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# Histological Study On Venom Gland Apparatus In Odontobuthus doriae (Scorpions:Buthidae), Scorpio maurus Towsendi (Scorpiones:Scorpionidea) And Hemiscorpius lepturus (Scorpions:Hemiscorpidea) From Iran

**Shahrokh Navidpour[1], Mohammad Mehdi Gharagozloyan[2] & Iraj Pousty[3]**

*[1,2] Razi Reference Laboratory of Scorpion Research, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.*
*[3]Department of Anatomy, Scinence and Reserch Branch, Islamic Azad University, Tehran, Iran*

***Corresponding Author:** Shahrokh Navidpour, Razi Reference Laboratory of Scorpion Research, Razi Vaccine and Serum Research Institute , Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran,*

## ABSTRACT

*Histological investigations were carried out on the venom gland of Hemiscorpius lepturus, Scorpio maurus townsendi, Odontobuthus doriae. The results revealed that the walls of H.lepturus poison sacs were single folded whereas those of both S. m. townsendi and O. doriae were complex folded. The number of folds O.doriae was higher than S. m. townsendi. It is notable that in O. doriae, there were two groups of cells in the poison sac. The venom-producing cells were visible on one side and the mucosal cells on the other side. The mucus cells observed by Masson trichrome staining were darker at the base of the cell than the apex.*

**Keywords:** *Odontobuthus doriae, Scorpio maurus townsendi, Hemiscorpius lepturus, Scorpion, Telson, Venom Gland, Histology.*

## INTRODUCTION

Scorpion sting has long been one of the problems in human societies and scorpion was considered as dangerous and harmful creature. Scorpions do not actually bite – they only sting. Many people use the word “bite” to describe a sting. The scorpion mainly stings in tropical and equatorial regions worldwide.

The snake antidote can easily neutralize the snake venom through the patient's body but there is a widespread disagreement among specialists regarding the neutralization of the scorpion venom by its antidote. Iran due to its proximity to the Equator and its climatic conditions is suitable for life and activities of more than 50 scorpions species. Despite their reputation and the great number of scorpion species, only a few, mostly belonging to the Buthidae family, are dangerous for humans. About 30 out of more than 1,500 described species worldwide have venom potent enough to be considered dangerous for human beings (Fet et al. 2000) [1]. Toxin-secreting cells can be identified with regards to the different nature of venom produced by these families using histological study. The aim of this study was to further characterize the venom gland cells of three scorpion families' in Iran. This study will be helpful to develop and produce an appropriate antidote to solve the scorpion sting related threats H. lepturus, S. maurus townsendi, O. doriae are considered as the most dangerous scorpions in Iran and neighboring countries (Navidpour et al. 2008) [2]

The sting or aculeus, which is a part of the scorpion venom apparatus, is situated on the final segment of the metasoma called the telson. The telson, as well as the entire body, is covered by cuticle, which has various types of sensory setae and pits on its surface (Brownell & Polis, 2001). [3] The venom apparatus of the H. lepturus, S. maurus townsendi, O. doriae like the rest of scorpions consists of paired venom glands, which are initially presented its along a canal. The venom apparatus of  is composed of two completely separate but similar glands, composing a single channel along the canal.

The telson is covered with cuticle constituting a sandwich-like superposition between itself and the secretory epithelium composed from a sheath of skeletal striated muscles that covers the base to apex. (Tabia and Jarrar .1993) [4]

Each gland is enclosed in a basal lamina, with a layer of loose connective tissue the lumen forms a single layer secretory epithelium glands located between the connective tissue. (Halse et al. 1980).

## Material & Methods

Scorpion species were identified and confirmed by the national scorpion reference laboratory located in Razi Vaccine & Serum Research Institute, Karaj, Iran. The venom apparatus of 30 adults O. doriae, S. maurus townsendi, H. lepturus were collected from scorpions found under the stones in Iran during April 2014. The telson was removed from each scorpion and quickly immersed duringfive days in the following fixatives: 10% neutral buffered formalin (pH 7.4) with 2% calcium acetate.

