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Original article

Influence of Methomyl (Copter 90%) on certain biochemical activities and histological structures of land snails *Monacha cartusiana*

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ABSTRACT

This manuscript was conducted to spotlight the toxic effect of two sub-lethal concentrations of Methomyl (Copter) LC_{20} (0.075 g/L) and LC_{40} (0.180 g/L) on some biochemical parameters and histological alterations for land snail *Monacha Cartusiana* (Muller, 1774). Land snails belong to the class *Gastropoda* and *Phylum Mollusca*. This study cleared that both the used concentrations (of Copter) caused a significant increase for activities of three enzymes: alkaline phosphatase (ALP), alanine amino transaminase (ALT), and Aspartate amino transaminase (AST) after 24, 48, and 72 h from exposure starting. In contrast, a total protein (TP) activity decreased at exposure for two concentrations at all lethality periods. Both concentrations of Copter (0.0.75 g/L and 0.180 g/L) have shown histological changes for land snail tissues after 96 h of exposure; digestive gland, hermaphrodite gland, foot, and mantle. Degeneration, rupture, and vacuolization for digestive cells have been shown; furthermore, hemolytic infiltration in connective tissue will be recognized for the digestive gland. The Oocyte and sperm show degenerated with deformation in the connective tissue of the hermaphrodite gland. Likewise, deformation in the muscle fiber layer of the foot in the land snail distorts the epidermis and mucus gland suffering from necrosis. Moreover, mantle shows rapture in epidermis layer, deformed in muscle fiber layer, and vacuolization and necrosis take place in mucus gland.

1. Introduction

Nowadays, land snails are considered the most injurious pest in stylommatophora in Egypt (Ali and Robinson, 2020; Heiba et al., 2002). Likewise, *Monacha cartusiana* (Müller, 1774), *Mollusca, Gastropoda, Pulmonata, Stylomatophora, Helicoidae, Monacheae* were widely distributed in the Egyptian Governorates. They are found in vegetable and field, fruit crops, orchards, ornamental and medical plants (Abou Senna et al., 2016; Heikal, 2015; Kadry et al., 2018; Rady et al., 2019; Shahawy, 2018; Shetaia et al., 2009). The control of snails with chemical compounds was widely distributed and more effective. Methomyl (Copter 90%) is a systemic chemical compound of monomethylcarbamate insecticide used for pest management in some field crops (Khalil, 2016; Khidr, 2019). Also, methomyl is the most pesticide used in the form of bait technic for terrestrial gastropods control (Abdallah et al., 2015). It is used as an abroad-spectrum insecticide in field crops, ornamental plants, vegetables, cotton, and fruit fields (Mortensen and Serex, 2014). The effects of pesticides on land snails will recognize physiologically by assaying the biochemical and histological changes (Abo Baker, 2011; Hamed et al., 2007; Khalil, 2016). Also, alterations in the biochemical parameters; alkaline phosphatase (ALP), alanine amino transaminase (ALT), Aspartate amino transaminase (AST), and total protein (TP); meanwhile, some histopathological alterations will be observed in many organs in the land snail *H. vestalis* were exposed to both methiocarb and Chlorpyrifos (Sharaf et al., 2015).

This study was planned to assay the effect of the methomyl (Copter 90%) pesticide with their sub-lethal concentrations LC_{20} (0.075 g/L) and LC_{40} (0.180 g/L) on some biochemical activities; alkaline phosphatase (ALP), alanine amino transaminase (ALT),

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Aspartate amino transaminase (AST) and total protein (TP) at three lethality exposure periods of *Monacha cartusina* land snail. Also, the effect of the above-mentioned sub-lethal concentrations on the histological structure of snails, digestive gland, hermaphrodite gland, foot, and mantle was studied.

2. Materials and methods

2.1. Experimented snails

Adult land snail *Monacha cartusiana* (shell size 10–13 mm) and (weight 3.7 g) were collected manually from infested field crops during the spring season at Ibshaway district El Fayoum Governorate (29 22°N: 30 51°E), middle Egypt. The adult snails were transferred in a muslin bag to the laboratory at Fayuom Agric. Res. Station, Egypt and then was washed with distal water; only the healthy breeder snails were used in the experiments and transferred into rearing plastic boxes, each one about 50 × 30 × 25 cm^3 in size, used as housing filled with moist sterilized loamy soil at 25C° ± 2C° and 75 % ± 5 % soil moisture. Each box was covered with a muslin cloth which was fitted with a rubber band to prevent snails from escaping. The snails were feeding on leaves of lettuce daily for two weeks.

