Histological study at apparatus of the venom gland in *Odontobuthus doriae* (Scorpiones:Buthidae) , *Scorpio maurus townsendi* (Scorpiones:scorpionidea) and *Hemiscorpius lepturus* (Scorpiones:Hemiscorpidea) from Iran

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Summary

Histological investigations were carried out on the venom gland of the ***Hemiscorpius lepturus***, ***Scorpio maurus townsendi***, ***Odontobuthus doriae* .** The results reveal that the walls form of the poison sacs ***Hemiscorpius lepturus*** half frizzy (Single Folded) , respectively. Poisonous sacs on the walls of the ***Scorpio maurus townsendi*** and ***Odontobuthus doriae*** complex folded and the number of folds is higher in comparison with ***Scorpio maurus townsendi.*** It is notable in ***Odontobuthus doriae***, the two parts of the cells in the poison sac is, so that the venom-producing cells in one side and the mucosal cells on the other side more apperanc. The mucus cells masson trichrome staining and at the base of cell darker than apex.

KEY WORDS: *Odontobuthus doriae*, *Scorpio maurus townsendi*, *Hemiscorpius lepturus*,scorpion, telson, venom gland, histology

Introduction

Scorpio the scorpion has long been one of the problems of human societies and Scorpio always as dangerous and insidious creature has learned. the scorpion stings in tropical and equatorial regions such problems in the world. Snake can easily snake venom antidote to neutralize the patient's body, but the scorpion venom neutralized by an antidote to the still widespread disagreement among experts that there is. Iran due to the proximity of the Equator and climatic conditions are suitable for life and activities in more than 50 species of scorpions that annually more than 60,000 people are leading to injury.

Despite their bad reputation and the great number of scorpion species, only a few, mostly in the family Buthidae, are dangerous to humans. About 30 out of more than 1,300 described species worldwide have venom potent enough to be considered dangerous to human beings (Fet et al., 2000; Brownell & Polis, 2001).

Histology of scorpion poison gland has not been investigated in Iran.

Since the production of toxic venom sac (Telson) is. Histological study of the organs of the scorpion toxin-secreting cells can be identified; possibly because of the different nature of venom belonging to these families is effective.

The aims of this study further understanding of the scorpion venom gland cells producing poisonous scorpions and three families in Iran, we can now step in the development of appropriate antidote and solve the problem survey**.**

Venom apparatus of scorpions has been described by many investigators (Pavlovsky 1912, Lourenco 1985, Polis 1990, Al-Asmari et al 2007, Taib & Jarrar 1993, Jarrar & Al-Rowaily 2008, Yigit & Benli 2008) for their histology, histochemistry and characters for higher-level taxonomy (Keegan & Lockwood 1971, Mazurkiewicz & Bertke 1972, Halse *et al.* 1980, Kanwar *et al.* 1981, Cebesoy & Ayvali 2003)

Hemiscorpius lepturus, *Scorpio maurus tonsendi*, *Odontobuthus doriae* are considered of the most dangerous scorpions in Iran and neighboring countries (Navidpour et al. 2008)

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).
the venom apparatus of the *Hemiscorpius lepturus*, *Scorpio maurus tonsendi*, *Odontobuthus doriae* like the rest of scorpions consists of paired venom gland, which initially presents its on canal.The venom apparatus of Leiurus quinquestriatus is composed of two completely. Separate but similar glands, each with it's on canal, which into a single canal. The telson is covered by cuticle like sandwiches between itself and the secretory epithelium a sheath of skeletal striated muscles that covers the base to apex. (TAIB and JARRAR)
Each gland is enclosed in a basal lamina, with a layer of loose connective tissue; between the connective tissue the lumen is a single layer of secretory epithelium gland. (Halse et al. 1980).

Material & Methods

Once identified and confirmed by a reference laboratory species of scorpions in scorpions Razi Vaccine Institute, The venom apparatus of 30 adult *Odontobuthus doriae*, *Scorpio maurus townsendi*, *Hemiscorpius lepturus* were collected from under thestones in Iran in April 2014.The telson was removed from each scorpion and quickly immersed for five days in one of 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, and cut serially sectioned at 5 μm. Paraffin sections were staining by haematoxylin-eosin and for better recognition differentiating mucus cells stained from Masson trichrome staining for histological examination.

