EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
41-50
Year: 2017

INFLUENCE OF IMMUNOSTIMULATORY β-GLUCAN ON *BIOMPHALARIA ALEXANDRINA* SNAILS UNDER LABORATORY AND SIMULATED FIELD CONDITIONS

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Article Received on 27/09/2017 **Article Revised on 17/10/2017** **Article Accepted on 07/11/2017**

ABSTRACT
β- glucans are natural biomolecules with immunomodulator activity. *Biomphalaria alexandrina* snails were immersed in β- glucan under laboratory conditions to evaluate the susceptibility of snails to infection with Schistosoma mansoni miracidia. The effect of β- glucan on infection rate of snails under simulated natural conditions was also evaluated. Exposure of snails to 1.0 mg/ml of β-glucan for 7 days recorded the most significant increase in hemocytes count compared to control snails. Spreading hemocytes were the most affected cell type after treatment with β- glucan recording 73% of total hemocytes after treatment with 1.0 mg/ml of β-glucan for 7 days. Infection rate of snails with *Schistosoma mansoni* miracidia was suppressed by exposure to β-glucan and this was associated with significant decrease in the total number of cercariae. Tested antioxidant enzymes after 1, 7, 14 and 21 days post immunostimulatory exposure and infection with *Schistosoma mansoni* miracidia recorded a significant increase. A pattern of retarded infection combined with deformation and degeneration in sporocysts was noticed in snails exposed to β- glucan compared to normally infected snails. Exposure of snails to β-glucan under simulated natural conditions reduced the infection rate of snails by 38.0%, 46.5% and 33.9% respectively. It is concluded that exposure of *Biomphalaria alexandrina* snails to β- glucan could enhance their immune ability to overcome infection with *Schistosoma mansoni* miracidia, so it is a promising candidate as biocontrol agent for schistosomiasis in Egypt.

KEYWORDS: β- glucan, *Biomphalaria* alexandrina, Susceptibility, Antioxidant enzymes, Infection dynamics and simulated natural conditions.

INTRODUCTION
The fresh water snail *Biomphalaria alexandrina* is an essential factor for transmitting *Schistosoma mansoni*, acting as intermediate host for the parasite in Egypt (WHO, 2016; Abdel-Hamid and Mekawey, 2014). A necessary element in schistosomiasis control strategy is snail control which can be managed through chemical, environmental and biological methods. During the last two decades increased attention has focused on immunostimulants and nucleotides to reduce susceptibility to various stressors and diseases, as well as enhance the overall health of invertebrates. The immune response can be modulated by many effectors like bacteria, yeast, glucans, peptidoglucans, lipopolysaccharides and other polysaccharides which have been widely studied in fish and crustaceans (Fujiki and Yano, 1997; Song *et al*., 2006).

β-1,3 glucans are structurally complex homopolymers of glucose, usually isolated from yeast and fungi. The number of individual glucans is almost as great as the number of sources used for isolation. Different physicochemical parameters, such as solubility, primary structure, molecular weight, branching, and polymer charge, influence the biological activities of β-1,3 glucans. Two different reasons for studying β-1,3 glucans in invertebrates exist, one of them is the general progress in our knowledge of the fundamental defense reactions in invertebrates, including phagocytosis, lectines or phenoloxidase system; the other reason is the ever increasing need to find more natural treatment for invertebrates stressed by extensive farming (Vetvicka and Sima, 2004).

Molluscs have an effective internal defense system consisting of cellular (Boehmler *et al*., 1996) and

humoral (Kofta, 1997) defense factors. Immune functions of molluscs are complemented by an array of killing mechanisms, which include release of degradative and oxidative enzymes (Renwrantz *et al.*, 1996) and the generation of highly reactive oxygen metabolites (Arumugam *et al.*, 2000). The production of reactive oxygen species by gastropod snails could be critical in determining susceptibility or resistance to *S. mansoni* and reflect their ability to kill the trematode parasites (Humphries & Yoshino, 2008).

The first line of defense against oxidative stress is provided by superoxide dismutases (SODs), (a group of metal-containing enzymes that catalyze the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide) 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 catalizes the conversion of hydrogen peroxide to water and oxygen, using an iron or manganese co-factor (Chelikani *et al*., 2004). Increase in 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 lethal effects of toxic carcinogenic compounds (Ketterer, 1988).

