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Effect of *Zingiber officinal* (ginger) on parasitological and biochemical parameters of mice infected with *Schistosoma mansoni* cercariae

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Abstract— The present study was undertaken to evaluate the antischistosomal properties of ginger (*Zingiber officinal*) against *Schistosoma mansoni* in infected mice, including determination of total protein , albumin levels, the activities of ALT, AST, ACP and AKP enzymes and the electrophoretic pattern of total protein in the serum of infected treated mice. The present results showed that treatment of infected mice with sublethal doses of ginger' extract significantly reduced the number of *S. mansoni* worms recovered from infected mice. Also, the data presented showed that mice treatment with this ginger 'extract reduced the number of ova/g tissue in each of intestine of infected mice in comparison with that of infected untreated group. Moreover, serum total protein and albumin levels and activities of ALT, AST, ACP and AKP enzymes of infected treated mice were improved in comparison with those of infected untreated ones. It is concluded that administration of ginger'extract extract could be valuable as antischistosomal agent.. Electrophoretic analysis serum's total protein showed there was a remarkable deviation in the serum homogenate of mice infected with *S. mansoni* and treated with ginger'extract in comparison with control non infected group.

Keywords: *Zingiber officinal* (ginger), *Schistosoma mansoni* cercariae, Male Swiss albino mice (Mus musculus

1 Introduction

Schistosomiasis is a public health problem in many developibg countries. An estimated 80% of all infected people are now concentrated in Africa (1,2). 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 (3,4). Chemotherapy of schistosomiasis is still one of the most effective methods for controlling this parasite (5). Some medically important plant species play a significant role in treatment of schistosomiasis (6,7,8,9 & 10). The medicinal plants have been used virtually in all cultures as a source of medicine and a natural basis for the maintenance of a good health, e.g., *Zingiber officinale*, *Nigella sativa* and *Asparagus officinalis* (11, 12).

Several studies had examined the influence of parasites on the host organisms, the mechanisms of host location and the molluskcs resistance to the parasites, that is incompatibility of the host (13). In case of the larvae of *S. mansoni* obtained their energy and growth substrates from the host, and released intermediate product of their metabolism into host's body. Organic acids are important component of parasite metabolism and participate in both catabolic (glycolysis) and anabolic (gluconeogenesis) pathways. Pyruvate and lactate are indicators of glycolytic processes under aerobic conditions, while fumarate, succinate and malate are indicators of the tricarboxylic acid cycle. The presence of ketone bodies, such as β-hydroxybutrate and acetoacetate, as well as fatty acids, such as acetate and propionate, are indicative for lipid metabolism (13).

Organic acids play a central role in the parasite of *S. mansoni* metabolism, as they serve as indicators of various metabolic reactions representing important components of energy and parasites metabolism. Thus, they may indicate the use of carbohydrates as an energy source in the flow of aerobic and anaerobic transition, the replacement of glucose through gluconeogenesis, and of protein via glucogenic amino acids or metabolism of lipids on a smaller scale via fatty acids and ketone bodies (14).

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. 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 (15). Acute schistosomiasis has a significant impact on specific liver functions and the alterations in specific protein isoforms and upregulation of unique proteins may be valuable as new markers of disease (16).

The drug of choice for schistosomiasis treatment is Praziquental (PZQ) (17). However, the possible emergence problems of drug tolerance or appearance of new resistant strains to PZQ (18). Especially with inevitable reinfection and retreatment makes the search for new antischistosomal drugs an essential target.

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Ginger (*Zingiber officinal* (ginger)) is widely used in traditional Chinese medicine (19). The medicines are purported to be effective treatment for inflammation, oxidant stress, helminthiasis and schistosomiasis (20, 21). It has also antischistosomal effect against S. mansoni miracidia and cercariae (22). 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 (23, 24). Another study has suggested that ginger free radical scavenging activity may reduce larvae survival (25, 26).

