Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 1255 – 1275 (2024)
www.ejabf.journals.ekb.eg

Sensitivity of *Biomphalaria alexandrina* Snails and *Schistosoma mansoni* Free Larval Stages to Ethanol Extract of *Jatropha gossypiifolia*

Samah Ibrahim Ghoname, Hanaa Mohamed Abu El Einin, Shereen Mahfouz Mansour*
Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt
Corniche El Nile St.,Warrak El – Hadar Imbaba P.O. Box 30
***Corresponding Author:** shery_83m@yahoo.com

ARTICLE INFO
Article History:
Received: Jan. 18, 2024
Accepted: Feb. 2, 2024
Online: Feb. 9, 2024

Keywords:
Biomphalaria alexandrina,
Jatropha gossypiifolia,
Schistosomiasis,
Hemocytes,
Comet Assay,
Histopathology,
Autophagy

ABSTRACT

The use of plant molluscicides for the control of *Biomphalaria alexandrina* snails is a critical part of schistosomiasis treatment in Egypt. The purpose of this research was to determine the molluscicidal activity of an ethanol extract of *Jatropha gossypiifolia* leaves against *B. alexandrina* snails and *Schistosoma mansoni* free larval stages. The current finding showed that ethanol extract was lethal for *B. alexandrina* snails, with LC_{50} at 51mg/ L and LC_{90} at 66.17 mg/ L. The lethal time for miracidia and cercariae after exposure to LC_{25} of *J. gossypiifolia* was 75 and 120 minutes, respectively. In addition, the hemocyte examinations of the treated snails showed that hyalinocyte had ramified membranes with short filopodia, a shrinkage nucleus and vacuoles, while granulocytes had more dense granules, two nuclei, and long filopodia. It caused a significant decrease in the total hemocyte count in all treated snails. Flow cytometry measurements revealed a significant increase in the expression of LC3B as a marker of autophagy at LC_{25} concentration compared to LC_{10} and control. In addition, the histopathology of snails exposed to LC_{25} of plant extract showed a necrotic alteration, which resulted in fiber tissue breakdown, increased empty spaces, the creation of large vacuoles in the head-foot area, and severe damage to gonadal cells. Furthermore, the comet assay indicates that *J. gossypiifolia* extract caused a genotoxic influence at concentrations LC_{10} and LC_{25}, where the tail length and the tail moment were significantly increased compared to untreated snails. In conclusion, *J. gossypiifolia* extract had potent molluscicidal activity against *S. mansoni* intermediate host and its free larval stages.

INTRODUCTION

Freshwater snails *Biomphalaria* spp. are intermediate hosts of *Schistosoma mansoni*, and population management is one of the key tactics in halting infectious agent transmission **(Rollinson *et al.*, 2013)**. *Biomphalaria alexandrina* snails are widely distributed in Egypt, and are susceptible to all *S. mansoni* strains, resulting in hundreds of cercariae shed to water daily **(Abou El Naga *et al.*, 2015)**. Furthermore, *Biomphalaria* snails have been used as an experimental model attributed to their broad geographic

distribution and characteristics that make them useful as environmental indicators in the lab, where various changes, including those in adult snail survival and reproductive capacity, as well as quantifiable cellular and structural DNA modifications, can be examined **(Morais *et al*., 2022)**.

The schistosomiasis control strategy relies on risk groups preventing and treating symptomatic individuals; however, these approaches are insufficient for limiting parasite transmission **(King & Bertsch, 2015)**. The lack of a viable and safe schistosomiasis vaccine, as well as the emergence of *S. mansoni* batches resistant to the antischistosomal medication Praziquantel, encourage snail control programs **(Hamed, 2010)**. Notably, niclosamide (Bayluscide) is effective in eliminating snails **(WHO, 2017)**. However, it is expensive, has secondary effects such as bioaccumulation, and is hazardous to non-target creatures such as fish, amphibians, and plants. Snails that are resistant to niclosamide have been discovered **(Dai *et al*., 2015; Wilkie *et al*., 2019)**. There is a need for novel and safer molluscicides, especially given the growing focus on disease prevention and elimination **(WHO, 2019)**. The hunt for new plant-derived molluscicides that are more selective for intermediate hosts while still being eco-friendly and biodegradable becomes necessary. Furthermore, plant extracts are generally less expensive and easier to obtain **(Jia *et al*., 2019)**.

Euphorbiaceae family is famous for species such as *Euphorbia milii var. splendens*, which has no cytological effect at quantities up to 200ppm **(Adewunmi, 1984)**. This family includes *Jatropha gossypiifolia*, also known as bellyache bush **(Abreu *et al*., 2003)**. Several plants in this group are being screened for molluscicidal activity, such as *Euphorbia royleana, E. antisyphilitica, E. lactea cristata,* and *J. gossypiifolia* were examined against *Indoplanorbis exustus* **(Singh & Agarwal, 1992)**. *J. gossypiifolia* is used as insecticides, pesticides, ornamentation, and religious rituals **(Felix-Silva *et al*., 2014)**. The primary biological activities of *J. gossypiifolia* that have been experimentally demonstrated include anti-microbial **(Nair *et al*., 2007)**, larvicidal **(Rahuman *et al*., 2008)**, and anti-Leishmania properties **(Martins *et al*., 2018)**. *J. gossypiifolia* is also non-toxic to humans since it is used to treat conditions such as gastric ulcers, cancer, diabetes, and diarrhea, as well as a scar-healing and diuretic agent **(Felix-Silva *et al*., 2014)**.

The biological reaction of an animal to xenobiotics begins with toxicant-induced alterations at the cellular and biochemical levels, which result in alterations in cell function and structure, tissues, physiology, and behavior of animals. These alterations may influence the ecosystem's and population's integrity **(Parvez & Raisuddin, 2005)**.

Autophagy is a lysosome-dependent intracellular breakdown process that is essential for a variety of physiological activities, and its disruption can cause diseases **(Levine & Kroemer, 2019)**. It is necessary not just for intracellular component constituent turnover, but also for the active removal of abnormal or probably damaging components, as well as fot the breakdown of by-products like protein. Throughout the initial phases of autophagy, an isolation membrane or phagophore encloses a piece of

cytoplasm and ultimately develops to become an autophagosome, which then combines with lysosomes to destroy components that have been sequestered **(Mizushima & Murphy, 2020)**. A fusion between autophagosomes and lysosomes can be visualized using microtubule-associated protein light chain 3 (LC3) **(Yoshii & Mizshima, 2017)**.

Therefore, in the current work, the molluscicidal effects of the *J. gossypiifolia* plant extract were investigated to detect how it impacted the biological system of *B. alexandrina* snails by analyzing its influence on histological, hematological, and molecular parameters of the treated snails.

MATERIALS AND METHODS

1. Experimental animals

Biomphalaria alexandrina snails, *Schistosoma mansoni* miracidia, and cercariae were obtained from the Schistosomiasis Biological Supply Center (SBSC), Theodor Bilharz Research Institute, Giza, Egypt. According to the method described by **Eveland and Haseeb (2011)**, these snails were kept under experimental laboratory conditions (25± 2ºC).

2. Plant collection and preparation of ethanolic extract

Jatropha gossypiifolia plant was collected from Al-Orman Garden (Egypt). The plant leaves were cleaned, dried, and pulverized after being harvested. Subsequently, they were placed in big glass flasks, and 95% ethanol was added. A glass rod was then used to macerate the substance. The plant material to solvent ratio was 1:7. The material was filtered in a simple funnel system utilizing filter paper after fifteen days of maceration, and the hydroalcoholic extracts were concentrated in a rotary evaporator to obtain the dry extracts **(Neiva *et al*., 2014)**.

3. Molluscicidal activity of *Jatropha gossypiifolia* leaves extract

A 1000ppm stock solution was prepared from an ethanolic extract of *J. gossypiifolia* leaves using dechlorinated tap water. A series of concentrations of plant extract stock solution (200, 150, 100, 75, 50, 35, 25mg/ L) were prepared to determine LC_{50}, LC_{90} **(WHO, 1983)**. Dechlorinated water was used for dilution, and ten snails were incubated for each concentration **(WHO, 1983)**. Another batch of snails of the same size was only dipped in dechlorinated water as a control (untreated) group. For each concentration, three replicates of ten snails (7- 9mm) were utilized. After 24 hours of exposure, these snails were withdrawn from the test sample solution and properly cleaned with dechlorinated water with a 24 hour recovery period. The dead snails were counted and verified using a wooden blunt nail to elicit typical pull movements of the foot. The mortality rate was determined **(WHO, 1965)**, and lethal concentrations were determined using probit analysis.

