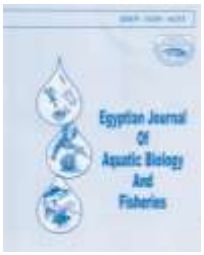
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 - 6131 Vol. 22(3): 55- 69 (2018)
ejabf.journals.ekb.eg



Effects of a plant product (Thymol) on the salivary gland of the giant slug *Limax maximus* in Egypt (Histological and Ultrastructural study)

Omaima. M. Mustafa
Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt
Corresponding Author: omayma_moustafa@yahoo.com

ARTICLE INFO

Article History:
Received: May 2, 2018
Accepted: June 30, 2018
Available online:July 2018

Keywords:
Limax maximus
Salivary gland
Thymol
Histology
Histochemistry
Ultrastructure

ABSTRACT

Thymol monotrpenoid compound is naturally found in many plants. It exhibites molluscicial activity against the land snails and slugs. The present study aims to describe histological, histochemical and ultrastructural investigations to display and clarify any deleterious effects probably evoked post- thymol application in the salivary gland of *Limax maximus*. To achieve the intended goal in this respect, the slugs were divided into three groups; the first group served as control, the second and the third groups received LC_{50} & LC_{90} of thymol for 48 hours in food. The histological results revealed that thymol had adverse effects on type's cells of salivary gland including vacuolated cytoplasm and deformed nuclei in different cells. The histochemical results indicated the presence of neutral mucopolysaccharides in the cells of salivary gland. The ultrastructural results revealed rough endoplasmic reticulum had been fragmented into small stacks and patches heterochromatin also appeared. Therefore, the treated slugs with LC_{90} of thymol can be recommended for controlling the target slugs and further studies are needed to evaluate their efficacy as safe economic molluscicides in the field, instead of using chemical pesticides that could pollute the environment.

INTRODUCTION

Since few years, certain terrestrial slugs became serious garden pests in Egypt infesting numerous agronomic, horticultural and ornamental plants. On the other hand, several wild plants have been screened for their molluscicidal activity against them. The plant molluscicides must have a high potency to slugs and low toxicity to non –tested living organisms. These plants must further be the source of cheap, effective and environmentally acceptable products, Fonghua *et al*. (2000). The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative. In Egypt, several local plant species screened and proved to have molluscicidal properties against different snail and slug species, Bakry (2009); Singh and Singh (2010); Al Daihan in the same year; Yousef *et al.* (2013) and Abdel-Haleem (2014). Although, some molluscs have been involved in the everyday life for humans in many cultures throughout, providing a source of shells, food, dyes and medicine Ahmed *et al*. (2018).

Thymol was tested as a molluscicide against snails such as *Bulinus truncates* by Lahlou and Barrada (2001); *Helix aspersa* by El-Zemity *et al*. (2001); *Biomphalaria*

alexandrina, *Bulinus truncates* and *Lymnneae natalensis* by Sharaf (2006). In addition, Radwan *et al*. (2008) proved that thymol exhibited high molluscicidal activity against *B. alexandrina*. Bakry (2009) examined the effect of exposure of *B. alexandrina* snails to LC_{25} methanol extract from the plants *Guayacum officinalis*, *Atriplex stylosa* and *Euphorbia splendens* for two weeks. Abnormalities appeared in the digestive gland were great damage of the epithelial tissues and revealed several cytoplasmic vacuolization. Singh and Singh (2010) established that in many plants; *Euphorbia splendens, Ambrosia maritime* and *Ziziphus spina-christi* the molluscicidal activity was due to the presence of saponins and alkaloid components. Also, thymol is a monoterpenoid obtained from the essential oil of laminacea species, such as *Monarda punctate* (mint), *Thymus vulgaris* (thyme) and *Thymus persicus* by Ferreira *et al*. (2011).

Yousef (2011) studied the effected of thymol and nicotine against *Eobania vermiculata* on the histology of digestive gland. The author showed vacuolated cytoplasm and different degrees of degeneration of their nuclei. Also, Yousef *et al.* (2013) studied the histopathological and ultrastructural effects of three Egyptian wild plants-extracts, as botanic toxic agents; namely *Euphorbia splendens, Ziziphus spina* and *Ambrosia maritime* on the digestive gland of freshwater snails; *Biomphalaria alexandrina* and *Bulinus truncates*. The author found also that the pathological damage reached its highest level after LC_{90} treatment for 48 hours that caused accumulation of the toxic agents of the plant inside digestive cells and numerous vacuoles in the digestive and excretory cells. The histological structure and ultrastructure of the salivary gland of the gastropods have been studied in pulmonates by Wagele *et al*. (2014). Lobo *et al.* (2016) studied the comparative of salivary glands in carnivorous and herbivorous cephalaspideans with histology, histochemistry and ultrastructure.

The present work was aimed to examine the adverse effects of thymol on the slug manifested by the histological, histochemical and ultrastructural changes in the cells of the salivary gland of this slug.

MATERIALS AND METHODS

Experimental animals

The healthy terrestrial slugs *Limax maximus* were collected from gardens near Nasr City, East Cairo in Egypt. Animals were then transferred to the laboratory and kept in plastic box covered with grating. The box was daily cleaned and provided with lettuce leaves, as a main source of food.

- Thymol monotrpenoid compound

Form: Yellowish crystals.
Molecular formula: 2- $\{(CH_3)_2CH\}$ C_6H_3 5-(CH_3) OH
Molecular weight: 150.22

- Determination of LC_{50} and LC_{90} of thymol

The LC_{50} and LC_{90} of thymol were estimated according to Abbott (1925).

Six concentrations of thymol for 30 slugs/ concentration (Mustafa 2018) were prepared and applied against the slugs for 48 hours, Regressing mortality lines and corresponding mortalities were established on log dose –mortality sheet. LC_{50} and LC_{90} values calculated according to the method of Finney (1971).

Experimental design

Healthy slugs were divided into three groups; the first untreated (control) group, the second LC_{50} group and the third LC_{90} group orally received thymol for 48 hours.

