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Impacts of the plant fertilizer, Caselio on the slug, *Deroceras reticulatum* (Gastropoda, Stylommatophora): laboratory studies.

Hoda H Abd-El Azeem, and Sherin K Sheir*.

Zoology Department, Faculty of Science, Menoufia University, Egypt.

ABSTRACT

The pest slugs have serious impacts on the world`s economy as a crop pests. So, the biological impacts of Caselio, the plant fertilizer (PMR) on the slug, *Deroceras reticulatum* were studied. Slugs were collected from infested places, El-Menoufia Province, Egypt. Slugs were exposed to 200, 500 and 800 µl/l of the fertilizer (PMR) for 14 days. Histology of the skin, foot, digestive gland, and intestine was investigated. In addition to cholinesterase in foot and lactate dehydrogenase activities in skin were measured. Histological alterations were observed in all investigated tissues/organs after exposure to all concentrations of PMR. Some of pathological signs were cellular degeneration, necrosis, inflammatory responses and leaky intestine (gaps). Exposure to PMR caused significant decrease in cholinesterase of slugs` foot than controls ($P = 0.01$) and significant increase lactate dehydrogenase in the skin of exposed slugs than the controls. In conclusion, *Deroceras reticulatum* were sensitive to PMR exposure and caused severe damage in the investigated tissues/organs and impacted the slugs' mobility and feeding. So, it can be used as a molluscicide for the slug, *Deroceras reticulatum*.

Keywords: *Deroceras reticulatum*, Caselio, histology, LDH and cholinesterase.

Corresponding author

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INTRODUCTION

The term slug is applied to molluscan animals that have no external shell. This species is a serious pest of global economic importance as it has adapted well to the varied environments around the world [1]. The field slug, *Deroceras reticulatum* (muller), is the most important pest species in a number of countries [1, 2]. It causes damage in arable crops such as winter wheat, oil seed rape, sugar beet, soybean and potato. It also attacks horticulture crops such as Strawberry, Brussels sprouts, Asparagus and Lettuce. In Egypt, the slugs are serious pests, where they cause damage to different agricultural crops in various governorates. They were recorded with high population density on the major economic crops; Egyptian clover, *Trifolium alexandrium*, cabbage, Brassica oleracea, lettuce, Lactuca sativa and guava, Psidium guajaava at Dakahlia and Giza governorate [3, 4 , 5].

Prevalent control strategies for slugs rely on methiocarb or metaldhyde pellets [6]. Chemical treatment are indiscriminate between species and can kill other species, especially natural slug predator and the control achieved with pellet bait has often been inadequate as result of poor treatment timing and pellet avoidance by slugs. These problems have introduced a sense of urgency into search for alternatives methods of pest management that are cheap and environmentally safe [7, 8].

Plant fertilizers are materials may be naturally in origin or synthetic. It is used to enhance the growth of the different parts of the plant. It is classified into main macronutrients Nitrogen (N): for leaf growth; Phosphorus (P): for roots, flowers, seeds, fruits development; Potassium (K): stem growth, movement of water in plants. The other three secondary macronutrients are calcium (Ca), magnesium (Mg) and sulphur (S). micronutrient such as copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Z), born (B), and of occasional significance there are silicon (Si), cobalt (Co), and vanadium (V) [9]. Fertilization may have adverse effects on the invertebrate species. Earthworm decreased in number with soil fertilization and disappeared at low soil pH where ammonium sulphate had been applied [10]. Beside the role of fertilizers in the growth of the plant it has a molluscidal effect. Sheir [11] found that Caselio (plant fertilizer) caused tissue damage in the digestive tubules as necrosis, fusion and increased lipofuscin pigment in the freshwater snail, *Lanistes carinatus*. El- Deeb et al., [12] exposed *Biomphalaria alexandrina* snails to the inorganic fertilizers (high phosphorus and high nitrogen content) and recorded significant decline in survivorship and egg laying capacity in addition to histological alterations in the digestive glands. Triebskorn [13] studied the effect of carbamate and metaldehyde molluscicides on the digestive tract of *Deroceras reticulatum*. He described that mucus deficiency in the digestive tract due to metaldehyde exposure. Dummee et al., [14] exposed the golden apple snail, *Pomacea canaliculata* to contaminated sediment with the metals (Fe, Mn, Cu and Zn) for two months. The results showed metals accumulation in the digestive tract and digestive gland higher than in the foot muscles. Yousef et al., [15] recorded significant increase in oxidative stress in males and females locusts, *Aiolopus thalassinus* collected from polluted areas with fertilizers industry.