After fixation, tissue samples were extracted from formalin and then thoroughly washed in running water, dehydrated, cleared, impregnated and embedded in paraffin wax. Samples are then cut in serial sections of 5 µm. Finally, paraffin sections were stained by hematoxylin-eosin and the Masson trichrome staining method was used in for the better recognition and differentiation of mucus cells histological examination.

## Results and Conclusions

Microscopy slides prepared from the three studied Scorpions (O.doriae, S. m. townsendi, H. lepturus) were studied. In all species, the venom apparatus was composed from completely separated bilateral venom glands covered by cuticle. Each gland had a canal that fuses into a single common canal in the acleus at the end of the curved needle-like sting ends. The common canal lacked musculature and was lined with a chitinous internal layer, followed by a non-excretory simple cuboidal epithelium.

Our observations showed that the secretory epithelium of the venom glands of was made up of three types of cells including venom-producing cells, mucus cells and supporting cells. The glandular epithelium consisted of venom-producing cells, mucus-secreting cells and non secretory supporting cells which were covered by a sheath of skeletal striated muscles. The venom-producing cells were apocrine and the high columnar cytoplasm presented small nuclei on its base.

The supporting cells were subcuboidal, located between the venom-producing cells and the basal lamina. These cells seemed to replace the venom-producing cells following their degeneration. The mucus cells were enclosed by the venom-producing cells. They were pyramidal or columnar with a nucleus located at the base of the cell. The poison was produced within the apical cytoplasm of the venom-producing cells, containing fine and coarse granules and exhibiting variable coloration patterns when using the same histological staining.

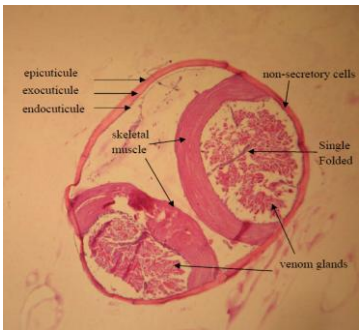


**Fig1.** *Cross-section of Hemiscorpius lepturus venom glands stained with hematoxylin-eosin ×40*

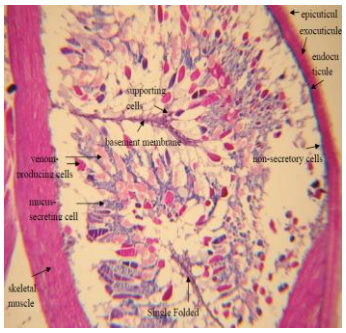


**Fig2.** *Cross-section of Hemiscorpius lepturus venom glands stained with Masson trichrome ×100*

### Hemiscorpius lepturus

The gland cuticle layers included epicuticule, exocuticule and endocuticule. Exocuticule was thicker than the other layers. Endocuticule was not dissociable from the exocuticule by hematoxylin-eosin staining. The endocuticule layer appeared indark blue by Masson staining. Immediately after this layer, the cuboidal cells with dark nuclei covered the whole internal part of the poison sac. Our sections clearly illustrated the single folded poison sac.

The connective tissue covered the inside of the poison sac and the fibroblast cells were located inside the gland wall. Toxic cells were in the basal lamina of the gland connective tissue looking like grape clusters.

Supporting cells exhibited cube-shaped with a spherical nucleus. The nucleus of supporting

cells was euchromatin in young cells and heterochromatin in older cells. These cells were sitting on the connective tissue.

The Poison-secreting cells were pyramid-cylindrical in the H. lepturus. At the top of the cells the cytoplasm was filled with a lot of granules. The nucleus was located in the bottom of the cell. The mucosal cells had pyramidal or columnar shapes and their nuclei were located at the base. They were located within the venom-producing cells. The skeletal muscle covered both poison sacs.