Histological preparations

Healthy normal slugs were rapidly dissected. Tissue samples of salivary gland were immediately dissected out and cut into small pieces. The tissues were fixed rapidly in aqueous Bouin's fixative for 24 hours and then kept in a mixture of 70% ethanol (95 parts) and glycerol (5 parts). The specimens were then dehydrated in ascending grades of ethanol. Then, the specimens were preserved and cleared in terpineol for about three days, then washed in benzene and embedded in three changes of pure paraffin wax (Merck, M.P. 54- 56 C°). Sections of 4-6 µm were prepared, mounted on clean glass-slides and stained with haematoxylin and eosin (H&E) Bancroft and Gamble, (2002). Finally, the tissues were examined using light microscope (Olympus CX 31, Tokyo, Japan) and photomicrographs were made as required.

Histochemical preparation

For the identification of mucopolysaccharides, specimens of the salivary were fixed in Carnoy's fluid, dehydrated, cleared and embedded in paraffin. Paraffin sections were stained with the Alcian blue-PAS method (Mowry, 1956).

Ultrastructural preparations

For ultrastructure evaluation by transmission electron microscopy (TEM) as described by Dykstra *et al*. (2002), at which the freshly excised salivary gland specimens were cut into small pieces measuring about 1 mm and immediately fixed in fresh 3% gluteraldehyde-formaldehyde at 4°C for 4 hours. The specimens were then washed in phosphate buffer (pH 7.4) and post- fixed in isotonic 1% osmium tetroxide for one hour at 4°C and processed. Semi-thin sections were cut at 1 µm thickness and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined on a Joel CX 100 TEM operated at an accelerating voltage of 60 kV at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

RESULTS AND DISCUSSION

Histological results

Examination of the histological sections obtained from the salivary gland of untreated (control) *Limax maximus*, showed that it divided into acini, held together by loose connective tissue (Fig. 1). Each acinus built up of various secretory types of cells. Each cell type possessed a basal nucleus. The lumen of the acinus appeared narrow and lined with a simple columnar epithelium, resting on a thin basal lamina (Fig. 2). These results agree with Lobo-da-Chuna (2009) on the salivary gland of the opisthobranches *Philinopsis depicta.*

The result obtained after exposure the slugs to LC_{50} of thymol showed certain histological changes in the salivary gland illustrated in Figures (3&4). The basal lamina encapsulating the salivary acini appeared rupture in some regions, vacuolated cytoplasm and deformed nuclei were observed in the different cell types, whereas the Figures (5&6) illustrated the histological changes appeared in the salivary of target slugs after exposure to LC_{90} of thymol. The secretory cells were seen widely separated from each other, highly vacuolated cytoplasm and severe affected nuclei including pyknosis and karyorrhexis.

The histological changes in the present work revealed that the slugs were received thymol the cells of salivary gland appeared with vacuolated cytoplasm and severe affected nuclei including pyknosis and karyorrhexis similar to the results obtained by Yousef (2011) on the digestive gland of land snail *Eobania vermiculata* by using the thymol and nicotine and Bakry (2009) who studied changes following exposure to three plants extracts*; E. splendens*, *Guayacum officinalis* and *Atriplex stylosa* on the digestive gland of *B. alexandrina.*

Histochemical observations

Examination of stained sections of the salivary gland of *L. maximus*, revealed that the secretory cells are intensely stained with red colour indicating their richness of neutral mucopolysaccharides with the absence of acid mucopolysaccharides. The acini of glands showed weak or almost negative reactivity (Figs. 7-9). At the histochemical level, the present study revealed that the secretory cells of salivary gland of the slug are intensely stained with red colour in Alcian blue (PAS) method indicating their richness of neutral mucopolysaccharides, this results agrees with those obtained by Lobo-da-Cunha *et al*. (2016) on their study on the salivary glands in carnivorous and herbivorous cephalaspideans (Gastropoda: Euopisthobranchia).

Ultrastructural studies

The ultrastructural results of the present investigation exhibited that the salivary gland of control slug, *L. maximus* possesses three types of the cells; ciliated, granular and mucous cells. The present work showed marked differences between the secretory cells of the salivary gland. The first type of cells; ciliated cells have microvilli and cilia on their apical membranes (Figs. 10&11) with wide apical surface and a very thin stalk reaches the base of the epithelium. These cells possess amount of glycogen granules abundant in the thin mid and basal portion of cells. The gap and tight junctions are obvious between adjacent cells (Fig. 11).

Furthermore, the present results showed that some cells of the salivary gland are held together tightly by a number of plasma membrane junction complexes. Cell junctions are important in characterizing a cells as well as tissue. Adherence junctions encircle a cell near its apical surface, allowing contact between the cell and all of its surrounding neighbors and provide a potential pathway for signals to be transmitted from the cell exterior to the cytoplasm, this result agrees with the result obtained by Bhattacharyya and Chaki (2012) on the ultrastructure of the salivary gland cell of active snail *Pila globosa.*

In addition numerous oval and spherical mitochondria are abundant in the upper portion of their cytoplasm (Fig. 11). Second type of cells; granular cells are distinguished by the abundance of secretory granules. These cells contain some electron- dense and light secretory material in the vesicles, which occupied the apical portion of these cells (Figs. 12, 14 &15). Their nuclei are oval in shape and present in the basal region (Fig. 10). The third type of cells; mucous cells are observed in the salivary gland with mucous secretion (Fig. 12). The present investigation showed that the salivary gland of *L. maximus* pointed to the difference in the granular cells was also possible to detect proteins in the secretory vesicles, which contain two distinct components: patches of secretion with higher electron- density containing protein and more abundant electron lucent component enclose polysaccharides, these results agree with Lobo-da- Cunha and Calado, (2008) and Lobo-da-Cunha *et al*. (2016) in their studies on the salivary glands in carnivorous and herbivorous cephalaspideans.

