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Control of the glassy clover snails *Monacha cartusiana* using *Zingiber officinale* extract as an ecofriendly molluscicide

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ABSTRACT

High population densities of *Monacha cartusiana* were observed to cause perdition of various economic crops at Sharkia Governorate. The present study was carried out control this harmful pest using ethanolic Ginger extract as a natural and environmentally safe molluscicide.Toxicological tests revealed that low concentration (20%) of ethanolic Ginger extract caused 10 % mortalityof cloversnails after one day of exposure and 66.7% mortalityafter28 days. The highest mortality percentage (90 %) was recorded after 28 days of treatment with40% Ginger extract. Biochemical investigations showed thatLC_{25} (7.04%) Ginger extract elevated ALT, AST, α & β esterases andphenoloxidase levels of treated snails compared to control.Histological inspections of the digestive gland and ovotestis of snails exposed to LC_{25}Ginger extract revealed that the digestive tubules showedvarious tubularanomalies, inflammatory hemocytes infiltration, excessive luminal secretionsand appearanceof necrotic areas. Acini of ovotestis weredeformedand comprised misshapen eggs and distorted sperms.TEM investigations showed cellular malformations in the digestive gland of treated snails including; severely disrupted microvilli, ruptured cell membranes, fractured RER and mitochondrial pyknosis.

Key words: Ecofreindly- Antioxidant Enzymes- Ginger extract-Histopathology- *Monacha cartusiana*-TEM.

INTRODUCTION

Land snails are considered as serious agricultural pests Egypt. They are herbivorous animals that devour mellow parts of vegetables, fruit trees and ornamental plants (Godan, 1983) and ruin mushy leaves, fruits, seeds and tubers (El-Okda, 1984). Undesirable smell and savor caused by mucous secretions left on plants during their movements, averts human and other animals from feedingon these contaminated plants (El-Deeb *et al.*, 1999). The glassy clover snails (*Monach cartusiana*) were recorded extensively at vast geographic areas at the Mediterranean regions and Southeastern Europe (Pieńkowska, 2018: Pieńkowska *et al*., 2019). High population densities were discoveredinfesting various economic crops at Sharkia Governorate, Egypt (Lokma, 2013). *M. cartusiana* reported to act also as an intermediate host for sheep lungworm and lancet liver fluke (Godan, 1983). Chemical control applications still the most commonly used for controlling land snailsalthough; they extensively prejudice non-target organisms including man (Gabr *et al.*, 2006). The high costs and resistance exerted against synthetic pesticides encourage the usage of naturally derived pest control strategies (Massoud and Habib 2003). Recently, several countries enhanced the use of plant extracts in pest control due to their low mammalian toxicity, low costs

and fast biodegradability (Singh *et al.*, 2000). Ginger extracts possess anti-inflammatory, analgesic, antioxidants and antimicrobial activities (Sharma *et al.*, 2013; Nikoli *et al.*, 2014; Amri and Touil-Boukoffa, 2016). Few studies have been carried out on the molluscicidal activities of Ginger (Ahmad *et al.*, 2013; and Edwin and Jacob 2017). Physiological and histopathological investigations give excellent indication about the hazards occurs within the organism because of exposure to toxicants. The present study aimed to control the glassy clover snail *Monacha cartusiana* using Ginger extract as a natural and alternative solution instead of using harmful chemical molluscicides.

MATERIALS AND METHODS

1. Snails collection and acclimation

Adult snails of *M. cartusiana* were collected from clover fields at Malames village, Meniet El-kamh district, Sharkia Governorate during spring 2018. Snailswere transferred to the laboratory and kept in glass cages (50 x 30 x 30 cm) contained moist soil and fed daily with fresh Lettuce for two weeks.

2. Ginger extracts preparation

Rhizomes of Ginger were purchased from the InternationalCompany (Cairo-Egypt). Rhizomeswere ground intoa fine powder using a pestle and mortar. The powder (30 g) was soaked in ethanol (600 ml) in a Sechelt apparatus for twodays. Ethanol was evaporated under reducedpressure until brown extract was obtained (Bakry *et al.*, 2013). Poisonous baits were formulated by mixing ethanolic Ginger extract with five grams of sugarcane syrup, completed with wheat bran to 100 grams and moisten with small amounts of water.

3. Experimental design

Clover snails were divided randomly into three main groups; the first group used for toxicological studies was subdivided into five sub groups and treated with different concentrations of Ginger extract for 28 days. Four replicates were applied for each subgroup.The second groupfed with baits containing LC_{25} of ginger extract for 14 days andused for biochemical, histopathological and ultrastructural investigations.The third group left as control group.

4. Biochemical measurements:

Digestive glands were dissected out from both control and treated snailsthen homogenized in distilled water using a Teflon homogenizer. The homogenates were centrifuged at 8000 r.p.m. for 15 minutes at 5ºC inrefrigeratedcentrifuge. Deposits were discarded and supernatants were kept in a deep freezer untiluse. Activities of AST and ALT enzymes were determined according to the procedure of Reitman and Frankle (1957). Alpha and Beta esterases assayed according to Van Asperen, (1962). Phenoloxidase activitymeasured using the method of Ishaaya (1971).All biochemical measurements were carried out in Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt.

