Biological and Biomedical Journal

Journal homepage: https://bbj.journals.ekb.eg

Immunomodulation of *Biomphalaria alexandrina* snails using sodium alginates as a novel method of schistosomiasis biocontrol in Egypt

Rasha M. Gad El-Karim*[1], Kamelia A. El Sayed[1], Maha G. Soliman[2], Nouran A. Abo Ouf[2], Faten A. Elfekky[2]

[1]Medical Malacology Department, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.
[2] Parasitology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.

ARTICLE INFO

Received:20/12/2024
Accepted:30/1/2025

Corresponding author:
Rasha M. Gad El-Karim, Ph.D
E-mail: r.gad@tbri.gov.eg
Mobile: 01067385565

P-ISSN: 2974-4334
E-ISSN: 2974-4324
DOI: 10.21608/BBJ.2024.312606.1038

ABSTRACT

Biomphalaria alexandrina snails are members of the phylum Mollusca and are known to have an effective innate defense system consisting of cellular and humoral defense factors which can be modulated by immunostimulation. The effect of sodium alginates on *B. alexandrina* snails and their hemocytes, antioxidant enzymes and infection dynamics was assessed under laboratory conditions. The influence of sodium alginates on the infection rate of snails with *Schistosoma mansoni* miracidia under simulated natural conditions was evaluated. Exposing snails to sodium alginates resulted in a considerable increase in the overall number of hemocytes and the most significant increase was 6650 hemocyte/ml after 7 days of exposure to 0.5 mg/ml sodium alginates, compared to 1950 hemocyte/ml in control snails. Superoxide dismutase, catalase, and glutathione activities were reduced. Lipid peroxide levels were significantly elevated after sodium alginates' exposure. Exposure of snails to sodium alginates under simulated natural conditions reduced the infection rate of snails by 28.3%, 18.5% and 55.4% after treatment with 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml respectively. In general, exposure to sodium alginates showed a pattern of delayed infection, as well as sporocysts distortion and degeneration when compared to normally infected snails. It is concluded that sodium alginate is a promising candidate as a biocontrol agent for schistosomiasis in Egypt as it enhanced *B. alexandrina* snails' immune ability to overcome infection with *S. mansoni* miracidia.

keywords: Antioxidants, *Biomphalaria alexandrina*, Hemocytes, Infection dynamics, *Schistosoma mansoni*, Sodium alginates

1. Introduction

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. Estimates show that at least 236.6 million people required preventive treatment in 2019 (WHO, 2023). Schistosomiasis remains one of the most prevalent infections in the world (King and Dangerfield-Cha, 2008). *Biomphalaria alexandrina* is the molluscan species responsible for *Schistosoma mansoni* transmission in Egypt (Abou-El-Naga, 2013; Mohamed et al., 2015).

Snail control is an important part of the schistosomiasis control approach, and it can be accomplished via chemical, environmental, and biological means. Immunostimulants and nucleotides have received more attention in the recent two decades as ways to lower vulnerability to various stresses and diseases, as well as improve invertebrates' overall health. Many effectors, such as bacteria, yeast, glucans, peptidoglycans, lipopolysaccharides, and other polysaccharides, have been examined extensively in fish and crustaceans to control the immune response (Fujiki and Yano, 1997; Song et al., 2006; Pereira et al., 2022).

Sodium alginate is a natural polysaccharide product derived from brown seaweed that grows in cold water regions. It had been used for a long time as an immunomodulator and supplementary diet for shrimps to boost their immune ability in aquacultures (Winton et al., 2005; Chun-Hung et al., 2006).

B. alexandrina snails as well as other molluscs have a well-developed internal defense system consisting of both cellular and humoral defense factors (Johnston and Yoshino, 1996; Kofta, 1997; Le Clec'h et al., 2016). Circulating hemocytes are the primary mediator of cellular defense reactions in snails. Several studies examining the composition of hemocyte populations of gastropods revealed that hemocytes are composed of a mixture of different types of cells (Adema et al., 1992; El-Karim et al., 2022). Gastropod hemocytes have been given a variety of functions, including an important involvement in defensive mechanisms like phagocytosis encapsulation and wound healing (Furuta et al., 1990; Wang et al., 2023). Immune utilities of molluscs are supplemented by an array of killing mechanisms including the release of degradative and oxidative enzymes (Renwrantz et al., 1996) and the generation of highly reactive oxygen metabolites (Arumugam et al., 2000). The ability of gastropod snails to kill trematode parasites may be reflected in their ability to produce reactive oxygen species, which could be important in determining susceptibility or resistance to *S. mansoni* (Humphries and Yoshino, 2008).

The first line of defense against oxidative stress is provided by superoxide dismutases (SODs), because of their intra- and extra-cellular localization at the same time (Stegeman et al., 1992; Johnson and Giulivi, 2005). Catalase (CAT) is a key component of the antioxidant defense system (Jamil, 2001). It catalyzes the conversion of hydrogen peroxide to water and oxygen, using an iron or manganese co-factor (Chelikani et al., 2004). An increase in the production of free radicals stimulates and increases antioxidant activities to cope with increased oxidative stress and protect the cells from damage (Torres et al., 2002). It is well known that glutathione reduced (GSH) plays a role in the protection of cells from the lethal effects of toxic carcinogenic compounds (Ketterer, 1988).

During the life cycle of trematodes, sporocysts larval stages develop in the molluscan intermediate hosts and there is an evolutionary battle that leads to an arms race between the host and parasites. Susceptibility or resistance to infection in snails is regulated genetically in a way that some susceptibility may be present in resistant snails (Carton et al., 2005). The significance of semi-field trials for effective laboratory investigations has been underlined by several scientists. According to Srivastava and Pandy (2015), a comprehensive research effort should be diverted to study their efficacy in large-scale field applications, and research efforts should also be directed to characterize receptors on target cells for the recognition of immunostimulatory substances. The present work was designed to evaluate the potential immunostimulatory effect of sodium alginate on *B. alexandrina* snails and its ability to reduce their susceptibility to infection with *S. mansoni* miracidia under both laboratory and simulated field conditions to plot the possibility to use it as a novel method of schistosomiasis biocontrol in Egypt.