Biochemical responses in organisms exposed to toxic contaminants have been used as biomarkers. Biomarkers measure the interaction between a biological system and an environmental agent, which might be chemical, physical, or biological [16]. In vivo inhibition or induction of biomarkers is a good environmental tool to assess the exposure and the effects of xenobiotics on organisms [17].

Acetylcholinesterase (AChE) is known as acetylhydrolase. It is an enzyme that catalyzes the breakdown of acetylcholine that function as neurotransmitters. Acetylcholine is the substance that the nervous system uses to activate skeletal muscles, a kind of striated muscle. These are the muscles used for all types of voluntary movement. AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type [18]. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides. Pessah and Sokolove [19] reported two types of cholinesterase in localized tissues of the terrestrial slug, Limax maximus, an acetylcholine-type enzyme (AChE, EC 3.1.1.7) occurs in the circumoesophageal ganglia and muscular foot of the animal. It exhibits novel properties, including appreciable activity towards propionyl esters, lower sensitivity to inhibition by the AChE inhibitor BW284C51. Acetylcholinesterase inhibition widely used in terrestrial and freshwater aquatic systems as an indicator of organophosphorus insecticides. Lundebye et al., [20] recorded that exposure of the crab, *Carcinus maenas* to four dimethoate treatments (0, 0.5, 1.0 or 2.0 mg/l) significantly reduced acetylcholinesterase activity in haemolymph samples. Coeurdassier et al., [21] found that Acetylcholinesterase (AChE) activity was strongly

decreased (80%) in the garden snail, *Helix aspersa* after exposure to organophosphorus pesticide, dimethoate at concentration 250 µg/g. Xuereb et al., [22] reported a relation between whole-body acetylcholinesterase (AChE) inhibition and changes in feeding and locomotor behaviors in the adult male, *Gammarus fossarum* during short-term exposure (96 h) to the organophosphorous pesticide, chlorpyrifos (CPE) and the carbamate pesticide, methomyl (MT). Significant mortality was observed from 50% AChE inhibition in MT exposure. The feeding rate and locomotion impairment were directly correlated to levels of AChE inhibition for both chemicals.

Lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes). Lactate dehydrogenase converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased and it converts NAD+ to NADH and back. It is released during tissue damage; it is a biomarker of common injuries and diseases [23]. LDH released from the foot mucosa of the slugs and the reduction in body weight can serve as a primary screening tool for the evaluation of the effect of various absorption enhancers and two β-blocking agents [24]. LDH used as biological marker to evaluate the biological effects of organophosphate pesticide chlorpyrifos in freshwater mussel, *Lamellidens marginalis* at concentration (5 ppm) of chlorpyrifos for 30 days. A significant increase in LDH activity in gill, hepatopancreas and muscles was observed [25].

The present study was designed to evaluate the adverse effects of Caselio, the plant fertilizer (PMR) on the slug, *Deroceras reticulatum* and in turn use it as a molluscicide. This evaluation can be done by measuring histopathological effects on skin; foot, digestive gland and intestine, as long as Cholinesterase in foot and Lactate dehydrogenase activities in skin.

MATERIALS AND METHODS

Animal:

The Pulmonate gastropod slugs used in the present study were *Deroceras reticulatum* (order: Stylomatophora, family: Limicidae). Slugs were collected by hand from the infested ornamental, fruit and grass plants from the ground surface and plant parts, El-Menoufia Province, Egypt. Collection was carried on early morning and late night where weather is cool and moist.

Stock culture:

Each 10-15 wild individuals (average weight 2 g each) were placed in glass containers measuring (15x15x22 cm) width, height and length, respectively. It was filled with moist soil (sandy loam) to 3 cm depth. The lid of each box is a shed material (muselin) for ventilation. Slugs were supplied with fresh lettuce leaves (Lactuca sativa) and sprayed water for soil moist daily. The remaining food and faecal matter were removed at the end of second day. The slugs were acclimatized under laboratory conditions with temperature 28 ± 2, relative humidity (RH) 85% and photoperiod 12 h light: 12 h dark at least for four weeks before being used in the experimental tests [5].