2.2. Methomyl

Methomyl (Copter 90% SP) is a Carbamate compound (S-methyl N (methyl carbamoyl oxy) thioacetimidate) with a structure formula ($C_5H_{10}N_2O_2S$). Methomyl is purchased from the chemical company of Egypt chem international Agricultural chemical (Egypt, Cairo). It is a broad-spectrum, systemic, anticholinesterase, carbamate insecticide. The pure chemical is colorless with a solid crystalline shape. Methomyl is used as foliar for the treated field, fruit, and vegetable crops, also used as fly bait (Mortensen and Serex, 2014)

2.3. Laboratory experiments

2.3.1. Determination of lethal concentrations

About six concentrations of methomyl were prepared by using dechlorinated tap water on the basis of weight/volume (Osman et al., 2011; Osman and Mohamed, 1991) as follows; (0.07, 0.14, 0.28, 0.56, 1.12, and 1.3 g/L). The experiments took place by the leaf-dipping technique. About 30 snails were placed into rearing plastic boxes and starved for two days (Souza, 2003) then Feeding the snails with fresh leaves of lettuce dipping in the tested concentration for 30 s (Ghamry, 1994) then outside and left it for one minute to dryness; fresh leaves were washed with distilled water used as control. Each concentration was applied with five replicates for treated and control replicates (30 snails for each one). The mortality percentages were recorded for 24, 48, 72, 96 h. The lethal concentrations of methomyl were computed according to probit statistical analysis (Finney, 1971). The computed sub-lethal concentrations LC20 and LC40 at 96 h were 0.075 g/L and 0.180 g/L, respectively.

2.3.2. Toxicity of the sub-lethal concentrations of the tested compound at 96 h

The present study was conducted to estimate the effect of two sub-lethal concentrations from Methomy (Copter 90%) on some biochemical and histological alterations for the land snail. The experiment was divided into three groups, each on about 10 snails, the first group used as control and the others exposed to LC20 (0.075 g/L) and LC40 (0.180 g/L) of sub-lethal concentrations of Methomyl at 96 h, as mentioned in Table 1.

2.3.3. Biochemical parameters

The snails from each group (0.075 & 0.18 g/L) of Copter and control were exposed for 24, 48, and 72 h of the tested concentrations. After exposure, the treated snails were removed and dissected out from the shell and quickly weighted, homogenized 50 mg/mL, and centrifuged at 8000 rpm for 10 min in the refrigerated centrifuge (El-Gohary and Genena, 2011). The deposits were discarded, and the supernatants were used to determine the levels of biochemical parameters; Alkaline phosphatase ALP (U/L) was determined kinetically by Biosystems company according to the method described by (Young, 2001), alanine amino transaminase ALT (U/L). Aspartate amino transaminase AST (U/L) was determined kinetically by a Human company using the method of (Young 2001). Total protein TP (g/dL) was determined by using Diamond company according to the method reported by (Burtis and Ashwood, 1999).

2.3.4. Histological preparation

The digestive gland, hermaphrodite gland, foot, and mantle tissues of *Monacha Cartusiana* snail were carefully dissected and fixed in 10 % formol saline for one day. The fixed samples were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax at 56° in a hot air oven for twenty-four hours. Sections of 4 μm thickness were cut. The obtained tissue sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin stain (Banchroft et al.,1996), examined with a light microscope, and photographed with a microscopic camera.

2.4. Statistical analysis

The statistical analysis was performed using IBM SPSS (Statistical Package for the Social Sciences, SPSS, version 20). One-way analysis of variance was applied to study the effect of two sub-lethal concentrations compared with control and their impact on levels of some biochemical parameters at the same experimental period in addition to; the experimental period 24, 48, and 72 h; tested by the post hoc analysis which used to compare the tested experimental periods at the same sub-lethal concentration and their effects on the same parameters. Data were portrayed as mean ± SE of five replicates. $P < 0.05$ was considered for significant.

3. Results

3.1. The lethal concentrations

The preliminary study aimed to determine the lethal exposure concentrations (g/L) (to choose the experimental LC values) provided; LC_1, LC_5, LC_{10},. . ..., and LC50 at exposure periods 24, 48, 72, and 96 h after exposing *Monacha Cartusiana* to the tested methomyl were observed in the gained data in Table 1 the tested sub-lethal concentrations of methomyl were LC_{20} and LC_{40} at 96 h (0.075 and 0.18 g/L), respectively.

3.2. Biochemical measurements

The obtained results showed the effects of two concentrations LC_{20} (0.075 g/L) and LC_{40} (0.18 g/L) of methomyl (Copter 90%) on some biochemical parameters; ALP, ALT, AST, and TP of land snail *Monacha cartusiana,* after three different exposure periods (24, 48, and 72 h) from exposure as shown in Tables 2–5.

Table 1
the lethal exposure levels of Methomyl (Copter 90 %) g/L on (*Monach Cartusiana)* land snail at four lethality periods.

LC_{values}	24 h	48 h	72 h	96 h
LC_{20}	0.29 ± 0.09	0.144 ± 0.04	0.09 ± 0.03	0.075 ± 0.02
LC_{40}	1.45 ± 0.63	0.49 ± 0.1	0.25 ± 0.05	0.18 ± 0.03

LC_{values}: is the log of concentration required to kill a certain percentage of exposed snails.

