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Molluscicidal Potency of Croton Crude Extracts on the Histological Changes of Terrestrial Snail, *Monacha obstructa* (L. Pfeiffer, 1842)

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Abstract

The present work performed to study the toxic effects of the crude plant extracts of *Croton tiglium* seeds on some histological changes of the terrestrial snail species *Monacha obstructa*. The molluscicidal efficacy of ethanol and acetone extracts of *Croton tiglium* seeds were tested against the adult snails of *M. obstructa* (L. Pfeiffer) (Gastropoda: Hygromiidae) at four concentrations using contact technique under laboratory conditions for seven days. Also, the impact of crude seed extracts was studied on some histological changes of the land snail, *M. obstructa*. The results revealed that the hexane and ethanolic extracts of croton exhibited molluscicidal activities against *M. obstructa* land snails. The ethanolic extract displayed the highest potency where LC_{50} was (0.08 %) while the LC_{50} of hexane extracts was (0.12 %). Histological inspections of the digestive gland and foot of snails exposed to LC_{50} croton extract showed that the digestive tubules showed various destruction, shrinkage in different types of cells lining the digestive tubules, marked increase in the width of most of the digestive tubules lumen and filled with secretory materials, rupture of basement membrane and the intertubular loose connective tissue between the digestive tubules showed necrosis. The histological alterations in the foot included rupture of the epithelial covering with necrosis, destruction of muscular tissue and necrosis of connective tissue with presence of dark brown pigment. Thus, these results indicated that croton seed crude extracts possessed molluscicidal potential for controlling the land snail with more future studies to develop and ensure its wider applicability as a molluscicide.

KEYWORDS
Monacha obstructa, Croton Crude Extracts, Terrestrial Snail

INTRODUCTION

Terrestrial snails are considered a serious agricultural pest that affects many important crops in many parts of the world including Egypt. The glassy clover snails, *Monacha obstructa* are important predominant agricultural pests, which infesting numerous agronomic, horticulture and ornamental plants causing serious economic damage reduces their yield, quality and marketing values (Ali-Asmaa, 2014; Ibrahim, 2017). The control of molluscan pests using synthetic molluscicides is still considered to be the most effective measures. However, these synthetic compounds may lead to problems of toxicity to non-target organisms in addition to deleterious long-term effects to the environment (Gabr *et al.*, 2006). The large-scale use of chemicals led to the development of resistance to target pests, and consequently also has a negative effect on natural enemies and other benefits causing disruption of biodiversity (Alghamdi, 2018).

Therefore, alternative environmentally friendly measures or compounds for effective molluscan control need to be developed. Currently attention is being drawn to the use of natural plant products for molluscan control because they are inexpensive and environmentally safe as an alternative approach (Marston and Hostettmann, 1985). Also, the World Health Organization recommends the use of plant molluscicide because of their lower toxic and residual effects (WHO, 1983). 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 (Singh *et al.*, 2000). Several histological studies were performed to determine certain histological changes resulting from toxic treatments, and to clarify the extent to which these terrestrial snails and slugs are affected (Yousef, 2011; Parvate and Thayil, 2017). Hence, the objectives of the current study were to assess the molluscicidal activity of ethanolic and hexane crude extracts of *C. tiglium* against the adult snails of *M. obstructa* and to further evaluate its toxic effect on histological changes of this snail.

MATERIALS AND METHODS

Experimental snails

Adult specimens of the glassy clover snail, *M. obstructa* were collected from infested field crops at El-Wasta village, El-Fath

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district at Assiut governorate during spring season. Healthy individuals were transferred in plastic bags to the laboratory of Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al-Azhar University Assiut Branch.

Then kept in glass boxes containing moistened soil and covered with mosquito netting secured with rubber band to provide ventilation and prevent snails from escaping. Also, fed on fresh leaves of lettuce for 14 days for acclimatization to retain only healthy individuals. The snails were selected for each treatment (Miller *et al*., 1988).

Plant material and extraction

Dried seeds of *Croton tiglium* L. (Euphorbiaceae) were extracted according to the method of Freedman *et al*. (1979) with a little modification. Dried seeds that used in the experiments were shade dried grounded into fine powder using an electrical grinder, 200 g from seeds powder was extracted with two solvents varied in polarity; hexane followed by ethanol 95%, by soaking in solvent and allowing standing for three 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 emulsification before using. Four concentrations per each plant extract were used to determine LC_{50} values of the plant extract.