Therefore, the present study was suggested to evaluate the antischistosomal properties of Ginger (*Zingiber officinal*) against *Schistosoma mansoni* in albino mice.

2 MATERIAL AND METHOD

2.1 Cercariae

Schistosoma mansoni cercariae were from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt

2.2 Experimental animals

Male Swiss albino mice (Mus musculus), 20-25 g were from SBSC, TBRI. They were maintained on standard diet 24% protein content.

2.3 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 (27).

2.4 Toxicity of the ginger' extract to albino mice:

The toxic effect of the ginger' extract to albino mice (20-25g) was recorded post 24 hours of oral administration via intragastric tube in an oil-form (Table 1 hree replicates, each of 6 mice, were used for each dose. Another 3 replicates were maintained without dosing as control. The lethal dose (LD100) was counted (28).

2.5 Mice infection

About 80 cercariae/mouse were injected subcutaneously into the abdomen using a syringe and a needle (29, 30).

2.5.1 Treatment of infected mice

Seven weeks post infection (PI), mice were orally administered the doses of the the ginger' extract for 2 successive days. The selected doses from the ginger' extract were 50, 75 and 100 mg/kg. Each dose was dissolved in sunflower oil as a vehicle. Three replicates, each of 6 mice, were used for each dose. The control replicates were 3 infected untreated and 3 uninfected untreated, both received only the vehicle.

2.5.2 Perfusion of infected mice

Two weeks post mice treatment; they were euthanized by decapitation and perfusion techniquedescribed by Smithers and Terry [31].. The mean number of worms/mouse was determined in each experiment (31, 32).

2.5.3 Egg developmental stages (Oogram)

The percentages of immature, mature and dead eggs from the liver and small intestinal wall of infected mice were computed from a total of hundred eggs per intestinal segment. Three segments per animal were examined. (33, 34).

2.5.4 Tissue egg load:

The number of eggs per gram tissue (liver and intestine) of infected mice was determined (35).

2.6 Biochemical parameters in serum of infected mice

2.6.1. Preparation of serum of mice

2-3 ml blood sample was taken immediately after scarification of mice in centrifuge tubes. The blood samples were centrifuged at 1500 Rpm for 10 min at + 4C°.The obtained serum was used for determination of functions enzyme and analysis of proteins (electrophoresis) (36).

2.6.2 Biochemical studies

The serum of sacrificed mice was collected for spectrophotometrically evaluation of total protein (37), albumin (38), and the activities of transaminases (AsT &AlT) (39), and phosphatases (ACP (40) and AkP (41)) enzymes. All physiological parameters determined in this study were determined spectrophometrically, using reagent kits purchased from BioMerieux Company, France.

2.6. 3 Electrophoretic analysis

The protein profiles were analyzed by SDS-PAGE electrophoresis for serum homogenate of mice infected with *S. mansoni* according to the procedure of Boswell et al. (42). Electrophoresis on SDS-PAGE a lab gel was fixed in 505 ml methanol hydrated in distilled water and stained with Commassie for 15 min. The gel was washed with distilled water and soaked in the developer until bands appeared. High and low molecular weight standards (marker 116= beta gluctosidase, marker 97.4 =phosphorylase B, marker 66.2= bovine serum albumin, marker 37.6 = carbonic anhydrase and marker 28.2= triose phosphate isomerase) were electrophoresed on the same gel to calculate the relative molecular weights of the examined antigens. The gel was dried at room temperature, photographed using Kodak Tri-X-pan films and the molecular weights and protein intensity were analyzed by using Gel Docu Advanced software program.

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 (43), 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.

2.7 Statistical analysis

The data are presented as mean ± standard deviation. The mean groups were compared by analysis of variance. Comparison of means was done by 2-tailed unpaired t-test (44). SPSS computer program

3 Results

It is found that LD100 of the ginger'extract to mice after 24 hours of oral administration was 3000 mg/kg. Therefore, sublethal doses of the ginger'extract were administered to infected mice groups to evaluate their antischistosomal properties.