The data in Table 2 computed by one-way ANOVA revealed that the activity of ALP enzyme for *Monacha cartusiana* was significantly affected and increased by exposure with both sub-lethal concentrations (0.075 and 0.18 g/L) of methomyl compared with the corresponding control. Also, the enzyme activity of the tested snails exposed to (0.18 g/L) concentration had a significant difference compared with 0.075 g/L of methomyl and the corresponding control at all experimental periods (24, 48, and 72 h).

In the experimental exposure periods, 24, 48, and 72 h, post hoc analysis show that at sub-lethal concentrations of 0.075 and 0.18 g/L, ALP activity at exposure periods 48 h and 24 h has no significant effect. In comparison, at exposure period 72 h, the activity of ALP was significantly affected and increased compared with the two corresponding exposure periods 24 and 48 h. As shown in Tables 3 and 4, one-way ANOVA analysis cleared that both enzymes ALT and AST increased with increasing concentrations of the methomyl. Moreover, ALT and AST activities significantly increased were exposed the snail to LC_{20} at all exposure periods compared with the corresponding control. Meanwhile, at exposure to LC_{40}, the activity of the enzyme was higher significant than the corresponding control and LC_{20} exposure concentration. Subsequently, the exposure periods 24, 48, and 72 h for the same test were analyzed by post hoc, which cleared that at both concentrations LC_{20} and LC_{40} of methomyl, the enzyme activity significantly increased at exposure period 48 h with corresponding the exposure period 24 h at the same concentration whereas, the lethal period 72 h significantly elevated in comparison with the corresponding exposure periods 24 and 48 h.

In contrast, as cleared in Table 5, the level of total protein in land snail *Monacha cartusiana* at both concentrations decreased significantly. However, it became less than the corresponding control at all exposure periods as shown by one-way ANOVA statistical analysis also showed that LC_{40} concentration causes a significant decrease for level TP with corresponding control and LC_{20} only at exposure periods 24 and 72 h. But the level of TP shows an insignificant decrease between LC_{40} and LC_{20} concentrations at 48 h. Also, post hoc analysis cleared that at LC_{20}, the lethal exposure period 72 h and 48 h decreased significantly related to 24 h whereas; at 72 h exposure period has no significance with the corresponding exposure period. On the other hand, at LC_{40} concentration, the exposure period 48 h not decreased significantly, neither 72 h nor 24 h. Nevertheless, the exposure period 72 h significantly decreased with exposure period 24 h only.

Table 2
Effect of Methomyl (Copter 90%) on the activity of alkaline phosphatase (AlP, U/L) of (*Monacha cartusiana)* land snail exposed to LC_{20} and LC_{40} concentrations at 96 h after 3 interval periods of exposure.

Chemical	Experimental Periods (hours)		
	24 h	48 h	72 h
Control	38.5 ± 1.6	38.2 ± 1.2	38.3 ± 0.7
LC_{20} (0.075) g/L	74.8 ± 1.1[a]	77.3 ± 2.2 [a]	86.2 ± 2.3[aAB]
% of change	(+94.49%) ❶	(+103.05%) ❶	(+125.18%) ❶
LC_{40} (0.180) g/L	84.8 ± 1.7 [ab]	86.6 ± 4.5[a]	126.3 ± 1.8[abAB]
% of change	(+120.27%) ❶	(+127.36%) ❶	(+229.94%) ❶

a, b: In the same column, a significant difference compared with the corresponding control, 0.075 g/L Copter at α = 0.05 (P < 0.05), respectively. ❶ In the column, percentage of change in relation to control.
A, B: In the same row, the significant difference compared with the 24 and 48 h of the experimental periods at α = 0.05 (P < 0.05).

Table 3
Effect of Methomyl (Copter 90%) on the activity of Alanine aminotransferase (ALT, U/l) of (*Monacha cartusiana)* land snail exposed to LC_{20} and LC_{40} concentrations at 96 h after 3 interval periods of exposure.

Chemical	Experimental Periods (hours)		
	24 h	48 h	72 h
Control	63.2 ± 0.97	63.5 ± 0.63	64.3 ± 0.61
LC_{20} (0.075) g/L	100.8 ± 2.9 [a]	115.4 ± 2.1 [a]	144.4 ± 3.0[aAB]
% of change	(+59.27%) ❶	(+81.73%) ❶	(+124.57%) ❶
LC_{40} (0.180) g/L	123.3 ± 1.6 [ab]	153.4 ± 2.4[abA]	174.5 ± 1.2[abAB]
% of change	(+95.40%) ❶	(+141.57%) ❶	(+171.38%) ❶

a, b: In the same column, a significant difference compared with the corresponding control, 0.075 g/L Copter at α = 0.05 (P < 0.05), respectively. ❶ In the column, percentage of change in relation to control.
A, B: In the same row, the significant difference compared with the 24 and 48 h of the experimental periods at α = 0.05 (P < 0.05), respectively.

Table 4
Effect of Methomyl (Copter 90%) on the activity of Aspartate aminotransferase (AST, U/l) of (*Monacha cartusiana)* land snail exposed to LC_{20} and LC_{40} concentrations at 96 h after 3 interval periods of exposure.