Results

Morphology and histology microscopy slids studied in three Scorpion(*Odontobuthus doriae*, *Scorpio maurus tonsendi*, *Hemiscorpius lepturus*).The venom apparatus completely separated bilateral venom glands covered by cuticle. Each gland has 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 lacks musculature and is lined with a chitinous internal layer, followed by a non-excretory simple cuboidal epithelium.The study shows that the secretory epithelium of the venom glands of *Hemiscorpius lepturus*, *Scorpio maurus tonsendi*, *Odontobuthus doriae* are made up of three types of cells: venom-producing cells, mucus cells and supporting cells. The glandular epithelium consists of venom-producing cells, mucus-secreting cells and non-secretory supporting cells and then covered by a sheath of skeletal striated muscles.The venom-producing cells are apocrine, high columnar cytoplasm present basally situated small nuclei. The supporting cells are subcuboidal, located between the venom-producing cells and on the basal lamina and seem to replace those following venom-producing cells degeneration.The mucus cells are between the venom-producing cells. They are pyramidal or columnar with the nucleus are located at the base of cell.
The poison produced, within the apical cytoplasm of the venom-producing cells, contains fine and coarse granules that showed variable coloration patterns when using the same histological staining.

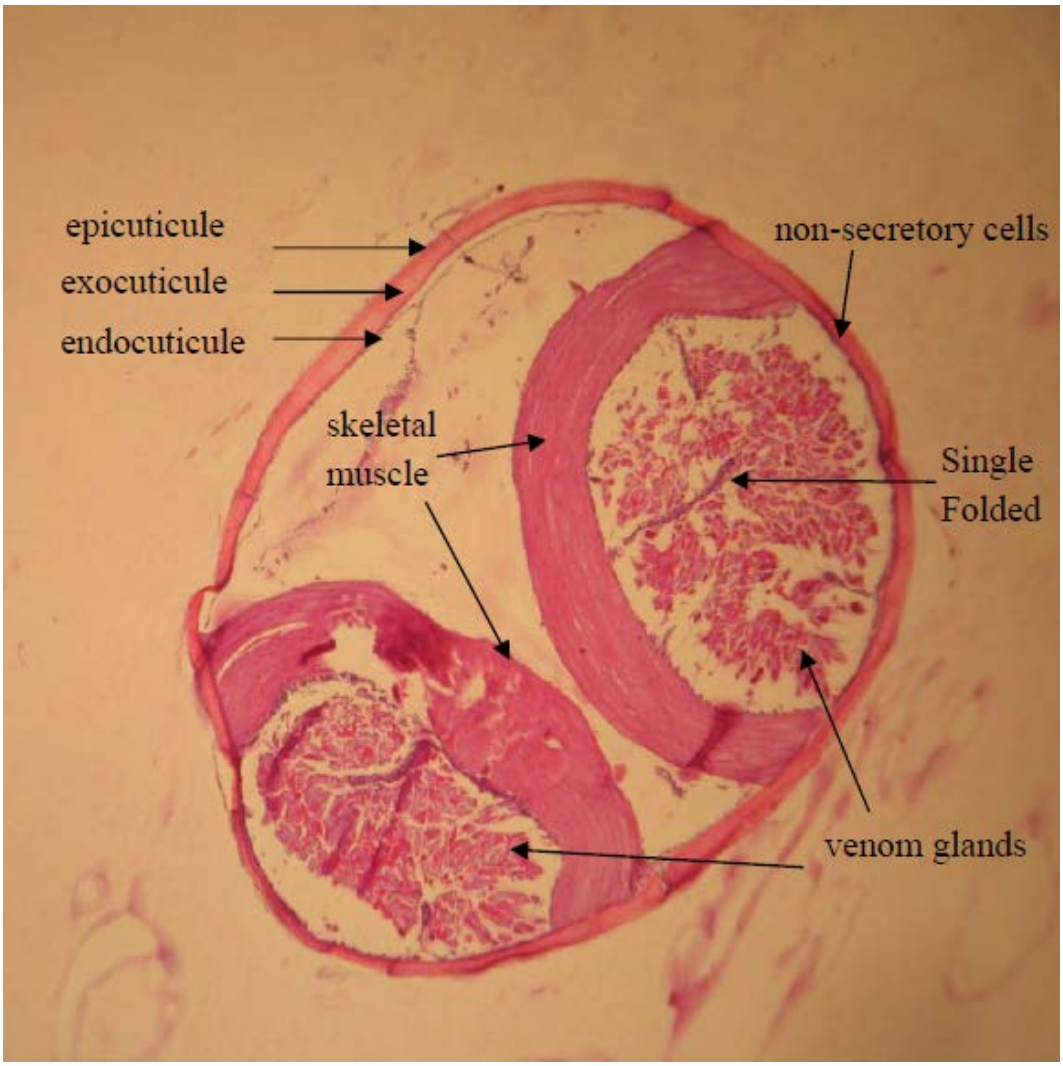

Figure 1. Cross-section of *Hemiscorpius lepturus* venom glands stained with hematoxylin-eosin ×40

Figure 2. Cross-section of *Hemiscorpius lepturus* venom glands stained with Masson trichrome ×100

Hemiscorpius lepturus

cuticle layers into the gland include: (epicuticule - exocuticule -endocuticule) are. exocuticule thicker than the other layers.endocuticule by hematoxylin-eosin staining are not inseparable the exocuticule.By masson staining, the endocuticule layer was appearing dark blue. Immediately after layer of cuboidal cells with dark nuclei, the whole bag of poison inside cover.The walls of poison sac covered by Single Folded.

The connective tissue covers the inside of the poison sac and the Fibroblast cells inside of the wall of gland, toxic cells in the basal lamina into the connective tissue of the gland, toxic landscape of grape clusters show

Supporting cells that cube-shaped with a spherical nucleus in younger cells are euchromatin and heterochromatin on older cells. . These cells are on the connective tissue.

The *Hemiscorpius lepturus* Poison-secreting cells are pyramid-cylindrical. On the top of cells the cytoplasm is filled by a lot of granules.The nuclei are located in the bottom of cells. Euchromatin nuclei are younger and Heterochromatin more the older one.The mucosal cells are located pyramidal or columnar cells and their nuclei are located at the base. They are among of the venom-producing cells.

the skeletal muscle coverd both poison sac definitely something the nerve fibers of the muscle layers are visible.