In the present study (Table1) treatment of infected mice with sublethal doses of ginger'extract significantly reduced the number of *S. mansoni* worms recovered from infected mice by 50.48%, 74.29 and 82.86 % for groups treated with 50,75 and 100 mg/kg, respectively (P<0.01).
The data presented in table 2 showed that mice treatment with this ginger'extract reduced the number of ova/g tissue in each of intestine of infected mice in comparison with that of infected untreated group. So, the reduction rates in the dose 50, 75 and 100 mg/kg were 29.3%, 51.73% and 60.17 %, respectively (P<0.001). Also, The reduction in the number of ova/g tissue in liver of infected mice was 29.62, 40.17% and 50.1, respectively. in the dose 50, 75 and 100 mg/kg
The current results in table 3 showed that infection of mice with *S. mansoni* reduced the serum total protein (42.6%) and albumin levels(30.77%), These data declared that treatment of infected mice with 50, 75 and 100 mg/kg of the ginger'extract decreased the concentrations of serum total protein and albumin in comparison with those of infected control ones. Thus, the serum total protein concentrations in mice treated with 50, 75 and 100 mg/kg of the ginger'extract were 56.38%, 65.96%and 77.66% g/dl, respectively, compared to of control group (P<0.001), while albumin concentrations in these mice were 57.7%,71.15% and 78.85%,respectively.

The current results in table 4 showed that infection of mice with *S. mansoni* increased the activities of the serum enzymes. AlT, AST, AkP and ACP enzymes compared to those of uninfected control group. The percentage of increased in the activities of AlT, AST, AkP and ACP enzymes was 49.43%,-130%,-59.84% and 68.65%. On the other hand, these doses (50, 70 and 100 mg/kg) decreased the activities of the serum enzymes, AlT, AST, AkP and ACP compared to those of infected control group.

The reduction of activity of AlT, AST, AkP and ACP enzymes for infected treated with the dose 100 mg/kg were were 46.37%,57.76%,40.81% and 39.74%,respectively. The same conclusion was recorded with treated with 50 and 70 mg/kg. Although the serum biochemical parameters of infected mice treated with the ginger'extract were ameliorated in comparison with those of infected untreated control group yet, they were still different from those of uninfected control mice.

Table (1):
Parasitological criteria after treatment of *Schistosoma mansoni* infected mice with ginger'extract: Worm burden, (Mean± SD).

Animal groups (doses)	Mean worm burden ± SD (liver and Porto-mesenteric)				% Total worm burden reduction
	Male	Female	Couples	Total	
Control infected	10.5+3.9	7.8+1.3	3.5+1.3	21+4.3	
50 mg/kg/2 days	5.2+1.2**	3.4+1.4 **	2.6+1.8	10.4+2.2*	50.48
75 mg/kg/2 days	2.2+0.6**	1.8+1.8 **	1.1+0.5	5.4+0.5**	74.29
100 mg/kg /2days	1.5+0.8**	1.1+0.5***	0.8+1.2	3.6+1.4**	82.86

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

Table (2)
Parasitological criteria after treatment of *Schistosoma mansoni* infected mice with ginger'extract: Tissue egg load and Eggdevelopmental stages, (Mean± SD).

Animal groups (doses)	Number of ova/g tissue		% Egg developmental stages ± SD		
	Intestine (Reduction %)	Liver (Reduction %)	Dead ova	Mature ova	Immature Ova
Control infected	4400+ 1123.2	1877+143	1+0.3	54+2.4	45+2.6
50 mg/kg /2 days	3111+ 3411.3* (29.3%)	1321+433* (29.62 %	7.3 + 1.4*	40.2+2.4*	51.2 +4.2*
75 mg/kg /2 days	2124+1543.2** (51.73%)	1123+322.4** (40.17%)	10 +1.1**	28+2.5**	62.5+1.6**
100 mg/kg /2days	1752.5+ 433.2*** (60.17%)	900+132.5*** (50.1%)	15+1.4***	15.1+2.4***	70.2+3.5***

*** P<0.001, ** P<0.01 and * P<0.05

Table (3)
Serum biochemical parameters (Total protein, Albumin) in mice treated with sublethal doses of ginger'extract post infection with *Schistosoma mansoni*.

