Effect of Zingiber officinal (ginger) on electrophoresis analysis and biochemical aspects of *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*

Fayez A.Bakry[1], Abd El-Atti M. S[2]., Somaya M. Ismail[3]

Abstract—Schistosomiasis is an important parasitic disease that infects humans. *Schistosoma mansoni* is one of the main species of schistosomes infecting humans. *Biomphalaria alexandrina* snails, act as an intermediate hosts and play a major role in the transmission of schistosomes. The effect of Zingiber officinal (ginger) on the survival rate, egg production, electrophoresis analysis, biochemical aspects of *B. alexandina* snails infected with *S. mansoni* were studied. The obtained result showed that a rapid decline in survival rate and egg production of infected snails with *S. haematobium* exposed to ginger. The present results also, showed that the glucose concentrations in uninfected and infected snails exposed to ginger were increased in hemolymph, while soft tissue glycogen decreased. The activities of glycogen phosphorylase, succinate dehydrogenase (SDH) and glucose-6-phosphatase in homogenate of snail's tissues of uninfected and infected snails exposed to ginger were reduced ($P< 0.001$) in response to infection and exposure to ginger. Qualitative and quantitative effects on the protein patterns have been revealed for uninfected and infected snails exposed to ginger. The electrophoresis pattern of total protein showed differences in number and molecular weights of protein bands.

Keywords — *Biomphalaria alexandrina* snails, *Schistosoma mansoni*, Zingiber officinal (ginger),

1 INTRODUCTION

Schistosomiasis is a public health problem in many developing countries. An estimated 80% of all infected people are now concentrated in Africa (1). Water resource schemes for power generation and irrigation have resulted in a tremendous increase in the transmission and out breaks of schistosomiasis in several African countries (2&3).

Most trematodes have a freshwater snail as intermediate host and a vertebrate as a definite host. The schistosomes that cause bilharziasis in man and in domestic animals belong to this group of parasites. Schistosomiasis is one of the most important parasitic diseases of man in Egypt. Much experimental work has been carried out on these schistosomes and on their intermediate hosts (4). Molluscs play a major role as intermediate hosts in the transmission of schistosomes. They are sites of the intense multiplication of the parasite larvae. The fresh water snail, *B. alexandrina* is an essential factor for transmitting *S. mansoni*, acting as intermediate host for the parasite.

Ginger (*Z. officinal L.*, Zingiberaceae) is widely used in traditional Chinese medicine (5). The medicines are purported to be effective treatment for inflammation, oxidant stress, helminthiasis and schistosomiasis (6). It has also antischistosomal effect against *S. mansoni* miracidia and cercariae (7). Phytochemical reports have shown that the main constituents of ginger are zingerone, paradol, gingerols and shogoals. These agents are known to have the ability to suppress the inflammatory and transformative processes of carcinogenesis. Some agents have been found to have antibacterial and antiprotozoae activities (8&9). Another study has suggested that ginger free radical scavenging activity may reduce larvae survival (10&11).

Electrophoresis is the ability to separate a polypeptide of interest and to have an indication of its molecular size .It is very important in any study involving mixtures of proteins (12). The most relatively simple and powerful technique involves sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). In this method, separation of proteins based on their molecular size where, the SDS –protein complexes are sieved through a polyacrylamide gel matrix. The combination of SDS and sieving properties in the molecular sized pores of the gel matrix, leads to an exceedingly high resolution of separation that is unattainable with any other separation method based upon protein size (13).

The present work aims to evaluate the effect of the effect of ginger on the survival rate, egg production, and electrophoresis analysis and biochemical aspects of *B. alexandrina* snails infected with *S. mansoni*

2 MATERIALS AND METHODS

2.1 SNAILS

The snail used in the present study was *B. alexandrina* that are the vector for *S. mansoni* in Egypt. The snails were obtained from the laboratory-bred stock in Medical Malacolo-

- *Medical malacology, Theodor Bilharz Research Institute (TBRI) ,Giza, Egypt, E-mail: bakeryFayez@yahoo.com
- ** Department of zoology, Faculty of Science, zagazig University, Egypt
- ***Zoology Department, Faculty of Science, Cairo Universty, Giza, Egypt.

gy laboratory, Theodor Bilharz Research Institute (TBRI) for this study. They were maintained in container (50 X 20 X 30 cm) where each 10 snails were placed in one liter of dechlorinated water (pH 7.0-7.5) to avoid crowding. Water temperature was adjusted to 25-27 °C and illumination was provided from 80 watts ceiling-level fluorescent lamps. Water was changed twice weekly and dead snails were removed every day. The snails were fed daily on lettuce leaves either fresh or boiled and oven dried. Commercial fish food (Tetramin D4520 Melle, Germany) was added once a week in the aquaria.

2.2 Ginger extract preparation

The rhizome of ginger was purchased from the International Company (Cairo-Egypt). The plant was authenticated and a specimen voucher was deposited (NRC-0234) at the Cultivation and Production of Medicinal and Aromatic Plants Department, National Research Centre, Dokki, Giza, Egypt. In order to prepare the ethanolic extract ginger was ground into a fine powder using a pestle and mortar. The powder (30 g) was refluxed in ethanol (600 ml) in a Sechelt apparatus for two days. Ethanol in the extract was evaporated under reduced pressure to give a brown extract (yield: 11%). The material was subsequently reconstituted in a known volume of sunflower oil (14).

2.3 Experimental test

These snails were randomly divided into 3 groups (100 snails each) the first group was used to study biology of exposed snails. The second group was used for Biochemical studies of snails. The third group was used for Molecular biological studies.