5. Histopathological studies

For light microscopy, digestive glands of both control as well as treated with LC_{25} Ginger extract for 14 days were dissected out and fixed in Bouin's fluid. Specimens dehydrated in a graded series ofethanol, cleared in Xylene for 20 minutes, impregnated in paraffin wax (three changes) at 60^{o}C for 2 hours and embedded in paraffin wax. Sections (4-5 µm thick) were cut by microtome, mounted on glass slides andstained with hematoxylin and eosin.

6. Ultrastructural studies

Dissected digestive glands of both control and Ginger treated snails were fixed in 2.5 % glutaraldehyde in 0.05 M cocodylate

buffer containing 0.15 sucrose at pH 7 for two hours. Specimens were fixed in 1% Osmium tetroxide at 4oC for an hour, dehydrated in an ascending series of ethanol and embedded in Araldite f Epon. Blocks were cut with diamond ultramicrotome knifes. Semithin sections stained with 1% Toluidine blue. Ultra-thin sections stained with aqueous Urinyl acetate and Lead citrate and examined under JEOL 100 CX TEM at the Electron Microscopy Unit, Faculty of Agriculture, El-Mansoura University, Egypt.

7. Statistical analysis:

Values of toxicological and biochemical studies were expressed as a mean ± SE. The obtained data were analyzed statistically for the significance of differences using student's t-test (Goldstein, 1964).

RESULTS

1. Toxicity tests

Figure (1) showed that snail mortalities increased with increasing of both Ginger extract concentrations and exposuretime. At the first day of exposure, all treatments caused low mortalities. Ethanolic extract of Ginger (20%) causes 10, 50, 56.6, 66 and 66.7% mortalitiesafter 1, 7, 14, 21 and 28 days of treatment respectively.Exposure to 30 % Ginger extract caused 6.6, 36.7, 70, 76.6 and 83.3% for the same days of exposurerespectively.Finally, 40% Ginger extract resulted in 10, 46.6, 76.6, 83.3 and 90% respectively, at the same time intervals. The highest percentage of mortalities (90%) occurred after treatment with40% Ginger extract for 28 days (Fig. 1).

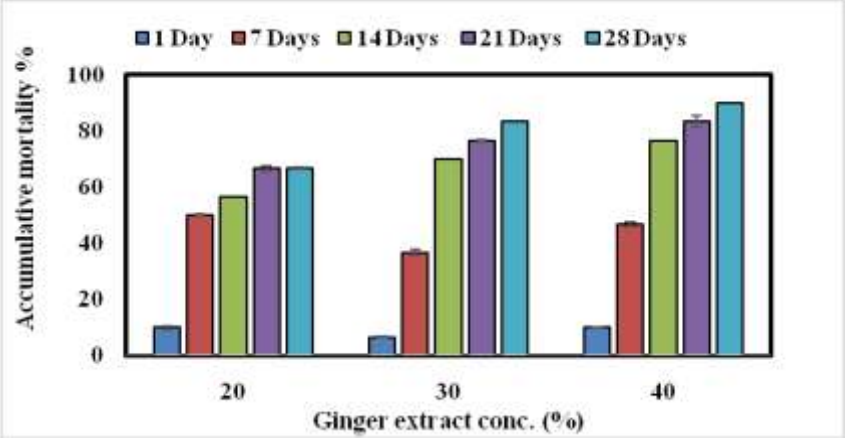

Fig.(1): Mortality percentages of *M. cartusiana* exposed to different concentrations of Ginger extract for 28 days.

2. Biochemical measurements

2.1. Activities of ALT and AST

Levels of AST were significantly elevated (P <0.05) after exposure to LC_{25} (7.04%) of *Z. officinale* extract and the highest level was recorded after two weeks of exposure compared to control. Activities of ALT were significantly increased(P < 0.05) after the first day of treatment, while their levels slightly decreased with increasing time of exposure, although levels were much higher than control (Fig. 2).

2.2. Activities of α and β-esterases

LC_{25} *of Z. officinale* extract caused a highly significant increase (P <0.05) of α and *β-esterases* activities after the first day of exposure. Values of both enzymes were

decreased insignificantly (P>0.05) after the 7th and 14th day of treatment (Fig. 2).

2.3. Activity of Phenoloxidase

Highly significant increase in the activity of Phenoloxidase was detected following the first day of treatment. Phenoloxidase levels decreased insignificantly (P>0.05) after one week of treatment. The lowest level of phenoloxidase was achieved after two weeks of exposure to Ginger extract (Fig. 2).

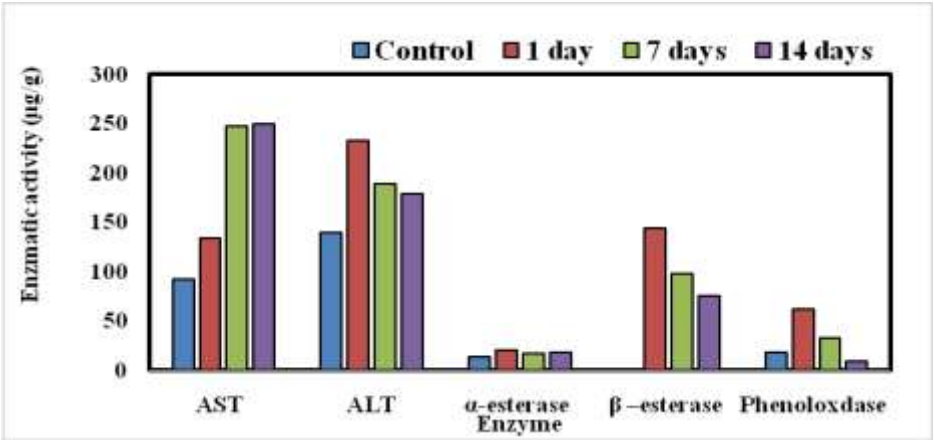

Fig. (2): Enzymatic activities of *M. cartusiana* treated with LC_{25} Ginger extract for 14 days.