Molluscicidal activity bioassays

A series of concentrations (0.125, 0.25, 0.5 and 1%) of the tested crude plant extracts were prepared for their contact toxicity using the treated surface exposure method which described by Ascher and Eliyahu (1981), where two ml of each concentration was deposited and distributed on the bottom of a petri dish that was moved 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, 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 and mortality percentages were estimated and corrected according to Abbott`s formula (Abbott, 1925).

Data analysis

Mortality data were used to estimate the median lethal concentrations (LC_{50}) values, as well as slope value of LCP lines for the used materials by probit analysis using LdP Line software and the toxicity index, was estimated according to Sun (1950).

Repellent activity bioassays

The repellent effects of croton plant extracts against adults of *M. obstructa* were evaluated using method described by McDonald *et al*. (1970) with certain modifications. Substrates were prepared from 9 cm diameter filter papers (Whatman No.1) which were cut into two halves. One ml of the four different concentrations of used plant extracts was applied to a half filter paper as uniformly as possible with a pipette. The treated half-discs were air-dried until the solvent was totally evaporated. Then, the treated and the untreated half-circles were placed contiguously on the petri dishes and ten adult snails were put at the center of each filter paper disc and the petri dish was covered. Care was taken so that an attachment did not prevent the free movement of snails from one half to another. For each treatment three replicates were used. The snails present in each half circle were counted after one and six hours post treatment. Data were converted to express percentage repulsion (PR) using the following formula of Talukder and Howse (1994). PR % = [(N- C)/c] x 100

Where: PR % = percentage repulsion, N= the number of snails present in the control half, C= the number of snails present in the treated half, c = the total number of snails in the Petri dish, Positive values (+) indicated repellency and Negative values (-) indicated attractancy.

Histological technique

The histological studies were carried out on *M. obstructa* to demonstrate the effect of the ethanolic and hexane extracts of croton. Snails were treated with LC_{50} values from the extracts. The histological studies were designed as four groups; the first group of snails was the control one without any treatment. The second and third groups of snails were treated with ethanolic and hexane extracts of croton. The fourth group of snails was treated with tween 80 and distilled water.

Dissection and tissue preparation

After 24 h exposure, snails' tissues were prepared for histological tests. 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 (Bancroft *et al*., 2013). The sections were examined for the histological and histopathological changes using an OLYMPUS BX51microscope and photographed with an OLYMPUSDP72 camera adapted to the microscope (Department of Anatomy and Embryology, Assiut University).

RESULTS

Data in Fig. 1 showed the efficacy of the ethanolic, and hexane crude extracts of croton seeds based on values of median lethal concentration (LC_{50}) which calculated for land snail species *Monacha obstructa* treated with extract concentrations when applied using the contact technique. Results showed that the ethanolic and hexane croton seed extracts proved to be effective against the tested land snail species. However, croton ethanol extract was most effective with LC_{50} value (0.08%) and toxicity index (100%). While it showed LC_{50} value (0.12%), and a toxicity index (66.7%) for croton hexane extract.

The repellent effect of the crude croton extracts

The repellency effects of ethanol and hexane crude extracts of croton on *M. obstructa* after one and six hours were illustrated in Fig. 2 and 3. Results revealed that the hexane croton extracts had a high repellency effect to snails after one and six hours with 58.3 % and 56.7 % repellency. While the ethanol croton extracts recorded 43.3 % and 46.7 % after one and six hours, respectively (Fig. 2). Also, it was found that the rate of repellency increased proportionally with the increases of concentrations where the

repellency effects of croton plant extracts showed the highest repellency action at 1% concentration with repellent percentage of 76.7 % and 70.00 % after one and six hours respectively (Fig. 3).