During the life cycle of trematodes, sporocysts larval stages develop in the mollusc intermediate hosts and there is an evolutionary battle leads to an arms race between 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 (Richards and Merritt, 1972; Richards, 1973; Carton *et al*., 2005).

The importance of semi-field trials of successful laboratory studies was recommended by several scientists, Srivastava and Pandy (2015) stated that a comprehensive research effort should be diverted to study their efficacy on large-scale field applications and the research efforts should also be directed to characterize the receptors on the target cells for recognition of immunostimulatory substances.

The aim of the present work was to evaluate the immunostimulatory effect of β- glucan on modulating the immune system of *Biomphalaria alexandrina* snails and reducing their susceptibility to infection with *Schistosoma mansoni* miracidia under both laboratory and simulated field conditions.

MATERIALS AND METHODS

Biomphalaria alexandrina snails (5-6mm 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 plates. Oven dried lettuce leaves were used for feeding. Water in the aquaria was continuously changed weekly, a photoperiodicity of 12hr. light/ 12hr. dark and water temperature of 25±1°C were kept. Dead snails were removed daily. *Schistosoma mansoni* ova used were obtained from CD1 mice livers previously infected with *Schistosoma mansoni* cercariae. The ova were allowed to hatch in small amount of dechlorinated water (25±1˚C) for about 15 minutes under a direct light (desk lamp). Then, the fresh hatched miracidia were collected by Pasteur pipette under a stereomicroscope and used in the experimental tests.

A commercial form of β–glucan from sigma-aldrish Company was provided by Egyptian International Center for Import.

Biomphalaria alexandrina snails were immersed in three different concentrations of β- glucan (0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml) for three time intervals (1 Day, 3 Days and 7 Days) to evaluate its effect on total and differential hemocytes count according to the protocol by Zanotti-Magalhães *et al*. (1997), also on their susceptibility to infection with *Schistosoma mansoni* miracidia.

For determination of antioxidant enzymes and histological studies snails were treated with 1.0 mg/ml of β-glucan for 7 days then exposed to *Schistosoma mansoni* miracidia.

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 activity by using superoxide dismutase assay kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. SD 25 21).
- CAT activity by using catalase assay kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. CA 2517).
- The concentration of GSH was estimated using colorimetric GSH kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. GR 2511).
- The concentration of malondialdehyde (MDA) (the end product of lipid peroxidation) was estimated using colorimetric method according to Satoh (1978) using lipid peroxide kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. MD 2529).

For 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 according to (Romeis, 1989) and photographed using Carl- Ziess, Germany microscope with digital camera.

For applying 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, previously described in detail by Yousif *et al.*, (1992). Snails were treated with 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml of β-glucan for 24 hrs. respectively according to the volume of the water, then snails were exposed to *Schistosoma mansoni* miracidia. After 24 Hours snails were returned to the laboratory to be maintained in aquaria for the development of the parasite inside the snail.

Results were expressed as mean ± SE and the obtained data were statistically analyzed using "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).

RESULTS

Data illustrated in Fig. (1) revealed that exposure of *Biomphalaria alexandrina* snails to 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml of β–glucan for 1 day results an increase in the number of hemocytes in hemolymph of snails being 2700 hemocyte/ml, 3400 hemocyte/ml and 4350 hemocyte/ml respectively in comparison with control ones being 1950 hemocyte/ml.

Elongation of exposure period to 3 days led to an increase in the count of hemocytes being 3050 hemocyte/ml, 3650 hemocyte/ml and 3700 hemocyte/ml for the same concentrations respectively in comparison with control groups. This feature was observed also by elongation of the exposure period to 7 days. It was noticed that, the number of hemocytes in hemolymph of exposed snails increased by increasing the concentrations and elongation of exposure periods.

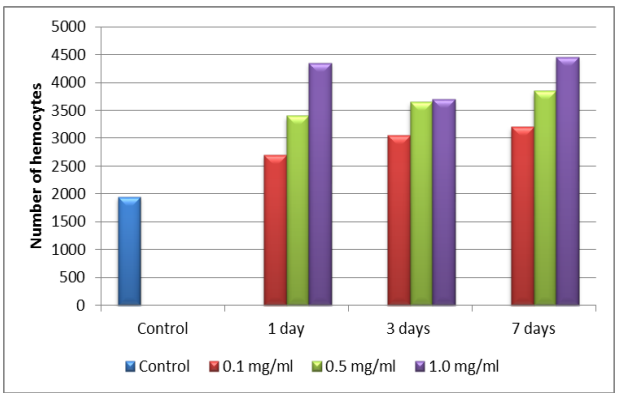


Fig. 1: Total hemocytes count/ml of *Biomphalaria alexandrina* snail's hemolymph exposed to β-glucan.

