

Protein profile analysis of scorpion (*Heterometrus swammerdammi*) venoms by two-dimensional gel electrophoresis

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Abstract—Scorpions are globally known to be venomous as well as medically important creatures. They possess millions of years of evolutionary history with considerable diversity. *Heterometrus swammerdammi* from the family Scorpionidae is one of the less harmful scorpions found in the Jaffna peninsula. There are no reports available about their protein profile from Sri Lanka. Therefore, the current research was undertaken to report their protein profile. The scorpions were sampled from selected locations of the Jaffna Peninsula and their venom was extracted using the electrical stimulation method immediately after the sampling. The sampled scorpions were housed in the Animal house of Department of Zoology, University of Jaffna with the supply of food and water *ad libitum*. The Sodium Dodecyl Sulphate - Polyacrylamide gel Electrophoresis (SDS-PAGE) of Laemmli was used in the profiling of scorpion venom with the 10% polyacrylamide gel and protein marker. The Coomassie brilliant blue stain was used to stain the gel and distilled water was used to de-stain the gel and then the gel was analysed. The protein marker consists of bands sized 225, 150, 100, 75, 50, 35, and 25 kDa, where 30, 70, 100, 110, and the bands above the size of 250 kDa were observed in the samples. The bands at 100 kDa and the bands between 60-75 kDa were the predominant in the samples and they were identified as hyaluronidase. The victims of scorpion bite are suffered from excessive sweating, agitation, tissue destruction at the biting site, and palpitation. Tissue destruction can be occurred due to the action of Phospholipase-A, Neurotransmitter can be a responsible molecule for the palpitation response. The 225kDa band and the band between 40-50 kDa would be the responsible bands for acute heart failure in the absence of proper treatment. Therefore, the functional assays should be done in the future to recognize the pharmacological activity of the above novel molecule in action.

Keywords – Scorpionvenom, Protein profile, SDS PAGE, *Heterometrus swammerdammi*, Black scorpion

I. INTRODUCTION

Scorpion is one of the most ancient arthropods with about 4300 million years of evolutionary history. There are 13 families and about 1400 described species and subspecies of scorpions (Kovarik, 2009). The venom of scorpion is primarily known for its ability to disturb the physiological activities of living beings and at times deems dangerous to humans. The venom of about 30 species of scorpions is capable of killing the human being and most of such venomous species belong to the family Buthidae (Keegan, 1980).

Almost all the medically important species of scorpions belong to the family Buthidae which is the largest and the most widely distributed family reported from all parts of the world. Family Scorpionidae includes seven genera of scorpions distributed among three subfamilies containing some of the world's largest living scorpions.

Heterometrus swammerdammi is one of the commonly found scorpions from the Jaffna Peninsula and it is believed to be a harmless species by the villagers. It is classified under the family Scorpionidae and it is the largest scorpion reported from Indian subcontinents. The scorpions occupy savannahs, humid forests, and rain forests, but some scorpions and many of the diplocentrids are found in drier habitats. Most species in this family are relatively harmless, but a serious case has

been reported for *Heterometrus* sp (Ehrenberg, 1828). In India, some species within the family Scorpionidae have also been reported as poisonous including *Heterometrus fastigiosus* (Chaubey, 2008).

During the past few years, several patients have been admitted to the Jaffna teaching hospital to receive treatments for scorpion stings. At present, the scorpion stings are reported frequently and about four patients are admitted to Jaffna hospital every week due to scorpion sting (Ranawana, 2013). The common victims of the scorpion bite are children and housewives; on many occasions, stinging occurs inside houses, where scorpions are found even among the clothes too. Also, these scorpions are found close to human dwelling among leaf litter, logs, and piles of firewood. Often, stinging scorpions are not brought along the patient (Ranawana, 2013). It is reported by the physicians that the patients would develop intense pain followed by benumbed feeling at the site of sting upon the sting. In severe envenomation cases, the patient develops excessive sweating, agitation, restlessness, and palpitation. The blood pressure shoots up and if not promptly treated, it leads to acute heart failure (Ranawana, 2013).

The venom consists of the components responsible for a mixture of activities such as neurotoxic, cardiotoxic, coagulant, haemostatic and hepatotoxic actions (Chippaux *et al.*, 1991). Venoms are complex cocktails of proteins, toxins,

and low molecular weight components where scorpion venom contains many proteins, peptides, and other compounds. Several of these compounds are biologically active and found to be particularly useful in physiological and pharmacological research as an investigatory tool (Oukkache *et al.*, 2008).