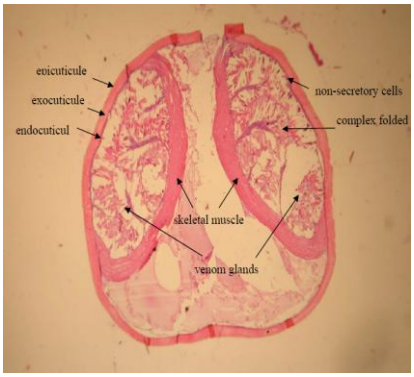


**Fig3.** *Cross-section of Scorpio maurus townsendi venom glands stained with hematoxylin-eosin ×40*

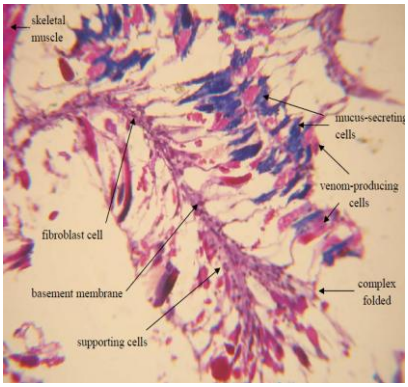


**Fig4.** *Cross-section of Scorpio maurus townsendi venom glands stained with Masson trichrome ×400*

## Scorpio maurus towsendi

The external cuticle layer surrounding the gland was similar to that of H. lepturus comprising epicuticule exocuticule and endocuticule. Exocuticule appeared thicker than the other layers. The endocuticule layer was not dissociable from the exocuticule layer by hematoxylin-eosin staining. The endocuticule appeared uncolored by Masson staining.

The poison sachad a complex folding and the non-secretory cells fixed the inner gland and

In some parts of the poison gland, folds were divided into two parts.

Connective tissue cells were abundant in the folds of this scorpion. The non-secretory supporting cells and venom-producing cells were similar to those of H. lepturus.

The mucus-secreting cells had basal nucleus and were enclosed by the venom-producing cells by Masson staining. The skeletal muscle covering both poison sac and nerve fibers was visible and formed the skeletal muscle layers. The attachment of muscle bundles to the telson cuticle was mediated by dense intercalated tendons.

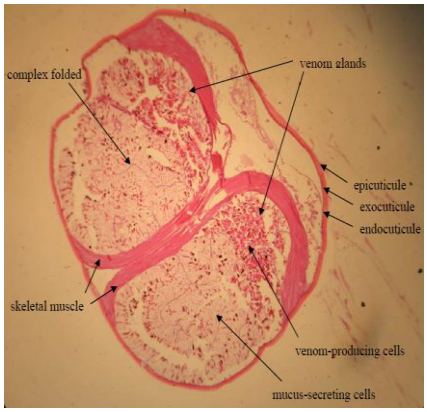


**Fig5.** *Cross-section of Odontobuthus doriae venom glands stained with hematoxylin-eosin ×40*

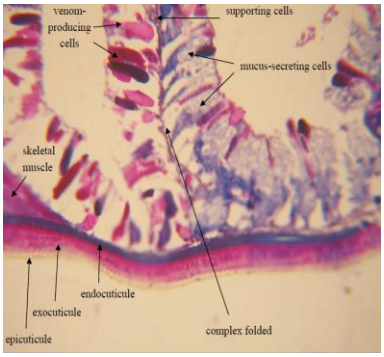


**Fig6.** *Cross-section of Odontobuthus doriae venom glands stained with Masson trichrome ×200*

## Odontobuthus doriae

The middle layer of exocuticule has less thickness to compare by H. lepturus and S. maurus townsendi. The endocuticule appeared indark blue by Masson staining.Connective tissue cells occurred in the folds of this scorpion and seemed to be more adundant than those observed in S. maurus towsen. The poisonous glands had a complex-folded form. Those folds were higher than S. maurus towsendi.

It is notable that this scorpion exhibits two groups of cells in its poison sac a similar

arrangement is also observed in the O. poison sac. The venom-producing cells were visible on one side and the mucosal cells on the other side. The mucus cells observed by Masson trichrome staining are darker at the base of the cell than the apex**.** The skeletal muscle is similar to the one of abovementioned scorpions.

