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Morphology, histology, histochemistry and fine structure of venom apparatus of the medically relevant Scorpion, *Leiurus quinquestriatus*

Mahmoud Abd El-Atti[1], Jihad A El-Qassas[1], Ali Gamal Gadel-Rab[2], Moustafa Sarhan[2] and Mahmoud Desouky[1]*

[1]Department of Zoology, Faculty of Science, Zagazig University, Zagazig, **Egypt**
[2]Department of Zoology, Faculty of Science, Al-Azhar University (Assuit Branch), Assuit, **Egypt**

***Correspondence:** mdesouky0@yahoo.com **Received 17-05-2020, Revised: 08-06-2020, Accepted: 10-06-2020 e-Published: 14-06-2020**

Leiurus quinquestriatus is one of the dangerous scorpions all over the world and represents a health hazards in Egypt. It has garnered a lot of attention after the isolation of a peptide called chlorotoxin from its venom which used for identification and treatment of gliomas. The present study was carried out to critically describe the morphology, histology, histochemistry and ultrastructure of its venom gland before and after milking to understand the mode of venom formation and secretion in this medically important scorpion. Microscopical examination revealed that the venom apparatus of *L. quinquestriatus* is composed of paired venom glands situated inside the telson's vesicle. Two venom ducts extend from these glands to open at the subterminal sides of the stinger. SEM revealed the presence of many sensory setae over the cuticle of venom apparatus. The venom epithelium is highly folded and has three types of cells: venom-producing cells, mucous (goblet) cells and supporting cells. Three types of distinct secretory granules were found in venom-producing cells that gave a variable positive response with histological and histochemical stains reflecting their constituent's richness. The herein results revealed also that these granules are distinct types and no transitional stages reflecting that they are at a maturation stage .The mode of venom secretion from venom glands of the studied species were found to be of apocrine type. Ultrastructural architecture of these cells and their granules were described.

Keywords: *L. quinquestriatus*, Venom apparatus, Morphology, Histology, Histochemistry, Ultrastructure

INTRODUCTION

Scorpions are very successful arachnids that inhabit all major terrestrial habitats (Possani LD, et al., 1999). Scorpion's venoms are known for their harmful effects and may cause severe health problems (Veiga ABG, et al., 2009). In spite of their negative effects, scorpion venoms are a rich source of bioactive ingredients that are extensively used as anti-parasitic (Adade CM, and Souto-Padrón T, 2015), insecticidal (Juichi H, et al., 2019), anti-bacterial (Amorim-Carmo B, 2019) and anti-viral agents (El-Bitar AMH, et al., 2019). In addition, recent researches proved that these toxins are effective in cancer therapy (Nafie MS, et al., 2020) including; glioma (Perumal SR, et al., 2017), breast adenocarcinoma (Crusca EJR, et al., 2018), prostate cancer (Ben Aissa R, et al., 2020).

Most of the previous studies on Egyptian scorpions were focused on their morphology and taxonomy (e.g. Kaltsas D, et al., 2008; El-Hennawy HK, 2014; Badry A, et al., 2018). In recent years, some peptides of medical importance were isolated from Egyptian scorpions

(El-Bitar AMH, et al., 2019; Elrayess, R.A. et. al. 2019). However, works on the morphological, histological and fine structures of Egyptian scorpion venom glands are very rare (Soliman BA, et al., 2013).

Leiurus quinquestriatus represents one of the most dangerous species of scorpions all over the world and it is encountered in Egypt, especially in Upper Egypt and Sinai (El-Hennawy HK, 2014). It represents a health hazards in Egypt and was reported to be more toxic than *Androctonus crassicauda* (Abd El-Aziz FE, et al., 2019). It has garnered a lot of attention after the isolation of a 36-amino acid peptide called chlorotoxin (CTX) from its venom by DeBin JA, et al., (1993). CTX has been used as a potential agent for diagnosis and treatment of gliomas (Lyons SA, et al., 2002; Biswas A, et al., 2012). Moreover, several bioactive toxins were isolated from the venom of this scorpion. For example, Bradykinin Potentiating Factor (BPF) normalized the hepatic injury induced by CCl_4 (Salman MMA, 2018). Furthermore, the crude venom of this scorpion induced cytotoxicity, elevated the reactive oxygen species and enhanced apoptotic pathways in some cancer cell lines (Salama W, and Geasa N, 2014; Al-Asmari AK, et al., 2018). The venom of this species has also antimicrobial activity against different types of pathogenic bacteria (Alajami R, et al., 2020). However, sufficient gaps exist in the literatures regarding the structure and function of venom glands of this species.

For all the above aforementioned information, the present study was carried out in order to describe the morphology, histology, histochemistry and ultrastructure of venom glands of *L. quinquestriatus*. In this study venom gland was observed before and after milking to understand the mode of venom formation and secretion in this medically important scorpion.

MATERIALS AND METHODS

Collections and Maintenance of Scorpions

L. quinquestriatus specimens were collected by hunters during the months (May-September 2018) from different geographical wild infested area in Egypt. Specimens were brought to Invertebrate Lab., Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt. They were placed in fairly small transparent plastic boxes (20 × 10 × 10 cm) with 5–6 cm of sand at the bottom. The lid was perforated to provide proper ventilation. Scorpions were fed with grasshoppers.

Ethical Approval

All protocols and experimental procedures were performed as per the norms of the Institutional Animal Care and Ethics Committee, Zagazig University, (ZU-IACUC) Zagazig, Egypt (Research protocol No., ZU-IACUC/1/F/178/2019).

Venom Extraction

For this purpose, Electrical method (Yaqoob RHM, et al., 2016) was used. Scorpion was placed on a Petri plate with sticky tape. With the help of pointed electrode rinsed in saline solution, electric current (20 V) was applied at the base of telson for 5 s until the venom was released.

Histological and Histochemical preparations

The telson was cut at its articulation with the last abdominal segment and quickly fixed in 10% neutral buffered formalin (pH 7.4) with 2% calcium acetate. Bouin's fluid, Gendre's fluid and Zenker's fluid were also used for histochemical preparations. Specimens were then treated with formic acid for 2h for softening of their hard cuticle. Tissue samples were then dehydrated through ascending series of ethanol (60- 100%), cleared in xylol and finally embedded in paraffin wax (Gabe M, and Saint-Girons H, 1969). Sections were then cut at 5-7 µm with a rotary microtome. Sections were stained by H&E (Drury RA, and Wallington EA, 1980). Periodic Acid Schiff (PAS) technique (Gurr E, 1962) was used for recognition and differentiation of carbohydrates while mercuric bromophenol blue method (Mazia D, et al., 1953) was used for recognition of proteins.