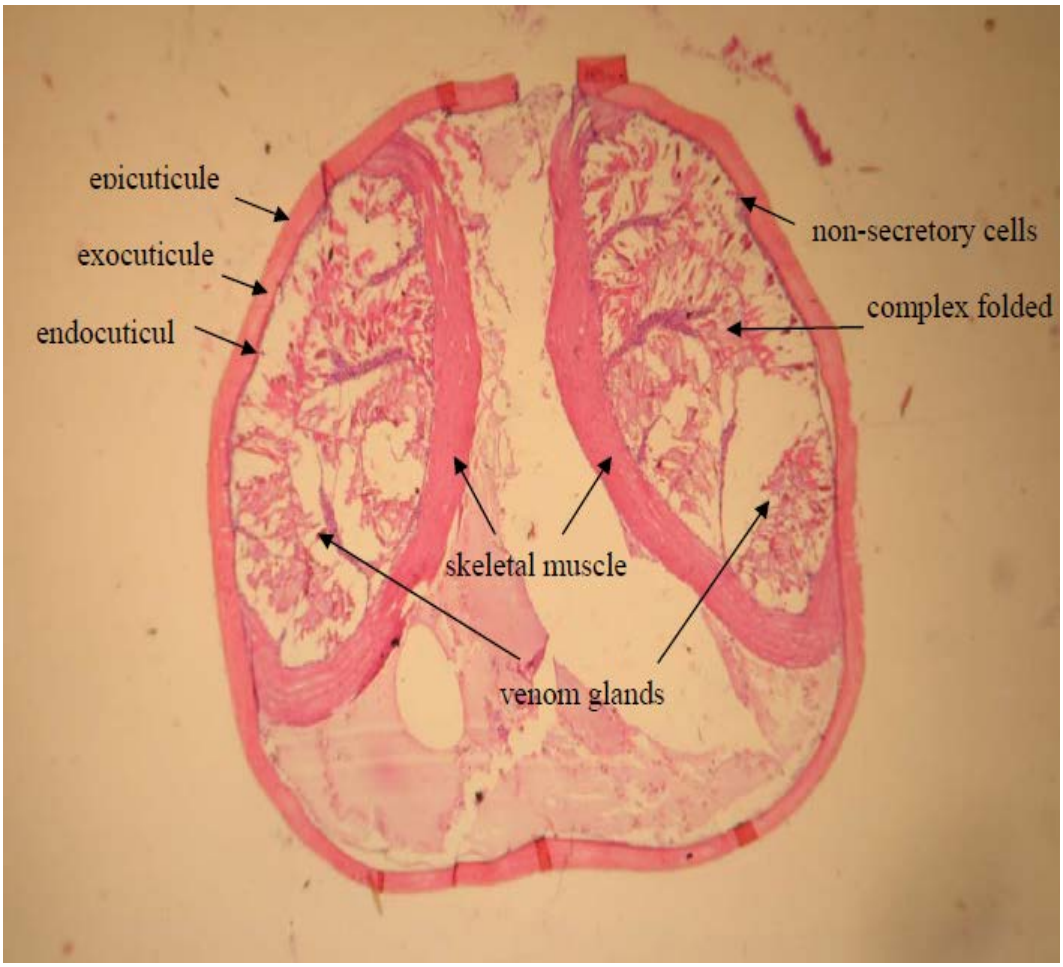

Figure 3. Cross-section of *Scorpio maurus townsendi* venom glands stained with hematoxylin-eosin ×40