Each group divided into four subgroups. The first subgroup was exposed to miracidia obtained from *S.mansoni* ova (10 miracidia/ snail) for 24 hours at 24°C and ceiling illumination. The second subgroup was exposed continuously to concentration (LC_{10}) of ginger. The third subgroup was individually exposed to freshly hatched *S. mansoni* miracidia (10miracidia/ snail) for 24 hours and then exposed continuously to concentration (LC_{10}) of ginger. The fourth subgroup was maintained unexposed under the same laboratory conditions as control, the tested compound was changed with new by prepared ones every 3 days. The snails were daily fed boiled lettuce leaves.

2.3.1 Biological studies

For studying the survival rate, egg lying of *B. alexandrina* (8-10 mm in diameter) and infection rate. Each aquarium from first group was provided with polyethylene sheets for oviposition. The egg masses and eggs laid on these sheets were counted using a binocularn microscope. Dead snails were daily removed from the aquaria and the mortality rate was calculated. After three weeks post miracidial exposure, surviving snails were individually examined for cercarial shedding in separate petri-dishes under artificial light for 3 hours. The produced cercarial suspension was poured in a graduated Petri-dish and all cercariae were counted after adding few drops of Bouin's fluid using a dissecting microscope. Infected snails were isolated and kept in special aquaria in complete darkness and examined for cercarial production twice weekly (3hours each) till all snails died. Prepatent period, total cercarial production/snail and duration of cercarial shedding were calculated. The period between miracidial exposure and the first shedding of cercariae for each snail was considered as the incubation period (prepatent period). The infection rate of snails was based on the total number of positive snails and those survived at the first shedding. The stimulant was calculated by summation of cercariae shed per snail through every stimulant period (3hours/3days) for all shedding snails divided by the number of stimulant periods throughout the experiment.

2.3.2 Biochemical studies

Four subgroups snails from second group used for Biochemical studies. Haemolymph samples were collected after 3 weeks in accordance with techniques described by Michelson (15) by 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. For preparation of tissue of both exposed and unexposed snails, one gram of snail's soft tissues from each snail group was homogenized in 5 ml distilled water pH 7.5. A glass homogenizer was used and the homogenate was centrifuged for 10 minutes at 3000 rmp the fresh superant was used.

All physiological parameters determined in this study were determined spectrophometrically, using reagent kits purchased from BioMerieux Company, France. Kits purchased from BioMerieux Company, France. Determination of tissues glycogen was evaluated according to Carrol et al, (16). Haemolymph glucose concentrations were determined according to the glucose oxides method of Trinder (17).

Glycogen phosphorlase was assayed according to the method of Hedrick and Fischer (18). Glucose-6-phosphatase (G-6-Pase) was assayed according to the method of Swanon (19). Succinate dehydrogenase (SDH) was determined according to Shelton and Rice. (20).

2.3 3. Molecular biological studies

These snails of four subgroups from fourth group used for Molecular biological studies After 3 weeks the snail's soft tissues were homogenized in double volume of 10 mM potassium phosphate buffer (pH 6.8) and centrifuged for 20 minutes at 25000 g (21). The supernatant was frozen at -20 °C for electrophoresis. SDS-Page electrophoresis was done according to the method of Laemmli (22). The wide range SDS-Page molecular weight pre-stained standard mixture (Bio-Rad) was applied to the first well. Scanning was applied using Gel pro software (Ver.3.0, USA, 1998), Media Sci. Image densitometry 700 Biorad).

Data analysis: To calculate percentage band sharing, the bands observed in a given lane were compared with those in other lanes of the same gel. Enlarged photographs of the gels were

examined and the principal bands were scored. A similarity matrix was constructed on the basis of the presence/absence of bands. This based on between all possible pairs in an analysis group and was constructed using the Dice similarity coefficient (Dice (23), using the formula: S = 2a/2a + b + c where a = the number of bands shared between organisms 1 and 2, b = the number of bands present in 1 but not in 2 and c = the number of bands present in 2 but not in 1.

2.4. Statistical analysis

The obtained data were analyzed by the student"s t-test for comparing means of the experimental and control groups (Spiegel (24).

3. RESULTS

The molluscicidal activity of ginger on *B. alexandrina* snails after 24 h of exposure under the present laboratory conditions is presented in Table 1. The data obtained clearly indicate that the recorded LC_{50} and LC_{90} values were 28 and 48 ppm of ginger, respectively. The results Table (2) showed that there is a rapid decrease in survival rate of infected snails exposed to LC_{10} of ginger. The decrease in this rate throughout 2th week of experiment was 30% which is significantly lower than that of control group ($p<0.001$).

Moreover, the infected snails exposed to ginger died at 3th week, while the infected snails showed gradual decrease in their survival rate up to the 4th week of the experiment reaching 8% which is significantly lower than control group ($p<0.001$) and all snails died by 5th week. In the groups of non- infected snails exposed to LC_{10} of ginger, a rapid decrease in the survival rate occurred throughout 2th & 3th weeks being 38% and 20% respectively which were significantly lower than that of control group 58 and 74% ($p<0.001$). All non- infected exposed continuously to this concentration died by 4th week.

Table (1) Molluscicidal activity of *Zingiber officinal* against *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions.

LC_{50} ppm	Confidence limit of LC_{50} (ppm)	LC_{90} ppm	LC_{10} ppm	Slope
28	18.7 - 42	48	12	2.25

It is clear also from results in Table (2) that groups of non-infected and of infected snails exposed to LC_{10} of ginger affected negatively the egg laying capacity of *B. alexandrina* snails. Thereafter, survived snails in these treatments complete stopped egg laying was observed 4th week of the experiment in the group of infected snails. Regarding all exposed groups and control snails began to lay eggs in the first week of the experiment. The total mean numbers of eggs laid by non- infected snails (17.89%) was higher than that laid by the snails exposed to LC_{10} of ginger either infected or non- infected (2.46, 1.5, respectively). The percent reduction in the egg laying capacity of infected or non-infected exposed to LC_{10} of ginger compared to that of the control, was 86.25, 91.62, respectively.