Scanning Electron Microscopy (SEM)

The telson was fixed in 3% glutaraldehyde in 0.1 ml sodium phosphate buffer (pH 7.2) for three hours. Specimens were then washed two times in sodium phosphate buffer, post fixed in 1% osmium tetroxide (OSO_4) in the same buffer for 2 hours at +4 ºC and then washed four times in sodium phosphate buffer. Telsons were then dehydrated in a graded series of ethanol (40%–100%). The last stages of dehydration were performed with propylene oxide. The specimens were dried and coated in a Polaron SC 500 sputter coater with a thin layer of gold (Hayat MA, 2000). The materials were examined with a Joel JSM 5800 Scanning Electron Microscope (EM

Unit, Faculty of Agriculture, El-Mansoura University, Egypt).

Transmission Electron Microscopy (TEM)

The specimens were fixed in 3% glutaraldehyde for 3 hours at 4°C with 0.1 ml sodium phosphate buffer (pH 7.2) and then washed three times with the same buffer. Specimens were then post fixed in 1% OSO_4 for one hour at room temperature and then washed three times in sodium phosphate buffer to remove OSO_4. They were then dehydrated in ascending series of ethanol (40%–100%). After dehydration, the specimens were embedded in Araldite CY 212 (Agar Scientific Ltd., UK) (Hayat MA, 1981). Thin sections (60 - 70-nm-thick) were cut with glass knife on RMC MT-X ultra-microtome (Boeckeler Instruments, USA) and mounted on 100-mesh copper grids. Sections were stained with uranyl acetate followed by lead citrate and examined under a Joel JEM 100 SX TEM (Jeol Ltd., Japan) at 80 kV (EM Unit, Faculty of Agriculture, El-Mansoura University, Egypt).

RESULTS

Morphology of Venom Apparatus of *L. quinquestriatus*

The venom apparatus of *L. quinquestriatus* is composed of a bulbus vesicle and a stinger or aculeus. The vesicle is yellowish and globose while the brownish aculeus is longer than vesicle, sharply pointed and shallowly curved (Plate I: A). A small venom opening situated at the lateral subterminal end of the aculeus (Plate I: B). Under light microscope, the surface of the venom apparatus seems smooth and devoid of any sensory setae (Plate I: A). However, under SEM, few small sensory setae emerge from the cuticle of the vesicle (Plate I: C).The diameter of these setae decreased along its length (Plate I: D). The telson is covered by a cuticle that consists of two layers: a homogeneous outer exocuticle and lamellar endocuticle. (Plate I: E). When the end of the sting tip is cut off with fine scissors and looked under the SEM, two separate venom ducts were observed embedded in a spongy cuticle (Plate I: E). In sagittal section, SEM micrographs showed a sheath of muscle fibres enclosing the venom glands from its mesal side (side away from the cuticle) (Plate I: F).

Histology and Histochemistry of Venom Glands of *L. quinquestriatus*

Before milking

Light microscopical examination of the venom apparatus revealed that two completely separated bilateral venom glands are found extended one on each side of the midline in the vesicle (ampulla) of telson (Plate II: A). The telson is covered by a hard, thick cuticle that composed of two layers: an outer homogeneous thin exocuticle and an inner thick lamellar endocuticle (Plate II: B). Each gland is surrounded by a layer of striated muscular tissue covers the gland from its mesal and mid-ventral sides (Plate II: C). The muscle fibrils appear striated with alternating light (I) and dark (A) bands and have oval nuclei (Plate II: C). A layer of dense connective tissue appeared purple in colour underlying the cuticle and the muscular tissue with trabeculea that extends inside the gland dividing it into lobules (Plate II: A,B&D).

Each venom gland comprises a sac-like structure with lumen (Plate II: A). The glandular epithelium lies between the connective tissue and lumen and thrown into a multitude of irregular folds and finger-like structures (Plate II: D, E&F). Three types of cells can be recognized: the goblet (mucous) cells, the venom-producing cells and supporting cells. The mucous cells are found in separate folded epithelium and occupy most of the stroma on the dorsal side of the gland while the venom-producing cells are concentrated in the ventral region (Plate II: A).

The goblet cells (mucous cells) are pyramidal and their nuclei situated at the base. Large oval to elongated basophilic secretory vesicles are found near the tips of these cells (Plate II: F).

The venom-producing cells are high columnar, bottle-shaped with small, rounded basal nuclei. They are filled with apical granular vesicles of various sizes, shapes and staining properties (Plate II: E). Three types of secretory granules can be recognized within the venom-producing cells; named A-type, B-type and C-type. Type A-granules are filled with rather large circular contents (Plate II: E&H). Type-B granules are filled with small oval contents (Plate II: E&G) while Type C-granules are filled with irregularly shaped basophilic contents (Plate II: G).

The supporting cells (Non-secretory cells) are sub-cuboidal cells attached to the basement membrane of the epithelial folding in between the secretory cells. (Plate II: D&E).