2. Materials and methods

Snails and *Schistosoma mansoni* ova

B. alexandrina snails (5-6 mm in diameter) used in the present studies were laboratory produced and obtained from colonies maintained in the Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt. They were maintained in plastic aquaria (16 x 23 x 9 cm), provided with dechlorinated aerated tap water (10 snails / L) and covered with glass. Oven-dried lettuce leaves were used for feeding. Water in the aquaria was continuously changed weekly, with a photoperiodicity of 12hr. light/ 12hr. dark and water temperature of 25±1°C were kept. Dead snails were removed daily. *S. mansoni* ova used were obtained from CD1 mice livers previously infected with *S. mansoni* cercariae. The ova were allowed to hatch in a small amount of dechlorinated water (25±1˚C) for about 15 minutes under a direct light (desk lamp). Then, the freshly hatched miracidia were collected by Pasteur pipette under

a stereomicroscope and used in experimental tests.

Experimental material

A commercial form of sodium alginates from Sigma-Aldrish Company was provided by the Egyptian International Center for Import.

Experimental groups

B. alexandrina snails were immersed in three different concentrations of sodium alginates (0.1, 0.5, and 1.0 mg/ml) for three-time intervals (1, 3, and 7 Days) to evaluate its effect on total hemocytes count according to the protocol by Zanotti-Magalhães et al. (1997). For the determination of antioxidant enzymes and histological studies, snails were treated with 1.0 mg/ml of sodium alginate for 7 days and then exposed to *S. mansoni* miracidia.

Hemolymph sampling for hemocytes count

The collection of hemolymph of *B. alexandrina* snails from different groups was performed as described by Negm et al. (1995). Water adhering to the snail was removed and the head foot was cleaned with tissue paper. By touching the foot with the point of the micropipette, the snail was forced to retract deeply into its shell and extruded hemolymph. Thus, as much as 30 µl of hemolymph was obtained from each snail.

Total hemocytes count

For total hemocytes count, 20 µl of hemolymph were individually collected from at least 5-10 snails/group for each experimental treatment. The number of cells in every experimental and control group was counted by diluting freshly collected hemolymph in leucocytes count solution in a 1:20 ratio. Using a Bürker- Turk hemocytometer, the hemocytes were counted for 3 replicates, and the mean number of circulating hemocytes was calculated.

Antioxidant assay

The soft parts of snails were dissected out from their shells after gentle crushing, and then the tissues were weighed and homogenized in phosphate buffer at a ratio of 1:10 W/V using a glass homogenizer for 5 minutes. The homogenates were centrifuged for 15 minutes at 3000 rpm at 4°C and the fresh supernatant was then used in antioxidant parameters assays as followed, SOD and CAT activities were measured by commercial kits (Biodiagnostic Company, Dokki, Giza, Egypt, Cat. No. SD 25 21, and CA 2517, respectively). The concentration of GSH was estimated using a colorimetric GSH kit (Cat. No. GR 2511). The concentration of malondialdehyde (MDA) (the end product of lipid peroxidation) was estimated using a colorimetric method according to Satoh (1978) using a lipid peroxide kit (Cat. No. MD 2529).

Infection dynamics by histological study

Randomly selected snails from the experimental groups were dissected. Shell was gently crushed between two glass slides, the shell fragments were removed using pointed forceps under a dissecting microscope. Snails' soft tissues were routinely processed for histological examination (Romeis, 1989) and photographed using Carl-Zeiss, Germany microscope with a digital camera.

Application of the tested material under simulated natural conditions

The experiment was carried out in the Snails Research Station of TBRI, El-Qanater El-Khayria, Qalubia Governorate, Egypt, described in detail by Yousif et al. (2013). Snails were treated with 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml of sodium alginates for 24 hours (hrs), respectively, according to the volume of water, then snails have been exposed to *S. mansoni* miracidia. After 24 hrs, snails were returned to the laboratory to be maintained in aquaria for the development of the parasite inside the snail. After 21 days post miracidial exposure, surviving snails were individually examined for cercarial shedding in multi dish plates. Post 3 hrs of exposure to light (desk lamp) using 2 ml dechlorinated water v each snail/well, positive snails were removed, marked and transferred to clean aquaria with dechlorinated water and maintained in the dark under laboratory conditions and the infection rate was calculated.

Statistical analysis

Results were expressed as mean ± SE and the obtained data were statistically analyzed using the t-test (Spiegel, 1981) and "chi-square" values

of contingency tables to determine the significant differences in means between the control and the experimental groups, Statistical analysis was performed by the SPSS computer program (version 20 for windows).

4. Results

Total counts of the hemocytes

The results in (Table 1) revealed that exposure of *B. alexandrina* snails to different concentrations of sodium alginates at different times led to a significant increase in the number of hemocytes in experimental groups in comparison with control groups. Exposure of *B. alexandrina* snails to 0.1 mg/ml for one day, increased the number of hemocytes from 3750 ± 87 at 0.1 mg/ml to 3425 ±78 at 0.5 mg/ml, and to 4525 ±90 at 1 mg/ml. Exposure of *B. alexandrina* snails to 0.1 mg/ml for one day, increased the number of hemocytes from 3750 ± 87 at 0.1 mg/ml to 3425 ±78 at 0.5 mg/ml and to 4525 ±90 at 1 mg/ml.

