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Alternations in parasitological, biochemical and molecular parameters of *Biomphalaria alexandrina* snails, intermediate host of *Schistosoma mansoni*, induced post exposure to the proposed snail biocontrol agent *Phasmarhabditis hermaphrodita* nematode

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ABSTRACT

Objective: To elucidate the effect of the parasitic nematode *Phasmarhabditis hermaphrodita* (*P. hermaphrodita*) as snail biocontrol agent on biological, parasitological, biochemical and molecular parameters of *Biomphalaria alexandrina* (*B. alexandrina*) snails, the intermediate host of *Schistosoma mansoni* (*S. mansoni*).

Methods: *B. alexandrina* snails (5–6 mm) were exposed to the 3rd stage larvae of the nematode *P. hermaphrodita* and/or *S. mansoni* miracidia for 24 h. Thereafter, they were thoroughly washed with clean dechlorinated water and transferred to clean aquaria for recovery and recording deteriorations in their biological, parasitological, biochemical and molecular parameters.

Results: The results showed that snails were dead after 24 h of exposure to 100 nematode larvae/snail. Cercarial production/infected snail was reduced to 68 cercariae/snail post simultaneous exposure to 40 nematode larvae and 10 *S. mansoni* miracidia compared to control (783.7 cercariae/snail) ($P < 0.001$). Similarly, the total hemocytes of snails increased to 3 800 hemocytes/mL hemolymph by the 3rd day post exposure compared to 2 100 hemocytes/mL for control group ($P < 0.001$). The results, also, elicited a significant elevation in activities of the enzymes aspartate transaminase, alanine transaminase, acid phosphatase and alkaline phosphatase in hemolymph of snails exposed to nematode larvae. Similar pattern was recoded for activities of catalase, glutathione reduced and nitric oxide in snails' digestive gland. Meanwhile total protein and albumin concentrations in snails' hemolymph and lipid peroxide in digestive gland tissues were decreased. The electrophoretic analysis of ovotestis-digestive gland complex of snails exposed to the nematode larvae indicated an increase in RNA intensity, while no significant changes were recorded in the molecular weight of intact DNA compared to control snails.

Conclusions: It is concluded that exposure of *B. alexandrina* snails to *P. hermaphrodita* nematode deteriorates their biological, parasitological, biochemical and molecular parameters, rendering them to be unsuitable for schistosomiasis transmission. However, comprehensive tests are needed to determine efficacy of *P. hermaphrodita* as a schistosomiasis biocontrol agent aiming to incorporate in control program of this parasite.

1. Introduction

There are multiple parameters affecting schistosomiasis transmission. The snail intermediate hosts are one of these parameters[1]. Snail control could be one of the methods of choice for schistosomiasis control[2]. A new approach to control the snail intermediate host of *Schistosoma mansoni* (*S. mansoni*) is using the bacterial-feeding nematode *Phasmarhabditis hermaphrodita* (*P. hermaphrodita*)[3]. It is a facultative parasite that significantly reduced terrestrial snails and slugs populations[4,5]. It is, also, associated with the bacteria *Moraxella osloensis* which are released into the slugs' body, multiply and eventually kill the host allowing the nematodes larvae to feed on the cadaver[6]. The nematode is hermaphrodite and self-fertilizing with a short life cycle, around 5 days, in which the non-feeding 3rd stage larvae are commercially

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mass produced as nemaslug and used for snails and slugs control operations[7].

In Egypt, *P. hermaphrodita* nematode has been recovered for the first time in Dakahlia Governorate associated with cadavers of the terrestrial snail *Monacha cantiana* and the slug *Deroceras reticulatum*[8]. In addition, the snail parasitic nematode *Phasmarhabditis tawfiki* Azzam was recorded and described for the first time from the terrestrial snail *Eobania vermiculata*[9]. Moreover, the later nematode species was capable of killing some terrestrial snails and slugs, some aquatic snails and insects larvae[9].

Although *P. hermaphrodita* nematode has been studied against terrestrial snails, yet few records are available on fresh water snails[10]. To control slugs and terrestrial snails, the commercial product nemaslug is mixed with water and applied as a drench to surface of soil, so, it can also be effective when applied in streams and water bodies where the snail *Biomphalaria alexandrina* (*B. alexandrina*) breed.