The effect of LC_{10} of ginger on infection of *B. alexandrina* with *S. mansoni* miracidia was presented in (Table 3). The infection rate was significantly lower than that of control snails (24%), being 11% for snails exposed to LC_{10} of ginger with a reduction rate 54.17%. Prepatent period (Table 3) of exposed snails to LC_{10} of ginger was prolonged to be 34±1.2 days compared to 36±1.4days for the control group. Meanwhile, the duration of cercarial shedding was significantly shortened among these snails, being 4.2±1.6 days for LC_{10} of ginger, compared with 10±1.2 days for control snails. Highly significant reductions of total cercarial production per snails were also detected in experimental snails in comparison with the control group.

The results in Table 4 show a clear reduction ($P < 0.001$) in the glycogen content in soft tissues of snails infected with *S. mansoni* compared to control group. The reduction rate was 30.21%. This reduction increased in non- infected and of infected snails exposed to LC_{10} of ginger. The reduction rate was 38.3% and 51.1%, respectively. On the other hand, glucose concentration in haemolymph of infected snails, snails of non- infected and of infected snails exposed to LC_{10} of ginger showed a marked increase ($P< 0.05$) in comparison with the control group. The rate of increase was 11.81%, 25.27% and 41.21%, respectively.

The results (Table 4) showed that a clear inhibitory effect in activities of glycogen phosphorylase, G-6-Pase and SDH in snail's soft tissues. The glycogen phosphorylase activity in tissues of snails infected snails of non- infected and of infected snails exposed to LC_{10} of ginger was 12.77, 38.3 and 51.1% compared to control group ($P< 0.01$). The reduction rates in activities of G-6-Pase and SDH in tissues of infected snails exposed to LC_{10} of ginger were 41.46 and 43.66%, respectively.

The pattern of protein profile identified by SDS-PAGE electrophoresis for *B. alexandrina* snails infected with S. *monsoni* and exposed to LC_{10} of ginger was shown in Fig. (1). Data in tables 5 and 6 as well as illustrated in fig 1 show that the protein profile of Non-infected snails (A) composed of 12 protein bands. This profile decreased to 10 bands after Non-infected snails exposed to ginger. The molecular weights of these bands for snails infected with *S. mansoni* ranged from 17 to 36 Kda. This profile decreased to 12 bands after infected snails exposed to ginger. The molecular weights of these bands for snails infected with *S. mansoni* ranged from 198.5 to 36 Kda. The protein profilen of untreated *B. alexandrina* (A) composed of 12 protein bands. This profile increased to 14 bands after snails infected with S. *mansoni*. The molecular weights of these bands for snails infected with S. *mansoni* ranged from 198.5 to 36 Kda.

The present data (Table 5 and Fig 1) showed the appearance of four bands in infected snail groups and disappearance of others in comparison with unifected snails. The disappeared bands are 198.5, 91.5, 79 and 76 Kda, while six bands appeared in snails infected with S. *mansoni* were 198.5, 93.26, 79.578, 74and 62 Kda.

Table (2) Effect of prolonged exposure to LC_{10} of *Zingiber officinal* on the survival and egg laying production of *Biomphalaria alexandrina* infected with *Schistosoma mansoni*

Plant extracrt	Snails	1week		2 week		3 week		4 week		5 week		R0	% change	P
		Survival %	Mean No. of eggs/snail	Survival %	Mean No. of eggs/snail	Survival %	Mean No. of eggs/snail	Survival %	Mean No. of eggs/snail	Survival %	Mean No. of eggs/snail			
Control	Non-infected snails	96	4.45	74	6.8	58	4.2	41	2.44	22	0	17,89		
	Infected snails	80	1.44	42	2.8	24	1.2	8	0.48	0	0	5.92	66.91	P<0.5,
Zingiber officinal	Non- infected snails exposed to LC_{10}	76	0.82	38	1.24	20	0.4	4	0	0	0	2,46	86.25	P<0.01
	Infected snails exposed to LC_{10}	80	0.62	30	0.88	0	0	0	0	0	0	1.5	91.62	P<0.001

Table (3) Effect of LC_{10} of *Zingiber officinal* (Ginger) on repatent period, duration of cercarial shedding and cercarial production (days) of *Biomphalaria alexandrina* infected with *Schistosoma mansoni*

Treatment	Infection rate	Prepatant periods (days)		Duration of cercarial shedding (days)		Total number of cercarial / snail	
		Rang	Mean±S.D	Rang	Mean±S.D	Rang	Mean±S.D
Infected snails	24%	28-42	36±1.4	4-16	10±1. 2	340-680	420.66±32.4
Infected snails exposed to LC_{10} of Ginger	11%(54.17)	30-40	34±1. 2	3-10	4.2±1.6**	98-280	124.6±42.22**

*P<0.5, **P<0.01, ***P<0.001

Table 4 Effect of LC_{10} of *Zingiber officinal* on glucose level in haemolymph, glycogen content and the activities of some enzymes in in soft tissues of *Biomphalaria alexandrina* infectedwith *Schistosoma mansoni* (40 days post exposure).

	soft tissue				haemolymph
	Glycogen hosphorlase	glucose-6 phospha-tase (G-6-Pase)	Succinate Dehydrogenase (S(SDH)	Glycogen content (mg/g tissue)	Glucose level (mg/ml)
Non-infected snails	9.4±0.24	0.82±0.44**	0.71±0.8	42.2±3.3	36.4±1.6
Infected snails	8.2±0.11** 12. 77%	0.74±0.34* 9.76%	0.59±0.13 16.9%*	36.2±2.1** 14.12%	32.1±3.1** 11.81%
Non- infected snails exposed to LC_{10} of Ginger	5.8±0.56 38.3%	0.61±0.16 25.61%	0.48±0.44 32.39%	31±0.42 26.54%	27.2±0.33 25.27%
Infected snails exposed to LC_{10} of Ginger	4.6±0.82 51.1%	0.48±0.22 41.46%	0.40±0.72 43.66	26.2±0.33 37.91%	21.4±0.34 41.21%

*P < 0.05&** P< 0.01 .