3. Microscopical Examination

3.1. Histological studies on control digestive and hermaphrodite glands

The digestive gland of control *M. cartusiana*comprises mainly manynumerous blind digestive tubules separated by intertubular connective tissue containing hemolymphatic vessels and disparate hemocytes. Each tubule encircled with circular muscle layer (Plate I, Fig. a). Three different cell types constitute the epithelial lining of digestive tubules: digestive, excretory and calcium cells (Figs. b- d).

Digestive cells*:* Columnar cells, possessed many vacuolesaccommodating yellowish brown granules and basally located rounded or oval nuclei.

Excretory cells: Rounded cells incorporate large dark brown excretory granule and small basally locatednuclei.

Calcium cells: Occur singly or in groups, pyramidal-shape, stacked with rounded calcium spherules (appeared as light bodies) and have centrally located globular nuclei.

On the other hand, the hermaphrodite gland (ovotestis) is embedded in the digestive gland and consists of many ovoidacinieach is bounded by interacinar connective tissue containing interstitial cells. Each acinus isborderedby germinal epithelial layerand many Sertoli cells in between. Their lumens embrace developmental stages of spermatogenesis and oogenesis (Figs. e &f).

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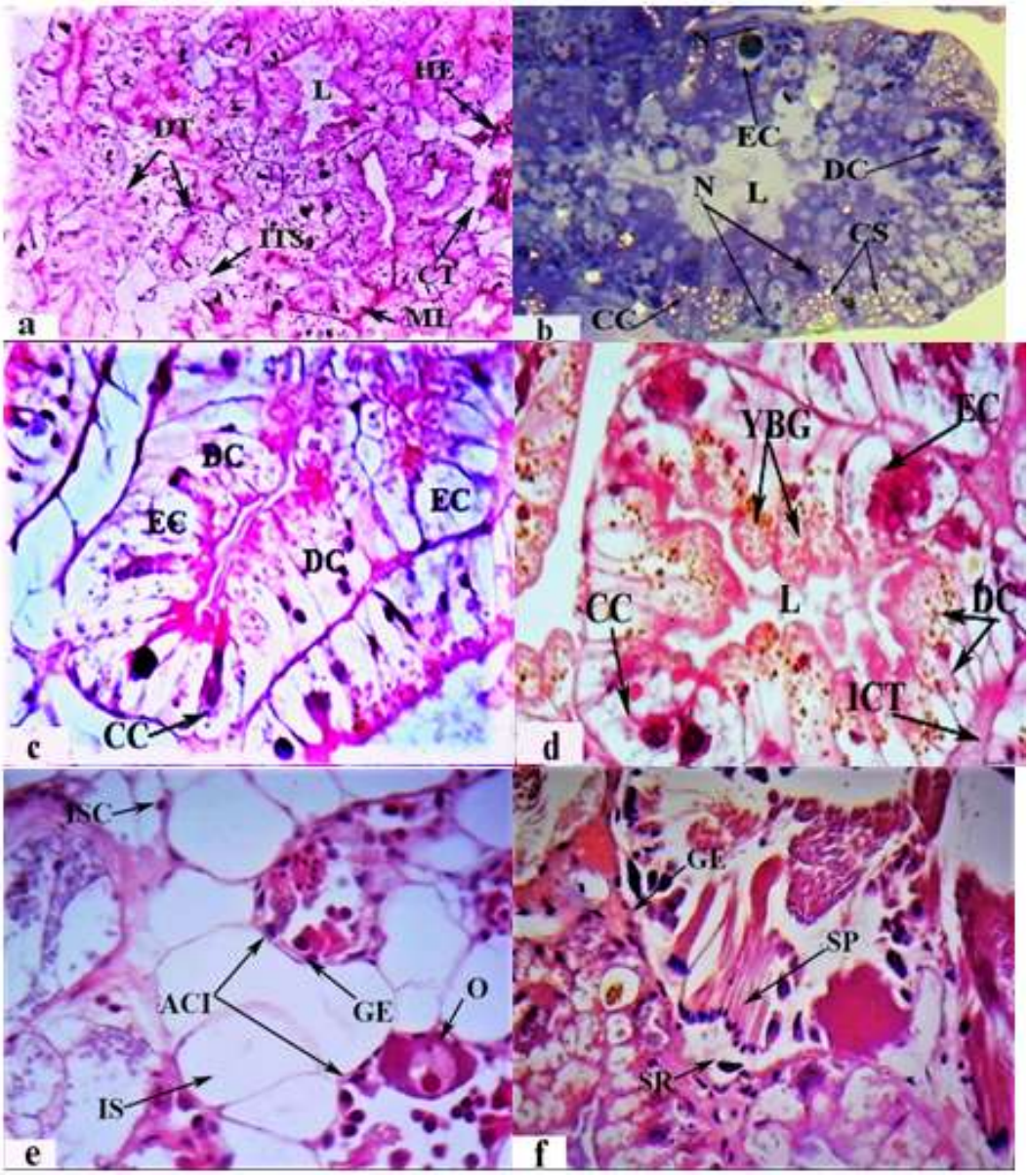

