Parasite Epidemiology and Control 23 (2023) e00331

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Molluscicidal activity of sodium hypochlorite against *Biomphlaria alexandrina* snails: Immunological and hepato-endocrine alterations with in silico docking study

Amina M. Ibrahim [a], Mohamed T. Hamed [b], Manal F. EL-Khadragy [c,*], Mostafa Y. Morad [b]

[a] *Medical Malacology Department, Theodor Bilharz Research Institute, Giza 12411, Egypt*
[b] *Zoology and Entomology Department, Faculty of Science, Helwan University, Helwan 11795, Egypt*
[c] *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia*

ARTICLE INFO

Keywords:
Schistosoma mansoni
Biomphlaria alexandrina snails
Sodium hypochlorite
Biochemical analysis
Endocrine disruption
Molecular docking

ABSTRACT

Schistosomiasis is a tropical disease that widely neglected. *Schistosoma mansoni* reproduce asexually within the freshwater snail, *Biomphlaria alexandrina.* Sodium hypochlorite (NaOCl) is a widely used disinfectant, so its effect against gainst *B. alexandrina* snails was evaluated. The present results showed that NaOCl has a molluscicidal activity against adult *B. alexandrina* snails at LC_{50} 1.25 ppm. Hemocytes displayed varied morphological forms after being exposed to the LC10 and LC25 concentrations of NaOCl in *B. alexandrina* snails, and the phagocytic index of *B. alexandrina* snail's hemocytes significantly increased. The phagocytic potency of exposed hemocytes to charcoal showed ruptured plasma membrane, engulfed particles, vacuolation in the cytoplasm and degeneration of nuclei. When *B. alexandrina* snails were treated with sublethal concentrations of NaOCl, transaminases (AST & ALT), alkaline and acid phosphatase activities were significantly increased. In contrast, the total protein, albumin concentrations, Testosterone (T) and 17β Estradiol (E) showed a significant decrease ($p \leq 0.05$) as compared to the control groups. The molecular docking interaction showed high efficiency for the ligand, NaOCl against the receptor binding sites of the acid phosphatase, alanine aminotransferase, estrogen and testosterone. The present results showed that NaOCl could be used as an effective molluscicide against *B. alexandrina* snails but more attention should be paid to investigate the side effects on the non-target organisms living in the freshwater environment.

1. Introduction

Trematode infections include Schistosomiasis and fascioliasis (Ibrahim and Bakry, 2019). According to recent estimates, 243 million persons globally have infected with *Schistosoma* species (WHO, 2022). The parasite infection may have systemic impacts on a patient's metabolism, seriously affecting their ability to grow, eat, and develop cognitively (Ezeamama et al., 2005). In terms of morbidity and mortality in humans, it is one of the most significant helminthic illnesses (Friedman, 2005). Schistosomiasis is the infection with the trematode worm, *Schistosoma* spp. that transmitted through cercarial penetration of human skin, around the world

* Corresponding author.
E-mail address: Mfelkhadragy@pnu.edu.sa (M.F. EL-Khadragy).

https://doi.org/10.1016/j.parepi.2023.e00331
Received 12 April 2023; Received in revised form 18 July 2023; Accepted 29 November 2023
Available online 4 December 2023

(Ibrahim and Kamal, 2019; Ibrahim and Nasr, 2019). Certain freshwater snail species, such as *Biomphalaria* spp. snails served as the intermediate hosts in the life cycles of *Schistosoma* spp. The role of *Biomphalaria* spp. snails as intermediate hosts for *Schistosoma mansoni* is extremely important in medicine (Ibrahim et al., 2023; Morad et al., 2023) because it helps in the disease transmission. The interactions between *Biomphalaria* spp. snails and *Schistosoma* spp. have drawn a lot of attention in this regard (Morad et al., 2023) because the infection of *Biomphalaria* spp. with schistosomes caused loss of fertility or even parasitic castration (Alberto-Silva et al., 2015).

Praziquantel is a widely used anthelmintic against parasites that proved efficiency against trematodes and cestodes and is the only approved drug against schistosomiasis (Ibrahim et al., 2019; Ibrahim et al., 2022b). Praziquantel has efficacy against adult worms, but poorly active against juvenile stages and for that reason, it must be periodically applied for effective treatment and control (Ibrahim et al., 2019). The heavy use of Praziquantel for treatment of schistosomiasis, resulted in high-resistance as reported in the study conducted by (Ismail et al., 1994) who reported that despite the use of praziquantel in some cases, the infection persisted. So, the huge uses of Praziquantel in humans and domestic animals treatments (Xavier et al., 2020) raise a considerable issue regarding the need for finding out a new drug for these parasites (Lago et al., 2017).

Despite molluscicide use is an efficient, rapid, and practical intervention technique to limit the spread of these infectious species in endemic areas (Ibrahim et al., 2022a; Ibrahim and Abdalla, 2017; Zheng et al., 2021), But on the other side, the widespread usage of various synthetic molluscicides was accompanied by serious environmental contamination and safety issues (Coelho and Caldeira, 2016). Sodium hypochlorite (NaOCl), which is chemically created from aqueous sodium chloride solutions, is the most practicable source of active oxygen. It can be used in drinking water without being hazardous (Taha et al., 2014). It can easily pass cellular membranes and is quickly expelled due to its small size and low molecular mass (Eventov et al., 1998). NaOCl is commonly known as powerful disinfectant, and its remedies successfully lessen the contagious potential of a variety of viruses, bacteria, and fungus (Peeters et al., 2008). Sodium hypochlorite has effective antimicrobial mechanisms, tissue dissolution capacity and showed a biological compatibility in less concentrated solutions (Abadias et al., 2008; Gerba and Denise, 2007). The study aimed to investigate the action of sodium hypochlorite against snails, *B. alexandrina* the intermediate host of *S. mansoni*, and elucidate its role as a control agent against *B. alexandrina* snail's immunological and hepato-endocrine alterations with *in-silico* molecular docking.

2. Materials and methods

2.1. Experimental animals

Biomphalaria alexandrina (9–10 mm) snails were reared in Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. It was housed in 15 × 24 × 10 cm plastic aquariums with blue green algae (*Nostoc muscorum*) and dechlorinated aerated tap water and oven dried lettuce leaves (10 snails/ L). Egg masses were collected using polyethylene sheets, and the water in the aquariums was changed once a week.

2.2. Sodium hypochlorite (NaOCl)

A commercial bleaching solution Clorox® (6.0% NaOCl solution) was used in this study. The stock solution was prepared on the basis of *V*/V using distilled water (1000 ppm).

2.3. Molluscicidal effects

To determine LC50 and LC90, serial dilutions (0.5, 1, 1.5, and 2 ppm) of the NaOCl stock solution were made with dechlorinated tap water. For each concentration, ten snails (9-10 mm) were maintained in each aquaria (WHO, 1983). Control snail group of the same size in dechlorinated water was assessed side by side with the tested solutions. Each concentration and control group underwent three replications, after 24 h of exposure and another 24 h of recovery. The percentages of mortalities were monitored and calculated by Probity analysis (Finney, 1971).