Disappearances of two bands are 106 and 69 Kda in uninfected snails exposed to ginger than uninfected snails. Also, Disappearance of three bands is 116, 69 and 43 Kda in infected snails exposed to ginger than infected snails

4. DISCUSSION

The present study showed that ginger have a considerable killing effect against *B. alexandrina* snails with LC_{50} & LC_{90} 1.25 and 3.15 ppm, respectively Similar results were obtained by Bakry et al. (25) using Atrazine and Roundup, Bakry et al. (12) using Deltamethrin and Malathion pesticide, Hasheesh and Mohamed (26) using Chlorpyrifos and Profenophos pesticides and El-Fiki and Mohamed (27) using the herbicides Gramaxone, Preforan and Treflan. In the present study the survival rate of infected *B. alexandrina* had decreased compared to the control snails. This was in accordance with the finding of El- Saadany (28) and El-Sayed et al.
(29), who mentioned that the mortality of *B. truncatus* snails was higher after infection than that in the control snails. This may indicate that the parasites compete with the host for the essential haemolymph-born nutrients as supposed by Becker (30). During the patent period, when the snails showed shedding of Cercarae, the mortality rates had increased probably due to production of an inhibitory compound in the infected snails which may reduce the survival of snails.

The present results showed that there is a rapid decrease in survival rates of snails infected as well as uninfected exposed to LC_{10} of ginger which are significantly lower than that of control group. This finding is supported by Rawi et al. (31) using *Calendula micrantha* and *Ammi majus* plant extracts, Tantawy et al.(32) using *Solanium lubium* extract , Bakry et al. (33) using *Agave franzosini* extract and Bakry et al (34) using plant extracts of *Calotropis procera, Euphorbia nubia*, and *Atriplex halimus*, Bakry et al.(25) using Atrazine and Roundup, They found that there was also a marked reduction in the survival rate of *B. alexandrina* snails treated with sublethal concentrations of molluscicides. The present studies of egg production in *S. mansoni* infected *B. alexandrina* confirm those of Thornhill et al (35); El- Saadany (28) and El- Sayed et al .(29) in the combination *B. glabrata-S.mansoni* and *B. alexandrina S.mansoni*, respectively

Agave franzosini extracts on *B. alexandrina.* Hasheesh and Mohamed, (26) found that the egg production of *B. truncatus* treated with LC_{25} of Chlorpyrifos and Profenophos pesticides was significantly reduced compared to that of the control group. Ibrahim et al. (39) stated that low concentrations of the organophosphorus pesticide Chlorpyrifos (Dursban) suppressed the egg production of *B. alexandrina* snails. Also, Abdel Kader et al. (40) studied the interruption of several synthetic and natural molluscicides on egg production, egg abnormality and egg masses of *B. alexandrina*. They found that the long term exposure to low concentration of different molluscicding agents markedly induced inhibition in egg production and increased abnormal eggs and egg masses rates.

Table (5): SDS-PAGE of soluble proteins in tissue of uninfected and infected *Biomphalaria alexandrina* with *Schistosoma mansoni* exposed to Infected snails exposed to LC_{10} of *Zingiber officinal* (Ginger)

Fraction	Marker KDa	Non-infected snails	Non- infected snails exposed to LC_{10} of Ginger	Infected snails	Infected snails exposed to LC_{10} of Ginger
1	205				
2				198.5	198.5
3		171	171	171	171
4	116	116	116	116	
5		106		106	106
6		101	101	101	101
7				93.26	93.26
8		91.5	81.5		
9				79.5	79.5
10	79	79	79		
11				78	78
12		76	76		
13				74	74
14		69		69	
15	66				
16				62	62
17		60	60		
18		54	54	54	54
19		43	43	43	
20		36	36	36	36
21	29				
Number of bands		12	10	14	11

The infected snails produced a significantly lower number of egg duction of sexually mature *B. glabrata* infected with *S.mansoni* decreased with decreased of the albumin gland reserves and ceased upon the depletion of protein and galactogen acting as limiting factors in egg production (36). Also, McClelland and Boums (37) and Meier and Meier-Brook (38) stated that by 4-6 weeks post-infection, schistosomes inhibited the host egg production either partially or totally.

The results indicate that non- infected and infected snails exposed to LC_{10} of ginger hindered the egg laying capacity. Few eggs were laid and complete stopped egg laying was observed from 3th and 4th week of experiment respectively. This may be due to the active substance of ginger which could affect the internal mechanism inside the snails to lay eggs. Similar results were obtained by Tantawy et al. (32) and Bakry et al. (33), respectively, using *Agave franzosini* extracts on *B. alexandrina* snail.

Similar results were obtained by Bakry et al.(12) using Deltamethrin and Malathion with *H. duryi*, Tantawy et al. (32) and Bakry et al. (33) , respectively, using *Salanum lubium* and

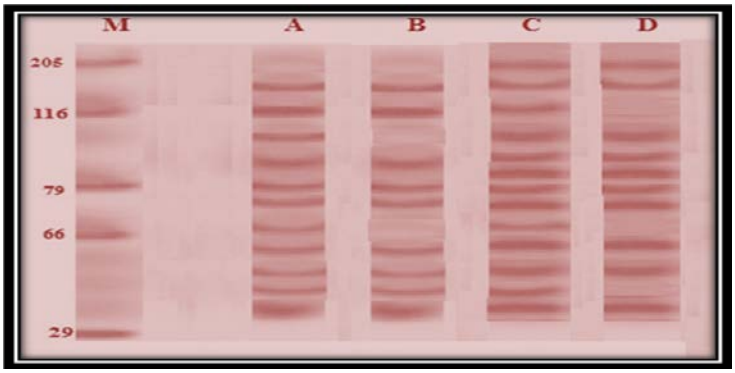

Fig (1): SDS-PAGE of soluble proteins in tissue of *Biomphalaria alexandrina* snails A= uninfected snails, B= uninfected snails exposed to Infected snails exposed to LC_{10} of Zingiber officinal (Ginger) C= infected snais with *Schistosoma mansoni* D= infected snails exposed to Infected snails exposed to LC_{10} of Zingiber officinal(Ginger) and M= Molecular Weight KD Lane (1)

