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Fine structure of the stinger, histology and histochemistry of the venom gland in the scorpion *Androctonus amoreuxi* (Buthidae)

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Light microscope;
Electron microscope

Abstract The venom apparatus of the scorpion *Androctonus amoreuxi* has been identified histologically and histochemically in the present study. The results showed that this apparatus composed of a pair of venom glands and a stinger located in the terminal segment called telson. The stinger of the venom apparatus has been studied by the light microscope and SEM. The stinger, located at the end section of the telson, is sickle-shaped. The venom is ejected through a pair of venom pores on its subterminal portion. Both venom ducts extend along the stinger without contact with each other since they are separated by connective tissue cells. The stinger is covered by cuticle and spines. Each venom gland is covered by a sheath of striated muscle and is lined with extensively folded secretory epithelium that consists of non-secretory and secretory venom-producing cells. The venom-producing cells reacted positively to histochemical tests for carbohydrates and proteins. The outcomes also revealed that the venom-producing cells of both glands produce neutral mucosubstances. The structure and secretion of scorpion venom glands are discussed within the context of the present results.

Introduction

Androctonus amoreuxi is a scorpion found abundant in Egypt (Balozet, 1975). It belongs to the family Buthidae. The body of the Scorpion *A. amoreuxi* can be divided into two separate parts: the cephalothorax (head and thorax) and the abdomen. The abdomen is divided into 12 distinct segments with the last five segments forming the metasoma, most often referred to as the tail. The tail usually curves upward and toward the body

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and contains the venom glands. When a Scorpion captures a prey in its claws, it strikes the prey with the tail. At the end of the metasoma is the telson, which is a bulb-shaped structure containing the venom gland and a sharp, curved stinger, which is also known as the aculeus to deliver the venom. The venom of scorpions is used for both prey capture and defense. Worldwide, scorpion stings are the most important cause of arachnid envenoming and are responsible for significant morbidity and pediatric mortality in many parts of South America, the Middle East, Asia, and both Northern and Southern Africa (Freire Maia et al., 1994; Ismail, 1995). Human deaths from scorpion bites normally occur among the young, elderly or infirm. Scorpions are generally unable to deliver enough venom to kill healthy adults. Some people, however, may be allergic to the venom of some species. Although all scorpions are venomous, the most diverse and wide spread family, Buthidae, includes the majority of medically significant scorpion species. About 50 species worldwide have venom sufficiently potent to be considered dangerous to human beings (Brownell and Police, 2001). The other scorpion species, however, are only venomous enough to affect small vertebrate animals and insects that are their own prey. In the present study, the histology and some histochemistry of the venom apparatus and the morphology of the stinger in the scorpion *A. amoreuxi* is studied (Fig. 1a and b).

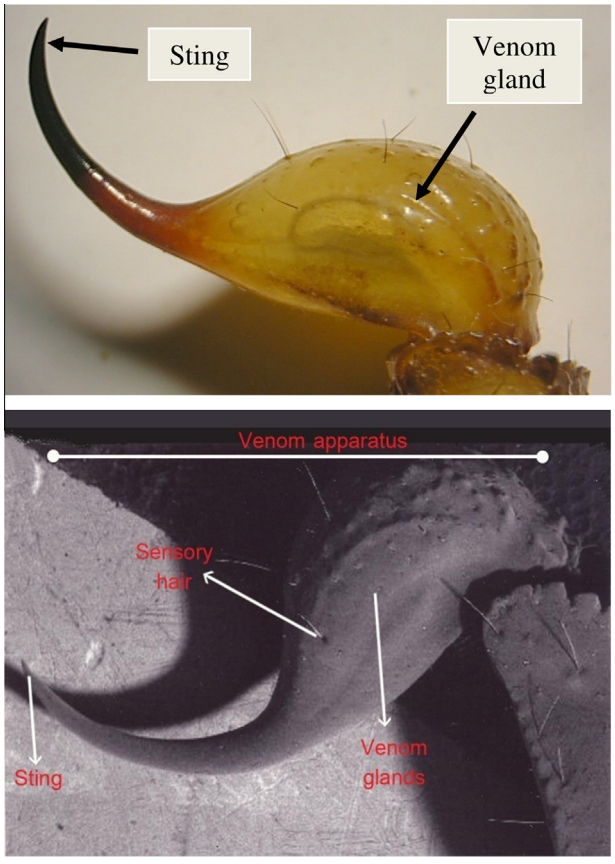

Fig. 1 (a) Photomicrograph showing the morphology of venom apparatus of *Androctonus amoreuxi* (60×). (b) Scanning electron micrograph showing the overall lateral view of the telson of *Androctonus amoreuxi*.