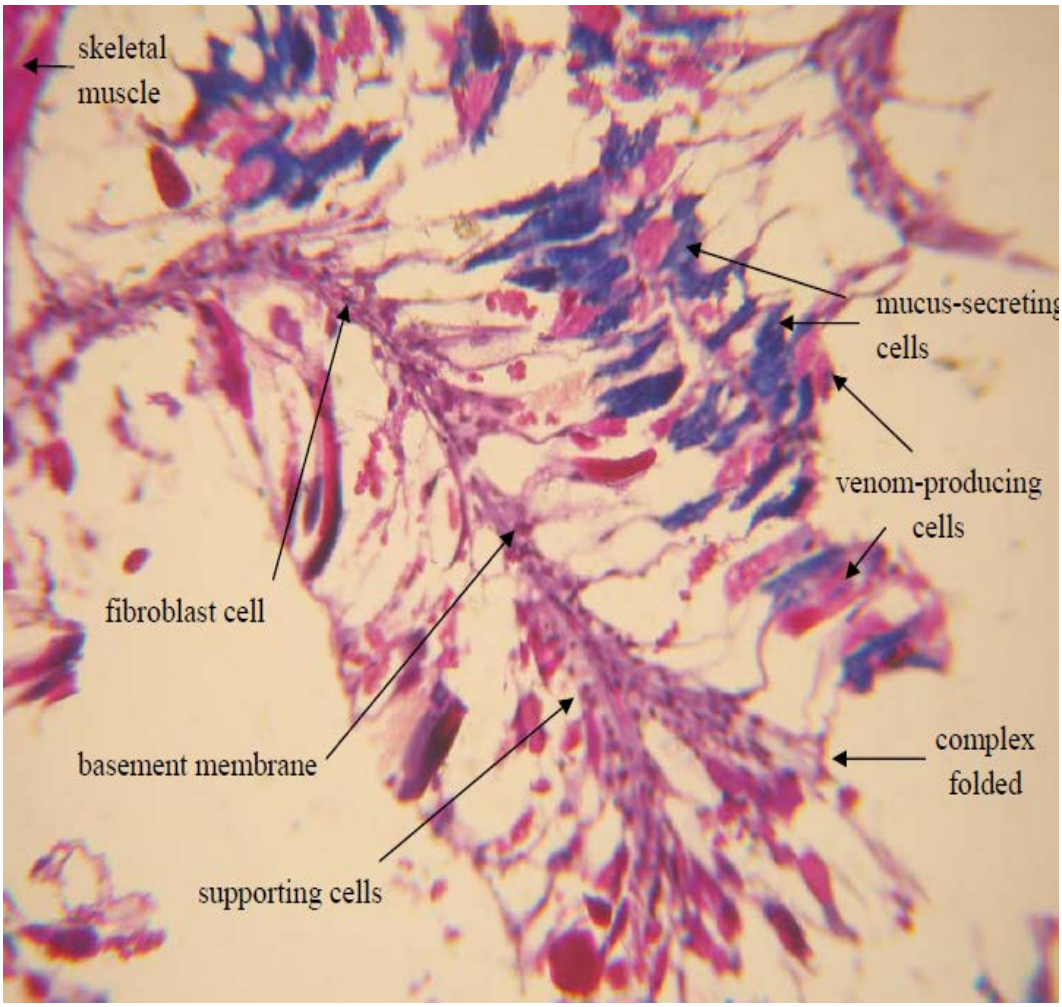

Figure 4. Cross-section of *Scorpio maurus townsendi* venom glands stained with Masson trichrome×400