The infectivity of *S. mansoni* miracidia to *B. alexandrina* was greatly reduced by LC_{10} of ginger. This was supported by the interruptions in biochemical parameters, as well, the activities of enzymes of treated snails that render their physiological processes unsuitable for the parasite development and reduce cercarial production (12&50). Comparable results were obtained in literature (41&42) using the plants, *Zygophyllum simplex, O. reticulum, F. selloea* and *A. celsii.*

However, there was no significant difference between the prepatent period of the snails exposed to LC_{10} of ginger and the control. Despite that, a highly significant reduction in the duration of cercarial shedding and total cercarial production per infected snails were detected. These phenomena were stated by many authors using different plant species as molluscicidal agents. Gawish (43) found that the period of cercarial shedding in snails treated with the experimental molluscicides during their exposure to miracidia is significantly shorter than that for control snails. This reduction in cercarial shedding period is probably due to rupture of snails' tissues through miracidial penetration in the presence of those molluscicides which increased the harmful effects of this plant Thus Badawy (44) Gawish (45) and Bakry (46) found that the plants *V. tinus, S. jambos, E. splendens* and *A. stylosa* have a remarkable decrease in the duration of cercarial shedding and cercarial production/snail from *B. alexandrina* infected with *S. mansoni* miracidia. The authors attributed this, probably, to the disturbances in the activities of snails' enzyme system, and the total protein concentration in their hemolymph that negatively affects the developmental stages of the parasite within their tissues. These observations are in accordance with many authors using different plant species as molluscicides. Thus, El-Ansary et al. (47) reported that a remarkable decrease in cercarial shedding in *Biomphalaria* snails treated with plant powder. Sharaf El-Din et al. (48) obtained similar reduction in cercarial production from *B. alexandrina.*

In the present study, *S.mansoni* infection led to a significant depletion of glycogen content in the tissue of exposed snails. The decrease of glycogen content in the tissues of snails was accompanied by a marked increase in haemolymph glucose concentration post *S. mansoni* miracidial exposure. This reduction may be attributed to uptake and utilization by the parasite (49).

These finding are in accordance with Bakry et al. (33) and Bakry and Abou El- Elanin (50) 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. (51) attributed the degradation of glycogen in *B. alexandrina* infected with *S. mansoni* mainly to dependence on the anaerobic glycolysis because the parasite destroys LD11 a lactate dehydrogenase isoenzyme associated with the aerobic respiration and stimulates LD5 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.

In the present study *S. mansoni* infection led to a significant reduction in glucose concentration in haemolymph. These finding are in accordance with Sharaf El-Din and El-Sayed (49) who found that exposure of *B. alexandrina* snails to *S. mansoni*, *Echinostoma liei* and to both parasites miracidia simultaneously led to a significant reduction in glucose concentration in haemolymph after two and three weeks of miracidial exposure.The primary source of nutrition for the sporocysts and developing cercariae is glucose, which is absorbed from the haemolymph. El-Ansary et al (47). Also, Etges et al. (52) found that the decrease in hemolymph glucose resulted by daughter sporocysts which migrate to the hepatopancreas 13 to 16 days post-infection.

Table 5 Dice's similarity coefficient (*S) of the protein profile bands between unifected and infected snails with *Schistosoma mansoni* exposed to *Zingiber officinal* (Ginger).

	Non-infected snails	Non- infected snails exposed to LC_{10} of Ginger	Infected snails	Infected snails exposed to LC_{10} of Ginger
Non-infected snails	1	0.91	0.62	0.43
Non- infected snails exposed to LC_{10} of Ginger	0.91	1	0.5	o.38
Infected snails	0.61	0.5	1	0.88
Infected snails exposed to LC_{10} of Giger	0.43	0.38	0.88	1

S = 2 a / 2 a + b + c, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.

Regarding the sources of energy for snails, LC_{10} of the ginger significantly decreased the glycogen content in soft tissues of non- infected and infected snails, while the glucose level in hemolymph increased. This may be attributed to the activity of the ginger that impedes oxygen consumption of snails, thus inducing anaerobic respiration.Under hypoxic conditions; animals derive their energy from anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis [Vincent et al., (53) and Sambasiva, (54). Nakano and Tomlinson (55) have suggested that catecholamine levels rise under stressful environmental conditions, enabling the increased utilization of glycogen for energy production. To restore its energy requirements, the snail has to increase the rate of glycolysis thus bringing about a reduction of the glycogen content and increase glucose level in the hemolymph (56&57).

In the present study, the levels of glycogen phosphorylase and glucose-6-phosphatase (G-6-Pase) in the soft tissues of normal and snails infected with *S. mansoni* were also significantly reduced in response to infection. This reduction is increased in the uninfected and infected snails exposed to ginger. With respect to G-6-Pase as glycogenolytic enzyme, it showed a reduced activity in treated snails which was attributed to either synthesis and/or degradation of glycogen (58). Increasing the glucose concentration stimulated glycogen synthesis and decreased the activity of glycogen phosphorylase. Glucose wreporated into glycogen during period of net glycogen breakdown, and vice versa; glycogen degradation occurred during periods of net glycogen as inco synthesis which depends on glucose concentration (59).