Animal groups (doses)	Total protein g/dl		Albumin g/dl	
	Mean± SD	%Change	Mean± SD	% Change
Control uninfected	9.4 ± 1.2		5.2 ± 0.3	
Control infected	5.4+1.2	42.6%	3.6+0.8	30.77%
50 mg/kg /2 days	4.1+1.1	56.38%	2.2+0.7	57.7%
75 mg/kg /2 days	3.2+0.1	65.96%	1.5+0.4	71.15%
100 mg/kg/2 days	2.1+0. 5*	77.66%	1.1 +0.7*	78.85%

*** P<0.001, ** P<0.01 and * P<0.05

Table (4)
The activity of enzymes (AlT, AsT, AcP and AkP) in mice treated with sublethal doses of ginger'extract post infection with *Schistosoma mansoni*.

Animal groups (doses)	Alanine amino transferase(ALT)U/		Aspartate amino transferase (AST) U/L		Alkaline phosphatases (AKP)U/L		Acid phosphatases (ACP U/L)	
	Mean± SD	% Change	Mean± SD	% Change	Mean± SD	% Change	Mean± SD	% Change
Control uninfected	26.5 ± 4.2		21+2.1		55.6± 4.2		18.5± 1.6	
Control infected	52.4+2.1	49.43%-	48.3+2.7	-130%	88.87+23.3	-59.84%	31.2 ± 11.2	68.65%
50 mg/kg /2 days	44.3+2.4	15.46%-	36.4+1.8	24.64%	75.5+14.5	15%	25.4+ 2.8	18.59%
75 mg/kg /2 days	36.8+4.5	29.78%	27.3+2.3*	43.48%	62.21+ 8.1*	30%	22.1 + 4.1 *	29.16%
100 mg/kg/2 days	28.2+ 3.3	46.37%	20.4+2.1**	57.76%	52.6+ 18.6	40.81%	18.8+ 2.1 **	39.74%

*** P<0.001, ** P<0.01 and * P<0.05

Table (5)
Protein fractionation of the serum of mice treated with sublethal doses of ginger'extract post infection with *Schistosoma mansoni*. Molecular Weight (KD)

Lane 1 (Mol.w.) Marker	amount	Lane 2 (Mo .w.) Serum con	amount	Lane 3 (Mol.w.) Serum 1	amount	Lane4 (Mol.w.) Serum 2	amount
				126.02	6.5303		
		120.91	3.8434			120.91	4.7282
				118.89	5.5334		
116	19.947						
		114.06	3.8733			115.55	5.4394
				112.16	14.093	112.16	.97488
		108.59	7.3391				
				102.05	2.6037	102.31	2.0303
						98.797	.38643
97.40	12.365	97.40	1.9998				
				87.443	1.3419		
						84.747	1.7609
		83.868	2.6285				
						73.738	5.1742
				71.964	6.4926		
		70.233	5.3747				
66.20	19.818						
						50.334	18.084
				45.269	35.296		
		42.553	12.641				
37.60	37.026	37.60	17.433				
						36.188	13.954
		34.638	32.367	34.638	7.7603	34.638	11.931
		32.903	9.2470				
		31.563	3.0993	31.563	20.160	31.563	35.358
28.20	10.8 44						
	99.999		99.846		99.863		99.822
	100		100		100		100

The pattern of protein profile identified by SDS-PAGE electrophoresis for serum homogenate of mice infected with *S. mansoni* was shown in Fig. 1. Data in Table 5 as illustrated in Fig. (1) showed that the protein profiles of serum of normal mice (lane1) and serum of infected mice (lane 2) are composed of 11 protein bands. This profile was reduced to 9 bands in the serum of infected mice treated with 100 mg/kg (lane 3). The molecular weights of these bands for serum of normal mice ranged from 120.91 to 31.563 KDa. Those for infected mice ranged from 126.02 to 31.563KDa and serum of infected mice treated with 100 mg/kg ranged from 120.91 to 31.563 KDa.

