



Research Article

# Acetylcholinesterase Inhibitory Activity of Venom of *Hottentota tamulus* (Fabricius) and *Androctonus finitimus* (Pocock) (Scorpiones: Buthidae) in *Musca domestica*

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## Article History

Received: October 19, 2019  
Revised: November 14, 2019  
Accepted: December 03, 2019  
Published: December 24, 2019

## Authors' Contributions

NR and AH conducted the experiments. HMT and SA designed the study. HMT provided the research materials and lab facilities and approved the manuscript. MKM analysed the data. SA, SYK and MKM wrote the manuscript.

## Keywords

Scorpions, Venom, Fractions, HPLC, *Musca domestica*

**Abstract** | In the present study, different venom fractions were isolated from the crude venom of *Hottentota tamulus* (Fabricius, 1798) and *Androctonus finitimus* (Pocock, 1897) by HPLC. The toxicity and acetylcholinesterase activity of selected venom fractions were determined using of *Musca domestica* adults. Only one fraction (out of 7) from *H. tamulus* venom and one fraction (out of 5) of *A. finitimus* venom were selected on the basis of their maximum yield. The approximate molecular weights of these selected venom fractions were determined by SDS Gel electrophoresis. The molecular weight of venom fractions of *H. tamulus* and *A. finitimus* used in the study were 36kDa and 54kDa, respectively. The doses of venom fractions used during the study ranged from 10-30µg/ml. Both venom fractions significantly inhibited the activity of AChE. However, 30µg/ml dose of both scorpion venom caused 100% mortality in adult *M. domestica*. It was concluded that the venom of scorpions contains peptides fractions that may be used as potential bio-pesticides.

**To cite this article:** Riaz, N., Tahir, H.M., Mukhtar, M.K., Hassan, A., Ali, S. and Khan, S.Y., 2019. Acetylcholinesterase inhibitory activity of venom of *Hottentota tamulus* (fabricius) and *Androctonus finitimus* (pocock) (scorpiones: buthidae) in *Musca domestica*. *Punjab Univ. J. Zool.*, **34(2)**: 207-211. <https://dx.doi.org/10.17582/journal.pujz/2019.34.2.207.211>

## Introduction

Scorpions are included in the list of earliest arachnids on earth (Dunlop and Selden, 2009) with almost 2000 species (Cordeiro *et al.*, 2015). Out of 18 scorpion families, Buthidae with nearly 500 species has a central position (Cordeiro *et al.*, 2015; Diaz-Garcia *et al.*, 2015). Scorpions are the potent arachnids and able to survive in harsh environmental conditions and the presence of venom in their body is one of the reasons behind their success

(Newton *et al.*, 2007). The telson of scorpions contains a pair of venom secreting glands.

A number of neurotoxins are involved in the composition of scorpion venom, that are interacting with different neurotransmitters of nervous system and effecting them in various manners (Ucar and Tas, 2003). The neurotoxins are proteinaceous in nature and are also target specific, so can be used against harmful insects (Bertazzi *et al.*, 2003). These insect toxins depolarized the membrane potential and results in continuous firing of action potential (Petricevich *et al.*, 2013). Scorpion venom enzymes comprises on proteolysis, PLA2, hyaluronidases,

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phosphatases and acetylcholinesterase inhibitors (Khodadadi *et al.*, 2012; Jalali *et al.*, 2012). These enzymes play a major role in various morbid variations in circulatory system, central nervous systems and skin (Seyedian *et al.*, 2010).

AChE, an ammonium containing enzyme, was the first neurotransmitter which was separated in 1920 (Mushigeri and David, 2005). Its presence is observed in many types of conducting tissues like nerves and muscle tissue as well as central and peripheral tissues. Acetylcholine is a major neurotransmitter at the neuromuscular junctions (Lopez and Pascual-Villalobos, 2010; Colovic *et al.*, 2013). Acetylcholinesterase, a serine hydrolase, is involved in hydrolysis of the ACh into acetate and choline. When ACh binds with AChE, the hydroxyl group of acetyl reacts with the serine at the ester site of AChE and loses its acetyl group (O'Brien, 1976). It assists in the conduction of nerve impulse in the organisms. Paralysis and death are ultimately caused due to reduction of AChE activity.

