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# Histopathological effects of methomyl and crude extracts of *Jatropha curcas* against the terrestrial snail, *Monacha obstructa* (Gastropoda:Hygromiidae)

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## Abstract

**Background:** The negative effect of over-reliance on the use of synthetic pesticides have led to the search for natural alternatives to pest control. This study was designed to evaluate the toxicity effect of the crude plant extracts of *Jatropha* (*Jatropha curcas* Linn.) seeds in comparison with methomyl on some histopathological changes of the terrestrial snail species *Monacha obstructa* (Pfeiffer, 1842). The toxicity effect of methomyl and crude extracts of ethanol and acetone extracts of *Jatropha curcas* seeds were determined on some histological changes of the land snail, *Monacha obstructa* at four concentrations using the contact technique under laboratory conditions.

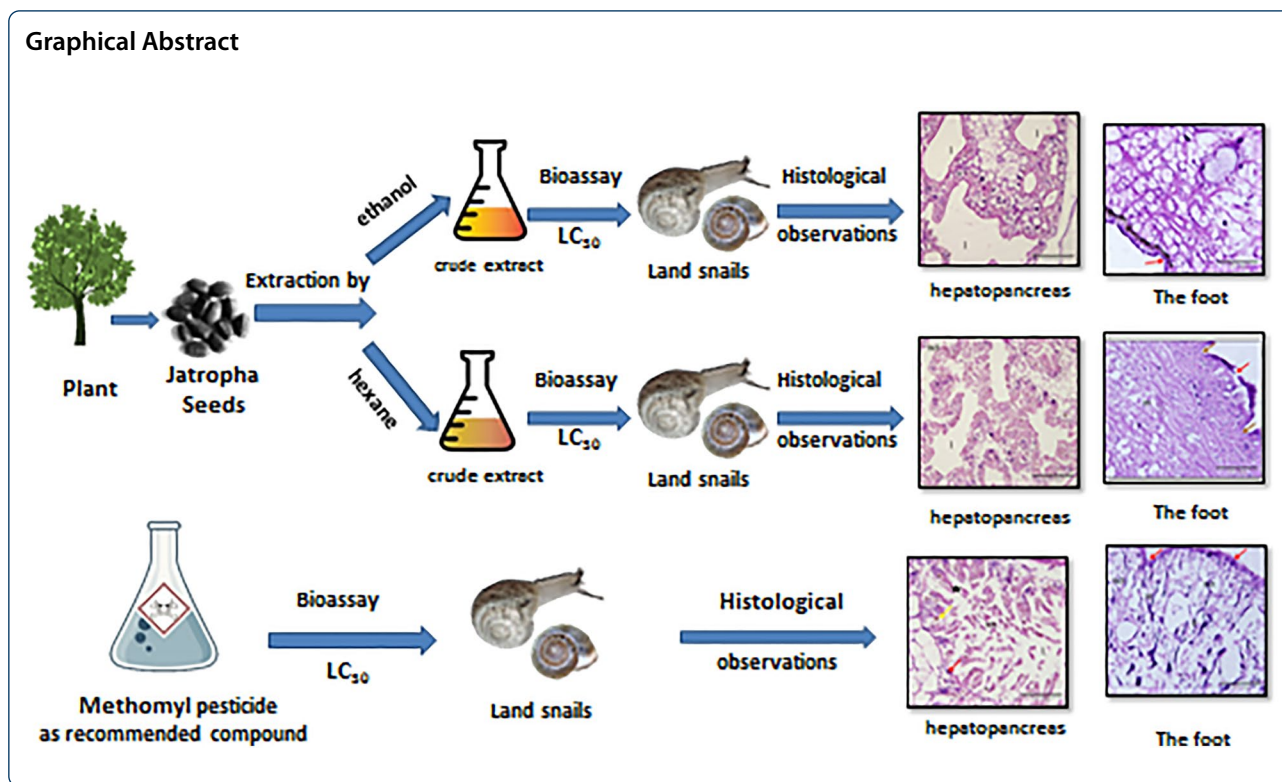
**Results:** The results revealed that the crude extracts of *Jatropha* exhibited obvious adequate effects compared with methomyl against *M. obstructa* land snails. The highest toxicity effects were obtained by methomyl followed by the ethanolic extract of *Jatropha* and hexane extract of *Jatropha*. The histopathological effect of LC<sub>50</sub> of *Jatropha* crude extract and methomyl on the digestive gland and foot tissues were examined in the land snails *M. obstructa*. The treated snails showed several histological changes in the digestive gland and foot compared with the control group snails. The histological examinations of the digestive tubules included destroyed and detached in the outer layer covering digestive tubules. In addition, marked increase in the width most of the digestive tubules lumen. The intertubular connective tissue between the digestive tubules showed great destruction, while the foot of treated snails showed rupture of the epithelium covering the foot and desquamation of the epithelium. We also observed the presence of areas of connective tissue necrosis and destruction of the muscular tissue.

**Conclusions:** It has been found that extracts of *Jatropha* seeds have a toxic activity that caused histopathological damage that led to the death of land snails, and thus it can be recommended as a source for the development of molluscicides.

**Keywords:** *Monacha obstructa*, *Jatropha curcas*, Histopathological effects, Methomyl, Terrestrial snail

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## Background

Terrestrial molluscs including snails and slugs belonging to class Gastropoda, are increased greatly in their economic importance value, whereas some gastropods became serious pests in many parts of the world. From the important species, the herbivorous land snail species which are considered one of the most serious pests of many crops and vegetables causing heavy economic damages as a result of feeding the plant's leaves, roots, and fruits [1]. Economic damage caused by these pests is due to not only feeding but also to contamination with their feces, bodies or slime leading to deterioration of their qualities and financial loss [2, 3]. In Egypt, the terrestrial snails especially the glassy clover snail, *Monacha obstructa* attacking many agronomic, horticulture and ornamental plants causing serious economic damage represented in nibbling the margins the leaves of the plants on which they feed and in some cases they bore into other parts as the roots, tubers and fruits [4, 5]. Control of snails on different crops is heavily dependent on the use of pesticides, which cause environmental contamination [6]. Synthetic chemical molluscicides had a toxic effect on non-target organisms, where they contaminate soil and water and may consequently affect local populations of humans and other animals [7]. The carbamate compounds appeared to have a high toxic effect

on the land snails *Eobania vermiculata* and *Monacha cantiana* under laboratory and field conditions. Among carbamates, methomyl had the highest molluscicidal activity [8, 9]. Several researchers reported that methomyl (The compound recommended by the Egyptian Ministry of Agriculture against land snails) was the most potent compound in reducing the population density of land snails [10–14]. The high cost of synthetic molluscicides and their negative impacts on the environment, as well as fear of emergence of snail resistance to these compounds have given a new impetus to the study of molluscicidal plants [15]. Thereby, using natural molluscicides for snail control is considered to be the most pragmatic approach [2]. Several countries have promoted the use of plant products due to their wide range of ideal properties, such as high target toxicity, low mammalian toxicity, low cost, solubility in water, easy biodegradability, abundant growth in endemic areas and operator safety [16]. *Jatropha curcas* and *Taxodium distichum* stand out as promising means for biofertilizer function and manage pests and diseases that affect agriculture output [17].

