**Acetylcholinesterase inhibitory potential of scorpion venom in Aedes aegypti (Diptera: Culicidae)**

Potencial inibidor da acetilcolinesterase do veneno de escorpião em Aedes aegypti (Diptera: Culicidae)

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**Abstract**

Scorpion venom contains a variety of neurotoxins which interact with ion channels and affect their activities. The present study was designed to evaluate the potential of scorpion venom as acetylcholinesterase (AChE) inhibitor by using Aedes aegypti as model organism. Venoms of two species, *Hottentota tamulus* (Fabricius, 1798) and *Androctonus finitimus* (Pocock, 1897) were selected for this study. Two peptides (36 kDa from *H. tamulus* and 54 kDa from *A. finitimus*) were separated from scorpion venom by using HPLC. Selected peptides caused significantly higher mortality in larvae and adults of *Aedes aegypti* than control (no mortalities were observed in control groups). Significant acetylcholinesterase (AChE) inhibitory potential of both peptides was recorded by spectrophotometer. The peptide of *A. finitimus* caused significantly higher mortality (95±1.53% in larvae and 100% in adults) than the peptide of *H. tamulus* (84.33±2.33% in larvae and 95.37±1.45% in adults). While *H. tamulus* peptide was more efficient in reducing AChE activity (0.029±0.012 in larvae and 0.03±0.003 in adults) than the peptide of *A. finitimus* (0.049±0.005 in larvae and 0.047±0.001 in adults). It was concluded that *H. tamulus* venom peptide was more efficiently reducing AChE activity, thus it could be a potential bio-insecticide which can be synthesized at industrial scale for the control of harmful insects.

**Keywords:** scorpions, peptides, HPLC, AChE activity, *Aedes aegypti*.

**1. Introduction**

Being ecofriendly in nature, bio-pesticides are getting popular for the control of target insect populations (Ortiz and Possani, 2015). Scorpion venom, due to its specific toxins against insects, has become the remarkable candidates for the production of bio-pesticides (Tahir et al., 2015). Scorpions use their venom for self-defense and to subdue prey. Scorpions of Buthidae family contain distinctive bio-active neurotoxins (Gwee et al., 2002; Tan et al., 2006; Radha, 2014; Díaz-Garcia et al., 2015). Many neurotoxins of venom target the nervous system of insects, disturbing their ion channels and neural activity (Radha, 2014). The excitatory insect toxins may be helpful...
in designing new insect selective bio-pesticides because of their special modes of action like they target only the nervous system of insects. The neurotoxins present in the scorpion venom are also involved in disturbance of neurotransmitters released at synaptic junctions of nerves (Theakston et al., 2003; Ozkan and Kat, 2005).

A variety of compounds such as hyaluronidase, lipids hydrolyzing enzymes, mucopolysaccharides, serotonin, histamine, proteinases, histamine releasers and different polypeptide compounds like discreplasmin are present in the scorpion venom (Valdez-Cruz et al., 2007; Feng et al., 2008). Scorpion venom also contain PLA2 (phospholipase A2), phosphatases and acetylcholinesterase inhibitors ( Jalali et al., 2012). These enzymes play a major role in various morbidity alterations in circulatory system, central nervous systems and skin (Seyedian et al., 2010). The estimated number of components in scorpion venom range from 72 in Androctonus spp. to 600 in Hottentotta spp. (Batista et al., 2007; Oukkache et al., 2008). The neurotoxins in venom divert the action potential, resulting in the release of neurotransmitters from cholinergic and adrenergic neurons (Theakston et al., 2003). They also block the neuromuscular transmission by stopping the ACh release (Gwee et al., 2002; Ozkan and Kat, 2005; Cordeiro et al., 2015).

Keeping in view, these specific properties of scorpion venom, the current study was intended. The plan was to separate the peptides from the venom of H. tamulus and A. finitimus venom and use them against Aedes aegypti, not only to evaluate their mortality but also their AChE inhibitory potential.

