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RESEARCH ARTICLE

Impact of *Moringa oleifera* seed aqueous extract on some biological, biochemical, and histological aspects of *Biomphalaria alexandrina* snails

Amina M. Ibrahim[1] · **Aboelfetoh M. Abdalla**[2]

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Abstract Schistosomiasis is one of the neglected tropical diseases. It is a snail-borne trematode infection, and *Biomphalaria alexandrina* snails are the intermediate host of *Schistosoma mansoni* in Egypt. The objective of this study is to evaluate the molluscicidal activity of the aqueous seed extract of *Moringa oleifera* against *B. alexandrina* snails. The results showed that this aqueous extract was lethal for *B. alexandrina* snails (LC_{50} 0.27 g/l; LC_{90} 0.41 g/l). Exposure of snails to the sublethal concentrations of this aqueous extract caused a considerable reduction in survival rates and hatchability rates of eggs of these snails. Moreover, it negatively affected some biochemical aspects, where it increased the levels of transaminases (ALT and AST), while it decreased the concentrations of total protein, albumin, and globulin concentration. Histological examinations of the digestive gland of snails exposed to the sublethal concentrations of aqueous seed extract of *M. oleifera* revealed severe damage in the digestive cells, where they lost their tips and some were degenerated, while the secretory cells increased in number. Regarding the hermaphrodite gland, there were losses of connective tissues and irregular sperms, and the eggs were degenerated. These findings prove the potent activity of aqueous seed extract of *M. oleifera* against the intermediate hosts of *Schistosoma mansoni* and provide a considerable scope in exploiting local indigenous resources for snails' molluscicidal agents.

Keywords Schistosomiasis · *Moringa oleifera* · *Biomphalaria alexandrina* · Hatchability rate · Biochemical aspects · Histology

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✉ Amina M. Ibrahim
aminamd.ibrahim@yahoo.com

Aboelfetoh M. Abdalla
Aboelfetoh2015@yahoo.com

[1] Environmental Research and Medical Malacology Department, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt

[2] Technology of Horticulture Crops Department, National Research Center, Giza, Egypt

Introduction

Schistosomiasis is the second neglected tropical parasitic infection after malaria (Kiros et al. 2014; Rizk and Aly 2015). *Biomphalaria alexandrina* snails are the intermediate host of *Schistosoma mansoni* (Le Clec'h et al. 2016) with high prevalence in Egypt (El-Sheikh et al. 2012). Several strategies have been used to control snail populations, since it breaks the life cycle (El-Ghany and El-Ghany 2017). Manufactured molluscicides are an imperative part in the incorporated schistosomiasis control programs (Abdel-Ghaffar et al. 2016), but because they have high cost and being poisonous to creatures of land and water (WHO 2014), they have stimulated interest for quest for plant molluscicides (Kiros et al. 2014).

Medicinal plants are promising choices that may grow the scope of molluscicides accessible for controlling of *B. alexandrina* snails as these plants are less costly, safer, and having a high level of degradability (Salawu and Odaibo 2011). *Moringa oleifera* Lamarck (family: Moringaceae) is an important medicinal plant referred as a miracle tree (Radovich 2011), widely distributed in tropical and subtropical regions (Okonkwo et al. 2014). All parts of this plant possess nutritional and medicinal properties (Nair and Varalakshmi 2011). The aqueous extract of *M. oleifera* seeds contain bioactive molecules including saponins, lectins, (Araújo et al. 2013),

and volatile oils (Kayode and Afolayan 2015). There are two types of lectins: coagulant *M. oleifera* lectin (cMoL) which is a basic protein and water-soluble *M. oleifera* lectin (WSMoL) which is an acidic protein (Santos et al. 2005; Santos et al. 2009). These proteins have ability to agglutinate erythrocytes (Weis and Drickamer 1996) and show coagulant activity that reduces water turbidity (Ferreira et al. 2011). Recent studies showed that *M. oleifera* lectins had ovicidal effects on *Aedes aegypti* (de Lima Santos et al. 2012) and insecticidal potential against *Anagasta kuehniella* (especially cMoL) (De Oliveira et al. 2011). Also, the seed powder of *M. oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physa marmorata* (Silva et al. 2013). The point of this study was to assess the deleterious effect of the aqueous seed extract of *M. oleifera* against *B. alexandrina* snails and how it is mirrored on survival rate, hatchability of eggs, biochemical, and histological aspects of these snails.