Materials and methods

Scorpions

Twelve adult *A. amoreuxi* scorpions were used in the present study. They were collected from Baltium, Egypt. In June 2010, scorpions were identified and then reared in special cages where they were fed insects at the Zoology Department, Faculty of Science, Suez Canal University, Ismailia. The telson, which is the last portion of the metasoma, was removed and studied.

Light microscope

The venom apparatus of the 12 captured, adult male and female *A. amoreuxi* scorpions, was utilized. The telson was removed from each scorpion and quickly immersed for 5 days in the following fixatives; 10% neutral buffered formalin (pH 7.9) with 2% calcium acetate. The cuticles of the fixed telson were removed with a sharp blade, by scratching, then thoroughly washed in running water, dehydrated, cleared, and embedded in paraffin wax, sectioned at 4–5 μm thickness and stained with hematoxylin–eosin and Masson trichrome stains for histological examination Fig. 2. The mucosubstances and proteins were histochemically identified in the telson using the periodic acid Schiff reagent for mucosubstances and mercuric bromophenol blue for proteins.

Scanning electron microscope

The stingers were fixed in 3% glutarldehyde buffered with 0.1 ml sodium phosphate buffer (pH 7.2) for 2 h and then washed four times with buffer post fixed in 1% osmium tetroxide (OSO_4) for 2 h at +4 °C and then washed four times with buffer. They were then dehydrated in graded ethanol series (40–100% ethanol) Fig. 3. The last stages of dehydration were performed with propylene oxide (Hayat, 1981).

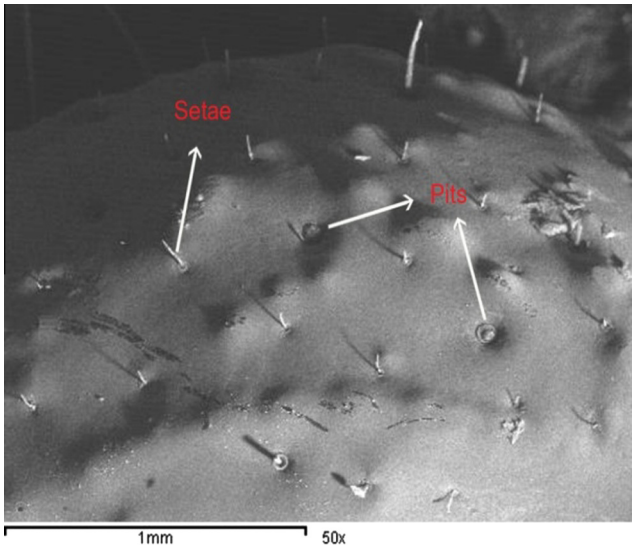

Fig. 2 Scanning electron micrograph showing pits and setae on the surface of telson.

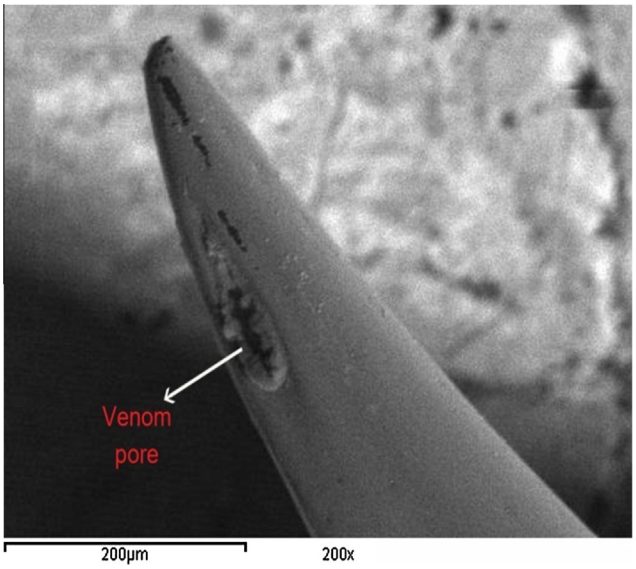

Fig. 3 Scanning electron micrograph of venom pore at a higher magnification (200 µm).