The results in Fig (2) indicated that three cell types named as granular (spreading) hemocytes, round small hemocytes and haylinocytes were observed in all experimental groups but varied in their relative proportions according to the stressor. Exposure to 0.1 mg/ml of β-glucan for 1 day resulted in an increase in the percentage of granular (spreading) hemocytes recording 72% compared to 62% of control snails. Also, exposure of snails to 1.0 mg/ml of β-glucan for 7 days raised percentage of granular (spreading) hemocytes being 73% compared to 62% of control snails.

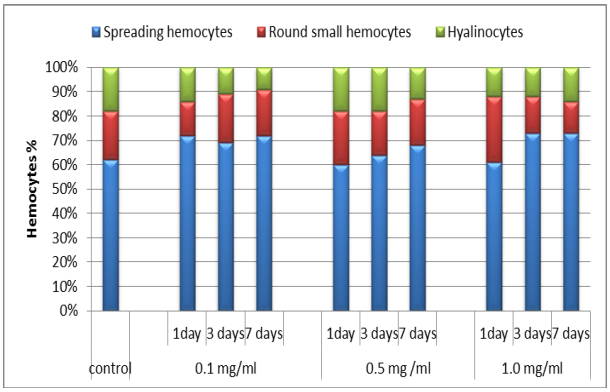


Fig. 2: Differential hemocytes count/ml of *Biomphalaria alexandrina* snail's hemolymph exposed to β-glucan.

As shown in Fig.(3), exposure of *Biomphalaria alexandrina* snails to 0.1 mg/ml of β-glucan for (1 day, 3 days and 7 days) resulted a significant suppression in the infection rates of the snails to be reduced by (18.3%, 35.4% and 23.1%) respectively. It was also noticed that increasing the concentration of β-glucan to 0.5 mg/ml led to a significant decrease in the infection rates by (36.8%, 26.9% and 25.7%) after treatment for (1 day, 3 days and 7 days) respectively. Also, it was noticed that the most significant reduction in the infection rate was recorded after exposure of snails to 1.0 mg/ml of β-glucan for 7 days being 61.0%.

Results in Fig.(4) and Fig.(5) revealed that exposure of snails to 0.1 mg/ml of β-glucan for (1 day, 3 days and 7 days) resulted in significant reduction in the mean total number of cercariae/shedding snail by (31.0%, 8.4% and 36.7%) respectively. Similar results were obtained for snails exposed to 0.5 mg/ml of β-glucan leading to a percent of reduction being (38.5%, 28.6% and 29.5) respectively. Raising the concentration of β-glucan to 1.0 mg/ml for 1 day, 3 days and 7 days resulted in a dramatic decrease in the mean total number of cercariae/shedding snail by (49.8%, 63.3% and 69.6%) respectively.

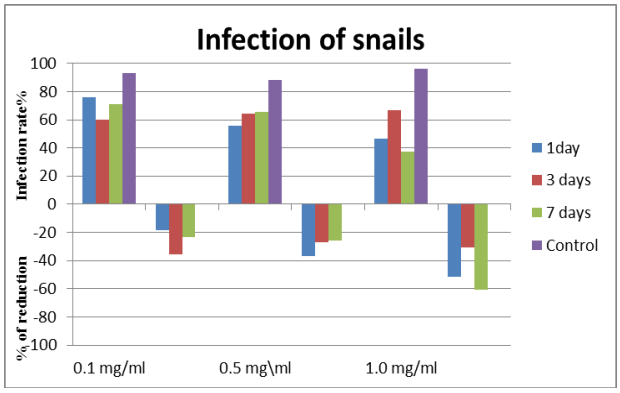


Fig. 3: Effect of β-glucan on Infection rate of *Biomphalaria alexandrina* exposed to *Schistosoma mansoni* miracidia.