Therefore, the present study aimed to evaluate the potential role of the parasitic nematode *P. hermaphrodita* as a biocontrol agent against *B. alexandrina* snails, the intermediate host of *S. mansoni*. Moreover, some parasitological, biochemical and molecular parameters of snails exposed to this nematode were determined.

2. Materials and methods

2.1. Snails and S. mansoni ova

Laboratory bred *B. alexandrina* snails (5–6 mm) and *S. mansoni* ova used were from Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt. *S. mansoni* ova were introduced into Petri dish with dechlorinated water and exposed to desk lamp for hatching to miracidia which were used in the present tests.

2.2. P. hermaphrodita nematode

The commercial product of *P. hermaphrodita*, nemaslug, was purchased from Becker Underwood Company (now a part of BASF Chemical Company), United Kingdom. So, 250 mg were introduced into Petri dish with 50 mL distilled water and the fresh active 3rd stage larvae were collected by Pasteur pipette under stereomicroscope for this study.

2.3. Bioassay test

B. alexandrina snails were individually exposed to the larval nematode at levels of 10, 20, 40, 60, 80 and 100 larvae/snail for 24 h. Thereafter, they were thoroughly washed and transferred to clean aquaria with 10 snails/L dechlorinated water for recovery. Thirty snails, in three replicates, were used for each nematode level. Snails were fed oven-dried lettuce leaves and water was changed weekly during the recovery period that extended to 28 days. Dead snails were daily removed and the long-lasting effect of this nematode on snails' survival rate was recorded at the 28th day post exposure by using a solution of sodium hydroxide (15%–20%) for determining snails death[11].

2.4. Snails exposure to nematode larvae and S. mansoni

Three nematode levels (10, 20 and 40 larvae/snail) were chosen according to the data of bioassay test. So, three methods of snails exposure to this nematode were followed regarding to their exposure to *S. mansoni* miracidia, *i.e.* pre-miracidial exposure, during miracidial exposure and post-miracidial exposure by 1, 7 and 21 days. In each method, 3 groups each of 30 snails were prepared. For pre-miracidial exposure, each group was exposed to one of the chosen nematode levels for 24 h, then washed and re-exposed to miracidia for another 24 h followed by transferring to clean dechlorinated water. They were maintained under laboratory conditions till shedding *S. mansoni* cercariae (patency). In simultaneous exposure, snails were exposed to nematode larvae and miracidia together for 24 h followed by transferring to clean water as previous groups. In post-miracidial exposure, snails were exposed firstly to miracidia for 24 h followed by transferring to clean water and after 1, 7 and 21 days they were re-exposed to nematode larvae for 24 h followed by transferring to clean water till shedding cercariae as previous groups. Snails were individually exposed to miracidia (10/snail) and/or chosen nematode levels at (25 ± 1) ℃. During maintenance of exposed snails till shedding cercariae (prepatency period), they were fed oven-dried lettuce leaves and dead snails were removed daily, and water was changed weekly. Snails infection rates, prepatent period, duration of shedding and cercarial production/snail were recorded.

2.5. Biochemical and immunological assays

Four snail groups were performed as follows: Group 1 for *S. mansoni* miracidial exposure only; Group 2 for simultaneous exposure to *S. mansoni* and nematode (60 larvae/snail); Group 3 for nematode exposure only; and Group 4 was unexposed snails (control). Hemolymph samples were collected from snails after 3, 7, 14 and 21 days post exposure to determine total and different types of hemocytes by using a Burker-Turk hemocytometer[1]. Spectrophotometric evaluations of total protein and albumin levels and the activities of transaminases aspartate transaminase (AST) and alanine transaminase (ALT) and acid phosphatase (ACP) and alkaline phosphatase (ALKP) enzymes were done, also, in these hemolymph samples[12].

The oxidative parameters glutathione reduced (GSH), catalase (CAT), lipid peroxide (LPO) and nitric oxide (NO) were determined in homogenate of snails' digestive gland tissues[13].

2.6. DNA and RNA electrophoresis

The effect of snails exposure to nematode larvae and/or *S. mansoni* miracidia on the nucleic acids DNA and RNA was evaluated in their ovotestis-digestive gland complex tissue homogenate. Intensity and analysis of RNA were done by using tissue lysin technique. DNA and RNA were separated by agarose gel electrophoresis and stained with ethidium bromide[14].