4. Miracidicidal and cercaricidal effect of leaves of *J. gossypiifolia* ethanolic extract

Three millilitres of dechlorinated tap water that included 20 freshly hatched miracidia or cercariae were mixed with three millilitres of double concentrations of the

plant extract's LC_{10} and LC_{25} values. As a control (untreated) group, three millilitres of dechlorinated water that included 20 freshly hatched miracidia or cercariae were used. Microscopical observations on the movement and mortality of miracidia and cercariae were made at time intervals of 15, 30, 60, 75, 90, and 120 minutes during the exposure period **(Eissa *et al.*, 2011)**.

5. Hemolymph collection

After the exposure of *B. alexandrina* snails (7- 9mm) to plant extract concentrations LC_{10} and LC_{25} for 3 days, *B. alexandrina* snails' shells were cleaned with 70% alcohol and dried, and the hemolymph from each group was collected from 5– 7 snails/ group by a cardiac puncture **(Martins-Souza *et al.*, 2006)**.

6. Hemocytes count and differentiation

An amount of 20µl of hemolymph was used for total hemocyte count, and the number of hemocyte cells in each examined group was determined by diluting newly obtained hemolymph with a leucocyte count solution at a 1:20 ratio. A Bürker-Turk hemocytometer was used to count the total number of hemocytes three times, and the mean number of circulating hemocytes was calculated. For differential hemocyte analysis, hemocyte monolayers were formed by depositing 10µl of hemolymph on a slide of glass and allowing the hemocytes to stick to the glasses surface for 15 minutes at the room temperature. The hemocytes were fixed with absolute methanol for five minutes before being stained with 10% Giemsa stain (Aldrich) for 20 minutes and investigated under a microscope **(Abdul-Salam & Michelson, 1980)**.

7. Measurement of autophagy by flow cytometry

Single cell suspensions were prepared from homogenized snail tissues in tris EDTA buffer pH 7.5. One hundred microlitter of cell suspension was blocked with 200µl of protein block solution (2% BSA; Merck KGaA) for 20min at room temperature. Then samples were treated using 200µl (0.05% saponin in PBS) for 20min at room temperature, as directed in the instructions for the IntraPrep permeabilization reagent. The samples were washed twice with PBS/saponin. They were incubated with 10µl of primary antibody (primary antibody (mouse anti LC3B, (1251A{NBP2-46892) diluted (1:20 in 0.05% saponin) for 15min in the dark at room temperature. Samples were washed twice with PBS/0.05%-saponin and centrifuged at 2000rpm for 5min. The supernatant was discarded and resuspended in 0.5ml of 0.05% saponin (diluted in PBS and vortex briefly). Afterward, 3ml of PBS was added per tube and spinned for 5min at 1600rpm, then the supernatant was discarded by tipping the liquid off (there should still be a small amount of liquid left in the tube, around 50ul) 10µl of secondary Polyclonal antibody (IgG) goat anti mouse IgG-RPE, Southern Biotech 1030-09S labeled with flourchrome (FITC) diluted at a 1:20 ratio in 0.05% saponin. 1µl of the 1:20 dilution was added to each tube, vortexed and incubated for 20min at room temperature in the dark. Cells were washed twice with PBS/BSA as mentioned above. Finally, the labeled cells were fixed with 200µl of 0.5% paraformaldehyd at 37˚C overnight and acquired on

Accuri C6 flow cytometer (Becton Dickinson, Sunnyvale, CA, USA). Histogram derived from flow cytometry was obtained with a computer program AccuriC6 software. Staining values were calculated as the percentage of the total number of cells counted. All experiments were repeated three times. Note the -ve control of stain of the same sample was treated with saponin only without LC3B to improve the cut off real +ve stain.

8. Histological study

Ten adult snails (7- 9mm) were subjected for 3 days (exposure) to *J. gossypiifolia* extract (LC_{10}, LC_{25}). The snails were then taken from the testing solution to study the histopathology in the head foot region, reproductive system, and digestive gland of treated versus untreated snails **(Mohamed & Saad, 1990)**. Each group had three replicates. After gently separating the soft parts of the snails from the shells, they were fixed in Bouin's solution, coated with paraffin wax, sectioned, and stained in hematoxylin as well as eosin. The sections have been investigated by a light microscope and were photographed with a Zeiss Video camera.

9. Comet assay

After *B. alexandrina* snails (7- 9mm) were exposed to LC_{10} and LC_{25} of *J. gossypiifolia* extract for 3 days, the head-feet of ten snails from every group were separated and stored at $-80^{o}C$ until they were used. The comet assay for detecting DNA breaks was measured using the single-cell gel assay, as described by **Grazeffe *et al.* (2008)**. The slides were independently coded and scored.

10. Statistical analysis

Using SPSS v. 17.0 for Windows (SPSS Inc. 2008), the sub-lethal concentration (LC_{10} and LC_{25}) and lethal concentrations values were calculated by applying the regression equation analysis to probit transformed mortality data **(Finney, 1971)**. The percentages were analyzed using chi-square, and the means were compared using the student's t test **(Goldstein & Goldstein, 1967)**.

RESULTS

1. Toxicity of *Jatropha gossypiifolia* extract

From Table (1), it is observed that the *Jatropha gossypiifolia* extract is lethal for *B. alexandrina* snails (LC_{50} at 51mg/ L and LC_{90} at 66.17mg/ L).

Table 1. Molluscicidal activity of *J. gossypiifolia* ethanolic extract for adult *Biomphalaria alexandrina* snails after 24 hours of exposure, followed by 24 hours for recovery

LC_{10} (mgL^{-1})	**LC_{25} (mgL^{-1})**	**LC_{50} (mgL^{-1})**	**confidence limits of LC_{50} (mgL^{-1})**	**LC_{90} (mgL^{-1})**	**Slope**
35.8	43	51	45.15 - 56.17	66.17	1.27

2. Larvicidal activity of *J. gossypiifolia* ethanolic extract

Mortality rate of *Schistosoma mansoni* miracidiae: *Jatropha gossypiifolia* extract exhibited a larvicidal activity, where, after 60 min of exposure of miracidiae to LC_{10} of plant extract, mortality rates of *S. mansoni* miracidia were observed (40%), while the miracidial mortality rate for those of LC_{25} was 66.6%, compared to only 5% for the control group. Furthermore, prolonging miracidial exposure to LC_{10} and LC_{25} concentrations resulted in 100% mortality after 90 and 75min, respectively, compared to 6.6 and 10% for the control group (Fig. 1a). For the mortality rate of *S. mansoni* cercariae, the mortality rate of cercariae after 120 min of exposure to LC_{10} and LC_{25} was 100% compared to 10% of control group (Fig. 1b).

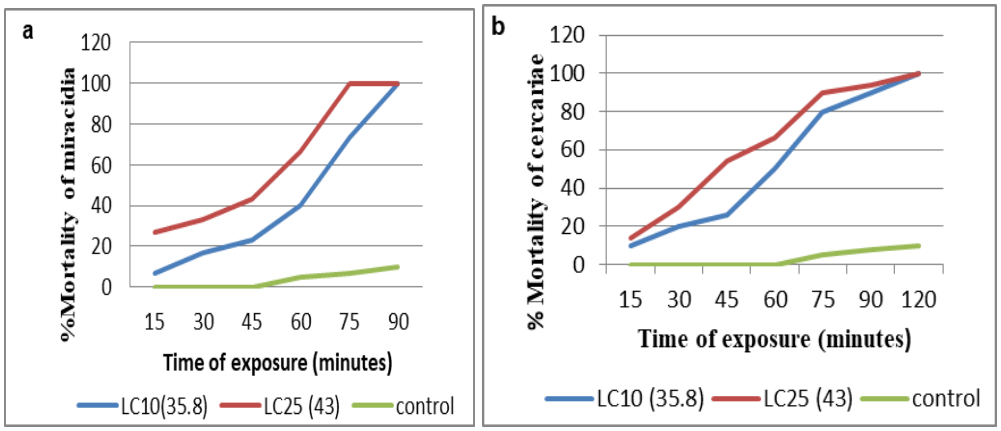

Fig. 1. The miracidicidal and cercaricidal activities of *J. gossypiifolia* ethanolic extract

3. Effect of sub-lethal concentrations of *J. gossypiifolia* extract on hemocytes

Fig. (2) shows a noticeable significant decrease ($P< 0.05$) in total hemocytes count in all treated snails with *J. gossypiifolia* ethanolic extract comparable to the control group. The normal *B. alexandrina* hemolymph contains three types of different cells classified according to their shape and granular contents. These cells are hyalinocytes, amoebocytes and granulocytes. Hyalinocytes have transparent cytoplasm (Fig. 3a). Granulocytes have granules in the cytoplasm (Fig. 3b). Amoebocytes have clear pseudopodia (Fig. 3c). After snails exposure to *J. gossypiifolia* extract at sub-lethal concentration (LC_{25}), the most affected cells are the hyalinocyte which showed ramified membrane with short filopodia (Fig. 4a- c), shrinkage nucleus and vacuoles (Fig. 4d), sometimes with eccentric nucleus (Fig. 4e). Granulocytes have more dense granules, two nuclei, and form long filopodia (Fig. 4f- g). Amoebocytes were granulated and other vacuolated (Fig. 4h- j). However granulocytes percentage of treated snails were lower than that of the control group, and both hyalinocytes and amoebocytes percentages of treated snails were higher than that of the control (Table 2).