2.3.1. Immunotoxin effects

Hemolymph was collected after *B. alexandrina* snails were treated with sublethal concentration of NaOCl according to (Nduku and Harrison, 1980). Where, a capillary tube was used to draw hemolymph from a small opening above the snail's heart.

a- Hemocytes monolayers:

A glass slide with 10 µl of hemolymph was placed on it, and it was left at room temperature for 15 min to dry. Absolute methanol was used to fix hemocytes for 5 min, and 10% Giemsa stain (Aldrich) was used to stain them for 20 min., then examined under light microscope (Abdul-Salam and Michelson, 1980).

b- Phagocytic activity

Hemocyte suspension (100 µl) was collected from each exposed and control groups, then It was applied on glass slides and left to sit for one hour at 37 °C in a humid environment to test for cell adhesion. Hemocytes were challenged with activated charcoal particles and incubated for 1 h at 37 °C in order to measure their phagocytic efficacy. The cells were then stained with Giemsa after incubation, air dried, fixed with methanol, and viewed under a light microscope. Both in control and exposed snails, various stages

Table 1
Molluscicide effect of NaOCl against *B. alexandrina* snails (24 h exposure, followed by 24 of recovery).

Concentration (ppm)	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
Biomphalaria alexandrina	0.78	1.004	1.25	1.71	1.1

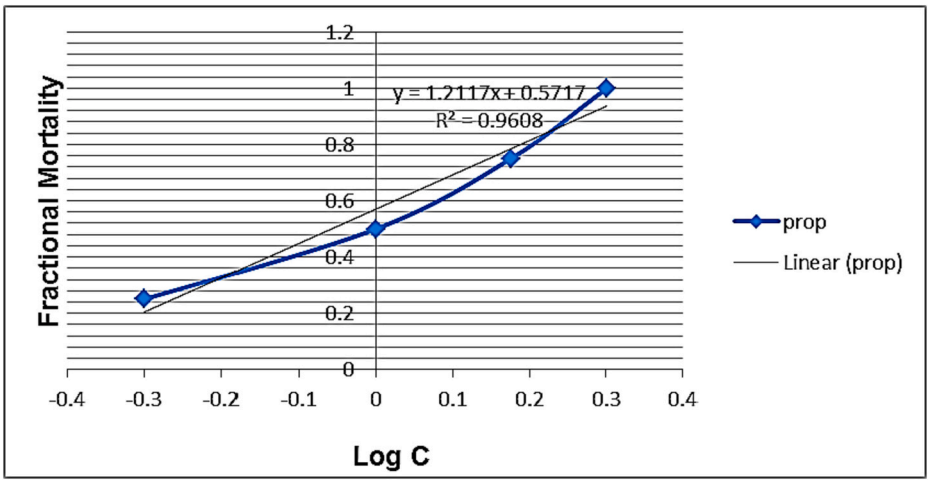

Fig. 1. Molluscicide activity of NaOCl against *B. alexandrina* snails by Probity analysis.

of hemocyte phagocytosis of charcoal particles were identified. To estimate phagocytic index, the following formula was applied according to (Guria, 2018).

$$\text{Phagocytic index} = \frac{\text{Counted cells with phagocytosed charcoal particle}}{\text{Total number of cells}} \times 100$$

2.3.2. Biochemical examinations

2.3.2.1. Tissue preparation. Snails were dissected from each control and exposed groups to the sublethal concentrations of NaOCl, then forceps were used to separate the soft tissues of digestive and the hermaphrodite glands from the shells. After weighing the soft tissues (1 g tissue/10 ml phosphate buffer), they were homogenised in a glass Dounce homogenizer. The supernatants from the centrifugation of the homogenates at 3000 rpm for 10 min were employed in the tests. The aspartate and alanine aminotransferase (AST; ALT) activities were measured using the (Reitman and Frankel, 1957) technique. Total protein was determined according to (Lowry et al., 1994). Albumin levels were measured according to (Gustafsson, 1976). Acid and alkaline phosphatase (ACP/ALP) were measured by the method of (Bergmeyer, 1985) modified by (Singh and Agarwal, 1991).

2.3.3. Endocrine disruption

Tissues of the hermaphrodite glands of ten snails were used in this experiment. Testosterone (T) and estradiol hormones (E) concentrations were measured for all groups according to the manufacturer's recommendations, for testosterone EIA kit (Enzo Life Science, Michigan, USA, ADI-900-065) and for estradiol EIA kit (Cayman Chemical Company, Michigan, USA, item no. 582251). 25 µl of each standard, control, and samples (serum) in duplicate were dispensed with new disposable tips into appropriate wells on a microplate coated with T or E monoclonal antibody. After that, each microplate well was filled with 200 µl enzyme conjugate, thoroughly mixed, and then incubated for 60 min at room temperature. Following this, the microplates were treated and absorbance was measured (Gholib et al., 2020).

2.3.4. The molecular docking interaction analysis

To study the action of sodium hypochlorite on snail exposure, acid phosphatase, ALT, estrogen and testosterone activities were selected to predict the action of NaOC The structure of several compounds, such as acid phosphatase (1D2T) from *Escherichia blattae* (Ishikawa et al., 2000) and ALT (1XI9) from *Pyrococcus furiosus* (Zhou et al., 2004) Estrogen (1iol) (Azzi et al., 1996), and Testosteron (1I9J) (Valjakka et al., 2002) were collected and encoded using Protein Data Bank (PDB). The programme, Molecular Operating Environment (MOE 2014.09) was used to conduct this investigation. After selecting the appropriate order for each molecule, and hydrogens were added then, the energy of the ligand, NaOCl, was minimized, and the partial charges were determined.