2.7. Statistical analysis

The data were analyzed by *Chi*-square test or by using *t*-test to determine the significant differences in means between control and the experimental groups. Statistical analysis was performed with the aid of the SPSS computer program (version 13.0 for Windows). Electrophoretic density of RNA and DNA were measured by Gel-Pro computer program.

3. Results

As shown in Table 1, the survival rate of *B. alexandrina* snails was reduced post exposure for 24 h to the 3rd infective larval stage of the nematode *P. hermaphrodita*. More reduction in snails survival rates was recorded by increasing the number of nematode larvae/snail. At the 28th day post exposure, it was reduced from 96.7% to 73.6% by increasing the number of nematode from 20 larvae/snail to 60 larvae/snail, respectively. Moreover, snails exposed to 100 larvae/snail suffered from hemorrhage and were dead after one day of exposure.

Table 1
Survival rates of *B. alexandrina* snails after 24 h exposure to the nematode *P. hermaphrodita*. %.

Period of recovery (day)	10 larvae/snail	20 larvae/snail	40 larvae/snail	60 larvae/snail	80 larvae/snail	100 larvae/snail	Control
1	100.0	100.0	100.0	100.0	66.7	0.0*	100.0
3	100.0	100.0	100.0	100.0	0.0*		100.0
7	100.0	100.0	100.0	86.8			100.0
14	100.0	100.0	100.0	76.9			100.0
21	100.0	96.7	100.0	76.9			100.0
28	100.0	96.7	100.0	73.6			100.0

*: Snails suffered from hemorrhage.

For infection of *B. alexandrina* snails with *S. mansoni* under stress of their exposure to the nematode *P. hermaphrodita*, Figure 1A indicates that exposure of snails to 20 and 40 nematode larvae/snail for 24 h at pre-, during and 1 day post-miracidial exposure, significantly reduced their infection rate ($P < 0.001$). Thus, at 40 larvae/snail in during miracidial exposure group, it was sharply reduced to 21.7% compared to 89.3% for control group. However, this parameter for snail groups exposed to nematode larvae at 7 and 21 days post-miracidial exposure did not significantly different from control group ($P > 0.05$).

Data of *S. mansoni* cercarial production/infected snail exposed to *P. hermaphrodita* nematode (10, 20 and 40 larvae/snail) showed a significant reduction compared to control group (Figure 1B) at pre-, during and 1 and 7 days post-miracidial exposure. The least number of cercariae/snail was observed for snail groups exposed to the nematode and *S. mansoni* miracidia simultaneously. In this case snails exposed to 40 larvae/snail shed only 68 cercariae/snail compared to 783.7 cercariae/snail in control group ($P < 0.001$).

Concerning the number of hemocytes in hemolymph of *B. alexandrina* snails exposed for 24 h to *S. mansoni* and/or *P. hermaphrodita* nematode, data in Figure 2 reveals an increase in this parameter for treated snail groups compared to their corresponding controls. For snails exposed simultaneously to both parasites, the number of hemocytes/mL hemolymph was significantly higher than that of control group throughout the experimental period ($P < 0.01$). Thus, 3 100 hemocytes/mL were recorded at 21 days post exposure to the parasites compared to 1 900 hemocytes/mL of control snails. It was, also, noticed that the number of hemocytes in hemolymph of snails exposed to nematode larvae alone was decreased by elongation of observation period from 3 to 14 days after exposure, being 3 300 and 2 300 hemocytes/mL, respectively. However, the vice versa was seen with snails exposed to *S. mansoni* alone, as the number was 2 800 and 3 700 hemocytes/mL through the same observation period.

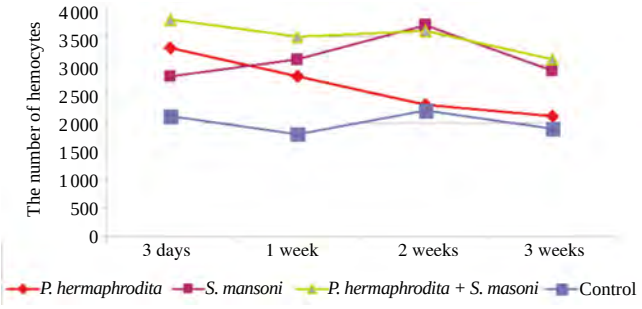

Figure 2. The number of total hemocytes/mL in hemolymph of *B. alexandrina* snails post 3, 7, 14 and 21 days of exposure to *S. mansoni* and/or *P. hermaphrodita*.