The available percentage of protein molecules were reported from the venom of eight species of Malaysian snakes. Inter-family, inter-sub family, inter-genus, and inter-species level variations in the banding patterns were also documented in the venom of snakes with the results from the analysis of two-dimensional electrophoresis. Inter-species variation in the banding pattern of two species of snakes belongs to the genus *Naja* was held at the approximate size of 15kDa and between 15-20 kDa (Vejayan *et al.*, 2010).

Isolation of protein molecules and peptide molecules were also implemented by various scientists from different parts of the world with different venomous creatures. The electrochemical and statistical evaluation of venom proteins and available peptides were documented in the honeybee (*Apis mellifera*). The biologically active peptides “Melittin” of molecular weight 2846.46 Da and “Apamin” of molecular weight 2027.34 Da were isolated using fast protein liquid chromatography and differential pulse voltammetry method adsorptive transfer technique respectively (Hoai Viet *et al.*, 2015).

Significant variations on the protein bands of the venom samples were documented from Colombian spider (*Phoneutria boliviensis*) with SDS page analysis. It revealed that the presence of bands in the range from 14 to 45 kDa. In that specific range, a significant concentration of band close to 14 kDa was observed in both venoms, but they were appeared to be lighter in colour in the venom of males. Three bands above 25kDa and below 45 kDa can be observed in Female venom, but they appeared to be absent from male venom (Sebastian *et al.*, 2015).

It was reported that the snake venom consisted of the protein molecules in the range from 6-100 kDa. But, the smaller low molecular weight polypeptides were the lethal components out of all the enzymes that contribute to the deleterious effects of the venom (Barry *et al.*, 2002).

Variation in the composition, lethality, and the quantity of the venom can be correlated with the geographical location of habitat, age of the animal, time of the year, and prey capture strategies (Barry *et al.*, 2002). Therefore, there is a need to investigate these scorpion species and the biochemical and other characters of their venom. As a preliminary work, the present study was under taken to analyse the composition of the scorpion venom from the species; *Heterometrus swammerdammi*.

II. MATERIALS AND METHODS

A field survey was carried out in selected places of the Jaffna Peninsula (Figure: 01). Random sampling was implemented during the field survey. Scorpions were searched under rocks, gaps of soil, leaf litters, under barks, and within vegetation. The scorpions were collected with the help of forceps and tongues. The collected scorpions were brought to the animal house where the venom was immediately extracted with an adaptive method in the Department of Zoology, Faculty of Science, University of Jaffna. After that, the scorpions were housed in an artificially constructed environment for future works. The housed scorpions were fed with 1-2 live crickets, locusts, and cockroaches twice a week and were provided with water *ad libitum* appropriately for their survival. The dead scorpions were used in the taxonomic identifications using standard taxonomic keys.