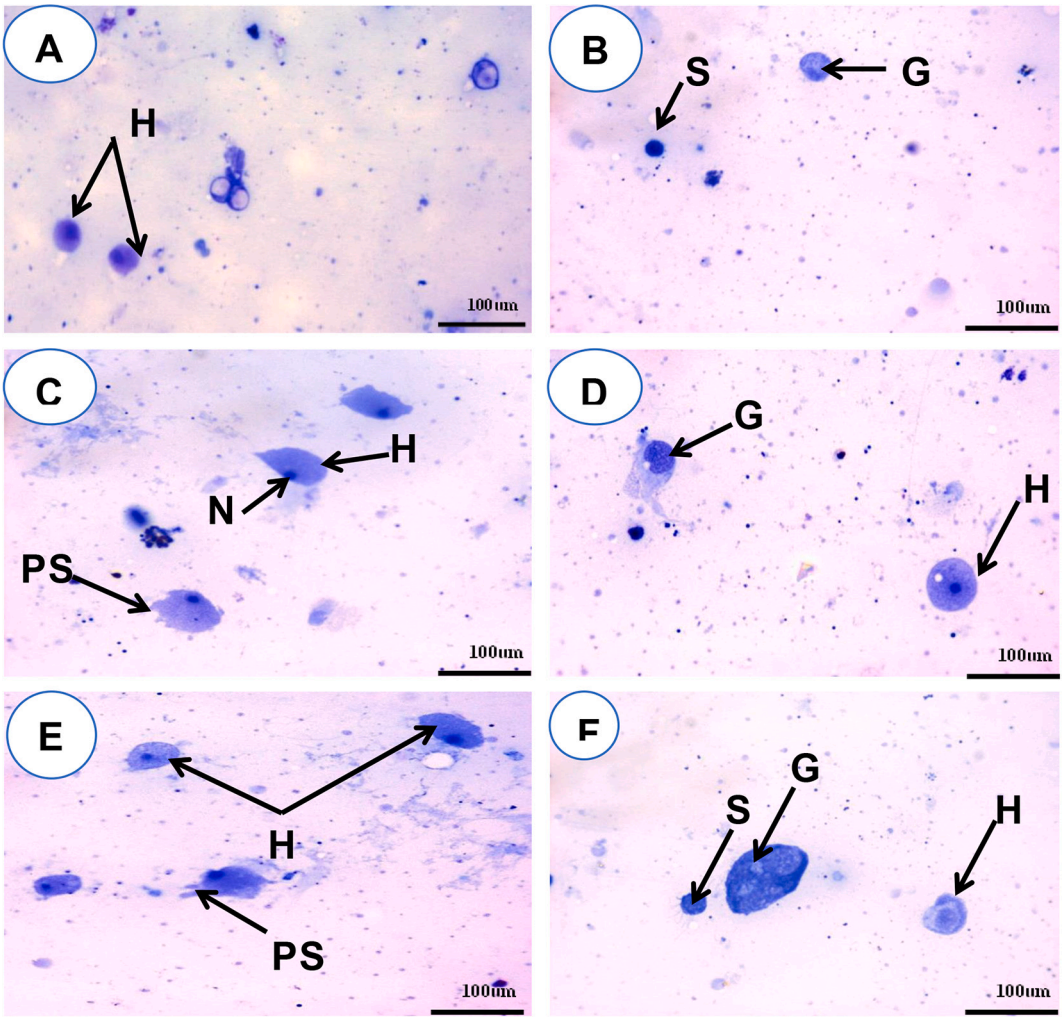

Fig. 2. Photomicrographs show *B. alexandrina* snails hemocytes. A,B) Control *B. alexandrina* snails hemocytes showing small hemocytes (S), granulocytes (G) and hyalinocytes(H). C, D) *B. alexandrina* snails hemocytes exposed to LC_{10} of NaOCl showing granulocytes (G) with numerous granules, and hyalinocytes had shrunk nucleus and small extending pseudopodia (PS). E, F) *B. alexandrina* snails hemocytes treated with LC_{25} of NaOCl revealing hyalinocytes with many pseudopodia, the granulocytes showed granulation. G: Granulocyte, H: Hyalinocyte, N: Nucleus, S: Round Small, PS: Pseudopodia. Magnification power (x40).

2.4. Statistical analysis

Using probability analysis, lethal concentration values were established (Murray, 1981). Student's *t*-test was used for data analysis in order to compare the means of the experimental and control groups (Finney, 1971).Values were expressed as mean ± S.D.

3. Results

The present study elucidated the molluscicide activity of 24 h of exposure NaOCl against adult *B. alexandrina* snails followed by another 24 h for recovery (Table 1). It showed the toxic effect of NaOCl on *B. alexandrina* snails at LC_{50} (1.25 ppm) (Fig. 1).

In the current study, there are three hemocytes types detected in the untreated group; small (undifferentiated); granulocytes and hyalinocytes (Fig. 2). After treatment of *B. alexandrina* snails with the LC_{10}, the granulocytes showed a large number of granules and many pseudopodia with shrunk nucleus were formed. Also, some hyalinocytes' nuclei have shrunk, while others have deteriorated (Fig. 2. C; D). The extensive morphological alterations were detected after exposure to LC_{25} where hyalinocytes enlarged in their size with irregular cell membrane and had many pseudopodia. The granulocytes were more active, enlarged, and more granulation appeared (Fig. 2.E, F).

The current findings demonstrated that exposure to LC_{10} and LC_{25} of NaOCl considerably raised the phagocytic index of the hemocytes of *B. alexandrina* snail (Fig. 3).

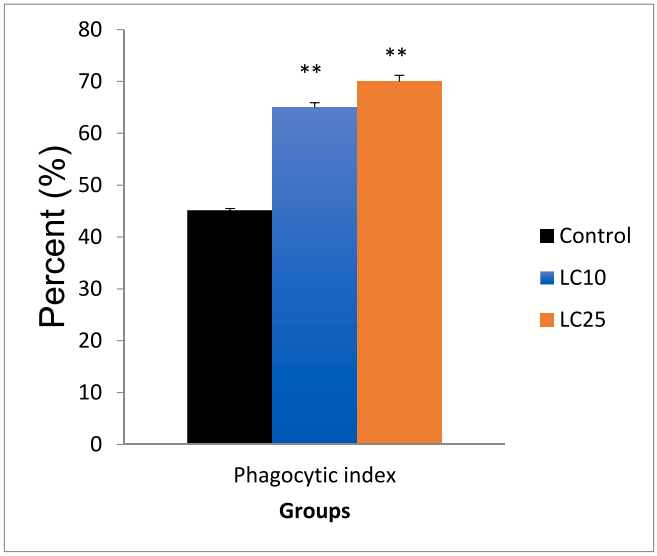

Fig. 3. Phagocytic percent of *B. alexandrina* snail's hemocytes after exposed to sublethal concentrations of NaOCl. * Significantly different as compared to control at $p < 0.05$.

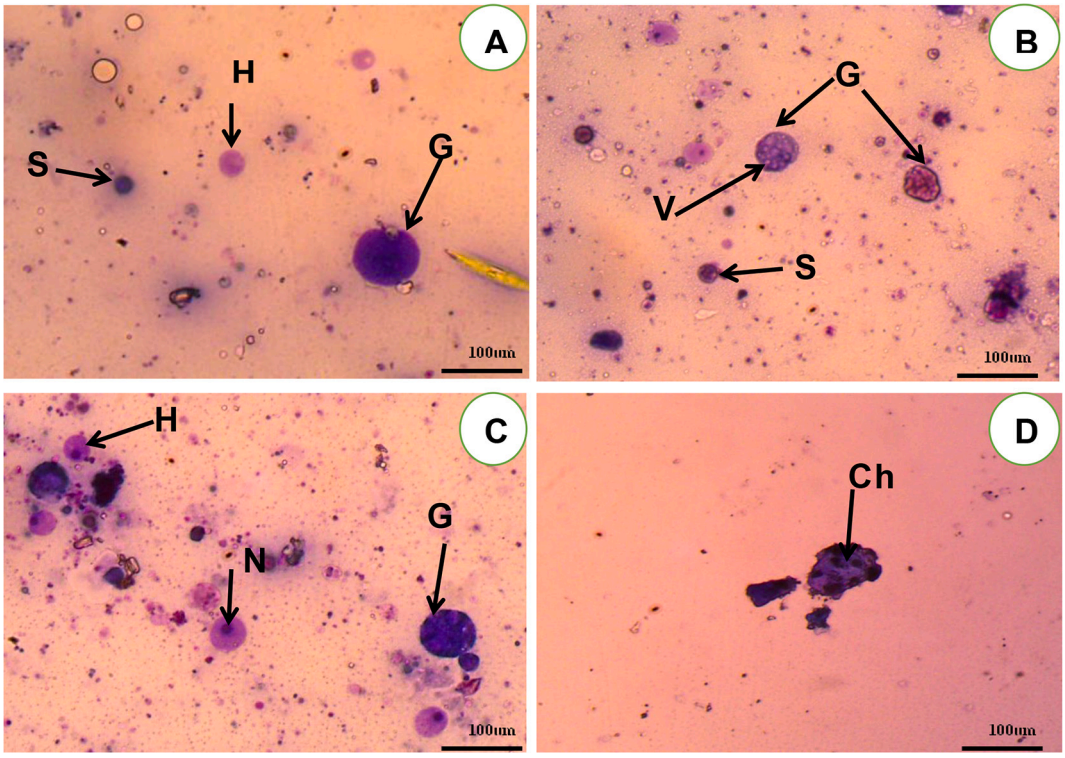

Fig. 4. Photomicrographs showing phagocytic activity of *B. alexandrina* snails. (A), Control snail hemocytes challenged with charcoal only. (B) *Biomphlaria alexandrina* snail hemocytes exposed to LC_{10} of NaOCl and then challenged with charcoal showing many vacuolation (V) in the granulocytes. (C, D) *Biomphlaria alexandrina* snail hemocytes exposed to LC_{25} of NaOCl showing enlarged granulocytes, with the engulfed charcoal particles and formed membrane blebs. H: hyalinocytes; G: granulocytes; S: small cells; Nucleus (N), Ch (charcoal) and vacuoles (V). Magnification power (x 40).