## DISCUSSION

Our results showed that venom apparatus of O.doriae were composed of two completely separate but similar glands, each one with its own canal. These observations are in agreement with those of Taib & Jarrar 1(993). [4]

The difference between the venom glands located in the lobes were observed by (Kanwar et al. 1981) [6]

These results are also in line with those reported for Buthus tamulus where the glands were divided longitudinally in two parts by a septum. (Quiroga et al. 1998). [7]

The cuticle can be also divided in two different sections and three main layers. (Hjelle, 1990). [8]

As in all other arachnids, the bodies of scorpions are covered with cuticle. The cuticle covering the telson is composed of three main layers: an outermost epicuticle, which is like a waxy layer, an exocuticle layer composing the homogenous middle layer, and the innermost endocuticle, which is the thickest layer. The endocuticle is made from alternating layers of chitin. (Mazurkiewicz & Bertke, 1972). [9] The external cuticle layers of the gland are divided into epicuticule - exocuticule - endocuticule. The structure of these three layers composing the cuticle can be distinguished in the sting cuticle of all 3 studiedscorpions as well as in the one of Mesobuthus gibbosus (Yigit & Benli, 2007) [10]. The ducts and the cuticle can be seen in both venoms. The epicuticle appeared as a thin transparent layer with an amorphous appearance, while the exocuticle and endocuticle constituted a thick homogenous middle layer and e a thick lamellar layer, respectively. The cuboidal cells were located immediately after the endocuticule layer in the H. lepturus, S. maurus towsendi and O. doriae species. They exhibited basophilic and dark nuclei that make the whole Telson. These cells have not secretory properties.

The present study showed that the secretory epithelium of the venom glands in all three studied species is made up of three types of cells: venom-producing cells, supporting cells and mucous cells. This is an agreement with the findings of Karsch 1879 on Buthus martensi (Keegan & Lockwood 1971) [11]. Taib & Jarrar 1993[4] reported that the secretory epithelium in the venom glands of L.quinquestriatus is made up of three type cell: venom-producing cells, mucous cells and supporting cells. The venom producing cells are apocrine and filled with granular droplets. They form high columnar and bottle shaped cells with a nuclei situated near to the basal lamina. The supporting cells are subcuboidal and attached to the basal lamina. They are arranged between venom producing cells and seem to replace them following their degeneration.

Yigit & Benli 2008 [12] have also shown that Euscorpius mingrelicus venom gland is composed of different cell types. One type represents the venom producing cells that have several granules of different sizes, shapes and electron densities. Another type is the supporting cells, which are found between the glandular epithelium and the cuticle or between the glandular epithelium and the last muscle bundles. The third type is goblet cells secreting mucus, which were enclosed by secretory epithelial cells.

Goyffon & Kovoor (1978) [13] have described only two types of epithelial cells lining the venom glands of Pandinus imperator Koch 1842. The same observations have been reported by Keegan & Lockwood (1971) [11] in the case of both Centruroides limpidus Hoffman 1932 and C. vittatus Say 1821.

Halse et al. (1980) [5] found less extensive folding in the secretory epithelium of Urodacus, occurring only on the medial side of each gland. The same authors also described two types of secretory epithelial cells including goblet and columnar cells with a nucleus situated in their basal regions. The secretory products were stored in the apical region of the goblet cells, whereas in the columnar cells they were more evenly distributed throughout the cell.

However, Junqua & Vachon 1968[14] concluded that there is only one type of secretory cell in the venom glands of scorpions.

Quiroga et al. 1998 [7] determined that T. Caripitensis's venom glands are made of a simple, pseudostratified epithelium. The epithelium contains secretory cells that have either coarse-grained or thin granules, basal cells or non secretory cells.

Jarrar & Al-rowaily 2008[15] showed that Androctonus crassicauda glands exhibited extensively folded secretory epithelium composed from both non-secretory and secretory venom-producing cells.