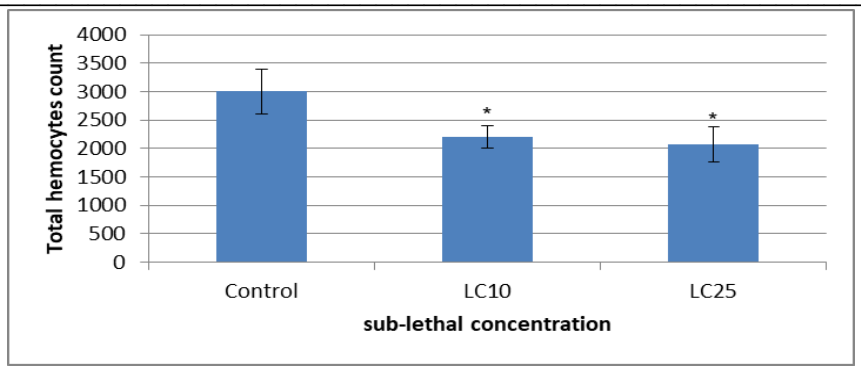

Fig. 2. Total hemocyte count of *B. alexandrina* snails exposed to *J. gossypiifolia* extract for 3 days (data are presented as mean ± standard deviation), * Significantly different at $P < 0.0.5$

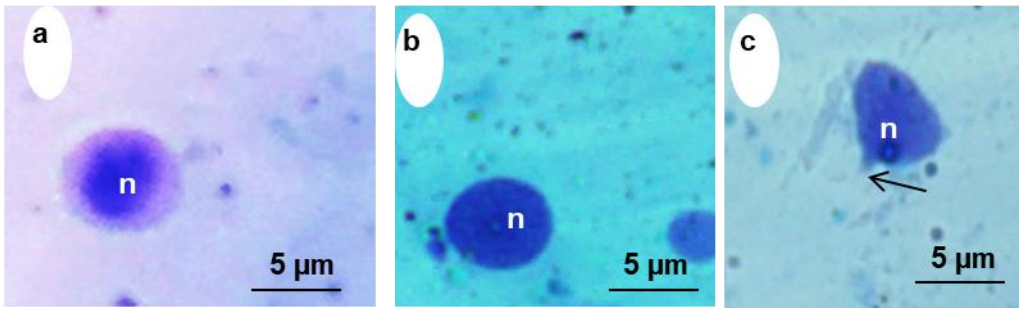

Fig. 3. Light photomicrographs of hemocytes from control *B. alexandrina* snails showing: **(a)** A hyalinocyte, **(b)** Granulocyte, and **(c)** An amoebocyte with pseudopodia (arrow) , (n) Nucleus

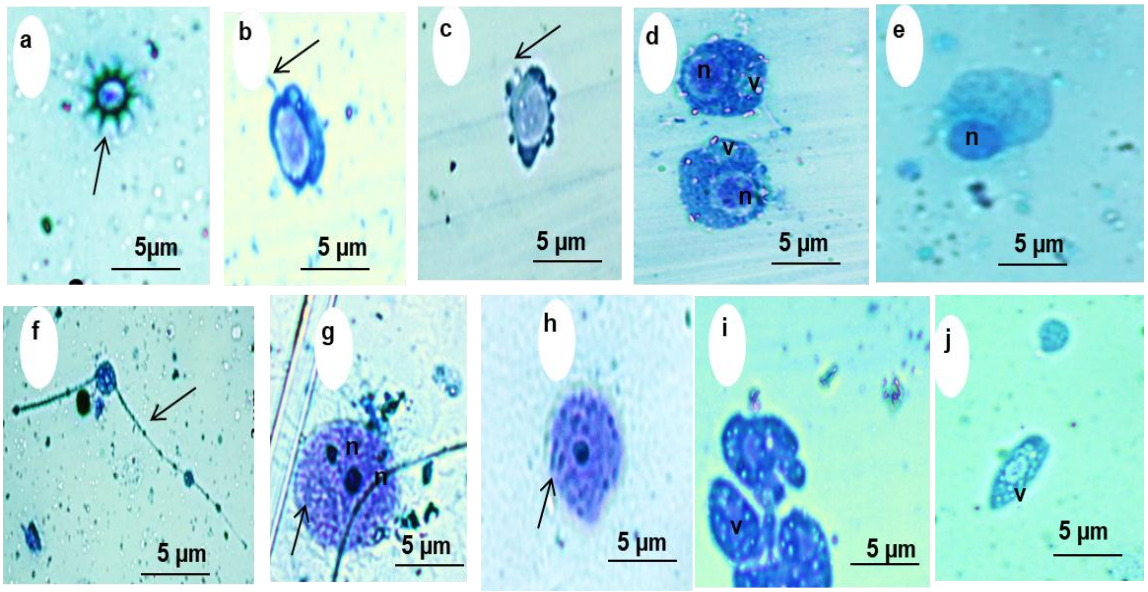

Fig. 4. Light photomicrographs of hemocytes from *B. alexandrina* snails exposed to LC_{25} of *J. gossypiifolia* for 3 days showing: **(a- e)** hyalinocyte with **(a- c)** many short filopodia (arrow), **(d)** shrinkage nucleus (n) and vacuoles (v), **(e)** protruded nucleus. **(f- g)** Granulocyte with **(f)** long filopodia (arrow), **(g)** two nuclei (n) and more dense granules (arrow). **(h- j)** Amboecyte with **(h)** granules, **(i-j)** vacuoles

Table 2. Differential hemocyte percentages in *B. alexandrina* snails exposed to *J. gossypiifolia* extract for 3 days

	Granulocytes	Hyalinocytes	Amoebocytes
Control	74	20	6
LC_{10}	48***	40**	12
LC_{25}	36***	50***	14*

* Significantly different at $P< 0.05$, ** *at* $P< 0.01$, *** *at* $P< 0.001$.

4. Flow cytometry

Flow cytometric analysis illustrated the measurement of autophagy process that occur in *B. alexandrina* snails tissues. A significant increase ($P< 0.01$) in the expression of LC3B in treated snail groups at concentration LC_{10} of *J. gossypiifolia* ethanolic extract compared to un-treated control group (Figs. 5, 6). The concentration of the extract increased at LC_{25}, LC3B expression was significantly increased ($P< 0.001$) compared to concentration LC_{10} and un-treated control groups (Figs. 5, 6).

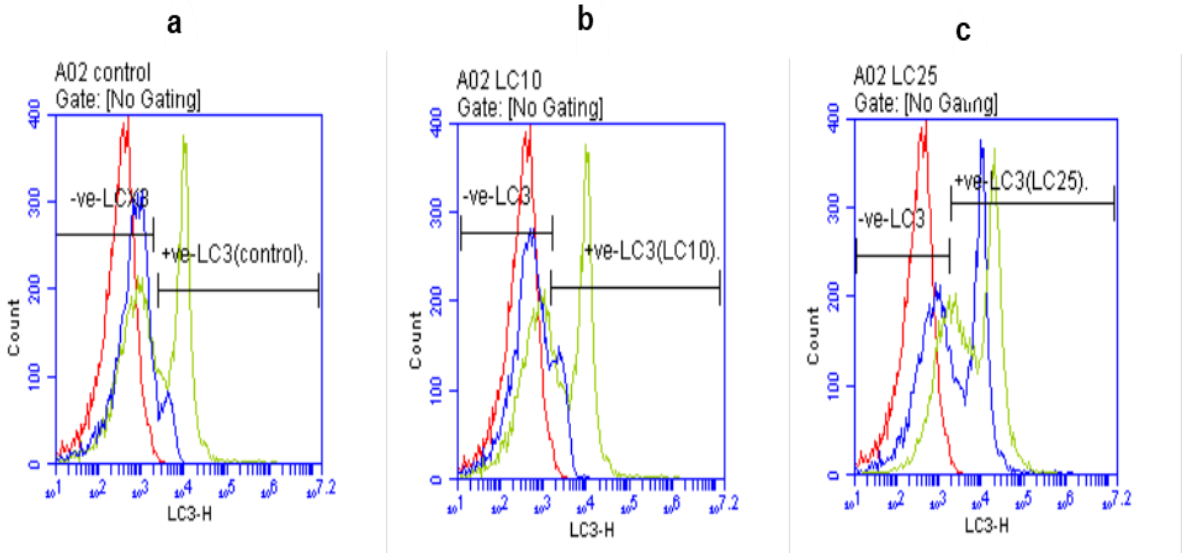

Fig. 5. Flow cytometry overlapping histogram was performed with intracellular LC3B-Antibody on *B. alexandrina* snails tissues showing the expression of LC3B in **(a)** untreated control cells, **(b)** treated cells with LC_{10} or **(c)** treated cells with LC_{25}. An intracellular stain was performed LC3B antibody (blue) and a matched isotype control polyclonal IGg (green) and the negative control of stain (treated cells with saponin only red)