The effect of sublethal concentrations of LC_{10} and LC_{25} of NaOCl on in vitro phagocytic activity of *B. alexandrina* hemocytes against charcoal after two weeks of the exposure are presented in Fig. 4. Results showed that cells of exposed snails to LC_{10} and LC_{25} of NaOCl showed ruptured plasma membrane, vacuolation in the cytoplasm and degeneration of nuclei (Fig. 4 B, C, and D). Some granulocytes

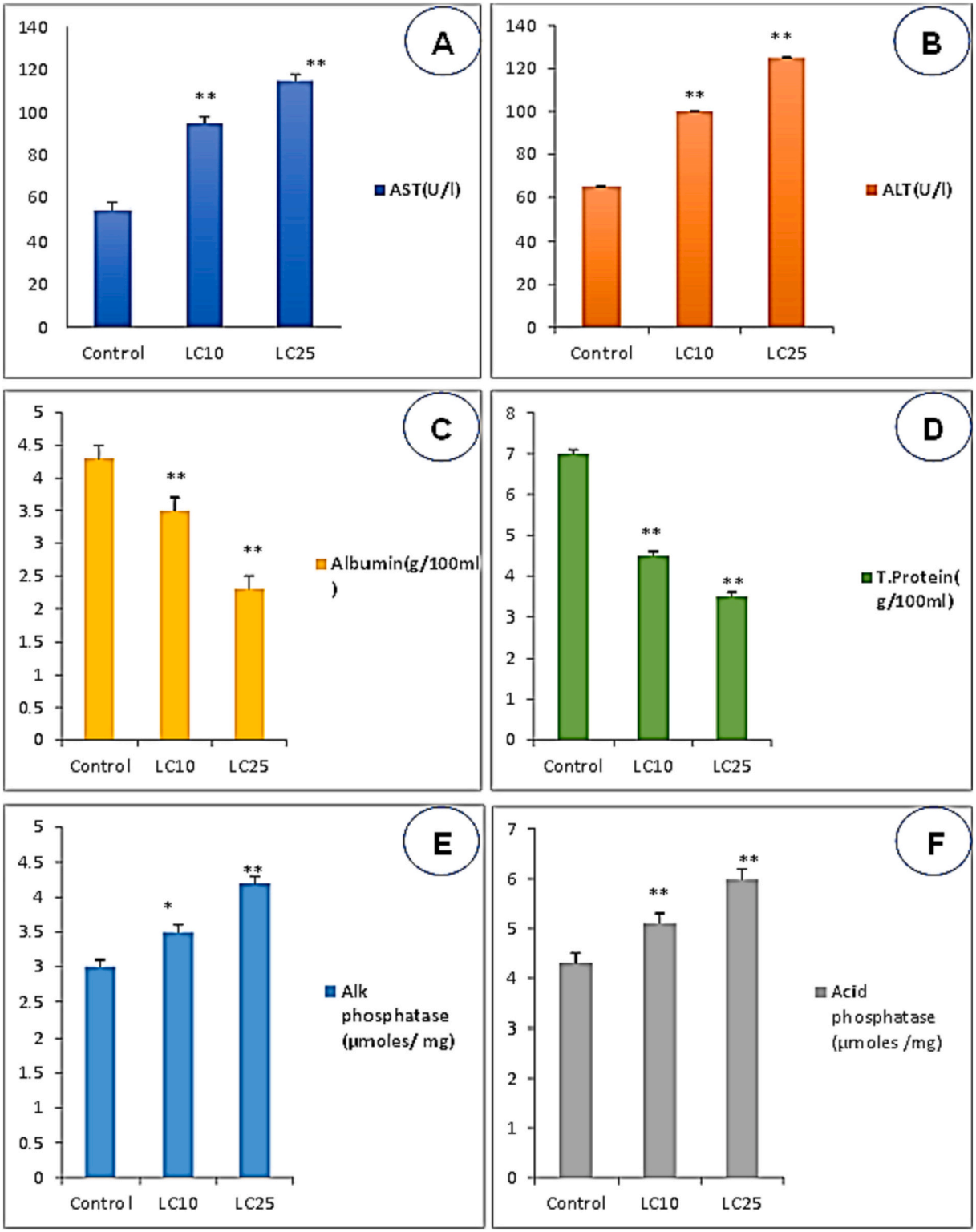

Fig. 5. The effect of LC_{10} and LC_{25} NaOCl exposure on *B. alexandrina* snails. A, B) transaminases (AST and ALT) concentrations after exposure to LC_{10} and LC_{25} NaOCl. C, D) the total protein, and albumin concentrations after exposure to LC_{10} and LC_{25} NaOCl. E, F) alkaline and acid phosphatases levels after exposure to LC_{10} and LC_{25} NaOCl. * = significant compared to control group at $p < 0.05$. ** = highly significant compared to control group at $p < 0.01$.

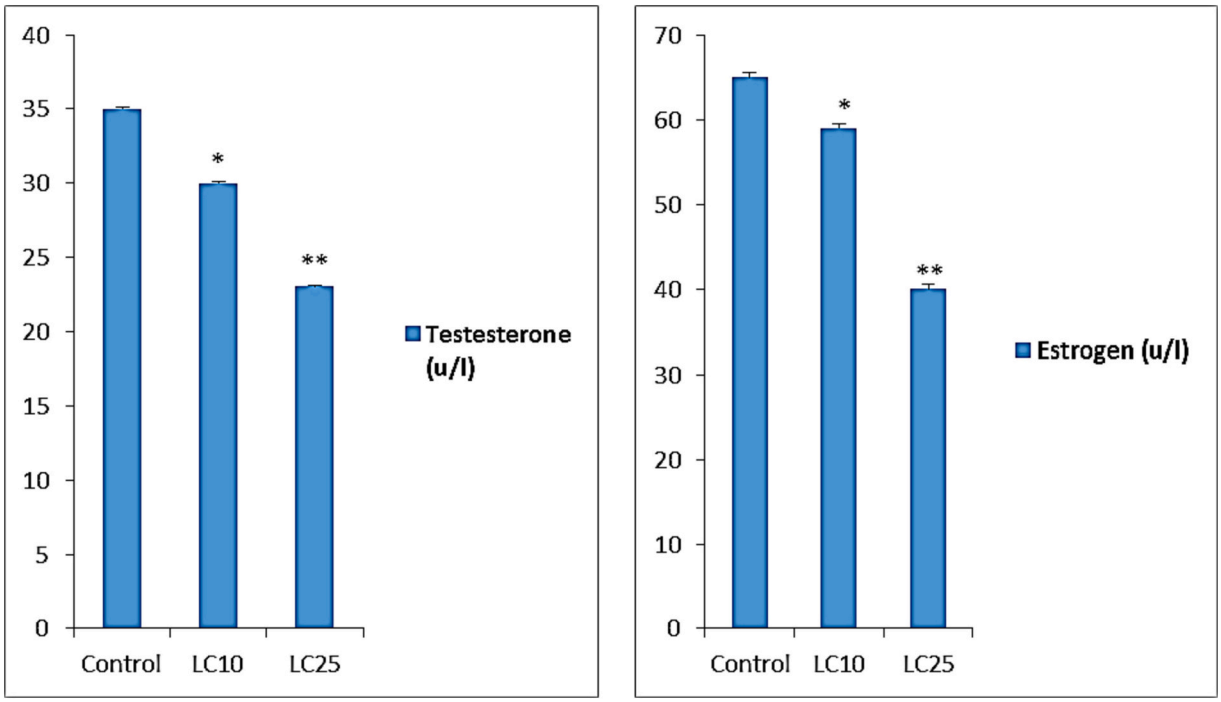

Fig. 6. The endocrine level changes, testosterone (T) and 17β Estradiol (E) in the tissue homogenate of *B. alexandrina* snails after exposure to sub lethal concentrations of LC_{10} and LC_{25} of NaOCl. Values are represented as mean ± S.D.

enlarged, had engulfed the charcoal particles and some formed membrane blebs. Hyalinocytes had shrunk nucleus with vacuoles in the cytoplasm (Fig. 4C) than that of the control group (Fig. 4.A).

