Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

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

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

N. Riaz[a]*, F. Zubair[a], N. Amjad[b], S. Ashraf[b], S. Asghar[b], M. Z. Awan[b] and S. Javaid[a]

[a]University of Sargodha, Department of Zoology, Sargodha, Pakistan
[b]University of Lahore, Department of Zoology, Sargodha, Pakistan

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* (Fabricus, 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.*

Resumo

O veneno do escorpião contém uma variedade de neurotoxinas que interagem com os canais iônicos e afetam suas atividades. O presente estudo foi desenhado para avaliar o potencial do veneno de escorpião como inibidor da acetilcolinesterase (AChE) usando o *Aedes aegypti* como organismo modelo. Venenos de duas espécies, *Hottentota tamulus* (Fabricus, 1798) e *Androctonus finitimus* (Pocock, 1897) foram selecionados para este estudo. Dois peptídeos (36 kDa de *H. tamulus* e 54 kDa de *A. finitimus*) foram separados do veneno de escorpião usando HPLC. Peptídeos selecionados causaram mortalidade significativamente maior em larvas e adultos de *Aedes aegypti* do que o controle (não foram observadas mortalidades nos grupos controle). O potencial inibitório significativo da acetilcolinesterase (AChE) de ambos os peptídeos foi registrado por espectrofotômetro. O peptídeo de *A. finitimus* causou mortalidade significativamente maior (95 ± 1,53% em larvas e 100% em adultos) que o peptídeo de *H. tamulus* (84,33 ± 2,33% em larvas e 95,37 ± 1,45% em adultos). Enquanto o peptídeo de *H. tamulus* foi mais eficiente na redução da atividade da AChE (0,029 ± 0,012 em larvas e 0,03 ± 0,003 em adultos) do que o peptídeo de *A. finitimus* (0,049 ± 0,005 em larvas e 0,047 ± 0,001 em adultos). Concluiu-se que o peptídeo do veneno de *H. tamulus* foi mais eficiente na redução da atividade da AChE, podendo ser um potencial bioinseticida que pode ser sintetizado em escala industrial para o controle de insetos nocivos.

Palavras-chave: escorpiões, peptídeos, HPLC, atividade da AChE, *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; Diaz-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

*e-mail: naila.riaz@uos.edu.pk; nailariaz35@yahoo.com
Received: December 23, 2021 – Accepted: September 9, 2022

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 discreplasminin 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 morbid 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 *Hottentota spp.* (Batista et al., 2007; Oukkache et al., 2008). The neurotoxins in venom divert the action potential, resulting in the release of neurotransmitters from cholinergeic 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 larvae 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.

Ae. aegypti 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-303S). 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 LC_{50} 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 venoms 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 *Ae. 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).

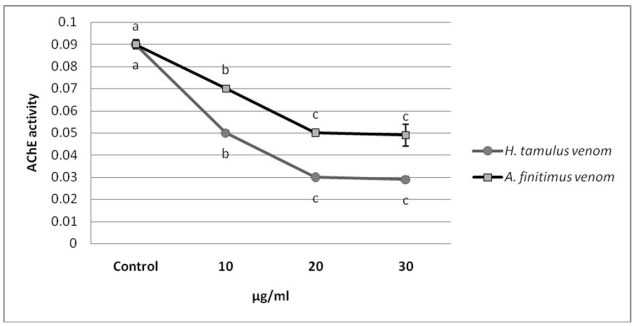

Figure 1. Effect on AChE activity in *Aedes aegypti* larvae when exposed with different doses of selected peptide fractions of *H. tamulus* and *A. finitimus*. Each value point represents Mean of three replicated with N = 10 larvae mosquitoes. **Note:** Error bars are in figure are representing the standard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

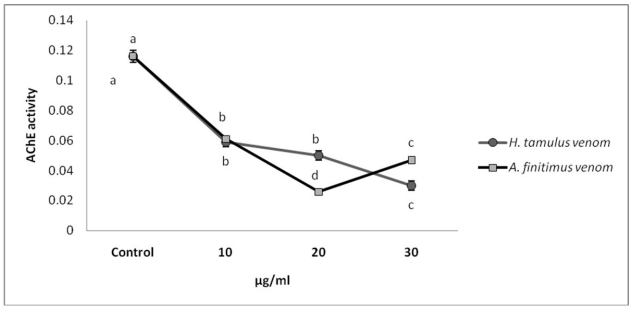

Figure 2. Effect on AChE activity in *Aedes aegypti* adults when treated with different doses of the selected peptide fractions of *H. tamulus* and *A. finitimus*. Each value point represents Mean of three replicates with N = 10 adult mosquitoes.

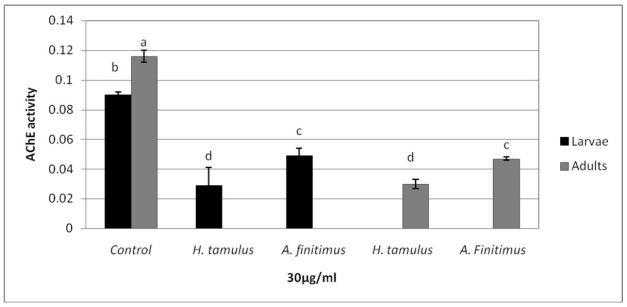

Figure 3. Comparison of AChE activity in *Ae. aegypti* when treated with specific peptide venom dose (30µg/ml) of both scorpion species. Each bar represents Mean ± SEM of three replicated with N = 10 for larvae and adult mosquitoes.