Also, in the present study, SDH activitiy in the in the soft tissues of normal and snails infected with *S. mansoni* significantly reduced in response to infection. This reduction is increased in the

uninfected and infected snails exposed to ginger. In the present study, SDH presented the lowest activity after exposure to ginger. This could be due to swollen, disrupted mitochondrial membranes and fewer crystals with subsequent leakage of enzymes as reported by Aboul-Zahab and El-Ansary (60) SDH is an important active regulatory enzyme of the tricarboxylic acid cycle (TCA), the common pathway for carbohydrates (61).

The present results indicated that infection of snails with *S. mansoni*, uninfected and infected exposed to ginger had qualitative and quantitative effect on the protein patterns of the studied snails. The shared bands of 171, 101, 54, 43 and 36 Kda seemed not to be affected by infection of snails or exposed to ginger. The electrophoretic pattern of the native proteins revealed difference in the number and molecular weights of protein bands compared to the control snails. These differences indicated that infection of snails with *S. mansoni* and exposed to ginger caused intensive effects which induced fractionation of the native protein. A protein change was previously detected by Rawi et al. (62), Aly et al., (63) and El- Sayed et al. (64).

Disappearances of two bands are 106 and 69 Kda in uninfected snails exposed to ginger than uninfected snails. Also, Disappearance of three bands is 116, 69 and 43 Kda in infected snails exposed to ginger than infected snails. This same report was mentioned with Bakry et al. (12), who found that the pesticides Malathion and Deltamethrin had qualitative and quantitative effect on the protein patterns of *B. alexandrina* snails. Furthermore, treated groups with the pesticides showed less number of protein bands indicating that the pesticides were thought to induce damage for these snails. It seemed appropriate to suggest that snails that gave similar type of protein bands to that of the control were able to resist molluscicidal effect. Protein changes due to snail treatment with plant extracts were previously detected by many authors (Rawi et al., (62), Aly et al., (63) and El sayed (64).

The present results (Table 6) indicated that the similarity index (S) was higher in case of unifected snails treated with ginger than infected snails treated with ginger (0.38 and 0.43,respectively) indicating that infected snails treated with Ginger had strong effect on protein pro-file of soft tissues of snails.

References

[1] World Health Organization, World health report on Schistosomiasis. Wikipedia, the Free Encyclopedia.mht, (2010)

[2] Y.G. Yapi, O.G. Briet, S. Diabate, P.E. Vounatsou, E. Akodo, M.T. Tanner, T. Teuscher, Rice irrigation and schistosomiasis in Savannah and forest areas of Cote d'Ivoire. Acta Trop., 93: 201-211, 2005.

[3] F. Sarkinfade, A. Akande, I. Abubakar, Z. Ilyasu, Urinary schistosomiasis in the Danjarima community in Kano, Nigeria. J. Infect. Dis. Contr., 3:130-135, 2009.

[4] K. El-Sayed,; S. El-Dafrawy, A. Sharaf El-Din, Influence of Schistosoma mansoni infection on *Biomphalaria alexandrina* snails under laboratory conditions. J. Zool. Egypt., 33: 343-354, 1999

[5] C. Goto, S. Kasuya, K. Koga, H. Ohtomo, N. Kagei, Lethal efficacy of extract from *Zingiber officinale* (traditional Chinese medicine) or [6]-shogaol and [6] gingerol in Anisakis larvae in vitro. Parasitol. Res.76:653–656, 1990.

[6] Z. Iqbal, M. Lateef, M. S. Akhtar, M. N. Ghayur, A.H. Gilani, In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. J. Ethnopharmacol. 106:285–287, 2006.

[7] C. O. Adewunmi, B. O. Oguntimein, P. Furu, Molluscicidal and antischistosomal activities of *Zingiber officinale*. Planta Med. 56: 374–37, 1990

[8] B.White, Ginger ;an overview .Am. Fam. Physician . 75(11): 1689-1691,2007.

[9] B. H. Ali, G. Blunden, M. O. Tanira, A. Nemmar, Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. Food Chem. Toxicol. 46 :409–420,2008.

[10] N. Lopes, P. Chicaro, M.J. Kato, S .Albuquerque, M .Yoshida, Flavonoids and lignans from Virola surinamensis twigs and their in vitro activity against Trypanosoma cruzi. Planta Med. 64: 667–668, 1998

[11] I. Hierro, A. Valero, P. Perez, P. Gonzalez, M.M. Cabo , M.P. Montilla, M.C. Navarro, Action of different monoterpenic compounds against Anisakis simplex s.l,L3 larvae. Phytomedicine 11, 77–82, 2004.

[12] F.A. Bakry, W.S. Hasheesh, S.A.H. Hamdi, Biological, biochemical, and molecular parameters of *Helisoma duryi* snails exposed to the pesticides malathion and deltamethrin, Pest. Biochem. Physiol. 101: 86–92.2011

[13] D. Rollinson, A. J. G. Simpon, The Biology of Schistosomiasis. Parasitic Infections.,3rd edn, ppts: 387 – 437, 1987.

[14] M.L.B. Ahui,P. Champy,A. Ramadan,L. Pham Van,, L Araujo, S. André K, Diem, D. Damotte, S. Kati-Coulibaly,M.A. Michel Atté Offoumou M. Dy, N Thieblemont, A Herbelin, Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. Int. Immunopharmacol. 8 :1626–1632, 2008.

[15] E. H. Michelson, Specificity of haemolymph antigens in taxonomic discrimination of medically important snails. J. Parasitol., 52: 466-472, 1966.

[16] N. V.Carroll, R. W. Longley, and J. H. Roe, The determination of glycogen in the liver and muscles by use of anthrone reagent. J. Biochem. 220: 583-593, 1956.

[17] P. Trinder, (Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem, 6: 24-27, 1969.

[18] H. Hedrick, and E.H.Fischer, On the role of pyridoxal 5- phosphate in phosphorylase, absence of classical vitamin B6 dependent enzymatic activities in muscle glycogen phoshorylase. Biochem.4,1337, 1965.