Materials and methods

Snails

Adult *B. alexandrina* snails (8–10 mm) from Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt, were used. Pieces of polyethylene sheets were put into the aquaria to collect egg masses. Water in the aquaria was changed weekly.

Plant materials

Seeds of *Moringa oleifera* (Moringaceae) were collected from the farm of Egyptian Scientific Society of *Moringa*. It was identified by Prof. Dr. Aboelfetoh Mohammed Abdelalla, National Research Center, Giza, Egypt. The seeds were air-dried, powdered, weighed, and added directly to filtered water in individual beakers.

Molluscicidal screening

A stock solution of seed aqueous extract was prepared by adding 1.0 g of *M. oleifera* seeds in 1 l of distilled water according to (Olaifa et al. 2003). To calculate LC_{50} and LC_{90}, five serial dilutions were prepared (0.15, 0.2, 0.25, 0.38, and 0.5 g/l). Ten *B. alexandrina* snails (8–10 mm) were placed in beakers for each concentration (Litchfield and Wilcoxon 1949) and another snail group of the same size was dipped in dechlorinated water only as control. Three replicates were used for each concentration. The exposure period was 24 h; after that, the snails were removed from the experimental test solution and washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water for another 24 h of recovery, and then, the percentages of observed mortalities were recorded. LC_0 is the concentration of a toxicant, below which no measurable effects take place (Warren 1900), and is estimated as 1/10 LC_{50} value (WHO 1965; El-Gindy et al. 1991).

Effect on survival rate of snails

B. alexandrina snails (8–10 mm) were divided into four groups (30 snails each); three groups were exposed to the aqueous seed extract at LC_0, LC_{10}, and LC_{25} for 24 h (exposure), then the snails were removed from the experimental test solution and washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water for another 24 h of recovery, and this was done for 2 weeks. The fourth group was left in dechlorinated water as control. After recovery, snails were observed daily to record the survival rate. All experiments were repeated three times.

Assay for ovicidal activity

Egg masses on the sheets were transferred to petri dishes, where they were exposed to the tested concentrations. For each concentration, 100 eggs were used and assays were repeated three times. At the end of exposure (24 h), egg masses were transferred to petri dishes with dechlorinated water and were examined daily under a stereomicroscope up to the seventh day.

Biochemical assays

Ten snails (8–10 mm) were subjected to each sublethal concentrations for 24 h (exposure), followed by another 24 h of recovery, and this method is done for 2 weeks. Three replicates of each concentration were prepared. Unexposed snails (control) were assayed side by side with the experimented groups. To collect the hemolymph, a small portion of the shell which situated directly above the heart of snails was removed and a capillary tube was inserted into the heart (Nduku and Harrison 1980). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the Reitman and Frankel (1957) technique. Total protein was determined according to the method of Doumas (1975). Albumin was determined according to Gustafsson (1976). Calculation of globulin was determined by subtracting the amount of albumin from the total protein and then calculating the albumin/globulin (A/G) ratio.

Histological study

Adult snails (8–10 mm) were exposed to the aqueous seed extract sublethal concentrations (LC_0, LC_{10}, and LC_{25}) for 24 h/week for two successive weeks. At the end of the exposure period, snails were removed from the

Table 1 Molluscicidal activity of *M. oleifera* seeds against adult *B. alexandrina* snails (24 h exposure). *Conc* concentration

Plant	Conc					
	LC_0	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
M. oleifera seeds	0.027	0.14	0.21	0.27	0.41	1.4

experimental aquaria, washed thoroughly with water, and dried using smooth tissue. Changes in histology of digestive and hermaphrodite glands of treated snails compared with control snails were done according to Mohamed and Saad (1990).

Statistical analysis

Lethal concentration values were defined by Probit analysis (Finney 1971). The data were analyzed by chi-square; comparison of means was carried out using Student's *t* test (Goldstein and Goldstein 1967). Values were expressed as mean ± SD.