Chemical	Experimental Periods (hours)		
	24 h	48 h	72 h
Control	603.3 ± 0.8	604.7 ± 1.66	603.3 ± 1.28
LC_{20} (0.075) g/L	819.5 ± 1.9 [a]	838.8 ± 2.67[aA]	915.7 ± 3.96[aAB]
% of change	(+35.84%) ❶	(+38.71%) ❶	(+51.78%) ❶
LC_{40} (0.180) g/L	839.5 ± 5.3[ab]	1013.1 ± 2.10[abA]	1126.2 ± 4.67[abAB]
% of change	(+39.15%) ❶	(+67.54%) ❶	(+86.67%) ❶

a, b: In the same column, a significant difference compared with the corresponding control, 0.075 g/L Copter at α = 0.05 (P < 0.05), respectively. ❶ In the column, percentage of change in relation to control.
A, B: In the same row, the significant difference compared with the 24 and 48 h of the experimental periods at α = 0.05 (P < 0.05), respectively.

Table 5
Effect of Methomyl (Copter 90%) on level of Total protein (TP, mg/dl) of (*Monacha cartusiana)* land snail exposed to LC_{20} and LC_{40} concentrations at 96 h after 3 interval periods of exposure.

Chemical	Experimental Periods (hours)		
	24 h	48 h	72 h
Control	1.16 ± 0.067	1.16 ± 0.040	1.14 ± 0.051
LC_{20} (0.075) g/L	0.96 ± 0.037 [a]	0.72 ± 0.017[aA]	0.70 ± 0.018[a A]
% of change	(-17.24%) ❶	(-37.93%) ❶	(-38.59%) ❶
LC_{40} (0.180) g/L	0.68 ± 0.021[ab]	0.61 ± 0.027 [a]	0.51 ± 0.025[ab A]
% of change	(-41.37%) ❶	(-47.4%) ❶	(-55.26%) ❶

a, b: In the same column, a significant difference compared with the corresponding control, 0.075 g/L copter at α = 0.05 (P < 0.05), respectively. ❶ In the column, percentage of change in relation to control.
A, B: In the same row, the significant difference compared with the 24 and 48 h of the experimental periods at α = 0.05 (P < 0.05), respectively.

3.3. Histological alterations

A histological section of the control digestive gland was observed by light microscopy (Fig. 1a.), showing that it consists

of digestive tubules (DT) lined with three different simple epithelium cells resting on a thin basement membrane. These cells were observed and differentiated into; Digestive cells (DC), Excretory cells (EC), and calcium cells (CC). Digestive cells are simple columnar cells constituting the most component of the tubular structure characterized by containing cytoplasm granules and rounded basally located nuclei. Excretory cells occurred with a small number in the tubular gland comparing with a digestive cell having pyramidal or conical shape with small basally flattened nuclei. Calcium cells are smaller in number than digestive cells with a pyramidal shape containing large, rounded nuclei and calcium granules. After treatment by Copter, the histological examination of the treated digestive gland of the snail shows some changes for the tissue. As in concentration 0.075 g/L LC_{20} of the Copter (Fig. 1.b), a remarkable degenerative and ruptured are shown for digestive cells. Some excretory cells show ruptured while large vacuoles replace the cytoplasm of others. Subsequently, at concentration 0.18 g/L LC_{40} of the Copter (Fig. 1.c), rapture and damage for the most digestive and excretory cells in the tissue. Hemocyte infiltration will recognize inside the intertubular connective tissue.

The normal histological structure of the control hermaphrodite gland of land snail is composed of a large number of acini having a round or oval shape in which oocytes and sperm and their supporting cells are formed, all separated by connective tissue (Fig. 2a.). After 96 h of exposure to both LC_{20} and LC_{40} of the copter, the histological examination of the hermaphrodite gland of the treated snailsshows degenerated oocytes (DOC), degenerated sperm (DSP), and deformation in connectivetissue.The untreated foot of Monacha cartusiana by light microscope examination is shown as in (Fig. 3a) composed of an outer epithelial ciliated cell (epidermis layer) followed by columnarepithelial pigmented cells unicellular (mucus gland) the innermost layer was longitudinal musclefibers (MF). Exposure for 96 h to copter makes histological alteration in the foot tissue of the snail. At 0.075 g/L of copter the, deformation of muscle fiber takes place, and slight distortion of the outerlayer (epidermis) and unicellular mucus gland suffered necrosis (Fig. 3b). At the 0.18 g/Lconcentration shown in (Fig. 3c), the outer layer is completely destructive and lysis. The musclefiber layer suffers from deformation and vacuole formation.

Control mantle of a land snail under the light microscope (Fig. 4. a) shows that the outer layer differentiated into simple cuboidal epithelial cells (epidermis), while the inner one is composed of unicellular mucus. Treated mantle of snail for 96 h of methomyl (Copter 90 %) makes changes in the histological structure of tissue shown at treatment with LC_{20} of copters in (Fig. 4.b) observed that the muscle fiber layer had been deformed and mucus gland cells become enlarged and empty. Rupture in the epidermis layer, and its cells become disintegrated. While, in (Fig. 4.c) the tissue treated with LC_{40} of the Copter, shown that the muscle fiber will be deformed and the mucus gland suffered from necrosis and vacuolization, glands containing basophilic secretion and occupied the most tissue, and other cells containing acidophilic secretions occur with a small number (mucus glands), the innermost layer is muscle fiber (MF).