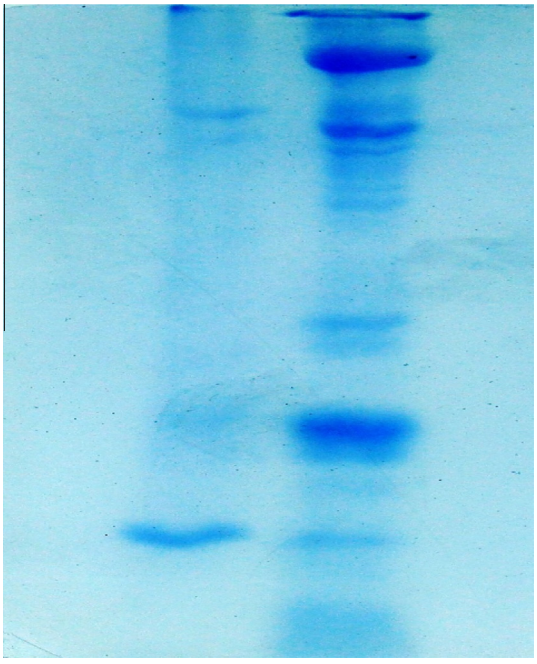

Fig. 4 Electrophoreses of venom gland.

Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) method for protein analysis of venom gland

Venom proteins were separated on the basis of molecular weight by SDS–PAGE according to the methods of Laemmli (1970) and Hames and Rickwood (1981). After electrophoresis, the gel was transferred to the staining solution until the appearance of protein band(s). For destaining (removing the excess stain), the gel was washed once with distilled water and soaked in the destaining solution for 2 h with shaking and the solution was replaced every 30 min until the clearance of the background.

Results

The venom gland of *A. amoreuxi* scorpion is invested by a capsule of fibrous connective tissue surrounded by a layer of smooth muscular tissue which encircles the gland. The connective tissue of the capsule extends to the inside of the gland in the front of thin branching trabecula of loose connective tissue upon which a layer of simple columnar epithelial cells is located (Fig. 5). The epithelial cells are engorged with density stained secretory materials that occupy most of the cell vicinity (Fig. 5). The discharge of these materials occurs by the holocrine mode of secretion in which the cells are completely ruptured and granules are discharged to the glandular lumina (Fig. 6). However, some of these cells were intact and appeared to contain their cytoplasmic components together with numerous small secretory vesicles. The tubular lumina also contain discharged secretory materials.

The glandular tissue stained with Masson trichrome technique (Fig. 9) indicated the presence of two types of secretory materials, stained with either red or blue coloration. The color intensity of the secretory materials also varied also. The red stained materials are in the form of small rounded vesicles (Fig. 9). The glandular epithelial cells exhibited variable PAS reactivity that differs from one cell to another (Figs. 7 and 8). The dense connective tissue capsule and the glandular

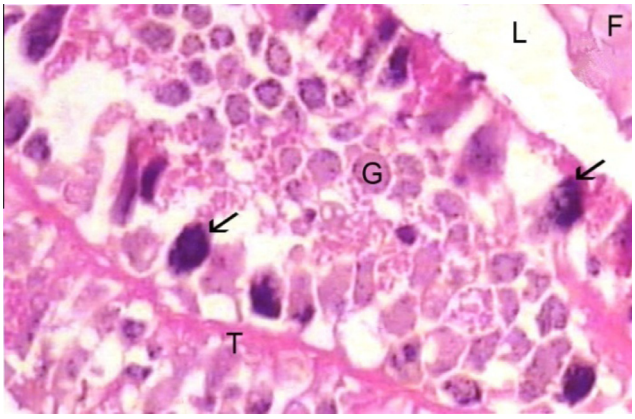

Fig. 5 A section of the venom gland of *Androctonus amoreuxi* Scorpion showing thin trabecula of connective tissue (T) upon which the glandular epithelia (G) are located. Note the presence of skeletal muscle fibers (F) at the periphery together with the glandular capsule. Note also the density stained secretory materials within the secretory cells (arrows). (H& E; ×100).

trabecula revealed dense PAS reactivity (Fig. 7), while the muscular tissue revealed weak reactivity (Fig. 8). The glandular epithelium consists of venom producing cells and non-secretory supporting cells. The venom-producing cells are apocrine high columnar in shape, filled with coarse granules in the apical portion. The supporting cells are subcuboidal in shape, located between the venom-producing cells and the underlying basement membrane (Fig. 7). The common canal of *A. amoreuxi* venom apparatus lacks musculature and is lined with an achitinous internal layer, followed by a non-excretory simple cuboidal epithelium. The extruded venom, within the gland lumen and within the apical cytoplasm of the venom producing cells contains fine and coarse granules, with discrete