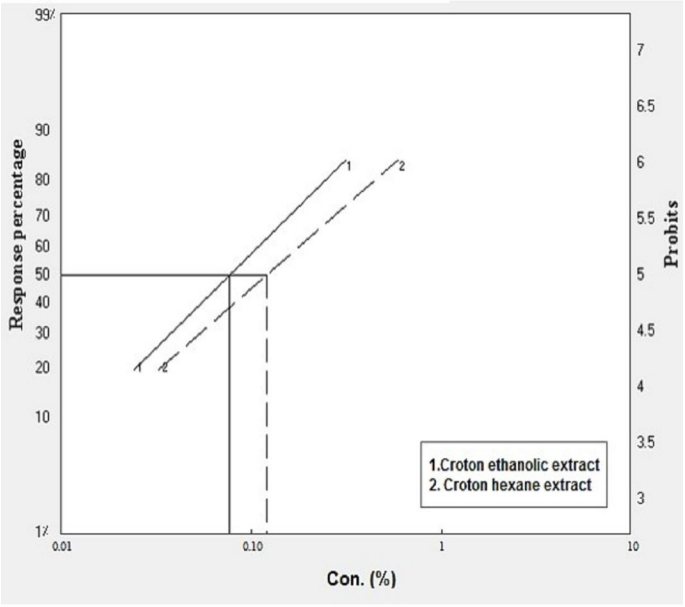

Fig. 1. LC- P Lines of ethanolic and hexane crude extracts of croton on *Monacha obstructa* using contact technique under laboratory conditions.

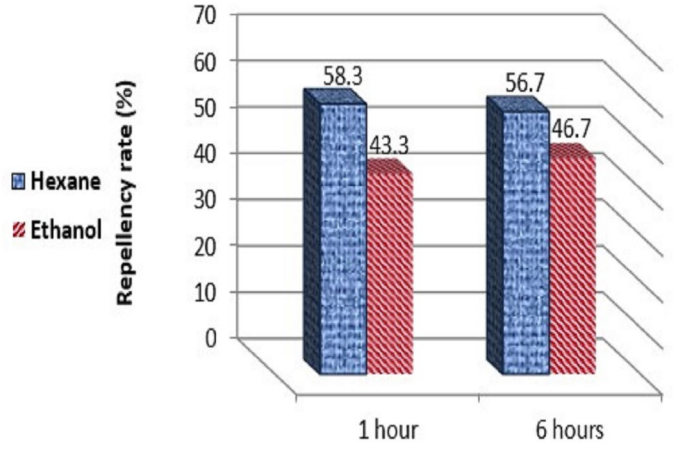

Figure 2. Repellency effect of two solvents of plant extracts on the glassy clover snail, *Monacha obstructa.*

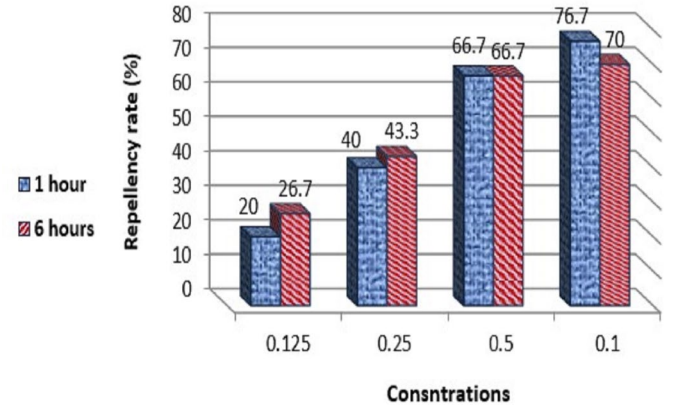

Fig. 3. Repellency effect of different concentrations of plant extracts against the glassy clover snail, *Monacha obstructa*.

Histological observations of snails

The digestive gland of the land snail *M. obstructa*

The histological study showed that the digestive gland of land snail, *M. obstructa* is composed of bilobed tubulo-acinar gland found in the dorsal portion of the animal body. The digestive gland is surrounded by an outer layer which is lined by a single layer of simple columnar cells resting on a basement membrane which underlined with circular muscle fibers. Each lobe consists of numerous tubules, which appear spherical or oval and are separated by intertubular loose connective tissue. Each tubule is surrounded by circular muscle fibers. The tubule of the digestive gland is lined with columnar cells resting on thin basement membrane.

There are four types of cells lining each tubule. They are digestive cell, calcium cells, excretory cells and thin cells. The abundant cell type lining the digestive tubule was the digestive cell. Their cytoplasm was highly vacuolated, and the nucleus was rounded and basally located. The cytoplasm of the digestive cells has a variable number of small granules. The calcium cells are found in the tubules either a single cell or sometimes in groups of two or three cells. The calcium cells are characterized by the presence of calcium spherules. The excretory cells usually contain large vacuole surrounded by thin layer of cytoplasm. There are only one or more large yellow granules. The thin cells are randomly distributed in between the other cell types. They are narrow and extend to the height of the epithelium (Fig. 4).