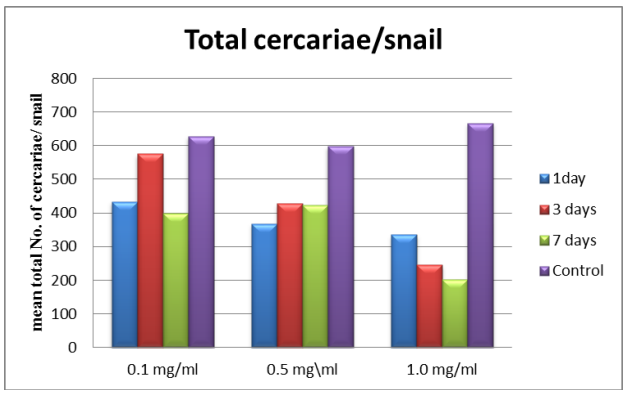


Fig. 4: Effect of β-glucan on mean total cercarial production of *Biomphalaria alexandrina* exposed to *Schistosoma mansoni* miracidia.

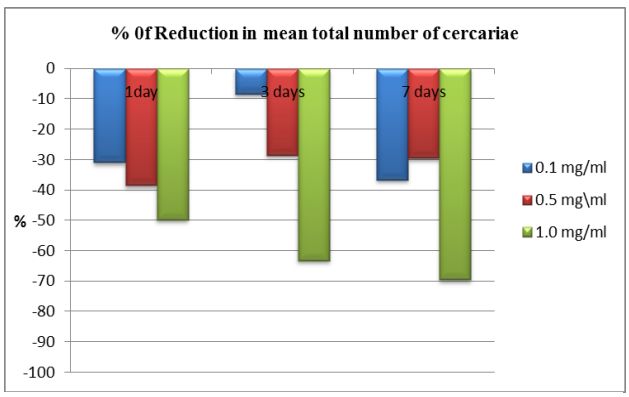


Fig. 5: Effect of β-glucan on % of reduction in the mean total number of cercariae from *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia.

Concerning the effect of β-glucan on antioxidant enzymes data illustrated Fig. (6) revealed that *Biomphalaria alexandrina* snails exposed to β-glucan only (group 1) exhibited no significant difference in SOD activity in comparison with control group, while *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia (group 2) showed a significant increase in SOD activity after 1, 7, 14 and 21 days being 319.5±3.3, 313±3.8, 354.9±3.8 and 299.1±11.1 respectively compared to 220.7±2.3 of control group.

Snails exposed to 1.0 mg/ml of β-glucan for 7 days followed by exposure to *Schistosoma mansoni* miracidia (group 3) exhibited much higher increase in SOD activity after 1, 7, 14 and 21 days recording 333±9.4, 336.2±7.5, 368.4±8.8 and 316.1±5.1 respectively compared to 220.7±2.3 of control group.

Results in Fig. (7) revealed that exposure of *Biomphalaria alexandrina* snails to β-glucan led to a significant increase in CAT activity after 7 and 21 days being 0.93±0.04 and 0.74±0.02 respectively compared to 0.3±0.02 of control group.b The effect of β-glucan on CAT activity in the tissue of *Biomphalaria alexandrina* snails after 1, 7, 14 and 21 days from exposure to *Schistosoma mansoni* miracidia showed a significant increase being 1.96±0.07, 3.3±0.07, 4.6±0.07 and 2.4±0.16 respectively.

As shown in Fig. (8) *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia showed a pattern of increase in GSH activity after 1, 7, 14 and 21 days recording 19.3±0.4, 22.4±0.64, 19.7±0.44 and 14.3±0.44 respectively compared to 9.5±0.27 of control group. The effect of β-glucan on GSH activity in the tissue of *Biomphalaria alexandrina* snails after 1, 7, 14 and 21 days from exposure to *Schistosoma mansoni* miracidia showed a significant increase being 23.2±04, 24.6±5.1, 24.6±0.5 and 20.2±0.6 respectively in comparison with 9.5±0.27 for control snails.

Data in Fig. (9) illustrated that LPO activity showed a significant increase after 1, 7, 14 and 21 days from exposure of snails to *Schistosoma mansoni* miracidia recording 81.8±3.2, 91.8±2.11, 106.3±3.5 and 118.7±3.3 respectively. While higher levels activity of LPO were recorded in snails previously exposed to 1.0 mg/ml of β-glucan being 149.1±3.7, 198.7±2.04 and 265.8±8.3 respectively then a decrease occurred after 21 days of infection recording 161±3.0 compared to 42.5±1.3 of control snails.