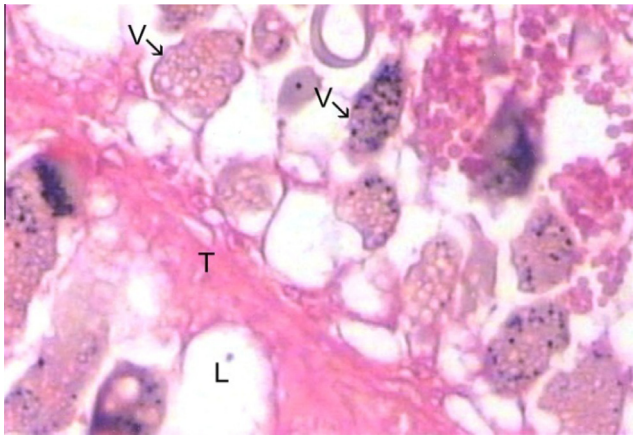

Fig. 6 Photomicrograph of the glandular epithelia of the venom gland of *Androctonus amoreuxi* scorpion resting upon the connective tissue trabecula (T). Note the secretory materials (V) within the epithelial cells and those released to the lumen (L). (H & E; ×400).

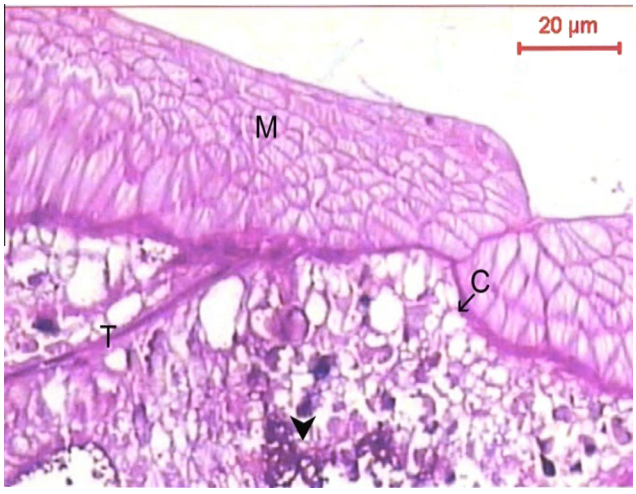

Fig. 7 Photomicrograph of the secretory epithelial cells of the venom gland of *Androctonus amoreuxi* scorpion showing variable PAS reactivity. Note the presence of positively stained vesicles within the secretory cells. The outer capsule and its branching trabecula are densely stained with PAS. (PAS; ×400).

morphologies, that show variable coloration patterns when using the same histological stain. The venom-producing cells reacted positively to PAS (Fig. 8).

Gel electrophoresis of the gland protein

The electrophoretic pattern of the protein marker included six protein bands with molecular weights of 212, 120, 97.7, 45, 31 and 20 kD base (Fig. 4). By comparing the electrophoretic pattern of the gland tissue of *A. amoreuxi* scorpion with that of the marker table (Table 1), fifteen protein bands were identified with the molecular weights of 189.4, 111. 91, 98, 73.3,

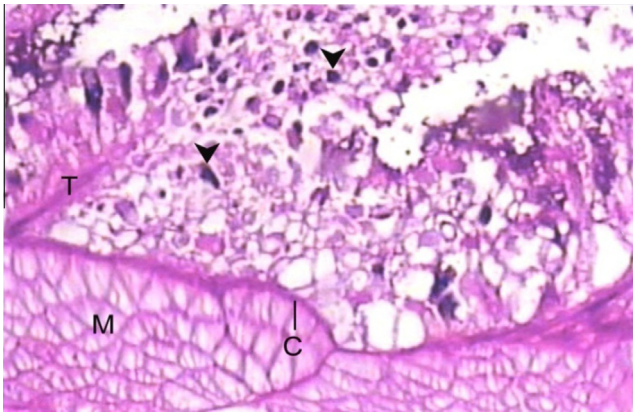

Fig. 8 Photomicrograph of the polysaccharide rich components in the glandular epithelial cells (arrows) and the tubular lumina (arrow heads) in the gland of *Androctonus amoreuxi* scorpion. The muscle fibers (M) show weak polysaccharide contents. Glandular capsule (C) surrounding the venom gland showing dense PAS reactivity. T: Trabecula. (PAS; ×400).