Result

From Table 1, it is observed that the aqueous seed extract of *M. oleifera* was lethal for *B. alexandrina* snails (LC_{50} 0.27 g/l; LC_{90} 0.41 g/l). By exposing these snails to sublethal concentrations (LC_0, LC_{10}, LC_{25}), its survival rates were highly significantly reduced ($p < 0.001$) than the control group (Table 2). Also, the hatchability rates of eggs exposed to these sublethal concentrations were highly significantly reduced ($p < 0.001$) compared to the control group (Table 3) and this reduction was concentration dependent.

Table 2 Survival rate of *B. alexandrina* snails exposed to sublethal concentrations of *M. oleifera* seeds for 2 weeks of exposure followed by 2 weeks of recovery

Weeks	Survival rate (%)			
	Groups			
	Control	LC_0	LC_{10}	LC_{25}
1	100	90.9[a]	82[a]	63.3[a]
2	100	81.7[a]	63.3[a]	54.5[a]
3	98	72.2[a]	55[a]	45[a]
4	97	63.6[a]	52.2[a]	36.4[a]

[a] Very highly significant compared to control at $p < 0.001$

Studying the ovicidal effect of these concentrations by light microscope reveals that there are alterations in the embryonic development of *B. alexandrina* snails' eggs (Fig. 1(B–D)) compared with control group (Fig. 1(A)), where all embryos are at the last stage (pre-hatching).

The activities of the transaminases (AST and ALT) in snails subjected to the sublethal concentrations of *M. oleifera* aqueous seed extract were highly elevated ($p \leq 0.001$) in comparison with those of control ones, while there was a reduction in total protein concentrations and these results reflected on the levels of albumin and A/G ratio in hemolymph of treated snails (Table 4).

Normal digestive gland of the adult *B. alexandrina* snails consists of a number of tubular glands which were lined with one layer of two types of cells: digestive cells (DC) and secretory cells (SC) (Fig. 2(E)). Exposing these snails to LC_0—the sublethal concentration—of *M. oleifera* aqueous seed extract showed that some cells were ruptured and lost their nuclei, while there was a marked increase in the number of the secretory cells (Fig. 2(F)). At LC_{10}, most of these cells became vacuolated, degenerated, and ruptured, and the lumen (L) increased (Fig. 2(G)). The most severe damage occurred at LC_{25}, where most of cells lost their identical shape resulting from dissolution of their cell membrane and tips of some digestive cells were ruptured (Fig. 2(H)). Regarding the hermaphrodite gland of the normal *B. alexandrina* snails, the male reproductive cells are differentiated in clusters forming primary and secondary spermatocytes. On the other hand, the female oogenic cells filled the acinar lumen and the mature ova are surrounded by follicular membrane (Fig. 3(I)). Exposing snails to the LC_0 sublethal concentration caused an increase in number of dead eggs with malformation in their shapes accompanied with degenerated nuclei (Fig. 3(J)). While exposure of snails to LC_{10} showed shrinkage and destruction in sperms and degeneration in eggs (Fig. 3(K)), the great damage in gonadal cells occurred at LC_{25}, where eggs lost their shapes and degenerated. Sperms were reduced in number and the connective tissue was dissolved and replaced by vacuoles (Fig. 3(L)).

Table 3 Hatchability and mortality rates of *Biomphalaria alexandrina* snail's eggs exposed to sublethal concentrations of *M. oleifera* seeds for 24 h

Concentration	% hatchability	% mortality
Control	99	1
LC_0	69[a]	31
LC_{10}	45[a]	55
LC_{25}	36[a]	64