PLATE I

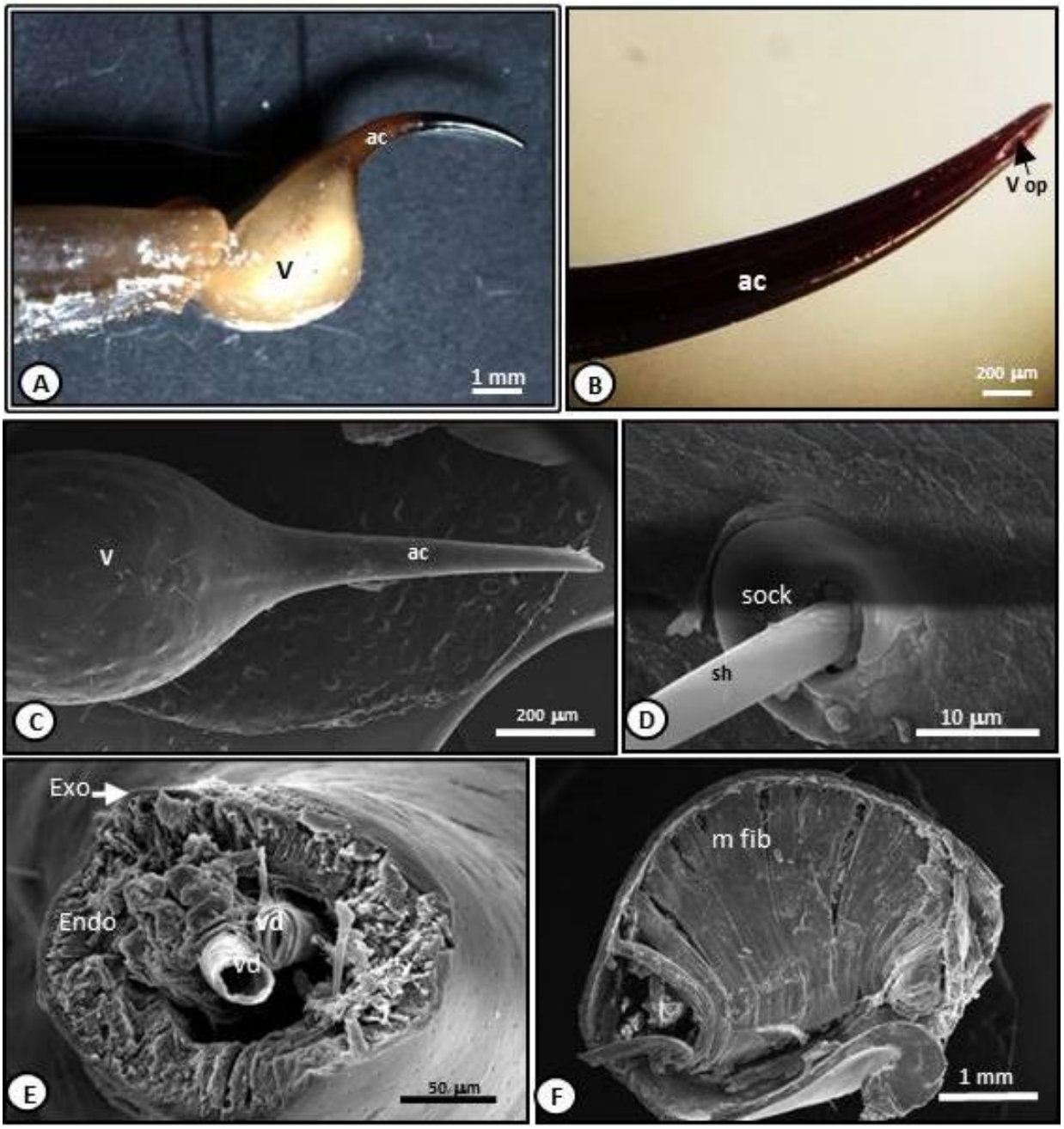

PLATE I: Morphology of venom apparatus of the scorpion: *L. quinquestriatus*. A: Photograph showing lateral view of telson. X, 10. **B**: Photograph of sting (aculeus) showing lateral venom opening. X, 50. **C**: Scanning electron micrographs of telson showing vesicle and aculeus. X, 25. **D**: High mag. of sensory hair projecting from distinct socket. X, 1500.**E**: The very tip of the sting snipped off with scissors, showing the two venom ducts surrounded by a spongy cuticle. X, 25. **F**: Sagittal section of telson under SEM showing a sheath of muscle fibres lined the gland from its mesal side. X, 25. ac: aculeus or stinger; Endo: endocuticle; Exo: exocuticle; m. fib: a sheath of muscle fibres; sh: sensory hair; sock: socket; V: vesicle; v. op: venous opening; Vd: venom duct.