The present data (Table 5 and Fig.1) showed the appearance of bands in infected groups and disappearance of others in comparison with control group. The disappearing 4 bands are 38.271, 78.777, 105.54 and 109.58KDa, while 5 bands appeared in serum infected mice 1and 2. .e.g. 45.269, 71.964, 87.443, 118.89 and 126.02KDa for serum infected mice1 and 36.188, 50.334, 73.738, 84.747 and 98.797for serum of infected mice treated with 100 mg/kg

Figure.1
Protein fractions of serum of mice treated with sublethal doses of ginger'extract post infection with *Schistosoma mansoni*. M= Marker, A = Control (serum of normal hamster), B= serum of infected mice 1 and F= serum of infected mice treated with 100 mg/kg of dinger

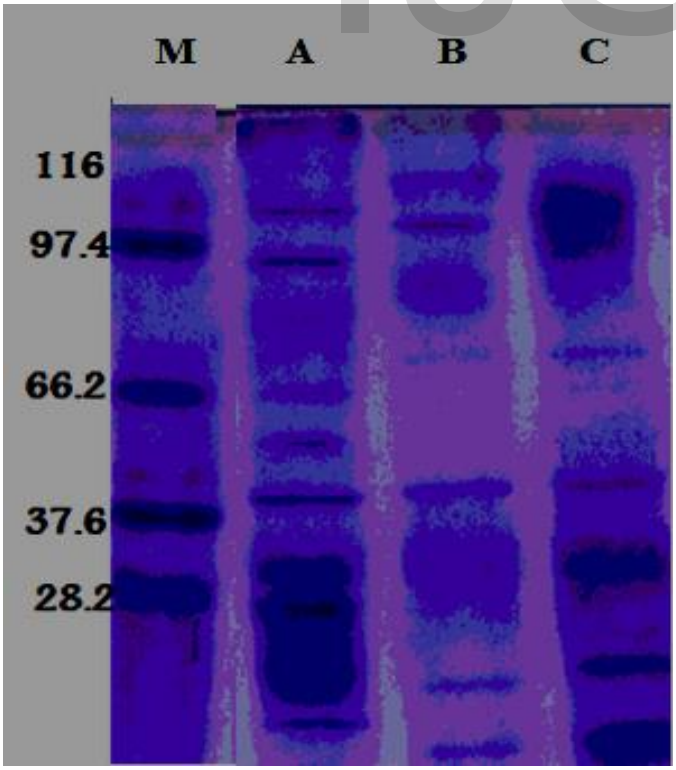

Table (6)
Dice's similarity coefficient (*S) of the protein profile bands between unifected, infected snails with *Schistosoma mansoni* and infected mice treated with *Zingiber officinal* (Ginger).

	Non-infected snails	infected snails	Infected snails exposed to LC_{10} of Ginger
Non-infected snails	**1**	**0.2**	**0.36**
infected snails	**0.2**	**1**	**o.40**
Infected snails exposed to LC_{10} of Giger	**0.36**	**0.40**	**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.

Two shared bands (31.563 and 34.638 KDa) appeared in protein profile of control and serum infected mice while three shared bands (31.563, 34.638 and 120.91KDa) appeared in protein profile of control and serum of infected mice treated with 100 mg/kg shared bands seemed not to be affected by infection in spite of the variation shown in their amount of protein. The present results showed qualitative and quantitative differences in protein expression and banding pattern between infected, infected treated with 100 mg/kg with and control mice.