The present investigation showed that the entire secretory of the ciliated cells, filled with vesicles are discharged into the lumen characterizing a holocrine process of secretion (Fig. 16) as well as the merocrine mechanism, the secretory vesicles

leave the cell by exocytosis, involving membrane fusion between the vesicles limiting membrane and the plasma membrane (Fig. 17). There are three main cellular mechanisms of secretion; merocrine, apocrine and holocrine depending on the way of the secretory products are released from cells, Moura *et al*. (2004) and Lobo-da-Cunha *et al*. (2016). In the merocrine mechanism, the secretory vesicles leave the cell by exocytosis, involving membrane fusion between the vesicle limiting membrane and the plasma membrane. In the process of apocrine secretion, the secretory product is discharged along with parts of the apical cytoplasm, whereas, in the holocrine mechanism, the product of secretion is eliminated with the whole cell involving destruction of the secretion filled cells. The present study investigated that the salivary gland of *L. maximus* exhibited two mechanisms of secretion were detected in the secretory cells: merocrine and holocrine.

Electron microscopically observations of the cells of the salivary gland of slugs received LC_{50} of thymol revealed ultrastructural changes in these cells. These changes including, presence of some lipid droplets (Fig. 18), some of rough endoplasmic reticulum fragmented to small stacks (Fig. 21) and the heterochromatin accumulated in patches in the nucleus (Fig. 22). Whereas, glycogen granules (Fig. 16), mucous secretion (Figs. 18&20) and light and dense electron materials (Fig. 20) appeared mild changes and like those observed in the salivary gland of the control slugs group. However, LC_{90} of thymol exhibited severe changes in the cytoplasm and the nuclei of all different cell types of salivary gland of the slug *L. maximus*. These changes including presence of wide spaces between the cells (Fig. 23), large vacuoles and fibers observed between the cells (Figs. 21&28), appearance of numerous lipid droplets (Fig. 26), reduction of glycogen granules (Fig. 27), rough endoplasmic reticulum fragmented into small stacks (Fig. 29) and the heterochromatin accumulated in patches in the nucleus (Figs. 21,27&30). The nuclear envelope of some nuclei exhibited irregular shape (Fig. 28). These results agree with the results observed by the authors Sharaf, 2006; Radwan and El-Zemity, 2007; Radwan *et al.*, 2008 and Yousef *et al*., 2013 on the digestive gland of *Biomphalaria alexandrina*.

CONCLUSIONS

The present observation revealed that the salivary gland of *L. maximus* are only secretory in nature enclose various types of vesicles. The secretory cells of the salivary gland are variable; these are cleared as three types; ciliated cells, granular cells and mucous cells. It was recommended that the application of LC_{90} of thymol in a trial to open new areas of application of monotrpenoid as eco-friend molluscicide.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol.18:265-267.

Abdel-Haleem, A. A. (2014). Cytotoxicity of plant extracts *Origanum syriacum* on gametogenesis of two terrestrial slugs, using TEM. Cytology and Histology. 5: 220-224.

Ahmed, T. B.; Michaelkotiw, L. and Benkendorff, L. (2018).Review of anti-inflammatory, immune-modulatory and wound nealing properties of molluscs. J.of Ethnopharmacol. 210: 156-178

Al-Daihan, S. (2010). Effect of plant molluscicides on selected enzymes related to energy metabolism in *Biomphalaria arabica* snails, molluscan hosts to *Schistosoma mansoni* in Saudi Arabia. J. Egypt.Soc. Parasitol. 40: 187–195.

Bakry, F. A., Mohamedm, R. T. and El-Hommossany, K. (2009). Biological and biochemical responses of Biomphalaria alexandrina to some extracts of the plants *Solanum siniacum* and *Artemisia judaica*. Pest. Biochem. Physiol. 99:174-180.

Bancroft, J. D. and Gamble, M. (2002). Theory and practice of histological techniques, 5TH ed. Churchill, Livingstone New York, Philadelphia, 109-136.

Bhattacharyya, K. N. and Chaki, K. K. (2012). Ultrastructure of the salivary gland cells in active and aestivated mollusk, *Pila globosa* (Gastropoda-Orthrogastropoda- Ampularidae). Proc. Zool. Soc. 65 (1): 64-69.

Dykstra, MJ.; Mann, PC.; Elwell, MR. and Ching, SV. (2002). Suggested standard operating procedures (Scps) toxicology / pathology studies in a GLP Environmental Toxicology &Pathology, 30 (6):735-743.

El- Zemity, S. R.; Mohamed, Sh. A.; Radwan, M. A. and Sherby, S. M. (2001). Molluscicidal efficacy and repellency of some naturally occurring monoterpenoids against the land snail, *Helix aspersa*, Muller (Mollusca: Pulmonata). Annals Agric. Sci. Ain Shams Univ., Cairo 46 (1): 339-346.

Ferreira, P.; Geraldo, L.; Davila, S.; Elisabeth, C. and Bessa, A. (2011). The influence of thymol& DMSO on survival, growth and reproduction of *Bradybaena similaris* (Mollusca-Bradybaenidae). Zoological 48: 145-150.

Finny, D. J. (1971). Probit analysis Cambridge University Press, 3rd, London, pp. 318-329.

Fonghua, WEI.; Xingjian, XU.; Yuhai, DAI. and Jianbin, LIU. (2000). Study of plant molluscicide from *Solarium xanthocarpum* extraction. Hubi, Institute of schistosomiasis control, Wujian, China.

Lahlou, M. and Berrada, R. (2001). Potential of essential oils in schistosomiasis control in Morocco. Int. J. Aromatherapy, 11(2): 87-96

Lobo-da-Cunha, A. and Caladog, G. (2008). Histology and ultrastructure of the salivary glands in *Bulla striata* (Mollusca- Opithobranchia). Invertebrate Biol., 127: 33-44

Lobo-da-Cunha, A.; Alves, A.; Oliveira, E. and Calado, G. (2016). Comparative study of salivary glands in carnivorous and herbivorous cephalaspideans (Gastropoda-Euopistho-branchia). J.of Mollus. Stud., 82:43-54.

Lobo-da-Cunha, A.; Ferreira, I.; Coelho, R. and Calado, G. (2009). Light and electron microscopy study of the salivary glands of the carnivorous opithobranch *Philinopsis depicta* (Mollusca-Gastropoda). Tissue and cell, 41: 367-375.

Moura, K. R.; Terra, W. R. and Ribeiro, A. F. (2004). The functional organization of the salivary gland of *Bimophalaria straminea* (Gastropoda- Planorbidae). J.of Mollus. *Stud.* 70: 21-29.