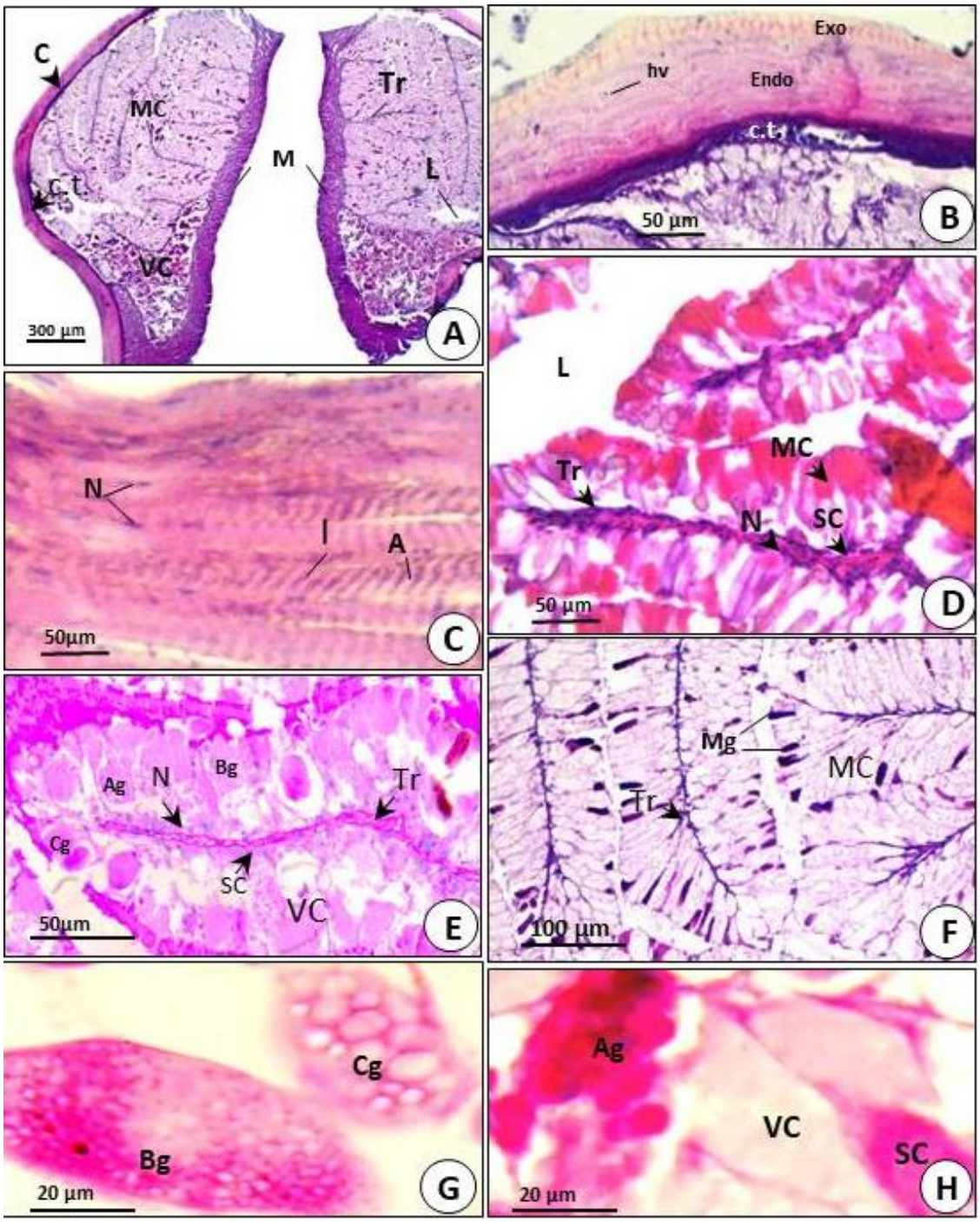

PLATE II: Light micrographs of venom glands of *L. quinquestriatus* showing their histological structures (Stained H&E). A: T.S. of venom glands showing their histological structure. X, 40. **B**: T.S. of venom gland showing two-layered cuticle. X, 250. **C**: T.S. of venom gland showing structure of muscles. X, 250. **D**: T.S. of venom gland showing folding of venom-producing cells. X, 250. E: T.S. of venom gland showing venom-producing cells with different types of granules. X, 400. F: T.S. of venom gland showing mucous cells. X, 250. **G&H:** Different types of venom granules. X, 1000. A: light band; Ag: A-type venom granules; Bg: B-type venom granules; C.t.: connective tissue; C: cuticle; Cg: C-type venom granules; Endo: endocuticle; Exo: exocuticle; hv: hemolymph vessel; I: dark band; L: lumen; M: muscle; MC: mucous cells; Mg: mucous granules; N: nucleus; SC: supporting cells; Tr: trabecula; VC: venom-producing cells.

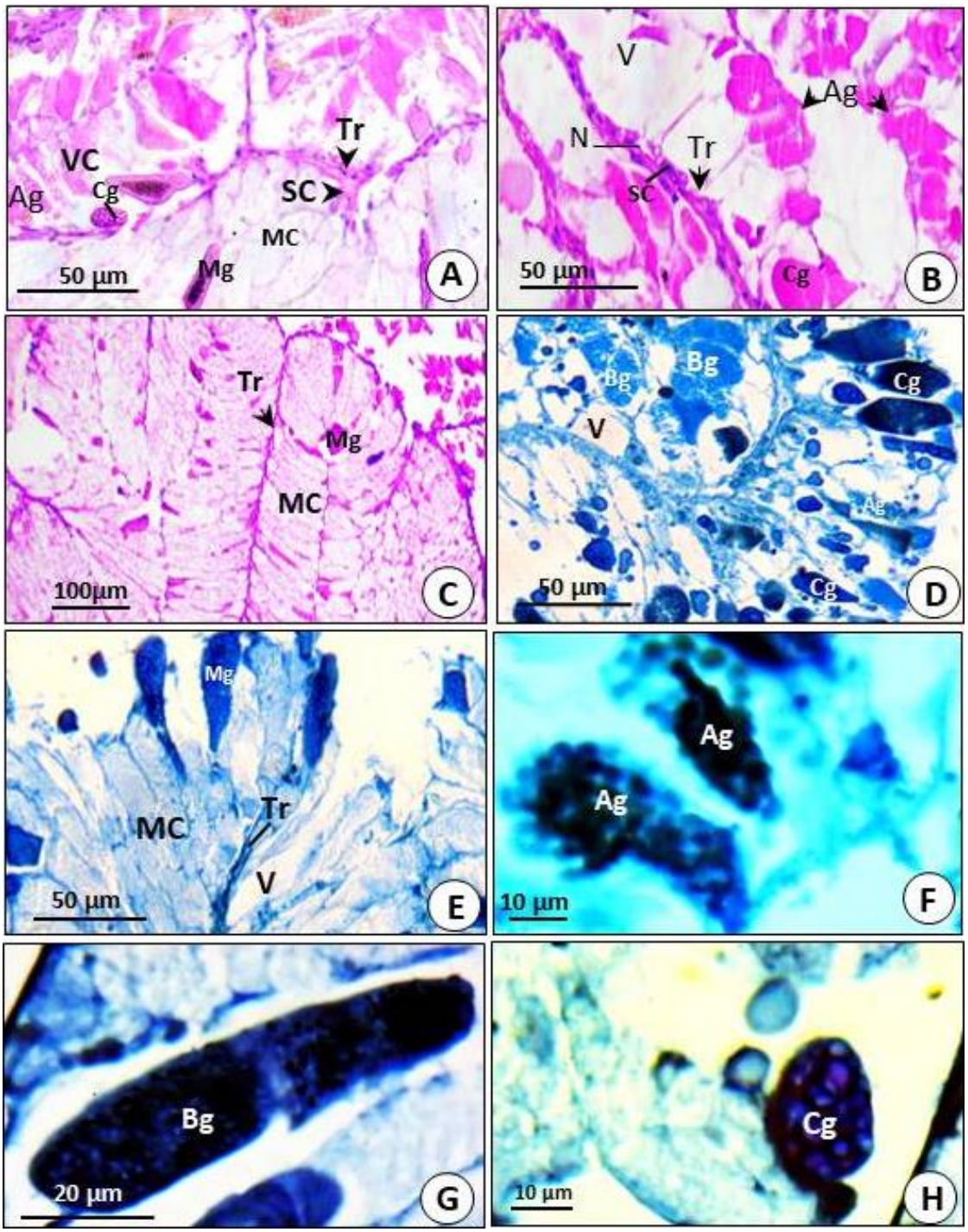

PLATE III: Light micrographs showing hitochemistry of the venom glands of *L. quinquestriatus*. A: T.S. of venom gland showing carbohydrate histochemistry of glandular secretory epithelium. (Stained PAS) X, 500. **B**: T.S. of venom gland showing carbohydrate histochemistry of venom-producing cells. (Stained PAS). X, 500. **C**: T.S. of venom gland showing carbohydrate histochemistry of mucous cells. (Stained PAS). X, 150. **D**: T.S. of venom gland showing protein histochemistry of venom-producing cells (Stained mercuric bromophenol-blue). X, 500. **E**: T.S. of venom gland showing protein histochemistry of mucous cells (Stained mercuric bromophenol-blue). X, 400. **F,G&H**: Protein histochemistry of different types of venom granules. (Stained mercuric bromophenol-blue). X, 1000. Ag: A-type venom granules; Bg: B-type venom granules; Cg: C-type venom granules; MC: mucous cells; Mg: mucous granules; N: nucleus; SC: supporting cells; Tr: trabecula; V: vacuole; VC: venom-producing cells.