*Jatropha* seeds contain numerous components, such as cuminaldehyde, thymol,  $\gamma$ -terpinene, phytic acid, toxic compounds known as phorbol esters, and a high protein content, all of which have a significant activity, such as potent cytotoxic, molluscicidal, insecticidal,

and fungicidal properties [18, 19]. The aim of this work is to evaluate the toxic effect of ethanolic and hexane crude extracts of *Jatropha curcas* seeds and methomyl on some histopathological changes of the adult snails of *M. obstructa*.

## Materials and methods

### Collecting and rearing animals

Live adult individuals of the glassy clover snail, *M. obstructa* were collected in early morning from heavily infested fields cultivated with Egyptian clover located in El-Wasta village (27°10'54.6"N, 31°12'58.2"E), El-Fath district at Assiut governorate during spring season (April, 2020) and transferred in plastic bags to the laboratory of Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al-Azhar University Assiut Branch. Then, they were kept in glass boxes (70 cm long × 40 cm wide and 40 cm deep) containing moistened soil and covered with insect netting to provide ventilation and secured with a rubber band to prevent snails from escaping and fed on fresh leaves of lettuce *Lactuca sativa* L. for 2 weeks for acclimatization to retain only healthy individuals [20] and water was sprayed on the soil layer daily to provide suitable humidity for snail activity. The snails were selected for each treatment.

### Preparation of *Jatropha curcas* crude extracts

The part used for the extraction was dried seeds as the physic nut of *J. curcas* (family: Euphorbiaceae). The seeds were extracted according to the method of Freedman et al. [21] with a little modification (where tested samples were soaked in the chosen solvent instead of using soxhlet procedure as mentioned by Mourad [22]). Dried seeds that were used in the experiments were shade dried grounded into fine powder using an electrical grinder, 200 gm from seeds powder was extracted with two solvents varied in polarity hexane followed by ethanol 95% by soaking in solvent and allowing to stand for 3 days. The produced extracts were filtered using filter paper, concentrated using a rotary evaporator and the remainder crude extracts were kept in the refrigerator until use. The obtained plant extracts were diluted with distilled water. Tween 80 was added for emulsified before using. Four concentrations (0.125, 0.25, 0.5 and 1%) of crude plant extract were used to determine LC<sub>50</sub> median lethal concentrations values of the plant extract.

### Contact toxicity test

Molluscicidal potential of the crude plant extracts compared with methomyl (S-methyl-N-[(methylcarbamoyl)oxy] thioacetimide) were tested by preparing a series of concentrations for their contact toxicity using the treated

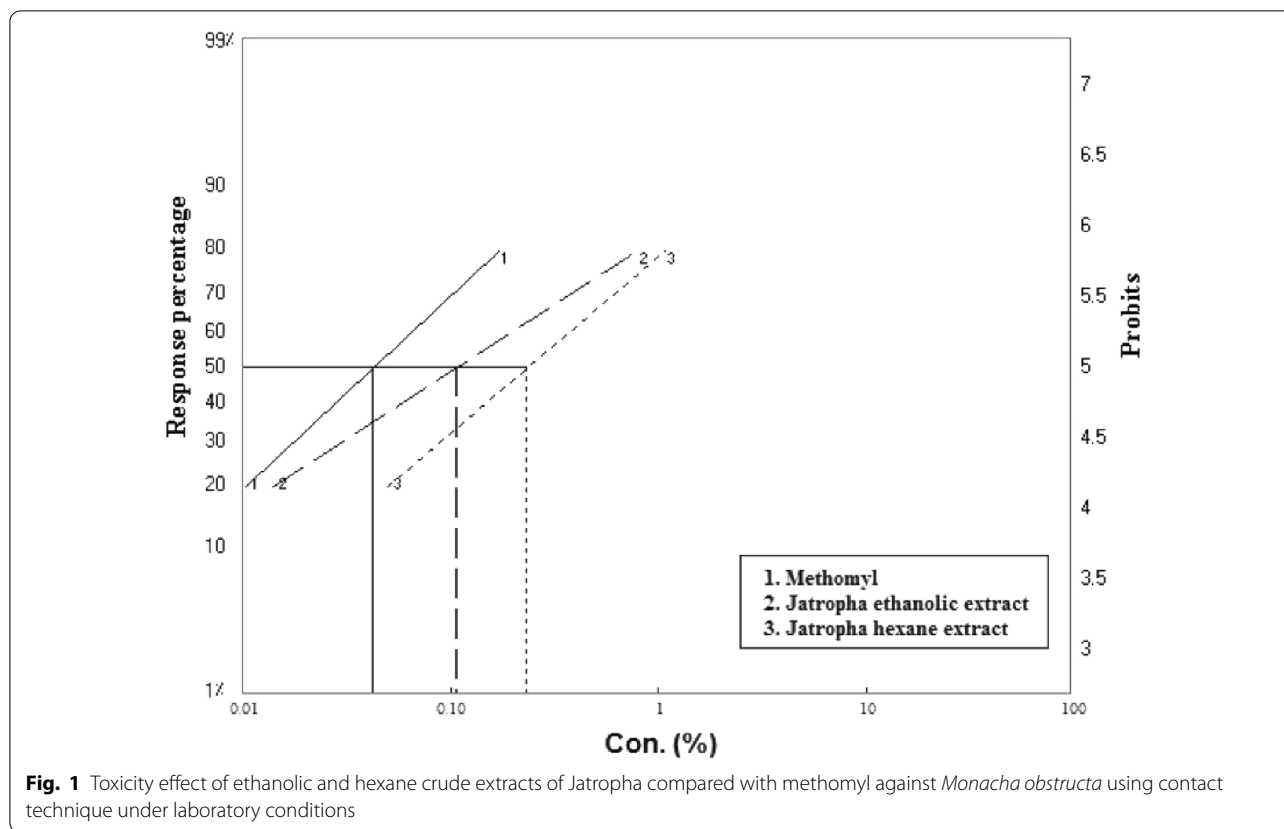
surface exposure method which was described by 2 ml of each concentration was deposited and distributed on the bottom of a petri dish then moving the dish gently in circles. Water was evaporated under room conditions in a few minutes leaving a thin layer film of the applied concentration of the tested plant extracts. Five healthy adult snails of the tested species were placed and exposed to the candidate concentration of the tested extract for 72 h [22], then transferred to another plastic box (10 cm diameter), closed with muslin cloth containing optimal soil (3–5 cm) and provided with fresh lettuce leaves. Three replicates were used for each treatment in addition to untreated check. Dead snails were counted daily for 7 days. Probit analysis was done to determine the median lethal concentrations (LC<sub>50</sub>) values, as well as slope value of LCP lines for the used materials using LdP Line software.

### Histological studies of snails

The histological studies were carried out on the specimens of the adult snails. The adult snails were treated with LC<sub>50</sub> values of the ethanolic and hexane extracts of *Jatropha* and methomyl compound to determine their effect on some histological changes in the digestive gland (hepatopancreas) and the foot of these snails. Our histological studies were designed as five groups, the first group of snails was the control one without any treatment. The second and the third groups of snails were treated with ethanolic and hexane extracts of *Jatropha*, respectively. The fourth group was treated with methomyl as recommended pesticide. The fifth group of snails was treated with tween 80 and distilled water. Specimens from the snails which were treated with previously mentioned extracts of *Jatropha*, methomyl and tween were fixed after 24 h from the first treatment.