Mustafa, O. M. (2018). Toxicity of thymol on the ultra-scanning structure of skin and digestive gland proteins of the two slugs (*Limax maximus* and *Lehmannia marginata),* The Egyptian Journal of Hospital Medicine 71 (6): 3405-3415.

Mowry, R. W. (1956). Alcian blue technique for the histochemical study of acidic carohydrates. J. Histochem. Cytochem., 4: 407-409.

Radwan, M. A. and El Zemity, S.R. (2007). Naturally occurring compounds for the control of harmful snails.Pakistan J. Zool., 39 (5): 339-344.

Radwan, M. A.; El-Wakil, S.R.; Mohamed, S.A. and Sherby, S. M. (2008). Potential of some monoterpenoids and their new N-methyl carbamate derivatives against

Schistosomiasis snail vector, *Biomphalaria alexandrina*. Ecotox. Environ. Saf. 71: 889-894.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain electron microscopy. J. Cell Biology, 17:208-212.

Sharaf, E. A. T. (2006). Molluscicidal effect of three monoterpenes oils on schistosomiasis and fascioliasis vector snails, in Egypt. J. Egypt. Soc. Parasitol., 36 (2): 599-612.

Singh, A. and Singh, V. (2010). Molluscicidal activity of *Saraca asoca* and *Thuja orientails* against the freshwater snail *Lymnaea acuminate*. Vet. Parasitol. 164: 206-210.

Wagele, H.; Klussmann-Kolb, A.; Verbeek, E. and Schrodel, M. (2014). Flashback and foreshadowing a review of the taxon Opithobranchia Organisms Diversity and Evolution, 14:133-149.

Wicht, M. J.; Haak, H.;Schutt-Gerowitt, S.; Kneist, S. and Noack, M. J. (2004). Suppression of caries-related microorganisms in dentine lesions after short-term chlorhexidine or antibiotic treatment. Caries Research 38: 436-441.

Yousef, A.; Naaema, B. and El-Kassas, B. (2013). Ultrastructure and histopathological effects of some plant extracts on digestive gland of *Biomphalaria alexandrina* and *Bulinus truncates* J. of Bas. and Appl. Zool. 66: 27-33.

Yousef, H. N. (2011). Effect of certain plant products on some physiological and histological aspects of the terrestrial snail *Eobania vermiculata* in Egypt. Ph. D. Faculty of Education, Ain Shams University.

EXPLANATION OF FIGURES

Figures 1-6: Photomicrographs of the salivary gland sections of *L. maximus* stained with H &E.

Fig. 1: Illustrating the salivary gland of a control slug which consists of acini (A) surrounded by connective tissue (CT) and secretory cells (SC) with nuclei (N). (X 400)

Fig. 2: Enlarged portion from the previous figure showing acinus of the salivary gland of a control slug with different secretory cells (SC) surrounded the lumen (L) and possess the nuclei (N). (X 1000)

Fig. 3: Showing the salivary gland of slug exposure to LC_{50} of thymol for 48 hours, Notice presence of many vacuoles (V) in the cytoplasm and rupture in some regions of the basal lamina of some cells (arrowhead). (X 400)

Fig. 4: Enlarged portion from the previous figure of the salivary gland of the slug exposure to LC_{50} of thymol for 48 hours showing vacuoles (V) in the cytoplasm. (X 1000)

Fig. 5: Illustrating the salivary cells of the salivary gland of slug exposure to LC_{90} of thymol for 48 hours with many vacuoles (V) in the cytoplasm and pyknotic nuclei (P). (X 1000)

Fig. 6: Showing the salivary gland of slug exposure to LC_{90} of thymol for 48 hours. The cells were seen widely separated from each other (asterisks) and many vacuoles (V) in the cytoplasm with karyorrhexis (K) in the nuclei of the salivary cells. (X 1000)

Figures 7-9: Photomicrographs of the salivary gland sections of *L. maximus* stained with Alcian blue (PAS)

Fig. 7: The Alcian blue revealing red colour stained of neutral mucopolysaccharides contents in the salivary gland of control slug. (X 400)

Fig. 8: Showing the salivary gland of slug received LC_{50} of thymol for 48 hours. The cells appear nearly control in the stainability with Alcian- blue (PAS). (X 400)

Fig 9: Illustrating the salivary gland of slug received LC90 of thymol for 48 hours. The cells appear weak stainability with Alcian-blue (PAS). (X 400)

Figures 10-17: Transmission electron micrographs of the salivary gland of the control *L. maximus*

Fig. 10: Showing cell types of salivary gland; ciliated cell (CC) with microvilli (Mv) and glycogen granules (GG), granular cell (GC) and mucous cell (MC). (X 6000)

Fig. 11: Showing ciliated cells (CC) with wide apical region, microvilli (Mv), mitochondria (M). Presence of gap junction (GJ) and tight junction (TJ) between the cells. (X 12000)

Fig. 12: Granular cell containing many vesicles filled with highly electron dense secretion (arrows) and mucous cell containing mucous secretion (MS). (X 6000)

Fig. 13: Illustrating the tubules of cilia (C) around the lumen (L) and nucleus (N) with nucleolus (Nu) in the ciliated cell. (X 15000)

Fig. 14: Showing light and dense electron materials (asterisks) and glycogen granules (GG) are observed in the ciliated cell. (X 12000)

Fig. 15: Showing rough endoplasmic reticulum (RER) and large vesicles of dense electron materials (asterisks) in the granular cell. (X 12000)

Fig. 16: Illustrating the entire secretory cells, filled with vesicles are discharged which their secretions into the lumen (asterisks). (X 5000)

Fig. 17: Showing secretory vesicles are discharged in the gland lumen with their limiting membranes and parts of the apical cytoplasm (asterisks). (X 5000)

Figures 18-22: Transmission electron micrographs of the salivary gland of *L. maximus* exposure to LC_{50} of thymol for 48 hours.