Similarly, exposure to 0.1 mg/ml for 3 days, increased the number of hemocytes in concentration dependent manner from 3300 ± 176 at 0.1 mg/ml to 4875 ±120 at 0.5 mg/ml, and to 3375 ±101 hemocyte/ml at 1.0 mg/ml. After 7 days of exposure to different concentrations of sodium alginates, the number of hemocytes increased dramatically (table 1).

Table 1: Total hemocytes count/ml *B. alexandrina* snail's hemolymph exposed to sodium alginates.

Period of exposure	The concentration of Sodium alginates			Control (unexposed)
	0.1 mg/ml	0.5 mg/ml	1.0 mg/ml	
1 day	3750 ± 87**	3425 ±78**	4525 ±90***	1950±43
3 days	3300 ± 176*	4875 ±120***	3375 ±101**	
7 days	4350± 59***	6650 ±177***	4800 ±93***	

Data expressed as mean ± SE,*, **and *** significantly different from control at $p<0.05$, $p<0.01$, and $p<0.001$.

Antioxidant assay in the tissue of *B. alexandrina* snails

Superoxide dismutase activity

The results revealed that there was no significant difference in SOD activities in the tissue of *B. alexandrina* snails exposed to sodium alginates only while exposure of *B. alexandrina* snails to *S. mansoni* miracidia led to a significant increase in SOD activity after 1, 7, 14 and 21 days recording 319.5±3.3, 313±3.8, 354.9±3.8 and 299.1±11.1 respectively compared to 220.7±2.3 of the control group (Fig. 1).

The effect of sodium alginates on SOD activity in the tissue of *B. alexandrina* snails after 1, 7, 14, and 21 days from exposure to *S. mansoni* miracidia showed a significant increase being 324.6±7.2, 359.2±3.0, 383.9±4.3, and 313.2 ±1.2, respectively in comparison with 220.7±2.3 for the control snails.

Catalase activity

Data in Fig. (2) showed that CAT activity increased significantly after 1, 7, 14, and 21 days from exposure to *S. mansoni* miracidia recording 1.43±0.11, 2.7±0.07, 3.2±0.06 and 2.05±0.07,respectively, compared to 0.3±0.02 of the control group. Data revealed also that the effect of sodium alginates on CAT activity in the tissue of *B. alexandrina* snails after 1, 7, 14, and 21 days from exposure to *S. mansoni* miracidia exhibited a much higher increase being 2.01±0.09, 4.0±0.2, 4.69±0.3 and 2.7±0.2, respectively.

Glutathione reduced activity

Regarding GSH activity under the effect of sodium alginates and/or *S. mansoni* infection, it was observed in Fig. (3) that GSH activity exhibited a gradual increase in all experimental groups during short (1 and 7 days) and long (14 and 21 days) terms from exposure to *S. mansoni* miracidia. The highest activity was noticed in the tissue of snails exposed to 0.5 mg/ml of sodium alginates after 14 days from exposure to *S.*

mansoni miracidia being 33.0±2.0 compared to 9.5±0.27 of the control group.

Lipid peroxide levels

Lipid peroxidation (LPO) was assayed by measuring the end product malondialdehyde (MDA) in the tissue homogenate of *B. alexandrina* snails. It was obvious that there was a significant increase in LPO activity after 1, 7, 14, and 21 days from exposure to *S. mansoni* miracidia being 81.8±3.2, 91.8±2.11, 106.3±3.2 and 118.7±3.3, respectively, compared to 42.5±1.3 of the control group. The addition of sodium alginates caused a much higher significant increase in the activity of LPO, as shown in fig. (4), after 1, 7 and 14 days, recording 174.7±5.1, 230.4±4.5 and 290.3±3.8, respectively then a decrease occurred after 21 days from exposure to *S. mansoni* miracidia recording 160.6±2.8 compared to 42.5±1.3 of the control group.

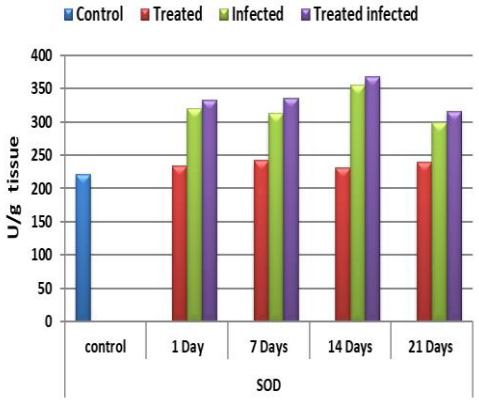


Fig. 1. Activity of SOD in the tissue of *B. alexandrina* snails.

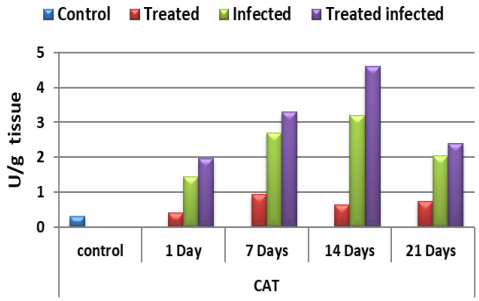


Fig. 2. Activity of CAT in the tissue of *B. alexandrina* snails.

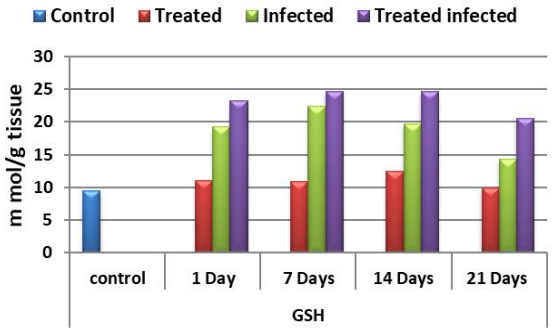


Fig. 3. Activity of GSH in the tissue of *B. alexandrina* snails.