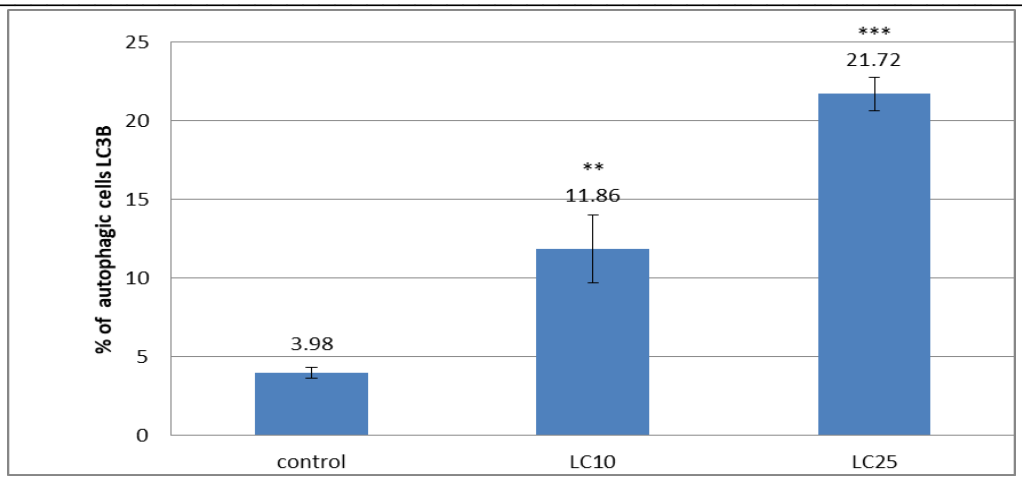

Fig. 6. Percent of autophagic cells LC3B prepared from *B. alexandrina* snails tissues of untreated control and autophagic cells treated with the two concentrations LC_{10} and LC_{25}. Data presented as mean ± standard deviation. ** significant at $P< 0.01$, *** at $P< 0.001$

5. Effect of *J. gossypiifolia* on the head-foot, digestive and hermaphrodite glands of adult *B. alexandrina* snails

The normal head-foot area of *B. alexandrina* contains a layer of ciliated cylindrical epithelial cells, unicellular mucous glands, and it is supported by strong connective tissue and longitudinal muscle fibers that compose the majority of the foot muscles (Fig. 7a). Chronic exposure to sublethal concentrations (LC_{10}) of *J. gossypiifolia* caused histopathological changes, such as damaged cilia, focal destruction of the epithelial covering and muscular fiber, and the degeneration of connective tissue, shrinkage mucus gland, and formation of vacuoles (Fig. 7b). Snail exposure to *J. gossypiifolia* (LC_{25}) caused necrotic alteration, which resulted in fiber tissue breakdown, increased empty spaces, and the creation of large vacuoles (Fig. 7c).

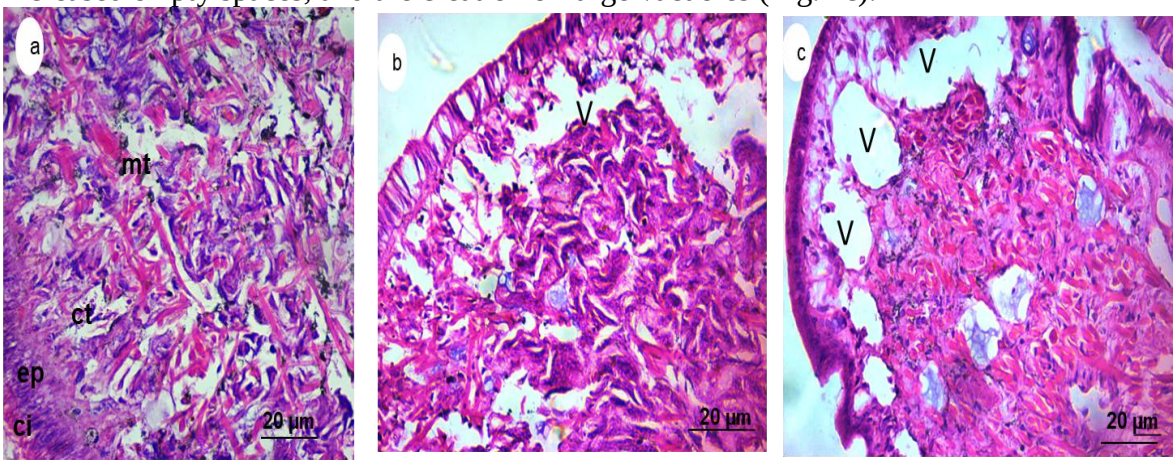

Fig. 7. Light micrographs of a section through the head-foot showing: **(a)** Ciliated (ci) epithelial cells (ep) and dense connective tissue (ct) followed by muscular tissue (mt) in normal snails, **(b)** The snails exposed to LC_{10} of *J. gossypiifolia* extract show epithelial cells lost its cilia and nucleus, degeneration of connective tissue, and formation of vacuole (v), and **(c)** The snails exposed to LC_{25} of plant extract show large vacuolations (v) and fiber tissue breakdown in the head-foot

B. alexandrina digestive glands were made up of a number of tubes linked together by a connective tissue and padded with two types of cells in one layer: digestive cells (the most common cell type), and secretory cells (Fig. 8a). Exposure of the snails to LC_{10} of *J. gossypiifolia* extract showed that several digestive cells ruptured and vacuolated, as well as secretory cells and connective tissue degeneration (Fig. 8b). At LC_{25}, the most severe damage occurred, where the most cells lost their walls, vacuolated, and some degraded, and the lumen expanded (Fig. 8c).

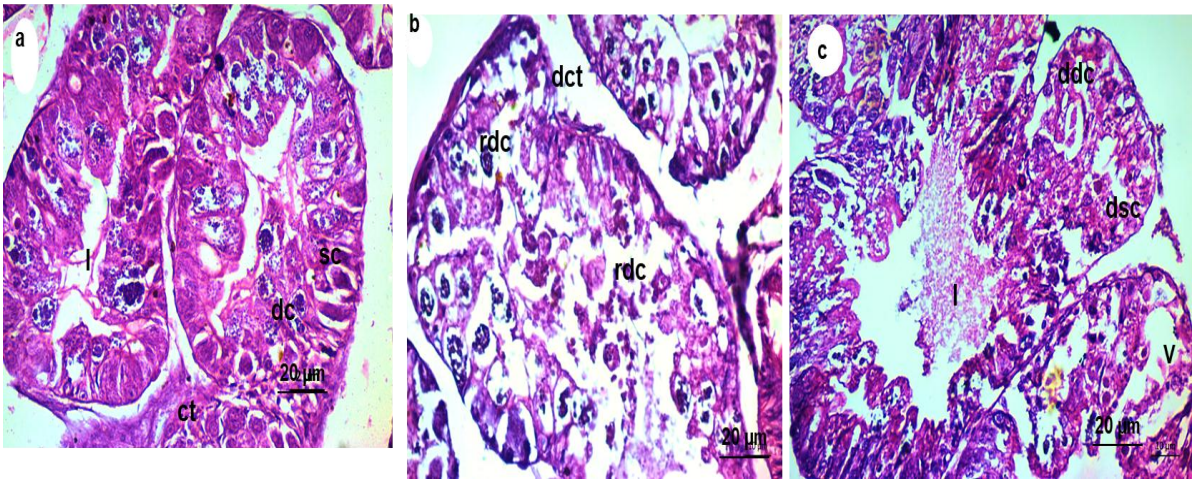

Fig. 8. Light micrographs of a section through the digestive gland of *Biomphalaria alexandrina* showing: **(a)** Control group, **(b)** Snails exposed to LC_{10} of *J. gossypiifolia* extract, and **(c)** Snails exposed to LC_{25} concentration of the extracted plant for 3 days. l: Lumen, sc: Secretory cells, dc: Digestive cell, ct: Connective tissue, rdc: Ruptured digestive cells, dct: Degenerative connective tissue, ddc: Degenerated digestive cells, dsc: Degenerated secretory cells, and v: Vacuolated digestive cell