Scorpio maurus townsendi

External cuticle layers around the gland is the same as *Hemiscorpius lepturus* containe (epicuticule - exocuticule - endocuticule) is .Exocuticule is appear and thicker than the other layers. by hematoxylin-eosin staining the endocuticule layer is not separable the exocuticule by masson staining the endocuticule is color loss.

The un-secretory cells which make up the inner gland. poison sac in spirit frizzy (complex folded), respectively. also folds in some parts of the poison gland is divided into two parts.

Connective tissue cells are abundant in the folds this poison sac in this scorpion . un-secretory supporting cells and venom-producing cells, are such as *Hemiscorpius lepturus* are.

by masson staining the mucus-secreting cells located basal nuclus appear between the venom-producing cells.

the skeletal muscle covered both poison sac and nerve fibers are visible the skeletal muscle layers.

The attachment of muscle bundles to the telson cuticle is mediated by dense intercalated tendons that firmly attach the muscle bundles to the cuticle**.**

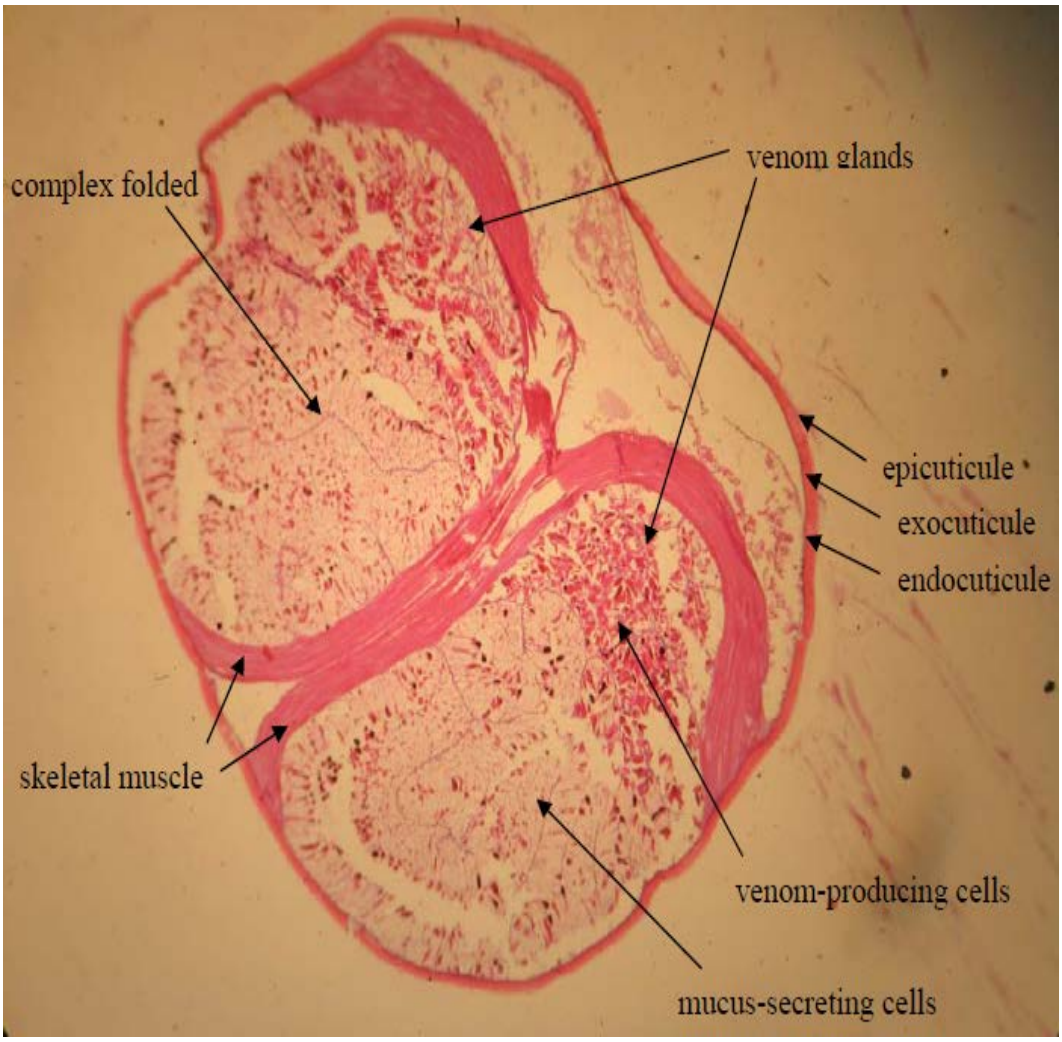

Figure 5. Cross-section of *Odontobuthus doriae* venom glands stained with hematoxylin-eosin ×40

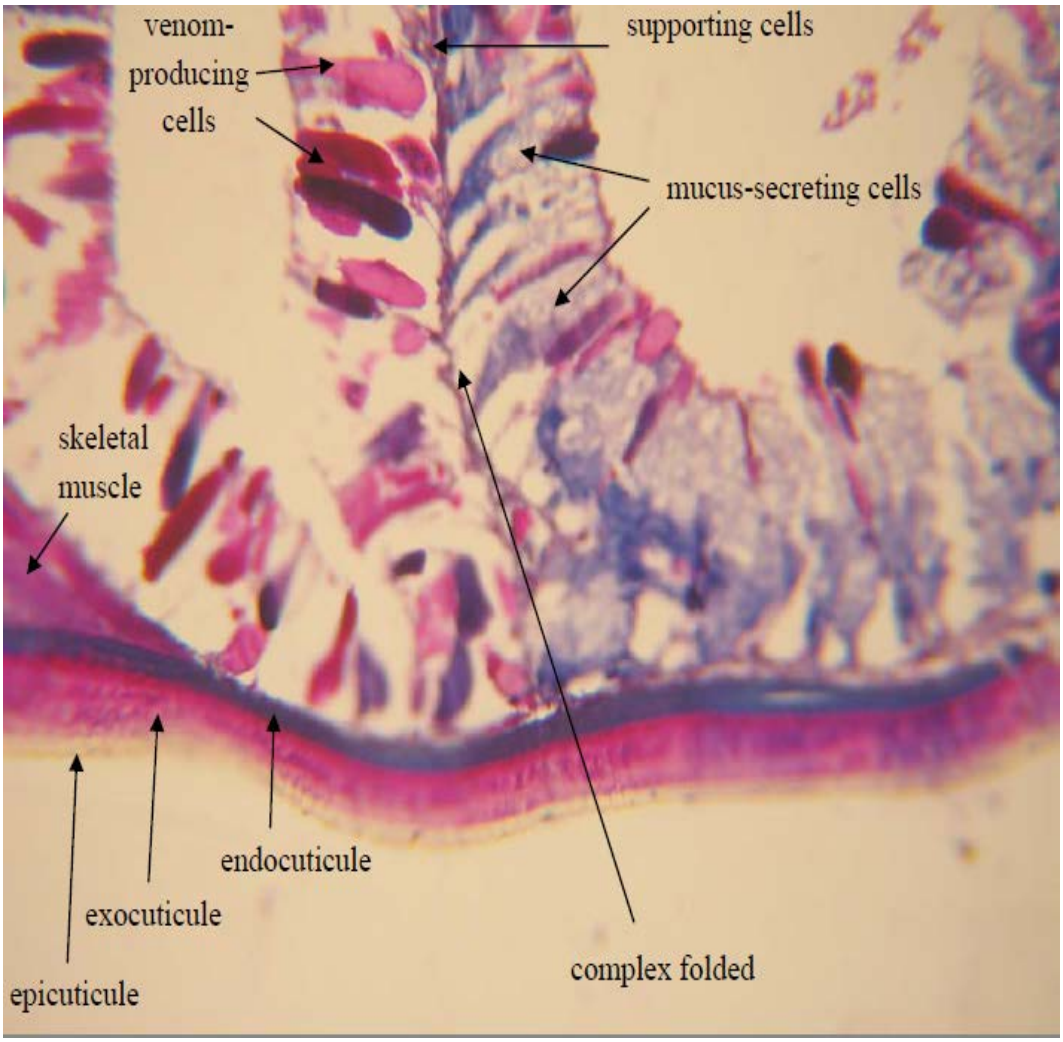

Figure 6. Cross-section of *Odontobuthus doriae* venom glands stained with Masson trichrome ×200