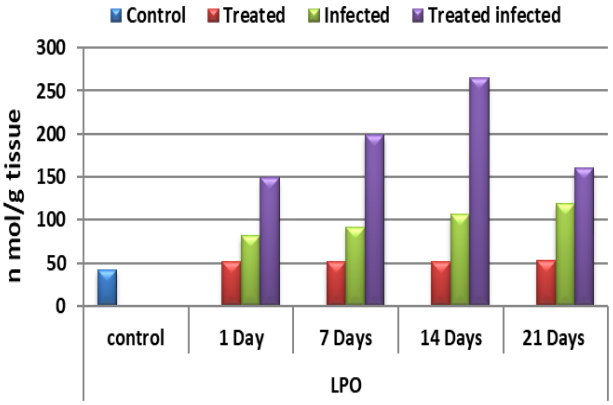


Fig. 4. Level of LPO in the tissue of *B. alexandrina* snails.

Infection dynamics by histological study

Fig. (5A) showed that after successful penetration, miracidia were observed within the head and tentacles of the snails during the first 24 hrs of infection (arrow), while the apical papilla, penetration gland and accessory glands of the penetrating miracidia were still obvious The photomicrograph in the foot region showed also the presence of individual hemocytes (arrowheads) in the proximity of the penetrating miracidia. As shown in Fig. (5B) the effect of sodium alginate on infection dynamics in snails was noticed regarding the deformation of miracidia (arrow) that occurred through degeneration of the miracidial soma (*e.g*, eye spot and neural mass), also it was obvious that there was an extensive hemocyte proliferation at

the site of penetration (arrowheads). Fig. (6A) showed the development of miracidia which took place and transformed into mother sporocysts after o week of infection through losing the apical papilla, penetration gland, and accessory glands and increase in size. Sporocysts surrounded with a cyst in the muscle fibers (arrow) were also observed. From Fig. (6B) it was clear that after one week of infection and treatment with sodium alginate, some miracidia were able to transform into mother sporocysts and all of them could be observed in the head-foot region, but it was noticed that the sporocyst has degenerated (arrow).

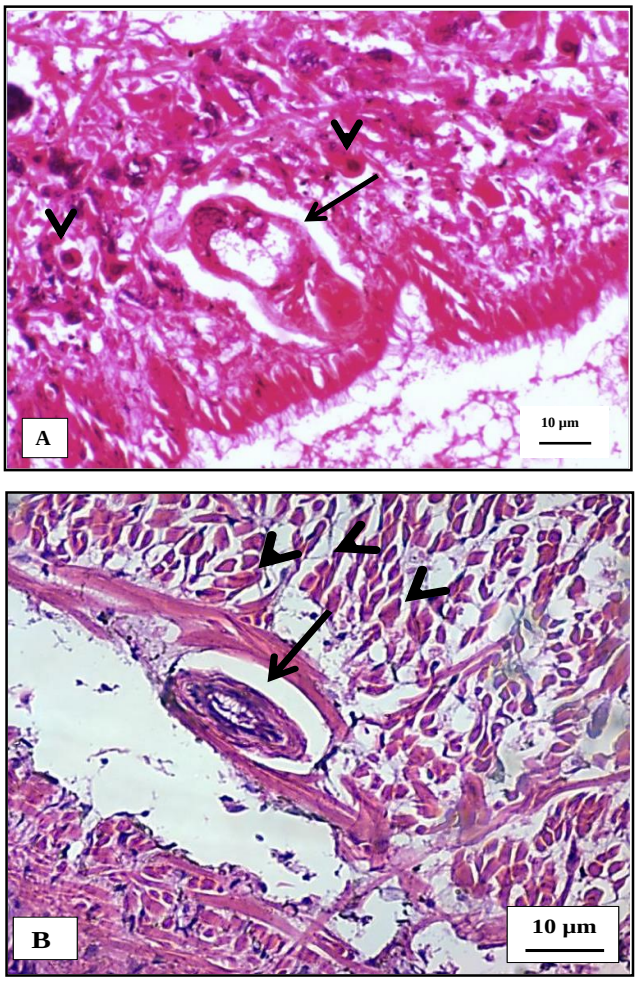


Fig. 5. Light photomicrograph in the head-foot region of *B. alexandrina* snail 24 h post infection. (A) Infected control and (B) treated with sodium alginate and then infected.

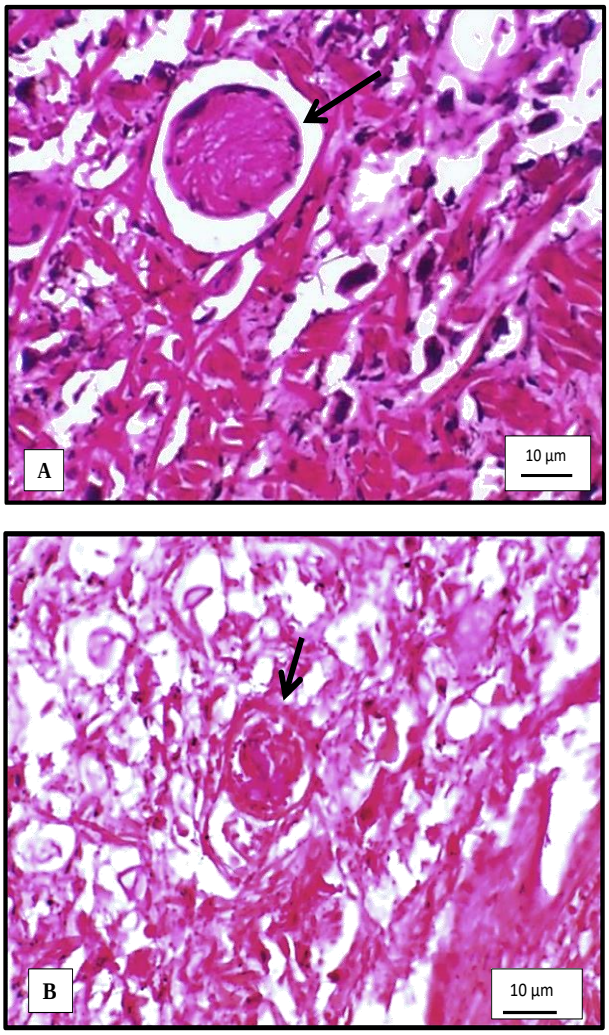


Fig. 6. Light microscope in the head-foot region of *B. alexandrina* snail one week post infection. (A) infected control and (B) treated with sodium alginate and then infected.