### Tissues preparation and examinations

To examine the histological soft parts of *M. obstructa*, the snails' tissues were prepared for histological tests after 24 h exposure. The soft tissue of the control group as well as treated ones of *M. obstructa* was dissected carefully from the outer shell. The digestive gland and the foot were immediately immersed and fixed in 10% formalin for 24 h. The fixed specimens were dehydrated in ascending grades of ethyl alcohol, then cleared with methyl benzoate, and embedded in paraffin wax. Samples were cut at 4–6 μm thickness and stained with Harris haematoxylin and eosin [23]. The sections were examined for the histological and histopathological changes using an OLYMPUS BX51 microscope and photographed with an OLYMPUS DP72 camera adapted to the microscope (Department of Anatomy and Embryology, Assiut University).



## Results

The toxicity effect of both ethanolic and hexane crude extracts of *Jatropha* seeds and methomyl compound on the land snails *M. obstructa* when applied using the contact method was determined under laboratory conditions. The ethanolic and hexane *Jatropha* seed extracts exhibited adequate results compared with methomyl against the tested land snail species (Fig. 1). The highest effectiveness was obtained by methomyl followed by the ethanolic extract of *Jatropha* and hexane extract of *Jatropha* with  $LC_{50}$  values of (0.05%, 0.11% and 0.23%), respectively.

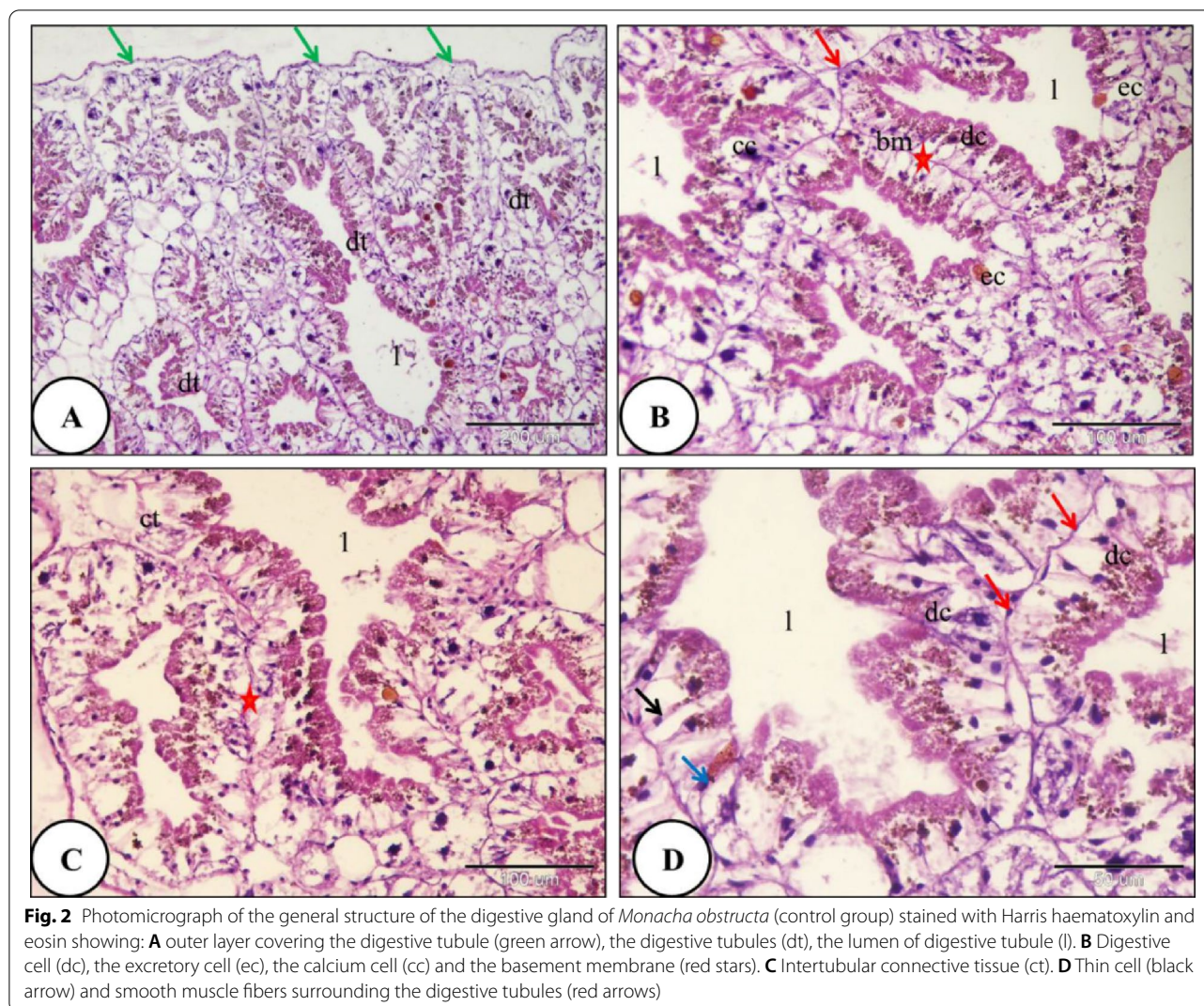
### Histological studies of the digestive gland (hepatopancreas) of the land snail *Monacha obstructa*

The histological studies in control group showed that the digestive gland of land snail *M. obstructa* is composed of digestive tubules. The digestive tubules are separated with connective tissue fibers. The shape of these tubules are variable, either oval or spherical. Each digestive tubule is lined with different types of cells. The first predominant cells type were the digestive cells which characterized are simple columnar. The digestive cells characterized by vacuolated cytoplasm and the rounded

and basally located nucleus. The second cells type are the excretory cells. The excretory cells usually contain large vacuole surrounded by thin layer of cytoplasm. The excretory cells are small in number when compared with the digestive cells. The third type is the calcium cells. The frequency of these type of cells are smaller than digestive cells. The calcium cells are pyramidal in shape and have large nucleus which is rounded. The fourth type of cells which had been observed were the thin cells. The thin cells are randomly distributed in between the other cell types (Fig. 2).

On the other hand, the digestive gland of *M. obstructa* treated group with  $LC_{50}$  of extract of ethanolic *Jatropha*. After 24 h exposure, the digestive tubules represented moderate changes in digestive tubules were observed. The apical part of the digestive cells was detached and formed blebs inside the lumen. The lumen of digestive tubules showed marked increased in width and some were some filled with secretory materials. Great destruction of the intertubular connective tissue between the digestive tubules has been observed (Fig. 3).

While the digestive gland of *M. obstructa* treated group with  $LC_{50}$  of hexane extract of *Jatropha* some tubules were as that treated with  $LC_{50}$  of extract of ethanolic



Jatropha but some digestive tubules showed great destruction (Fig. 4).