[a] Very highly significant compared to control at $p < 0.001$

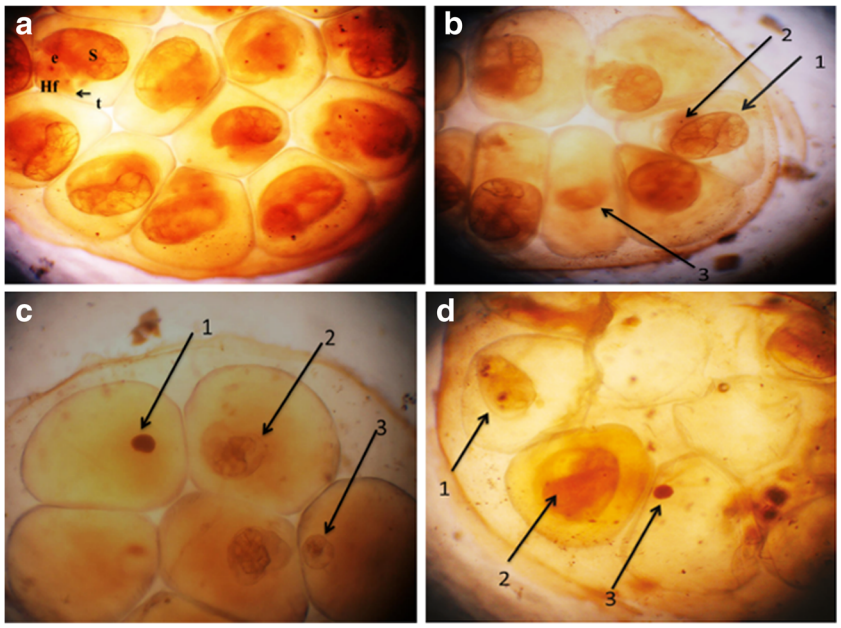

Fig. 1 *B. alexandrina* embryos after exposure to sublethal concentration of *M. oleifera* seeds. (A) Control embryos (e: eye; HF: head foot; S: shell; t: tentacle). (B) At LC_0: 1: normal embryo, 2: eye of the embryo, 3: embryo with indeterminate malformations. (C) At LC_{10}: 1: dead embryo, 2: normal delayed embryo, 3: malformed embryos. (D) At LC_{25}: 1: malformed embryo, 2: embryo with indeterminate malformation, 3: dead embryo at blastula

Discussion

On the premise of the institutionalized technique for World Health Organization, the median lethal concentration (LC_{50}) for any molluscicidal material must not surpass 100 ppm (WHO 1993). The present results showed that the aqueous seed extract of *M. oleifera* was toxic to *B. alexandrina* snails at LC_{50} 0.27 g/l. This agrees with findings of Silva et al. (2013), who confirmed that the seed powder of *M. oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physa marmorata* and stated that the snails were retracted into shell and suffered hemorrhage after treatment.

The present results showed that survival rates of adult *B. alexandrina* snails were markedly reduced post their exposure to sublethal concentrations of the aqueous seed extract. Comparable perceptions were recorded by Bakry (2009) who stated that exposure of *B. alexandrina* snails to methanol extracts of *Euphorbia splendens*, *Atriplex stylosa*, and *Guayacum officinalis* led to a significant reduction in their survival and growth rates. The reduction in survival rate was due to these snails could overcome the destructive impact of these toxic compounds through discharging it to the surrounding media or by biodegrading it to non-poisonous by-products (Mohamed et al. 2012).

Concerning the hatchability rates of eggs exposed to sublethal concentrations (LC_0, LC_{10}, LC_{25}) of the *M. oleifera* aqueous seed extract, they were highly significantly reduced compared to the control group and this reduction was concentration dependent, while the mortality rate of eggs increased with increasing the concentration. By light microscope, there were alterations in the embryonic development of *B. alexandrina* snails' eggs compared with control group. These results agree with that of Ferreira et al. (2009) who stated that water extract of *Moringa oleifera* seeds had lethal

Table 4 Effect sublethal concentrations of *M. oleifera* seeds on some biochemical parameters in hemolymph of adult *B. alexandrina* snails

Conc. (ppm)	AST (U/l)	ALT (U/l)	T. protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	A/G ratio
LC_0	76.5 ± 0.5***	102.1 ± 1.9***	5.86 ± 0.35	2.8 ± 0.1	3.05 ± 0.35	0.921
LC_{10}	98 ± 2.6***	110.7 ± 2.5***	5.96 ± 0.2	2.1 ± 0.35**	3.82 ± 0.4	0.560
LC_{25}	151.3 ± 6***	129.7 ± 1.5***	4.6 ± 0.13***	1.81 ± 0.12**	2.84 ± 0.17*	0.63
Control	58.2 ± 2.8	61.2 ± 0.8	6.12 ± 0.2	2.9 ± 0.11	3.22 ± 0.11	0.925

