

## Effect of Infection with *Shistosoma mansoni* on Some Biological Parameters in *Biomphalaria alexandrina* Snails

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**Abstract:** Histological alterations in digestive gland as well as hematological and biochemical changes in haemolymph of *Shistosoma mansoni* infected *Biomphalaria alexandrina* snails were studied. The results showed great histological damages in tissues of the digestive gland of infected snails with large numbers of sporocysts and cercariae at several stages of development encircled by cysts in tissue of digestive gland. A detailed light (LM) and scanning electron microscopy (SEM) of the haemocytes in infected snails were described to evaluate the capability to respond to parasitic infections. Three main haemocytes categories could be distinguished namely: hyalinocytes, agranulocytes and granulocytes according to the presence or absence of granules. Phagocytic ability and pseudopodial formation were obviously increased in infected snails. The results showed a significant decrease in total protein and albumin content with elevation of total lipids content and glucose concentration in the haemolymph of infected snails. It was concluded that haemocytes (SEM) in infected snails have large cells with a large imagination, smooth surface and secretion particles adherent to these cells to respond to parasitic infections.

**Key words:** *Biomphalaria alexandrina* • *Schistosoma mansoni* miracidia

### INTRODUCTION

*Biomphalaria* snails are the most common freshwater intermediate host snail prevailing in developing countries and have been recognized as the intermediate host for *Schistosoma mansoni*, which is responsible for wide spread schistosomiasis infections in humans. *B. alexandrina* snails play an increasingly important role in transmission of *S. mansoni* in Egypt and they are considered to be potential hosts of the schistosome parasite [1].

Molluscan innate immunity comprises humoral and cellular elements, with the cellular component considered to be more important for defence. Mobile cells called haemocytes, which are functionally analogous to mammalian monocytes and macrophages [2]. The haemocytes may be circulating in hemolymph or fixed in tissues. All molluscan species possess granular or agranular haemocytes [3-5]. A second type of haemolymph cells are small cells called hyalinocytes which may be agranular or slightly granular [6,7].

Gastropod haemocytes take part in cell defence reactions, such as phagocytosis [8] and recognition and

elimination by oxidation of nonself particles. This process is accomplished by phagocytic haemocytes within the blood and haemolymph of both vertebrate and invertebrate animals [9]. Snail haemocytes are able to mount a cytotoxic response against parasites [10] and are apparently involved in the destruction of the sporocysts of *S. mansoni* [11]. Many workers studied haemocytes as molluscan internal defence or immune system [12-15].

Some biochemical studies on the organic composition of the haemolymph of some snails had been carried out by Mobarez [16] and Moura *et al.* [17]. Also, plasma protein fractions were studied in a variety of freshwater and marine species by employing a number of methods of electrophoresis [18].

The digestive gland of mollusks is involved in extracellular and intracellular digestion of food material, absorption of nutrients, storage of lipids, glycogen and minerals and it plays also a major role in detoxification [19]. The snail's digestive gland (hepatopancreas) was described by Brackenbury [20].

The present work investigated the histological alterations in the digestive glands of *Biomphalaria alexandrina* infected snails. Also, the changes in

hemocytes of *B. alexandrina* in infected snails were followed up using light and scanning electron microscopy to evaluate the capability of the snail to respond to parasitic infections.

## MATERIAS AND METHODS

Laboratory bred *B. alexandrina* snails from Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Egypt, were used. *S.mansoni* eggs were obtained from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute, Cairo, Egypt.

Two groups (each of 50 snails) of *B. alexandrina* snails were prepared. The first group was individually exposed to 10 miracidia /snail for 24 hours at  $24 \pm 1$  °C and ceiling illumination. After exposure to miracidia, snails were maintained in dechlorinated tap water. The second group was not exposed to miracidia (the control group). Three weeks post miracidial exposure, surviving uninfected and infected *B. alexandrina* snails were used for the present work.

**Histological Study:** The digestive tracts of surviving uninfected and *S. mansoni* infected *B. alexandrina* snails were removed from their shells, fixed in Bruin's fluid for 5 hrs, then transferred to 70% alcohol. Further procedures included dehydration in 100% alcohol, clearing in xylol and paraffin embedding were followed. Five im sections were stained with hematoxylin and eosin. Stained slides were examined under polarized light microscope [21].