4. Discussion

4.1. Biochemical measurements

For sub-lethal concentrations LC_{20} (0.075) and LC_{40} (0.180) of methomyl experiments, our data cleared that ALP, ALT, and AST activities increased significantly compared with the corresponding control at all experimental exposure periods (24, 48, and 72 h) of methomyl at both sub-lethal concentrations (LC_{20} and LC_{40}). The

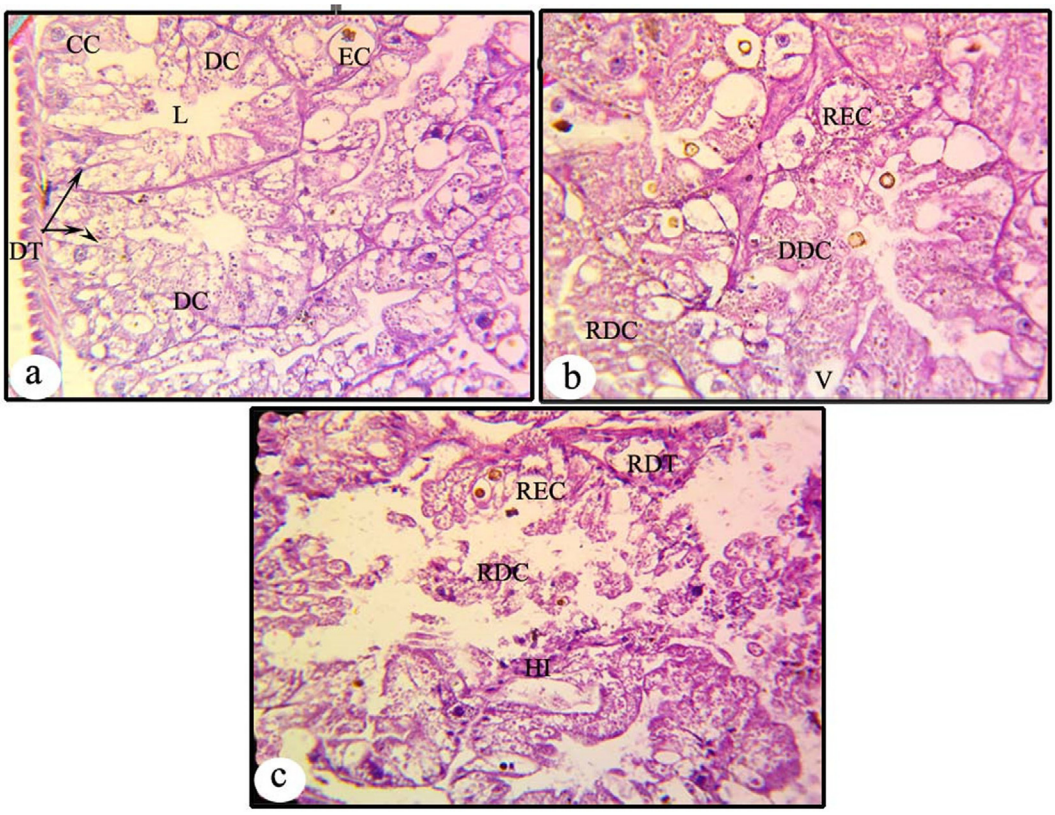

Fig. 1. Light micrograph of the digestive glands of *Monacha cartusiana* snails. (a) Normal digestive gland, (b) Snails exposed to LC_{20} of Methomyl, (C) Snails exposed to LC_{40} of Methomyl. DC: Digestive cells, CC: calcium cell, EC: excretory cells, L: Lumen, RDC: Ruptured digestive cells, DDC: Degenerated digestive cells, DEC: Degenerated excretory cells, V: Vacuole, RDC: Ruptured digestive cells, HI: Hemocyte infilteration. H&E; ×40.