As noticed in Fig. (7A) the digestive gland after 2 weeks of infection showed considerable changes in the morphology including the elongation of the body and an increased number of dividing germ cells. The migrated sporocysts away from the site of penetration were observed at the base of the digestive gland while masses of daughter sporocysts were also observed in the tissues around the digestive gland acini. Numerous sporocysts scattered in the digestive tubules showed different developing stages (arrow) with an increase in the size and number of dividing germ cells (GC) inside it. Fig. (7B) indicated that cellular responses continued to increase after 2 weeks of infection resulting in a

degeneration of the sporocysts which still settled in the head-foot region. Degenerated sporocysts surrounded with atrophy and necrotic changes of connective tissues were also seen (arrow).

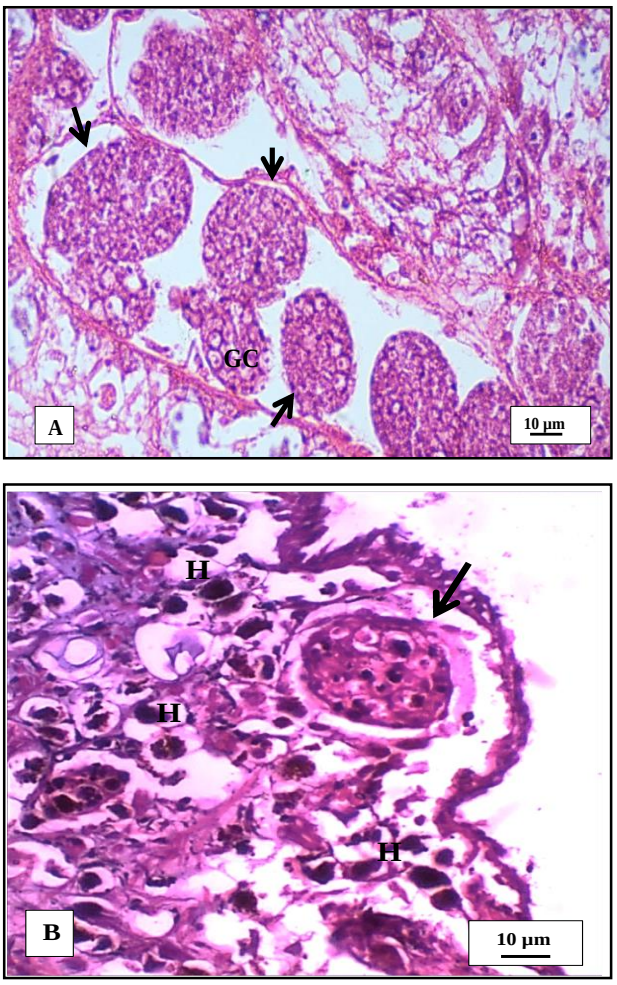
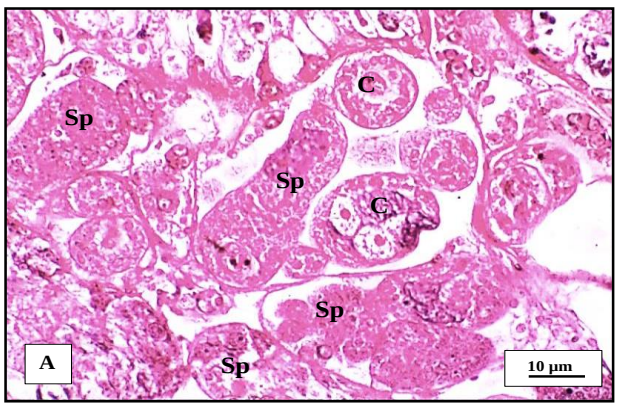


Fig. 7. Light photomicrograph in the tissue of *B. alexandrina* snail 2 weeks post infection. (A) Infected control (in the digesteve gland) and (B) treated with Sodium alginate and then infected (in the head-foot region).



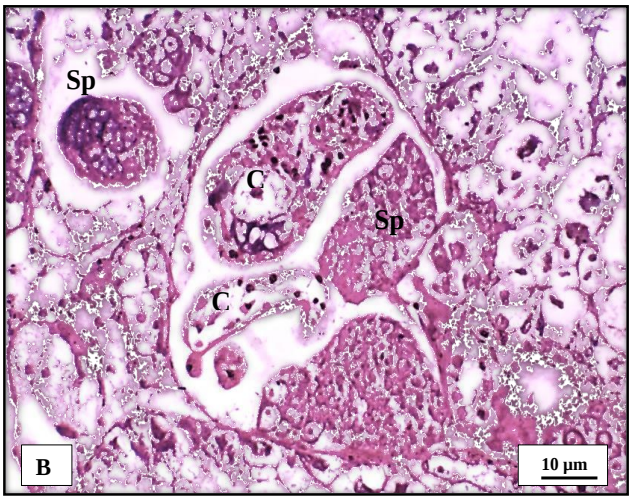


Fig 8. Light photomicrograph in the tissue of *B. alexandrina* snail after cercarial shedding. (A) Infected control (in the ovotestis) and (B) treated with Sodium alginate and then infected (in the digestive gland).