### Haemtological Studies

**Collection of Haemolymph:** Haemolymph samples were collected from uninfected and infected as outlined Michelson [22] via removing a small portion of the shell and inserting a capillary tube into the heart. Haemolymph was pooled from 10 snails collected in a vial tube (1.5 ml) and kept in ice-bath. The collected haemolymph from each snails group was divided into 3 volumes. The first one used for haemocytes count, the 2<sup>nd</sup> for light and scanning electron microscopic examinations and the 3<sup>rd</sup> for biochemical analysis.

The number of haemocytes/mm<sup>3</sup> of hemolymph was counted in a Burker-Turk haemocytometer [23]. Monolayers of haemocytes were prepared and stained with Giemsa's stain for 20 min. according to the method of Abdul-Salam and Michelson [24].

**Light Microscopy Preparation:** Haemolymph smears were fixed in methanol and stained with Giemsa stain [6] Differential haemocyte counts were performed according to Brown [25].

**Scanning Electron Microscopy Preparation:** Haemolymph smears were fixed in methanol [26]. Fixed smear were subjected to a sputter coating with gold palladium and then examined in Jeol JSMT220 SEM that operates at 10-15kv.

**Biochemical Analysis:** Total protein, Albumin, Lipid and glucose content in haemolymph were determined spectrophotometrically using commercially available reagent kits purchased from BioMerieux Company, France.

Total protein concentration was determined according to Lowry *et al.*[27]. Albumin was determined with bromocresol green as described by Doumas *et al.* [28]. Lipids, including free fatty acids, esterified, non saturated fatty acids and cholesterol were determined according to the method of Cantarrow and Trumper [29]. Haemolymph glucose concentrations were determined according to the glucose oxidase method of Trinder [30].

## STATISTICAL ANALYSIS

Analysis of data was carried out by student's *t*-test for comparing the means of experimental and control groups [31].

## RESULT

Non-infected snails (Fig.1) showed normal histology of the digestive tract with appearance of normal columnar epithelia (Ec) and secretary (Sc) cells. Cells are settled on the basal portion of the cell. Fig. 2 shows obvious histological changes in the digestive gland of snails infected with *S. mansoni* with large numbers of sporocysts and cercariae at different developmental stages encircled by cycts. Infection of snails with *S. mansoni* miracidia caused great damage in the epithelial region of *B. alexandrina*, the cells seemed to be empty, secretary cells disappeared and connective tissue was damaged.

The different shapes of haemocytes observed through the scanning electron microscopy did not wholly confirm those reported under the light microscopy, so special emphasis was given to the surface structure.

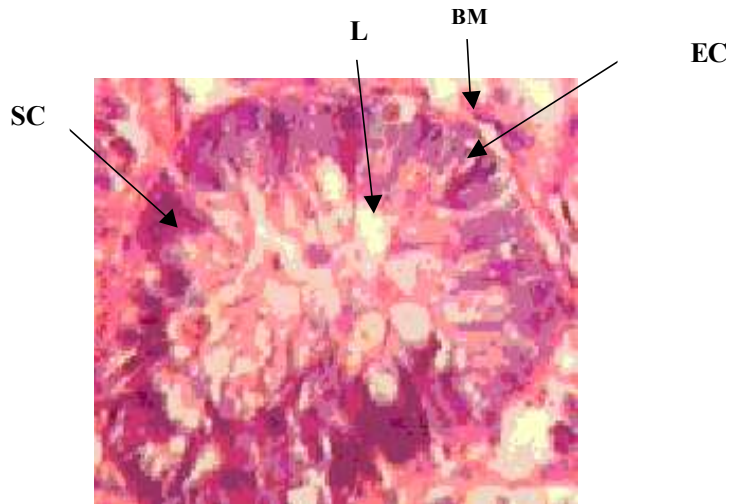


Fig. 1: T.S. in normal *Biomphalaria alexandrina* showing the digestive gland Ec: epithelial cells, SC: secretory cells, L: lumen, BM: basement membrane. (Hematoxylin and eosin, X=400.)

