MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Immunomodulation of *Biomphalaria* snails against *Schistosoma mansoni* infection using the parasite's antigens as a novel method of schistosomiasis control in Egypt

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Received:27/8/2025
Accepted:15/9/2025

Abstract: Schistosomiasis is a neglected tropical disease and affects over 200 million people worldwide. The snail *Biomphalaria glabrata* is one of the intermediate hosts of *S. mansoni*. This work aimed to verify the potential immunostimulatory effect of *Schistosoma mansoni* antigens on *Biomphlaria* snails and their capacity to decrease the snails' susceptibility to infection with *S. mansoni* miracidia. Uninfected untreated snails were used as control -ve. All infected untreated and infected treated snails were challenged by *Schistosoma mansoni* miracidia (25 miracidia/ snail) after injection with the *Schistosoma* egg antigens (SEA) and the Schistosoma whole antigen product (SWAP). The tested antigens affected the snail's immune response to *S. mansoni* as evidenced by a significant increase in the survival rate and a decrease in infection rate and cercariae production. In addition, an increase in total hemocyte count, and the phagocytic activity of infected snails treated with SEA and SWAP. In conclusion, it was confirmed that using the parasite's antigens can act as an immunostimulant in the prevention and control of *S. mansoni*.

Keywords: *Biomphalaria*, *Schistosoma*, antigens, immunomodulation, susceptibility

1.Introduction

Schistosomiasis is an extremely serious disease that impacts more than 200 million individuals globally. The illness affects at least 240 million people worldwide, while more than 700 million people live in 78 endemic countries [1]. *S. mansoni* and *B. glabrata* begin interacting when the free-living forms (miracidia) infect the snails and develop into sporocysts. The balance between the trematode's infectious mechanism and the snail's internal defense system (IDS) defines whether an infection is successful or not [2].

Hemocytes are the defense cells of snails that work with the cellular response system [3]. Numerous studies have detailed the function of snail hemocytes in identifying, eliminating, and killing invasive pathogens [4, 5]. The production of cytotoxic compounds, reactive oxygen and nitrogen intermediates, antimicrobial peptides, and pathogen recognition receptors (PRRs) are examples of humoral reactions [6,7]. Phagocytosis is essential for the respiratory burst, a significant defense mechanism in freshwater snails that generates reactive oxygen species (ROS). ROS which are produced during oxidative damage have been demonstrated to be essential for the death of sporocysts in incompatible snail–trematode interactions [8].

Immunological memory and specificity have been established employing various pathogen strains, despite the fact that it is typically impossible to differentiate between a short-term memory and a continuing response [9,10,11,12,13, 14]. The specificity of the snail *Biomphalaria's* immunological memory response was examined previously [11], especially in relation to a particular genotype-dependent immune response. Host response was investigated after homologous versus heterologous parasite exposures.

In order to plot the possibility of using it as a novel method to control schistosomiasis in Egypt, this work was designed to assess the potential immunostimulatory effect of *Schistosoma mansoni* antigens on *Biomphlaria* snails and their capacity to decrease the snails'

susceptibility to infection with *S. mansoni* miracidia.

2. Materials and methods

2.1. Experimental design

Biomphlaria glabrata snails (12-14 mm shell diameters) as well as the *Schistosoma mansoni* antigens (*Schistosoma* egg antigens (SEA) and *Schistosoma* whole antigen product (SWAP)) were obtained from the Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The snails were kept in plastic aquaria (16 × 23 × 9 cm) under constant aeration for at least a period of 4 weeks before immunization (injection). The aquaria were provided with dechlorinated aerated tap water (10 snails/L). They were maintained in air-conditioned room at 25 °C. Oven-dried lettuce leaves and blue-green algae (Nostoc muscorum) were used for feeding and water in the aquaria was changed twice weekly.

For experimental design, four snail groups with 100 specimens each were used: Control negative group- not injected & not infected; Control positive group- injected with 10 µl of PBS (phosphate buffer solution); SEA group- injected with 10 µl of (Schistosoma egg antigen) SEA in the head foot region; SWAP group- injected with 10 µl of (Schistosoma whole antigen product) SWAP in the head foot region.