Experimental material:

Caselio is a plant metabolism regulator (PMR) in solution form contains (7% N, 20% P_2O_3, 4% Zn, 1.8% Fe, 1.3% Mn and 4.5% amino acids (w/v). It is a Product of international Egypt chem, registry license No. 2593.

Experimental design:

The concentrations used in this study were sublethal, 200, 500 and 800 µl/l of Caselio/PMR. 120 individuals of mature slugs were randomly divided into 4 groups, 3 replicates/group. The 1st group was kept as non-treated control, 2ndgroup was treated with Fertilizer (PMR, EC 200 µl/l), the 3rd group was treated with Fertilizer (PMR, EC 500 µl/l), and the 4th group was treated with Fertilizer (PMR, EC 800 µl/l). The application method was carried out by spraying, in which each 10 individuals were sprayed with 10 ml of their specific concentration. The exposure was done as intermittent (not continuous) design, where concentrations were

sprayed four days and stopped for the rest of the week (3 days) for two weeks (sprayed with water instead). Food was added as fresh lettuce leaves.

The assessed parameters were histology of skin, foot, digestive gland and intestine of slugs. In addition, cholinesterase and lactate dehydrogenase were analyzed in foot and skin, respectively.

Histology:

For the histological examination, three slugs were randomly selected from each concentration as long as the control group. The skin, foot, digestive gland and intestine were collected at the end of the experiment (14 days). Investigated tissues were dissected and fixed in Bouin's fluid immediately. After 24 h of fixation, specimens were dehydrated in alcohol (ascending series). Then specimens were cleared in xylene and embedded in melted paraplast at 60 °C. Serial sections were cut at 6 µm thickness for digestive gland, intestine, skin and foot, then stained with Ehrlich's Haematoxylin and counterstained by Eosin [26]. Sections were then mounted and covered with glass cover. Histological sections were photographed using Carl Ziess, Germany microscope with photo-automated digital camera.

Enzymes assay:

Cholinesterase (ChE):

Slugs` foot (0.08 g) was homogenized in 300 µl cold phosphate buffered saline (PBS), centrifuged at 4,000 rpm for 10 minutes at 4 °C. The supernatant was collected for the assay. ChE activity was determined according to King [27] using cholinesterase (Butyrylthiocholine. Kinetic kit, Spain). The unit of cholinesterase was expressed as the amount of enzyme that transforms 1µmol of 5,5-dithiobis-2-nitrobenzoic ac. (5,5 DTNB) per minute at 37°C and PH 7.7. Optical density (OD) was measured at 405 nm. The concentration was expressed in units per liter of samples (U/L).

Lactate Dehydrogenase (LDH):

Slugs` skin (0.08 g) was homogenized in 300 µl cold phosphate buffered saline (PBS), centrifuged at 4,000 rpm for 10 minutes at 4 °C. The supernatant was collected for the assay. LDH activity was determined according to Pesce [28] using LDH.LQ Kinetic kit (Pyruvate. Kinetic UV. DGKC. Liquid, Spain). The unit of lactate dehydrogenase was expressed as the mount of NADH per minute at 37°C and pH 7.7. Optical density (OD) was measured at 340 nm. The concentration is expressed in units per liter of samples (U/L).

Statistical analysis:

Enzymes data were analyzed using Statgraphics (v5.1 software). LC values were calculated using the plot of fitted model of simple regression. Data were expressed as mean ± SEM. Statistical analysis was carried by One-way ANOVA to set the difference between the control and treated groups, setting the probability level to $P \leq 0.05$. Where ANOVA could not be applied, Kruskal Wallis test was used.

RESULTS

PMR toxicity/molluscicidal effect and pathological signs

Molluscicidal/toxicity effect of the plant fertilizer, PMR against adult *D. reticulatum* slugs after 120 h of exposure was measured. The results showed that LC_{50} and LC_{90} values were 934 and 1300 µl/l, respectively with slope value 5.04 (Fig. 1). The control groups recorded no mortalities or pathological signs during the toxicity test or the experimental period. At the end of the experiment, thinning of the body and darkening of the skin of 20, 43 and 60 % for 200, 500 and 800 µl/l exposed slugs, respectively were recorded.