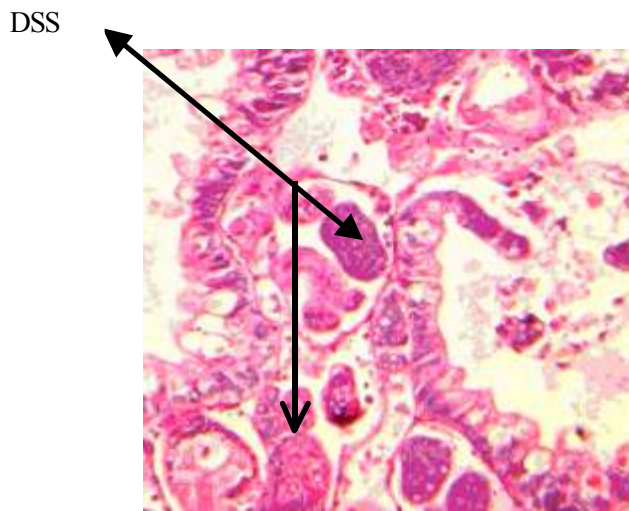


Fig. 2: T.S. in *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni* showing developmental stages of sporocysts (DSS). (Hematoxylin and eosin, X=400.)

**Light Microscopy Observations(LM):** Data in Table 1. Show that a total of 446 cells were counted from uninfected snails.39.23% of these cells were hyalinocytes, 21.3% were agranulocytes with acidophilic cytoplasm, 30.27% were agranulocytes with basophilic cytoplasm, 3.15% were granulocytes with acidophilic cytoplasm and 6.05% were granulocytes with basophilic cytoplasm.

A total of 312 cells were counted from infected snails. 69.23% of these cells were hyalinocytes, 21.15% were agranulocytes with acidophilic cytoplasm,4.49% were agranulocytes with basophilic cytoplasm,1.92% were granulocytes with acidophilic

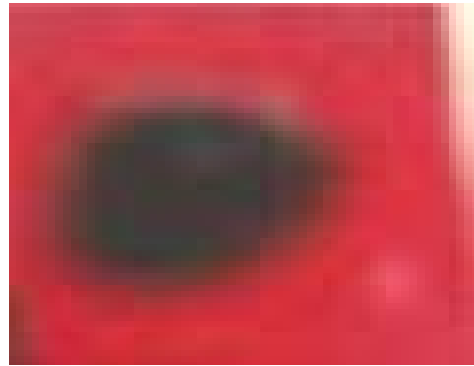
cytoplasm and 3.21% were granulocytes with basophilic cytoplasm.

Hyalinocytes of uninfected snails (Fig. 3) have a spherical (Fig. 3a) or an oval shape (Fig. 3b). Their cytoplasm is agranular and basophilic. The amount of cytoplasm is very small (Fig. 3a) and sometimes could not be distinguished (Fig. 3b). The nucleus is central and rounded.

Hyalinocytes of infected snails (Fig. 6) may be rounded (Fig. 6a) or oval shape (Fig. 6b). The cytoplasm is agranular and basophilic; very small in amount (Fig. 6a). Pseudopodia may be present (Fig. 6b). The nucleus is round (Figure 6a), oval (Fig. 6b).

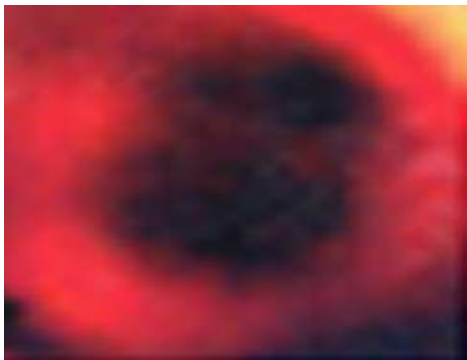


A- Cells have a spherical shape

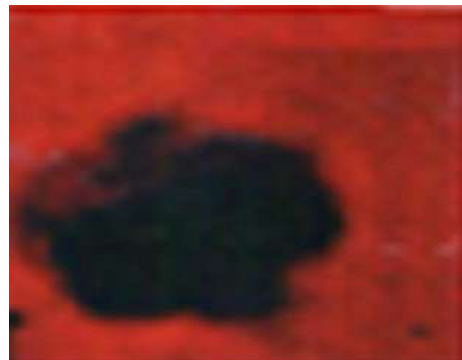


B- Cells have an oval shape

Fig. 3: Uninfected *Biomphalaria alexandrina* haemocytes, A&B (Hyalinocytes) (Giemsa's stain, X=400)