Regarding to types of hemocytes, there were two types in the tested and control snails, *i.e.* hyalinocytes and granulocytes which were differentiated to small and large cells (Table 2). The small granulocytes were dominant hemocytes. They accounted for 94% of total hemocytes after 7 days of exposure to the nematode alone compared to 81% for control group ($P < 0.01$). Also, their precentages were 95% and 97% from hemocytes after 14 days of exposure to *S. mansoni* alone or combined with *P. hermaphrodita* nematode, respectively, compared to 82% for control snails ($P < 0.01$). Meanwhile, the percentages of hialynocytes were very low for the test and control snails, throughout the observation period (21 days).

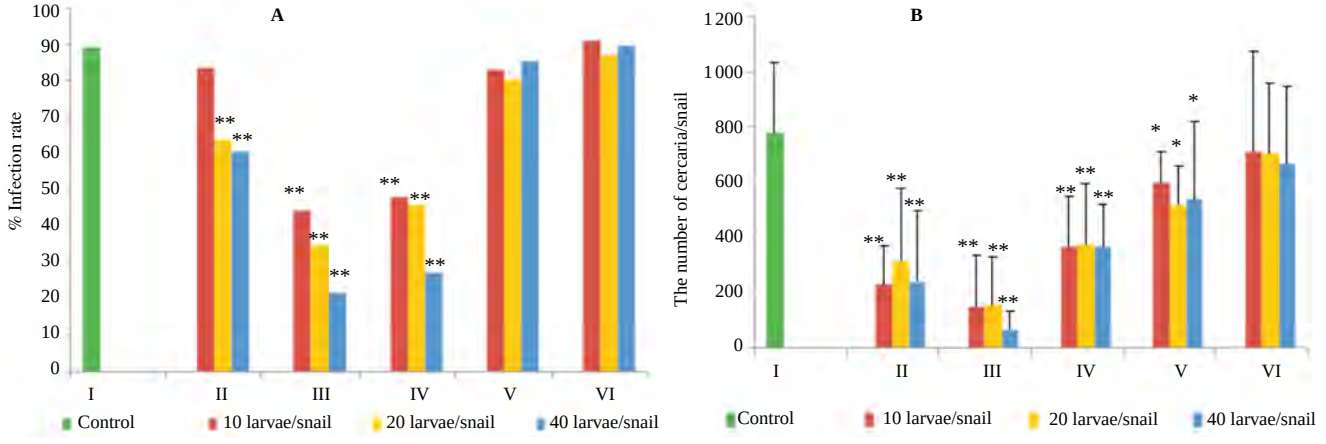

Figure 1. Infection rate of *B. alexandrina* snails exposed to the nematode *P. hermaphrodita* as well as *S. mansoni* and the number of cerarial production/snail.

A: Infection rate; B: Cerarial production/snail; I: Control; II: 1 day pre-miracidial exposure; III: During miracidial exposure; IV: 1 day post-miracidial exposure; V: 7 days post-miracidial exposure; VI: 21 days post-miracidial exposure. *: $P < 0.01$ compared with control; **: $P < 0.001$ compared with control.

Table 2
The percentages of types of haemocytes in hemolymph of *B. alexandrina* snails exposed to *S. mansoni* and/or *P. hermaphrodita* nematode (60 larvae/snail). %.

PAE	Type of cells	*P. hermaphrodita*	*S. mansoni*	*P. hermaphrodita* + *S. mansoni*	Control
3 days	Large granulocytes	10	11	13	13
	Small granulocytes	88	83	83	80
	Hyalinocytes	2	6	4	7
7 days	Large granulocytes	4	5	6	11
	Small granulocytes	94**	92**	91*	81
	Hyalinocytes	2	3	3	8
14 days	Large granulocytes	10	3*	2*	12
	Small granulocytes	85	95**	97**	82
	Hyalinocytes	5	2	1*	6
21 days	Large granulocytes	10	10	9	12
	Small granulocytes	86	86	88*	80
	Hyalinocytes	4	4	3	8

PAE: Period after exposure; *: $P < 0.05$ compared with control; **: $P < 0.01$ compared with control.