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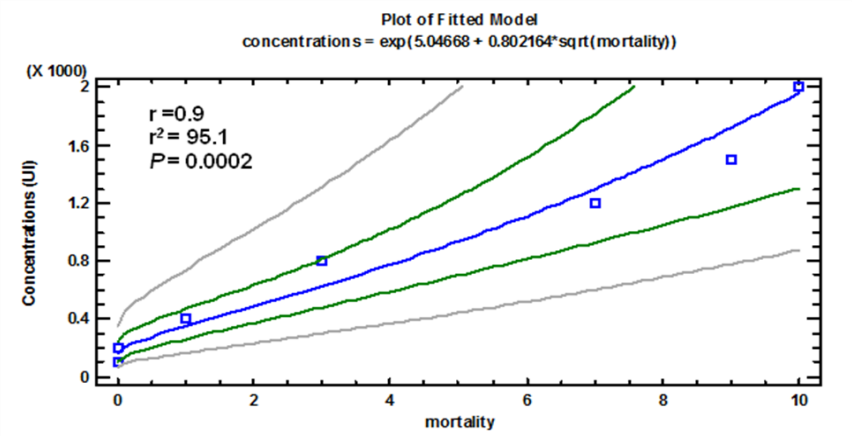

Fig 1: Simple regression representing concentrations of the fertilizer (PMR) versus slugs` mortality.

Histological alterations

Skin and foot

Skin is the outer layer of the slug`s body. Histologically, this area of skin is consisted of an outer layer of columnar epithelia, invaded by intercellular spaces, gland cells which rest on connective tissue. The later is connected to the muscle layer of the foot (Fig. 2a).

The effect of exposure to 200 µl/l PMR in slugs` skin and foot appeared as cellular degeneration in the epithelial layer which devoid of nuclei, detachment of connective tissue from the foot muscles and separation and formation of intercellular spaces between muscle fibers (Fig. 2b). Exposure to 500 µl/l of PMR caused appearance several vacuoles, intercellular spaces in the muscle layer and cellular degeneration in the epithelial layer without nuclei and covered by mucous secretion (Fig. 2c). The degenerative effect of 800 µl/l of PMR became more sever and appeared in the complete necrosis of the columnar epithelia and the underlying layer of connective tissue. Also, some cracking like areas in the muscle layer was observed (Fig. 2d).

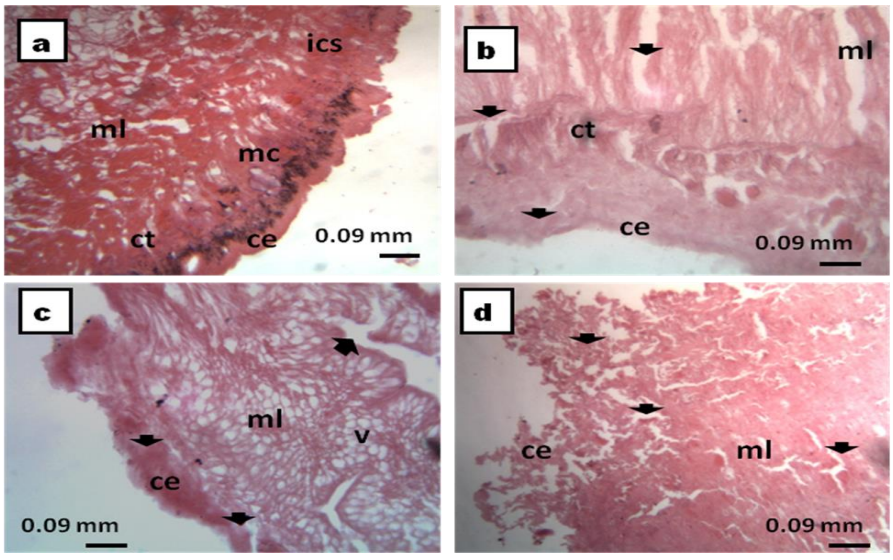

Fig. 2. Effect of PMR exposure on skin and foot. (a) control; (b) 200 µl/l; (c) 500 µl/l and (d) 800 µl/l. ct, connective tissue; ics, intercellular spaces; mc, mucous cell; ml, muscle layer; ce, columnar epithelia; solid arrows, degenerative tissue sites; and v, vacuoles.