Odontobuthus doriae

The middle layer of exocuticule has less thickness to compare by *Hemiscorpius lepturus* and *Scorpio maurus townsendi*.by masson staining the Eendocuticule is more dark blue.Connective tissue cells in the folds occurred in this scorpion, which seems more severe *Scorpio maurus townsendi*.Poisonous glands in spirit form is complex those folds are higher than *Scorpio maurus townsendi*.It is notable this scorpion are two parts of the cells in the poison sac, so that the venom-producing cells in a part and the mucosal cells are on the other parts.
by masson staining the mucosal cells, were staining darker at the base than the apex appear**.** The skeletal muscle is the same as other pervious scorpions were study in our survey that is taken separately at each poison sac**.**

Discussion

Findings venom apparatus of *Hemiscorpius lepturus, Scorpio maurus tonsendi, Odontobuthus doriae* is composed of two completely separate but similar glands, each one with its own canal is in occordence with Taib NT & Jarrar BM 1993 of *Leiurus quinquestriatus* (Hemprich et Ehrenberg, 1827)
The difference between of the venom glands into lobes were observed by Kanwar *et al*.
Similar to this research in *Buthus tamulus* gland where the glands were divided longitudinally into parts by a septum. Quiroga *et al*.
The cuticle can be also divided into different sections in three main layers (Hjelle, 1990).
As in all arachnids, the bodies of scorpions are covered by cuticle. The cuticle covering the telson is composed of three main layers: an outermost epicuticle, which is like a waxy layer; an exocuticle, the homo- genous middle layer;

and the innermost endocuticle, which is the thickest layer. The endocuticle is constructed of alternating layers of chitin (Mazurkiewicz & Bertke, 1972)
External cuticle layers into the gland are epicuticule - exocuticule - endocuticule.
The structure of these three-layers cuticle can be distinguished in the sting cuticle in all three scorpions in this study as of M. gibbosus (Yigit & Benli, 2007). The ducts and the cuticle can be seen in the both venom. epicuticle, a thin transparent layer with an amorphous appearance. exocuticle, a thick homogenous middle layer and endocuticle, a lamellar layer thicker than the epicuticle.
at the *Hemiscorpius lepturus*, *Scorpio maurus tonsendi*, *Odontobuthus doriae* non-toxic cells immediately after endocuticule layer are cuboidal with basophilic nuclei and dark that makes the whole Telson from the inner .These cells have secretory properties.
The present study shows that the secretory epithelium of the venom glands in all three cases of *Hemiscorpius lepturus*, *Scorpio maurus tonsendi*, *Odontobuthus doriae* is made up of three types of cells: venom-producing cells, supporting and cells mucous cells. This is an agreement with the findings of KUBOTA (1918) on *Buthus martensi* Karsch 1879 (cf. KEEGAN & LocKwooD 1971) and Taib and Jarrar reported that the secretory epithelium of *L. quinquestriatus* venom glands are made up of three types cell: venom-producing cells, mucous cells and supporting cells.The cells that are venom producing apocrine and filled with granular droplets. They are high columnar, bottle shaped cells that have small, round nuclei and 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 and Benli at Similarly found that *E. mingrelicus* venom gland is composed of different cell types. One type is the venom producing cells that have several granules of different sizes, shapes and electron densities. Another type are the supporting cells, which found between glandular epithelium and cuticle or between glandular epithelium and the last muscle bundles. Type is goblet cells, where found among secretory epithelium cells, its function to secrete mucus**.**
while GoYFFON & KovooR (1978) have described only two types of epithelial cells lining the venom glands of Pandinus imperator Koch 1842 and so did KEEGAN & LocKWOOD (1971) in the case of both Centruroides limpidus Hoffman 1932 and C. vittatus Say 1821.
Halse et al. (1980) found less extensive folding of the secretory epithelium in *Urodacus,* although the folding occurred only on the mesal side of each gland. There were one large fold, and two to four smaller folds. They also described two types of secretory epithelial cells goblet and columnar and in both types of cell the nucleus lay in the basal region. However, 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) concluded that there is only one type of secretory cell in the venom glands of scorpions.
Quiroga *et al*. 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 nonsecretory cells.
JARRAR and AL-ROWAILY at black Saudi scorpion family Buthidae, of *Androctonus crassicauda* have been expressed lined with extensively folded secretory epithelium (formed of non-secretory and secretory venom-producing cells).
The venom droplets are seen inside the cytoplasm at the apical parts of the
Venom producing cells are 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 one size. These granules are vary in their stain reactions with the same stain and can be grouped accordingly into at least five types as a reflection to their contents or the degree of maturity**.** (TAIB and JARRAR)
However, in this study, the mucus cells are stained darker with Masson of mucus cells at the base and more faded than were headed. Color due basophilic cells at the base of concentration due to higher material and color of the mucous secreting cells and basophiles less than at the head of the lower concentration of mucous.
Perhaps the reason of this difference between the sexes and subspecies is the family Buthidae and their different habitats.Cuticle structure and the arrangement of layers at *Scorpio maurus townsendi* , is such as *Hemiscorpius lepturus* is. Also Non- secretory cells, similar family *Hemiscorpidea* that cover inside the telson.
in *Scorpio maurus townsendi* form of poison sac is complex folded. This folds and in is the some as parts of the gland due to toxic cells also divided into two branches. The connective tissue cells due to the wide distribution of this family in folds are higher than the as *Hemiscorpius lepturus.*
Al- Asmari et al. (2009) at Scorpio maurus kruglovi sections, Scorpionidae family, showed simple or no folding, with one layer of a very thick cuticle. That the scorpions used in this study in contradiction . Perhaps this difference may be due to differences in the species and their habitat.