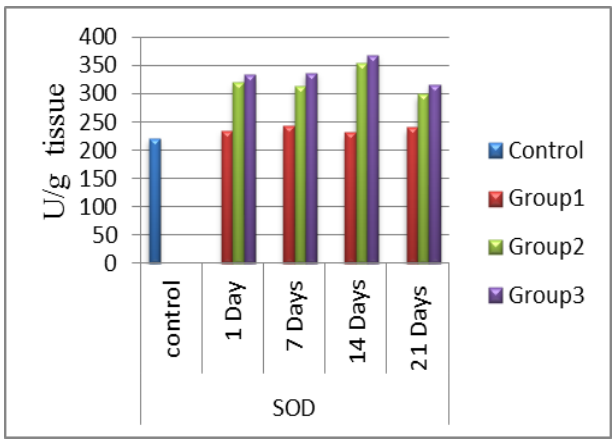


Fig. 6: Activity of SOD in the tissue of *Biomphalaria alexandrina* snails.

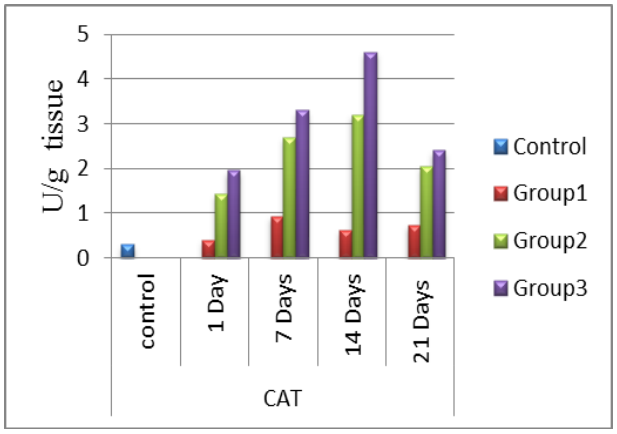


Fig. 7: Activity of CAT in the tissue of *Biomphalaria alexandrina* snails.

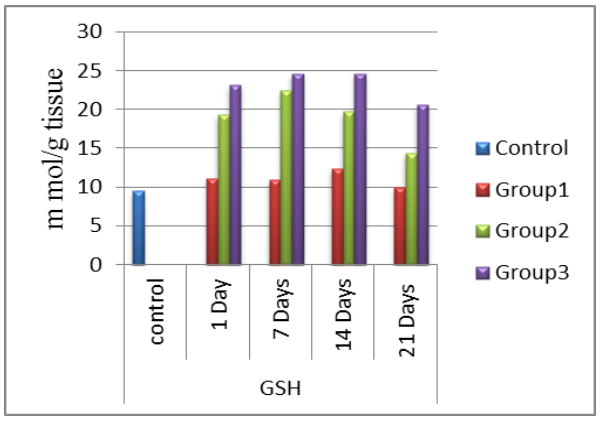


Fig. 8: Activity of GSH in the tissue of *Biomphalaria alexandrina* snails.

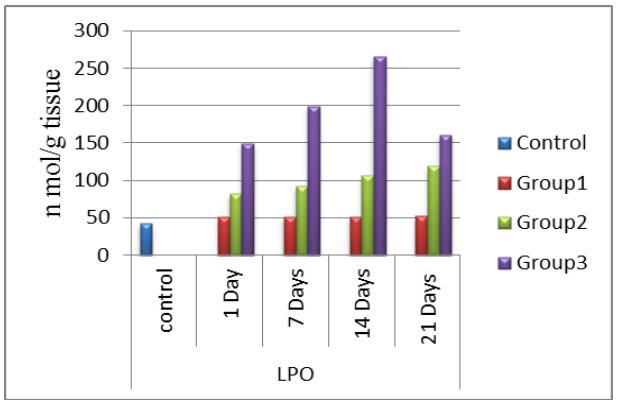


Fig. 9: Activity of LPO in the tissue of *Biomphalaria alexandrina.*

Group 1: exposed to 1.0 mg/ml of β-glucan for 7 days.
Group 2: exposed to *Schistosoma mansoni* miracidia.
Group 3: exposed to 1.0 mg/ml of β-glucan for 7 days followed by exposure to *Schistosoma mansoni* miracidia.

Fig. (10A) showed that after successful penetration, miracidia were observed within the head and tentacles of the snails during the first 24 hours 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 (arrow heads) in the proximity of the penetrating miracidia. As shown in Fig. (10B) the effect of β-glucan on infection dynamics in snails was noticed regarding deformation of miracidia (arrow) occurred through degeneration of the miracidial soma (e.g., eye spot and neural mass), also it was obvious that there was extensive hemocytic proliferation at the site of penetration (arrow heads).