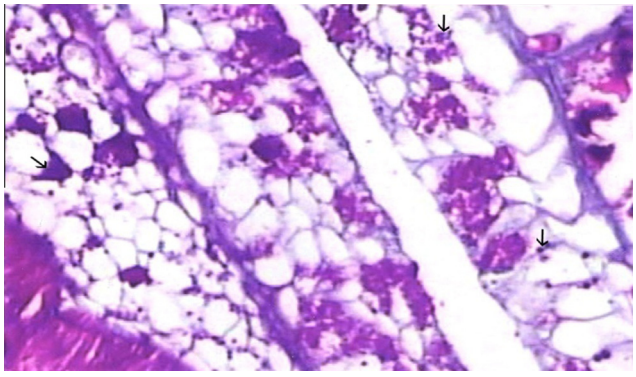

Fig. 9 Masson reaction in a section of the venom gland of *Androctonus australis* scorpion revealing red and blue colored secretory materials with variable color density. The red stained materials are in the form of small rounded vesicles (arrows). (Masson; ×400).

64.64, 62.36, 59.7, 57.6, 46.4, 45, 29.56, 27.06, 24.79, 19.39 and 5.91 kD base.

Discussion

The venom-producing cells of *A. amoreuxi* were found to be PAS-positive and diastase-resistant, which indicated that this scorpion species contains neutral mucosubstances and are devoid of glycogen. Neutral mucosubstances have been found in the venom of some other scorpion species and are reported to be abundant in the most dangerous ones (Goyffon and Kovoor, 1978; Taib and Jarrar,1993). According to Pearse's interpretation (Pearse, 1985) *A. amoreuxi* contained neutral mucosubstances. The nature of neutral mucosubstances, which are polymers of glucosamine, may indicate a possible role in osmoregulation and in the transfer of venom protein fragments to the victim's tissue. The present study showed that the histological structure of the venom glands of *A. amoreuxi*

Table 1 The marker table.

Band	Marker						Lane 2					
	Rf	Mol Wt	Intensity	Area	%	Conc.()	Rf	Mol Wt	Intensity	Area	%	Conc.()
Standard												
1	0.01	250	13,158	306	3.61	3.61	0.05	109.52	33,800	440	6.04	6.04
2	0.03	150	22,100	272	6.07	6.07	0.24	31.44	49,560	400	8.86	8.86
3	0.06	100	32,776	306	9	9	0.27	28.02	41,800	360	7.47	7.47
4	0.1	70	34,510	340	9.48	9.48	0.49	16.59	171,560	920	30.67	30.67
5	0.15	50	33,762	340	9.27	9.27	0.58	14.42	123,280	760	22.04	22.04
6	0.19	40	46,444	340	12.76	12.76	0.68	4.75	139,400	760	24.92	24.92
7	0.25	30	41,208	340	11.32	11.32						
8	0.34	20	33,252	340	9.13	9.13						
9	0.58	15	43,622	340	11.98	11.98						
10	0.63	10	31,688	340	8.7	8.7						
11	0.66	5	31,586	340	8.67	8.67						

is similar to the observations reported by Hasle et al. (1980). They found that each gland is enclosed in a basement membrane followed by a layer of connective tissue that extends in between a single layer of glandular secretory epithelium. Keegan and Lockwood (1971) reported that in most of the scorpions, the secretory epithelium is thrown into more or less extensive folding, which varies in complexity depending on the genus and family. Moreover, the glandular tissues stained with Masson technique indicated the presence of two types of secretory materials exhibiting different staining colorations. Mohallal and Rahmy (1990) revealed that the presence of differently stained secretory materials might indicate that the glandular epithelial cells secrete different components that vary in their stainability. There are setae and cuticular pits scattered all over the telson of *A. amoreuxi*. Cuticular sensory organs are common in all arachnids. In scorpions, short, curved chemosensory setae are scattered all over the animal's body. Fet et al. (2000) studied metasoma or Orthochirus. A peculiar array of over 1000 cuticular pits was found ventrally and laterally on the posterior segments of metasoma and telson. Scanning electron microscope (SEM) showed those pits adorned with variable size setae, which exhibited microanatomical features characteristics for chemoreceptors.