Supporting cells and secretory cells in *Scorpio maurus townsendi* are the same as *Hemiscorpius lepturus* exocuticule layer at *Odontobuthus doriae* Less than extent, compared with *Hemiscorpius lepturus* and *Scorpio maurus townsendi* . Which may be due to progression of gland and increased toxic cell.
in *Odontobuthus doriae* the form of poison sac is complex folded and it folds higher than the *Scorpio maurus townsendi*. Probably due to differences in the type and amount of secreted toxin.
histological profiles shows scorpion venom glands that were collected from the Riyadh region. Telson cross sections of Buthidae family: Compsobuthus arabicus, Compsobuthus werneri, Leiurus quinquestriatus, Androctonus crassicauda, Androctonus bicolor, Buthacus yotvatensis nigroaculeatus, Buthacus leptochelys and Orthochirus innesi reflect complex folded glands.In fact, telson sections of Leiurus quinquestriatus, Androctonus crassicauda and Androctonus bicolor present very distinct and densely folded glands. (Al-Asmari et al)
Lourenço (24) and Pavlovsky (40) have extensively studied scorpion venom glands. It was found that the complexity and effectiveness of the venom relies highly on the simplicity or folding of venom glands, which is related to the scorpion family and phylogeny. Furthermore, scorpions whose telson sections showed very distinct and densely folded glands (Leiurus quinquestriatus,Androctonus crassicauda and Androctonus bicolor) are the most toxic and medically important. The venom gland morphology presents constant generic characteristics that could be useful and applicable in higher level scorpion taxonomy.
Pawlowsky (1913) reported on six of seven known families and found that the morphology followed a generalized scheme, with the major differences consisting of the presence versus the absence of folds in the secretory epithelium. Two main types were listed: type I (primitive gland) possesses 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. Still another one is electron lucent with transparent granules (Yigit and Benli)
In *Odontobuthus doriae* seems that the connective tissue cells are in the folds higher than those of *Scorpio maurus townsendi*. the frequency and extent of the tumor is probably due to the folds.
Significant differences in the microscopic structure of the cells were not observed at all three cases of *Hemiscorpius lepturus*, *Scorpio maurus tonsendi*, *Odontobuthus doriae* in male and female Scorpio and it seems like there is no difference between males and females.
Additional histochemical investigations are needed on the venom of scorpions to identify their toxic and nontoxic antigenic components.

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