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. Tahir 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 abnormal nerve impulse transmission and enhanced the levels of neurotransmitters like adrenaline, nor-adrenaline, aspartate and acetylcholine. As AChE enzyme is involved in the hydrolysis of ACh, so inhibition of AChE causing the accumulation of ACh at nerve synapses. According to the results presented here, scorpion venom showing its anti-AChE activity effectively. Ozkan et al. (2007) found the sharp decline in AChE level after 12th hour of venom introduction in rats as compared to control group. They used the venom of *Androctonus spp.* and found it to target the ACh receptors and increasing ACh concentration, thus acting as neurotoxic agent. These results are in accordance to our present findings that AChE activity was significantly lower in venom treated group as compared to the control. Likewise, Vatanpour (2003) and Abdel-Rahman et al. (2010) reported reduction in AChE activity in chicks and cockroaches when exposed with scorpion venom respectively.

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.

Acknowledgements

We are thankful to University of Sargodha, GC University, Lahore for their support to conduct this research.

References

ABDEL-RAHMAN, M.A., OMRAN, M.A.A., ABDEL-NABI, I.M., NASSIER, O.A. and SCHEMERHORN, B.J., 2010. Neurotoxic and cytotoxic effects of venom from different populations of the Egyptian *Scorpio maurus palmatus*. *Toxicon*, vol. 55, no. 2-3, pp. 298-306. http://dx.doi.org/10.1016/j.toxicon.2009.08.003. PMid:19682484.

BATISTA, C.V.F., ROMÁN-GONZÁLEZ, S.A., SALAS-CASTILLO, S.P., ZAMUDIO, F.Z., GÓMEZ-LAGUNAS, F. and POSSANI, L.D., 2007. Proteomic analysis of the venom from the scorpion *Tityus stigmurus*: biochemical and physiological comparison with other Tityus species. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, vol. 146, no. 1-2, pp. 147-157. http://dx.doi.org/10.1016/j.cbpc.2006.12.004. PMid:17270501.

CORDEIRO, F.A., AMORIM, F.G., ANJOLETTE, F.A. and ARANTES, E.C., 2015. Arachnids of medical importance in Brazil: main active compounds present in scorpion and spider venoms and tick saliva. *The Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 21, no. 1, pp. 24. http://dx.doi.org/10.1186/s40409-015-0028-5. PMid:26273285.

DÍAZ-GARCÍA, A., RUIZ-FUENTES, J.L., YGLESIAS-RIVERA, A., RODRÍGUEZ-SÁNCHEZ, H., RIQUENES GARLOBO, Y., FLEITAS MARTINEZ, O. and FRAGA CASTRO, J.A., 2015. Enzymatic analysis of venom from Cuban scorpion *Rhopalurus junceus*. *Journal of Venom Research*, vol. 6, pp. 11-18. PMid:26605039.

ELLMAN, G.L., COURTNEY, K.D., ANDRES JUNIOR, V.J. and FEATHERSTONE, R.M., 1961. A new rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, vol. 7, no. 2, pp. 88-95. http://dx.doi.org/10.1016/0006-2952(61)90145-9. PMid:13726518.

FENG, L., GAO, R. and GOPALAKRISHNAKONE, P., 2008. Isolation and characterization of a hyaluronidase from the venom of Chinese red scorpion *Buthus martensi*. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, vol. 148, no. 3, pp. 250-257. http://dx.doi.org/10.1016/j.cbpc.2008.06.003. PMid:18611448.

GHANE, M., ZARE MIRAKABADI, A., RABEI, H., MOHAMADPOUR, N. and EBRAHIM HABIBI, A., 2008. Identification and Purification of two mammalian nourotoxins from Iranian Scorpion *(Buthotus schach)* venom. *Archives of Razi Institute*, vol. 63, pp. 39-45.

GWEE, M.C., NIRTHANAN, S., KHOO, H.E., GOPALAKRISHNAKONE, P., KINI, R.M. and CHEAH, L.S., 2002. Autonomic effects of some scorpion venoms and toxins. *Clinical and Experimental Pharmacology & Physiology*, vol. 29, no. 9, pp. 795-801. http://dx.doi.org/10.1046/j.1440-1681.2002.03726.x. PMid:12165045.

JALALI, A., BAVARSAD-OMIDIAN, N., BABAEI, M., NAJAFZADEH, H. and REZAEI, S., 2012. The pharmakokineticc of *Hemiscorpius lepturus* scorpion venom and Razi antivenom following intramuscular administration in rat. *Journal of Venom Research*, vol. 3, pp. 1-6. PMid:22826800.