The biochemical and oxidative parameters of *B. alexandrina* snails exposed to the nematode *P. hermaphrodita* and/or *S. mansoni* (Figure 3) showed that glucose levels in hemolymph of snails were significantly raised to 25 and 34 mg/100 mL after 7 and 21 days of exposure to *S. mansoni* alone, respectively, compared to 11 mg/100 mL for control group ($P < 0.001$). Similar observation was recorded for snail groups simultaneously exposed to the nematode and *S. mansoni*, but those exposed to nematode alone had values almost as control snails ($P > 0.05$). Although total protein and albumin concentrations for snails exposed to either nematode and/or *S. mansoni* were significantly less than those of control group, the activities of AST, ALT, ACP and ALKP enzymes were elevated ($P < 0.001$), except snails at 21 days post exposure to nematode alone the activities of these enzymes were less than control ones ($P < 0.05$).

The levels of CAT, and GSH as oxidative parameters in digestive gland tissues of *B. alexandrina* snails were significantly higher than those of control group after 21 days of simultaneous exposure to the nematode and *S. mansoni* ($P < 0.001$). CAT recorded 80 IU/mg tissue for treated group compared to 55 IU/mg tissue for control group. On the other hand LPO levels at 21 days post snails exposure to either *S. mansoni* alone or combined with the nematode were significantly less than that of control group ($P < 0.001$).

Molecular data (Table 3 and Figure 4) indicated an increase in RNA intensity in the ovotestis-digestive gland complex of *B. alexandrina* snails post exposure to the nematode and/or *S. mansoni* compared to control snails. This increase in RNA intensity was higher after 7 days of snails' exposure to the parasites than that after 21 days. However, no changes were observed in DNA of treated snails compared to control group (Figure 4).

Table 3
Quantitative analysis of electrophoretic patterns of RNA optical density at 100 bp in ovotestis-digestive gland complex of *B. alexandrina* snails exposed to *S. mansoni* and/or *P. hermaphrodita* after 7 and 21 days of exposure.

Period after exposure	Parasites	Optical density at 100 bp
7 days	*P. hermaphrodita*	120
	S. mansoni	110
	P. hermaphrodita + *S. mansoni*	104
21 days	*P. hermaphrodita*	90
	S. mansoni	100
	P. hermaphrodita + *S. mansoni*	87
Control		75
Control + RNase		3