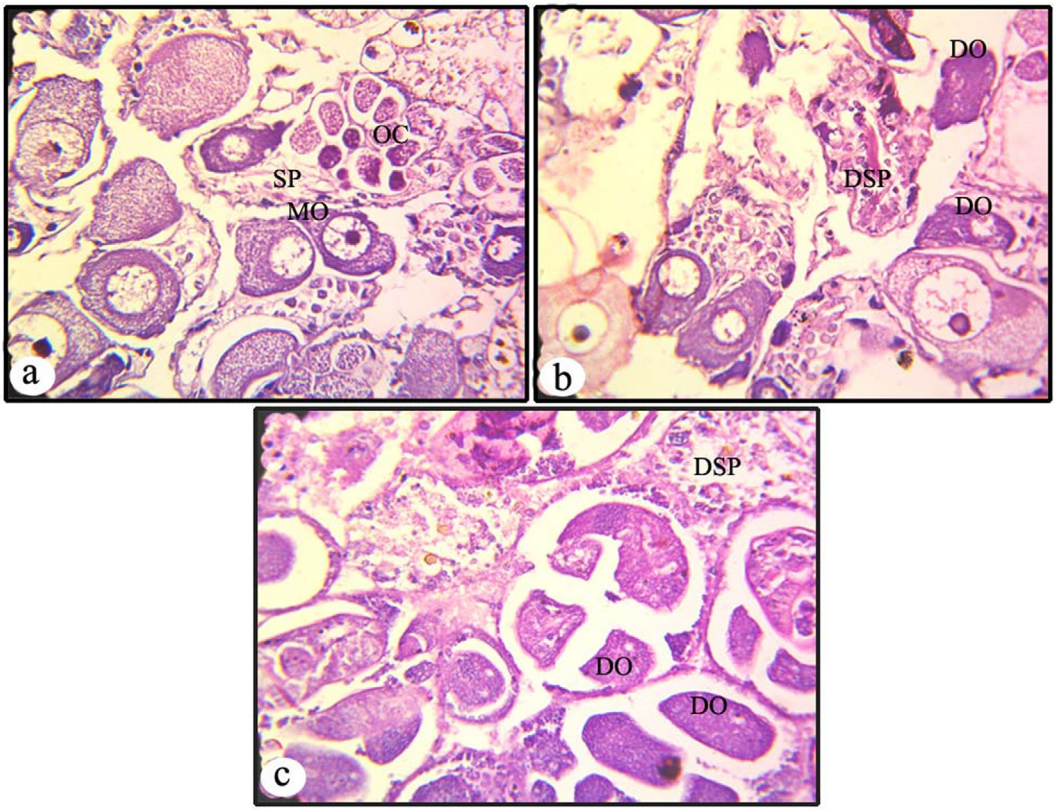

Fig. 2. Light micrograph of the hermaphrodite of *Monacha cartusiana* snails. (a) Normal hermaphrodite gland, (b) Snails exposed to LC_{20} of Methomyl, (C) Snails exposed to LC_{40} of Methomyl. MO: Mature Ovum, OC: Oocytes, SP: Sperms, DO: Degenerated Ovum, DSP: Degenerated Sperms, DOC: Degenerated Oocytes, H&E; ×40.

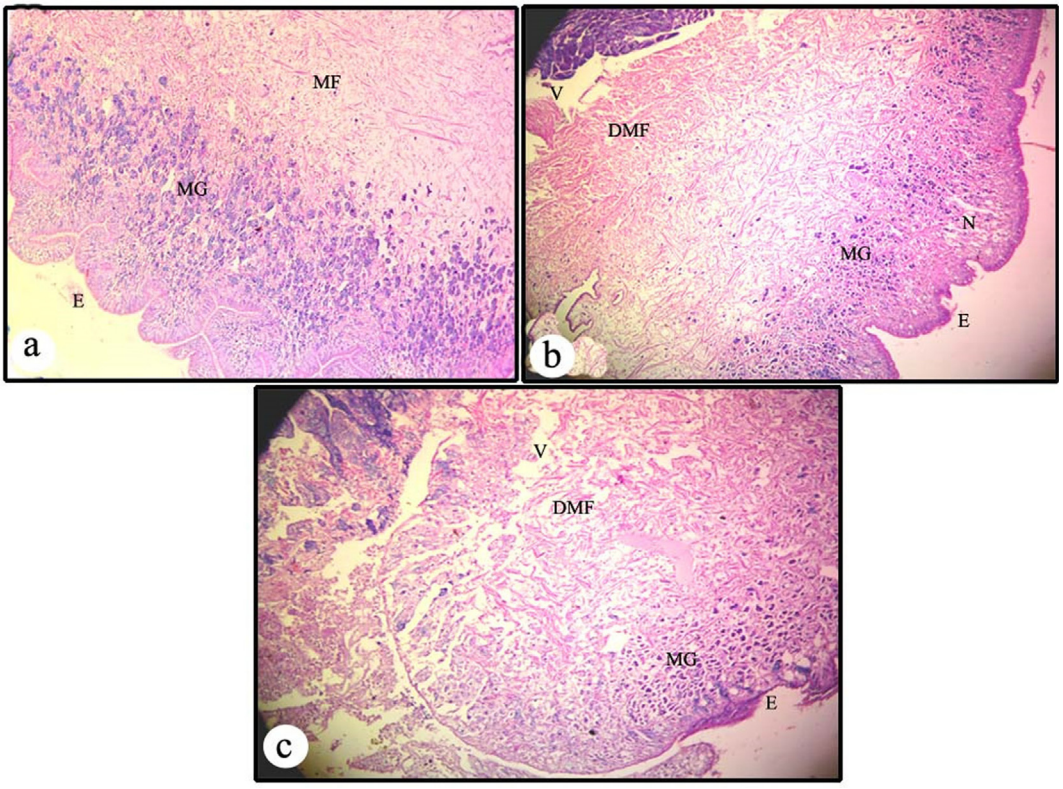

Fig. 3. Light micrograph of the foot of *Monacha cartusiana* snails. (a) Normal foot, (b) Snails exposed to LC_{20} of Methomyl, (C) Snails exposed to LC_{40} of Methomyl. E: Epidermis, MG: Moucus Gland, MF: Mussel Fiber, DMF: Deformed Mussel Fiber, N: Necrosis, V: Vacuoles. H&E; ×10.