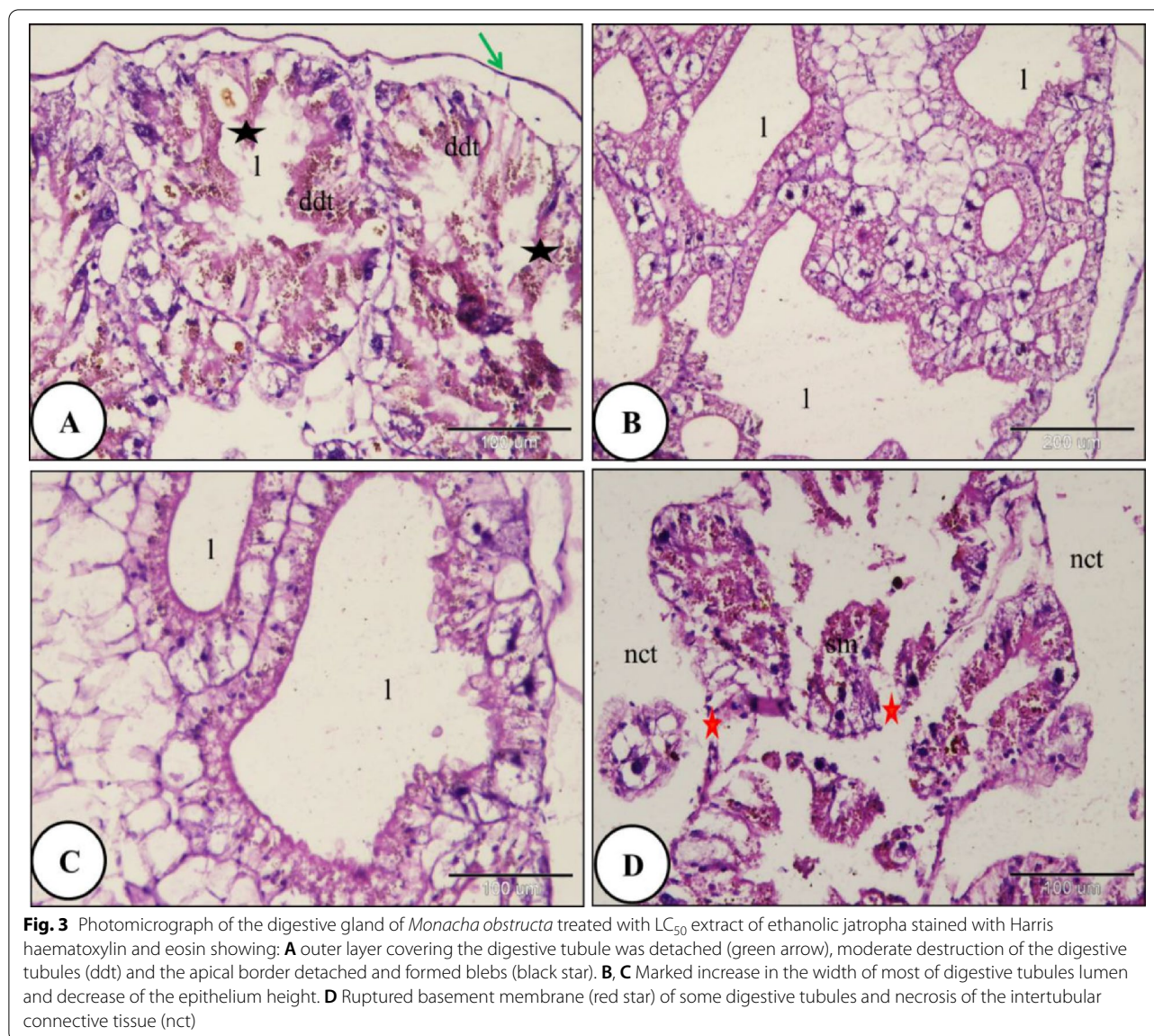
In comparison, it has been found that the digestive gland that treated with  $LC_{50}$  of methomyl. After 24 h exposure, the outer layer covering digestive tubules was detached and destroyed. The digestive tubules showed breakdown, vacuolization and increased number of large granules. Some of calcium cells showed pyknotic nuclei. There is also increase in the number and size of excretory granules in the excretory vacuoles of excretory cells (Fig. 5).

#### Histological studies of the foot of the land snail *Monacha obstructa*

In control group, the foot, the histological observation revealed that the foot of *M. obstructa* represented an external single layer of epithelial tissue that covers an

internal layer of connective tissue. The foot is covered with pseudostratified columnar epithelium. The foot had two parts the sole and sides. The connective tissue layer beneath the epithelial layer contains many glands. Regarding to the glands of the foot, at the sole are greater in number and deeply embedded within the connective tissue as well as in the muscular layer than those at the sides which are lesser in number and superficially located (Fig. 6).

concerning, the foot of *M. obstructa* treated with  $LC_{50}$  of extract of ethanolic Jatropha showed ruptured epithelial layer of the foot sole, and the folds became more deep with undifferentiated epithelial cells also the folds of the sides of the food became deeper and the epithelial layer that covers the sides became ruptured, and the connective tissue are wider (Fig. 7). While, the foot of *M. obstructa* treated with  $LC_{50}$  hexane extract of Jatropha



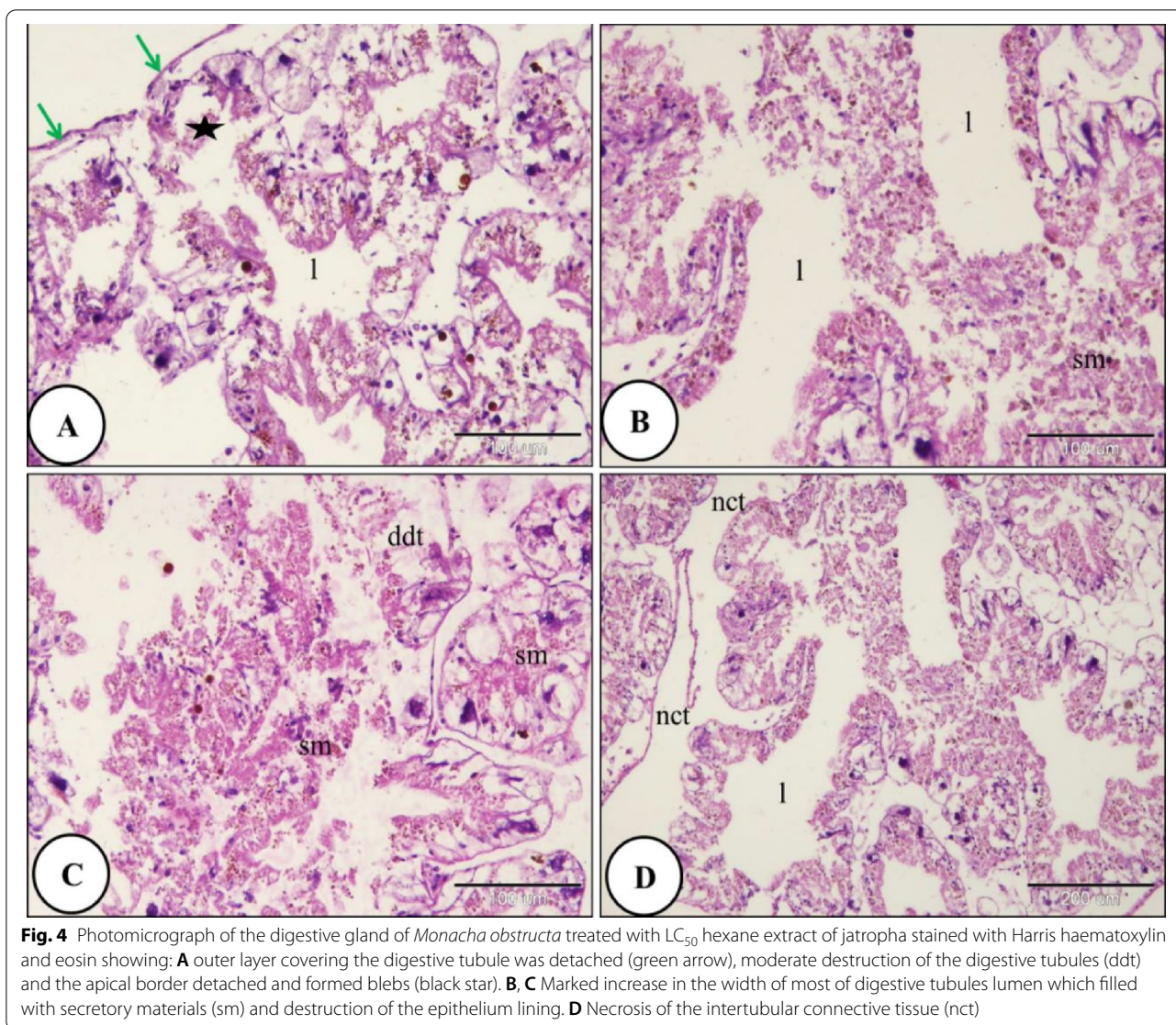
showed rupture of the epithelium covering the foot, desquamation of the epithelium and presence of areas of connective tissue necrosis and destruction of the muscular tissue (Fig. 8).