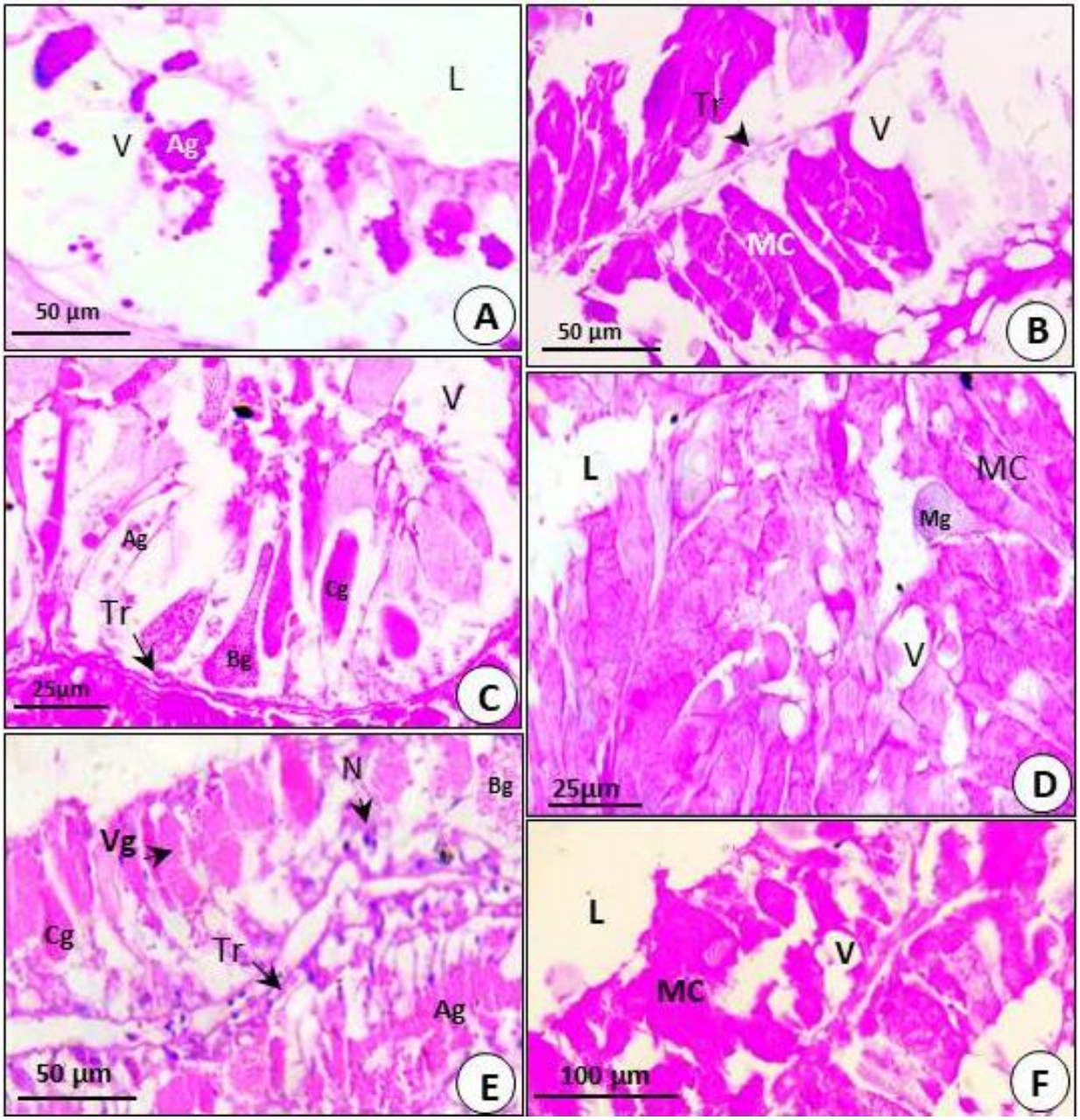

PLATE IV: Histological structures of the venom glands of *L. quinquestriatus* after different times of milking (Stained H&E). A: T.S. of venom gland showing venom-producing cells just after milking. X, 400. **B**: T.S. of venom gland showing mucous cells just after milking. X, 400. **C**: T.S. of venom gland showing venom-producing cells after one day of milking. X, 600. **D**: T.S. of venom gland showing mucous cells after one day of milking. X, 600 **E**: T.S. of venom gland showing venom-producing cells after three days of milking. X, 400**. F**: T.S. of venom gland showing mucous cells after three days of milking. X, 250. Ag: A-type venom granules; Bg: B-type venom granules; Cg: C-type venom granules; L: lumen; MC: mucous cells; Mg: mucous granules; N: nucleus; Tr: trabecula; V: vacuole; Vg: venom granules.