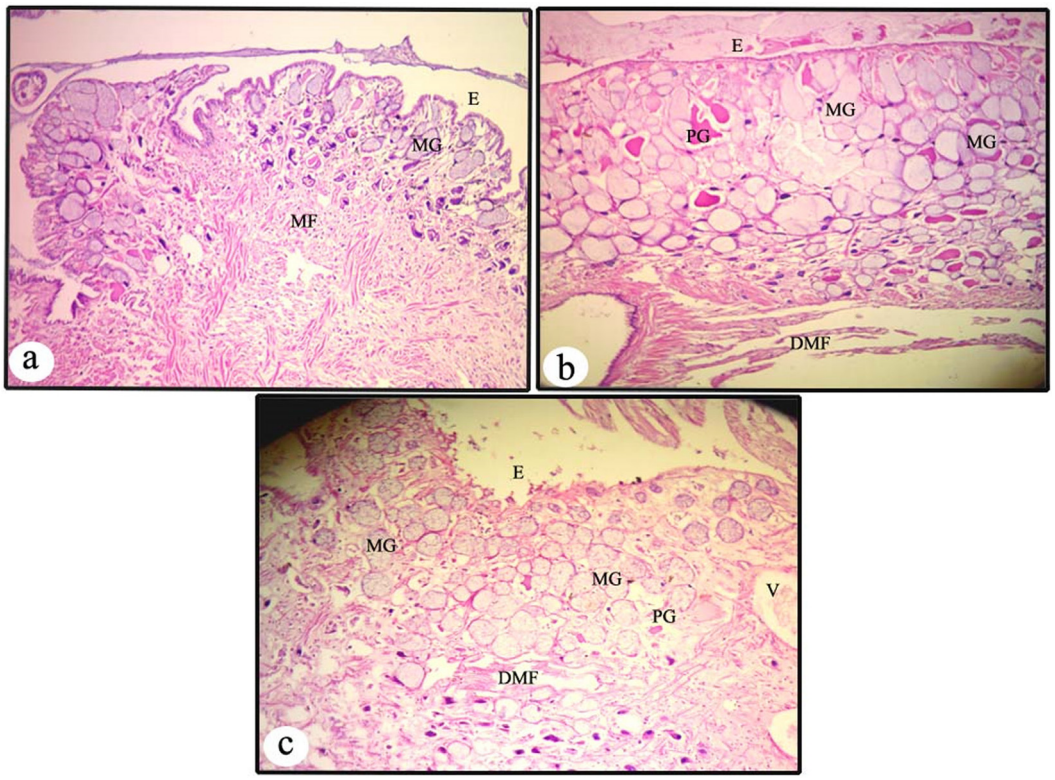

Fig. 4. Light micrograph of the mantle of *Monacha cartusiana* snails. (a) Normal mantle, (b) Snails exposed to LC_{20} of Methomyl, (C) Snails exposed to LC_{40} of Methomyl. E: Epidermis, MG: Moucus Gland, PG:Protein Gland, MF: Mussel Fiber, DMF: Deformed Mussel Fiber, N: Necrosis, V: Vacuoles. H&E; ×40.

increase in ALP, ALT, and AST activities may be due to damage of the digestive gland and cell necrosis caused by the effect of insecticides and caused leakage out the enzymes out the cells (Kammon et al., 2010). The alterations in biochemical parameters can be used as an indicator for environmental conditions and risk measurements of land snails (Khalil, 2016). The increase in activities of enzymes may be due to necrotic changes and degeneration in the liver caused by pesticides through which the enzymes leakage out the cells (Arfat et al., 2014). These results are supported by (Al-Attar, 2005) who recorded that; ALP, ALT, and AST significantly elevated the fish exposed to cadmium. AST, ALT, and ALP enzymes are located in hepatic cells and in somebody tissues; intestine and muscle, gill, and heart. Thus, the direct exposure of the animal to the toxic effects makes it requires adequate energy to get over the toxic stress. This toxic stress is obtained via gluconeogenesis during breaking down the free amino acid to overcome the requirement energy, leading to increased transaminase enzyme activity (Neelima et al., 2013; Samanta et al., 2014). The toxic stress leads to alteration in cell permeability, which causes elevations in enzymes activity due to leakage of the enzymes out of the damaged cells (Meenakshi et al., 2020). (Banaee et al., 2016) agree with our results and stated that the increase in activities of AST and ALT enzymes might occur as a result of an increase in permeability of cell membrane or damage of cell membrane of hepatocyte but disagreement with our results in decreasing the activity of ALP lower than normal which may be due to reduction in synthesis of ALP and tissue damage. (Naveed et al., 2010) cleared that activity of ALT and AST were enhanced when exposed *Channa Punctatus* fish to the toxic effect of triazophos pesticides. In contrast, our observations agree with (El-Gohary and Genena, 2011) that the activity of AST significantly increased in *M. cantiana* and *E. vermiculta* under exposure to three different tested molluscicides and increased the activity of ALT in *M. cantiana* only but disagreement with our that in *E. vermiculata* ALT significantly decreased at the three tested molluscicides.