The adult *B. alexandrina* hermaphrodite glands are made up of cub-shaped acini that are joined to each other by a connective tissue. Male reproductive cells defined into primary, secondary spermatocytes and sperms. Fully formed sperms were discovered in the lumen or adhering to sertoli cells. The female oogenic cells fill the acinar lumen as primary, secondary oocytes and mature ova (Fig. 9a). Snails treated with sub-lethal concentration LC_{10} of extracted *J. gossypiifolia* induced an increase in the number of dead ova with malformed forms, destruction of sperms, and connective tissue degeneration (Fig. 9b). The exposure of the snails to LC_{25} of *J. gossypiifolia* causes significant damage to gonadal cells (Fig. 9c), where the oocytes were atretic in which the oocytes were destroyed, not developed to mature ova, and surrounded by phagocytic hemocytes. The sperms were degenerated and most of the spermatogenic disappeared (Fig. 9c).

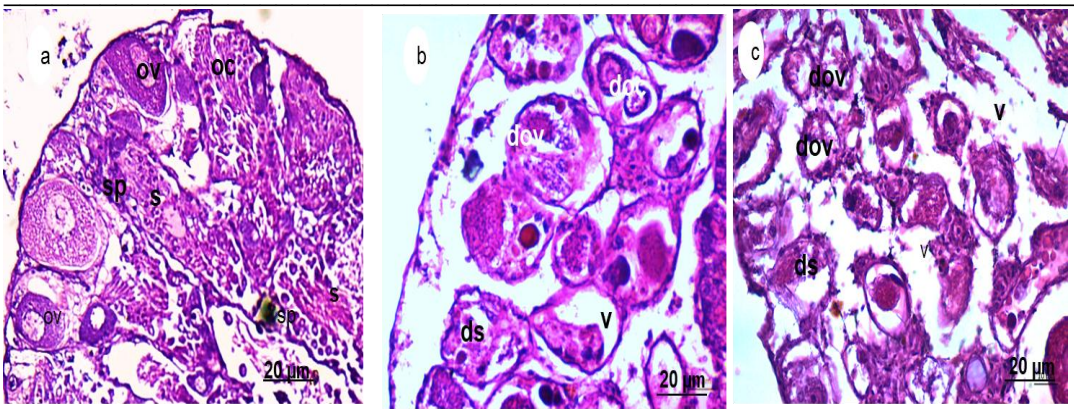

Fig. 9. Light micrographs of a section through hermaphrodite gland of adult *B. alexandrina* snails showing: **(a)** Normal snails, **(b)** Snails exposed to LC_{10} of *J. gossypiifolia* extract, and **(c)** Snails exposed to LC_{25} of plant extract. Ov: Mature ovum, s: Sperms, sp: Spermatocytes, oc: Oocyte, dov: Degenerated ovum, doc: Degenerated oocyte, ds: Degenerated sperms, and v: Vacuole

6. Effect of *J. gossypiifolia* extract on DNA of *Biomphalaria alexandrina* snails

The current results showed that after snails exposure to *J. gossypiifolia* sub-lethal concentrations (LC_{10} and LC_{25}), there were DNA breakage where the percentage of the comet, tail length, percent of DNA in tail and tail moment were significantly increased (P<0.05, 0.01 and 0.001) compared to control snails (Table 3 & Fig. 10).

Table 3. DNA breakage after *B. alexandrina* exposure to *J. gossypiifolia* extraction

	% of comet	**Tail length (px)**	**%DNA in tail**	**Tail moment**
Control	11.4 ± 0.51	7 ± 1	2.61±1.2	0.5±0.17
LC_{10}	15.2 ±0.26***	10.95±1.94*	4.74±1.8	1.1±0.02**
LC_{25}	21.8± 0.76 ***	16± 2.3**	13.3±3.3**	2±0.06***

*= Significantly different at P< 0.05, ** at P< 0.01 , *** at P< 0.001.

1 px= 0.264mm.

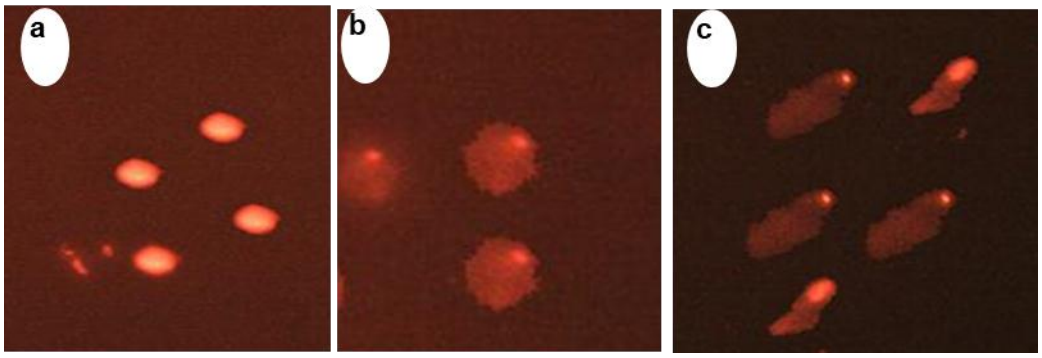

Fig. 10. Light micrographs show the extent of DNA migration by comet assay in **(a)** Normal *B. alexandrina*, and **(b, c)** Snails exposed to sub-lethal concentrations of *J. gossypiifolia* (LC_{10}, LC_{25}) for 3 days, with high DNA migration than normal snails

DISCUSSION

Jatropha gossypiifolia leaves are considered to have anti-inflammatory and insecticidal properties, moreover the latex is bactericidal and molluscicidal **(Wu *et al.*, 2019)**. According to the World Health Organisation, LC_{50} of any molluscicide shouldn't go above 100ppm **(WHO, 2019)**. In the present study, the molluscicidal activity of *J. gossypiifolia* extract on *B. alexandrina* snails after 24 hours of exposure, followed by 24 hours of recovery, revealed an LC_{50} at 51mg L^{-1}. **Filho *et al.* (2014)** indicated the molluscicidal action of *J. gossypiifolia* extract (LC_{50}> 100mg L− 1) and attributed its impact to the occurrence of alkaloids and saponins. According to its phytochemical constitution, the components determined from different parts of *J. gossypiifolia* were saponins, tannins, flavonoids, alkaloids, lignoids, coumarins, phenols, steroids, and terpenoids **(Zhang *et al.*, 2009)**. The plants with more content of tannin and saponin could be more effective in mollusk control. Saponins' molluscicidal activity is owing to their unique detergent impact on snail epithelial tissues **(De Souza *et al.* 2014, Abou El-Nour , 2021)**.

The majority of the plants tested for *Schistosoma* cercariae and miracidia were generally efficacious at lower concentrations than their molluscicidal counterparts. In terms of the miracidicidal and cercaricidal activities, the current study indicated that 100% death rate for miracidia and cercarial after 75 and 120 minutes exposure to the concentration of LC_{25} of *J. gossypiifolia*, respectively. This is consistent with the findings of **Sulaiman *et al.* (1988)** who reported that, LC_{100} (10mg L^{-1}) of plant *Balanites aegyptiaca* has miracidicidal and cercaricidal activities due to the existence of the saponin fraction. Hydroethanolic extract of *Pedilanthus tithymaloides* stem bark killed all cercariae within 60min of incubation at various concentrations from 125 to 1000µg/ mL **(Nkondo *et al.*, 2022)**.