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Digestive gland

The digestive gland is located in the visceral hump and is consisted of several closely compacted tubules, differs in shape and size and separated from each other by a thin layer of connective tissue. The tubule itself is surrounded by a layer of thin basement membrane. Each tubule is consisted of a layer of different types of epithelia surrounding a small lumen which differentiated into digestive cells, calcium cells and excretory cells (Fig. 3a).

Under the effect of 200 µl/l exposure, slugs` digestive gland showed severe degenerative histological architecture. There were no cellular boundaries inside the tubule and numerous vacuoles appeared (Fig. 3b). Exposure to 500 µl/l of PMR caused necrosis in the digestive tubules with continuous appearance of vacuoles (Fig. 3c). The degenerative effect of 800 µl/l of PMR became obvious, where the epithelia of the tubules turned completely necrotic with no lumen or clogged at some cases. Also, some inflammatory responses appeared in some areas as haemocytes infiltration (Fig. 3d).

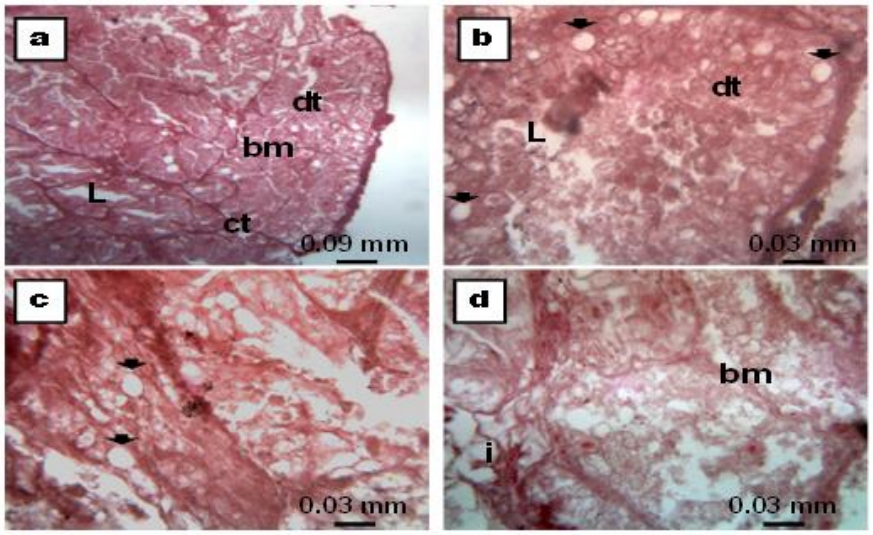

Fig 3: Effect of PMR exposure on digestive gland. (a) control; (b) 200 µl/l; (c) 500 µl/l and (d) 800 µl/l. dt, digestive tubule; bm, basement membrane; ct, connective tissue; L, lumen; I, inflammation arrows; and v, vacuoles.

Intestine

The intestine is surrounded externally by a layer of circular muscles which connected to the intestinal epithelia by loose connective tissue. It is composed of ciliated columnar epithelia which folds internally. In the intestinal epithelia, there are mucus and secretory cells (Fig.4a).

Exposure to 200 µl/l of PMR caused hyperplasia of columnar epithelial layer with disappearance of its columnar design. In addition, cilia distributions were irregular on the epithelial cells. Gaps between the intestinal folds and connective tissue were appeared (Fig.4b). After exposure to 500 µl/l of PMR, the height of the intestinal folds was severely shrunk when compared to the control or the former concentration. Epithelial layer of the intestinal folds became degenerative, sloughed of cilia and pathological change in overall shaping (Fig.4c). More degeneration of the shape and architecture of the cellular structure recorded after exposure to 800 µl/l of PMR, as thinner muscular layer than the control, necrotic epithelia with cilia abrasion (Fig.4d).