The biochemical analysis of LC_{10} and LC_{25} of NaOCl exposure to *B. alexandrina* snails showed a significant elevation of transaminases (AST & ALT), alkaline and acid phosphatase ($p \leq 0.05$) in contrast, the activities of total protein and albumin showed a considerable decrease ($p \leq 0.05$) as compared to the control groups (Fig. 5).

After two weeks of exposure to sub lethal concentrations (LC_{10} and LC_{25}) of NaOCl, the levels of testosterone (T) and 17 estradiol (E) were dramatically decreased ($p < 0.05$) as compared to control group (Fig. 6).

The molecular docking interactions were performed to investigate the action of NaOCl. Acid phosphatase (AP), alanine aminotransferase (ALT), Estrogen (E), and testosterone (T) were chosen. The tested docking interaction showed high efficiency for the ligand, NaOCl against the receptor binding sites of the selected structures. The docking score, also known as interaction-free energy, was used to analyse the inhibitory effect. NaOCl showed docking interaction ability through 2H-acceptor and 2 metals scores (−6.1,-0.9, −1.1 and − 4.6 Kcal/mol) against acid phosphatase (Fig. 7A), and through 2 metals score (−0.9, and − 2.4 Kcal/mol) against ALT (Fig. 7B), while this interaction appeared through 2 metals score (−1.1, and − 3.6 Kcal/mol) with estrogen (Fig. 7C), and finally through 2 metals scores (−0.8, and − 1.5 Kcal/mol) (Table 2) with testosterone (Fig. 7 D).

4. Discussion

The snail population control is the key factor in the limiting spreading of schistosomiasis worldwide (Ibrahim and Hussein, 2022). The purpose of this study was to detect the molluscicide action of high biocide activity, sodium hypochlorite that was widely used for water disinfection as well as residential, industrial, medicinal, and research purposes (Emmanuel et al., 2004). The present study indicated a molluscicide ability for NaOCl against *B. alexandrina* snails with LC_{50} at 1.25 ppm and LC_{90} at 1.71 ppm. The present results are in agreement with the findings previously obtained by (Taha et al., 2014) who found that effects of NaOCl on the mortality of the *L. natalensis* snails were concentration- and time-dependent and after 30 min of incubation, fatal effects at concentrations of 20, 30, 40, and 50 ppm ranged from 30% to 60%. None of the snails treated lived after 4 days.

Hemocytes, which are in charge of phagocytosis, make up the majority of the snail immune system in mollusks (Baroudi et al., 2020). In the present study, when hemocyte monolayers were examined, three different cells were detected; round, tiny, hyalinocytes, and spreading hemocytes and there were many alterations specially pseudopodia formation after exposure of *B. alexandrina* snails to NaOCl. These pseudopodia are working with other snail's innate immune system to eliminate any aquatic contaminants (Donaghy et al., 2010).

The present results are in accordance with the investigations obtained by (Morad et al., 2022) who found that various changes, including the development of pseudopodia and an increase in granules, were seen in the hemocytes of treated snails with selenium nanoparticles. Such adaptations could result from the insecticide's molluscicide properties as well as from adjustments to intercellular

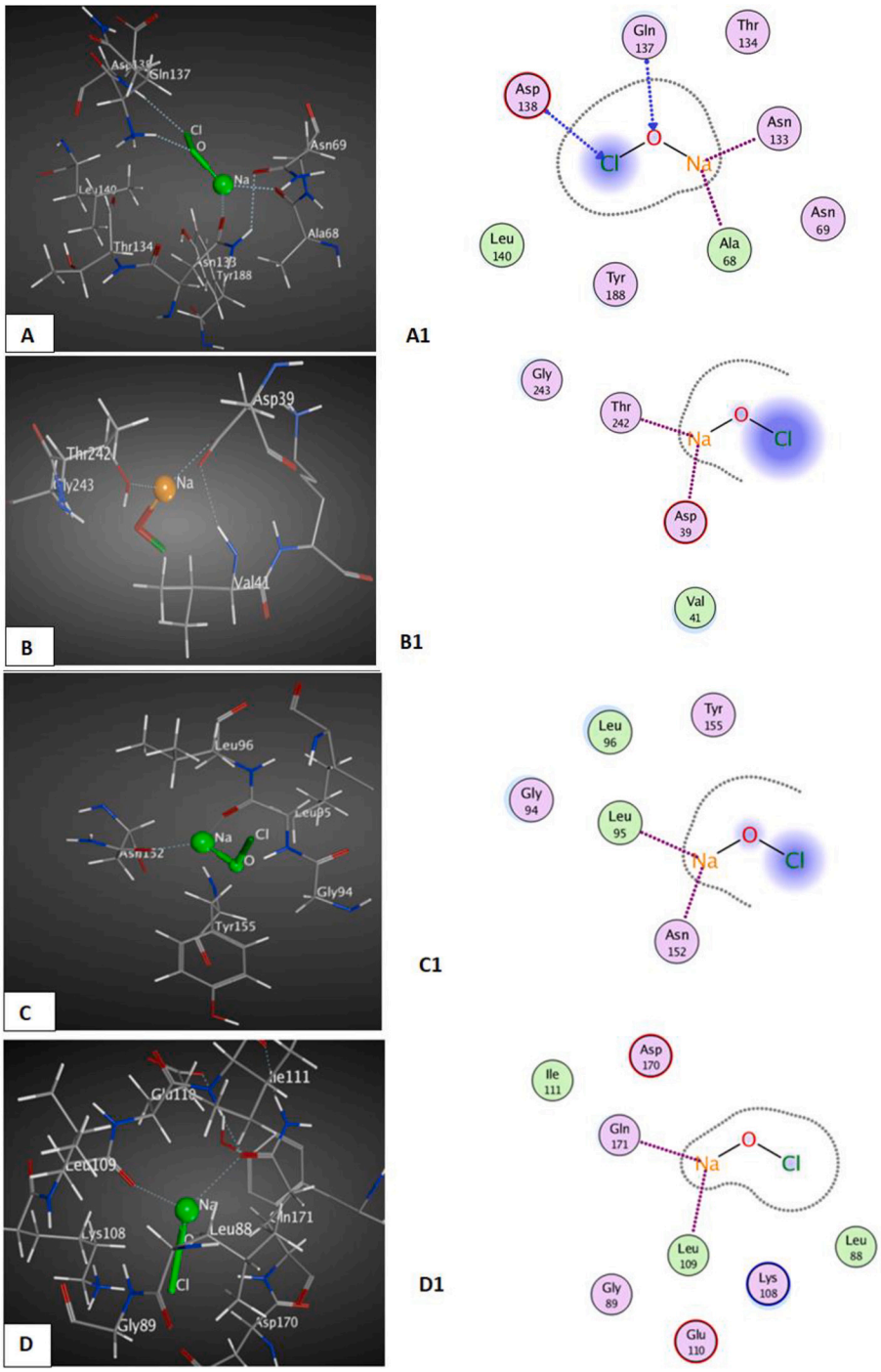

Fig. 7. 3D and 2D docked interaction map for the ligand (NaOCl) with the binding sites of Acid phosphatase (A, A1), alanine aminotransferase (B, B1), Estrogen (C, C1), and testosterone(D, D1), respectively.

adhesion molecules that are sensitive to the absorption of foreign materials, increasing granulocytes' phagocytic activity (Matricon-Gondran and Letocart, 1999).