This study revealed that the foot of *M. obstructa* treated with LC<sub>50</sub> of methomyl after 24 h exposure, showed rupture of the epithelium covering the foot and presence of dark brown pigment within the epithelial covering and connective tissue. The foot sides showed intensive lysis and necrosis of the connective tissue. Moreover, there were focal necrosis of the gland and the muscular tissue underneath the epithelium (Fig. 9). In addition, it was found that the digestive gland and the foot of snails treated with tween 80 and distilled water nearly showed

the same histological structure of the untreated ones (Figs. 10, 11).

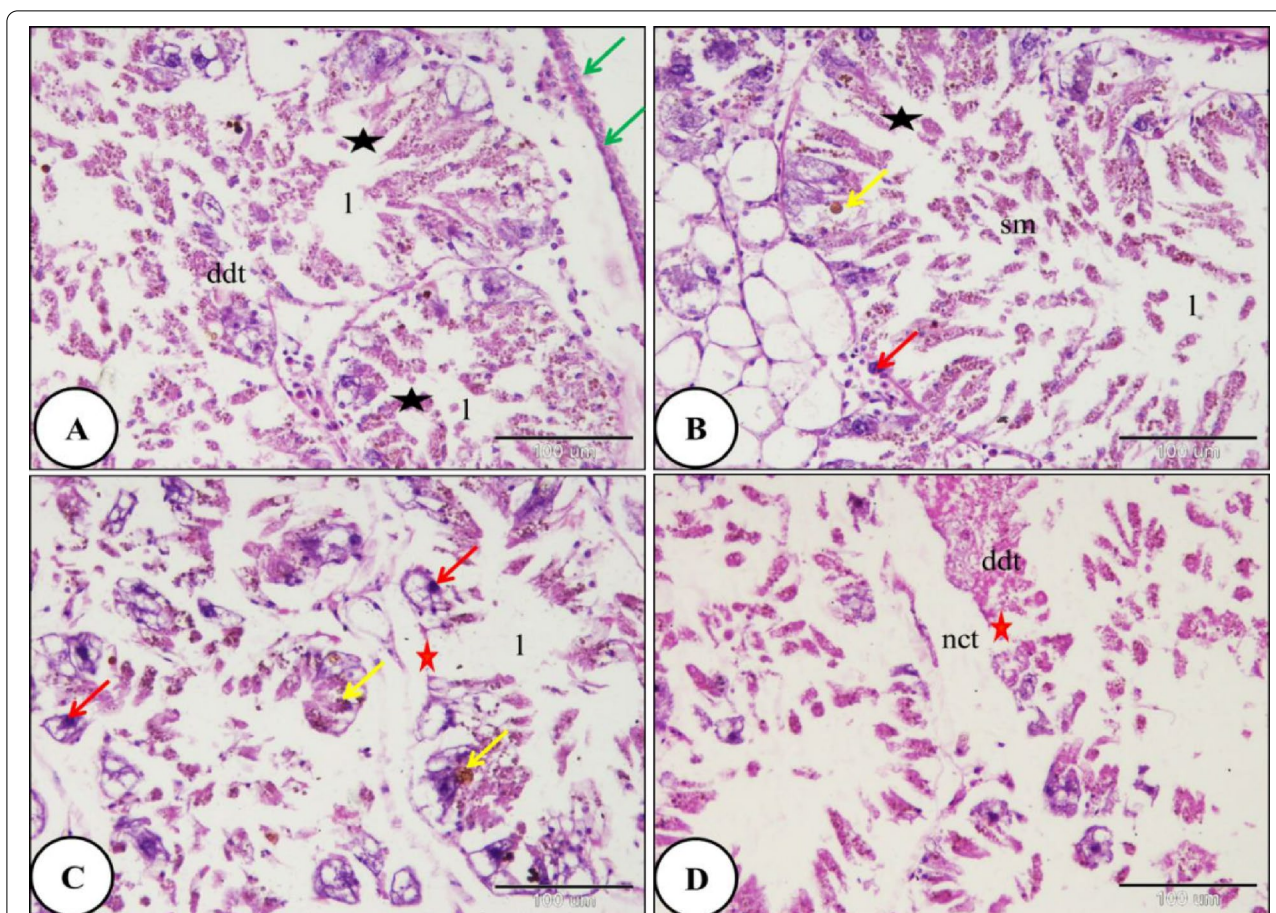
### Discussion

The results of this study can be summarized that the tested jatropha extracts are toxic to the harmful land snail *M. obstructa* compared to methomyl and the histopathological alterations caused were proportionate to the potential of the tested extracts. Some plant parts are toxic and their extracts studied and found that they possess biological activity to the molluscs and recorded positive results for controlling them. Thus, Jatropha seed extracts have clear effects, as it was observed that the



activity and movement of the treated snails were inhibited, after the first day of treatment, and this is explained by the histological changes of the treated snails compared to the control group. Molluscicide activity is prevalent in Euphorbiaceae family, although the activity varies from one species to another and even between different parts of the same plant, as *Euphorbia royleana* [24]. The previous studies have shown that *Euphorbia helioscopia* has a toxic molluscicide effect [25, 26], they mentioned that toxic pellets formulated from roots, stems, leaves or flowers of *E. helioscopia* showed molluscicide activity against both tested molluscs, *Theba pisana* and *Arion hortensis* and especially stems and leaves were found to have potential molluscicide properties against *T. pisana* and *A. hortensis*.

Regarding the histological structure of digestive gland of *M. obstructa* of control group. Ali [27] and Ali and Said [28] stated that digestive gland of unexposed snails consists of many tubules, each of them lined with four types of epithelial cells, digestive cells (elongated cells with many small granules), calcium cells (triangular in shape with large basic nucleus), excretory cells (elongated cells with large vacuole) and thin cells (very thin and long cells). Digestive tubules are separated by intertubular connective tissue and surrounded by a thin layer of circular muscle fibers. Hamed et al. [29] observed that thin cells were hardly identified in histological sections and are considered undifferentiated precursors of the other cell types, digestive cells; excretory cells and calcium cells (in *Agriolimax*) or secretory cells (in *Lymnaea*).