Plate I. Photomicrographs showing sections of the digestive and hermaphrodite glands of control *M. cartusiana*. **(a)**:T.S. of untreated digestive gland showing the normal histological architectureof digestive tubules (X100). **(b)**: Semi thin section showing different types of cells forming digestive tubule and calcium spherules of calcium cells (X400). **(c & d)**: Sections of the digestive tubules showing calcium cell at the corners, yellowish brown granules of digestive cells and large excretory dark granule of excretory cells (X400). **(e & F)**: Cross sections showing ovotestis acini of control *M. cartusiana* containing different developmental stages of oocytes and sperms (X400). ACI: Acini of the ovotestis; CC: Calcium cell; DC: Digestive cell; DT: Digestive tubules; EC: Excretory cell; GE: Germinal epithelium; HE: Hemocytes; ICT: Intertubular connective tissue; IS: Interacinar space; ISC: Interstitial cell; L: Lumen; ML: Muscle layer; PO: Primary oocyte; SO: Secondary oocyte; Sd: Spermatids; SP: Sperms; SR: Sertoli cell; YBG: Yellowish brown granules.

3.2. Ultrastructural studies of control digestive gland

Transmission electron microscopic studies revealed that the hepatic ducts of M.cartusiana digestive glandarelined with a ciliated columnar epithelium resting on a thin basement membrane andsupported by connective tissue layer and circular muscle bundles. These cells have oval nucleiwith prominent nucleolus and surrounded with thin layer of rough endoplasmic reticulum. Their cytoplasm is homogenous and contains many pinocytotic vesicles, whichwere noted also in the underlying connective tissue layer. Mitochondria are oval and more concentrated at the apex of these cells (plate II, Figs. a&b).Electron micrographs confirmed the presence of three main cell types constituting the epithelial lining of the tubules which are differentiated into; digestive, excretory and calcium cells.

Digestive cells: Their outer borders provided with well-developed microvilli projecting towards the lumenand havebasally ovalnuclei. Mitochondria and rough endoplasmic reticulum are scattered throughout cytoplasm. The cytoplasm contains alsomany electron- dense lysosomalgranules showing different states of activities. Adjacent digestive cells are interconnected by inter inter-cellular junctions (Fig.c).

Calcium cells: Pyramidal cells have numerous apical microvilliand rounded nuclei. Their cytoplasm possesses many electron- lighted calcium spherules, electron dense granules and oval mitochondria which are more concentrated at the apex (Fig. d).

Excretory cells: Rounded cells contain massive microvilli in their free border. The nucleus is rounded, basally located and surrounded bydense layer of rough endoplasmic reticulum and numerous oval mitochondria. The cytoplasm filled with dark osmiophilic excretory granules. A smaller excretory cell (regenerative) was noted beside the mature one that has a large globular nucleus containing an eccentric nucleolus. Its cytoplasm is condensed, containing few mitochondria and devoid of excretory granules (Figs. e& f).

3.3.3. Treated digestive gland

Light microscopic examinations of the digestive gland treated with LC_{25}ofGinger extract revealed various tubular deteriorations, inflammatory hemocytic infiltrations, tissue exudates and excessive luminal secretions. The epithelial lining undergoes vacuolization, extensive cell lysis and necrotic areas. The basement membranes and muscular layers surrounding tubules were lacerated. The digestive cells have accumulations of large numbers of darkly stained granules and pyknotic nuclei. Excretory cells showed decreased amount of dark brown granules. Calcium cells were disrupted and have few number of calcium spherules (Plate III, Figs. a-d).