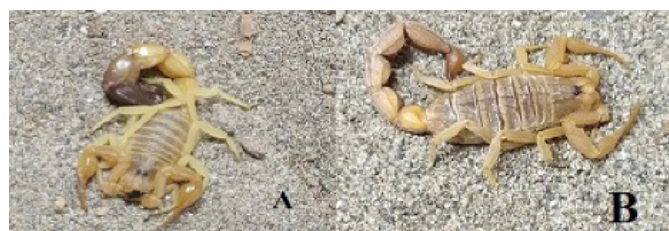
The AChE inhibition was reported in many different organisms when exposed with the scorpion venom (Isbister *et al.*, 2003; Ozkan and Filazi, 2004). Keeping in view, this capability of scorpion venom, the present study was designed with a purpose to control the high population of disease causing insect i.e., *Musca domestica*. The aim was to expose the *M. domestica* adults with selected doses of scorpion venom peptides and to check its effect on the AChE activity. To achieve this goal, two scorpion species were selected including *Hottentota tamulus* and *Androctonus finitimus*. The effect caused by these selected venom peptides on AChE activity was also compared with an insecticide, Chlorpyrifos, used as positive control in the study.

## Materials and Methods

### Scorpion collection

The study was conducted from May 2016 to August 2017 in Punjab, Pakistan. The scorpions were collected from different areas of Sargodha including mostly rural areas. As scorpions are nocturnal in nature, so most of the collection was done at night and battery operated UV lamps were used for the collection as they glow at night. Total 100 scorpions belonged to species of *H. tamulus* and *A. finitimus* were collected and only adult scorpions were included in this study (Figure 1). The two species of scorpions were examined properly and then identified as *H. tamulus* and *A. finitimus* on the basis of their specific characters. The scorpions were shifted to laboratory in plastic boxes (5"H×3"W. A 2") thick layer of sand was spread at the bottom of the box, to make a proper habitat for scorpion. Water was provided to these scorpions on daily basis in a small sized container. The boxes were designed in such a way so that proper oxygen was supplied to scorpions. Living grass hoppers, cockroaches, houseflies and crickets were provided to scorpions as food once in a week. After 5-6 days of providing food, venom was

extracted by electrical stimulation.



**Figure 1: Scorpion species used in the study. A: *Androctonus finitimus* and B: *Hottentota tamulus*.**

### Venom extraction

Electrical stimulation method was used for extraction of venom as described by Ozkan and Filazi (2004) and Yaqoob *et al.*, 2016. Venom was extracted by placing the scorpion on a flat surface and tape was adjusted on its body to keep it immobile. Then the scorpion stinger was adjusted in the tip of capillary tube. Approximately 22V electric current was applied at the base of telson with the help of pointed electrodes to shock the animal until venom was ejected. Venom droplet was collected in a capillary tube and then supernatant was collected after centrifugation for 15 minutes at 14000rpm in a refrigerated centrifuge (MPW-352R) with temperature maintained at -4°C. Supernatant was chilled at -40°C till additional use.

### HPLC for venom characterization

HPLC (LC.20 AT SPD- M20A) was performed to separate the peptide fractions from the crude venom. For that purpose, running buffer (10% acetonitrile and 0.1% trifluoroacetic acid (TFA)) was prepared. The 50µl of crude venom was taken. The sample was applied to C18 column. Venom fractions were eluted and collected in an Eppendorf's tube. The fractions with the highest peak and with more concentration were selected and further lyophilized. Gel electrophoresis was performed further to determine the molecular weight of selected peptide fractions.

### Gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was used for determination of approximate molecular weight of selected peptides. For this, 12% resolving gel (15m1) and 5% stacking gel (3m1) was used according to the method of Sambrook and Russle (2001). A standard protein marker with known molecular weight (12 to 160 kDa) (Novex, BenchMark™ protein ladder) was used to compare it with the molecular weight of the peptides. Approximately 70V electric current was adjusted in the electric supply initially and then raised up to 85V when dye was seen to enter in resolving gel till the process ended.

### Bioassay

*Musca domestica* were collected from local areas of Sargodha city, Punjab, Pakistan (32° 5' 1" North, 72° 40'

16" East) and shifted in the laboratory in plastic bottles. Because scorpions deliver a small amount of venom each time when extracted, so it was not possible to use residual bioassay method. Only topical bioassay test was performed. *M. domestica* adults were kept in freezer for few seconds to immobilize them. After that these were divided in to one control group (10 flies) and three experimental groups, each group containing 10 flies. Each fly in negative control group was treated simply with water, positive control group flies were treated topically with chlorpyrifos (0.1µl/ml). The flies in experimental groups were treated with 10µg/ml, 20µg/ml and 30µg/ml dose of the selected peptide fractions topically. The mortality rates were assessed after 24 hours. After that time period, heads of these flies were removed and homogenized in sodium phosphate buffer (0.1M, pH: 8.0. The homogenate was centrifuged at 15000rpm for 15 minutes. The resulting supernatant was collected and stored at -20 °C for enzyme estimation. The experiments were repeated thrice to obtain the best results.

#### Acetylcholinesterase estimation and statistical analysis

Ellman *et al.* (1961) method was used for the estimation of AChE and reaction mixture was processed through spectrophotometer (APELDP-303S) at 412nm and 28 °C. The readings were taken thrice for each sample. One-way ANOVA followed by Tukey's test was applied to compare the AChE activity among different groups.

## Results

Results of HPLC are shown in Figure 2. In the figure different peaks are representing different fractions. From the venom of *H. tamulus*, 7 major fractions were obtained numbered from 1-7 (Figure 2A). However, fraction 4 was selected for further study as its yield was maximum. The number of prominent fractions obtained from the crude venom of *A. finitimus* were 5 (numbered from 1-5) but I selected fraction 4 (Figure 2B). Approximate molecular weight of fraction 4 of *H. tamulus* was 36kDa while molecular weight of fraction 4 of *A. finitimus* was

54kDa. Molecular weights were determined by SDS Gel electrophoresis.

Figure 3 depicts the mortality rate in *M. domestica* adults when treated with the selected peptide fraction of the scorpion's venom and chlorpyrifos. Negative control group of *M. domestica* showed  $5 \pm 0.57\%$  mortality. An efficiency of 54kDa of *A. finitimus* venom peptide was noted because it caused high mortalities ( $55 \pm 1.73\%$  with 10µg/ml dose and  $86.67 \pm 1.45\%$  with 20µg/ml dose) in *M. domestica* as compared to the 36kDa of *H. tamulus* venom ( $34.33 \pm 2.33\%$  with 10µg/ml dose and  $71 \pm 2.08\%$  with 20µg/ml dose). With increase in the concentration of venom, increased mortality was observed with both scorpion species. The 100% rate of mortality was observed in *M. domestica* adults when exposed with 30 µg/ml of both scorpion species venom fraction while  $96.89 \pm 0.95\%$  mortality was recorded in Chlorpyrifos treated group (+ve control).