The immune response of *Biomphalaria* snail is mediated by both cellular effectors called humoral factors **(Mitta *et al.*, 2012)** and hemocytes **(Nacif-Pimenta *et al.*, 2012)**, which operate independently or together to combat invading microorganisms or parasites. Snail hemocytes are involved in a variety of mollusk physiological processes **(Fried, 2016)**. The hemocytes have an important role in phagocytosis and encapsulation reactions. *B. alexandrina* hemocyte monolayers revealed three distinct cell morphologies: granulocytes, hyalinocytes, and amoebocytes **(Mansour *et al.*, 2021, 2023)**. Granulocytes are the common type of cells and perform the majority of phagocytosis although hyalinocytes have a role in wound repair and amoebocytes create pseudopodia **(Barçante *et al.*, 2012)**. In the current work, the light microscopy of the hemocyte of *B. alexandrina* snails exposed to LC_{25} of *J. gossypiifolia* extract revealed some morphological changes, with the granulocytes percentage of exposed snails being lower than that of the untreated group, while the percentages of hyalinocytes and amoebocytes were higher than the control group. Granulocytes had more dense granules and developed long filopodia.

Hyalinocytes showed a shrinking nucleus and formed short filopodia. **Ibrahim *et al.* (2019)** found that following exposure to butralin, glyphosate isopropyl ammonium, or Pendimethalin herbicides, hyalinocytes showed a shrunken nucleus and granulocytes developed pseudopodia with many engulfed particles. Amoebocytes have a significant role in phagocytosis and encapsulation processes and have dense granules that have been regarded as lysosomes containing hydrolase enzymes that perform intracellular digestion of extracellular materials **(Barçante *et al.*, 2012)**. Several pseudopodia, or filopodia, were expanded by the hemocytes, causing cell-to-cell clustering **(Mahilini & Rajendran, 2008)**. Pseudopodia participates in the phagocytic process in the atrium to remove these foreign particles and cell debris **(Donaghy *et al.*, 2010)**, but hyalinocytes are largely important for tissue healing, coagulation, and immunological response **(Yoshino & Coustau, 2011)**. Moreover, hyalinocytes can phagocytose to a lesser amount than granulocytes **(Osman *et al.*, 2003)**. In the present results, the plant extract induced more damage in snail tissues; this explain the higher percentage of hyalinocytes to repair it.

In the current study, there was a detectable decrease in total hemocyte counts in all snails treated with *J. gossypiifolia* ethanolic extract, as compared to untreated snails. This decrease could be due to snails becoming weak and changing in physiological parameters, as a result of continuous exposure to *J. gossypiifolia* extract, a direct action of the plant extract on hemocytes, or the flow of the originated hemocytes with the hemolymph to concentrate themselves at sites of tissue damage to repair it. On contrast, the number of circulating hemocytes from *Biomphalaria glabrata* infected with *S. mansoni* and exposed to *E. milii* latex was significantly higher than the uninfected and exposed snail groups **(Friani *et al.*, 2022)**. Furthermore, *Biomphalaria glabrata* infected with *E. milii* latex and exposed to 1.0mg/ L showed enhanced hemocytes proliferation in the tentacles, mantle, digestive gland and kidney. Moreover, the sporocysts were destroyed in the digestive gland and the kidney due to granulomatous formation around it **(Friani *et al.*, 2022)**.

Autophagy is one of the primary processes for maintaining cellular homeostasis; it uses cellular machinery to degrade and recycle old proteins and organelles **(Oczypok *et al.*, 2013)**. This process can promote cells to survive during times of stress, but excessive autophagy can also cause cell death **(Degterev & Yuan, 2008)** and apoptosis **(Han *et al.*, 2015)**. LC3A/B and p62 expression levels have been used as autophagy markers **(Schmitz *et al.*, 2016)**. The current study found a significant increase in the expression of LC3B in the treated snail groups, indicating enhanced autophagosome formation and consequently an increased autophagy process to remove damaging material caused by plant extract. Morevoer, the plant has a potent molluscicidal agent for *B. alexandrina* snails, similar to the findings of **Shao *et al.* (2016)**, who reported that autophagic cell death can be induced by natural pesticides. Our findings are consistent with those of **Xu *et al.* (2017)** who used Pyrethrum extract to characterize the insecticidal mechanism in *Spodoptera frugiperda* and revealed cell death via autophagy and up-regulation of LC3-II

and beclin-1 expression. **Sethuraman *et al.* (2017)** proved that curcumin has a cytotoxic effect, as well as inducing autophagic cell death in *Spodoptera frugiperda* cells .

The current results found histological changes in the head-foot region of snails subjected to *J. gossypiifolia* extract, such as damaged cilia, focal destruction of the epithelial covering and muscle fiber, degeneration of connective tissue and mucus gland, and the presence of vacuoles. This finding coincides with that of **Ibrahim and Abdel-Tawab (2020)** who demonstrated that, the ethanol extract of *Cystoseira barbata* caused histopathological alterations in the head-foot region of *B. alexandrina* snails. **Wang *et al.* (2018)** stated that damage to the mantle, foot plantaris, and tentacles could cause the snail to die.

According to the current findings, the digestive glands of normal *B. alexandrina* snails are made up of several tubular structures that are lined by two different cell types in one layer: digestive cells (the most common cell type), and secretory cells. When the snails were exposed to LC_{10} from the extracted *J. gossypiifolia*, it was observed that some digestive cells had ruptured and vacuolated, as well as connective tissue and secretory cells experienced a degradation. The most serious harm was caused by raising the extracted plant's content. These results match those of **Saad *et al.* (2017)** who detected the vacuolization and rupturing of the digestive cells in the digestive glands of snails treated with LC_{25} (280mg L-1) of *Cystoseira barbata* aqueous extract. They attributed these changes to the existence of saponins, phenolic compounds, and alkaloids, which increased and accelerated the penetration rate of algal products via the epidermis of snails, hence increasing their detrimental effects **(Saad *et al.*, 2019)**.

After exposure of snails to the extracted *J. gossypiifolia* in the current study, the histological evaluation of the exposed hermaphrodite gland revealed dead ova, destruction of sperms, and degeneration of connective tissue. These findings agree with those of **Mossalem *et al.* (2013)**, who observed the full death of gametogenic cells and severe damage to hermaphrodite gland tissues of *B. alexandrina* snails exposed to the anthelmintic plant derivative (artemether).

The comet assay, which identifies DNA single-strand breaks by measuring DNA migration from immobilized individual cell nuclei, is one of the most sensitive techniques for detecting DNA abnormalities **(Fairbairn *et al.*, 1995)**. **Sarkar *et al.* (2015)** used it on a variety of aquatic animals, including gastropods. This approach is regarded as one of the most promising genotoxicity indicators since it requires a minimal number of cells and identifies damage at the single-cell level **(Bolognesi & Cirillo, 2014)**. The current findings demonstrated that DNA breaks where the percentage of the comet, tail length, percent of DNA in the tail in addition to the tail moment were all significantly higher compared to control snails. Furthermore, the researchers hypothesized that such DNA strand breaks were caused by an oxidative stress or a covalent binding to DNA (**Ye *et al.*, 2012; Bolognesi & Cirillo, 2014; Sarkar *et al.*, 2015)**. In line with the current findings, **Ibrahim and Ghoname (2018)** elucidated that, the exposure of *B. alexandrina* snails to

aqueous extracts of *Anagalis arvensis* leaves caused DNA breakage, as shown by the comet assay. According to **Ye *et al*. (2012)**, the number of DNA strand breakages increased following exposure to DNA-damaging agents compared to the control.

CONCLUSION

Based on the current findings, the ethanolic extract of *J. gossypiifolia* can be used as a highly effective molluscicidal agent against *S. mansoni* free larval stages (miracidia and cercariae) and its intermediate host. Therefore, it can be used for the application in *schistosomiasis* control to reduce water pollution while conserving non-target creatures.

REFERENCES

Abdul-Salam, J.M. and Michelson, E.H. (1980). *Biomphalaria glabrata* amoebocytes, effect of *Schistosoma mansoni* infection on in vitro phagocytosis. Journal of Invertebrate Pathology, 35:241–248.

Abou-El-Naga, I.F. ; Sadaka, H.A. ; Amer, E.I.; Diab, I.H. and Khedr, S.I. (2015). Impact of the age of *Biomphalaria alexandrina* snails on *Schistosoma mansoni* transmission: modulation of the genetic outcome and the internal defence system of the snail. Mem Inst Oswaldo Cruz, 110:585–595

Abou El-Nour, M.F. (2021). Evaluation of molluscicidal, miracicidal and cercaricidal activities of crude aqueous extracts of *Origanum majorana, Ziziphus spina-christi* and *Salvia fruticosa* on *Schistosoma mansoni* and *Schistosoma haematobium*. Egyptian Journal of Aquatic Biology & Fisheries, 25(2): 913 – 933

Abreu, I.C.; Marinho, A.S.; Paes, A.M.; Freire, S.M.; Olea, R.S.; Borges, M.O.; et al. (2003). Hypotensive and vasorelaxant effects of ethanolic extract from *Jatropha gossypiifolia* L. in rats. Fitoterapia, 73:650–7

Adewunmi, C.O. (1984). Natural products as agents of schistosomiasis control in Nigeria: a review of progress. Int J Crude Drug Res., 22:161-6.