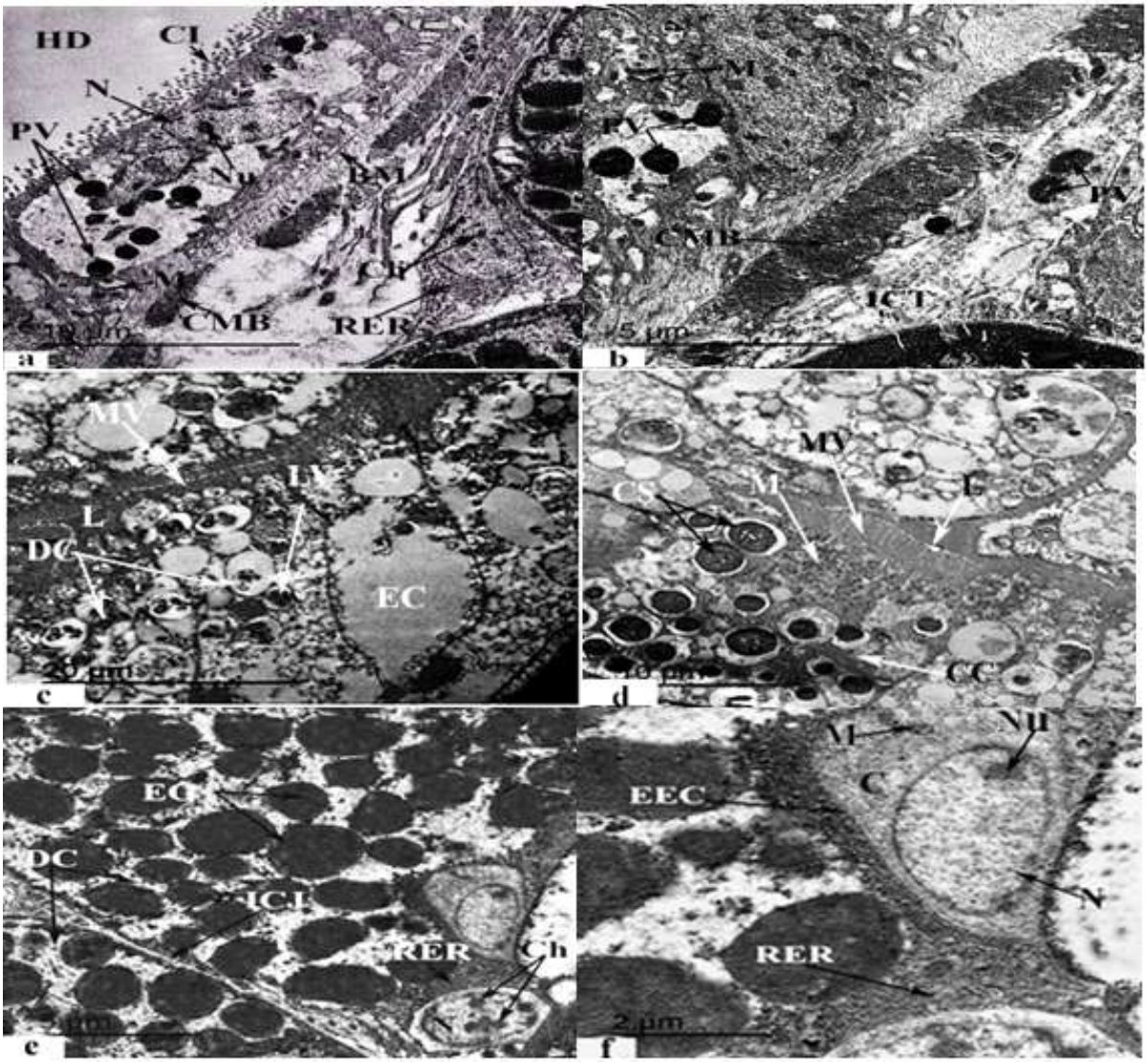

Plate II. Electron micrographs showing normal cellular structures of the digestive gland of *M. cartusiana*.**(a):** Ciliated columnar cells lining the hepatic duct (X13600). **(b):** Connective tissue layer and circular muscle bumbles surrounding digestive tubules (X17000). **(c):** Cellular lining of digestive tubule showing group of digestive cells (X 6800). **(d)**: Calcium cells containing characteristic calcium spherules (X6800). **(e**): The basal region of excretory cell and secretory granules (X17000). (**f):** Small regenerative excretory cell (X17000). BM: Basement membrane; C: Cytoplasm; CC: Calcium cells; CH: Chromatin material ; CS: Calcium spherules**;** CI: Ciliated columnar epithelium; CMB: Circular muscle bundles; CT: connective tissue; DC: Digestive cell ; DV: Digestive vesicles; EC: Excretory cells; EG: Excretory granules; EEC: Regenerative (Embryonic) excretory cell; HD: Hepatic duct; ICJ: Inter cellular junctions; ICT: Intertubular connective tissue**;** L: Lumen; LV: Lysosomal vesicles; M: Mitochondria; MV: Microvilli; N: Nucleus; NU: Nucleolus; PV: Pinocytotic vesicles; RER: Rough endoplasmic reticulum.