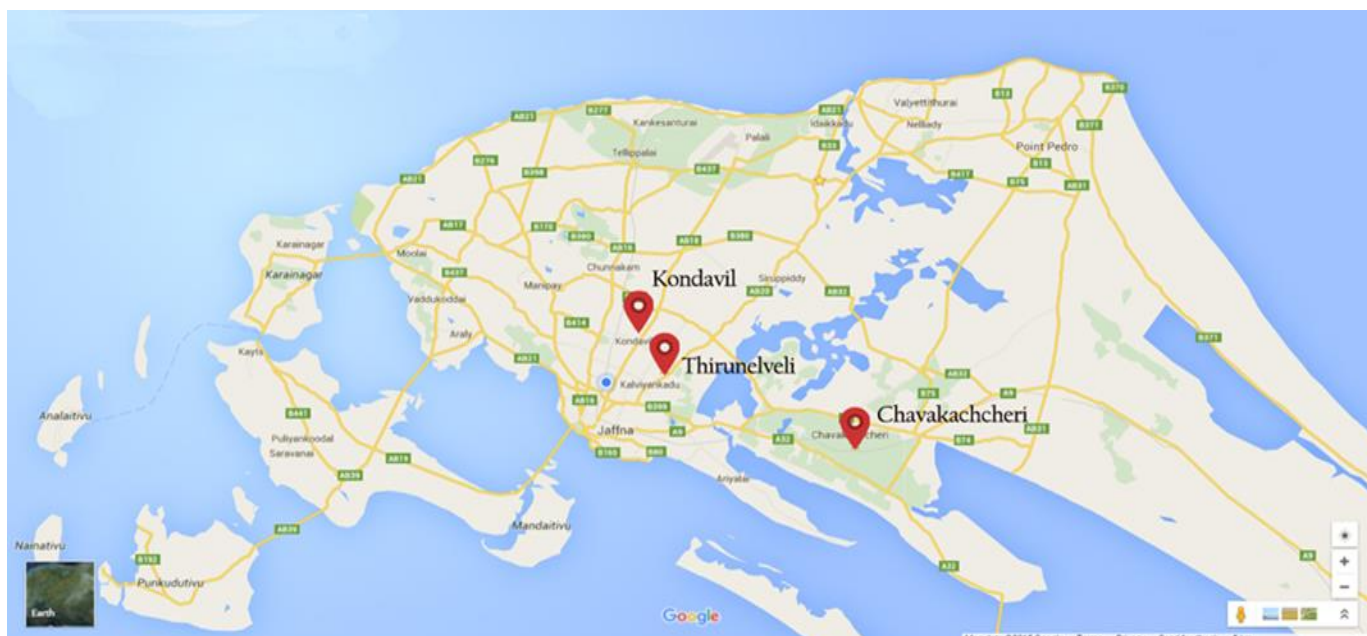


Figure 01: Collection sites of the scorpion *Heterometrus swammerdammi* within Jaffna Peninsula Source: Google Earth map.

A small box with cushion was prepared where the scorpion was kept and tied up with a cloth and the tied tape. Then, 5V of shock treatment was given in between the 4th and the 5th metasomal membrane, and venom was collected by placing a completely labeled Eppendorf tube at the telson region (Gobalakrishnakone, 1995). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate protein on a 10% polyacrylamide gel by following the methods of Laemmli. Coomassie Brilliant Blue stain (0.125%) was used to stain the gel and gel was de-stained using double distilled water. The molecular weights of venom protein were estimated in accordance with the obtained band patterns.

Institutional ethical clearance was obtained for this study from the ethical review committee of University of Jaffna

III. RESULTS AND DISCUSSION

Clear banding of the electrophoretic patterns of scorpion venom proteins was observed (Figure:02). The molecular weights of the venom proteins are given (Table 01) where a maximum of nine protein bands was appeared in the sample L1 (Figure: 02). Considerably narrow variations were observed in molecular weights in the individual venom samples of the species.

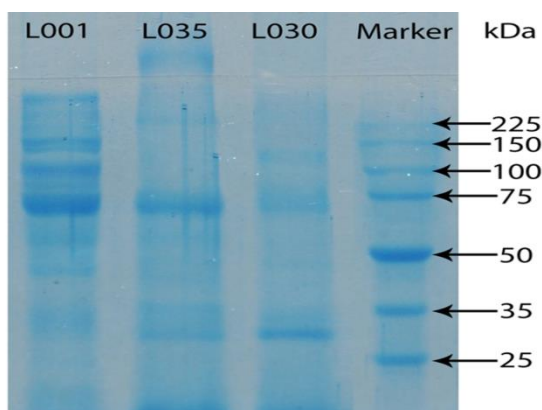


Figure 02: Molecular weight determination of purified scorpion venom by SDS-PAGE. Lane 01- L001 Crude venom of scorpion collected from Thirunelveli, Lane 02- L035 Crude venom of scorpion collected from Kondavil, Lane 03- L030 crude venom of scorpion collected from Chavakachcheri area and the Lane 04- protein marker.

Table 01: The molecular weights of resolved protein bands from samples of *Heterometrus swammerdammi* collected from different locations of Jaffna Peninsula, Where √, √√, and – indicate the presence of the single band, double bands and the absence of bands within the range respectively.

	225 kDa	101-150 kDa	100 kDa	60-75 kDa	40-50 kDa	25-35kDa
Sample L001	√	√√	√	√√	√√	√
Sample L035	–	–	–	√	–	√
Sample L030	–	–	–	√	–	√
Marker	√	√	√	√	√	√
Reported molecules	Not known	Neurotransmitters At 115kDa	Hyaluronidase	Hyaluronidase	Not known	Phospholipase A

225, 150, 100, 75, 50, 35 & 25 kDa sized bands were predominantly observed in the protein marker (Figure: 02). Bands between 60-75 kDa and the bands between 25-35 kDa were predominant in all the 3 venom samples. The bands in 225, 101-150, 100, and 40-50 kDa were absent in two venom samples namely L035 and L030. But the band between 100-150 kDa was present only in sample L001.