The present study demonstrated that the level of total protein was significantly decreased in the snail exposed to two sub-lethal concentrations of methomyl than the control one. Our results follow several previous observations. (El-Shenawy et al., 2012) stated that TP level decrease in the digestive gland of *E. vermiculata* snails collected from two pollutant areas compared to snails collected from un pollutant areas. The decrease in TP level may result from the imbalanced rate of degradation with the rate synthesis of total protein in tissues of the body. (El-Gohary and Genena, 2011) agree with our result that the level of TP decreased in *M. cantiana* were treated with three different molluscicides. But disagree with our results in treating *E. vermiculata* with the same three different molluscicides. The level of TP significantly increased. (Shahawy, 2018) reported that the level of TP reduced in the tissues of land snails *H. vestalis* and *T. pisana* after exposure to two pesticides, Agrinate and Bio magic. Also, (El-Khayat et al., 2018) incorporated our observations, who cleared that the TP increase for *Biomphalaria Alexandrian* snails in the more pollutant lacks as an ecosystem than the corresponding snail inside high water quality lacks.

4.2. Histological alterations

The results obtained also revealed that many histological alterations in *monacha cartusiana* tissues, digestive gland, hermaphrodite gland, the foot, and the mantle under exposure to Copter with both sub-lethal concentrations LC_{20} (0.075 g/l) and LC_{40} (0.18 g/L) after 96 h of toxic exposure. The alterations in the digestive gland are shown as remarkable degeneration and rupture for

digestive and execratory cells and vacuolization and hemolytic infiltration. The histological changes in the digestive gland may be caused by enzymatic activity disturbances in different species (Heiba et al., 2002; Sharaf et al., 2015). Those observations are corporate with those of (Ali and Said, 2019; Peña et al., 2017) observed that the treated digestive gland showed rupture in the digestive envelope of tubules that disrupted columnar digestive cells and appeared without its content. (Hamlet et al., 2012) reported that *Helix aspersa* snail, when treated with thiamethoxam, observed digestive gland suffered from fragmentation the digestive cells with rupture for the outer epithelial layer, and degeneration of the digestive tubules in a dose-dependent manner, subsequently, at higher concentration, the tissue suffers from several deteriorations. This is in agreement with the results of (Attia et al., 2021), who reported that; weatfert, an inorganic fertilizer caused significant hyperplasia of the digestive gland of *Eobania vermiculata* snail, also having cell congestion and fat cell. Our results indicated that the hermaphrodite gland showed severe damage under copter effect with both concentrations; male and female gametes are observed degenerated within acini with deformed connective tissues surrounding them. These observations are in harmony with those of (Sharaf et al., 2015) reported that the hermaphrodite gland suffers from increased damage after treatments with diazinon and some acini deprived of Oocytes and male gamete inhibited from developed. Also, (Heiba et al., 2002) found that the lannate insecticide caused pathological alteration in gonads of both *E. vermiculata* and *M. cantiana* after treatment. It observed that inhibition of maturation male gamete and absence the female gamete inside the acini. Moreover, Copter with both concentrations affected the foot of *Monacha Cartusiana* snail where muscle fiber deformed, a rupture in the epidermis, necrosis of mucus gland, and vacuole formation. The obtained results are in agreement with previously published studies (Ali and Said, 2019; Cofone et al., 2020; Peña et al., 2017). They reported that after the treatment, snail foot pesticides suffered from similar changes like a rapture in epithelium with narrow and deeper folds containing undifferentiated cells, reduction in mucus glands, and the epidermal layer disintegrates disorganization of muscle fiber. Also, observed the histopathological alterations in mantle tissue after treatment with both concentrations; deformation in muscle fiber and enlargement and empty mucus gland cells containing vacuoles, rupture in the epidermis layer, and its cells disintegrated. These results are in harmony with those of (Ünlü et al., 2005) observed an increase in protein cells, disorganization in epithelial cells, concentrated in mucus cells when the mantle of *Galba truncatula* freshwater snail was exposed to thiodan. These same findings were observed by (Ali and Said, 2019) reported that rupture the outer epithelial layer in the mantle, empty the unicellular gland containing basophilic secretory material for *monacha obstructa* were exposed to UV-A.

5. Conclusions

The biochemical and histopathological changes observed in the present study cleared that methomyl (Copter 90%) with both sublethal concentrations LC_{20} (0.075 g/l) and LC_{40} (0.18 g/l) at different experimental periods (24, 48, and 72 h) have significant toxic effects on enzymatic activities and structure of the tissues for the *Monacha cartusiana* land snail.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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