Fig. 18: Showing the salivary cells are nearly control; some lipid droplets (LD), mucous secretion (MS) and glycogen granules. (X 6000)

Fig. 19: Illustrating the light and dense granules (asterisks) and glycogen granules (GG). (X 6000)

Fig. 20: Showing granular cell with light and dense electron materials (asterisks) and the nucleus (N) is also observed. Mucous secretion (MS) is also appeared. (X 8000)

Fig. 21: Illustrating some rough endoplasmic reticulum (arrows) fragmented into small stacks. (X 15000)

Fig. 22: Showing numerous stacks of rough endoplasmic reticulum (RER) and patches of heterochromatin in nucleus (N) and abundant of scattered fibers. (X 15000)

Figures 23-30: Transmission electron micrographss of the salivary gland of *L. maximus* exposure to LC_{90} of thymol for 48 hours.

Fig. 23: Showing the different types of cells far from each other's (arrows). (X 6000)

Fig. 24:Showing many large vacuoles (V) in-between different cells. (X 12000)

Fig. 25: Illustrating wide space between the cells (asterisk), the mucous secretion (MS) possesses more electron dense material on the peripheral surface (arrows). (X 10000)

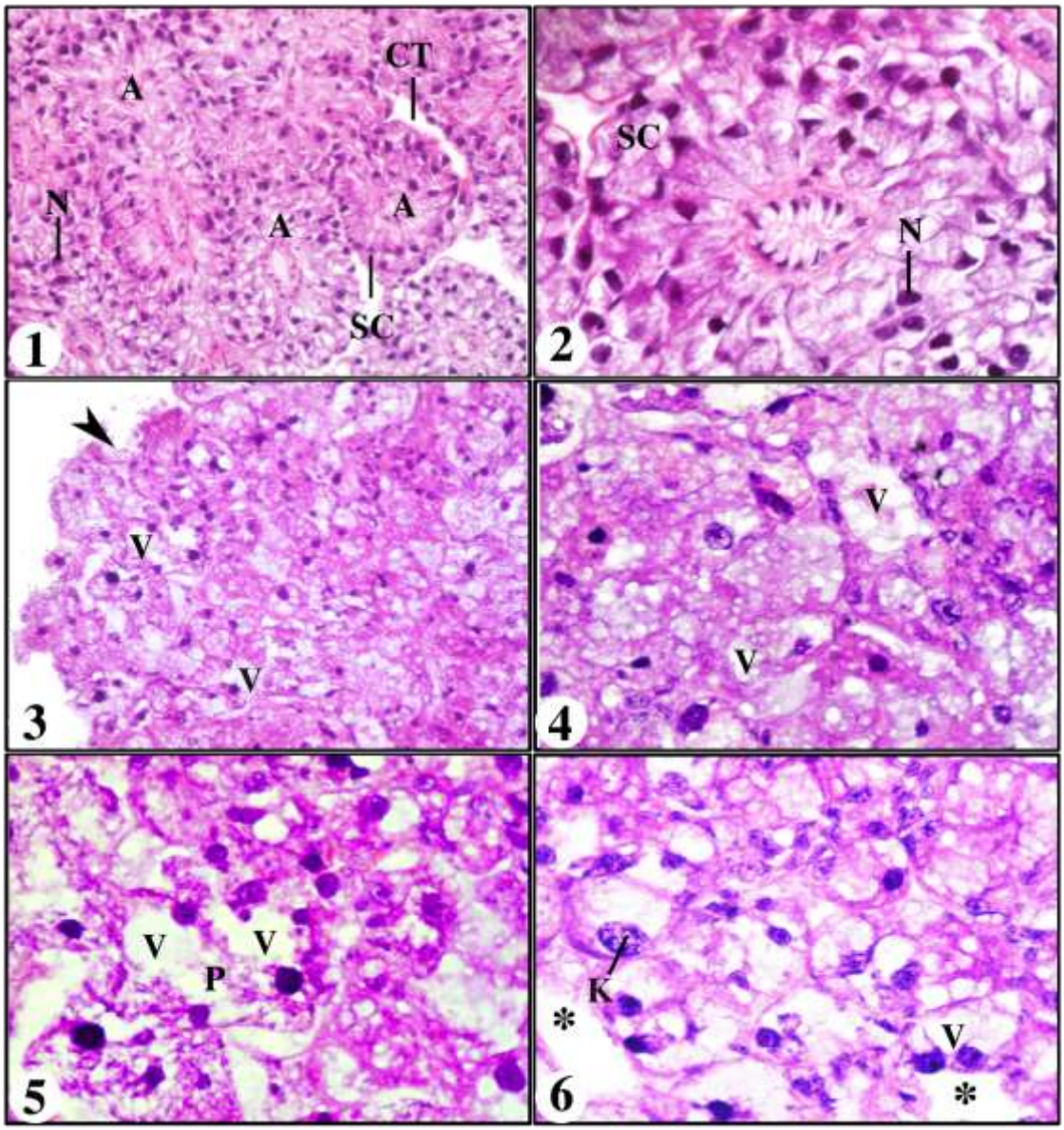
Fig. 26: Showing numerous of lipid droplets (LD) and few fibers (F) in-between the cells. (X 10000)

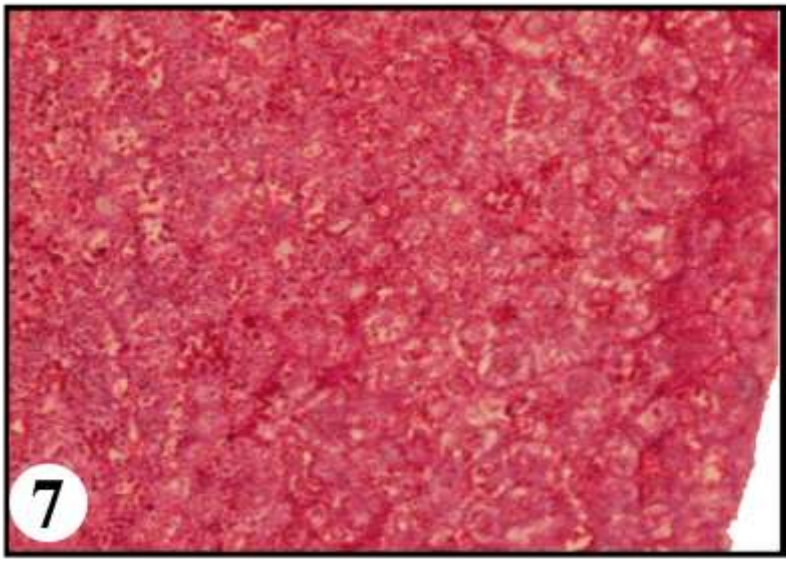
Fig. 27: Illustrating the reduction of glycogen contents (asterisks).The basal nucleus (N) is also observed. (X 8000)