The venom apparatus of *A. amoreuxi* is composed of a pair of venom glands that produce the venom and the sting is used for injecting the venom. The venom apparatus of *Leiurus quinquestriatus* is composed of two completely separated but similar glands, each with its own canal, which fuse into a single common canal (Ref. The differentiation of the venom gland into the lobes was observed by Kanwaru etal. (1981) in the venom gland of *Buthus tamulus* where the glands are divided longitudinally into parts by septum. Quiroga et al. (1998) studied the histology of the venom gland of adult female *Tityus caripitensis*. The venom gland of *T. caripitensis* is made of two ovoid lobes that fill the vesicle except a small cavity (lumen) where the venom accumulates. These characters are similar to *Euscorpius mingrelicus* and the others. Samano-Bihop and Ferriz (1964) studied the Mexican scorpions from three genera: *Vejovis, Diplocentrus* and *Centaurus*, they concluded that the morphology of the venom gland presents highly constant generic characteristics which could be used to classify the scorpions into families.

Gel electrophoresis was among the widely used techniques for the determination of the venom proteins against a standard protein. In this technique, identical proteins migrate to the same distance under electrical forces, while non-identical proteins usually migrate to different distances (Lodish et al., 2004). In the present study SDS–PAGE gel electrophoresis technique separated from the venom of the fat tailed scorpion revealed molecular weight that ranged between 14.4 to 212 KDa and from 5.9 to 189 to the gland. Similar results were published by Badahe et al. (2006) in their electrophoretic studies on the venom of the red scorpion *Mesobuthus tamulus*. The band 30–80 may be phospholipase A2 (Sabotka et al., 1976; Badahe et al., 2006). And the band 26.8 was previously identified as hemorrhagic toxin (Badahe et al., 2006). However, the band 50.22 was not yet identified (Maeda et al., 1991; Badahe et al., 2006).

The knowledge about the enzyme of scorpion venom is meager. Only few reports are available on the presence of specific enzymatic activities in scorpion venom (Minton, 1974; Amir et al., 1994). Isolate at least seven protein fractions with proteins and alkaline phosphomonoesterase activities from Isometrus vittatus scorpion.

No data were available in the literature concerning the high molecular weight bands of more than 100 kDa such as bands of 212 kDa and 170 kDa of the present study. Generally, scorpion venom consists of a variety of compounds which include several neurotoxins, histamine, serotonin, enzymes, enzyme inhibitors and other unidentified compounds that produce large variety of poly-peptidic toxins (Amir et al., 1994). Abbas et al. (2009) isolated three major α-type toxins from *A. amoreuxi* scorpion AaH1, AaH2 and AaH3 which were previously described as putative toxins from CDNAs (Chen et al., 2003). According to Abbas et al. (2009) two main families of scorpion toxins have been described; long chain and short chain scorpion toxins. Long chain toxins are peptides made of about 60–76 amino acid residues whereas short chain toxins contain only 25–40 residues. The so-called long chain toxins are mainly responsible for the neurotoxic symptoms developed during scorpion envenomation. The neurotoxic effect is related to the impairment of the function of Na channels which act as molecular batteries permitting excitable cells to produce and propagate electrical impulses (De la Vega and Possani,

2005). They may also contain mucus, various salts, peptides, nucleotides and amino acids (Froy et al., 1999).

In this concern Amir et al. (1994) isolated four fractions (IA, IB, IVA, and IVB) from the crude venom of *Isometrus vittatus* with molecular weights of 80.0, 75, 19 and 17 kDa. The four fractions have protein alkaline phosphomonoesterase activities. These bands that molecular weights are in agreement with bands that were isolated from the crude venom and the venom gland of *A. amoreuxi*. In the higher molecular weight region, the venom protein bands might correspond to the high molecular weight hyaluronidase that was isolated from the venom of *Tityus serrulatus* (Alagon et al., 1978).

Recently six toxins with varying lethality were isolated from the venom of *Androctonus mauritanicus* by HPLC technique (Zerrouk et al., 1991). Abbas et al. (2009) isolated fractions containing peptides with average masses of 6936, 6997 and 7048 kDa from the crude venom of *A. amoreuxi*. The composition of the three peptides revealed by amino acid analysis was in perfect concordance with that calculated from CDNA in the venom gland (Chen et al., 2003). AamH1, AamH2 and AamH3 are composed of 63, 64, 66 amino acids and constitute 0.59%, 1.32% and 0.885% in the weight from the whole crude venom, respectively (Abbas et al., 2009). This result may explain appearance of the bands 62.36, 59.7 and 64.64 in the venom gland protein electrophoresis and bands.

The various constituents of the venom may act directly and individually or synergistically to manifest their effects (Amir et al., 1994). In addition, differences in the amino acid sequence of each toxin account for their differences in the function and immunology (Froy et al., 1999). The presence of cytotoxic components in the venom of snake and arthropod, including scorpion is not uncommon and responsible for mild to severe tissue and organ damage.

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