2.2. Injection of snails

Snails were injected in the head-foot region with 10 µl of Schistosoma mansoni antigens according to the groups described previously [15].

2.3. Infection of snails

Under a dissecting microscope, 25 freshly hatched miracidia were selected from the glass Petri dish using a Pasteur pipette with a rubber bulb. Then, in a 24-well culture plate, the miracidia were added to each well. Individual snails were placed in these wells, and the plate was covered to keep the snails from escaping. After allowing the miracidia to penetrate for two hours, the snails were moved into freshly set aquarium tanks [16].

2.4. Hemolymph Collection

Hemolymph samples were collected from all experimental group outlined. Snails underwent bleeding on days 1, 7, 14 post immunization and days 1, 7, 14, 21 and 35 after infection. 70% alcohol was used to clean each snail's shell. Using a 3 mL 24G syringe, the snail was punctured close to the innermost coil near the heart, and fresh hemolymph was extracted. From each snail, roughly 10 µl of hemolymph was extracted. Hemolymph was pooled from 3 snails collected in each vial tubes (1ml) containing anticoagulant and kept in ice-bath [17].

2.5. Total hemocytes count

The freshly collected hemolymph was diluted in a leucocyte count solution 1:20 ratio. Next, 10 µl of hemolymph was placed on a Neubauer hemocytometer to determine the total hemocytes count under light microscope at 40x. The total hemocytes count (THC) was expressed as cells/mm3 [18].

2.6. Examination of snails for cercarial shedding

Starting from day 21 post miracidial exposure, the snails were examined individually and repeatedly for cercarial shedding in multi dishes under artificial light for 4 hours (stimulant period) and 2 ml of dechlorinated tap water/snail. After initial shedding was observed, snails were screened individually twice weekly till the end of the experiment [19]. The snail's infection rate was calculated at the end of the experiment by dividing number of shedding snails by the total number of exposed snails and the survival rate was calculated by dividing the number of alive snails at the end of the experiment by the total number of exposed snails [20].

2.7. Hemocytes phagocytosis

Approximately, 100 µl of pooled hemolymph was superimposed with an equal volume of yeast suspension on a clean glass slide. Three slides were prepared for each experimental group. Slides with experimental mixture were incubated in a humid chamber for 60 min. The phagocytic reaction was stopped using absolute methanol after washing with PBS (pH 7.4). The fixed monolayers were stained with Giemsa stain for 15 min, rinsed with tap water, air dried and mounted in DPX. then examined under light microscope for phagocytosis of yeast at day 14 post injection and day 1 and 21 post infection [21].

2.8. Statistical analysis

All results were expressed as means ± SD, and any statistical significance was determined using one-way ANOVA, followed by Tukey test at $P<0.05$ by using GraphPad Prism software version 5.

3. Results

3.1. Survival rates

Results showed that the survival rate of snails challenged with *Schistosoma* was greatly affected, being 86% at the 4th week of exposure compared to 96% for the control group. However, snails immunized with the parasite's antigens considerably restored their survival rate to be 94% for the SEA-treated group and 91% for the SWAP-treated group Fig.(1) .

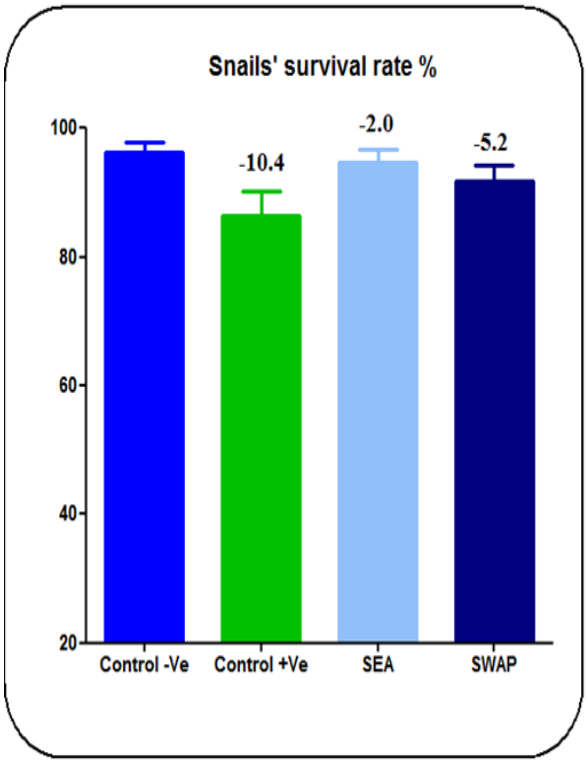

Figure (1): The survival rate (%) for all investigated groups at the end of the experiment. Results are presented as mean ±SD and % of change to control -ve group. Significant change at $P<0.05$.