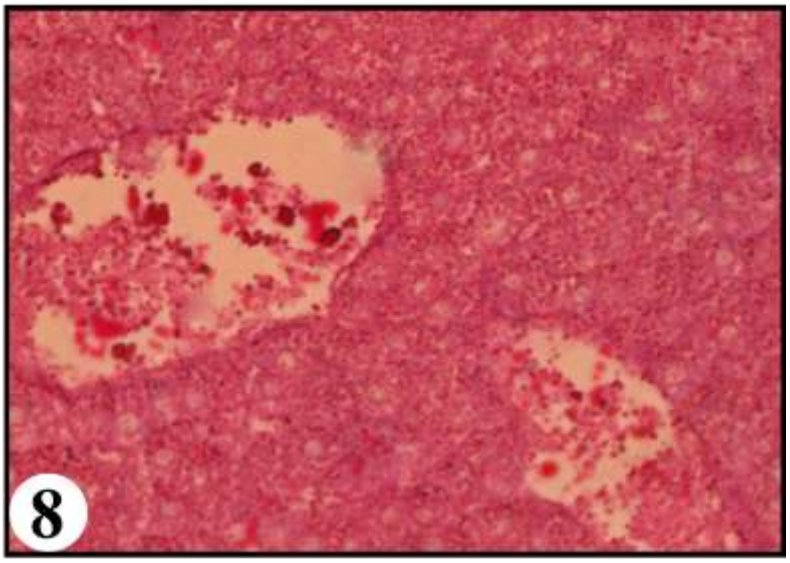
Fig. 28: Showing scattered many fibers (F) in-between the cells, lipid droplets (LD) and appearance of cilia (C). (X 15000)

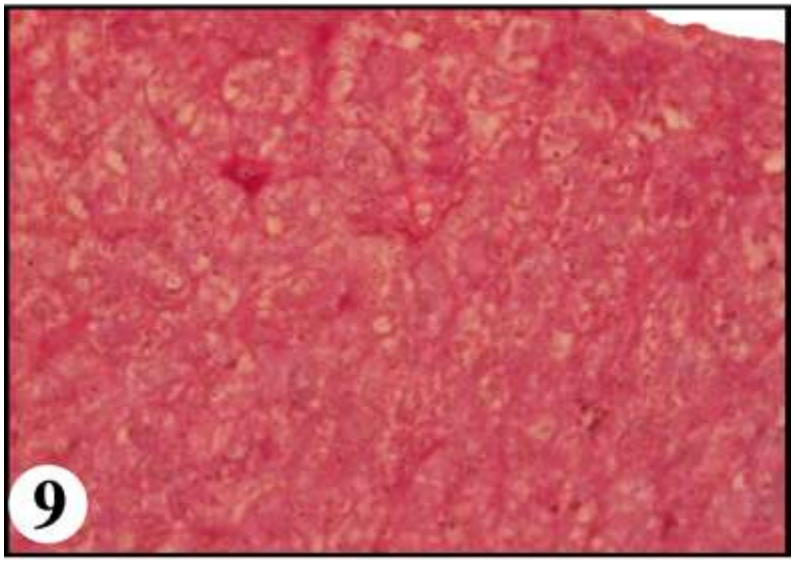
Fig. 29: Showing rough endoplasmic reticulum (RER) fragmented into numerous small stacks. (X 12000)

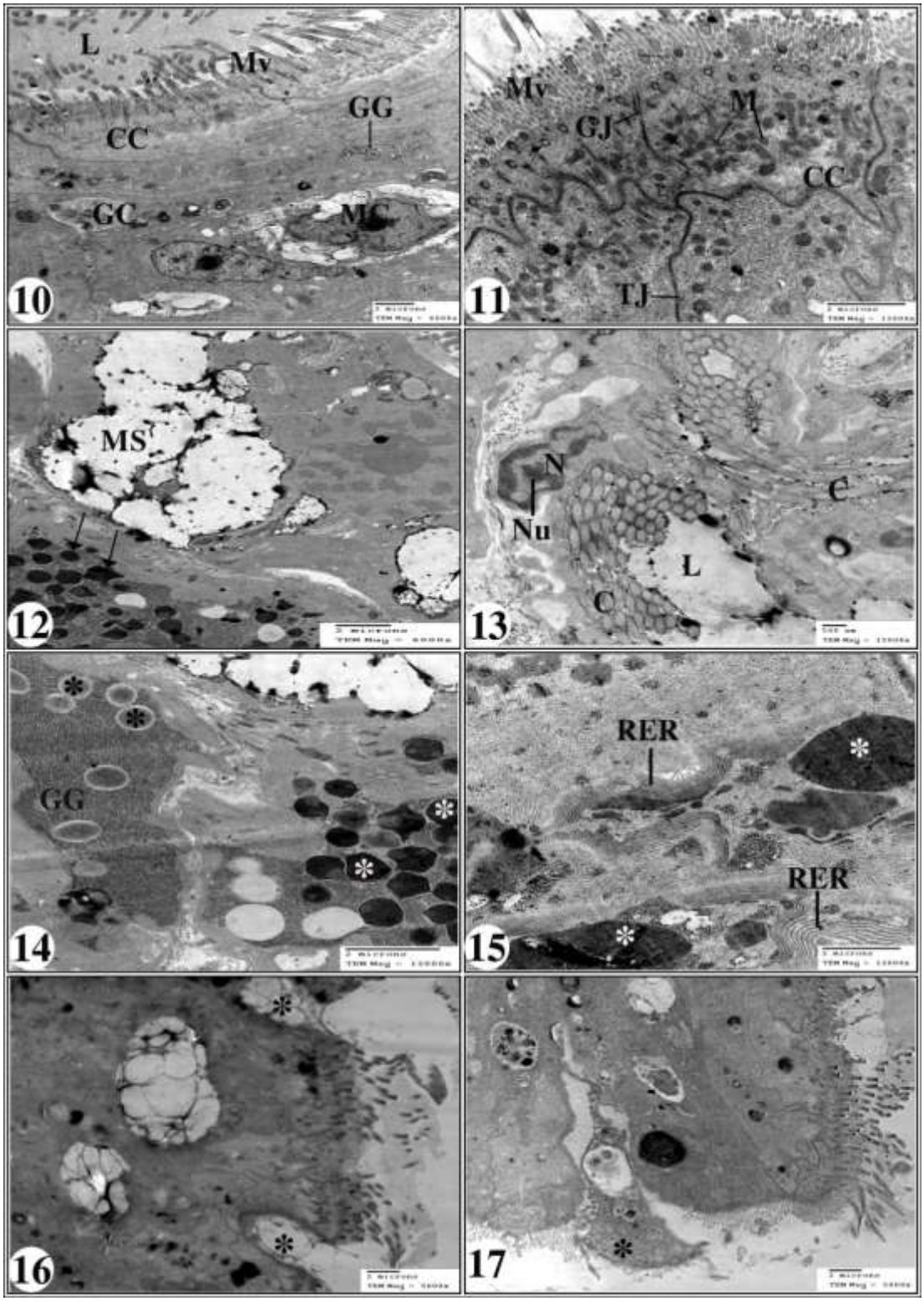
Fig. 30: Displaying a heterochromatin which appear large patches in the nucleus (N) and mucous secretion (MS) is also observed. (X 8000)