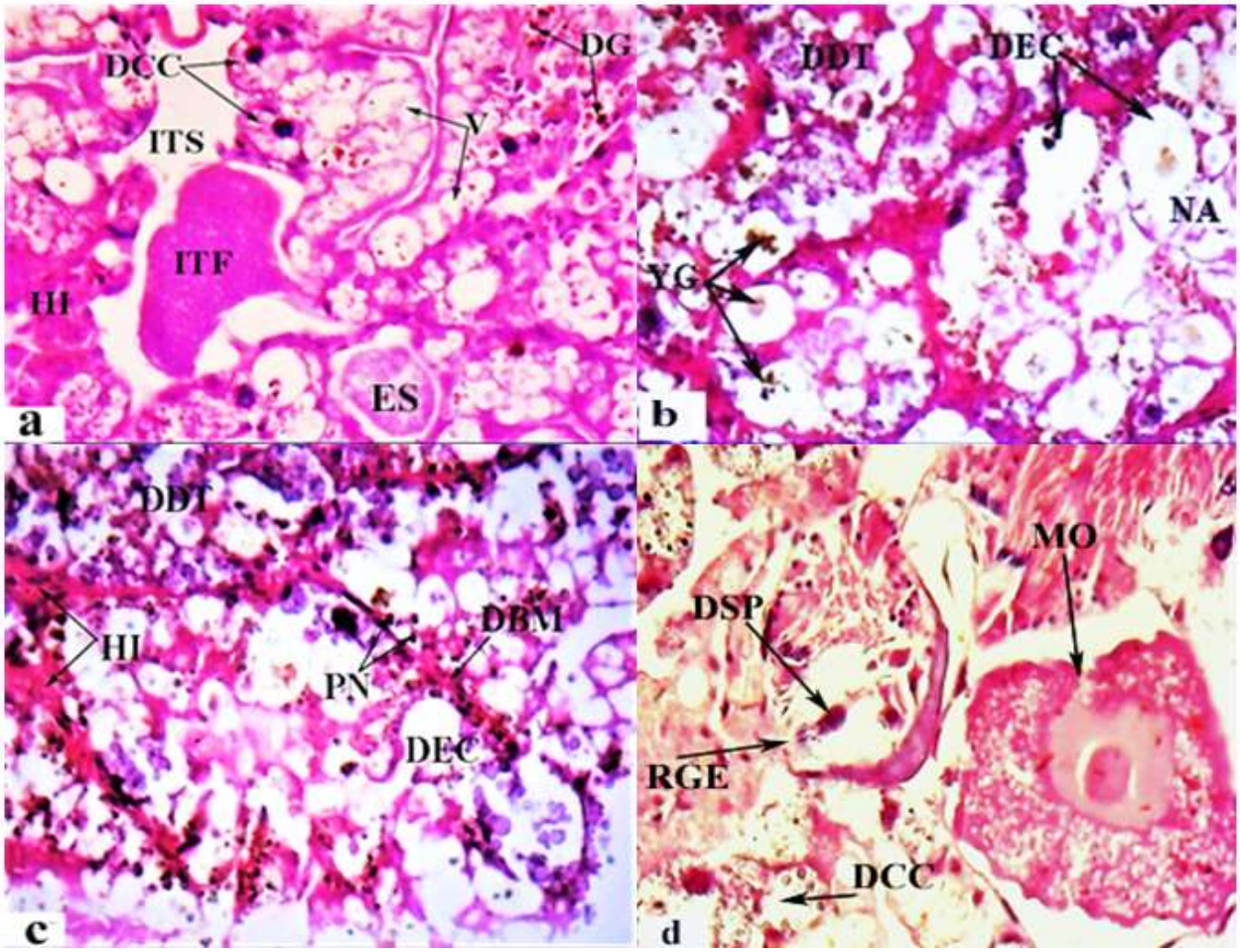

Plate III. (a- c): Photomicrograph showing cross sections through the digestive gland of *M. cartusiana* treated with LC_{25} of ethanolic Ginger extract for 2 weeks(X 400). **(d):** T. S. of treated ovotestis showing deformed oocyte and sperms(X 400). CL: Cell lysis; CS: Calcium spherules; DBM: Degenerated basement membrane; DCC: Deformed calcium cells; DEC: Deformed excretory cells; DDT: Deformed digestive tubules; DG: Dark granules; DSP: Deformed spermatozoa; EC: Excretory cell; ES: Excessive secretions; ITF: Intertubular filtrate; HA: Hermaphroditic acinus; HI: Hemocytic infiltration; N: Nucleus; NA: Necrotic area; PN: Pyknotic nuclei; S: Secretions; YG: Yellowish brown granules.

3.4. Ultrastructural abnormalities

Electron micrographs of the digestive gland of *M. cartusiana* treated with LC_{25} Ginger illustrated many ultrastructural abnormalities as severely ruptured microvilli of digestive and calcium cells.Excessive cloudy luminal secretions were noted. Cell membranes of various cells were lacerated. The cytoplasm was highly vacuolated inclosing denselyosmiophilic granules and lysosomal vesiclescomprising cellular debris. RER of excretory and calcium cells were ruptured in the form of disorganized tubules. .Mitochondria showed condensation of their matrix (mitochondrial pyknosis) andsprinkled cristaeand the nuclei became pyknotic. Intercellular junctions of adjacent cells were deformed (Plate IV, Figs. a- d).

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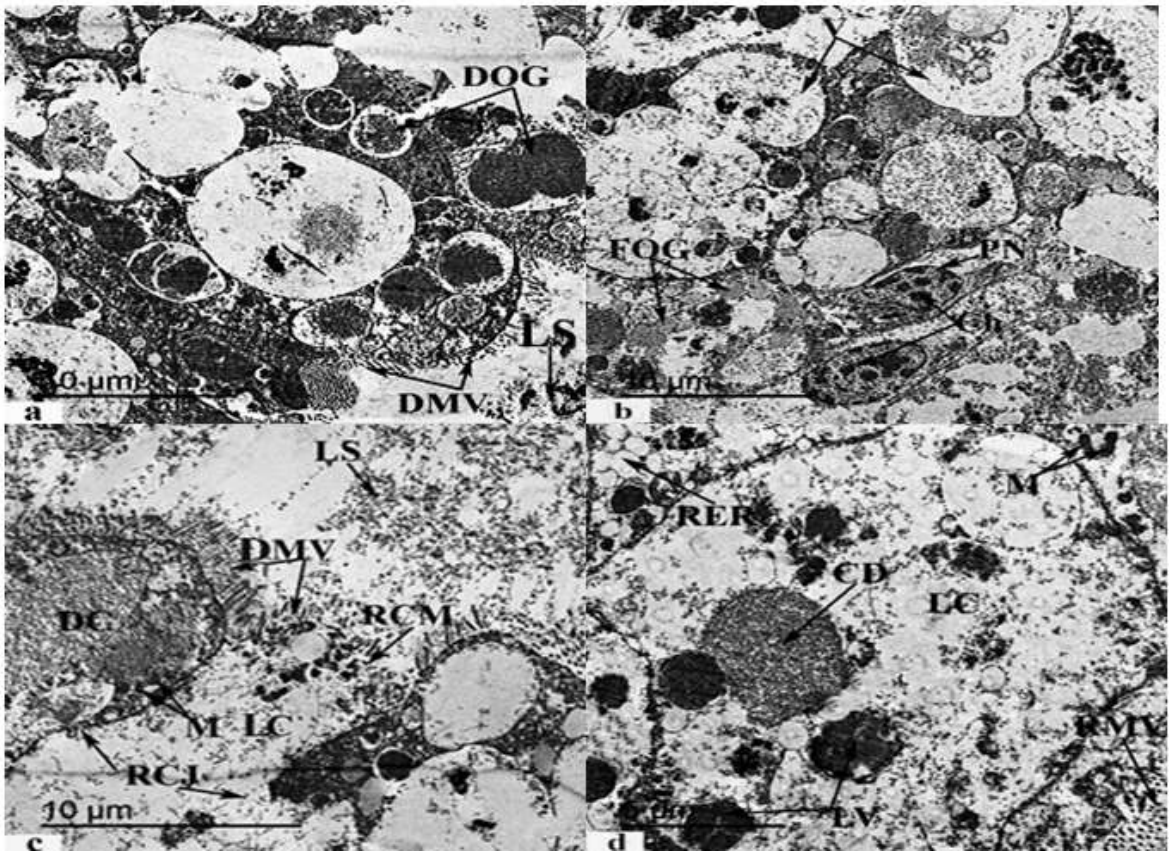