3.2. Infection rate

Infection levels were 72% in the control +ve group. It had been found that larger specimens had higher rates of infection. Large numbers of trematode larvae were observed to typically reside in the snail's digestive gland. Fig. (2) showed the impact of immunization on *Biomphalaria* infection with *Schistosoma mansoni* miracidia. The rate of infection was considerably reduced to 45% for snails subjected to SEA while for the snails subjected to SWAP, the infection rate was 61% with a decrease rate of 27% and 11% less than that of control +ve snails respectively.

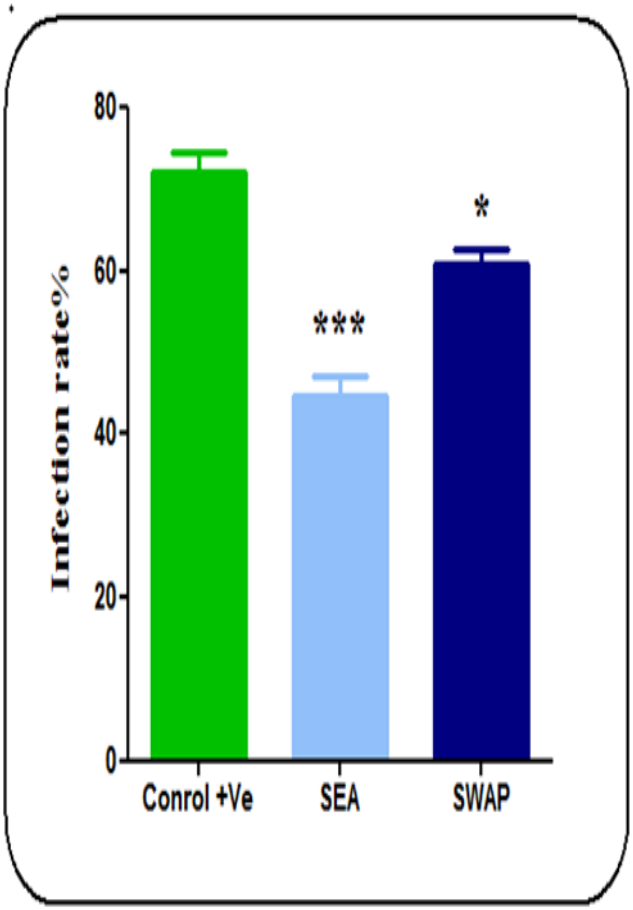

Figure (2): The infection rate (%) for all investigated groups starting from day 21 post miracidial exposure. *: significance of SWAP vs control +ve, ***: significance of SEA vs control +ve. Significant change at $P<0.05$.

3.3. Hemocyte count

During the time of immunization, a slight increase occurred in the number of circulating hemocytes from day 1 to day 14 after exposure to *Schistosoma* antigens. This increase was gradual through the 2 weeks after injection. From day 1 to day 14 after infection, the number of circulating hemocytes showed a dramatically rise in the control +ve group compared to the control -ve snails. While in the immunized groups, the numbers of hemocytes displayed a gradual and smooth increase when compared with the infected but not immunized snails.