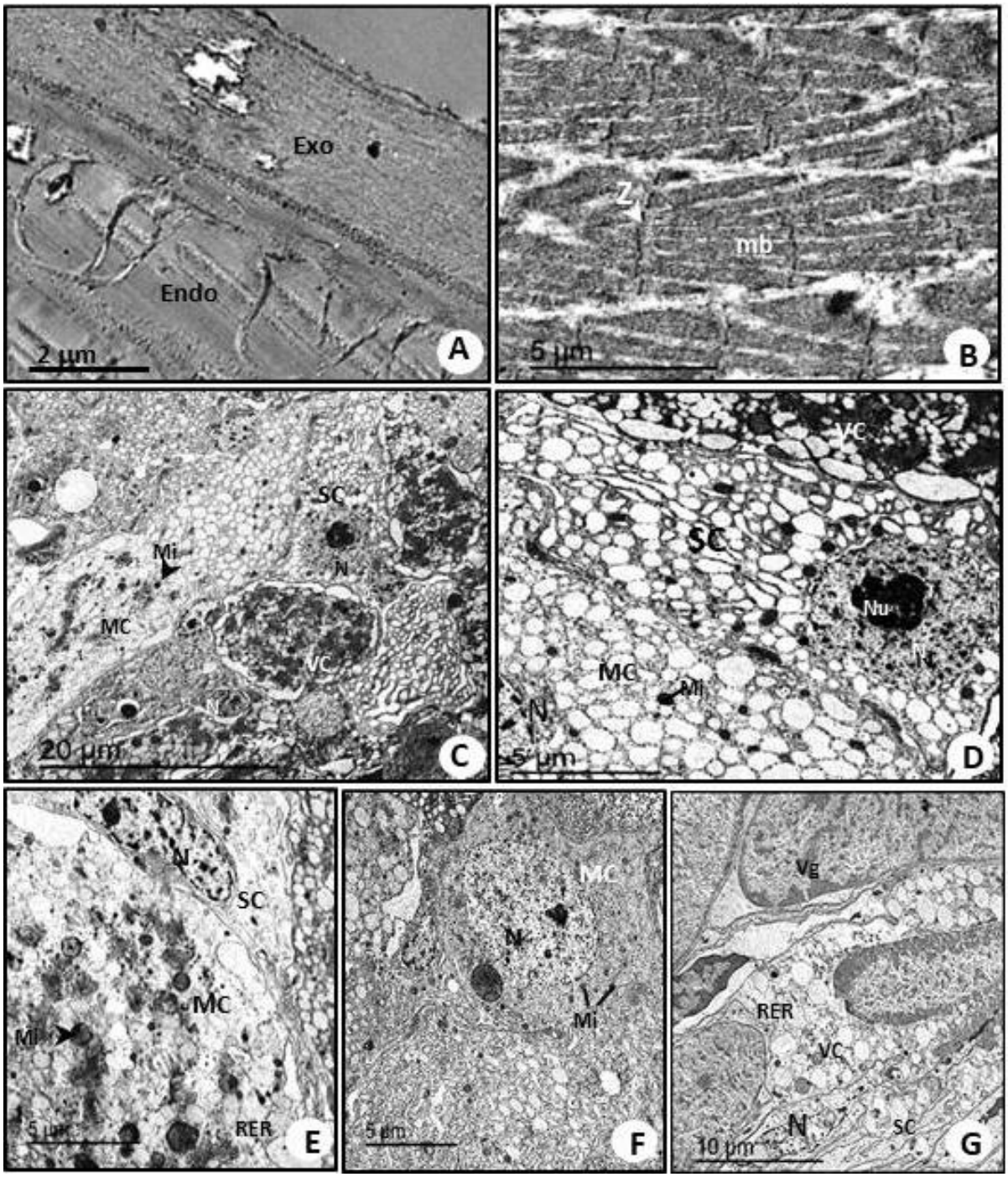

PLATE V: Transmission electron micrographs of the venom glands of *L. quinquestriatus.* A: The ultrastructure of cuticle. X, 10,000. **B**: The ultrastructure of muscles. X, 7,000. **C**: Different types of cells. X, 2,250. **D**: Different types of cells. X, 5,400. **E:** Non-secretory cell and venom-producing cells. X, 6,000. **F**: Mucous cell showing round nucleus, many mitochondria and cisternae of RER. X, 5,000. **G**: Different types of cells. X, 3,900. Endo: endocuticle; Exo: exocuticle; mb: muscle bundles; MC: mucous cells; Mi: mitochondria; N: nucleus; Nu: nucleolus; RER: rough-endoplasmic reticulum; SC: supporting cells; VC: venom-producing cells; Vg: venom granules; Z: Z-line.