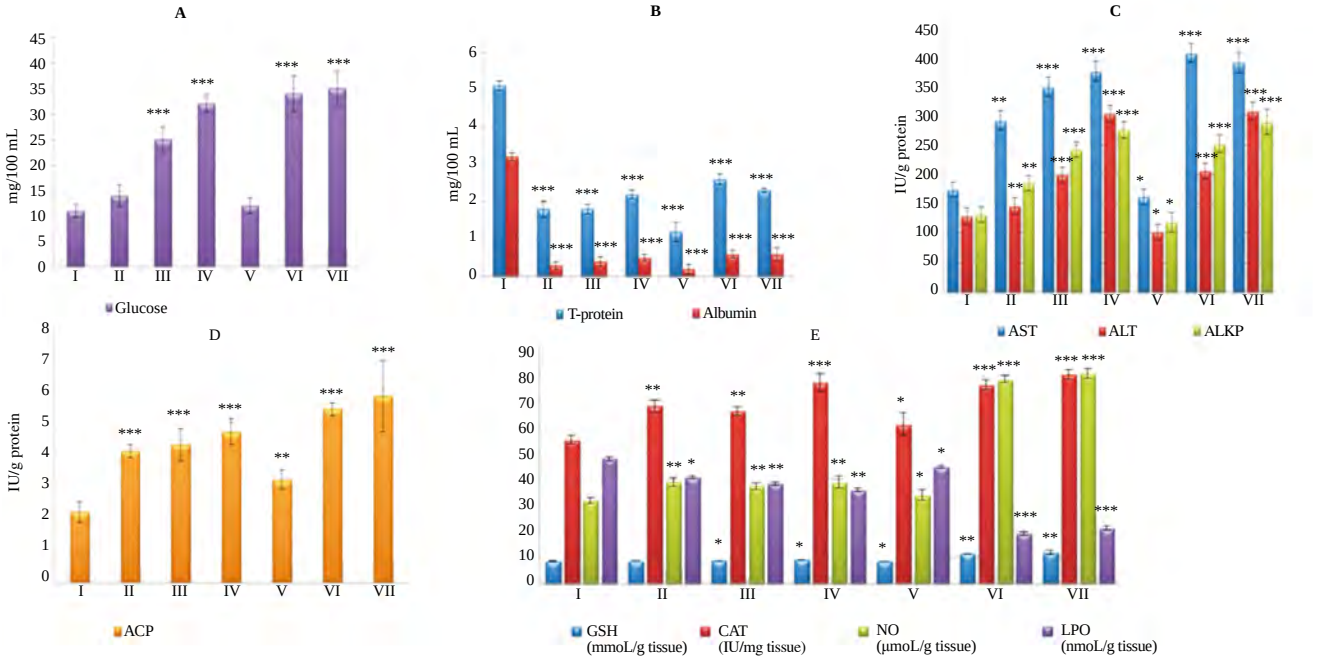

Figure 3. Biochemical and oxidative parameters of *B. alexandrina* snails exposed to the nematode *P. hermaphrodita* and/or *S. mansoni*.
A: Glucose level; B: Total protein and albumin concentrations; C: Activities of AST, ALT and ALKP; D: Activity of ACP; E: Activities of GSH and CAT and concentrations of NO and LPO.
I: Control; II: *P. hermaphrodita*, 7 days; III: *S. mansoni*, 7 days; IV: *P. hermaphrodita* + *S. mansoni*, 7 days; V: *P. hermaphrodita*, 21 days; VI: *S. mansoni*, 21 days; VII: *P. hermaphrodita* + *S. mansoni*, 21 days. A–D were evaluated in hemolymph of *B. alexandrina* snails, and E in tissue homogenate of digestive gland of *B. alexandrina* snails. *: $P < 0.05$ compared with control; ***: $P < 0.01$ compared with control; ***: $P < 0.001$ compared with control.

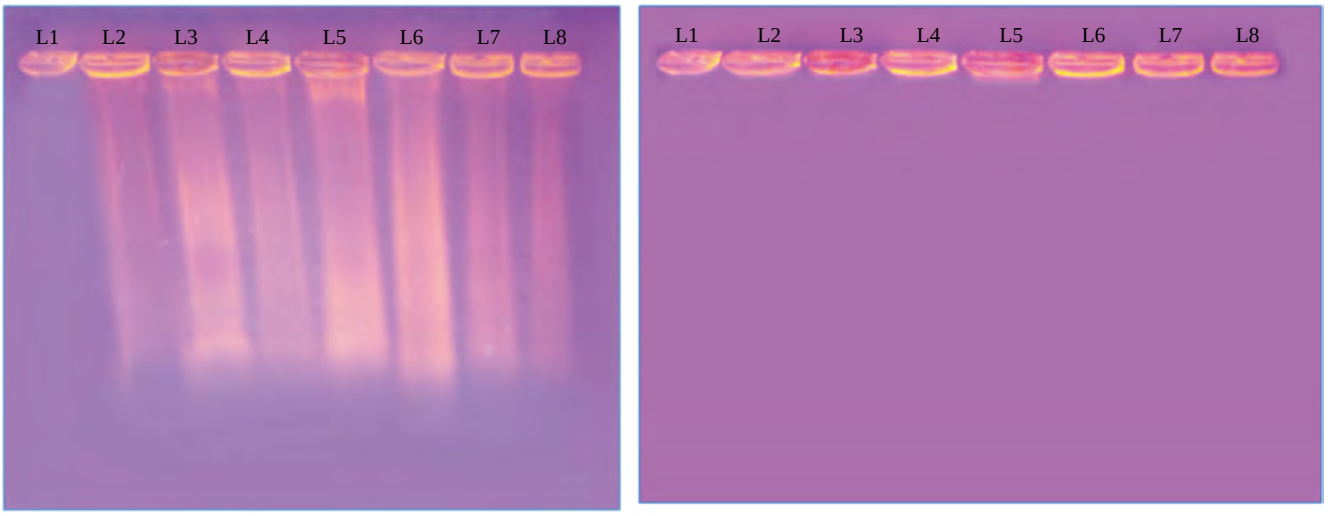

Figure 4. Electrophoretic patterns of RNA (left) and DNA (right) in ovotestis-digestive gland complex of *B. alexandrina* snails post 7 and 21 days of exposure to *S. mansoni* and/or *P. hermaphrodita*.
L1: Control + RNase; L2: Control; L3: Exposed to *P. hermaphrodita* only, 7 days; L4: Exposed to *S. mansoni* only, 7 days; L5: Exposed to *S. mansoni* + *P. hermaphrodita*, 7 days; L6: Exposed to *P. hermaphrodita* only, 21 days; L7: Exposed to *S. mansoni* only, 21 days; L8: Exposed to *S. mansoni* + *P. hermaphrodita*, 21 days.