A -Agranulocytes with acidophilic cytoplasm

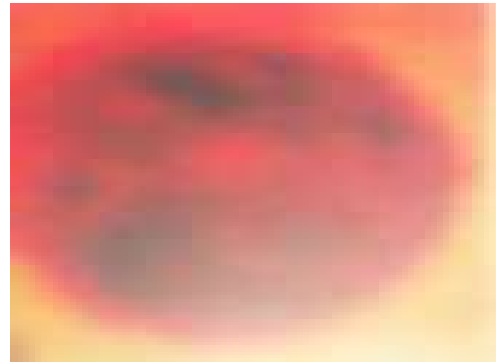


B-Agranulocytes with basophilic cytoplasm

Fig. 4: Uninfected *Biomphalaria alexandrina* haemocytes (Agranulocytes) (Giemsa's stain, X=400)

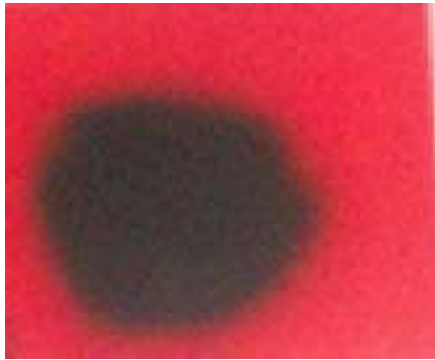


A-Granulocytes with acidophilic granules and cytoplasm



B- Granulocytes with basophilic granules and cytoplasm

Fig. 5: Uninfected *Biomphalaria alexandrina* haemocytes (Granulocytes) (Giemsa's stain, X=400).



A- Cells have a rounded shape

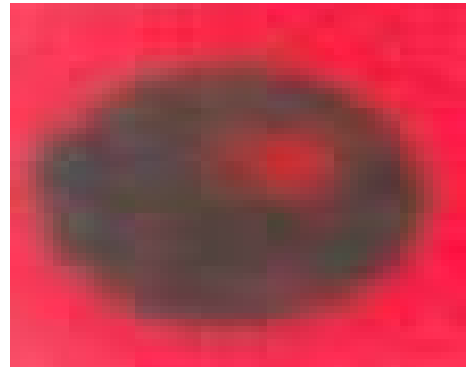


B- Cells have an oval shape

Fig. 6: Infected *Biomphalaria alexandrina* haemocytes, A&B (Hyalinocytes) (Giemsa's stain, X=400)



A - Agranulocytes with acidophilic cytoplasm



B- Agranulocytes with basophilic cytoplasm

Fig. 7: Infected *Biomphalaria alexandrina* haemocytes (Agranulocytes) (Giemsa's stain, X=400)

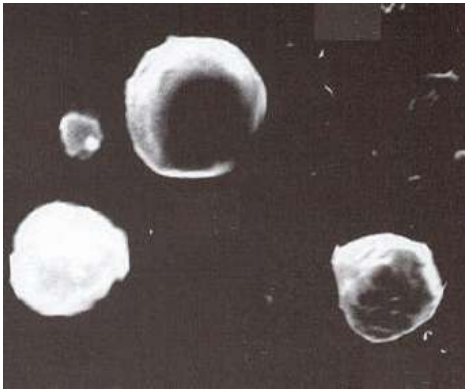


A-Granulocytes with acidophilic granules and cytoplasm

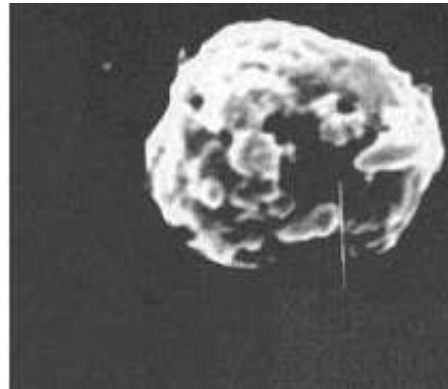


B- Granulocytes with basophilic granules and cytoplasm

Fig. 8: Infected *Biomphalaria alexandrina* haemocytes (Granulocytes) (Giemsa's stain, X=400)