The present results (Table 6) indicated that the similarity index (S) was higher in case of infected mice treated with ginger than infected mice (0.36 and 0.2, respectively) indicating that infection with *S. mansoni* and treated with ginger had strong effect on protein profile of mice.

DISCUSSION

In this study, ginger 'extract orally administered to *S. mansoni* infected mice exhibited a moderate antischistosomal effect as the reduction rates of worm load/mouse. The same trend was recorded for the number of ova/g tissue in the intestine and liver of treated mice. However, the percent of dead ova in the intestinal wall of treated mice and the immature oval stage, also, deteriorates compared to those of infected control groups. These observations are met with the criteria for the assessment of the antischistosomal compounds and/or drugs (45, 46).

The antischistosomal activity of natural products was previously recorded. Thus, several plant species were screened in vitro against *S. mansoni* worms, some possessed a strong ac-

tivity (LD50 < 15 µg/ml), e.g. *Agave americana*, *A. lophantha*, *Furcaraea selloa*, *Solanum nigrum* and Pinus canariensis (47). As well, an oral dose 200 mg/kg methanol extract of the plants *Viburnum tinus* and *Draceana draco* significantly reduced the MT mesocarp of the plant *Balanites aegyptiaca* suppressed the oval number/g faeces of mice infected with a *S. mansoni* Sudanese strain (49). Moreover, the antischistosomal drug artemether from the leaves of the plant *Artemisia annua*, exhibited a promising effect at an oral dose 6 mg/kg in randomized clinical trials (17, 50).

The same trend was stated for the drug mirazid (Myrrh resin and oil) from the plant Commiphora molmol, as oral dose 10 mg/kg for 3 and 6 consecutive days (51, 52). However, Botros et al., 53 proved that this drug (mirazid) has very poor antischistosomal properties (< 20% cure rate) after several tests on experimental animals and patients.

Regarding the biochemical parameters, the present study declared that infection of mice with *S. mansoni* decreased the levels of serum total protein and albumin, but the activities of transaminases (AlT &AsT) and phosphatases (AlT & AsT) enzymes were elevated. Then, treatment of the infected mice with methanol extract of the tested plant species decreased the levels of total protein, albumin and the activities of the tested enzymes in comparison with those of untreated infected ones; however, these ameliorated levels of the biochemical parameters still different from that of uninfected control mice. This could be attributed to the deteriorations in cells' metabolic processes exerted by the parasites' ova in the liver and intestine of the infected hosts (48 and 54). As well, improvement the levels of the tested biochemical parameters in the infected treated mice agrees with that of mice groups infected with S. mansoni and treated with either thymoquinone (55) or artemether (46).

SDS-PAGE of whole cell proteins is a useful technique for identification of isolates complex. The present results indicated that infection of mice with *S. mansoni* treated with ginger'extract had qualitative and quantitative effect on the protein patterns of the liver tissues and serum of infected mice. The electrophoretic pattern of the native proteins revealed difference in the number and molecular weight of protein bands compared to the control mice. These differences indicated that infection of mice with *S. mansoni* and treated with ginger'extract caused intensive effects that induced fractionation of the native protein. This agrees with Bakry et al. (57), who observed two characteristic bands (140.82KD&14.42KD) in the electrophoretic patterns of tissue proteins from *B. truncatus* snails infected with *E.recurvatum* at intervals of two and four weeks post infection, but there is only one band (100.9KD) characteristic for *B. truncatus* snails infected with *S. haematobium* snails. Also, EL-Dafrawy et al. (58) showed qualitative and quantitative differences in the protein expression and banding patterns between non-infected and infected *B.alexandrina* and *B.truncatus* snails. However, in *Biomphalaria* snails, the molecular weight of the plasma proteins found to be ranged between 10 and 450 KDa, this may be due to, all strains show protein patterns, although minor inter-and intrastrain differences occur. Accordingly, the fractionation of native proteins into bands different from that of the control may be attributed similar to changes occurred in DNA of the treated snails (59).

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