One of the many activities of hemocytes is phagocytosis, a general immunological response to foreign substances (Hooper et al., 2007). Hemocyte activity for immunological response includes the ingestion of foreign particles into an endocytic vacuole by ingestion of the particle, creation of pseudopods, and invagination of the cell membrane (Ladhar-Chaabouni, 2016).The present investigation showed that NaOCl treatment significantly increased the mean phagocytic index of the hemocytes of exposed snails challenged with

Table 2
In-silico docking study of the investigated molecules, Acid phosphatase (AP), alanine aminotransferase (AST), Estrogen (E), and testosterone (T), with the ligand, NaOCl. Abbreviation; ALA: Alanin; ASN: Asparagine; ASP: aspartic acid; GLN: Glutamin; LEU: Leucine; THR: Threonine;

PDB molecules	Docking energy score (Kcal/mol)	Kind of interaction	residue of interacting amino acids
Acid phosphatase (AP)	−6.1	H-acceptor	GLN 137
	−0.9	H-acceptor	ASP 138
	−1.1	Metal	ALA 68
	−4.6	Metal	ASN 133
alanine aminotransferase (ALT)	−0.9	Metal	ASP 39
	−2.4	Metal	THR 242
Estrogen (E)	−1.1	Metal	LEU 95
	−3.6	Metal	ASN 152
Testosterone (T)	−0.8	Metal	LEU 109
	−1.5	Metal	GLN 171

charcoal that may indicate the increase in the mortality index is indication of the Cell death or apoptosis (Guria, 2018). After exposure to different particles, the phagocytic capability of hemocytes from different mollusks species can increase, decrease, or stay the same (Ladhar-Chaabouni, 2016). Our results are consistent with a recent study found that the phagocytic index of hemocytes increased after the treatment of *Planorbarius corneus* and *Biomphalaria glabrata* with various chlorpyrifos (Garate et al., 2020).

Aspartate and Alanine Aminotransferases (AST and ALT) were important in various stressful situations and were utilised as biomarkers for water pollution (Masola et al., 2003)(Khalaf, 2008). The results of the current study revealed that these two enzymes were significantly more active in all groups who received NaOCl treatment. This is a reflection of the harm NaOCl has done to liver cells. These findings are consistent with a prior study (El-Deeb et al., 2017) that discovered an increase in AST and ALT activity in the hemolymph of five species of fresh water snails subjected to LC10 and LC25 Nicodamid The observed modifications could be the result of animal experiments to balance amino acids in various body parts. Similarly, Beltagi et al., 2010 reported a significant increase in the levels of ALT and AST in the exposed land snails to LC25 and LC50 thymol. On contrary, (Abdel Salam et al., 2022) found that thymol could inhibit the release of ALT and AST in the hemolymph of exposed snails. According to the effectiveness of molluscicides as a toxic agent that induced rupturing and inflicted injuries in many snail organs, may explain the changes in the activities of AST and ALT exposed *B. alexandrina* snails (Ahmed et al., 2012).

According to the current investigation, the contents of total protein and albumin in the hemolymph of the snails subjected to sublethal doses of NaOCl (LC10 and LC25) were lower than those in the control group. This investigation was similar to a study conducted by (Mohamed, 2012) who mentioned that total protein and albumin concentrations in hemolymph significantly decreased after *B. alexandrina* snails were exposed to sublethal doses of the insecticides Regent and Mimic for 4 weeks. This decrease was brought on by the destruction and cellular degeneration of the treated snails' ovotestis and digestive glands.

Invertebrate endocrine systems control activities like development, growth, and reproduction that are also present in vertebrates and there are many particles that may lead to disrupting development and reproductive functions of invertebrate endocrine systems (Ford and LeBlanc, 2020). The current findings demonstrated that, in comparison to the control group, both testosterone (T) and estradiol (E) levels considerably decreased following exposure to LC10 and LC25 of NaOCl. This effect could be explained based on the damages occurred by NaOCl in hermaphrodite glands of treated snails (Ibrahim et al., 2018). Our results are in agreement with the study of (Omran and Salama, 2016) who investigated that the treatment of *B. alexandrina* snails with atrazine and glyphosate herbicides caused an inhibition in the levels of Testosterone (T) and Estradiol (E). In contrast, (Ibrahim and Hussein, 2022) Found that the exposure of *B. alexandrina* snails to organophosphorus Chlorpyrifos 48%EC pesticide could considerably increase levels of Testosterone (T) and Estradiol (E) in comparison to the control group.

Molecular docking is a potential method for determining how well ligand molecules counteract the effects of specific proteins through receptor-ligand interactions. Acid phosphatase enzyme is involved in lysosomes, autolysis and necrosis in gastropods (Ibrahim and Bakry, 2019). The present study examined the interaction of NaOCl with acid phosphatase, ALT, estrogen, and testosterone. It revealed interactions between the ligand under study, NaOCl and acid phosphatase, ALT, estrogen, and testosterone. The interactions between NaOCl and acid phosphatase, ALT might resulted in their elevation. In contrast, this interaction could lead to inhibition of the endocrine enzymes, estrogen and testosterone.

5. Conclusion

Sodium hypochlorite affected the physiological and immunological characteristics of *B. alexandrina* snails, which is a good sign that it could be used as a powerful molluscicide. The freshwater zooplanktonic species as a non-target organism for toxicity evaluation in aquatic ecosystems located in habitat as *B. alexandrina* should be further studied to identify their influence on the genotoxic levels.

Funding

This research has been funded by Princess Nourah bint Abdulrahman University Researchers Supporting project number (PNURSP2023R23), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

CRediT authorship contribution statement

Amina M. Ibrahim: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Mohamed T. Hamed:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Manal F. EL-Khadragy:** Software, Formal analysis, Writing – original draft, Writing – review & editing. **Mostafa Y. Morad:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research has been funded by Princess Nourah bint Abdulrahman University Researchers Supporting project number (PNURSP2023R23), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

References

Abadias, M., Usall, J., Oliveira, M., Alegre, I., Vinas, I., 2008. Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. Int. J. Food Microbiol. 123, 151–158. https://doi.org/10.1016/j.ijfoodmicro.2007.12.008.

Abdel Salam, H., Maghrabi, O., Al-Malki, S., Albenasy, K., 2022. Thymol as molluscicidal agent against biomphalaria alexandrina, intermediate host of *Schistosoma mansoni*. J. Essent. Oil Bear. Plants 24, 1–12. https://doi.org/10.1080/0972060X.2021.2003255.

Abdul-Salam, J.M., Michelson, E.H., 1980. Biomphalaria glabrata amoebocytes: effect of Schistosoma mansoni infection on in vitro phagocytosis. J. Invertebr. Pathol. 35, 241–248.