Regarding, the digestive gland of *M. obstructa* treated group with LC_{50} of extract of ethanolic croton. After 24 h exposure, some digestive tubules showed slight destruction and others showed major destruction, shrinkage, and great destruction in different types of cells lining the digestive tubules were observed. Moreover, marked increase in the width of most of the digestive tubules lumen, which was filled with secretory materials. There was a rupture in the basement membrane. The intertubular loose connective tissue between the digestive tubules showed massive necrosis (Fig. 5).

The digestive gland of *M. obstructa* treated group with LC_{50} of hexane extract of croton

After 24 h exposure, some tubules were greatly destructed, and the basement membrane was destroyed. The apical border of most digestive cells showed remarkable disruption. The detached apical surface formed blebs inside the lumen of the digestive tubule. The lumen of digestive tubules was filled with the secretory materials. There was necrosis of the intertubular loose connective tissue between the digestive tubules (Fig. 6).

The foot of the land snail *M. obstructa*

The histological observation revealed that the foot of *M. obstructa* of control group 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. 7).

The foot of *M. obstructa* treated with LC_{50} of extract of ethanolic croton showed rupture of the epithelial covering with ne-

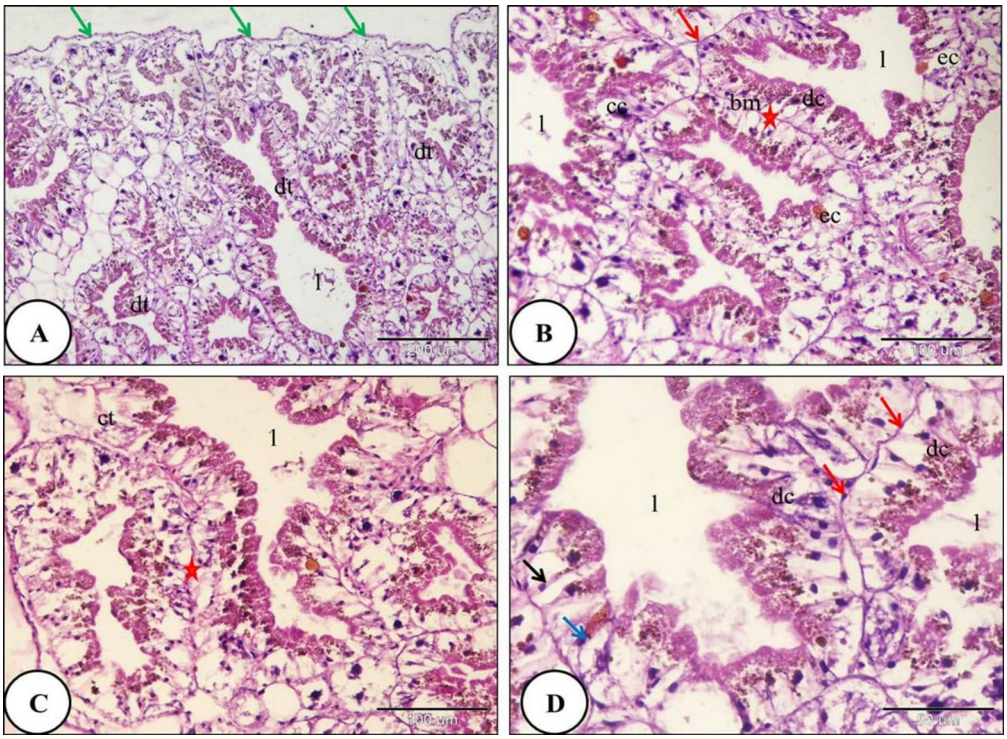

Fig. 4. Photomicrograph of the general structure of the digestive gland of *Monacha obstructa* (control group) stained with H&E showing: (A) An outer layer covering the digestive tubule (green arrow), the digestive tubules (dt), the lumen of digestive tubule (l). (B) The digestive cell (dc), the excretory cell (ec), the calcium cell (cc) and the basement membrane (red stars). (C) The intertuubular connective tissue (ct). (D) The thin cell (black arrow) and smooth muscle fibers surrounding the digestive tubules (red arrows).