[19] M. A. Swanson, Glucose-6- phosphatase from liver .In: Methods in Enzymology Vol.2:pp 541-543, 1955.

[20] E. Shelton, and M.E.RiceComparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates .J.Nat.cancer Inst.18,117, 1957.

[21] L., P.M., Marchat, C. Loiseau, Pous and F. Petek, Isoenzymatic diagnosis of filariae: a method for separation of lactate dehydrogenase isoenzymes from Molinema dessetae (Nematoda: Filarioidea). Comp. Journal of Physiology and Biochemistry, 109 (2/3): 451-457, 1994.

[22] U.K., Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685, 1970.

[23] L .R. Dice, Measures of the amount of ecological association between species. Ecol., 26: 297-302, 1945.

[24] R. M. Spiegel, Theory and problems of Statistics, Schaum's outline series. McGraw-Hill, Singapore, 1981.

[25] FA. Bakry, H. A. Abdelsalam b, M. B. Mahmoud a, S. A.H. Hamdi, Influence of Atrazine and Roundup pesticides on biochemical and molecular aspects of *Biomphalaria alexandrina* snails. Pest. Biochem. Physiol. 104 9–18, 2012.

[26] W.S. Hasheesh, R.T. Mohamed, Bioassay of two pesticides on Bulinus truncates snails with emphasis on some biological and histological parameters, Pest. Biochem. Physiol. 100 1–6, 2011.

[27] S.A. El-Fiki, A.M. Mohamed, Effect of some herbicides on the toxicity of certain molluscicides against *Biomphalaria alexandrina* snails, Egypt. J. Bilharz. 5 91–100 1978.

[28] M. M. El-Saadany, Effects of *Schistosoma mansoni* infection on survival and fecundity of the fresh water snail *Biomphalaria alexandrina*,

with special reference to *Schistosoma mansoni*. Proc. Zool. Soc. A. R. Egypt., 27: 171-184, 1996.

[29] K. El-Sayed, S. El-Dafrawy, and A.Sharaf El-Din, Influence of *Schistosoma mansoni* infection on *Biomphalaria alexandrina* snails under laboratory conditions. J. Zool. Egypt., 33: 343-354, 1999.

[30] W.Becker, Microcatorimetric in *Biomphalaria glabrata*, the influence of *Schistosoma mansoni* on the basal metabolism. J. Comp. physiol., 135(B): 101-105, 1980.

[31], S. M. Rawi; H. El-Gindy, A. M. Haggag, A.Abou El Hassan, and A. Abdel Kader, Few possible molluscicides from *calendula Micrantha officinalis* and *Ammi majus* plants. I. Physiological effect on B. alexandrina and B. 1154runcates. J. Egypt. Ger. Soc. Zool. 16(D): 69-75, 1995.

[32] A. Tantawy, A. T. Sharaf El-Din, and F. A. Bakry, Laboratory evaluation of the molluscicding activity of *Solanum lubium* (Sclanaceae) against *Biomphalaria alexandrina* snails. J. Inter. Cong. Biolog. Sci. (ICBS) 1(2): 307-318, 2000.

[33] F. A. Bakry, A. A. Tantawy, F. M. A. Ragab, and A. Abdel Kader, Effect of sublethal concentrations of *Agave franzosini* plant (Agavaceae) on the infectivity *of Schistosoma man*soni to *Biomphalaria alexandrina* snails. J. Egypt. Ger. Soc. Zool. 15: 129-141, 2001.

[34] F.A.Bakry, A. M. Sakran, B.B. Mostafa, The effect of *Schistosoma haematobium* infection on Biological and physiological parameters of *Bulinus truncatus* snails. J. Inter. Cong. Biolog. Sci. (ICBS) 2(2): 835-846, 2002.

[35] J. A. Thornhill, J. T. Jones, and J. R. Kusel, Increased oviposition and growth in immature *Biomphalaria glabrata* after exposure to *Schistosoma mansoni*. Parasitology, 93: 443-450, 1986.

[36] D. L. Looker, and F. J. Etges, Effect of *Schistosoma mansoni* infection on fecundity and perivitelline fluid composition in *Biomphalaria glabrata.* J. Parasitol., 65: 880-885, (1979.

[37] G. McClelland, and T. K Bourns, Effects of tricholbilharzia ocellata on growth, reproduction and survival of Lymnaea stagnalis. Exp. Parasitol., 24: 137-146, 1969.

[38] M. Meier, and C. Meier-Brook *Schistosoma mansoni*: Effect on growth, fertility and development of distal male organs in *Biomphalaria glabrata* exposed to miracidia at different ages. Z. Parasitenkid., 6: 121-131, 1981.

[39] W.L. Ibrahim, P. Furu, A.M. Ibrahim, N.O. Christensen, Effect of the organophosphorous insecticide, chlorpyrifos (Dursban), on growth, fecundity and mortality of *Biomphalaria alexandrina* and on the production of *Schistosoma mansoni* cercariae in the snail, J. Helminthol. 66 : 79–88, 1992.

[40] A. Abdel Kader, S. A. H. Hamdi, and S. M. Rawi, Biological and biochemical studies on *Biomphalaria alexandrina* snails treated with low concentrations of certain molluscicides (synthetic and of plant origin). J. Egypt. Soc. Parasitol., 35: 841-858, 2005.

[41] F.A. Barky Effect of methanol extracts of *Furcraea gigantean* and *Lampranthus spectabilis* plants on *Biomphalaria alexandrina* infection by *Schistosoma mansoni* and on energy metabolism indicator, Egypt. J. Schist. Infect. Endemic Dis. 28 1–14, 2006.

[42] F.A. Bakry, S.A.H. Hamdi, Effect of different plant molluscicides on bioiogical and biochemical of *Biomphalaria alexandrina* snails, Egypt. J. Exp. Biol. 2 99–106, 2006.