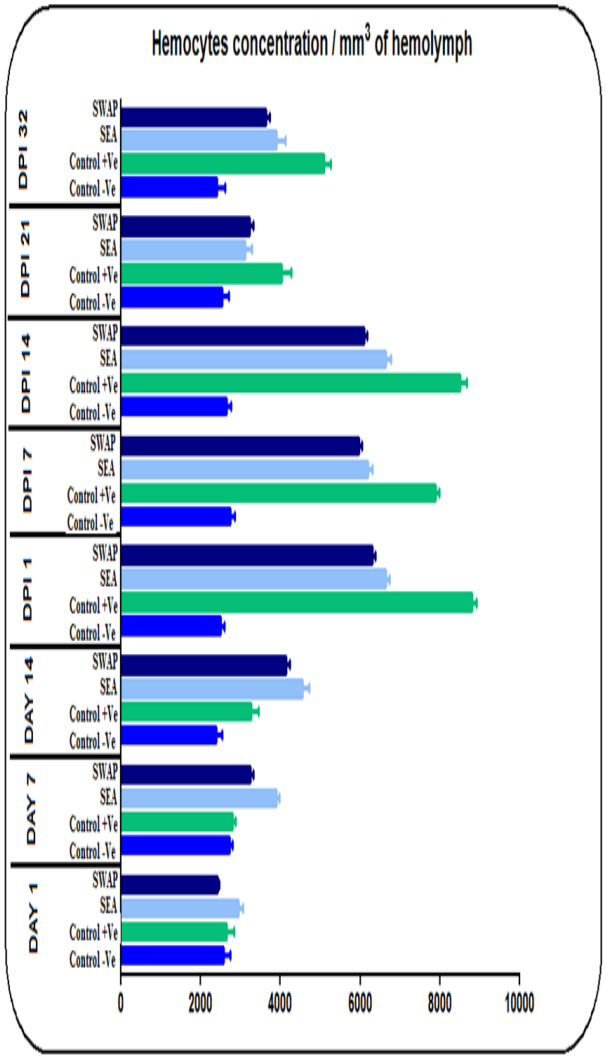

Figure (3): Mean number of circulating hemocytes/mm^3 of hemolymph in three snails from each experimental group. Control -ve: *Biomphalaria glabrata* free from infection with *Schistosoma mansoni*, Control +ve: *B. glabrata* exposed to (25 miracidia\ snail) of *Schistosoma*, SEA gp.: *B. glabrata* immunized with *Schistosoma* egg antigens 2 weeks before infection, SWAP gp.: *B. glabrata* immunized with *Schistosoma* whole antigen product 2 weeks before infection. Data are presented as mean number ± standard deviation.

3.4. Phagocytic activity

Nearly 62% of the infected snails' hemocytes had undergone phagocytosis after 15 hours after the challenge. In contrast, the treated infected snails showed between 80 and 72 percent phagocytic activity. Only 86% of the hemocytes in the infected untreated group were capable of phagocytosing at day 21 after infection. But in the infected snails that received treatment, 92 and 87% of the hemocytes phagocytosed Fig.(4) .

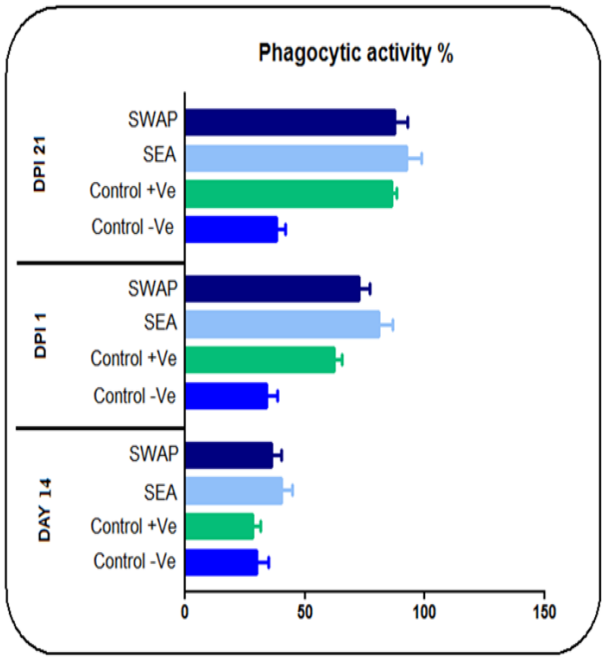

Figure (4): Percentage phagocytosis of hemocytes of *Biomphalaria glabrata* in three snails from each experimental group. Control -ve: *Biomphalaria glabrata* free from infection with *Schistosoma mansoni*, Control +ve: *B. glabrata* exposed to (25 miracidia \ snail) of *Schistosoma*, SEA gp.: *B. glabrata* immunized with *Schistosoma* egg antigens 2 weeks before infection, SWAP gp.: *B. glabrata* immunized with *Schistosoma* whole antigen product, at day 14 after injection with *Schistosoma* antigens, day 1 post infection and day 21 post infection.