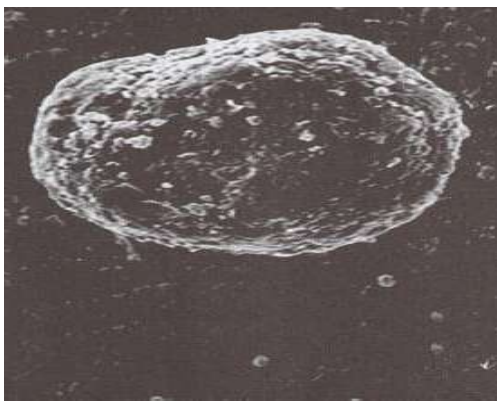


A- haemocytes with smooth surface

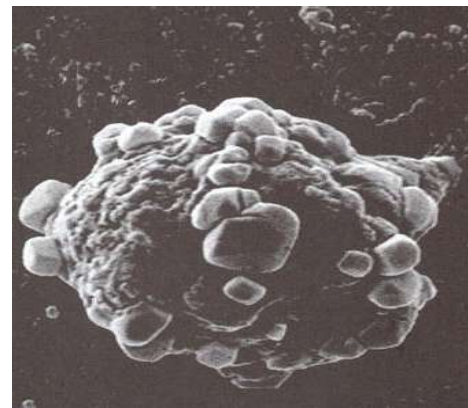


B- haemocytes with rough surface, numerous pseudopods

Fig. 9: Scanning electron micrograph showing haemocytes of uninfected *Biomphalaria alexandrina* (A&B) (15kv, X=5000)



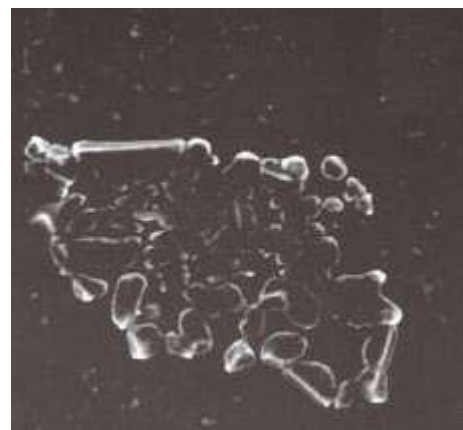
A- Large cells with a few tiny particles



B- large club shaped cells with large particles



C- Large cells with large invagination



D- Very small cells may be aggregated in a polygonal shape

Fig. 10: Scanning electron micrograph showing haemocytes of infected *Biomphalaria alexandrina* (15kv, X=5000)

Table 1: Number of haemocytes/mm<sup>3</sup> and percentage of different categories in haemolymph of *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*

	Total number of counted cells	Cell type				
		Agranulocytes		Granulocytes		
		Hyalinocytes	Acidophilic cytoplasm	Basophilic cytoplasm	Acidophilic granules	Basophilic granules
Uninfected snails	446	175(39.23%)	95(21.3%)	135(30.27%)	14(3.15%)	27(6.05)
Infected snails	312**	216***(69.23%)	66**(21.15%)	14***(4.49%)	6***(1.92%)	10***(3.21%)

\* p< 0.05,\*\* p< 0.01 and \*\*\* p< 0.001

Table 2: Total protein, Albumin, lipid and glucose content in haemolymph of *Biomphalaria alexandrina* infected with *Schistosoma mansoni*

Treatment	Total protein (mg/ml)	Albumin (mg/ml)	Total Lipid (mg/ml)	glucose (mg/ml)
Control snail	5.6±1.5	1.4 ± 0.54	3.1±0.86	24.2 ± 4.2
Infected snails	2.5±1.1	0.62±0.23	5.1±1.3	32±3.5
% change	55.4%	55.7%	-64.5%	-32.2%

\* p< 0.05,\*\* p< 0.01 and \*\*\* p< 0.001

Agranulocytes of uninfected snails appeared to have two subtypes (Fig. 4). Agranulocytes with acidophilic cytoplasm (Fig. 4a) have oval shape with a central oval nucleus. Agranulocytes with basophilic cytoplasm (Fig. 4b) may be polygonal shape and nucleus is peripheral and polygonal.

Agranulocytes of infected snails (Fig. 7) have two subtypes. Agranulocytes with acidophitic cytoplasm (Fig. 7a) are oval shape with a peripheral oval nucleus. Agranulocytes with basophilic cytoplasm (Fig. 7b) are rounded shape and Nucleus is small rectangular and peripheral.