2. Materials and Methods

2.1. Venom collection and characterization

Scorpions (total 60 scorpions) were collected from district Sargodha, Punjab, Pakistan and kept in the laboratory at Department of Zoology, University of Sargodha. Two species of scorpions, Hottentota tamulus (30 scorpions) and Androctonus finitimus (30 scorpions) were used for this study. These scorpions were maintained in the laboratory following the method described in Yaqoob et al. (2017). Venom was collected by electrical stimulation method described by Ozkan and Filazi (2004) and Yaqoob et al. (2017). From this venom, 6 mg venom was dissolved in 0.05% trifloroacetic acid (TFA) in graded water of HPLC. It was centrifuged for 15 mins at 14000 rpm and supernatant was collected. The peptide fractions were separated by HPLC (LC 20 AT SPD- M20A) from the crude venom on C18 column at the flow rate of 1ml/minute. Fractions were collected manually and stored at -20°C. The fractions with the highest peak and in more amount were selected. Approximate molecular weights of two selected fractions were determined by SDS-PAGE by comparing with standard protein markers.

2.2. Model organisms and toxicity assay

Aedes aegypti (larvae and adults) were collected from Insectary, GC University, Lahore, Pakistan. The larvae were kept in plastic cups containing 7 ml water. Larvae were divided into seven groups, each group containing 10 larvae. No treatment was applied to the control group; however, the larvae 1st, 2nd and 3rd experimental groups were treated by mixing 10µg/ml, 20 µg/ml and 30 µg/ml of venom of H. tamulus in the water, respectively. Similarly, larvae of experimental groups 4th, 5th and 6th were respectively treated with 10µg/ml, 20 µg/ml and 30 µg/ml of A. finitimus venom. The mortality rate in control and experimental groups was assessed after 24 h post treatment. After that, each larve was homogenized in 600 µl sodium phosphate buffer (0.1 M; PH 7.0) containing 0.01% (w/v) of Triton X-100. This homogenate was centrifuged at 13500 rpm for five minutes. The supernatant was collected and used further for enzyme analysis.

A. finitimus adults (n=70) were divided into one control and six experimental groups. Control group was left untreated however, 1st, 2nd and 3rd experimental groups was topically treated with 10µg/ml, 20 µg/ml and 30 µg/ml venom of H. tamulus and experimental groups 4th, 5th and 6th were topically treated with 10µg/ml, 20 µg/ml and 30 µg/ml venom of A. finitimus. The mortality rate was assessed after 24 h post treatment. For enzyme estimation the head of each adult mosquito was removed and homogenized in 600 µl sodium phosphate buffer (0.1 M; PH 7.0). Further processing was same as described above.

2.3. Ellman’s assay and statistical analysis

The AChE activity was estimated following the method of Ellman et al. (1961). In this method, DTNB was used as a reagent and Acetylthiocholine iodide as substrate. The absorbance was recorded at 412 nm for 3 min using spectrophotometer (APELPD-3035). The whole experiment was performed in triplicate.

Normality of the data was assessed before data processing. One way-ANOVA followed by Tukey’s test was applied to compare the AChE activity among different groups. Probit analysis was used to compute LC50 values. Statistical Package for Social Sciences (SPSS version 16.0) and Minitab (13.4) were used for statistical analyses.

3. Results

The recorded molecular weights of peptide fractions separated from the venom of H. tamulus and A. finitimus were 36 KDa and 54 KDa respectively. With different concentration of these peptides, the significant mortalities were observed in Aedes aegypti larvae and adults (Table 1). Venom concentration-dependent mortality was observed. Maximum mortality rate in larvae (84.33±2.33%) and in adult (95.37±1.45%) were recorded with 30 µg/ml of venom peptide of H. tamulus. While 95±1.53% and 100% deaths were recorded with the same concentration of venom peptide of A. finitimus in larvae and adults of Ae. aegypti respectively.

A significant decline was found in AChE activity in Ae. aegypti larvae in venom treated groups than control (P< 0.05; Figure 1). It was depicted from the results that A. finitimus venom peptide has more inhibitory potential than H. tamulus venom peptide (Figure 1). The significant
difference in AChE activity between venom treated adults and control in Ae. aegypti was also observed (P< 0.05; Figure 2). Figure 2 showed that the least AChE activity was observed with 20 µg/ml of A. finitimus and with 30 µg/ml of H. tamulus venom peptide in Ae. aegypti adults. The comparison between the activities of AChE in larvae and adults, when treated with 30 µg/ml venom peptide respectively, was depicted in Figure 3. A significant difference between the activities of AChE was observed in the control and experimental groups while H. tamulus was found to be more effective in terms of reducing AChE activity.