Noteworthy result was obtained when the AChE activity of *M. domestica* adults was recorded after treating them with selected venom peptides. With *H. tamulus* venom peptide, a significant difference of the 1<sup>st</sup> experimental group (treated with 10µg/ml) was noted with other groups (including +ve and -ve control group, 2<sup>nd</sup> and 3<sup>rd</sup> experimental group treated with 20µg/ml and 30µg/ml of *H. tamulus* venom peptide fraction respectively;  $F_{3,8} = 127.75$ ,  $P < 0.01$ ). The 2<sup>nd</sup> and 3<sup>rd</sup> experimental groups differed non-significantly from each other (Figure 4). A significant dissimilarity was found between the +ve and -ve control groups with that of the three experimental groups treated with *A. finitimus* venom peptide fraction (Figure 4). The 4<sup>th</sup> experimental group (treated with 10µg/ml of *A. finitimus* venom peptide) differed considerably from 5<sup>th</sup> and 6<sup>th</sup> experimental groups (treated with 20µg/ml and 30µg/ml of *A. finitimus* venom peptide fraction respectively;  $F_{3,8} = 1.59$ ,  $P < 0.01$ ). Figure 4 illustrates that chlorpyrifos was efficiently inhibiting AChE activity followed by 20µg/ml and 30µg/ml dose of both scorpion species venom peptide fractions.

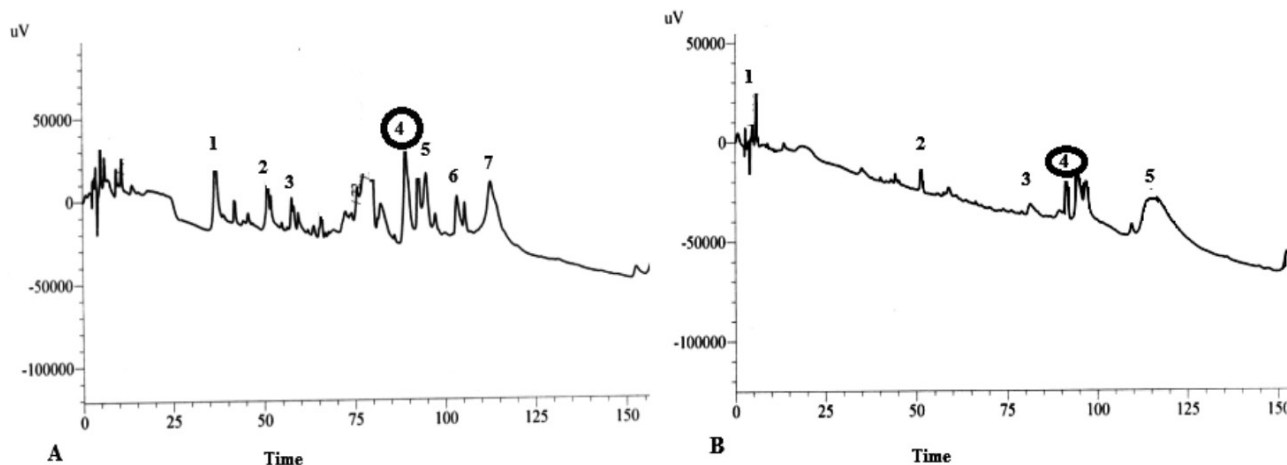
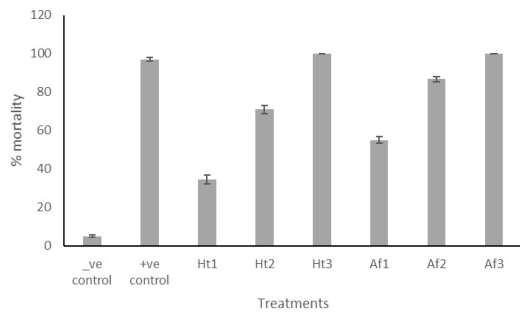
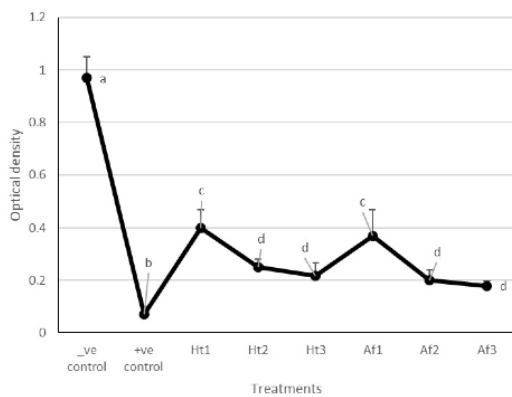


Figure 2: Peptide fractions of scorpion species (A= *Hottentota tamulus* and B= *Androctonus finitimus*) were separated by HPLC.