A/G ratio albumin/globulin ratio

*Significant compared to control at $p < 0.05$; **highly significant compared to control at $p < 0.01$; ***Very highly significant compared to control at $p < 0.001$

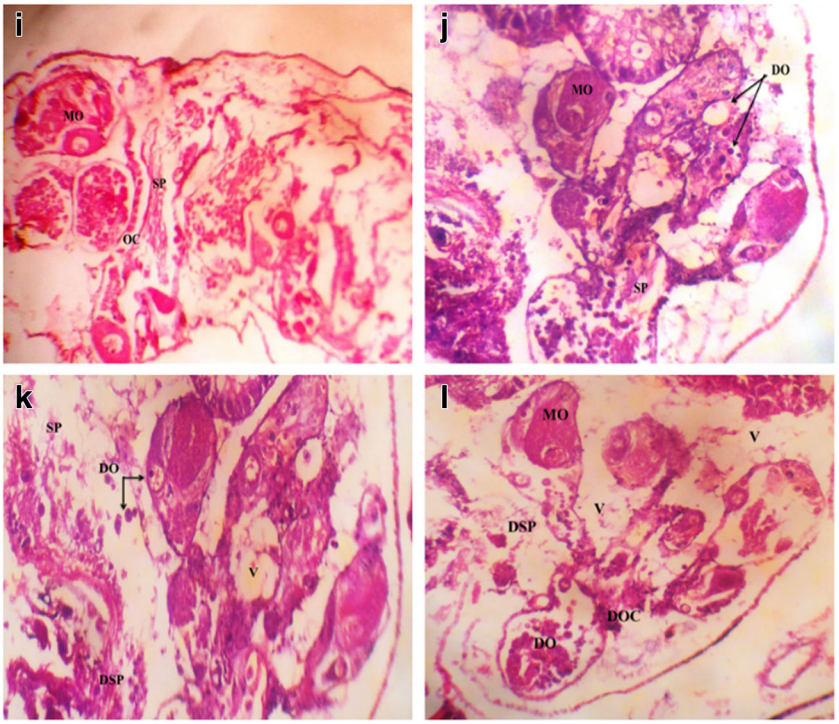

Fig. 3 Histological sections in hermaphrodite gland of adult *B. alexandrina* snails. (I) Normal control snails. (J) Snails exposed to LC_0 of aqueous extract of seeds. (K) Snails exposed to LC_{10} of aqueous extract of seeds. (L) Snails exposed to LC_{25} of aqueous extract of seeds. MO mature ovum, SP sperms, SPR spermatocytes, OC oocyte, DO degenerated ovum, DOC degenerated oocyte, DSP degenerated sperms, V vacuole (×100)

action against *Aedes aegypti* larvae and eggs. Also, Rocha-Filho et al. (2015) studied the ovicidal action of *M. oleifera* flower extract and stated that it had delayed the development of treated embryos and reasoned that the flower extract caused changes in the physiology of snails, which interfered with the production of eggs.

The present work showed marked elevation in the activity of AST and ALT enzymes in all the treated groups than control