Bands sized 30, 70, 100, 110 & greater than 250 kDa were observed from the sample L001. Among these bands, the 30 kDa band belongs to the phospholipase A, the 70 kDa band belongs to hyaluronidase. Bands with the sizes of 100kDa and 110 kDa belong to the neurotransmitters.

Among the banding patterns of the sample L035 70 kDa size band was the prominent one. Whereas the banding pattern of the scorpion L030 showed the prominent band at 30 kDa.

The sample L001 was identified as a female Scorpion and the other two samples (L030 and L035) were male scorpions as per the morphological identification. An important finding was made that the male and female scorpion species were sharing differences in their banding patterns.

The bands above 60kDa – 75kDa were prominent in the female scorpion sample L001, and the bands below this range were prominent in male scorpions L030 and L035. In these bands we could clearly see the differences on the intensity of the bands between male and female scorpion venom samples as the bands appeared to be lighter and less prominent in male venom samples. This observation would lead us to think that the venom of the female scorpion is more intense than the male scorpion venom as reported by Sebastian *et al* 2015 for spiders. Moreover, there are four bands above 60 kDa -75 kDa can be observed clearly in female venom samples, but they appeared to be absent from the male venom samples. The bands below 60-75 kDa were highly outstanding in male venom than female venom samples. Which indicates that the female scorpion is having a broad scale protein in their venom sample.

Among the banding patterns of male scorpions collected from two different locations namely, Kondavil and Chavakachcheri areas were sharing the same molecules. But 110 kDa molecules can be observed from the sample L035 from the Kondavil area. This observation indicates there is no variation in the composition of venom with the geographical distribution. But a low-flying band could be seen in the gel at 110 kDa size in the venom of the Kondavil sample. As per the review Barry *et al.*, 2002, major variation in the banding pattern can be possible with the age and the size of the scorpion and the period of collection. As per the morphometric measurements of the scorpions, the total length of the samples L030 and L035 were 82.2mm and 124.0mm respectively. The average total length of adults varies from 130–176mm in *Heterometrus swammerdammi*, the black scorpion. So, both samples are in the developing stages where L030 was in the early development stage, and L035 was in the late development stage. The venom was extracted on the same day for both scorpions at 10 minutes intervals. Therefore, it can be concluded that the variation in the banding pattern can be negligible since both extractions were performed at the same time. Therefore, it could be finalized that the variation was possible due to the variation in their developmental stages.

The bands in the smaller molecular weight region are known to be the lethal ones. Therefore, the bands at the bottom of the gel (Figure: 02) having molecular weights less than 50kDa were well-thought-out as the lethal proteins in this study. Those bands consisted of phospholipase-A and the unknown peptide molecule. The phospholipase-A of molecular weight in between 25-35 kDa region is the band with the least weight. This enzyme peptide can damage the phospholipid molecule in the cellular membrane which can be ultimately ended up in the destruction of tissue masses in the biting sites. The neurotransmitter molecules were observed in the upper portion of the bands at 115 kDa. This is a quite large compound, which could be responsible for the mild palpitation response. The neurotransmitter molecules can cause the palpitation when they reached to the cardiovascular system from the site. Since it is comparatively a large compound, their effective action could be mild. Therefore, they can be responsible for the mild palpitation resulting from the bites of Sri Lankan black scorpion *Heterometrus swammerdammi*. Through literature, it is revealed that different environmental and ecological parameters also have a high impact on the protein profile of venom. Therefore, this research should further be documented with the broad-scale profile with the respective conformational biochemical assays for the determination of the protein availability.

IV. CONCLUSION

It is found that novel molecules of 100 kDa and 70 kDa are hyaluronidase and bands between 25-35 kDa belongs to phospholipase-A which can be responsible for the tissue damage in the biting area, 115 kDa bands belong to the neurotransmitter molecule which can be conscientious for the palpitation and the molecule responsible for 225 kDa and 40-

50kDa band region are not known yet. These unknown molecules could be accountable for acute heart failure in the absence of proper treatment. It needs to analyse further to recognize the novel protein and peptide molecules through biochemical studies to ensure the nature of this scorpion venom in Jaffna. But the band pattern representing the neurotransmitter molecule falls in the 115 kDa region, which is considered as the low prospective molecule with less toxic nature, thus the people's belief that the black scorpion is to be as the less harmful scorpion might be true.

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