Barçante, T.A.; Barçante, J.M.; Fujiwara, R.T. and Lima, W.S. (2012). Analysis of circulating haemocytes from *Biomphalaria glabrata* following *Angiostrongylus vasorum* infection using fow cytometry. J Parasitol Res., 1–6.

Bolognesi, C. and Cirillo, S. (2014). Genotoxicity biomarkers in aquatic bioindicators, Current Zoology, 60(2): 273–284.

Dai, J.R.; Li, Y.Z.; Wang, W.; Xing, Y.T.; Qu, G.L. and Liang, Y.S. (2015). Resistance to niclosamide in Oncomelania hupensis, the intermediate host of *Schistosoma japonicum*: should we be worried? Parasitology, 142:332–40.

Degterev, A.; Yuan, J. (2008) Expansion and evolution of cell death programmes, Nat. Rev. Mol. Cell Biol., 9: 378–390.

De Souza, B.A.; da Silva, L.C.; Chicarino, E.D. and Bessa, E.C., de, A. (2014). Phytochemical and molluscicidal activity of *Mikania glomerata Sprengel* (Asteraceae) in different life stages of *Subulina octona* (Mollusca, Subulinidade). Brazilian Arch Biol Technol., 57:261–268. https://doi.org/10.1590/S1516-89132014000200015

Donaghy, L.; Hong, H.K.; Lambert, C. *et al*. (2010). First characterisation of the populations and immune-related activities of hemocytes from two edible gastropod species, the disk abalone, Haliotis discus and the spiny top shell, Turbo cornutus. Fish Shellfish Immunol., 28:87–97.

Eissa, M.M.; El Bardicy, S. and Tadros, M. (2011). Bioactivity of miltefosine against aquatic stages of *Schistosoma mansoni, Schistosoma haematobium* and their snail hosts, supported by scanning electron microscopy. Parasit. Vectors, 4, 73.

Eveland, L.K. and Haseeb, M.A. (2011). Laboratory rearing of *Biomphalaria glabrata* snails and maintenance of larval schistosomes in vivo and in vitro. In *Biomphalaria* snails and larval trematodes. Springer: New York, NY, USA, 33–55.

Fairbairn, D.W.; Olive, P.L. and O'Neill, K.L. (1995). The comet assay: A comprehensive review. Mutat. Res., 339: 37-59.

Felix-Silva, J.; Giordani J.; Brandt R.; Silva, J.r, Arnóbio, A.; Zucolotto, Silvana, M.; Fernandes, P. and De Freitas, M (2014). "*Jatropha gossypiifolia* L. (Euphorbiaceae): A Review of Traditional Uses, Phytochemistry, Pharmacology, and Toxicology of This Medicinal Plant". Evidence-based complementary and alternative medicine. 369204. doi:10.1155/2014/369204.

Filho, P. A.; França, C.R.; Oliveira, D.S.; Mendes, R.J.; Gonçalves, J.R.S and Rosa, I.G. (2014). Evaluation of the molluscicidal potential of hydroalcoholic extracts of *Jatropha gossypiifolia Linnaeus*, 1753 on *Biomphalaria glabrata* (Say, 1818). Revista do Instituto de Medicina Tropical de São Paulo, 56: 505–510.

Finney, D.J. (1971). Probit analysis, 3rd edn. Cambrige University Press, Cambrige

Friani, G.; Costa, A. V.; Mota, E.; Marta, J. F.; Souza, S.; Figueiredo, M.; Gôlo, S. P. and Mello-Silva. (2022): Destruction of *Schistosoma mansoni* sporocysts in

Biomphalaria glabrata after phytochemical exposure, An Acad Bras Cienc, 94(2):e20190676. doi: 10.1590/0001-3765202220200620

Fried, B. (2016). An update on hemocytes in *Biomphalaria* snails. J Hematol Oncol Res., 2: 26-30. https://doi.org/10.14302/issn.2372- 6601.jhor-14-401.

Goldstein, A. and Goldstein, A. (1967). Biostatistics: an introductory text.

Grazeffe, V.S.; De Freitas, T.L.; De Sa, P.A. *et al*. (2008). Establishment of the comet assay in the freshwater snail *Biomphalaria glabrata* (Say, 1818). Mutat Res Toxicol Environ Mutagen, 654:58–63.

Hamed, M.A. (2010). Strategic control of schistosome intermediate host. Asian J. Epidemiol., 3(3): 123-140.

Han, K.; Kim, J. and Choi, M. (2015). Autophagy mediates phase transitions from cell death to life. Heliyon 1: e00027.

Ibrahim, A.M and Abdel Tawab,H. (2020). *Cystoseira barbata* marine algae have a molluscicidal activity against *Biomphalaria alexandrina* snails supported by scanning electron microscopy, hematological and histopathological alterations, and larvicidal activity against the infective stages of *Schistosoma mansoni*. Biologia 75:1945–1954

Ibrahim, A. M.; Ahmed, A. K.; Bakry, F. A.; Rabei, I. and Abdel-Ghaffar, F. (2019). Toxicological impact of butralin, glyphosate-isopropylammonium and pendimethalin herbicides on physiological parameters of *Biomphalaria alexandrina* snails. Molluscan Research, 39(3), 224–233.

Ibrahim, A.M. and Ghoname, S.I. (2018). Molluscicidal impacts of *Anagallis arvensis* aqueous extract on biological, hormonal, histological and molecular aspects of *Biomphalaria alexandrina* snails. Exp Parasitol., 192:36–41.

Jia, T.W.; Wang, W.; Sun, L.P.; Lv, S.; Yang, K.; Zhang, N.M.; *et al*. (2019). Molluscicidal efectiveness of Luo-Wei, a novel plant-derived molluscicide, against *Oncomelania hupensis*, *Biomphalaria alexandrina* and *Bulinus truncatus*. Infect Dis Poverty, 8:27.

King, C.H. and Bertsch, D. (2015). Historical perspective: snail control to Prevent Schistosomiasis. PLoS Negl Trop Dis. 9(4): 1-6 e0003657. doi: 10.1371/journal.pntd.0003657.

Levine, B. and Kroemer, G.(2019). Biological functions of autophagy genes: a disease perspective. Cell, 76: 11-42.

Mahilini, H.M. and Rajendran, A.(2008). Categorization of hemocytes of three gastropod species *Trachea vittata* (Muller), *Pila globosa* (Swainson) and *Indoplanor bisexustus* (Dehays). J. Invertebr. Pathol., 97: 20–26.

Mansour, S.M.; Sayed, S. and Abel- Wareth. (2021). Efect of methyl gallate on immune response of *Biomphalaria alexandrina* (Ehrenberg, 1831) snails to infection with *Schistosoma mansoni* (Sambon, 1907). Parasitol Res., 120:1011–1023.

Mansour, S.M.; Taha, R.G.; Youssef, A.A. (2023). Assessment of *Amphora coffeaeformis* and *Scenedesmus dimorphus* algae as immunostimulant agents on *Biomphlaria alexandrina* snails against Schistosoma mansoni. Biologia, 78:737–748.

Martins, G.V.; Alves, D.R.; Viera-Araújo, F.M.; Rondon, F.; Braz-Filho, R.; Morais, S.M. (2018). Chemical Study and Evaluation of Antioxidant, Anti-acetylcholinesterase and Antileishmanial Activities of Extracts from *Jatropha gossypifolia L*. Revista Virtual de Química, 10: 21–36.

Martins-Souza, R.L.; Pereira, C.A.; Martins -Filho, O.A.; Coelho, P.M.; Correa, A. (2006). Differential lectin labeling of circulating hemocyte from *Biomphalaria glabrata* and *Biomphalaria tenagophila* resistant or susceptible to *Schistosoma mansoni* infection. Mem Inst Oswaldo Cruz, 101:185–192. https://doi.org/10. 1590/S0074-02762006000900029

Mitta, G.; Adema, C.M.; Gourbal, B.; Loker, E.S.; Theron, A. (2012). Compatibility polymorphism in snail/schistosome interactions: From field to theory to molecular mechanisms. Dev Comp Immunol., 37(1):1–8.