4. Discussion

Controlling the intermediate host snails is crucial to the success of Schistosomiasis control efforts. While the majority of methods rely on the use of natural or synthetic molluscicides, other efforts try to stop the spread of Schistosomiasis by focusing on the development of the parasite at the intramolluscan stage [22]. In this regard, boosting the snail's immunity to the parasite by employing the parasite's antigen itself appears to be a successful strategy. This study aims to clarify the influence of the *Schistosoma mansoni* antigens on *B. glabrata* snails' immune system particularly regarding the dynamics of infection with *S. mansoni*.

The snails' susceptibility pattern is influenced by a number of factors, including their internal defense system (IDS). The excretory secretory products of the penetrating miracidia trigger this mechanism [23]. The

internal defense system is comprised of cellular and humoral components [17]. Thus, the immune response is carried out by circulating hemocytes and soluble hemolymph factors [24].

For several animal models across different invertebrate phyla, an acquired resistance has been shown after a primary antigenic stimulation or contact with a pathogen. The group that has been studied the most is arthropods [10,25,26,27]. Additionally, mollusks have been studied [12, 9]. According to several of these researches, there is a particular immunological memory that protects against bacteria, yeasts, and viruses.

Here, after the initial contact with the parasite's antigens, a change in the immune response was noted. The current findings demonstrated distinct variations in the snails' susceptibility to infection with the *S. mansoni* strain.

Circulating hemocytes had a role in the parasite's eradication after the subsequent encounter with the same strain. The exposure to the parasitic antigens prior to infection stimulated the proliferation of hemocytes. Although the activation mechanism is unknown, a number of researchers working with the *B. glabrata- S. mansoni* system have hypothesized that hemocyte activation plays a significant role in schistosome resistance [28].

Consequently, when the snails were treated with the parasitic antigens, the phagocytic indexes of hemocytes increased noticeably, and the process of extracellular trapping and endocytosis of multiple yeast cells improved qualitatively, especially in the hours following treatment. Following bacterial, fungal, and viral infections, similar outcomes have been demonstrated, indicating a generally successful immune response in invertebrates [29].

It is evident from the examination of the earlier findings that treating *B. glabrata* snails with the *S. mansoni* antigens enhanced their overall immune responses. This, in turn, caused the infection rate of *B. glabrata* snails with *S. mansoni* miracidia to drop significantly compared to control values, which is regarded as the study's intended outcome. Several investigations examined the impact of immunostimulation in *B. glabrata* snails, yielding comparable findings. For instance, sodium alginates administered to *B. alexandrina* snails was found to improve their immune system's capacity to fight off *S. mansoni* miracidia infection [30]. Furthermore, the immunity of *B. alexandrina's* was boosted, and its infection rate with *S. mansoni* was decreased by antioxidants derived from plants. For instance, treatment with Eucalyptus camaldulensis EtOAc extract was found to cause a substantial decrease in the infection rate of *B. alexandrina* [22]. Furthermore, administering the antioxidant chemical methyl gallate to *B. alexandrina* decreased the snail's infection rate and raised the number of hemocytes in its hemolymph [31].

5. Conclusion

Taken together, our results show that *Schistosoma mansoni* antigens increase hemocyte proliferation and induce the death of the parasite. These antigens have an immunostimulatory effect and can be used as indirect therapy, because it can be used to enhance the resistance of the intermediate host snails against the parasite to control schistosomiasis transmission. The mechanisms of the *B. glabrata* immune response modulation by exposure to *Schistosoma mansoni* antigens are still poorly understood and deserve more investigation. Further studies of the parasite's antigens exposure and *S. mansoni* infection on the *B. glabrata* immune system are under consideration.

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