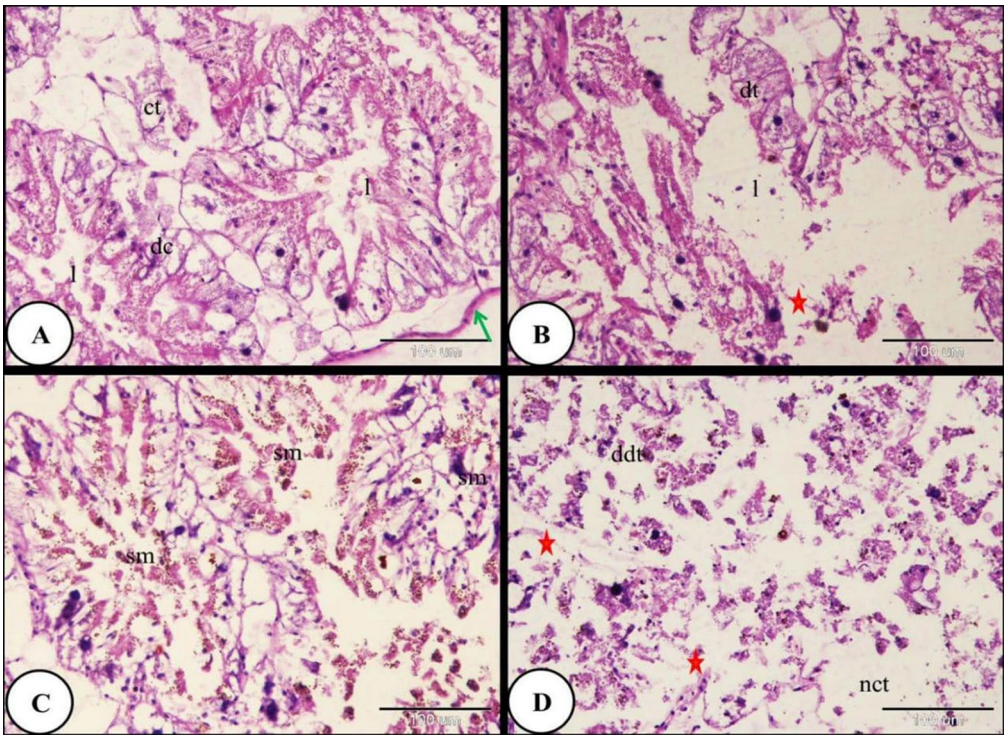

Fig. 5. Photomicrograph of the digestive gland of *Monacha obstructa* treated with LC_{50} ethanolic extract of croton stained with H&E showing: (A) The outer layer covering the digestive tubule was detached (green arrow), the digestive tubules (dt), the lumen of digestive tubule (l). (B) Mild destruction of the digestive tubules. The width of lumen of the digestive was increased. (C) The lumen filled with secretory materials (sm). (D) Massive destruction of the digestive tubules (ddt), ruptured basement membrane (red star) and necrosis of the intertubular connective tissue (nct).

crosis, destruction of muscular tissue and necrosis of connective tissue (Fig. 8). Furthermore, the foot of *M. obstructa* treated with LC_{50} hexane extract of croton showed similar histopathological changes, in addition to the presence of deep folds in the side of the foot and the necrosis of connective tissue, which became more obvious, also dark pigments appeared and the gland was empty (Fig. 9).

The digestive gland and the foot of snails treated with tween 80 and distilled water showed nearly the same histological structure of the untreated ones (Figs. 10 and 11).

DISCUSSION

Findings from this study indicated that the crude extracts of croton seeds had a toxic effect against *M. obstructa* and can introduce economic control of its population without causing environmental pollution. Since, most of plants of the Euphorbiaceae contain toxic compounds, as mentioned (King *et al.*, 2009).