Mizushima, N. and Murphy, L (2020). Autophagy Assays for Biological Discovery and Therapeutic Development. Trends in Biochemical Sciences Vol. 45 (12): 1080-1093 https://doi.org/10.1016/j.tibs.2020.07.006.

Mohamed, S.H. and Saad, A.A. (1990). Histological studies on the hermaphrodite gland of Lymnaea caillaudi and *Biomphalaria alexandrina* upon infection with certain larval trematodes. Egypt J Histol., 13:47–53.

Morais, V.H.; Filho, R.L.; Dos Santos, J.A.; *et al.* (2022). Use of *Biomphalaria glabrata* as a bioindicator of groundwater quality under the influence of norm. J Environ Radioact, 242. https://doi.org/10.1016/j.jenvrad.2021.106791.

Mossalem, H. S.; Abdel-Hamid, H.; El-Shinnawy, A. N. (2013). Impact of artemether on some histological and histochemical parameters in *Biomphalaria alexandrina*. African Journal of Pharmacy and Pharmacology, 7: 2220-2230.

Nacif-Pimenta, R.; Mattos, A.C.; Orfanó, A.S.; Barbosa, L.; Pimenta, P.F.; Coelho, P.M. (2012). *Schistosoma mansoni* in susceptible and resistant snail strains *Biomphalaria tenagophila*: In vivo tissue response and in vitro hemocyte interactions. PLoS ONE, 7(9):1–12. https://doi.org/10.1371/journal.pone.0045637.

Nair, R.; Kalariya, T.; Chanda, S. (2007). Antibacterial activity of some plant extracts used in folk medicine. Journal of Herbal Pharmacotherapy, 7:191–201.

Neiva, V.A.; Ribeiro, M.N.; Nascimento, F.R.; Cartágenes, M.S.; Coutinho-Moraes, D.F.; Amaral, F.M.(2014). Plant species used in giardiasis treatment: ethnopharmacology and in vitro evaluation of anti *Giardia* activity. Brazilian Journal of Pharmacognosy, 24: 215–224.

Nkondo, E.T.; Jatsa, H.B.; Feussom, N.G.; Kenfack, M.C.; Femoe, M.U.; Guetchueng, S.T.; Kowa, C.K.; Kamtchouing, P.; Tchuente, L. (2022). In Vitro Cercaricidal and Schistosomicidal Activities of the Raffia Wine and Hydroethanolic Extracts of *Pedilanthus tithymaloides Linn* (Poit). Stem Barks Evidence-Based Complementary and Alternative Medicine, ID 2672150.

Oczypok, E.A.; Oury, T.D.; Chu, C.T. (2013). It's a cell-eat-cell world: Autophagy and phagocytosis. Am J Pathol., 182: 612-622.

Osman, A.M.; Gomaa, M;, Saad, A.H. (2003). *Biomphalaria alexandrina*: cellular responses of susceptible and resistant snails to *Schistosoma mansoni* infection. J. Egypt. Soc. Parasitol., 33 (3), 805–827.

Parvez, S. and Raisuddin, S. (2005). Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctate (Bloch). Environ. Toxicol. Pharmacol., 20:112-117, doi:10.1016/j.etap.2004.11.002.

Rahuman, A. A.; Gopalakrishnan, G.; Venkatesan, P.; Geetha, K. (2008). Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. Parasitology Research, 102: 981–988.

Rollinson, D.; Knopp, S.; Levitz, S.; Stothard, J.R.; Tchuem Tchuenté, L.A.; Garba, A.; Mohammed, K.A.; Schur, N.; Person, B.; Colley, D.G.; Utzinger, J. (2013). Time to set the agenda for elimination. Acta Trop; 128(2):423-40. doi: 10.1016/j.actatropica.2012.04.013.

Saad, A.A.; Ragab, F. M.; AbdelFatah, H.M.; *et al*. (2017). Effect ot two algae; *Cystosei*ra *barbata* and *Dictyota dichotoma* on digestive gland of *Biomphalria alexandrina* snails. J Environ Sci., 37:37–59. https://doi. org/10.21608/jes.2017.19467

Saad, A.A.; Ragab, F. M.; AbdelFatah, H.M.; *et al.* (2019). Effect of *Cystoseira barbata* and *Dictyota dichotoma* algae on reproduction and protein pattern of *Biomphalaria alexandrina* snails. Molluscan Res., 39:82–88. https://doi.org/10.1080/13235818.2018.1524740

Sarkar, A.; Bhagat, J.; Ingole, B.S.; Rao, D.P.; Markad, V.L. (2015). Genotoxicity of cadmium chloride in the marine gastropod Nerita chamaeleon using comet assay and alkaline unwinding assay. Environ. Tox., 30(2):177-87. doi: 10.1002/tox.21883.

Schmitz, K.J.; Ademi, C.; Bertram, S.; Schmid, K.W.; Baba, H.A. (2016). Prognostic relevance of autophagy-related markers LC3, p62/sequestosome 1, Beclin-1 and ULK1 in colorectal cancer patients with respect to KRAS mutational status. World J Surg Oncol, 14: 189.

Sethuraman, V.; Benshui, S.; Gaofeng, C.; Shengjiao, F.; Guohua, Z. (2017). Curcumin induces autophagic cell death in Spodoptera frugiperda cells. Pesticide Biochemistry and Physiology, 139 79–86.

Shao, X.; Lai, D.; Zhang, L.; Xu, H. (2016). Induction of autophagy and apoptosis via PI3K/AKT/ TOR pathways by azadirachtin A in Spodoptera litura cells, Sci. Rep. 6

Singh, A. and Agarwal, R.A. (1992). Molluscicidal activity of Euphorbiales against the snail *Indoplanorbis exustus*. Acta hydrochimica et hydrobiologica, 20: 262-264 https://doi.org/10.1002/aheh.19920200502.

Sulaiman, S.M.; Bashir, A.K.; Mohamed, A.M. (1988). Effects of certain Sudanese plant extracts on egg-hatching, miracidia and cercariae of Schistosoma mansoni. Int J Crude Drug Res., 26:17–21. https:// doi.org/10.3109/13880208809053883.

Wang, W.; Mao, Q.; Yao, J. et al. (2018). Discovery of the pyridylphenylureas as novel molluscicides against the invasive snail *Biomphalaria straminea*, intermediate host of *Schistosoma mansoni*. Parasites Vectors, 11:291. https://doi.org/10.1186/s13071-018-2868-7.

WHO. (1965). Molluscicide screening and evaluation. Bull. World Health Organ., 33: 567–581.

WHO. (1983). Report of the Scientific Working Group on Plant Molluscicide & Guidelines for Evaluation of Plant Molluscicides; TDR/SCHSWE (4)/83.3; WHO: Geneva, Switzerland.

WHO. (2017). Field use of molluscicides in schistosomiasis control programmes: an operational manual for programme managers. ISBN 97

WHO. (2019). Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis.

Wilkie, M.P., *et al.* (2019). Control of invasive sea lampreys using the piscicides TFM and niclosamida: toxicology, successes & future prospects. Aquatic Toxicol., 211:235-252. doi: 10.1016/j.aquatox.2018.12.012.

Wu, Q.; Patocka, j.; Nepovimova, N.; Kuca, K. (2019). *Jatropha gossypiifolia L*. and its biologically active metabolites: A mini review, J Ethnopharmacol., 24:234:197-203. doi: 10.1016/j.jep.2019.01.022.8-92-4-151199-5.

Xu, Z. ; Lu, M.; Yang, M.; Xu,W.; Gao, J.; Zhang, Y.; Yang, Y; Tao, L. (2017). Pyrethrum-extract induced autophagy in insect cells: a new target? Pestic. Biochem. Physiol. 137 21–26.

Ye, J.; Wu, H. ; Wu, Y.; Wang, C.; Zhang, H.; Shi, X.; Yang, J. (2012). High molecular weight hyaluronan decreases oxidative DNA damage induced by EDTA in human corneal epithelial cells. Eye (Lond), 26 : 1012-1020

Yoshii, S.R. and Mizshima, N. (2017). Monitoring and Measuring Autophagy. Int. J. Mol. Sci., 18(9), 1865; https://doi.org/10.3390/ijms18091865.

Yoshino, T.P. and Coustau, C. (2011). Immunobiology of *Biomphalaria*-trematode interactions. In: *Biomphalaria* snails and larval trematodes. New York: Springer, 159-89.

Zhang, X.P.; Zhang, M.L.; Su, X.H.; Huo, C.H.; Gu, Y.C.; Shi, Q.W. (2009). Chemical constituents of the plants from genus J*atropha*. Chemistry and Biodiversity, 6(12):2166–2183.