Granulocytes of uninfected snails are also differentiated into two subtypes (Fig. 5). Granulocytes with acidophilic granules and cytoplasm (Fig. 5a) are rounded shape with peripheral nucleus. Granulocytes with basophilic granules and cytoplasm (Fig. 5b) are spherical shape with a rounded peripheral nucleus. Granules may be numerous.

Granulocytes of infected snails have two subtypes (Fig. 8). Granulocytes with acidophilic granules central basophilic cytoplasm and peripheral homogeneous acidophilic cytoplasm (Fig. 8a). Cells are rounded with peripheral small rounded nuclei, granules may be very small. Granulocytes with basophilic granules and cytoplasm (Fig. 8b). Cells may be rounded with peripheral rounded nuclei and one large granule near the nucleus.

**Scanning Electron Microscopic Observations (SEM):**

From Fig. 9 it is seen that Large and small rounded haemocytes of uninfected snails with smooth surface (Fig.9a) and haemocytes with rough surface, numerous pseudopod-like cytoplasmic projections (Fig.9 b).

From Fig. 10 it is seen that Large haemocytes of infected snails with a few tiny particles (Fig.10a) and large club shaped cells with large particles with expanses of smooth surface between short cytoplasmic projections (Fig. 10b). Large cells with a large invagination, smooth surface and secretion particles adherent to these cells.(Fig.10 c) and very small cells may be aggregated in a polygonal shape (Fig.10 d).

Table 2 shows that the total protein content was significantly reduced in infected snails (P< 0.01) than in control snails with 55.4% reduction. Albumin content in haemolymph of snails infected with *S. mansoni* (40 days post exposure) was significantly lower (P< 0.001) than that in the control group with 55.7% reduction. The total lipid and glucose concentration in haemolymph of snails infected to *S. mansoni* showed a significant increase (P< 0.05) in comparison with the control group with 64.5 and 32.2% increase respectively.

**DISCUSSION**

The histological results showed considerable changes in digestive gland of snails infected with *S. mansoni* with large numbers of sporocysts and cercariae at several stages of development encircled by cycts. This agrees with Bakry *et al.* [32, 33] who found degeneration and evacuation in the gametogenic stages of the exposed snails. Correa [34] indicated that miracidia respond to chemical snail host signals with different behavioral patterns, which lead to accumulation around and finally to contact with the snail.

The defense system is made by cellular and humoral elements. The cellular defense system is operated by

hemocytes (moving cells), which have phagocytic capacity. The humoral immunity is measured by lectins and opsonins, which are proteins synthesized by hemocytes with specific affinities to carbohydrates [35]. The results reported up till now suggest that the hemocyte could be the effector element in the destruction mechanism of trematodes, being directly involved in the death of some encapsulated parasites [36,37] or in the production of soluble factors which could be cytotoxic [38]. The majority of the authors [37, 38] agree that the snails' defense generally occurs by means of destruction, total or partial, of the primary sporocyst at the first few hours following the penetration of the miracidium.

In the present study, formation of vacuoles and pseudopodia in hyalinocytes, agranular and granular haemocytes with basophilic cytoplasm and granules in infected *B. alexandrina* may be a pathological response. Malek and Cheng [6] mentioned that all molluscan haemolymph cells are capable of pseudopodia movement. They classified molluscan haemocytes into large granular or agranular cells. Large granular cells can be distinguished into large granulocytes with acidophilic, basophilic, refractile granules. In addition to the large granulocytes, there is another large cell type called fibrocyte. The second type of haemolymph cells was small cells (hyalinocytes), which may be agranular or slightly granular. These findings disagree with the present results, where there were no refractive granules or fibrocytes.

The increase in hyalinocytes number in infected *B. alexandrina* compared to uninfected ones may be a pathological reaction. According to Ottaviani [39] the population of circulating hemocytes of the majority of gastropod molluscs is constituted by two cellular types: hyalinocytes that emit pseudopodes involved in phagocytosis and granulocytes. Several authors [40-44], also distinguished two sub-populations of circulating hemocytes in hemolymph of *B. glabrata*. Sminia [45] revealed that hyalinocytes are phagocytic. Zhang *et al.* [46] classified granules into acidophilic or basophilic. The present investigation differs from that of Zhang *et al.* [46] in that a third type, Agranulocytes, was detected.