4. Discussion

Schistosomiasis is an acute and chronic disease causing significant cases of morbidity and mortality worldwide[15]. 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. The rhabditid nematode *P. hermaphrodita* has been evaluated as a parasite capable of killing slugs and terrestrial snails from the families Arionidae, Limacidae and Helicidae and fresh water snails from Lymnaeidae and Planorbidae[3,4,16]. The present study demonstrated that *B. alexandrina* snails exposed to 100 nematode larvae/snail suffered from hemorrhage and were dead after 1 day of exposure. This could be due to attempts of nematode larvae to penetrate snails' skin, rupturing it, and hemolymph oozes out leading to snails death. This is in accordance with swollen mantle and death of the freshwater snails *Lymnaea stagnalis* and *Biomphalaria pfeifferi* after 14 days of exposure to the nematode *P. hermaphrodita*[3,4]. Similar results on death of the land snails *Monacha cantiana* and *Opeas pyrgula* and the slug *Deroceras reticulatum* were recorded post their exposure to this nematode[9,17,18]. However, exposure of the land snail *Achatina fulica* to the same nematode at 30 and 150 larvae/cm^2 for 70 days has no effect on the snails weight gain, and this was attributed to the snails' ability to encapsulate nematode larvae after 3 days of exposure and kill them[5].

The present data revealed a significant reduction in *S. mansoni* cercarial output from *B. alexandrina* snails exposed to the nematode *P. hermaphrodita* during miracidial exposure and at 1 day pre- or post exposure to miracidia. This agrees with Mossalem *et al.* and Abdel-Hamid *et al.* on double infection of *B. alexandrina* snails with *S. mansoni* and/or *Echinostoma liei* (*E. liei*) trematodes[19,20]. The present reduction in cercarial output from infected *B. alexandrina* could be due to interruption in their physiological and defense mechanisms that rendering them to be unsuitable for development of *S. mansoni* larvae within their tissues. This was supported by the present data on disturbances in liver and oxidative enzymes, number and types of hemocytes and the decrease in the levels of total protein and glucose in hemolymph of *B. alexandrina* snails exposed to the nematode and/or *S. mansoni* miracidia. It was reported that protein synthesis in snails' tissues is responsible for production of *S. mansoni* cercariae from infected *B. alexandrina* snails[21].

The present study disclosed a significant increase in the total number of hemocytes/mL hemolymph of *B. alexandrina* snails after exposure to the nematode and/or *S. mansoni* miracidia. This phenomenon was obvious for snails simultaneously exposed to the two parasites. This agrees with Abdel-Hamid *et al.* on exposure of *B. alexandrina* to *S. mansoni* and *E. liei* miracidia[20]. The authors added that this treatment could stimulate hematopoietic organs to produce a number of new undifferentiated hemocytes during first, second and third week post-miracidial exposure. Similarly, an increase in the percentages of hemolymph amoebocytes in *B. alexandrina* and *Biomphalaria glabrata* snails post exposure to *S. mansoni* miracidia was recorded[22].

From the present data, two types of hemocytes were observed in snails' hemolymph, hyalinocytes and granulocytes that differentiated to small and large granulocytes. This was in accordance with Bakry *et al.* on two types of hemocytes in *Bulinus truncatus* snails[23]. However, three types in *B. alexandrina* snails, hyalinocytes, granulocytes and amoebocytes were recorded[21,22]. It is well known that granulocytes have a critical role in defense mechanism within the immune system of snails against invading foreign biotic and abiotic agents[24].