Fig. 8(A) showed that after cercarial shedding, daughter sporocysst were found throughout the tissues around the gonads with developmental stages of cercariae. Well-developed different parasitic stages occupying most of the snail tissue were observed with the destruction of snail tissue becoming more evident. Although encapsulation of sporocysts was not recorded in infected snails, individual hemocytes could sometimes be observed in the proximity of well-developed sporocysts. Fig. 8(B), and after cercarial shedding, the presence of few cercariae (C) and a number of necrotic sporocysts (Sp) were observed. From the histological study, it was noticed that there were great differences between normally infected snails and previously treated snails with immunostimulant compounds. The first difference was regarding the site of daughter sporocysts which are found in the digestive gland and could not migrate to the gonads. The second difference was the presence of few cercariae compared to normally infected snails, also a number of residual sporocysts were noticed.

Application of the tested material under simulated natural conditions

The results in Table (2) show that there was a significant reduction in the survival rate of snails at first cercarial shedding with the exposure of snails to 1.0 mg/ml of sodium alginate to 86.2% in comparison with 96.7% in control snails. Data in table (2) revealed also that exposure of *B. alexandrina* snails to sodium alginates (0.1, 0.5 and 1.0 mg/ml) for 24 hours reduced the infection rate of snails by 28.3, 18.5 and 55.4%, respectively compared to 89.7% of the control group.

Table 2: Effect of Sodium alginate on the Survival rate of snails at first cercarial shedding and Infection rate of *B. alexandrina* snails exposed to *S. mansoni* miracidia under simulated natural conditions

Parameter		Sodium alginates			Control
		0.1 mg/ml	0.5 mg/ml	1.0 mg/ml	
Survival rate at 1st shedding	No. of exposed	30	30	30	30
	No. of survived	28	26	25	29
	%	96.5	89.7	86.2*	96.7
Infection of snails	Number	18	19	10	26
	%	64.3***	73.1**	40.0***	89.7
	% of reduction	-28.3	-18.5	-55.4	——

Data expressed as mean ± S.E. *, **and ***= significantly different from control at $p < 0.05$, $p < 0.01$ and $p < 0.001$

5. Discussion

Control of schistosomiasis could be achieved by chemotherapy, molluscicides and proper sanitation. The use of alternative biological control methods targeting the snail intermediate hosts needs to be exploited as an avenue for disease management. Based on these facts and due to the extending problem of schistosomiasis in terms of morbidity, mortality, treatment cost and side effects, it was decided to study the effect of sodium alginates as immunostimulant material on *B. alexandrina* snails. Natural immunostimulants like sodium alginates are biocompatible, biodegradable, cost-effective, and safe for the environment as well as human health and enhancing disease and stress resistance (Ortuno et al., 2002, Kadowaki et al., 2013, Mohamed, et al., 2012, Pereira et al., 2022).

The present results show that exposure of *B. alexandrina* snails to different concentrations of sodium alginates at different times led to a significant increase in the number of hemocytes in experimental groups in comparison with control groups, these results are in harmony with those (Tingjun, 2009) who reported that cell number counting results of the Crab *Charybdis japonica* indicated that the total number of hemocytes increased after treatment with sodium alginates and inactivated vibrios. The present results on the effect of sodium alginates on some antioxidant enzymes' activities showed that there was no significant difference in all antioxidant activities in the tissue of *B. alexandrina* snails exposed to sodium alginates only in all experimental groups in comparison with control snails. This comes following Cheng *et al*. (2005) who concluded that no significant differences in superoxide dismutase activity were observed among the white shrimp *Litopenaeus vannamei* injected with saline and those injected with sodium alginates at 10, 20 or 50 mg/ g. However, *L. vannamei* injected with sodium alginate at 50 mg/ g and then exposed to the pathogen *Vibrio alginolyticus* showed higher phagocytic activity and significantly elevated SOD activity after 4 days. Also, Chun-Hung et al. 2006 showed that shrimp fed a diet containing 1.0 and 2.0g kg^{-1} sodium alginates had significantly increased SOD activity and added that sodium alginates can be used as an immunomodulator for shrimp through dietary administration to modify immune genes expression of shrimp.

SOD, CAT, GSH and LPO values increased significantly after 1, 7, 14 and 21 days post-exposure of *B. alexandrina* snails to *S. mansoni* miracidia. These results agree with Maha et al.,

(2011) who noticed that SOD, CAT and GSH activities of *B. alexandrina* snails infected with *S. mansoni* miracidia recorded high significant elevation. They reported also that oxidative stress resulted in the formation of highly reactive hydroxyl radicals that could stimulate lipid peroxidation.

Previous results showed a significant increase in the number of hemocytes after exposure to sodium alginates and this may be related to the increase in antioxidant activities in harmony with Holmblad and Soderhall (1999) who stated that the activity of SOD, which is responsible for scavenging superoxide anion increased with increases in total hemocytes count. They found also that the activity of NADPH oxidase, released by hyaline cells is responsible for the reduction of O_2 increased for the shrimp fed a diet containing sodium alginate at 1.0 and 2.0 g/ kg and the increase of superoxide anion production in the shrimp fed sodium alginate containing diet is a consequence of increased number or activity in hyaline cells and total hemocytes count. From the histological study, it was noticed that there were great differences between normally infected snails and previously treated snails with sodium alginate mainly noticed as retarded infection dynamics. It was obvious that a little number of cercariae was found in the tissue of treated snails compared to normally infected snails with several residual sporocysts. These results come in agreement with Lewis et al. (1993) who found that the capacity of an infected snail to shed cercariae is well correlated with histopathological studies. In highly susceptible *B. glabrata*, for instance, sporocysts and cercariae are observed in great numbers, simply displacing the structures in the absence of any host tissue reaction. On the other hand, less susceptible snails, eliminating few cercariae exhibit marked focal and diffuse hemocyte proliferation in several organs and tissues, usually resulting in encapsulation of disintegrating sporocysts and cercariae. Also, this may be related to the high increase in total hemocytic count and increased proportion of granular (spreading) hemocytes which have a critical role in the defense mechanism within the immune system of snails against invading foreign biotic and abiotic agents according (Cavalcanti et al., 2012; Wang et al., 2023). This role may lead to a reaction against the sporocysts in the early stages of infection leading to a decrease in the number of sporocysts that finally could emit cercariae.