The venom droplets are located inside the cytoplasm, at the apical parts of the venom producing cells as well as inside the lumen of the gland. The majority of the venom-producing cells are filled with aggregates of granules and each cell contained granules of similar size. These granules vary in their reactions with the same stain and can be grouped into at least five types according their contents or degree of maturity**.** (Taib & Jarrar 1993) [4].

However, in this study, the mucus cells stained with Masson, appeared darker and more faded in their base than in their head. This differential coloration is due to the high concentration of basophilic cells located at the base of these cells and the higher presence of well-colorable mucous secreting cells.

Cuticle structure and layers at S. maurus townsendi , are similar to those of H. lepturus.

In the S. maurus towsendi, poison sac exhibits a complex folded form. Some parts of the gland, the folds are divided into two branches. In the connective tissue cells, the folds are more widely distributed in S. maurus towsendi when compared to H. lepturus. Supporting cells and secretory cells in S. maurus towsendi are similar to those of H. lepturus. The differences observed in this family could be linked to their habitats.

Al- Asmari et al. 2009[16] at S.maurus kruglovi sections, Scorpionidae family, showed simple or no folding, with one layer of a very thick cuticle. Thus, these results are in disagreement with those of the present study. However, several factors such as differences in the studied species and their respective habitats may partly explain these contradictory observations. The Exocuticule layer of O. doriae seemed to be less than extended, compared with those of H. lepturus and S.maurus towsendi . This could be due to the progression of gland and an increasing number of toxic cells.

In O. doriae the poison sac form is complex folded and it folds more extensively than the S. maurus towsendi. This fact is probably due to differences in the type and amount of secreted toxins.

Histological profiles show that scorpion venom glands collected from the Riyadh region belonging to the telson cross sections of Buthidae family and including Compsobuthus arabicus, Compsobuthus werneri, Leiurus quinquestriatus, Androctonus crassicauda, Androctonus bicolor, Buthacus yotvatensis nigroaculeatus, Buthacus leptochelys and Orthochirus innesi exhibit complex folded glands. In fact, telson sections of Leiurus quinquestriatus, Androctonus crassicauda and Androctonus bicolor are known for their distinct and densely folded glands. (Al-Asmari et al 2007) [16].

Lourenco 1985[17] and Pawlowsky 1912[18] have extensively studied scorpion venom glands. They have found that the complexity and effectiveness of the venom rely highly on the folding level of their venom glands, which is related to the scorpion family and phylogeny. Therefore, the venom gland histology presents constant generic characteristics that could be useful and applicable in higher level scorpion taxonomy.

The work of Pawlowsky 1913[19] on six of seven known scorpion families revealed that the morphology followed a generalized scheme and the scorpion families could be mainly differentiated according to ,the presence or the absence of folds in the secretory epithelium. Two main types were listed: type I (primitive gland) possess a smooth and indented epithelium and type II (complex gland) presents true folds.

Venomous secretory materials produced within the venom gland were subdivided into several types according to their locations and structures. These granules vary in their reactions to the same stain. One type is coarse-grained electron-dense granules. Another type of granule has a spongy structure and large electron-dense granules in secretory cells. The third type is electron lucent with transparent granules (Yigit & Benli 2008). [12]

In O. doriae, it was shown that the connective tissue cells are more extensively folded than those of S. maurus towsendi. The frequency and extent of the venom gland are probably due to these folds. To conclude, significant differences were observed in the microscopic structure of the cells of H. lepturus, S. maurus towsendi, and O. doriae . Moreover, in all three studied species males and females exhibited similar features. The venom gland histology performed in this study presents constant generic characteristics that

could be useful and applicable in the investigation of higher level scorpion taxonomy.

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## REFERENCES

[1] Fet V, Sissom WD, Loe G, Braunwalder ME. Catalog of the Scorpions of the World (1758–1998). New York, NY: New York Entomological Society. 2000; 690.

[2] Navidpour S, Kovarik F, Soleglad M E, Fet V. Scorpions of Iran (Arachnidda, Scorpiones). Part I. Khoozestan Province. Euscorpius. 2008; 65: 1-41.