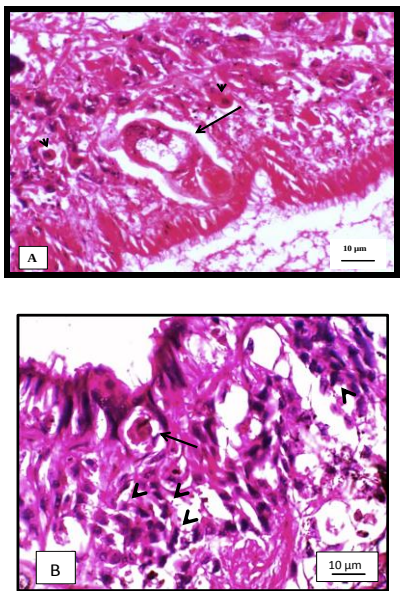
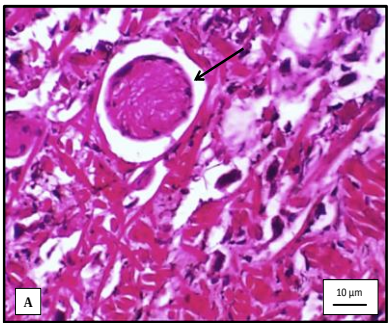


Fig. (10): Light photomicrograph in the head-foot region of *Biomphalaria alexandrina* snail 24 hours post infection. (A) Infected control and (B) treated with β-glucan then infected.

Fig. (11A) showed the development of miracidia which took place and transformed into mother sporocysts after 1 week of infection through losing apical papilla, penetration gland, accessory glands and increase in size. Sporocyst surrounded with a cyst in the muscle fibers (arrow) was also observed.

From Fig. (11B) it was clear that after 1 week of infection 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 was degenerated (arrow) with large number of fat vacuoles (FV) and degenerated muscle fibers (DM) and cilia.



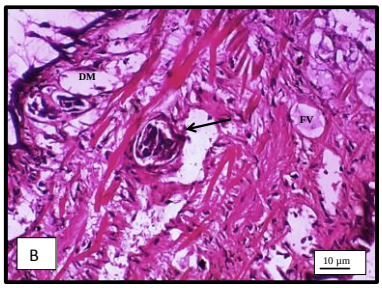
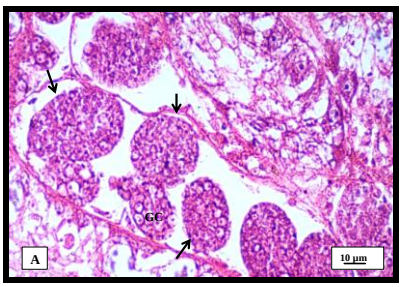


Fig. (11): Light photomicrograph in the head-foot region of *Biomphalaria alexandrina* snail 1 week post infection. (A) Infected control and (B) treated with β-glucan then infected.

As noticed in Fig. (12A) the digestive gland after 2 weeks of infection showed a 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 and 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 increasing in the size and number of dividing germ cells (GC) inside it.

Fig. (12B) revealed that exposure of snails to β-glucan resulted in degeneration of the sporocysts which still settled in the head-foot region due to retarded infection dynamics, sporocyst tegumental destruction (arrows) in the necrotic atrophied degenerated tissue muscles fibers (MF) including generative cells, numerous hemocytes were settled in the proximity of the sporocysts were also observed.



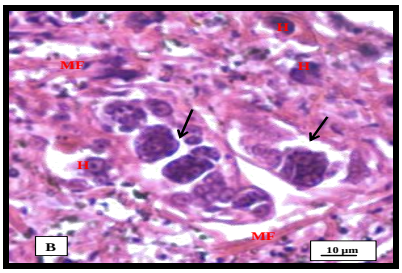
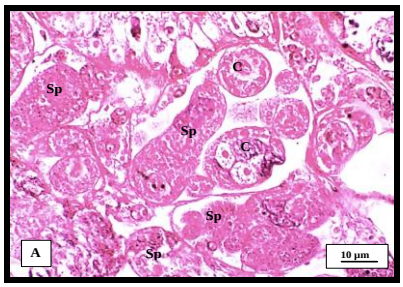


Fig. (12): Light photomicrograph in the tissue of *Biomphalaria alexandrina* snail 2 weeks post infection. (A) Infected control (in the digestive gland) and (B) treated with β-glucan then infected (in the head-foot region).