The present studies showed that, under simulated natural conditions, the infection rate of *B. alexandrina* snails was reduced by exposure to different concentrations of sodium alginate in all experimental groups compared to control ones. It appeared also that sodium alginate at a concentration of 1.0 mg/ml has a more reducing effect being 55.4%. This agree with Barman et al. (2013) who stated that it is expected during the coming years immunostimulants will find more applications to make aquaculture sustainable. Therefore, immunostimulants may be an effective tool for controlling infectious diseases in aquaculture.

Conclusion

It is concluded from this study that exposure of *B. alexandrina* snails to sodium alginates could enhance their immune ability to overcome infection with *S. mansoni* miracidia through the increase in total hemocytes count and antioxidant enzymes activities which have been proven to be linked to their ability to kill the trematode parasite *S. mansoni*. Also treatment with sodium alginates before infection led to retarded infection dynamics and the production of a low number of cercariae. Applying sodium alginates under simulated natural conditions was highly promising regarding the reduction of infection rates. Sodium alginates may be used as a biocontrol agent for *B. alexandrina*, the snail host of *S. mansoni* in Egypt and in evaluating their effect on schistosomiasis transmission.

5. Reference

Abou-El-Naga IF, 2013. *Biomphalaria alexandrina* in Egypt: past, present and future. J. Biosci. 38:665–672.

Adema CM, Harris RA, Van Deutekom-Mulder EC, 1992. A comparative study of hemocytes from six different snails: Morphology and functional aspects. J. Inverteb. Pathol. 59: 24-32.

Arumugam M, Romestand B, Torreilles J, Roch P, 2000. *In vitro* production of superoxide and

nitric oxide (as nitrite and nitrarte) by *Mytilus galloprovinciaUs* haemocytes upon incubation with PMA or laminarin or during yeast phagocytosis. Eur. J. Cell Biol. 79: 513-519.

Barman D, Nen P, Mandal SC and Kumar V, 2013. Immunostimulants for Aquaculture Health Management. J. Marine Sci. Res. Dev. 3: 134.

Carton Y, Nappi AJ, Poirie M, 2005. Genetics of anti-parasite resistance in invertebrates. Dev. Comp. Immunol. 29: 9-32.

Cavalcanti M, Filho F, Brayner F, 2012. Morphological characterization of hemocytes from *Biomphalaria glabrata* and *Biomphalaria straminea*. Micron, 43: 285-291.

Chelikani P, Fita I, Loewen PC, 2004. Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 61: 192-208.

Cheng W, Liu CH, Kuo, CM, Chen JC, 2005. Dietary administration of sodium alginate enhances the immune ability of white shrimp Litopenaeus vannamei and its resistance against Vibrio alginolyticus. Fish Shellfish Immunol. 18: 1-12.

Chun-Hung Liu, Shinn-Pyng Yeh, Chin-Ming,Kuo, Cheng, W and Chang-Hung Chou, 2006. The effect of sodium alginate on the immune response of tiger shrimp via dietary administration: Activity and gene transcription. Fish Shellfish Immunol. 21: 442-452.

El-Karim G, 2022. Assessment of the antioxidant capacity of *Lanistes carinatus* tissue extract and its immune-boosting influence on *Biomphalaria alexandrina* against infection with *Schistosoma mansoni*. Egypt. J. Aquat. Biol. Fish. 26: 361–376.

Fujiki K, Yano T, 1997. Effects of sodium alginate on the non-specific defence system of the common carp *Cyprinus carpio L.* Fish Shellfish Immunol. 7: 417-427.

Furuta E, Yamaguchi K, shomozawa A, 1990. Haemolynph cells and the platelet-like structures of the land slug, *Incilaria fruhstorferi* Collinge (Gastropoda: Pulmonata). Anatomi. Anz, 170: 99-109.

Holmblad T, Soderhall K, 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. Aquacul, 172: 111–123.

Humphries JE, Yoshino TP, 2008. Regulation of hydrogen peroxide release in circulating hemocytes of the planorbid snail *Biomphalaria glabrata.* Dev. Comp. Immunol. 32: 554-562.

Jamil K, 2001. Bioindicators and biomarkers of environmental pollution and risk assessment. Science Publishers, Inc, Enfield, NH & Plymouth, UK.pp 200

Johnson F, Giulivi C, 2005. Superoxide dismutases and their impact upon human health. Mol. Aspects Med. 26: 340-52.

Johnston LA, Yoshino TP, 1996. Analysis of lectin and snail plasma-binding glycopeptides associated with the tegumental surface of the primary sporocysts of *Schistosoma mansoni*. Parasitol, 112: 469-479.

Kadowaki T, Yasui Y, Nishimiya O, Takahashi Y, Kohchi C, Soma GI, Inagawa H, 2013. Orally administered LPS enhances head kidney macrophage activation with down-regulation of IL6 in common carp (Cyprinus carpio). Fish Shellfish Immunol, 34: 1569-1575.