Ahmed, M., El-Emam, M., Osman, G., Abdel-Hamid, H., Ezzat, R., 2012. Biological and biochemical responses of infected Biomphalaria alexandrina snails with Schistosoma mansoni post exposure to the pesticides Basudin and Selecron and the phytoalkaloid colchicine. J. Evol. Biol. 4 (2), 24–32.

Alberto-Silva, A.C., Santos, E.G.N., Santos, C.P., Mello-Silva, C.C., 2015. Changes in the locomotory and reproductive behavior of Biomphalaria glabrata infected with Schistosoma mansoni. Exp. Parasitol. 153, 68–74. https://doi.org/10.1016/j.exppara.2015.03.004.

Azzi, A., Rehse, P.H., Zhu, D.-W., Campbell, R.L., Labrie, F., Lin, S.-X., 1996. Crystal structure of human estrogenic 17β-hydroxysteroid dehydrogenase complexed with 17β-estradiol. Nat. Struct. Biol. 3, 665–668.

Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020. Snail as sentinel organism for monitoring the environmental pollution; a review. Ecol. Indic. 113, 106240.

Beltagi, S.M.H., Al-Shinnawy, M.S.A., Elkattan, N.A., Yousef, H.N., 2010. Effects of sublethal doses of selected botanical molluscicides on oxygen consumption of the brown garden snail, Eobania vermiculata. The Egypt. J. Hospital Med. 40, 402–410.

Bergmeyer, H., 1985. Methods of enzymatic analysis, methods of enzymatic analysis. In: Metabolites 3: Lipids, Amino Acids and Related Compounds, vol. 8.

Coelho, P.M.Z., Caldeira, R., 2016. Critical analysis of molluscicide application in schistosomiasis control programs in Brazil. Infect. Dis. Poverty 5. https://doi.org/10.1186/s40249-016-0153-6.

Donaghy, L., Hong, H.-K., Lambert, C., Park, H.-S., Shim, W.J., Choi, K.-S., 2010. First characterisation of the populations and immune-related activities of hemocytes from two edible gastropod species, the disk abalone, Haliotis discus discus and the spiny top shell. Turbo cornutus. Fish Shellfish Immunol. 28, 87–97. https://doi.org/10.1016/j.fsi.2009.10.006.

El-Deeb, F.A.A., Marie, M.-A.S., Hasheesh, W.S., Hussein, R.M.A., Sayed, S.S.M., 2017. Biomarkers of oxidative stress in Biomphalaria alexandrina snails for assessing the effects of certain inorganic fertilisers. Molluscan Res. 37, 289–294. https://doi.org/10.1080/13235818.2017.1357783.

Emmanuel, E., Keck, G., Blanchard, J.-M., Vermande, P., Perrodin, Y., 2004. Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. Environ. Int. 30, 891–900. https://doi.org/10.1016/j.envint.2004.02.004.

Eventov, V.L., MIu, A., Kukaeva, E.A., 1998. Detoxication and disinfection by sodium hypochlorite. Med. Tekh. 36–39.

Ezeamama, A., Friedman, J., Acosta, L., Bellinger, D., Langdon, G., Manalo, L., Olveda, R., Kurtis, J., McGarvey, S., 2005. Helminth infection and cognitive impairment among Filipino children. Am. J. Trop. Med. Hyg. 72, 540–548. https://doi.org/10.4269/ajtmh.2005.72.540.

Finney, D.J., 1971. Probit Analysis (3rdedn.). Combrige University Press.

Ford, A.T., LeBlanc, G.A., 2020. Endocrine disruption in invertebrates: a survey of research Progress. Environ. Sci. Technol. 54, 13365–13369. https://doi.org/10.1021/acs.est.0c04226.

Friedman Kanzaria, H., 2005. Relationship between Schistosoma japonicum and nutritional status among children and young adults in Leyte, The Philippines (vol 72, pg 527, 2005). Am. J. Trop. Med. Hyg. 73, 230.

Garate, O.F., Gazzaniga, S., Cochón, A.C., 2020. A comparative study of enzymatic and immunological parameters in Planorbarius corneus and Biomphalaria glabrata exposed to the organophosphate chlorpyrifos. Aquat. Toxicol. 225, 105544 https://doi.org/10.1016/j.aquatox.2020.105544.

Gerba, Charles P., Denise, K., 2007. Enteric virus survival during household laundering and impact of disinfection with sodium hypochlorite. Appl. Environ. Microbiol. 73, 4425–4428. https://doi.org/10.1128/AEM.00688-07.

Gholib, G., Wahyuni, S., Akmal, M., Hasan, M., Agil, M., Purwantara, B., 2020. The validation of a commercial enzyme-linked immunosorbent assay and the effect of freeze-thaw cycles of serum on the stability of cortisol and testosterone concentrations in Aceh cattle. F1000Research 8, 1220. https://doi.org/10.12688/f1000research.19804.3.

Guria, S., 2018. Lead (Pb) induced paraptosis like cell death in hemocytes of Lamellidens Sp: a preliminary study. World Sci. News 94, 163–172.

Gustafsson, Je, 1976. Improved specificity of serum albumin determination and estimation of" acute phase reactants" by use of the bromcresol green reaction. Clin. Chem. 22, 616–622.

Hooper, C., Day, R., Slocombe, R., Handlinger, J., Benkendorff, K., 2007. Stress and immune responses in abalone: limitations in current knowledge and investigative methods based on other models. Fish Shellfish Immunol. 22, 363–379. https://doi.org/10.1016/j.fsi.2006.06.009.

Ibrahim, A.M., Abdalla, A.M., 2017. Impact of Moringa oleifera seed aqueous extract on some biological, biochemical, and histological aspects of Biomphalaria alexandrina snails. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-017-0397-0.

Ibrahim, A.M., Bakry, F.A., 2019. Assessment of the molluscicidal impact of extracted chlorophyllin on some biochemical parameters in the nervous tissue and histological changes in Biomphalaria alexandrina and Lymnaea natalensis snails. Invert. Neurosci. 19 https://doi.org/10.1007/s10158-019-0230-1.

Ibrahim, A.M., Hussein, A.A.A., 2022. Toxicological impact of organophosphorus Chlorpyrifos 48%EC pesticide on hemocytes, biochemical disruption, and molecular changes in Biomphalaria alexandrina snails. Pestic. Biochem. Physiol. 186, 105154 https://doi.org/10.1016/j.pestbp.2022.105154.

Ibrahim, A.M., Kamal, A.A.K., 2019. Trematode cercarial fauna obtained from the field-collected freshwater snails Lymnaea natalensis in Egypt. Bull. Natl. Res. Cent. 43 https://doi.org/10.1186/s42269-019-0138-2.