ORTIZ, E. and POSSANI, L.D., 2015. The unfulfilled promises of scorpion insectotoxins. *The Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 21, no. 1, pp. 16. http://dx.doi.org/10.1186/s40409-015-0019-6. PMid:26085828.

OUKKACHE, N., ROSSO, J.P., ALAMI, M., GHALIM, N., SAÏLE, R., HASSAR, M., BOUGIS, P.E. and MARTIN-EAUCLAIRE, M.F., 2008. New analysis of the toxic compounds from the *Androctonus mauretanicus mauretanicus* scorpion venom. *Toxicon*, vol. 51, no. 5, pp. 835-852. http://dx.doi.org/10.1016/j.toxicon.2007.12.012. PMid:18243273.

OZKAN, O. and FILAZI, A., 2004. The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different

methods from scorpions, *Androctonus crassicauda* (Olivier 1807). *Acta Parasitol Turcica*, vol. 28, no. 1, pp. 50-53.

OZKAN, O. and KAT, L., 2005. *Mesobuthus eupeus* scorpionism in Sanliurfa region of Turkey. *The Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 11, no. 4, pp. 479-491. http://dx.doi.org/10.1590/S1678-91992005000400008.

OZKAN, O., ADIGUZEL, S., KAR, S., KURT, M., YAKISTIRAN, S., CESARETLI, Y., ORMAN, M. and KARAER, Z., 2007. Effects of *Androctonus crassicauda* (Olivier, 1807) (scorpiones: buthidae) venom on rats: correlation among acetylcholinesterase activities and electrolytes levels. *The Journal of Venomous Animals and Toxins Including Tropical Diseases*, vol. 13, no. 1, pp. 69-81. http://dx.doi.org/10.1590/S1678-91992007000100005.

RADHA, K.M., 2014. Enzymes and toxins in scorpions of Buthidae family Insulin-Glucose Administration reverses metabolic, cardiovascular, ECG Changes and pulmonary oedema in scorpion envenoming syndrome. *International Journal of Bioscience and Medicine*, vol. 3, pp. 9-25.

RIAZ, N., TAHIR, H.M. and SAMIULLAH, K., 2017. Effect of *Hottentotta tamulus* (Scorpiones: Buthidae) crude venom on *Rhopalosiphum erysimi* (Hemiptera: Aphididae) in the laboratory. *Punjab University Journal of Zoology*, vol. 32, pp. 9-14.

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 University Journal of Zoology*, vol. 34, no. 2, pp. 207-211.

SEYEDIAN, R., PIPELZADEH, M.H., JALALI, A., KIM, E., LEE, H., KANG, C., CHA, M., SOHN, E.T., JUNG, E.T., RAHMANI, A.H. and MIRAKABADY, A.H., 2010. Enzymatic analysis of *Hemiscorpius lepturus* scorpion venom using zymography and venom-specific antivenin. *Toxicon*, vol. 56, no. 4, pp. 521-525. http://dx.doi.org/10.1016/j.toxicon.2010.05.008. PMid:20493200.

TAHIR, H.M., ZAFAR, K., MISHAL, R., NASEEM, S., BUTT, A., YAQOOB, R., AHSAN, M.M. and ARSHAD, M., 2015. Potential use of venom of *Odontobuthus odonturus* (Arachnida:Buthidae) as bio-pesticide against *Rhopalosiphum erysimi* (Homoptera: Aphididae). *Pakistan Journal of Zoology*, vol. 47, pp. 37-40.

TAN, P.T., VEERAMANI, A., SRINIVASAN, K.N., RANGANATHAN, S. and BRUSIC, V., 2006. SCORPION2: a database for structure-function analysis of scorpion toxins. *Toxicon*, vol. 47, no. 3, pp. 356-363. http://dx.doi.org/10.1016/j.toxicon.2005.12.001. PMid:16445955.

THEAKSTON, R.D.G., WARRELL, D.A. and GRIFFITHS, E., 2003. Report of a WHO workshop on the standardization and control of antivenom. *Toxicon*, vol. 41, no. 5, pp. 541-557. http://dx.doi.org/10.1016/S0041-0101(02)00393-8. PMid:12676433.

VALDEZ-CRUZ, N.A., SEGOVIA, L., CORONA, M. and POSSANI, L.D., 2007. Sequence analysis and phylogenetic relationship of genes encoding heterodimeric phospholipases A2 from the venom of the scorpion Anuroctonus phaiodactylus. *Gene*, vol. 396, no. 1, pp. 149-158. http://dx.doi.org/10.1016/j.gene.2007.03.007. PMid:17466468.

VATANPOUR, H., 2003. Effects of black scorpion *Androctonus crasicuda* venom on striated muscle preparation in vitro. *Iranian Journal of Pharmaceutical Research*, vol. 2, pp. 17-22.

YAQOOB, R., TAHIR, H.M., RASHEED, A., SAMIULLAH, K., ZAHRA, K., AHSAN, M.M. and NASEEM, S., 2017. Partial characterization of venom from common buthid scorpions (Scorpion: Buthidae) of District Sargodha, Pakistan. *Biologia (Pakistan)*, vol. 63, pp. 13-19.