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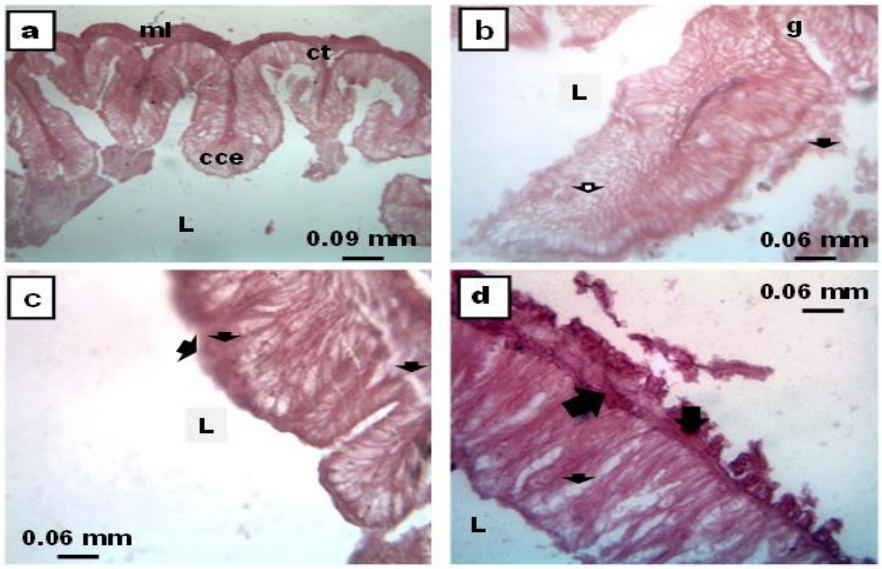

Fig 4: Effect of PMR exposure on intestine. (a) control; (b) 200 µl/l; (c) 500 µl/l and (d) 800 µl/l. ml, muscle layer; cce, ciliated collumnar epithelia; L, lumen; ct, connective tissue; hollow arrow, hyperplasia; solid arrow, degeneration; and g, gap.

Cholinesterase (ChE) activity

Cholinesterase activity in the control slugs' foot was 61.3 ± 3.8 U/mg tissue (Table 1). The activity of ChE was significantly inhibited after exposure to different concentrations of PMR (P = 0.01, Kruskal Wallis) when compared to the control and 200 µl/l of PMR. ChE activity in the slugs' foot recorded 23.2, 53.0 and 88.9 % reduction than the control levels for 200, 500 and 800 µl/l exposure to PMR, respectively (Fig. 5a).

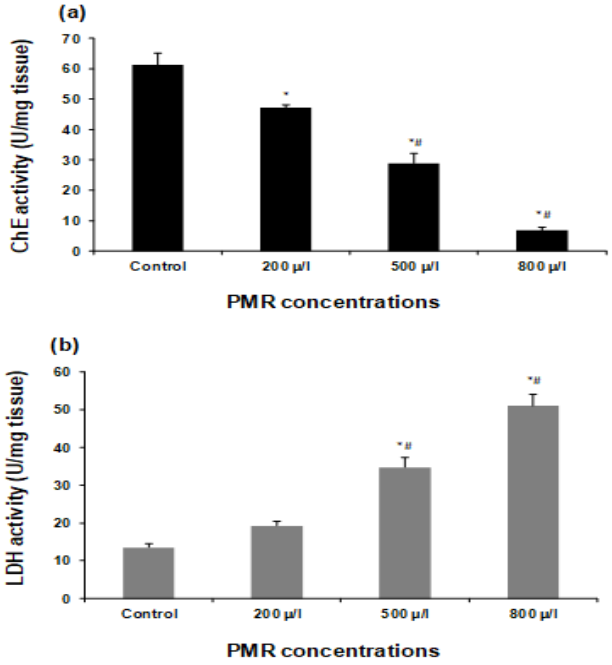

Fig 5: Effect of PMR exposure on (a) cholinesterase activity (U/mg tissue) in foot and (b) lactate dehydrogenase activity in skin (U/mg tissue) of the land slug, *Deroceras reticulatum*. # indicates a significant difference between treatments and 200 µl/l PMR for ChE and LDH, respectively (Kruskal-Wallis, P ≤ 0.05).

Lactate dehydrogenase (LDH) activity

Lactate dehydrogenase in the control slugs' skin was 13.5 ± 1.0 U/mg tissue (Table 1). The activity of LDH was significantly increased (P = 0.01, Kruskal Wallis) after exposure to different concentrations of PMR. LDH activity in the slugs' skin recorded 1.4, 2.6 and 3.8 folds of increase than the control levels for 200, 500 and 800 µl/l exposure to PMR, respectively (Fig. 5b).