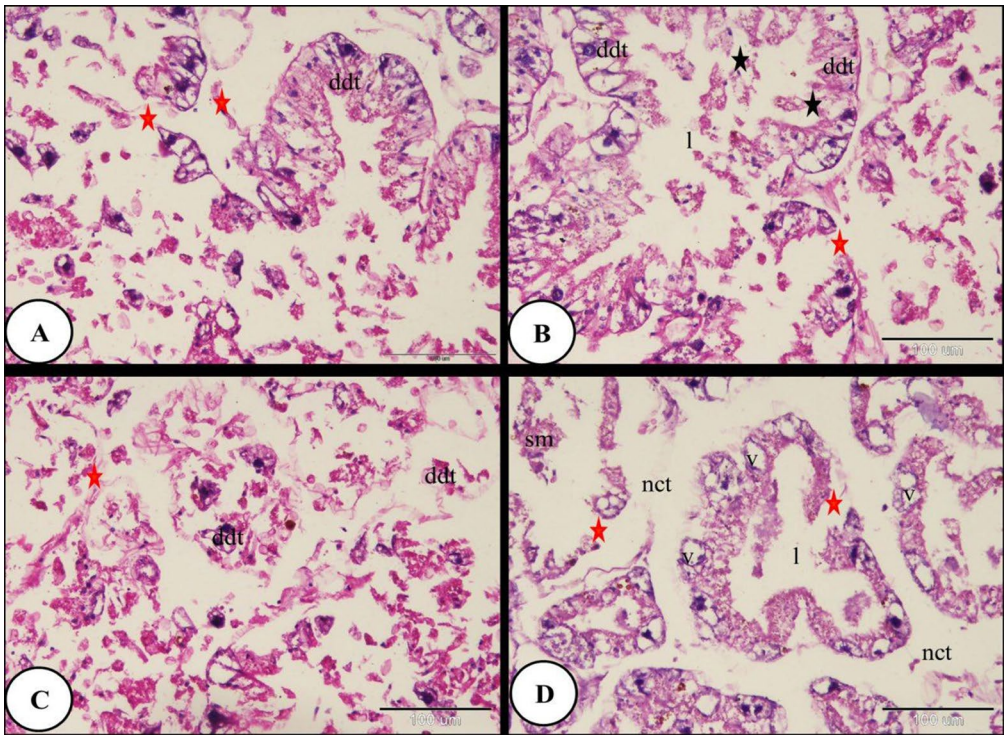

Fig. 6. Photomicrograph of the digestive gland of *Monacha obstructa* treated with LC_{50} hexane extract of croton stained with H&E showing: (A) Destructed digestive tubules and ruptured basement membrane (red star). (B) The apical order of digestive cells detached (black star) inside the lumen of digestive tubule (l). (C) The normal structure of the digestive tubules was completely changed. (D) Most of the epithelium lining the digestive tubules was vaculated (v) digestive tubules shrank with necrosis of the intertubular connective tissue (nct).

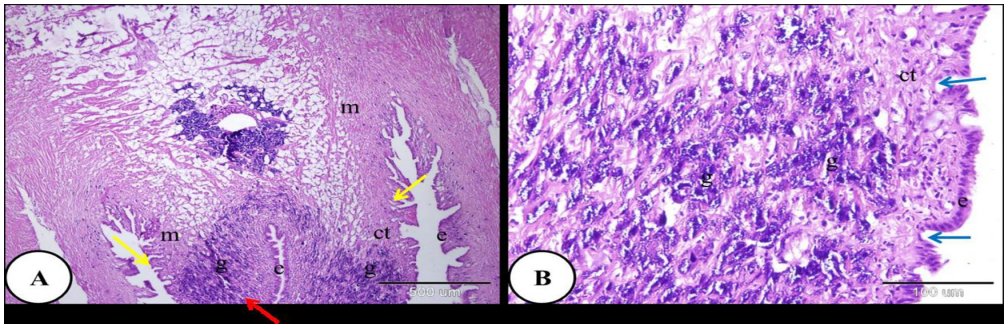

Fig. 7. Photomicrograph of the general structure of the foot of *Monacha obstructa* (control group) stained with H&E showing: (A) The side (yellow arrows) and the sole (red arrow) of the foot which covered with epithelial layer (e), the inner connective tissue layer (ct), glands embedded in the connective 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), and superficial folds in the epithelium covering the sole (blue arrows).

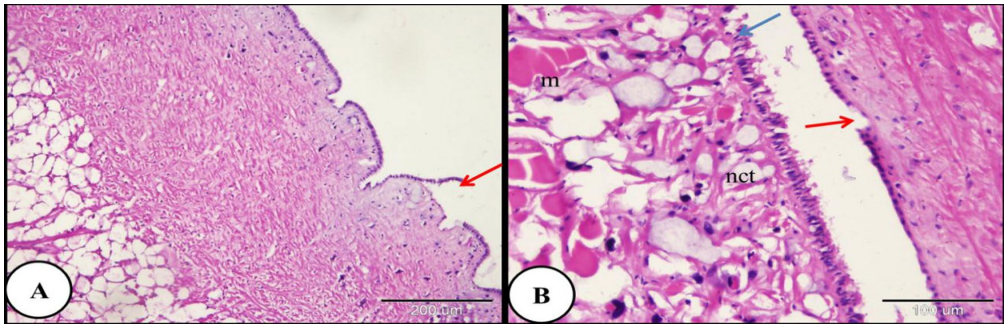

Fig. 8. Photomicrograph of the foot of *Monacha obstructa* treated with LC_{50} ethanolic extract of croton for 24 h stained with H&E showing: (A) the epithelium covering the foot is ruptured (red arrows). (B) The foot sides appeared with rupture of epithelial cells (red arrow), destruction of muscular tissue (m) and connective tissue necrosis (nct).