Plate IV. (a) Electron micrographs showing ultrastructural alterations in the digestive gland of *M. cartusiana* treated with LC_{25} Ginger ethanolic extract. **(a)**:Apical portion of digestive cellshowing disrupted microvilli(X 10200)**. (b)**: Basal region of excretory cellshowing nuclear pyknosis and vacuolization (X 13600). **(c)**: Digestive cell with distorted brush boarder and pyknotic mitochondria (X = 10200). **(d)**: Calcium cell with lytic cytoplasm, ruptured RER and microvilli (X = 17000). CD" Cellular debris; Ch: Chromatin materials**;** DMV: Disrupted microvilli; DOG: Darkly osmiophilic granules; FOG: Faint osmiophilic granules; LC: Lytic cytoplasm; LS: Luminal secretions; LV: Lysosomal vesicles; M: Mitochondria; PN: Pyknotic nucleus; RCM: Ruptured cell membrane; RCJ: Ruptured intercellular junctions**;** RER: Deformed rough endoplasmic reticulum; V: Vacuoles**.**

DISCUSSION

Several toxicological studies have been carried out to evaluate the molluscicidal activities of chemical pesticides, plant extracts and biocides against harmful land snails (Asran, 2001; Gabr *et al.,* 2006; El-Sherbini *et al*., 2009; Abdel-Haleem, 2013; EL-Sayed *et al*., 2013 Mwonga *et al.,* 2015; Prabhakaran *et al.,* 2017). The present study showed that low concentrations of ethanolic Ginger extract caused low mortality percentages after the first day of exposure while the highest mortalities of *M. cartusiana* snails occurred at high concentrations of Ginger at the third and fourth weeks.The presence of alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic derivativescaused the molluscicidal

potency of Ginger (Sharma *et al.,* 2016). This finding was in harmony with Singh *et al.* (2010) who revealed that ceniole, citral, 6 - gingerol and oleoresin extracted from *Z. officinale* rhizomes had potent molluscicidal properties and these active ingredients resulted in an inhibition in the reproductive capacity of *Lymnaea acuminate* snails. Bakry *et al.* (2013) showed a rapid decline in survival rate and egg production of *Biomphalaria alexandrina* snails exposed to Ginger. The obtained results showed that Ginger extract caused disturbancesin the enzymatic activities of treated clover snails. Highly significant elevationsof AST and ALT levelsrecorded after the first day of exposure may be attributed to the activation of defensive mechanisms inside the snail body to overcome the effect of toxicants. The gradual decrease happened in both enzymes later may be duo to cell injuries in digestive gland of treated snail and toxical hepatitis (Farkas *et al.,* 2004). Results demonstrated that Ginger extract caused a significant increase in α- esterase while it caused initial increase in β-esterase after one day of exposure then gradually decrease till the end of experiment. Increments in the activities of detoxification enzymes (αand β-esterases) may result from the stress occurred on enzyme expression system for synthesizing new and higher amounts of detoxification enzymes (Wheeler and Isman, 2000). Thisresultwas in agreement with the findings of El-Gendy (2015) who reported an elevated levelof α- esterase and decreased β-esterase levels of aphids treated with sublethal concentrations of *Origanum vulgare* extract. These results disagreed with the findings of Abd-El-Aziz (2014) who proved that the activity of α- esterase enzyme was inhibited while β-esterase activity was stimulated after treatment with Indoxacarb, Emamectin benzoate and Pyridalyl. Phenoloxidase is an antioxidant enzymeand plays an important role in the immune system of most invertebrates (Smith and Soderhall, 1991; Soderhall and Cerenius, 1998). The activity of Phenol oxidases were stimulated after the entrance of exotoxins inside organisms (Lee *et al*., 1998). Ginger ethanolic extract caused significant increase in the PO activity for a week then decreased at the second week. This result may attributed to the disturbances occurred in some defensive mechanisms of the snail as a result to the high stress induced by Ginger extract. These results are in conformity with Farrag *et al.* (2015) who stated that Phenoloxidase levels were significantly increased in treated 5^{th} nymphal instar haemolymph of desert locust after two dayswhile at the fourth days of treatment with *Metarhizium anisopliae*, their levels were decreased significantly compared to control.