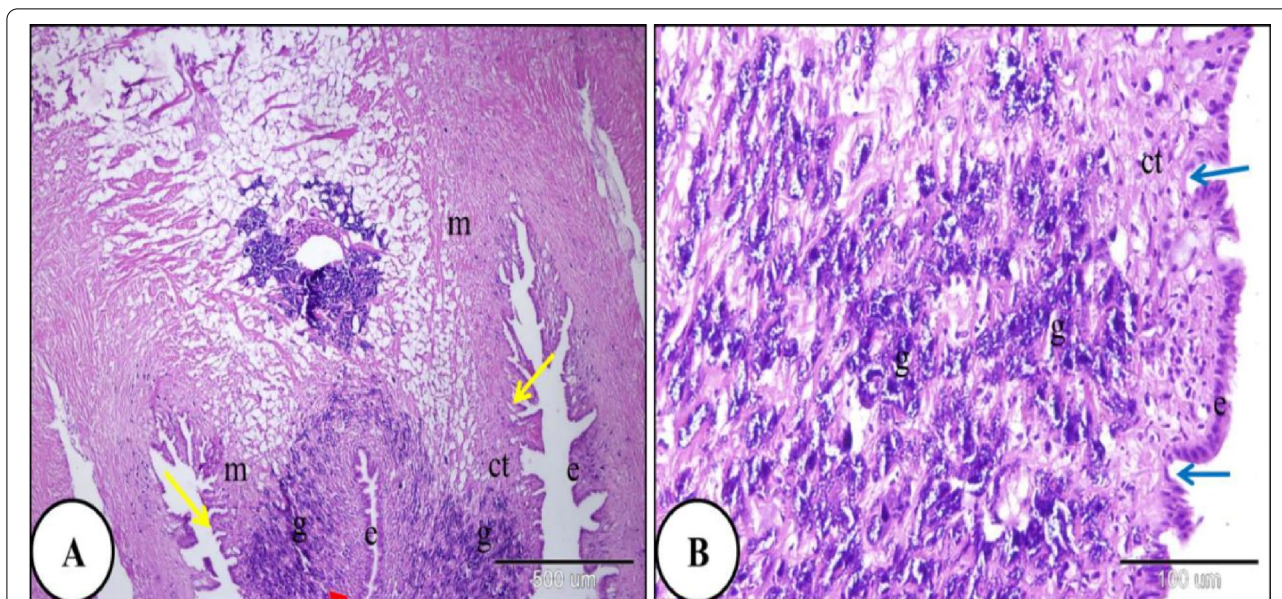
**Fig. 5** Photomicrograph of the digestive gland of *Monacha obstructa* with  $LC_{50}$  of methomyl stained with Harris haematoxylin and eosin showing: **A** outer layer covering the digestive tubule was detached (green arrow), the digestive tubules showed breakdown (ddt), the lumen of digestive tubule (l). **B** Detachment of apical border of the epithelium lining digestive tubules forming blebs (black star) and the lumen filled with secretory materials (sm). **C** Excretory cells had large brown granules (yellow arrow) and pyknotic nucleus (red arrow). **D** Great destruction of the digestive tubules (ddt), ruptured basement membrane (red star) and necrosis of the intertubular connective tissue (nct)

Moreover, Gaber et al. [12] found that the digestive gland of the land snail *M. cartusiana* composed of three types of cells digestive, excretory and calcium cells and the thin cells not observed in their study. The digestive gland performs several functions in intracellular and extracellular digestion, absorption of digested food materials, excretion, osmoregulation and detoxification and alterations in its structure may affect these functions Romeo et al. [30].

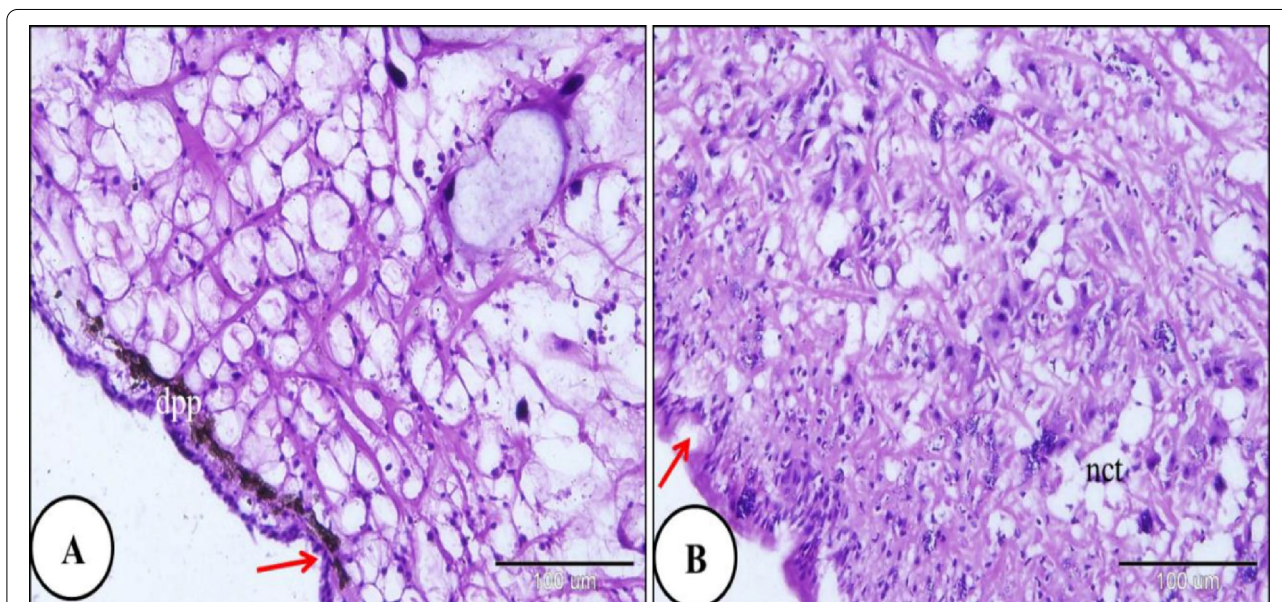
Our results indicated that both *Jatropha* and methomyl caused changes in the normal structure of the digestive gland. Ali et al. [31] observed that, the digestive gland of *Eobania vermiculata* snails treated with aqueous extract of *Solanum nigrum* showed histological alterations. The digestive cells were shrunk or completely degenerated. The excretory cells were significantly increased in number. Moreover, Ali [27] observed that, the digestive gland of *M. obstructa*, treated with the extract of *Acacia nilotica*

and *Cuminum cyminum*, the lumen of most digestive tubules, also showed a marked increase in their width and were filled with secretory materials, the intertubular connective tissue showed great destruction. Both extracts caused great destruction and shrinkage in all cell types of the digestive tubules or formation of blebs in the apical surface of many digestive cells especially in the case of snails treated with *C. cyminum*. In general, there is a great shrinkage in the digestive cells of the digestive gland of snails which treated with different plant extracts; however, Heiba et al. [32] found that the digestive cells in the digestive gland of *Eobania vermiculata* and *M. cantiana* became swollen and more vacuolated. Hamed et al. [29] obtained the same result in *Eobania vermiculata* using carbamate molluscicide. Sharaf et al. [33] cleared that many histological changes were observed in the digestive gland of *Helicella vestalis* after exposure to