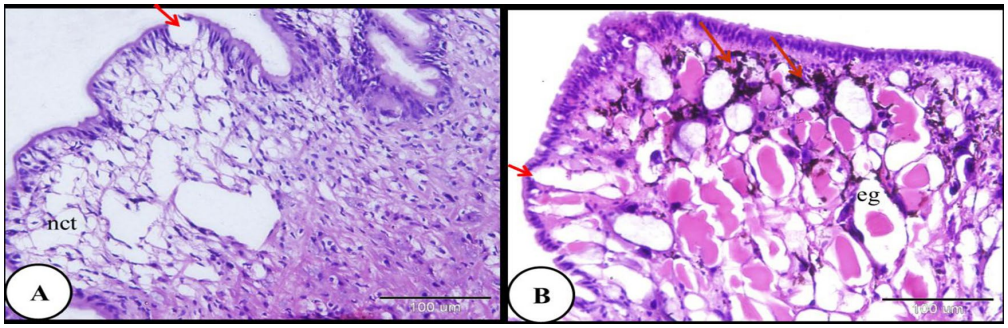

Fig. 9. Photomicrograph of the foot of *Monacha obstructa* treated with LC_{50} hexane extract of croton for 24 h stained with H&E showing: (A) the epithelium covering the foot is ruptured (red arrows) and connective tissue showed necrosis (nct). (B) The presence of dark brown pigment in the connective tissue (brown arrows) and the gland was empty (eg).

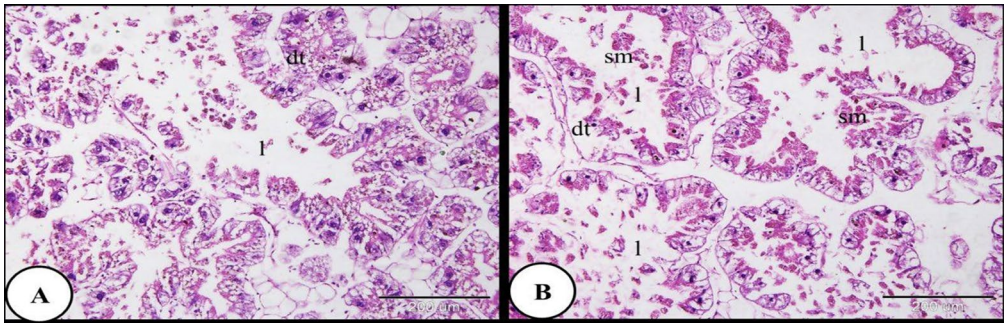

Fig. 10. Photomicrograph of the general structure of the digestive gland of *Monacha obstructa* treated with Tween 80 and distilled water stained with H&E showing: (A) The digestive tubules (dt)), the lumen of digestive tubule (l). (B) The lumen contained some secretory materials (sm).

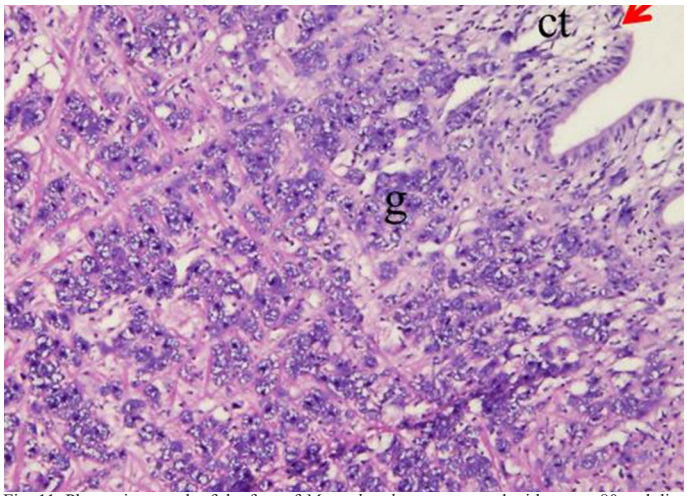

Fig. 11. Photomicrograph of the foot of *Monacha obstructa* treated with tween 80 and distilled water stained with H&E showing the epithelium covering the foot (red arrows), the connective tissue (ct) and the glands (g).