Fig. (13A) showed that after cercarial shedding, daughter sporocyst were found throughout the tissues around the gonads with developmental stages of cercariae. well-developed different parasitic stages occupying most the snail tissue were observed with destruction of snail tissue became 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. (13B), after cercarial shedding, presence of few number of cercariae (C), number of necrotic sporocysts (Sp) and number of residual sporocysts (arrows) were observed. From histological study it was noticed that there was a great differences between normally infected snails and previously treated snails with immunostimulant compounds. The first difference was regarding the site of daughter sporocysts which found in the digestive gland and could not migrate to the gonads. The second difference was the few number of cercariae present compared to normally infected snails, also number of residual sporocysts were noticed.



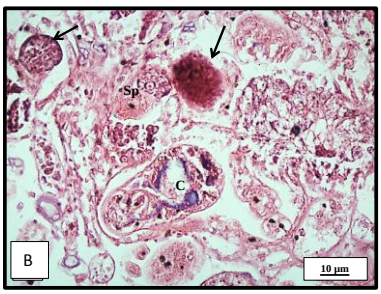


Fig. (13): Light photomicrograph in the tissue of *Biomphalaria alexandrina* snail after cercarial shedding. (A) Infected control (in the ovitestis) and (B) treated with β-glucan then infected (in the digestive gland).

Data in table (1) indicated that exposure of *Biomphalaria alexandrina* snails to β-glucan under simulated natural conditions led to reduction in the infection rate of snails by 38.0%, 46.5% and 33.9% respectively.

Table 1: Effect of β-glucan on infection rate of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia under simulated natural conditions.

Parameter		β- glucan			Control
		0.1 mg/ml	0.5 mg/ml	1.0 mg/ml	
Infection of snails	Number	15	12	16	26
	%	55.6***	48.0***	59.3***	89.7
	% 0f reduction	-38.0	-46.5	-33.9	——

DISCUSSION

Previous studies demonstrated that some natural immunostimulants are biocompatible, biodegradable, costeffective, safe for the environment as well as human health enhancing disease and stress resistance (Ortuno *et al*, 2002; Kadowaki *et al*, 2013). It is known that β-glucan has been reported to enhance the non-specific defense system of shrimp *Panaeus japonicus* against vibriosis (Itami *et al*., 1994). This effect was caused by direct impact on hemocytes via stimulation of phagocytosis, cell adhesion and superoxide anion (Chang *et al*., 2000) and superoxide dismutase production (Chang *et al*., 2003).

The present results show that exposure of *Biomphalaria alexandrina* snails to different concentrations of β-glucan for different times led to a significant increase in the number of hemocytes in all experimental groups in comparison with control groups. The most significant increase was noticed after exposure of snails to 1.0 mg/ml of β-glucan for 7 days being 4450 hemocyte/ ml compared to 1950 hemocyte/ ml of control group. This agrees with Suphantharika *et al*. (2003) who noticed that *In vitro* studies with β-glucan on black tiger shrimp for 3 days showed improved hemocytes number and enhanced bacterial killing activity against the pathogen *Vibrio harveyi*.

As observed in this study, exposure of snails to 0.1 mg/ml and 1.0 mg/ml of β-glucan for 1 and 7 days results an increase in the percentage of granular (spreading) hemocytes recording 72% and 73% compared to 62% of control snails respectively. These observations come in harmony with Seta *et al*., (1996) who reported that granulocytes are described as cells responsible for phagocytosis. For this reason, variations in the number of granulocytes in the face of stressors are more expected to occur than variations in the number of haylinocytes. Russo and Lagadic (2004) reported that, granular (spreading) hemocytes are usually the most numerous cell type, large and highly phagocytic. *In vivo* and *in vitro* experiments showed that these cells phagocytose small particles and encapsulate particles that are too large to be engulfed by an individual cell (Fryer and Bayne, 1996).

The capacity of molluscs to respond strongly to stimuli depends on hemocyte viability and functional capacity. Studies carried out on *Biomphalaria tenagophila* demonstrate that the temporary increase in the number of circulating granulocytes results in decreased susceptibility to infection by *Schistosoma mansoni* (Pereira CAJ, *et al*., 2006). This phenomenon was established in this study as the infection rate of snails with *Schistosoma mansoni* miracidia was suppressed by exposure to β-glucan and this was associated with significant reduction of cercarial production.