[43] F. M. Gawish, Evaluation of combination of certain molluscicide against *Biomphalaria alexandrina* and the free-living stages of *Schistosoma mansoni*. Ph.D. Thesis, Zool. Dept., Fac. Wom Arts, Sci. and Educ., Ain Shams Univ., Egypt, 1997.

[44] A.M.S. Badawy, Studies on *Dracaena draco* (Agavaceae) and *Viburnum tinus* (Caprifoliaceae) as Plant Molluscicides Against the Snail Vectors of Schistosomiasis and the Larval Stages of this Parasite, Ph.D. Thesis, Zoology Dept. Girls College for Arts, Science and Education, Ain Shams University,Cairo, Egypt, 2007.

[45] F.A. Gawish, Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails' reproduction and infection with *Schistosoma mansoni,* N. Egypt. J. Med. 39: 103–110, 2008

[46] F.A. Bakry, Impact of Some Plant Extracts on Histological Struture and Protein Patterns of *Biomphalaria alexandrina* Snails.Globul J. Molecul. Sci. 4(1): 34-41, 2009.

[47] A. El-Ansary, E. M. Sammour, and A. M. Mohamed, Susceptibility of *Biomphalaria alexandrina* to infection with *Schistosoma mansoni* correlation with the activity levels of certain glycolytic enzymes. Egypt. J. Parasitol., 30(2): 125-136, 2000.

[48] A.T. Sharaf El-Din, F.A. Bakry, A. Tantawy, Molluscicidal activity of *Zygophyllum simplex* (Family;Zygophyllaceae) against *Biomphalaria alexa*ndrina and *Bulinus truncatus*.Egypt. J.Aqual.Biol&Fish.Vol.4:131-143, 2001.

[49] A. T. Sharaf El-Din, and K. A. El-Sayed, Alteration in glucose, glycogen and lipid contents in *Biomphalaria alexandrina* 1154runca post-exposure *to Schistosoma mansoni* and *Echinostoma Liei* miracidia. J. Egypt. Ger. Soc. Zool., 23(D): 103-113, 2001.

[50] F.A Bakry and H.M, Abu-El-Einin, Estimation of free amino acids and protein in *Bulinus truncatus* snails infected with *Schistosoma haematobium* or *Echinoparyphium recurvatum*. 8th international conference on system biology. At October 1-6, 2007 Long Beach, California, USA.77-78, 2007.

[51] L. Nabih, R. Fattag, M. Rizk, and A. El-Ansary, Effect of magnesium and urea on glucose and glycogen content in schistosomiasis intermediate host. Pakistan. J. Biochem., 26(1-2): 9-14, 1993.

[52] F. J. Etges, O.S. Carter, and B. Webber, Behavioural and developmental physiology of *Schistosoma* larvae as related to the molluscan host. Ann. N. Y. Acad. Soc., 266: 430-496, 1975.

[53] S.Vincent, T. Ambrose, L. Cyrill, M. Selvanaygam, Biochemical responses of the Indian major carp, Catla catla (Ham.) to chromium toxicity, Indian J. Environ.Health 37 (1995) 192–196, 1995.

[54] R.K.R.S. Sambasiva, Pesticide Impact on Fish Metabolism, Discovery Publishing House, New Delhi, pp. 129–149, 1999.

[55] T. Nakano, N.Tomlinson, Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance, J. Fish Res. Board Can. 24: 1707–1715, 1967.

[56] P. Baskaran, S. Palanichamy, Impact of agricultural (ammonium chloride) fertilizer on physiology and biochemistry of fresh water teleost fish, *Oreochromis mossambicus*, J. Ecobiol. 2 : 97–106, 1990.

[57] R. Vasanthi, P. Baskaran, S. Palanichamy, Influence of carbofuran on growth and protein conversion efficiency in some fresh water fishes, J. Ecobiol. 2 (1990) 85–88, 1990.

[58] A.I. Michael, R.A. Wahab, N.M. Guriguis, I.M. El-Gazayerli, S.Hamza, Effect of *Schistosoma mansoni* infection in mice on hydrolytic enzyme activity of the liver, Egypt. J. Physiol. 6 : 37, 1979.

[59] A.G. Tielens, J.M. Van den Heuvel, J.H. Van Mazijk, E.J. Wilson, B.C.J. Shoemaker, Substrate cycling between glucose 6-phosphate and glycogen occurs in *Schistosoma mansoni*, Mol. Biochem. Parasitol. 39 : 109, 1990.

[60] S. M. Rawi, H. El-Gindy, & A.Abdel Kader, New possible molluscicides from calendula Micrantha officinalis and Ammi majus.II. Molluscicidal, physiological and egg laying effects against Biomphalaria alexandrina and Bulinus truncates. J. Eco. Toxical. Environmental Safety 35(3),261, 1996.

[61] M.Gonzalez, M.C. Tejedor, Variations of glycolytic kinases and pentosephosphate pathway dehydrogenase in response to lead accumulation in hemopoietic cells of rock doves (Colomba livia), Bull. Environ. Contam. Toxicol. 50: 779–786, 1993.

[62] S.M., Rawi, H.I. El-Gindy and A., Abd-Kader, The effect of some molluscicides on total protein, albumin, globulin and total lipid of the snail *Biomphalaria alexandrina*. *Bulletin* of the *Faculty of Science Cairo University,* 61:87-101**,** 1993.

[63] S.A. Aly, H.F. Aly, N. Saba-El-Rigal and R. E. M., Sammou, Induced changes in biochemical parameters of the molluscan tissues non-infected using two potent plants molluscicides. Journal of Egyptian Society of Parasitology, 34(2):527-542. 2004.

[64] K.A., El- Sayed, Effect of the plant *Cupressus macro-carpa* (Cupressacea) on some haematological and Biochemical parameters of *Biomphalaria alexandrina* snails. Journal of Egyptian Society of Parasitology, 36(3):911-924, 2006.