Table 1: Effect of PMR exposure on cholinesterase activity in foot and lactate dehydrogenase activity in skin of the land slug, *Deroceras reticulatum*

Groups	Mean		% reduction	fold of increase
	ChE (U/L)	LDH (U/L)	ChE	LDH
control	61.3 ± 3.8	13.5 ± 1.0	-	-
200 µ/l	47.1 ± 0.9*	19.3 ± 1.3	-23.2	1.4
500 µ/l	28.8 ± 3.3*#	34.7 ± 2.6*#	-53.0	2.6
800 µ/l	6.8 ± 1.0*#	50.9 ± 3.1 *#	-88.9	3.8

Note, n = 3 slugs per group. Data are means ± SEM, in U/mg tissue, * indicates a significant difference from the control, # indicates a significant difference between treatments and 200 µ/l PMR for ChE and LDH, respectively (Kruskal-Wallis, P ≤ 0.05).

DISCUSSION

The present study recorded interesting results about the responses of the slug, *Deroceras reticulatum* under the effect of the plant fertilizer (Caselio). The slug showed increase in mucous secretion as a first line of defense against that chemical substance. Then, under sever conditions (high concentration exposure) it reacted differently by increased muscle contraction, which approved by inhibition of ChE activity. LDH increased activity in the skin indicated cellular damage of the slugs as a response to the fertilizer. Histopathological observations of different organs can stand with the other physiological (ChE and LDH) disturbance caused by this chemical fertilizer.

The present results recorded molluscicidal effect of the fertilizer (PMR) on the slug, *Deroceras reticulatum*. Some of the obvious symptoms were that the body became thinner in shape and darker in color. It may be due dehydration or contraction of overall body muscles. It is well known that terrestrial gastropods face severe problems of water conservation due to using it for keeping their skin wet, and use of mucus in locomotion [29]. Consequently, terrestrial gastropods have evolved a variety of structural and behavioral adaptations to reduce the risk of dehydration. Selvi et al., [30] described that body fluid loss in the slug, Laevicaulis alte Férussac was 37%–62% after exposure to silica synthesized from rice husk ash (rha) coated with leaf extracts of *Azadirachta indica*, *Pongamia pinnata* (L.), *Nicotiana tabacum* (L.) and *Calotropis procera* (L.). Triebskorn [13] discussed deficiency of mucous production as a result of metaldehyde attack to the immature mucous cells in *D. reticulatum*. Increased mucus secretion is one of the first reactions of slugs to mechanical or chemical irritation caused by molluscicidal poisoning [31]. So, the intensified mucus secretion after exposure leads to an increased loss of liquid. The other explanation of body thinning may be due to the decrease of AChE activity. Acetylcholinesterase (AChE) functions as a neurotransmitter to activate skeletal muscles, which is a kind of striated muscle used for all types of voluntary movement. Several studies reported the inhibition of Acetylcholinesterase (AChE) after the exposure to different pesticides [21, 22, 32].

The results recorded cellular degeneration in the epithelial layer of slugs` skin and foot. The mantle is considered as an important organ for respiration in pulmonate gastropods [33]. Gupta and Durve [34] showed similar histopathological changes in the mantle of the freshwater snail, *Viviparus bengalensis.* Jonnalagadda and Rao [35] showed disorganization of the mantle tissues and swelling of the foot epithelium when exposed to endosulfan (35%) and other pesticides on the freshwater snail, *Bellamya dissimilis.* Ünlü et al., [36] recorded histopathological alterations of the freshwater snail, Lymnaea stagnalis after exposure to 0.36 % and 0.72 % endosulfan, for 96 such as irreversible necrotic changes occurred in the digestive gland. Degenerative changes in the muscle fibers, proteins and pigment cells of the foot and the connective tissue of the mantle.