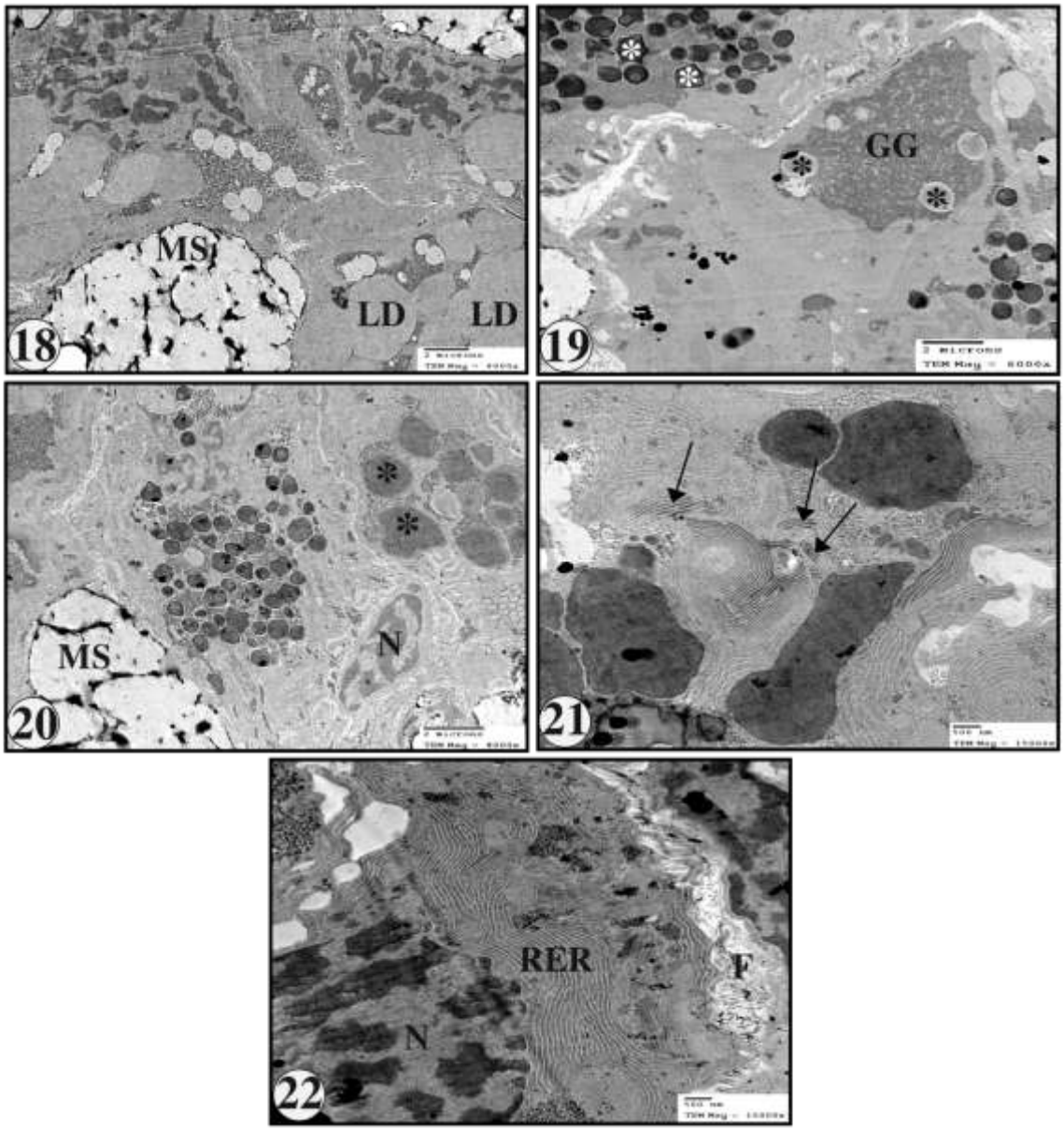

A
CT
N
A
A
SC
1
SC
N
2
V
V
3
V
V
4
V
P
V
5
K
*
V
*
6

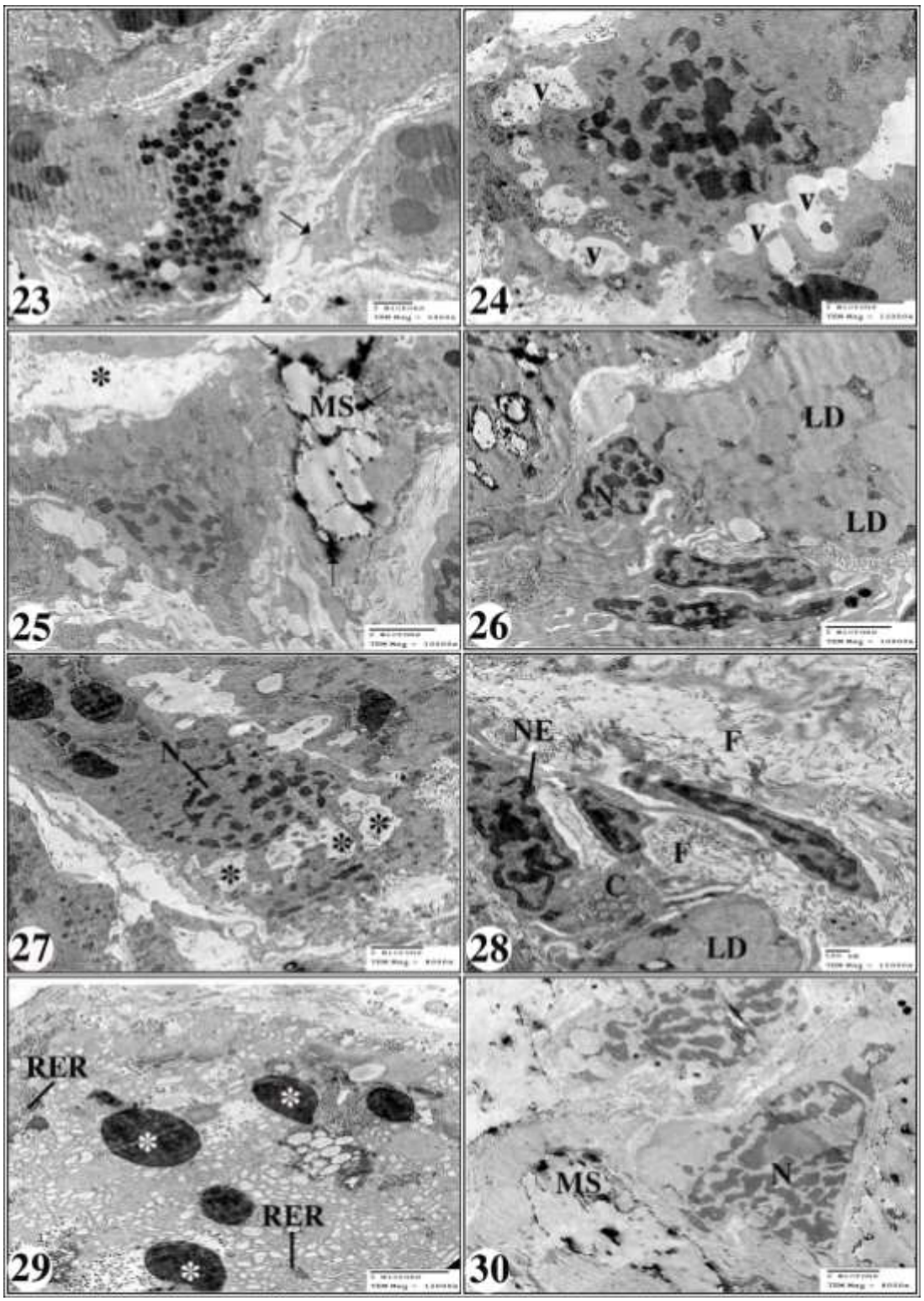

7


8


9


L
Mv
GG
CC
GC
MC
10
Mv
GJ
M
CC
TJ
11
MS
12
N
Nu
L
C
13
GG
14
RER
RER
15
16
17


MS
LD
LD
18
GG
19
MS
N
20
21
RER
F
N
22


23
24
V
V
V
V
25
MS
26
LD
N
LD
27
N
28
NE
F
F
C
LD
29
RER
RER
30
MS
N

ARABIC SUMMARY

تأثيرات منتج نبات (ثيمول) على الغدة اللعابية للبزاقة العملاقة ليماكس ماكسيمس في مصر (دراسة هستولوجية وتركيبية دقيقة)

أميمة محمد مصطفى
قسم العلوم البيولوجية والجيولوجية ، كلية التربية ، جامعة عين شمس ، القاهرة ، مصر

مركب الثيمول يوجد بشكل طبيعي في العديد من النباتات. وقد أظهر نشاطاً كمبيد للرخويات ضد القواقع الأرضية والبزاقات. تهدف الدراسة الحالية إلى وصف التاثيرات النسيجية وكيمياء الانسجة والتركيبية الدقيقة لتوضيح أي تأثيرات ضارة محتملة قد يكون استحضرها في مرحلة ما بعدالتعرض للثيمول في الغدة اللعابية لـلبزاقة.