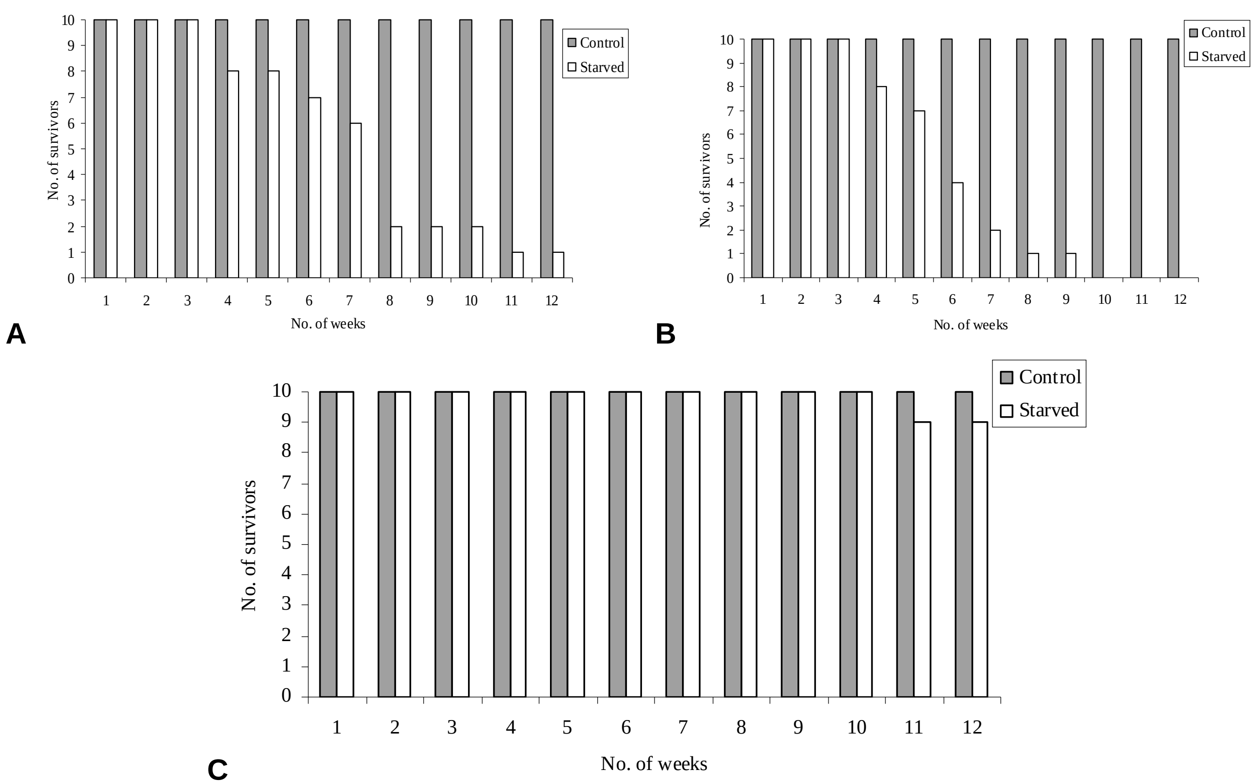

Fig. 1. Effect of desiccation and starvation on survival of *Odontobuthus odonturus* (A), *Androctonus finitimus* (B) and *Mesobuthus tamulus* (C).

We recovered different quantities of venom from different scorpion species by electrically stimulating the telson (Table III). When current of 25V was applied to scorpions of different species we get 5.21±0.36 (µl), 7.17±1.20 (µl) and 4.90±0.31 (µl) venom from *O. odonturus*, *A. finitimus* and *M. tamulus* respectively. However results of ANOVA followed by Tukey's test has shown that quantity of venom obtained from *A.*

finitimus was significantly higher than *O. odonturus* and *M. tamulus* ($F_{2,88}$ = 3.659; *P* = 0.03). Candido and Lucas (2004) used current of 1.250kV with 0.5 Hz frequency to get an average amount of 0.4 mg venom per scorpions of genus *Tityus.* However, the quantity of venom decreased with each consecutive extraction. Ozkan and Filazi (2004) has recovered venom from two different species of *Mesobuthus i.e*., *M. gibbosus* and *M. eupues* using electric stimulus of 24V. Gopalakrishnakone *et al.* (1995) has used current of 150V and 10Hz frequency for 10-15 seconds for successful extraction of venom from *Heterometrus longmanus*. According to them this current intensity do not harm the scorpions, however; average quantity of venom recovered by this method was not mentioned. Latifi and Tabatabai (1979) have recovered an average amount of 0.3mg venom per *A. crassicauda* by electric stimulation and 0.5g by maceration method. Nisani *et al.* (2007) have collected about 37.23 to 39.69µl venom from *Parabuthus transvaalicus* by manually stimulating the telson which was quite higher than the quantity reported by Inceouglu *et al.* (2003) *i.e*., 22µl per scorpion. Although quantity of venom recovered by using maceration or mechanical stimulation is always higher but according to various studies quality is poor in terms of toxicity and antibody production (Ismail *et al*., 1994; Inseouglu *et al*., 2003; Ozkan *et al*., 2007; Oukkache *et al*., 2013).

Table III.- Quantity (Mean±SE) of venom extracted by eclectic stimulation.

Species	No. of animals milked	Average weight (g)	Quantity of venom (µl)
Odontobuthus odonturus	44	0.87±0.12	5.21±0.37 [a]
Androctonus finitimus	12	1.25±0.25	7.17±1.2 [b]
Mesobuthus tamulus	35	1.23±0.15	4.90±0.31 [a]

P, 0.03; F, 3.659; df, 2, 88.

Decrease in venom production with 4-5 consecutive extraction has also been observed (data not documented). We also observed that few *M. tamulus* and *O. odonturus* do not yield venom after 5-6 consecutive extractions (gap between extractions was 15 days). Candido and Lucas (2004) has also associated lower yield of venom from scorpions of genus *Tityus* with consecutive extractions. However, to clarify this fact that whether this decrease in venom quantity is due to consecutive extractions, more investigations are required.

It is a well-known observation that scorpions can thrive harsh conditions, they can cope with hunger, desiccation and cold weather very well (Rochet *et al*., 1979; Zlotkin *et al*., 1978; Huber *et al*., 2005). But how long a scorpion can live without food, is unknown. In our study we tried to find surveillance ability of different scorpion species. *M. tamulus* was found more resistant to hunger and desiccation as they survive more than three months without food and water (Fig. 1C). They usually limit their activity and growth. While *O. odonturus* and *A. finitimus* are more sensitive to starvation and died within 8-9 weeks without food (Fig. 1A,B). We concluded that electric stimulation of venom gland is an efficient method for extraction of scorpion venom and base of telson is more appropriate site for current application. This method does not physically harm the scorpions. In addition, keeping scorpion in laboratory for investigatory studies requires less maintenance efforts.

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