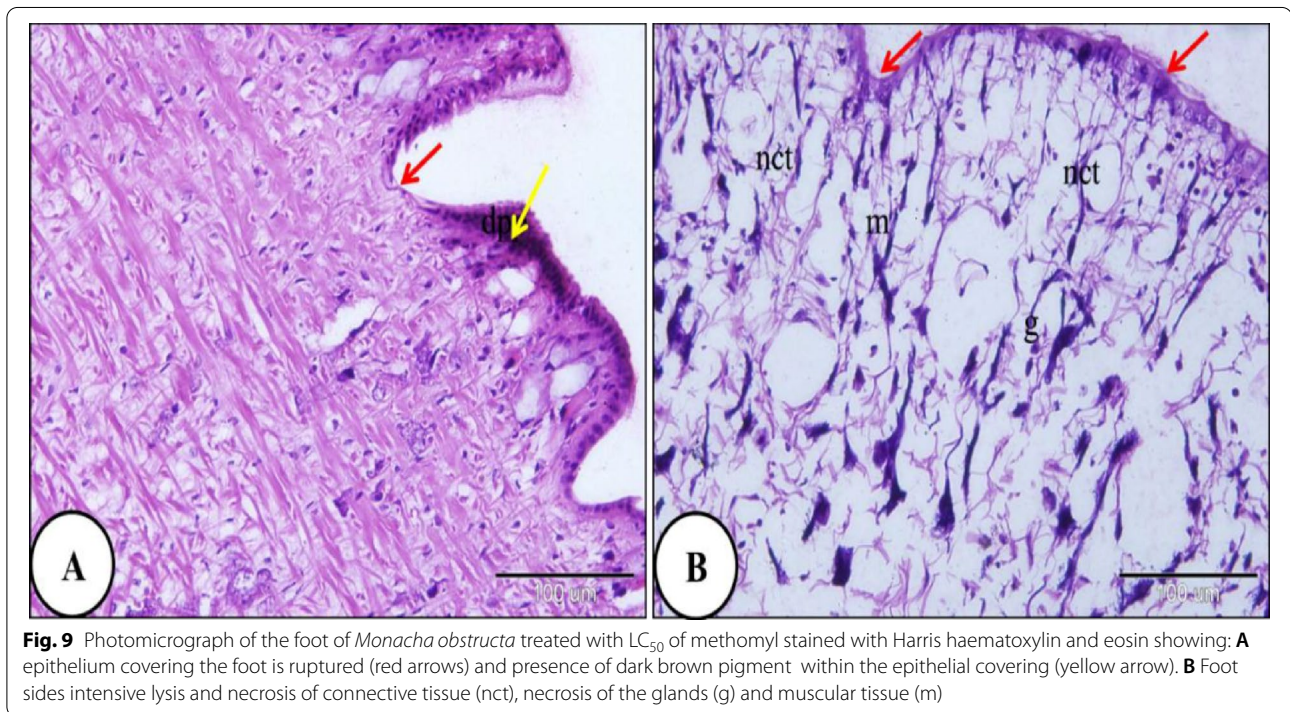
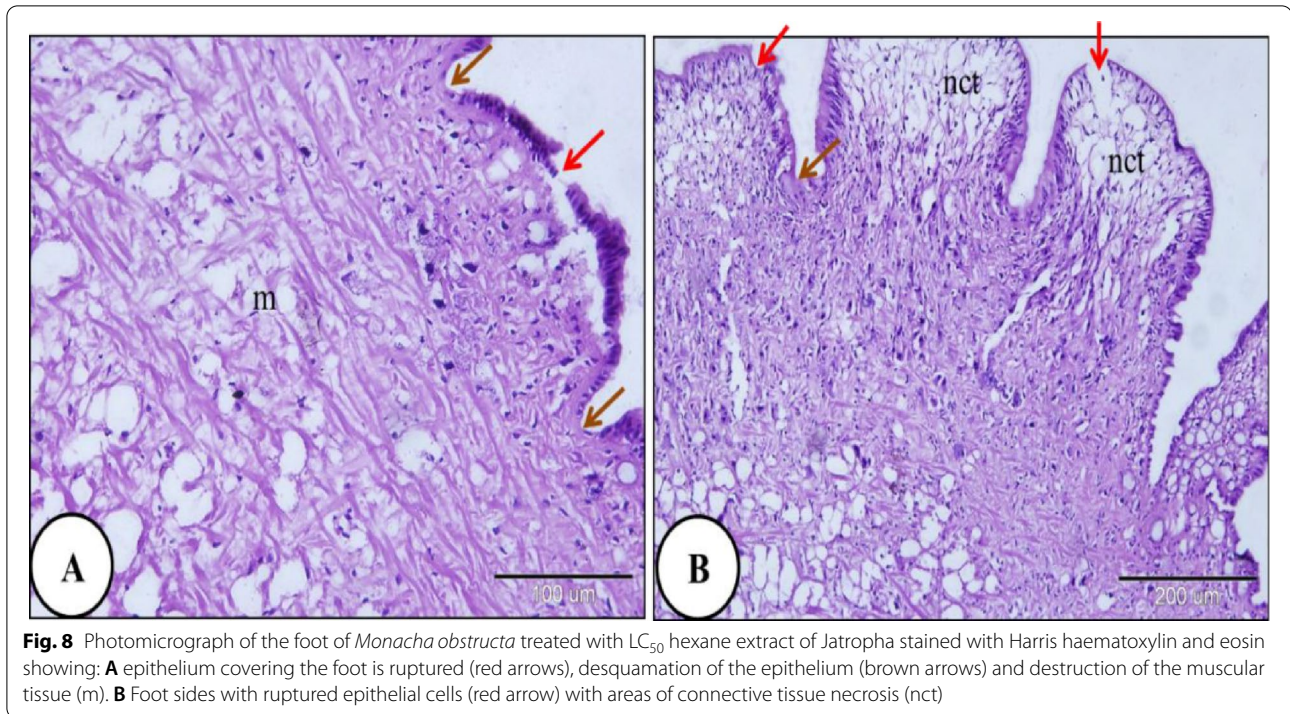
**Fig. 6** Photomicrograph of the general structure of the foot of *Monacha obstructa* (control group) stained with Harris haematoxylin and eosin showing: **A** side (yellow arrows) and the sole (red arrow) of the foot which covered with epithelial layer (e), the inner connective tissue layer (ct), glands embedded in the connective tissue (g) and the muscular tissue (m). **B** Photomicrographs of the foot of *M. obstructa* showing B the foot sole is covered with pseudostratified columnar epithelium (co), connective tissue layer (ct) with many deeply embedded glands (g), the and superficial folds in the epithelium covering the sole (blue arrows)



**Fig. 7** Photomicrograph of the foot of *Monacha obstructa* treated with LC<sub>50</sub> extract of ethanolic *Jatropha* stained with Harris haematoxylin and eosin showing: **A** epithelium covering the foot is ruptured (red arrows) and presence of dark brown pigment in the connective tissue (dbp). **B** Foot sides with ruptured epithelial cells (red arrow) with focal areas of connective tissue necrosis (nct)