Ali-Safaa *et al*. (2015) recorded the highest mortality rates at 25% concentration of *Acacia nilotica* for species 23% and 20% of *Cuminum cyminum* concentrations for the two species, *E. vermiculata* and *M. obstructa* respectively. The extract of *A. nilotica* caused the appearance of black material on the snail shell. The treated snails showed histological alterations in the digestive and salivary glands. Nnamdi *et al*. (2020) mentioned that the Aqueous *Moringa oleifera* Lam seed extract was toxic to *Bulinus* adult snails in a dose dependent manner and the total lethal concentration (LC_{50} and LC_{90}) determined after 24 h exposure from the whole streams were 468 and 813 ppm respectively. Ruma and Sanchez (2016) determined the toxicity of the extracts of *Jatropha curcas* against the golden apple snail (*Pomacea canaliculata*). It was shown that the *J. curcas* extracts exhibited molluscicidal effects on the tested *P. canaliculata* snails, which could be attributed to the presence of phenols, tannins, flavonoids, saponins, triterpenoids and sterols. Khalifa-Rasha (2015) cleared that four plant extracts (calendula, seena-P, black cumin and clove) have high repellency effect to snails after one and six hours: (93.33% and 100%), (86.67% and 80%), (80% and 93.33%), and then (40% and 80%), respectively. Six plant extracts (visnaga fruit, thyme, lavender, seena-L, belladonna, and garlic) were moderate in their repellency effect. Two plants (colophony and nutmeg) have low repellency (6.67% and 26.76%) was attractive after one hour. Mustard has high repellency after one hour but after six hours. Neither repellency nor attractancy effect was observed.

Also, these results indicated that the histopathological alterations caused were proportionate to the potential of the tested extracts. Same structure of digestive gland of *M. obstructa* was observed by Ali-Asmaa (2014) and Ali and Said (2019) they 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. Lopes *et al*. (2001) described three types of cells forming the digestive tubules namely digestive, calcium and excretory cells in the digestive gland of *Oxychilus atlanticus*. Also, Sharaf (2009) gave the same result when studying the digestive gland of *Monacha* cartusiana. Also, Sharaf *et al*. (2015) observed the presence of the previous three types of cells in the digestive gland of the land snail Helicella vestalis. Hamed *et al*. (2007) also suggested that the excretory cells originate from digestive cells during maturation, as the single large vacuole of the excretory cell seems to result from the fusion of all heterolysosomes and residual bodies of a digestive cell.

Regarding the histological observations of treated land snails. Similar observation was described on the digestive gland of *Eobania vermiculata* snails treated with the extract of *Solanum nigrum* (Sawasdee *et al*., 2011; Ali-Asmaa, 2014). Heiba-Fadia *et al*. (2002) obtained an opposite result during studying of the effect of Lannate insecticide on the land snails, *Eobania vermiculata* and *Monacha contiana*, as they found that the tubules were widely separated by connective tissue and the lumen of these tubules appear narrower and had irregular or branched shape.

Same structure of the foot of *Monacha* sp. was observed by Ali and Said (2019). These histopathological changes are in congruence with the results of a previous study using crude extracts of camellia seed and mangosteen pericarp, which altered cells in the foot by relaxing muscle fibers and forming gaps between epithelial cells and connective tissue, resulting in the derangement of the cilia in the snail *Bithynia siamensis* (Aukkanimart *et al*., 2013). Abdel-Rahman-Amal (2020) observed that the land snail, *Monacha* sp. (Gastropoda: Helicidae) in both control group and the vehicle (1% tween 80) treated group exhibited nearly the same normal histological structures of the examined tissues.

CONCLUSION

The current study showed that the crude extract of croton seeds gave promising results as a molluscicide and deserves further studies in order to identify and characterize the active ingredients in these plants as well as studying the selective toxicity of these extracts on non-target animals. Also, a practical method of preparing the compound from these plants to develop them as cheaper and better alternatives to chemical molluscicides.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest.

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