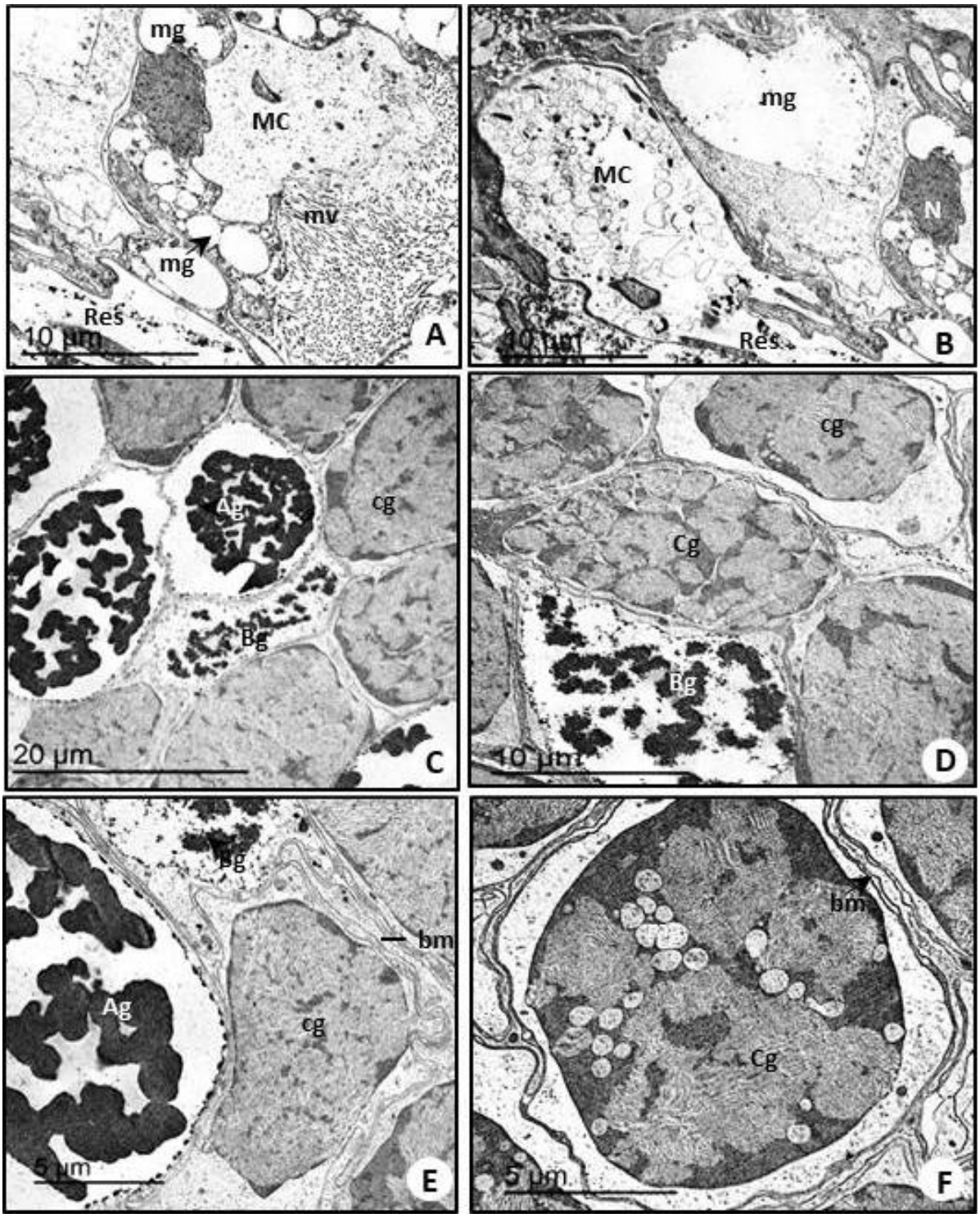

PLATE VI: Transmission electron micrographs of the venom glands of *L. quinquestriatus*. A: Mucous cells with microvilli at its apical portion. X, 3,400. **B**: Secretory cells showing discharge of venom granules. X, 2,800. **C**: Venom-producing cells with different types of venom granules. X, 2,250. **D**: Venom-producing cells with different types of venom granules. X, 3,800. **E**: Venom-producing cells with different types of venom granules. X, 5,000. **F**: C-type venom granules. X, 6,600. Ag: A-type venom granules; Bg: B-type venom granules; bm: bound membrane; Cg: C-type venom granules; MC: mucous cells; Mg: mucous granules; MV: microvilli; Res: reservoir.

Histochemical preparations of the venom - producing cells revealed the presence of rounded

granules of variable colour density upon staining with same stains. These granules showed high activity for periodic acid in the form of dark magenta-red granules scattered in the cytoplasm (Plate III: B). Moderate activity for periodic acid was determined in form of homogenous magenta-red granules in these granules (Plate III: A&B). Numerous light magenta-red granules were also observed scattered throughout these cells (Plate III: B).The mucous cells were found to contain large apical elongated mucous granules that deeply stained with both PAS (Plate III: C) and bromophenol blue (Plate III: E). Similarly, the glandular venom secretory cells gave a variable positive response to mercuric bromophenol blue that indicate the presence of variable amount of protein in these cells (Plate III: E-H). Microscopical preparations revealed that some of the three types of granules appeared dark blue in colour and others were observed in light blue colour by mercuric bromophenol blue stain (Plate III: D, F, G&H).

After milking

The histological architecture of the venom gland was observed after milking process to reveal the mode of venom secretions. The glands were stimulated with electric shock to release venom and then allowed to recover for three days.

Just after milking, the venom- producing cells seemed nearly empty with small amount of venom granules mainly of type A (Plate IV: A). On the other hand, some mucous cells were found almost empty while others were loaded with mucous granules (Plate IV: B).

After one day of milking, many granules started to appear in both mucous and venom-producing cells. The secretory cells appeared in an active phase of secretion. The apical surface of these cells appeared ruptured and membrane-bounded secretory granules of various shapes and staining properties appeared released into the lumen (Plate IV: C& D).

After three days of milking, the secretory epithelium became loaded again with secretory granules and the apical portion of cells became intact (Plate IV: E&F).

The microscopical slides stained by both PAS and bromophenol blue of the glands after milking support the above finding and revealed also that the secretion stages of venom and mucous occurred in progressive manner.

Ultrastructure of Venom Glands of *L. quinquestriatus*

Under TEM, the venom gland of *L. quinquestriatus* appeared surrounded by cuticle, which consists of two layers: an outer thin homogeneous exocuticle and an inner thick lamellar endocuticle (Plate V: A).The muscles occur in several bundles and separated by thin layer of connective tissue containing numerous vesicles. The Z lines (the borders of the sarcomeres) show irregular structure (Plate V: B). Three types of cells can be recognized in the folded epithelium of the glands: venom-producing cells, mucous cells and supporting cells.

A. Venom-producing cells

The secretory venom-producing cells are tall (Plate V: C). The nuclei and other organelles are found in the basal area (Plate V: C). In electron micrographs, these cells appear to be undergoing extensive protein synthesis where RER appear extensive, highly distended and filled with an amorphous substance (Plate V: C). Many mitochondria are interspersed throughout the ER (Plate V: E). Most of the cell is filled by vesicles of different sizes, shapes and electron densities. Three distinct types of membrane-bounded secretory granules can be distinguished with TEM: Type-A granules are filled with large electron-dense vesicles which appeared aggregated together (Plate VI: C&E). The diameter of the vesicles exceeds 3 µm and reaches up to 8 µm. Type-B granules are filled with small (1-4µm), irregular, electron-dense vesicles which less round than those of type A (Plate VI: D&E). Type-C granules are rounded, electron-lucent vesicles and filled with electron-lucent elongated rods (about 1µm long) (Plate VI: D, E&F).

B. Mucous cells (Goblet-cells)

They are columnar with irregular basal nuclei (Plate V: C).The mucous cell has electron-dense cytoplasm containing electron-lucent, confluent granules of mucous. A large number of rounded RER cisternae and mitochondria are found throughout the cytoplasm (Plate V: D).The apical surface of mucous cells is provided with long microvilli (Plate VI: A). Sometimes, the apical surface appeared ruptured and large vacuoles are seemed extruded into the lumen of the gland (Plate VI: A &B).

C. Supportive Cells (Regenerating-cells) Interspersed within the secretory epithelium few

numbered, squamous cells were observed. Supportive cell has a flat-shaped nucleus and devoid of cilia or secretory vesicles (Plate V: G). Sometimes, the cytoplasm of these cell may contain mesh of microfilaments and tubules (Plate V: G).

DISCUSSION

Scorpions are characterized by possession of venom apparatus located at the end of their telsons. Their success relies on the secretion of very potent neurotoxic venom that is used mainly for killing their preys and repelling their competitors. In addition to these functions, it was reported that venom glands play an important role during mating. According to Jiao GB, and Zhu MS, (2010) the male scorpion often stings the female during courtship. This sexual sting might represent a stimulating impulse (Inceoglu B, et al., 2003).

The present study confirmed that the venom apparatus of *L. quinquestriatus* comprises paired venom gland located inside the vesicle and a pointed stinger. Compared to other less active scorpion as *Scorpio maurus* (Navidpour S, et al., 2018), the bulb is larger and the stinger is taller and sharper. This may be correlated with fact that this species is a dangerous active hunter.