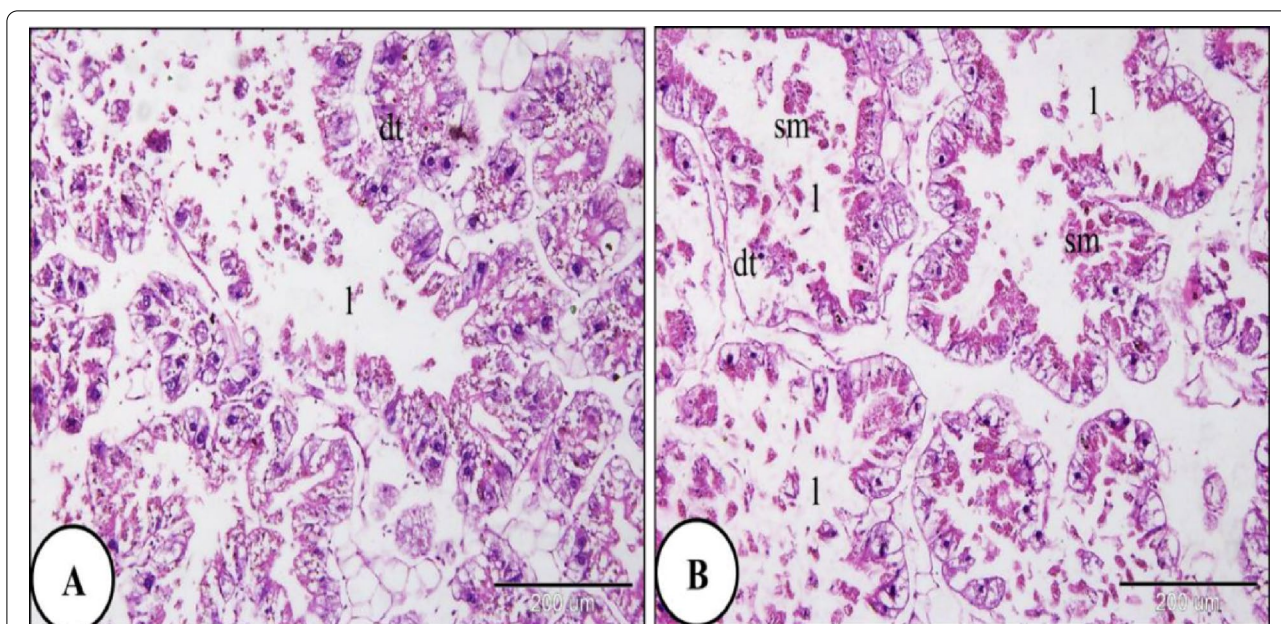
sublethal concentrations of both methiocarb and chlorpyrifos. These alterations included severe tubular disruption, vaculation, nuclear pyknosis and necrosis of tubules. Moreover, there study revealed that chlorpyrifos

was much more toxic to the tested snail than methiocarb. Abdel-Rahman [34] found that neomyl caused necrosis, degeneration of the digestive tubules and toxic precipitation in the land snail, *Monacha* sp. (Gastropoda).

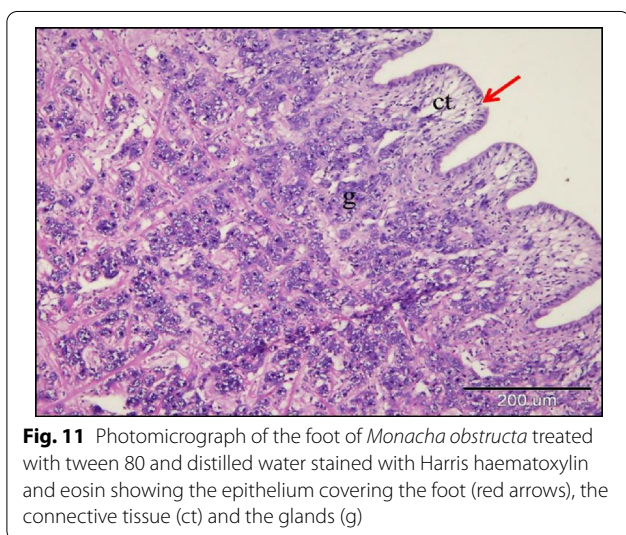


In agreement with Ali [35], Ali and Said [28] and Gaber et al. [12], the histological structure of the foot of *M. obstructa* represented an external layer of epithelial tissue which covers an internal layer of connective tissue.

The foot is covered with pseudostratified columnar epithelium, and had two parts the sole and sides. The connective tissue layer beneath the epithelial layer contains many glands. Regarding to the glands of the foot, at the



**Fig. 10** Photomicrograph of the general structure of the digestive gland of *Monacha obstructa* treated with Tween 80 and distilled water stained with Harris haematoxylin and eosin showing: **A** digestive tubules (dt), the digestive tubules (dt), the lumen of digestive tubule (l). **B** Lumen contained some secretory materials (sm)



**Fig. 11** Photomicrograph of the foot of *Monacha obstructa* treated with tween 80 and distilled water stained with Harris haematoxylin and eosin showing the epithelium covering the foot (red arrows), the connective tissue (ct) and the glands (g)

sole are greater in number and deeply embedded within the connective tissue as well as in the muscular layer than those at the sides which are lesser in number and superficially located.

Gaber et al. [12] mentioned that exposure of the *M. cartusiana* snail to methomyl (copter) for 96 h makes histological changes in the normal histological structure of foot of *M. cartusiana* snail. He showed that, deformity of muscle fiber with slight distortion of the outerlayer and

necrosis of mucus at 0.075 g/L of copter and by increasing the concentration to 0.18 g/L, he observed complete destruction and lysis of the outer layer. Moreover, deformity and formation of vacuole of the muscle fibers.

The foot of *M. obstructa* snails treated with  $LC_{50}$  of Jatropha showed rupture of the epithelium covering the foot, desquamation of the epithelium and presence of areas of connective tissue necrosis and destruction of the muscular tissue. Same results were previously recorded by Abdl-Kader [36]. She suggested that the death of snails is shown to be primarily correlated with the complete destruction of the foot tissues and the consequent great loss of the total body water content. In addition, Mitobe et al. [37] found that aspirin caused a dose-dependent reduction in mucous synthesis of rats. The acetylsalicylic acid caused intense effect on the mucous gland tissues of both snail species *E. vermiculata* and *M. obstructa*. This effect leads to inhibition of mucous synthesis which is very important for snail life [38].

### Conclusions

These results indicated that the tested jatropha extracts are highly effective against *M. obstructa* and the histopathological changes of hepatopancreas gland and the foot of *M. obstructa* treated with jatropha extracts

exhibited nearly the same histopathological alteration of methomyl. Thus, these extracts may be of great value in the field to control the population of the target herbivorous land snail *M. obstructa* being eco-friendly as safe and economic molluscicide, which no harm upon ecosystems instead of using chemical pesticides that could pollute the environment.

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#### Author contributions

All authors of this research contributed to the conception of the study; HAM, RE performed the experiment; HAM carried out the toxicity assay on snails; RE made the histological examination sections; HAM, SAS, RE contributed significantly to analysis and manuscript preparation and SAS, AFE revised and edited it. All the authors have read and approved the final manuscript.

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#### Availability of data and materials

The data sets applied during the current study are available on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Assiut University, Egypt.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

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