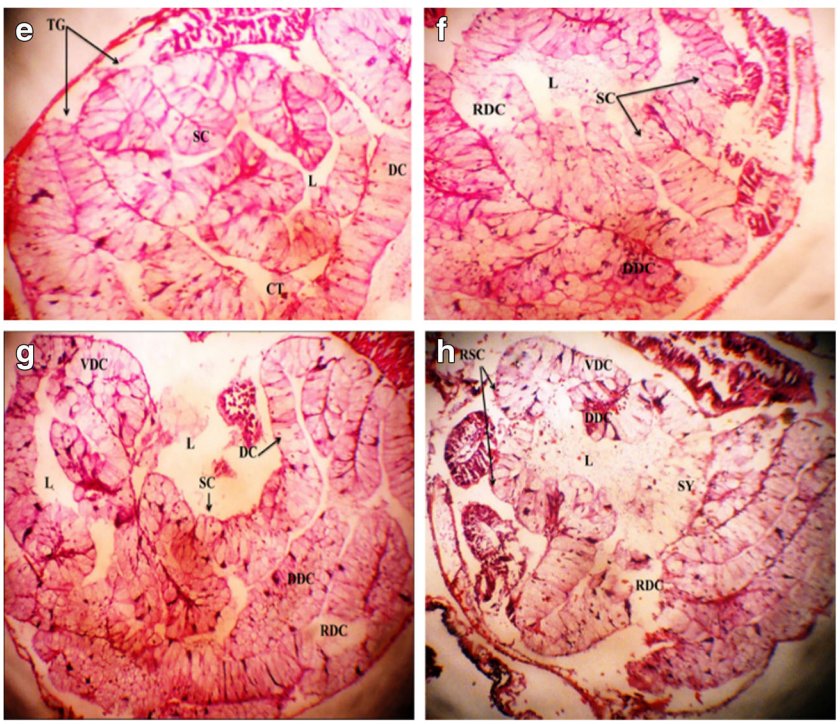

Fig. 2 Histological sections in digestive gland of adult *B. alexandrina* snails. (E) Normal control snails. (F) Snails exposed to LC_0 of aqueous extract of seeds. (G) Snails exposed to LC_{10} of aqueous extract of seeds. (H) Snails exposed to LC_{25} of aqueous extract of seeds. DDC degenerated digestive cells, RSC ruptured secretory cells, TG tubular gland, DC digestive cells, SC secretory cells, VDC vacuolated digestive cell, RDC ruptured digestive cells, L lumen, SY syncytium (×100)

group. This is mirroring the harms created by this aqueous seed extract on hepatic cells and these outcomes are in congruity with the past investigation of Abdel et al. (2004) who reported severe damages in these vital activities by exposure to low doses of synthetic and natural molluscicides. These changes were due to animal's trials to restore the amino acid balance in different body organs (El-Emam and Ebeid 1989).

The current study indicated that total protein and albumin concentrations in hemolymph of snails exposed to the tested concentrations were decreased compared to that of control group. This observation was previously recorded by Fahmy et al. (2014) that the total protein and albumin contents in hemolymph of *B. alexandrina* snails were reduced after their exposure to sublethal concentrations of zinc oxide nanoparticles (ZnONPs). This decrease might be due to physiological adaptability of the animal to compensate the toxic stress which led to the stimulation of protein catabolism (Hasheesh et al. 2011). The major cause for the decline of albumin level and albumin/globulin (A/G) ratio after the exposure may be due to the effect of the seed powder on liver parenchyma (Mohamed et al. 2012).

During the present study, aqueous seed extract induced histopathological changes in the digestive gland and these damaged increased with increasing the concentrations. The most prominent severe damage in the digestive cells was the presence of a great loss of identical shape of digestive cells. Its tips were ruptured and most of these cells are degenerated; also the secretory cells increased in number and the connective tissue between digestive tubules shrank. These outcomes concur with El-Deeb and El-Nahas (2005) on *Euphorbia nubica* and *Sesbania sesban* plants which caused epithelial necrosis and abnormal increase in the ratio of secretory to digestive cells.

In the present work, the histological examination of the treated hermaphrodite gland showed losses of connective tissues, irregular sperms, and degenerated eggs after exposure. These results agree with Mossalem et al. (2013) who stated that there was a complete destruction of gametogenic cells and severe damage of hermaphrodite gland tissues, by exposing *B. alexandrina* snails to the anthelmintic plant derivative (artemether).

Conclusion

From the prior results, aqueous seed extract of *M. oleifera* can be used as a potent molluscicidal agent for the intermediate host of *S. mansoni*. Therefore, new studies are needed to define the proper technique(s) for application of such tested agents in schistosomiasis control aiming to minimize water pollution and saving the non-target organisms.

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Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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

Abbreviations *A/G ratio*, albumin/globulin; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *B. alexandrina*, Biomphalaria alexandrina; *g/l*, gram per liter; *h*, hour; *LC*; lethal concentration; *mm*, millimeter; *M. oleifera*, Moringa oleifera; *ppm*, part per million

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