Ibrahim, A.M., Nasr, S.M., 2019. Cytochrome oxidase subunit and internal transcribed spacer molecular revelation of infection with Fasciola sp. in field- collected Lymnaea natalensis snails. J. Egypt. Soc. Parasitol. 49, 357–364.
Ibrahim, A.M., Ahmed, A.K., Bakry, F.A., Abdel-Ghaffar, F., 2018. Hematological, physiological and genotoxicological effects of match 5% EC insecticide on Biomphalaria alexandrina snails. Ecotoxicol. Environ. Saf. 147 https://doi.org/10.1016/j.ecoenv.2017.09.059.
Ibrahim, A., Abdel-Tawab, H., Hussein, T., 2019b. Pentoxifylline and/or praziquantel reduce murine schistosomiasis mansoni histopathology via amelioration of liver functions. Egypt. J. Aquat. Biol. Fish. 23 https://doi.org/10.21608/ejabf.2019.67229.
Ibrahim, A.M., Ahmed, A.K., Hammam, O.A., Abdel-Ghaffar, F., 2022a. Immunotoxical, neurotoxical, histopathological and immunohistopathological alterations of Nerium oleander and Tecoma stans methanolic extract on Biomphalaria alexandrina snails. Acta Trop. 230, 106405 https://doi.org/10.1016/j.actatropica.2022.106405.
Ibrahim, A.M., Hussein, T.M., Abdel-Tawab, H., Hammam, O.A., Ghareeb, M.A., 2022b. The ameliorative effects of Eremina desertorum snail mucin in combination with Silymarin against experimentally induced liver fibrosis. Egypt. J. Chem. 65, 181–190. https://doi.org/10.21608/EJCHEM.2021.84043.4114.
Ibrahim, A.M., El-karim, R.M.G., Ali, R.E., Nasr, S.M., 2023. Toxicological effects of Saponin on the free larval stages of *Schistosoma mansoni*, infection rate, some biochemical and molecular parameters of Biomphalaria alexandrina snails. Pestic. Biochem. Physiol. 191, 105357 https://doi.org/10.1016/j.pestbp.2023.105357.
Ishikawa, K., Mihara, Y., Gondoh, K., Suzuki, E., Asano, Y., 2000. X-ray structures of a novel acid phosphatase from Escherichia blattae and its complex with the transition-state analog molybdate. EMBO J. 19, 2412–2423.
Ismail, M., Attia, M., Metweally, A.A., Farghaly, A.M., Bruce, J., Bennett, J., El-Badawy, A.A., Hussein, M.H., 1994. Assessment of praziquantel therapy in treatment of Schistosoma mansoni infection. J. Egypt. Soc. Parasitol. 24, 231–238.
Khalaf, S., 2008. Measurement of selected enzymatic activities in Solanum nigrum -treated Biomphalaria arabica snails. J. Appl. Sci. 8 https://doi.org/10.3923/jas.2008.881.885.
Ladhar-Chaabouni, R., 2016. The cell cultures and the use of haemocytes from marine molluscs for ecotoxicology assessment. Cytotechnology. Cytotechnology 68, 1669–1685.
Lago, E., Xavier, R., Teixeira, T., Silva, L., Da Silva Filho, A., Moraes, J., 2017. Antischistosomal agents: state of art and perspectives. Future Med. Chem. 10 https://doi.org/10.4155/fmc-2017-0112.
Lowry, O., Schagger, H., Cramer, W.A., Vonjagow, G., 1994. Protein measurement with the folin phenol reagent. Anal. Biochem. 217, 220–230. https://doi.org/10.1016/0304-3894(92)87011-4.
Masola, B., Chibi, M., Naik, Y.S., Kandare, E., Zaranyika, M.F., 2003. Activities of glutamate dehydrogenase and aspartate and alanine aminotransferases in freshwater snails Helisoma duryi and Lymnaea natalensis exposed to copper. biomarkers 8, 33–42.
Matricon-Gondran, M., Letocart, M., 1999. Internal defenses of the snail Biomphalaria glabrata: I. Characterization of Hemocytes and fixed phagocytes. J. Invertebr. Pathol. 74, 224–234. https://doi.org/10.1006/jipa.1999.4876.
Mohamed, M., 2012. Biological and biochemical responses of infected Biomphalaria alexandrina snails with Schistosoma mansoni post exposure to the pesticides Basudin and Selecron and the phytoalkaloid colchicine. J. Evol. Biol. Res. 4, 24–32. https://doi.org/10.5897/JEBR12.006.
Morad, M.Y., El-Sayed, H., Elhenawy, A.A., Korany, S.M., Aloufi, A.S., Ibrahim, A.M., 2022. Myco-synthesized Molluscicidal and Larvicidal selenium nanoparticles: a new strategy to control Biomphalaria alexandrina snails and larvae of Schistosoma mansoni with an in silico study on induced oxidative stress. J. Fungi. https://doi.org/10.3390/jof8030262.
Morad, M.Y., El-Sayed, H., El-Khadragy, M.F., Abdelsalam, A., Ahmed, E.Z., Ibrahim, A.M., 2023. Metabolomic profiling, antibacterial, and Molluscicidal properties of the medicinal plants Calotropis procera and Atriplex halimus: in silico molecular docking study. Plants 12, 477. https://doi.org/10.3390/PLANTS12030477.
Murray, R., 1981. Speigel: Statistical estimation theory. In: Schaum's Outl. Ser. Theory Probl. Stat. SI Units, First ed. McGraw Hill B. Company, Singapore 156.
Nduku, W.K., Harrison, A.D., 1980. Cationic responses of organs and haemolymph of Biomphalaria pfeifferi (Krauss), Biomphalaria glabrata (say) and Helisoma trivolvis (say)(Gastropoda: Planorbirdae) to cationic alterations of the medium. Hydrobiologia 68, 119–138.
Omran, N.E., Salama, W.M., 2016. The endocrine disruptor effect of the herbicides atrazine and glyphosate on Biomphalaria alexandrina snails. Toxicol. Ind. Health 32, 656–665.
Peeters, E., Nelis, H.J., Coenye, T., 2008. Evaluation of the efficacy of disinfection procedures against Burkholderia cenocepacia biofilms. J. Hosp. Infect. 70, 361–368. https://doi.org/10.1016/j.jhin.2008.08.015.
Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63.
Singh, D.K., Agarwal, R.A., 1991. Action sites of Cypermethrin, a synthetic Pyrethroid in the snail Lymnaea acuminata. Acta Hydrochim. Hydrobiol. 19, 425–430. https://doi.org/10.1002/aheh.19910190415.
Taha, H.A., El-Shaikh, K.A., Al-Sadi, M.M., 2014. Effect of sodium hypochlorite on Fasciola gigantica eggs and the intermediate host, Lymnaea natalensis: a scanning electron microscopy study. J. Taibah Univ. Sci. 8, 75–83. https://doi.org/10.1016/j.jtusci.2013.12.003.
Valjakka, J., Takkinenz, K., Teerinen, T., Söderlund, H., Rouvinen, J., 2002. Structural insights into steroid hormone binding: the crystal structure of a recombinant anti-testosterone fab fragment in free and testosterone-bound forms. J. Biol. Chem. 277, 4183–4190.
WHO, 1983. Report of the Scientific working Group on Plant Molluscicide & Guidelines for evaluation of plant molluscicides. WHO (TDR/SCHSWE (4)/83.3, Geneva.
WHO, 2022. Schistosomiasis [WWW Document]. Organ. Fact sheet;Schistosomiasis, World Heal.
Xavier, R.P., Mengarda, A.C., Silva, M.P., Roquini, D.B., Salvadori, M.C., Teixeira, F.S., Pinto, P.L., Morais, T.R., Ferreira, L.L.G., Andricopulo, A.D., de Moraes, J., 2020. H1-antihistamines as antischistosomal drugs: in vitro and in vivo studies. Parasit. Vectors 13, 278. https://doi.org/10.1186/s13071-020-04140-z.
Zheng, L., Deng, L., Zhong, Y., Wang, Y., Guo, W., Fan, X., 2021. Molluscicides against the snail-intermediate host of Schistosoma: a review. Parasitol. Res. 120, 3355–3393. https://doi.org/10.1007/s00436-021-07288-4.
Zhou, W., Tempel, W., Shah, A., Chen, L., Liu, Z.J., Lee, D., Lin, D., Chang, S.H., Brereton, P.S., Izumi, M., 2004. Alanine aminotransferase from Pyrococcus furiosus Pfu-1397077-001. Southeast Collab. Struct. Genomics.