The digestive gland (liver) of molluscs is involved in digestion, absorption beside storage of lipids, glycogen and minerals (Beeby and Richmond, 1988), and plays a major role in detoxication (Henry *et al.,* 1991).The digestive gland of gastropod molluscs is the key organof metabolism serving also as the main site of accumulation and biotransformation of xenobiotics (Desouky, 2006). Histological and ultrastructural investigations on the digestive gland of *M. cartusiana* confirmed the presence of three main cell types; digestive, calcium and excretory cells forming the epithelial wall of digestive tubules. This finding was in harmony with the finding of Heiba*et al.* (2002); Ismail *et al.* (2013) and Sharaf *et al.* (2015) who reported only three cell types. However, Hamed *et al*. (2007) reported four different cell types.

The present histopathological examinations of the digestive gland treated with LC_{25} Ginger extract showed tubular deteriorations, tissue exudates, hemocytes infiltration, excessive luminal secretions,

cell lysis and some necrotic areas appearinside tubules. Acini of ovotestis were distorted and contained deformed egg and sperms. These findings were in agreement with Abdel-Haleem, (2014) who reported a marked inhibition of the various stages of gametogenesis of terrestrial slugs treated with *Origanum syriacum*.

Electronmicroscopic examinations showed severely ruptured microvilli, lacerated cell membranes, disorganized RER and condensation of the matrix of mitochondria. These damaged cellular alterations may be due to the direct toxic effect of Ginger extract. Mitochondrial shrinking might be due to toxicant-induced inhibition of Na^+/H^+ transporter and impairment of the overall osmoregulatory mechanism of the cell (Vilella *et al.*, 1991).In this concern, Hamed *et al*. (2007)showed disruption and reduction of microvilli, increased number of calcium spherules and presence of large numbers of excretory granules in the excretory cells in the digestive gland of *E. vermiculata*exposed to methomyl. Moreover, Sharaf El-Din *et al.* (2012) indicated severe ultrastructural alterations in the cerebral ganglia of treated with *Biomphalaria alexandrina* exposed to ethanolic extract of *Anagalis arvensis* in the form of, pyknotic nuclei, atrophy of the perikarya of some neurons, fragmentation or dilation of rough endoplasmic reticulum, damage of mitochondria, and vacuolation of cytoplasm. Furthermore, Ustina *et al.* (2018) investigated ultrastructural alternations in the digestive gland of the Egyptian slug, *Limax maximus* exposed to thymol pesticide and they reported cytoplasmic vacuolation, degeneration of some nuclei, rupture of microvilli and increasing of calcium spherules inside secretory cells.

Conclusion

Ginger extract was showed highly toxical degrees against the clover snails *M. cartusiana* and resulted in various biochemical, histopathological and ultrastructural disturbances. Ginger extract caused severe deformations of both eggs and spermsand this may inhibit the flourishing of clover snail's populations at Sharkia Governorate.Therefore, this natural product may be incorporated into apply the control programs of land snailsinstead of using harmful synthetic pesticides. Poisonous baits control technique used in this study is safe for non-target organisms and considered as asubstitutional method in spite of the classical spray control methods.

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Control of the glassy clover snails *Monacha cartusiana* using *Zingiber officinale* extract as an ecofriendly molluscicide

مكافحه قواقع البرسيم الزجاجي *موناكا كارتوزيانا* بإستخدام مستخلص الزنجبيل كمبيد رخوي صديق للبيئه

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المستخلص

لوحظت كثافات عاليه من قوقع البرسيم الزجاجي *موناكا كارتوزيانا* والتي تسببت في هلاك العديد من المحاصيل الإقتصاديه بمحافظه الشرقيه. أهتمت هذه الدراسه بمكافحه هذه الأفه الضاره بإستخدام المستخلص الإثيلي لنبات الزنجبيل كمبيد رخوي طبيعي أمن علي البيئه. وضحت دراسات السميه أن التركيز الأقل لمستخلص الزنجبيل (20%)سبب وفيات نحو 10% فقط من قواقع البرسيم بعد اليوم الاول من المعامله ووصلت نسبه الوفيات الي 66.7% بعد اليوم الثامن والعشرين من المعالجه . سجلت أعلي نسبه وفيات في قواقع البرسيم (90%) وذلك بعد تعرضها للطعوم السامه لمستخلص الزنجبيل بتركيز 40% لمده 28يوم.

أظهرت الفحوصات البيوكيميائيه أن تعرض القواقع لتركيز تحت مميت من مستخلص الزنجبيل LC_{25} (7.04%) قد أحدث إرتفاعا معنويا في مستوي إنزيمات اسبرتيت امينو ترانسفيريز و ألانين امينو ترانسفيريز والفا وبيتا إستيريز و الفينول أوكسيديز في القواقع المعالجه مقارنه بالقواقع غير المعالجه. كما أوضحت الفحوصات النسيجيه لكل من الغده الهاضمه والغده الخنثويه بعد تعرضها لتركيز تحت مميت LC_{25} من المستخلص العديد من الإختلالات التركيبيه في الأنيبيبات الهاضمه وإرتشاحات إلتهابيه لكريات الدم وظهور إفرازات كثيفه بداخل تجاويب الأنيبيبات الهاضمه كما ظهرت بعض المساحات النسيجيه المتحلله. تشوهت الجيوب الخنثويه و إشتملت علي خلايا بيضيه غير طبيعيه وحيوانات منويه مشوهه.