**Table 1.** Mortality rate in Ae. aegypti larvae and adults when exposed with different concentrations of selected peptide fraction of H. tamulus (36 kDa) and A. finitimus (54 kDa) venom.

| Aedes aegypti | Control group | H. tamulus venom dose | A. finitimus venom dose |
|---------------|---------------|-----------------------|------------------------|
|               |               | 10 µg/ml | 20 µg/ml | 30 µg/ml | 10 µg/ml | 20 µg/ml | 30 µg/ml |
| Larvae        | No mortality  | 48±0.577 | 74±0.20 | 84.33±2.33 | 47±2.52 | 84.67±2.6 | 95±1.53 |
| Adults        | No mortality  | 34±2.08 | 74±2.31 | 95.37±1.45 | 54±2.08 | 74.67±1.76 | 100±0.00 |

**Note:** Data is showing the mean percentage mortalities (Mean± SEM).

**Figure 1.** Effect on AChE activity in Aedes aegypti larvae when exposed with different doses of selected peptide fractions of H. tamulus and A. finitimus. Each value point represents Mean of three replicated with N = 10 larvae mosquitoes. **Note:** Error bars are in figure are representing the standard error.

**Figure 2.** Effect on AChE activity in Aedes aegypti adults when treated with different doses of the selected peptide fractions of H. tamulus and A. finitimus. Each value point represents Mean of three replicates with N = 10 adult mosquitoes.

**Figure 3.** Comparison of AChE activity in Ae. aegypti when treated with specific peptide venom dose (30µg/ml) of both scorpion species. Each bar represents Mean ± SEM of three replicated with N = 10 for larvae and adult mosquitoes.
4. Discussion

Scorpions with their neurotoxic venom also contain certain insect specific peptides thus behaving as bio-pesticide (Ghane et al., 2008). In view of this capability of scorpion venom, current study was focusing to control the population of *Ae. aegypti* by using scorpion venom. The idea was to target the AChE of *Ae. aegypti* that leads to accretion of ACh at synapse, thus upsetting the nerve impulse transmission. In the present study, the peptide fraction of *A. finitimus* seems to be more potent killer of *Ae. aegypti* as compared to *H. tamulus* venom peptide. It was found that maximum deaths in larvae were recorded against 30 µg/ml venom of *H. tamulus* and *A. finitimus* venom. Similarly, 30 µg/ml dose of each venom peptide caused maximum mortality in adult mosquitoes. Riaz et al. (2017) also reported that *H. tamulus* crude venom is highly effective against *Rhopalosiphum* *erysimi*. Selected scorpion venom peptides have potential to act as an insecticidal agents and showing significant mortalities as compared to control group. Taher et al. (2015) also reported higher mortality in organism treated with venom than control. Riaz et al. (2019) reported the efficacy of *A. finitimus* and *H. tamulus* venom peptides against *M. domestica*.

The current results suggested that with increased venom concentration, AChE activity decreases. Basically, neurotoxins in venom disturbing the voltage dependent sodium channels that results in repetitive firing of somatic, sympathetic and para-sympathetic neurons. It further leads to release the neurotransmitters like ACh. It was observed that the venom has have potential to target the ion-channels and properties have potential to kill the target insects. AChE activity decreases. Basically, neurotoxins in venom disturbing the voltage dependent sodium channels that results in repetitive firing of somatic, sympathetic and para-sympathetic neurons. It further leads to release the neurotransmitters like ACh. It was observed that the venom has have potential to target the ion-channels and properties have potential to kill the target insects. The current results suggested that with increased venom concentration, AChE activity decreases. Basically, neurotoxins in venom disturbing the voltage dependent sodium channels that results in repetitive firing of somatic, sympathetic and para-sympathetic neurons. It further leads to release the neurotransmitters like ACh. It was observed that the venom has have potential to target the ion-channels and properties have potential to kill the target insects.

5. Conclusions

Both peptide fractions due to their bio-insecticidal properties have potential to kill the target insects. The venom has have potential to target the ion-channels and to release the neurotransmitters like ACh. It was observed that *H. tamulus* venom peptide was more efficiently reducing AChE activity, thus can be used to control the harmful insects by targeting their neuromuscular junctions.

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