لتحقيق الهدف المقصود في هذا الصدد ،قد تم تقسيم البزاقات إلى ثلاث مجموعات. كانت المجموعة الأولى بمثابة المجموعة الضابطة، و تناولت المجموعة الثانية والثالثة تركيزات(LC_{50} , LC_{90}) من الثيمول لمدة ٤٨ ساعة في الغذاء. وتم الحصول على الغدد اللعابية ومعالجتها لإجراء الفحوصات. أظهرت النتائج النسيجية أن الثيمول له تأثيرات ضارة على انواع خلايا الغدد اللعابية بما في ذلك وجود فراغات في السيتوبلازم وتشوه في بعض انوية الخلايا المختلفة. وأشارت نتائج دراسة كيمياء الانسجة وجود عديدات التسكر المخاطية المحايدة في خلايا الغدة اللعابية. وكشفت النتائج التركيبية الدقيقة أن الشبكة الإندوبلازمية قد تم تجزئتها إلى جزيئات صغيرة ، كما ظهرت بقع هيتروكروماتين كثيفة في الانوية .

لذلك يمكن التوصية للبزاقات المعاملة (LC_{90}) من الثيمول لمكافحة البزاقات المستهدفة وهناك حاجة إلى مزيد من الدراسات لتقييم فعاليتها كمبيدات رخوية اقتصادية آمنة في الحقل بدلا من استخدام مبيدات الآفات الكيميائية التي يمكن أن تلوث البيئة.