Glucose levels in hemolymph of *B. alexandrina* snails exposed to *S. mansoni* alone or with *P. hermaphrodita* nematode in this study were significantly increased which could be partially due to

degradation of glycogen to supply the parasite requirements. This was stated by Abdel-Hamid *et al*. who found a significant reduction in glycogen content in tissues of *B. alexandrina* snails exposed to *S. mansoni* and *E. liei* miracidia accompanied by marked increase in hemolymph glucose levels at the first week post exposure[20]. Bakry *et al*.[25], also, have reported that the primary source of nutrition for *S. mansoni* sporocysts and developing cercariae is glucose which is absorbed from snails' hemolymph.

The total protein and albumin levels were significantly decreased in hemolymph of snails exposed to the nematode and/or *S. mansoni* miracidia. The marked reduction in the present study was observed for snails simultaneously exposed to the two parasites. This coincides with the study of Mohamed and Ali that after 30 days of infection, when *S. mansoni* cercariae were fully developed in *B. alexandrina* snails, amino acids profile was significantly deteriorated[26]. Parasite absorption and utilization of nutrients may explain the decrease in hemolymph protein concentration of *B. alexandrina* snails infected with *S. mansoni*[20].

The overall elevation recorded for activities of the enzymes ALT, AST, ALKP and ACP in hemolymph of *B. alexandrina* snails post exposure to the nematode and/or *S. mansoni* miracidia in this study could be due to destruction of several snails' cells and/or secretion from the tegument of *S. mansoni* sporocysts. These observations are in agreement with Abdel-Hamid *et al.* on significant elevation of enzymes activities in hemolymph of *B. alexandrina* snails infected with *E. liei* and/or *S. mansoni*[20]. The recorded disturbance in enzymes activities could be attributed to mechanical damage of snails' skin by nematode larval attempts to penetrate and the actual skin penetration of *S. mansoni* miracidia which may deteriorate metabolic processes of infected snails. This phenomenon was stated by Soliman and El-Ansary on alternations of metabolic and physiological processes in snails infected with schistosome and echinostome parasites, which grow and proliferate within snails' tissues and extract nutrients from and expel wastes into the host hemolymph[27].

GSH is an important cellular protectant against reactive oxygen metabolites in several cells by serving as a substrate of GSH peroxidase[20]. In the present study, the increase of GSH had been associated with a depletion of LPO in snails exposed to the nematode and/or *S. mansoni* miracidia. This was stated in a study on *B. alexandrina* snails exposed to zinc oxide nanoparticles[12]. Similarly, a correlation between enhancement of lipid peroxidation and consequence depletion of GSH was stated[13].

CAT is a haemoprotein, protecting the cells from accumulation of H_2O_2 by dismutating it to water and oxygen[28]. The present study recorded a significant increase in CAT activity after snails exposure to the nematode and/or *S. mansoni* miracidia. This agrees with Bakry *et al*. and Mahmoud as well as Rizk on elevation of CAT activity in *B. alexandrina* snails infected with *S. mansoni*[23,29]. This could be due to enhanced oxygen free radicals production by snails' infection, which may stimulate antioxidant activities to cope with increased oxidative stress and protect cells from damage[30].

NO is a highly reactive molecule synthesized by oxidation of L-arginine to L-citrulline, catalyzed by the enzyme NO synthase[31]. NO synthase is a conserved enzyme, which has been identified in the snails *Lymnaea stagnalis* and *Biomphalaria glabrata* defense cells[32]. The present investigation showed a significant increase in the activity of NO following snails exposure to the nematode and/or *S. mansoni* miracidia. The direct toxicity of NO is enhanced by reacting with superoxide radical forming peroxynitrite which is capable for oxidizing cellular structure and then causing lipid peroxidation[33].

Molecular data in this study indicated an increase in RNA intensity in the ovotestis-digestive gland complex of snails exposed to the experimental parasites. This finding supports the present deteriorations in protein levels and in activities of the tested biochemical parameters of treated snails which is in accordance with the results of Abdel-Hamid *et al*. on infection of *B. alexandrina* snails with *E. liei* and/or *S. mansoni* miracidia[20].

It is concluded that exposure of the fresh water snails *B. alexandrina* to the parasitic nematode *P. hermaphrodita* interrupted their biological and biochemical parameters that negatively reflect on their suitability for schistosomiasis transmission. However, more studies are needed about this nematode as suggested snail biocontrol agent to be considered in the strategy of schistosomiasis control.

Conflict of interest statement

We declare that we have no conflict of interest.

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