Concerning the effect of β-glucan on *Biomphalaria alexandrina* snails exposed to β-glucan only, there was a significant increase in CAT activity after 7 and 21 days compared to control group. Also, there was a significant increase in LPO activity after 1, 7 and 21 days after exposure compared to control group. These results agree with Orhan Veli Ozkan, 2010 who found that malondialdehyde (MDA) and nitric oxide (NO) levels and CAT activity were significantly increased in the stomachs of acetylsalicylic acid (ASA)–induced gastric damage treated rats as a response for exposure to β-glucan which is known for its antioxidant capacity.

SOD, CAT, GSH and LPO activities increased significantly after 1, 7, 14 and 21 days from exposure of *Biomphalaria alexandrina* snails to *Schistosoma mansoni* miracidia These results agree with (Maha *et al.* 2011) who noticed that SOD, CAT and GSH activities showed that *B. alexandrina* snails infected with *S. mansoni* miracidia demonstrated a high significant elevation while oxidative stress resulted in formation of highly reactive hydroxyl radical which stimulated lipid peroxidation. These results are compatible with the study of Bender *et al* (2005 and 2007) who stated that, the production of reactive oxygen species by *B. glabrata* snails has been linked to their ability to kill the trematode parasite *S. mansoni.*

The effect of β-glucan treatment and/or *S. mansoni* infection on antioxidant enzymes "SOD, CAT, GSH, LPO", demonstrated high increase in all antioxidant enzymes and this may also be related to the significant increase in hemocytes as reported by Suphantharika *et al*. (2003) who noticed that *In vitro* studies with β-glucan on black tiger shrimp for 3 days showed improved hemocytes number and enhanced bacterial killing activity against the pathogen *Vibrio harveyi*. This increment can be also related to the increase in granular (spreading) hemocytes percentage which is responsible for the production of reactive oxygen species as mentioned by Bender *et al*. (2005).

The present histological results using light microscopy showed that after successful penetration, miracidia were observed within the head and tentacles of the snails during the first 24 hours of infection. The miracidia

transformed into mother sporocysts after 1 week of infection then after 2 weeks considerable changes in the morphology were observed including the elongation of the body and an increased number of dividing germ cells with migration of the sporocysts away from the site of penetration at the base of the digestive gland. These observations agree with (Nacif-Pimenta *et al*., 2012) who described the entire life cycle of the parasite inside the snail beginning from penetration of the tegument until shedding of cercariae after approximately 30 days. Also Mattos et al. (2011) stated that after penetration of the snail tissue, *S. mansoni* miracidia undergo morphological and physiological changes, and become primary sporocysts, which then go on to complete their life cycle within the intermediate host.

The present study showed that, after cercarial shedding, daughter sporocyst were found throughout the tissues around the gonads and developmental stages of cercariae were present with well developed different parasitic stages. They occupied most the snail tissue and the destruction of snail tissue became more evident. This damage in the tissue may be due to mechanical compression, lysing of cells by enzymes some excretions from parasites with starvation autolysis of cells and reduction in the amount of glycogen stored (Malek and Cheng 1974). The present results agree with the work of Saad and Mohamed (1989) who found that the infection with *Schistosoma haematobium* increased the cellular vaculation of the tissue of *Bulinus truncatus* snails.

From histological study it was noticed that there were great differences between normally infected snails and previously treated snails with β-glucan mainly noticed as retarded infection dynamics. It was obvious that little number of cercariae was found in the tissue of treated snails compared to normally infected snails with a number of 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 number, 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 defense mechanism within the immune system of snails against invading foreign biotic and abiotic agents according to (Cavalcanti MG *et al*., 2012). This role may led to reaction against the sporocysts in early stages of infection leading to decrease in the number of sporocysts that finally had the ability to emit cercariae.

CONCLUSION

It is concluded from this study that exposure of *Biomphalaria alexandrina* snails to β-glucan could enhance their immune ability to overcome infection with *Schistosoma mansoni* miracidia, through the increase in total hemocytes count, percentage of granular (spreading) hemocytes and antioxidant enzymes activities which have been proved to be linked to their ability to kill the trematod parasite *Schistosoma mansoni*. Also treatment with β-glucan prior to infection led to retarded infection dynamics and production of low number of cercariae. Applying of β- glucan under simulated natural conditions is highly promising to use β-glucan as biocontrol agent for *Biomphalaria alexandrina*, the snail host of *Schistosoma mansoni* in Egypt and in evaluating their effect on schistosomiasis transmission.

ACKNOWLEDGMENTS

This research was founded by internal project No. 91M, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.

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