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

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**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.

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

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**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-García *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,
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 esteric 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 (Ibister 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×2”L) 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% trifloraacetic 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

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’ 2”) during May–August 2016. They were reared on a sugar solution. The flies were used for experiments when they were 3 days old. The flies were kept in small sized containers. The flies were exposed to the 50µg AChE inhibitory activity of scorpion venom peptide fractions for 2 hours. The flies were then checked for paralysis. The test was repeated 5 times.
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 ºC 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 (APELPD-303S) at 412nm and 28 ºC. 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±0.57% mortality. An efficiency of 54kDa of _A. finitimus_ venom peptide was noted because it caused high mortalities (55± 1.73% with 10µg/ml dose and 86.67± 1.45% with 20µg/ml dose) in _M. domestica_ as compared to the 36kDa of _H. tamulus_ venom (34.33± 2.33% with 10µg/ml dose and 71± 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±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 1st experimental group (treated with 10µg/ml) was noted with other groups (including +ve and –ve control group, 2nd and 3rd experimental group treated with 20µg/ml and 30µg/ml of _H. tamulus_ venom peptide fraction respectively; F3,8 = 127.75, P<0.01. The 2nd and 3rd 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 4th experimental group (treated with 10µg/ml of _A. finitimus_ venom peptide) differed considerably from 5th and 6th experimental groups (treated with 20µg/ml and 30µg/ml of _A. finitimus_ venom peptide fraction respectively; F3,8 = 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 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 species (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 scorpiions 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, Aandroctonus australis, Buthacus arescinola, Buthus judacus and Centurroides 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.

References