**Figure 3: Comparison of mean percentage mortality rate in *M. domestica* adults caused by specific doses of selected scorpion species venom peptides and Chlorpyrifos. (\*-ve control= Water, +ve control= Chlorpyrifos, Ht1= 10µg/ml of *H. tamulus* venom peptide, Ht2= 20µg/ml of *H. tamulus* venom peptide, Ht3= 30µg/ml of *H. tamulus* venom peptide, Af1= 10µg/ml of *A. finitimus* venom peptide, Af2= 20µg/ml of *A. finitimus* venom peptide and Af3= 30µg/ml of *A. finitimus* venom peptide). \* Data represented as Mean± SEM.**



**Figure 4: Comparison of AChE activity (Optical density) in *M. domestica* adults caused by specific doses of selected scorpion species venom peptides and Chlorpyrifos. (-ve control= Water, +ve control= Chlorpyrifos, Ht1= 10µg/ml of *H. tamulus* venom peptide, Ht2= 20µg/ml of *H. tamulus* venom peptide, Ht3= 30µg/ml of *H. tamulus* venom peptide, Af1= 10µg/ml of *A. finitimus* venom peptide, Af2= 20µg/ml of *A. finitimus* venom peptide and Af3= 30µg/ml of *A. finitimus* venom peptide). Different alphabets indicates the significant difference between the different groups, same alphabet indicates non-significant difference in the groups. Optical density was recorded with absorbance at wavelength of 412nm**

## Discussion

Scorpion venom comprises of some potent compounds with strong biopesticidal potential (Gurevitz, 2010; Leng *et al.*, 2011; Tahir *et al.*, 2015). Only single peptide fraction was selected from each scorpion specie (used in current study) weighing 36kDa (*H. tamulus* venom fraction) and 54kDa (*A. finitimus* venom fraction). The presence of sufficient amount of these two peptide fractions was also reported by Yaqoob *et al.*, 2016. According to present study, *A. finitimus* venom was proved to be more lethal

as compared to *H. tamulus* venom. The high toxicity of *Androctonus* genus was also reported by Ozkan and Filazi (2004). The venom of both scorpions with 30µg/ml concentration resulted in 100% mortality in adults *M. domestica*.

The insecticidal properties of scorpion venom were also observed by many researchers like Petricevich *et al.* (2013) observed the insecticidal properties of the peptides isolated from the venom of *Buthus martensii* Karsh, *Aandroctonu australis*, *Buthacus arenicola*, *Buthotus judacus* and *Centruroides noxius*. Likewise, Manzoli-Palma *et al.* (2003) reported the insecticidal ability of *Tityus serrulatus* in honey bees and aphids while the mortalities in aphids were also reported due to the venom of *H. tamulus* and *Odontobuthus odontorus* by (Riaz *et al.*, 2017; Tahir *et al.*, 2015) respectively. In the present study, the venom of the two-scorpion species successfully affecting on the activity of AChE and similar observations were noted by (Ozkan *et al.*, 2007) in rats. Similarly, increase in the level of ACh was noted in the organisms exposed with the scorpion venom (Baswaker and Baswaker, 1999; Cardoso *et al.*, 2004).

## Conclusion

It can be concluded from above findings that scorpion venom peptides have an ability to act as bio-insecticide and also have an ability to inhibit the AChE level in the target insect. So, it can be used to control harmful insects that are damaging the agriculture field badly.

### Statement of conflict of interest

Authors have declared no conflict of interest.

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