The present results showed that the different shapes of haemocytes observed through the scanning electron microscope did not wholly confirm to those recorded under the light microscope. This agrees with Zhang *et al.* [46] who found that different shapes of haemocytes observed under the SEM were not wholly the same as that recorded under the light microscope. On the other hand, no fundamental differences were found in the ultrastructure of the blood cells of *B.alexandrina*, *Lyminaea stagnalis*,

*B.glabrata* and *B. truncatus* [2, 47]. However, these authors concluded that only one type of blood cells, the amoebocyte, exists in the freshwater snails.

Regarding, classification of haemocytes studied by SEM depending on shape, size and surface structure, the results of the present study agree with those of Zhang *et al.* [46] in the presence of round cells, poly-angle shaped cells and the presence of surface secretion particles in some cell shapes. Moreover, haemocytes with pseudopodia and invaginations were detected in infected *B. alexandrina* which may reflect a pathological reflex.

Matricon-Gondran and Letocart [8] revealed that haemocytes in nonparasitized snails have globular shape (this was found in the present work with uninfected *B. alexandrina*) while stressed haemocytes were generally spherical with filopodia and vacuoles of various sizes (the present study showed that many haemocytes of *B. alexandrina* may have one or more pseudopodia).

The present results found that haemocytes (SEM) in infected snails have large cells with a large invagination, smooth surface and secretion particles adherent to these cells, that is a response to infection. *B. alexandrina* circulating haemocytes are known to be the major cellular effectors of the response against *S. mansoni* larval stages [11, 44 and 48]. In cooperation with humoral factor, these cells accomplish recognition, adhesion to the parasite surface and formation of a multilayer cellular capsule resulting in the death of the parasite after cytotoxic activation [23, 37, 49 and 50].

In the present study, *S. mansoni* infection led to a significant elevation of glucose concentration in the haemolymph of exposed snails. This elevation may be attributed to uptake and utilization by the parasite [51] findings are in accordance with Mohamed [52] and Ishak *et al.* [53] who stated that although schistosomes infection of *B. alexandrina* and *B. truncatus* snails stimulated lactic acid formation in their tissues yet it highly reduced the glycogen content and rate of gluconeogenesis in tissues of these snails. Nabih *et al.* [54] attributed the degradation of glycogen in *B. alexandrina* infected with *S. mansoni* mainly to dependence on the anaerobic glycolysis because the parasite destroys LD<sub>11</sub>, a lactate dehydrogenase isoenzyme associated with the aerobic respiration and stimulates LD<sub>3</sub>, the isoenzyme responsible for the maintenance of anaerobic respiration. As a consequence, food depletion of glycogen takes place in order to meet the energy requirements via the anaerobic respiration.

Regarding to the biochemical effects, the obtained results indicated significant declines in the albumin



content of the haemolymph. The drop in albumin content may also reflect damage in the hepatic parenchyma which are considered the site of albumin origin [55, 56]. Increase lipid content in haemolymph of infected snails is due to the interruption in lipid metabolism and accelerates the lipid synthesis caused by parasite. This finding agrees with the previous findings of Agrawal *et al.* [57] and Rawi *et al.* [56, 58].

The present results showed that the protein content was significantly reduced in haemolymph of infected *B. alexandrina* with *S. mansoni* than that of control snails. The cause of this decrease in protein content may be due to the presence of parasites which reproduce heavily in a short time, so extract much nutritive substances from their snail host [59]. This finding agrees with many investigators reported that infection of snails with schistosomes caused obvious changes in the protein metabolism [60-62] stated that the reduction in the protein content in infected *B. glabrata* and *B. truncatus* could be due to the proteolysis of tissue protein external to the parasite which are then absorbed as micromolecules by developmental stages of the parasite.

It was concluded that the histological results showed considerable changes in digestive gland of snails infected with *S. mansoni* with large numbers of sporocysts and cercariae at several stages of development encircled by cycts. In addition, different shapes of haemocytes observed through the scanning electron microscope did not wholly confirm to those recorded under the light microscope. Haemocytes (SEM) in infected snails have large cells with a large imagination, smooth surface and secretion particles adherent to these cells to respond to parasitic infections. In addition, a significant decrease in total protein and albumin content with elevation of total lipids content and glucose concentration in the haemolymph of infected snails.

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