The digestive gland (also known as hepatopancreas and liver) of gastropods is the largest organ in their body [37]. The present study recorded extensive vacuolization in the cells; this is confirmed by the investigations of Hamed et al., [38] who found severe vacuolization in digestive cells of Eobania vermiculata treated with molluscicidal carbamates; methiocarb and methomyl. They explained that by the interaction of the lipophilic properties of the molluscicide with cellular membranes as a result of direct contact of toxin and cell surfaces. In addition, this interaction might induce changes in composition, fluidity and stability of membranes by the toxin or its metabolite [39]. The current results recorded degenerative range of epithelia reached to necrotic stage. This is supported by Triebskorn and Künast [40] who reported that carbamate molluscicides caused gaps between the basal parts of the epithelial cells, the underlying connective and muscle tissues in the digestive gland of the land snail, Monacha obstructa treated with methomyl. Similar findings were reported in the land snail, *Eobania vermiculata* exposed to methiocarb [38]. Triebskorn [41] also reported degenerative effects in the hepatopancreatic cells of the slug, *Deroceras reticulatum* exposed to metaldehyde. In present study, necrosis in the lining epithelial cells in some digestive tubules was observed. Triebskorn et al., [42] reported that, necrosis was observed in the skin of the slug, D. reticulatum after oral or dermal application of metaldehyde. The necrotic effect is associated with oxidative stress after exposure and accompanied by the role of the lysosomal compartments. The lysosomal compartments are essential for cellular functions including the normal turnover of most long-lived proteins and all organelles [43]. Oxidative stress during apoptosis and lysosomal involvement in apoptosis due to stress is recognized [44, 45]. The oxidative stress determines the degree of lysosomal destabilization and consequences of events from the arrested growth, reparative autophagy, and apoptosis evolved to necrosis. Moderate lysosomal rupture induces apoptosis, while pronounced lysosomal leakage results in necrosis [46].

The intestine showed some histological alterations after exposure to the investigated concentrations of PMR as appeared in cellular degeneration, necrosis, inflammatory responses and leaky intestine (gaps). This was supported by Triebskorn [13] who described ulterastructurally changes of digestive tract of *Deroceras reticulatum* as the destruction of Golgi cisternae, rER, mitochondria and finally cell death (necrosis) are involved after metaldehyde and carbamate ingestion. Metaldehyde enhances destruction of the cellular secretory apparatus; especially in immature cells which resulting the desiccation of the animal. Dummee et al., [14] demonstrated histopathological alterations in the digestive tracts of golden apple snail, *Pomacea. canaliculata* including a loss of cilia and an increase in the number of mucous cells in P. canaliculata exposed to contaminated sediment by (Cr, Fe, Cu and Zn).

The inhibition of AchE activity in the present work under the effect of chemical substances was detected in several researches. Inhibition in AchE is a consequence of organophosphorous compounds exposure [21]. In addition, Riberaa et al., [48] mentioned the decrease in energy levels (cytochrome-P450-dependent and NADPH Red) in the earthworm Eisenia fetida andrei exposed to contaminated artificial soil with carbaryl. In addition, inhibition in glucose metabolism, O_2 consumption and glycogen levels in the digestive gland of the freshwater snails, B. alexandrina and Bolinus truncates was recorded after exposure to organophosphorus insecticides [47]. This kind of mode of action can lead to a decrease in the activities of AchE with/without interfere with its production and transfer to the target organs. The induction by low doses and inhibition by high doses of chemicals can be a pattern of some enzymes but not for all, and depends on the pollutants type, organism species and metabolic reactions.

The current data recorded significant increase in the activity of LDH. LDH was choosen as a biomarker of the oxidative stress induced by the tested material (fertilizer, PMR). Increased oxidative stress biomarkers as lipid peroxidase in fertilizers polluted areas was documented by Yousef et al., [15]. Salama et al., [49] recorded significant increase of the level of LDH in the land snail, *Helix aspersa* after exposure to several chemicals such as methomyl, carbofuran and chlorpyrifos. The increased release of LDH in mantle tissue is an indicative of cellular or membrane damage. The increasing demands of organisms to energy during stress to detoxify, bio-transform and excrete the toxicants is achieved by the use of carbohydrate as the principal and immediate energy source [50]. This can be accompanied by elevated level of LHD as result to the role of LDH in converting the pyruvate, the final product of glycolysis to lactae. The enhancement of LDH activity was recorded in other studies; El Gohary et al., [51] who used the commercial molluscides baits, Gastrotox, mlotov and mesurol against the two land snail *M. cantiana* and *E. vermiculata*; and Abd- El- All [52] who exposed *E. vermiculata* to niclosamid.

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CONCLUSION

Caselio exposure caused severe damage in the investigated tissues/organs and impaired the slugs' mobility and feeding by interfering with foot contraction/relaxation processes and flattening and gaping intestinal epithelia. So, Caselio can be used as a molluscicide against the slug, *Deroceras reticulatum* infested areas.

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