[3] Brownell P, Polis G. Introduction. In: Brownell, P. H. & G. A. Polis (eds.). Scorpion Biology and Research. New York, NY: Oxford University Press, 2001; 3–12.

[4] Taib NT, Jarrar BM. Histological and histochemical characterization of the venom apparatus of Palestine yellow scorpion, Leiurus quinquestriatus Hemprich & Ehrenberg 1828. Tropical Zoology, 1993; 6:143–152.

[5] Hasle SA, Prideaux PL, Cockson A, Zwicky KT. Observations on the morphology and histochemistry of the venom glands of a scorpion, Urodacus novaehollandiae Peters (Scorpionidae). Australian Journal of Zoology. 1980; 28. 185-194.

[6] Kanwar U, Sharma A, Nagpal N. Morphological and cytochemical studies on the venom secreting cells of the scorpion Buthus tamulus. Journal of Animal Morphology & Physiology 1981; 28(1): 206-209.

[7] Quiroga M, Marval MJ, Parilla-Alvarez P, Sousa L. Tityus caripitensis n. sp. Scorpion venom gland histology. Toxicon 1998; 36.1269.

[8] Hjelle J. T. Anatomy and morphology. Ppin: Polis, G.A. (ed.). Biology of Scorpions. Stanford, California: Stanford University Press. 1990; 9–63.

[9] Mazukiewicz J E, Bertke EM. Ultrastructure of the venom gland of the scorpion, Centruriodes sculpturatus (Ewing). Journal of Morphology. 1972; 137: 365–384.

[10] Yigit N, Banli M. The sting of Mesobuthus gibbosus (Scorpiones: Buthidae): morpholo gical and ultrastructural characterization Euscor pius. 2007; 61:1-5.

[11] Keegan HL, Lockwood WR... Secretory epithelium in venom glands of two species of the genus Centruroides. American Journal of Tropical Medicine and Hygiene 1971; 20 (5):770-785.

[12] Yigit N, Banli M. The venom gland of the scorpion species Euscorpius mingrelicus (Scorpiones: Euscorpiidae): morphological and ultrastructural characterization. Journal of Venomous Animals & Toxins including Tropical Diseases. 2008; 14(3): 466-480.

[13] Goyffon M, Kovoor J. Chactoid venoms. In: BETTINI S. Ed. Arthropod Venoms. Berlin: Springer-Verlag. 1978; 395-417.

[14] Junqua C, Vachon M. Les Arachnides venimeux et leurs venins. Etat actuel des recherches. Memoires Academie Royale des Sciences d'Outre-Mer (Cl. Sci. Nat. Med., N.S.) 1968; 17 (5): 1-136.

[15] Jarrar BM, Al-Rowaily MA. Histology and histochemistry of the venom apparatus of the black scorpion Androctonus crassicauda (Olivier, 1807) (Scorpiones: Buthidae). Journal of Venomous Animals & Toxins including Tropical Diseases 2008; 14(3): 514-526.

[16] Al-Asmari AK, Al-Saif AA, Abdo NM. Morphological identification of scorpion species from Jazan and Al-Medina Al-Munawwara Regions, Saudi Arabia. Journal of Venomous Animals & Toxins including Tropical Diseases 2007; 13(4):821-843.

[17] Lourenco WR. Essai d'interpretation de la distribution du genere Opisthocanthus (Arachnida, Scorpiones, Ischnuridae) dan les region Neotropicale et Afrotropicale : étude taxonomique, biogeographique, evolutive et ecologique. PhD Thesis, L'Universite Pierre et Marie Curie, Paris. 1985; 287.

[18] Pawlowsky E. Studies on the organization and development of scorpions. Quarterly Journal of Micro- scopical Science. 1912; 68: 615-643.

[19] Pawlowsky E. Scorpiotomische Mittei- lungen. I. Ein Beitrag zur Morphologie der Giftdrüsen der Skorpione. Zeitschrift für Wissen-schaftliche Zoologie. 1913; 105: 157–177.

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