Two-layered cuticle reported in the studied species may give the sting more flexibility and more resistance to mechanical stress that favour this dangerous scorpion. There are setae and cuticular pits scattered all over the telson of studied species. The microanatomical features of these setae are characteristics for mechanoreceptors or chemoreceptors. Similar setae were observed in other scorpions as *Euscorpius migrelicus* (Yigit N, and Bayram A, 2007., Yigit N, et al., 2007). A number of ducts were observed in the cuticle which discharges the secretion of dermal glands. Gaffin DD, and Brownell PH, (1992) stated that female cuticle extracts induce courtship patterns in male scorpions and suggested that these glands may produce a sex pheromone in females.

The paired venom gland in the present study was provided mesaly (side away from the cuticle) with a band of vertical striated muscle. This arrangement of muscles was observed in other scorpions as *Androctonus crassicauda* (Jarrar BM, and Al-Rowaily MA, 2008) and *Scorpio maurus townsendi* (Navipour S, et al., 2018) and may act to squeeze the gland against the cuticle for discharging of venom.

The present work revealed that the glandular venom epithelium of *L. quinquestriatus* belongs to Type II according to the classification of Pawlowsky EN, (1913) i.e. it is extensively folded and consists of a mass of secretory epithelium. These results are also in line with those reported for other members of family Buthidae (Al-Asmari AK, et al., 2007). The complex epithelial folding may be unsurprised in this dangerous scorpion since the amount, complexity and effectiveness of the venom rely highly on the folding level of venom glands. In addition, the level of venom epithelial folding in the present work may give further evidence that the complexity of scorpion venom glands is related to the scorpion family and phylogeny (Sherwan TA, 2015) and could be useful and applicable in higher level scorpion taxonomy.

The general histology, histochemistry and fine structure of venom glands of several scorpion species were studied by several researchers (Kanwar U, et al., 1981; Quiroga M, et al., 1998; Soliman BA, et al., 2013; Navidpour S, et al., 2018). However, the histology and fine structure of Egyptian scorpions was poorly investigated. Thus, further studies of scorpion venom gland morphology and ultrastructure are warranted. Based on microscopical examinations of the venom glands of the studied scorpion, three types of cells were recognized in the glandular epithelium of the studied scorpion which are: mucous cells, venom -producing cells and supportive cells. Similar findings were reported in different scorpions as *Euscorpius mingrelicus* (Yigit N, and Benli M, 2008), *L. quinquestriatus* (Taib NT, and Jarrar BM, 1993) and S. *maurus towsendi* (Navidpour S, et al., 2018). However, some previous studies reported only one (Junqua C, and Vachon M, 1968) or two (Keegan HI, and Lockwood WR, 1971; Quiroga M, et al., 1998) types of cells in the venom glands of other scorpions. The supportive cells arranged between venom-producing cells and seem to act as reserve cells responsible for regeneration of the damaged cells.

In the present study, three types of secretory granules were found in venom-producing cells according to their sizes, shapes and staining proprieties. Similarly, three types of secretory granules were described in different scorpion species (Halse SA, et al., 1980; Sentenská L, et al., 2017). However, Navidpour S, et al., (2018) reported that these granules are grouped into four types in *Euscorpius alpha* while Taib NT, and Jarrar BM, (1993) grouped them into five types in *L. quinquestriatus* according their contents or

degree of maturity.

The venom granules were found to give a variable positive response with both histological and histochemical stains. This may reflect the richness and variability of their constituents. Most of these granules were positively stained with bromophenol blue due to their protein contents. They were also found to be PAS-positive with variable intensity. This revealed that the venom of this species contains neutral muco-substances with variable quantity. Neutral muco-substances have been found in the venom of some other scorpion species especially in dangerous ones (Goyffon M, and Kovoor J, 1978; Hasle SA, et al., 1980; Taib NT, and Jarrar BM, 1993). Neutral muco-substances may have a possible role in the transfer of venom protein fragments to the victim's tissue (Jarrar BM, and Al-Rowaily MA, 2008).

According to Sentenská L, et al., (2017), the different types of granules in venom-producing cells may represent developmental stages where the content of the vesicles develops from a homogeneous state towards a finely one, forming a coarse to fine substance. Based on microscopical examination in the herein study, one could conclude that these granules are distinct types with different constituents and there are no transitional stages which reflecting that they are at a maturation stage.

In order to elucidate the mode of their secretion, the venom gland of *L. quinquestriatus* was stimulated electrically to discharge venom and examined it after different times of milking. Based on light and electron microscopic examination, the secretory cells seem to be of the apocrine type, i.e. the apices of the secretory cells break down during the secretion process and appear to pinch off "snouts", leading to a histologic picture of "decapitation secretion" into the glandular lumen. Venom gland of the studied scorpion has three phase of secretory cycle. 1.Elaboration phase (Just after milking): in which they contain fine uniform or empty granules. 2.Accumulation phase (from 1-3 days after milking): larger granules are seen in the epithelial cells. In this phase, the secretory cells appear very active. Large number of secretory granules of different size and shape accumulated in the apical region. 3.An expulsion phase (appeared also in unmilked glands): the venom granules and a portion of cytoplasm of the cell are extruded into the lumen of the gland. Most scorpion venom secreting cells were found to be of apocrine type (Taib NT, and Jarrar BM, 1993; Jarrar BM, and Al-Rowaily MA, 2008; Navidpour S, et al., 2018). However, Soliman BA, et al. 2013 revealed that the mode of venom secretion in *Androctonus amoreuxi* occurs by the holocrine mode.

CONCLUSION

The outcome results critically described the histology, and ultrastructure of *L. quinquestriatus* venom gland, as well as the histochemical nature of their constituents and the mode of venom secretions. Based on microscopic examination in the herein study, three types of cells were recognized in the venom glands. There are three types of venom granules in the venom-producing cells. These granules are distinct types with different constituents and there are no transitional stages which indicate that they are at a maturation stage.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mahmoud Abdel-Atti, Ali Gamal Gadel-Rub and Jihad Al-Qassas performed the practical part and participate in scientific writing. Moustafa Sarhan developed the idea and participated in scientific writing and discussion. Mahmoud Desouky developed the idea, fellow up the publication process and supervised the work. All authors read and approved the final version.

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