Ketterer B, Meyer D, Clark AG, 1988. Soluble glutathione transferase isozymes. In H Sies, Ketterer, eds, Glutathione Conjugation. Academic Press, London, pp 73-135.

King CH, Dangerfield-Cha M, 2008. The unacknowledged impact of chronic schistosomiasis. Chronic Illn. 20084(1):65-79.

Kofta W, 1997. Fluke invasion and the immune system of the snail. Wiad. Parazytol, 43 (1): 27-38.

Lewis FA, Richards CS, Knight M, Cooper LA and Clark B, 1993. *Schistosoma mansoni*: Analysis of unusual infection phenotype in the intermediate host snail *Biomphalaria glabrata*. Exp. Parasitol, 77: 349-391.

Maha Z, Rizk Mohamed B, Ahmed Hoda M, lfayoumy, Wagdy, K. Basaly, Nahla N, Kamel, 2011. Response of two vector snail species to infectivity with compatible and

incompatible Schistosoma parasites. Eur. J. of Exp. Biol. 1: 190-202.

Mohamed AH, Sharaf El-Din AT, Mohamed AM, Habib MR, 2012. The relationship between genetic variability and the susceptibility of *Biomphalaria alexandrina* snails to *Schistosoma mansoni* infection. Mem. Inst. Oswaldo. Cruz. 107: 326–337.

Mohamed H, Mona ET, Rizk WM, Mai LY, 2015. Efficacy of probiotics, prebiotics, and immunostimulant on growth performance and immunological parameters of *Procambarus clarkia* juveniles. J. Ba. Appl. Zool, 69: 17–25.

Negm H, Mansour M, Saad AH, Daoud S, 1995. Defense mechanisms in adult and juvenile *Biomphalaria alexanderina* towards selective *Schistosoma mansoni* glycoproteins. J. Egypt. Immunol. 1: 163-176.

Ortuno J, Cuesta A, Rodriguez A, Esteban MA and Meseguer J, 2002. Oral administration of yeast, Saccharomyces cerevisiae, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata L*.). Vet. Immunol. Immunopathol, 85: 41-50.

Pereira Moreira B, Weber MH, Haeberlein S, Mokosch AS, Spengler B, Grevelding CG, and Falcone FH, 2022. Drug repurposing and de novo drug discovery of protein kinase inhibitors as new drugs against schistosomiasis. Molecules 27: 1414.

Renwrantz L, Schmalmack W, Redel R, Friebel B and Schneewei H, 1996. Conversion of phenoloxidase and peroxidase indicators in individual haemocytes of *Mytilus edilis* specimens and isolation of phenoloxidase from haemocyte extract. J. comp. Physiol, 165: 647-658.

Romeis B, 1989. Mikroskopische Technik. Auflage, Urban & Schwarzenb-erg, Munchen-Wien-Baltimore. 17: 235-236.

Satoh K, 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica. Chimica. Acta. **90:** 37-43.

Song F, Wu TX, Cai LS, Zhang LJ, Zheng XD, 2006. Effects of dietary supplementation with *Clostridium butyricum* on the growth performance and humoral immune response in *Miichthys miiuy*. J. Zhejiang Univ. SCI. B. 7: 596-602.

Spiegel D, 1981. Vietman grief work using hypnosis. Amer. J. clini. Hyp, 24(1): 33-44.

Srivastava PK, Pandey AK, 2015. Role of immunostimulants in immune responses of fish shellfish. Biochem. Cell. Arch, 15: 47-73.

Stegeman JJ, Brouwer M, Di Giulio RT, Forlin L, Fowler BA, Sanders BM and Van Veld PA, 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett, R. J, Kimerle, R. A, Mehrle, P. M. Jr. and Bergman, H.L. (eds.), Biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers, MI, USA, Boca Raton, 253-336.

Tingjun F, 2009. Identification and characterization of a hemocyanin-derived phenoloxidase from the crab *Charybdis japonica*. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 152: 144-9.

Torres MA, Testa CP, Gaspari C, Masutti MB, Panitz CMN, Curi-Pedrosa R, Almeida EA, Di Mascio P and Wilhelm Filho D, 2002. Oxidative stress in the mussel *Mytella guyanensis* from polluted mangroves on Santa Catarina Island, Brazil. Mar. Pollut. Bull. 44: 923–932.

Wang X, Tang Y, Li Z, Wu Q, Qiao X, Wan F, Qian W, and Liu C, 2023. Investigation of immune responses in Giant African Snail, *Achatina immaculata*, against a two-round lipopolysaccharide challenge. Int. J. Mol. Sci. 24: 12191.

WHO, 2023. Schistosomiasis, https://www.who.int/news-room/fact-sheets/detail/schistosomiasis.

Le Clec'h W, Anderson TJ, Chevalier FD, 2016. Characterization of hemolymph phenoloxidase activity in two Biomphalaria snail species and impact of Schistosoma mansoni infection. Parasit. Vectors. 22: 9-32.

Winton Cheng, Chun-Hung Liu, Ching-Ming Kuo and Jiann-Chu Chen, 2005. Dietary administration of sodium alginate enhances the immune ability of white shrimp *Litopenaeus vannamei* and its resistance

against *Vibrio alginolyticus*. Fish and Shellfish Immunol, 18: 1-12.

Yousif F, Roushdy M, EL Dafrawy S, 2013. Schistosoma mansoni cercarial host location and infection under stimulated natural conditions in Egypt. J.Egypt.Soci.Parasitol.43 (2):.325-315

Zanotti-Magalhães EM, Magalhães L, Carcalho JF, 1997. Relationship between the pathogenicity of Schistosoma mansoni in mice and the susceptibility of the vector mollusk. IV - Infectiousness of the miracidia. Rev. Saúde Pública. 31: 488-494.