Effect of bee venom or proplis on molecular and parasitological aspects of *Schistosoma mansoni* infected mice

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Abstract The present study was performed to elucidate the efficacy of *Apis mellifera* L bee venom (BV) or proplis (200 mg/kg orally for three consecutive days) on *Schistosoma mansoni* infected mice. The results recorded reduction in the total worm burden, numbers of immature eggs and the ova count in hepatic tissue in BV (sting or injection) or proplis treated groups as compared to the infected group. Histological examination illustrated a significant increase ($P \leq 0.05$) in the diameter of hepatic granuloma in BV treated groups (272.78 and 266.9, respectively) and a significant decrease in proplis treated mice (229.35) compared with the infected group (260.67). Electrophoretic pattern of RNA showed a decrease in mean of maximal optical density in liver and intestine of *S. mansoni* infected mice treated with bee venom (sting or injection) as compared with infected group. Flow cytometry analyses of RNA or apoptotic percentage of worms recovered from BV sting (19 and 49 % respectively); BV injected (20.5 and 51.17 %, respectively) and proplis (35 and 23.93 %, respectively) groups were compared with *S. mansoni* infected group (37.87 and 39.21 %, respectively). It can be concluded that administration of bee venom or proplis are effective in case of *S. mansoni* infection. Although bee venom cause increase of granuloma diameter and this might be due to venom concentration and further studies are required to avoid such harmful effect.

Keywords *Schistosoma mansoni* · Bee venom · Proplis · Flow cytometry · RNA · Apoptosis

Introduction

Schistosomiasis is a parasitic disease caused by blood flukes (trematodes) of the genus *Schistosoma* (Marek et al. 2013; WHO 2014). It is estimated that 207 million people are infected in 74 countries throughout Latin America, Africa and Asia and more than 779 million people are at risk of infection, with mortality estimated at up to 280000 deaths annually in sub-Saharan Africa alone (Steinmann et al. 2006; Fenwick 2006). Several chemotherapeutic agents were used as antischistosomal drugs. Of these, praziquantel does not prevent reinfection.

As a complementary medicine approach, bee venom has been found to have several biological functions including an antinociceptive effect (Kwon et al. 2002), antibacterial action (Perumal Samy et al. 2007), anti skin photoaging (Han et al. 2007) and an immunity boosting effect. Several reports have indicated that bee venom administration can induce a significant anti-inflammatory response mediated by inhibition of inflammation mediators. Bee venom is a rich source of enzymes, peptides and biogenic amines. There are at least 18 components in venom which have some pharmaceutical properties, including melittin, apamin, peptide, enzymes (i.e., phospholipase A2), biologically active amines (i.e., histamine adolapin and MCD peptide have anti-inflammatory activities, these substances are present in very small quantities in the whole bee venom (Martin and Hartter 1980; Kwon et al. 2001).

Zieler et al. (2001) have demonstrated that venom phospholipases A2 (PLA2s) strongly inhibit oocyst formation of malaria when administered to mosquitoes with an infectious blood meal.

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Propolis is a natural product derived from plant resins collected by honeybees. Propolis has been used in folk medicine as a health aid since ancient times (Isla et al. 2001). In general, propolis contains a variety of chemical compounds including polyphenols (flavonoid aglycones, phenolic acids, and their esters, phenolic aldehydes, alcohols and ketones), terpenoids, steroids, amino acids and various inorganic compounds (Kartal et al. 2003). The antioxidants present in propolis play a great role in their immunomodulation properties. Therapeutic administration activates lymphocytic function. Propolis increases the cellular immune-response through the increase of mRNA for interferon- γ and activates these cells to produce cytokines (Fischer et al. 2007).

Apoptosis (programmed cell death) is one of the most interesting biological events which have received a great deal of attention for its role in regulating morphogenesis during embryonic stages of development and cellular turnover during post-natal life (Raff 1992; Williams and Smith 1993). It has been associated with tissue homeostasis and consequently involved in the pathogenesis of a large number of diseases (Miscia et al. 1997).

The amount of RNA in the cells reflects the activity of gene expression, which is responsible for cell differentiation. In the analysis of gene expression, the steady state level of RNA transcripts is one of the most convenient parameters used to monitor the gene activity in the cell lines and tissues. A variety of methods such as S1 hybridization, RNAs protection and Northern blotting can be used to measure RNA level (Williams et al. 1986; Belin 1994).

The aim of this study was to investigate the potential effect of honeybee workers (*Apis mellifera* L.) venom (sting or injection) or propolis on *Schistosoma mansoni* infected mice on some Molecular; Parasitological and Histological aspects.

Materials and methods

Bee venom sting

Craniolan honeybee workers (*Apis mellifera* L.) were obtained from the Apiary of the Faculty of Agriculture, Menoufia University, Egypt. Mice were stung with live bees by stimulating them to sting in the abdomen over the location of the liver with single sting/week for two weeks. For determination of bee venom sting dose, three groups (10 mice/group) were used to record mice mortality rate. First group exposed to one sting. Second mice group exposed to 2 stings (one/day). While, third group mice exposed to 3 stings for three consecutive days (one/day).

Bee venom injection

Bee venom powder obtained from Faculty of Agriculture, Menoufia University. Bee venom dissolved in distilled water (0.1 mg/kg body weight) and injection was subcutaneously (Huh et al. 2010) one dose weekly for two weeks.

Propolis

Propolis sample was supplied from Beekeepers Society, Gharbia Governorate, Egypt in powder form. Propolis administered to mice as aqueous suspension in 3 doses each of which 200 mg/kg orally for three consecutive days (Orsatti et al. 2010).

Experimental animals

Eighty six male Swiss albino mice (CD-1 strain) were obtained from Schistosome Biological Supply Program (SBSP) unit at Theodor Bilharz Research Institute (TBRI) Giza, Egypt. Mice were transported to Zoology Department, Faculty of Science, Menoufia University and caged separately in groups in an environmentally controlled animal facility operating on a 12 h dark/light cycle at 24–26 °C and were given access to water and standard rodent food pellets ad libitum. All experiments were done in compliance with the guide lines for the care and use of laboratory animals (National Research Council 1985).

S. mansoni cercariae were hatched from *Biomphalaria alexandrina* infected snails obtained from TBRI. Each mouse was injected subcutaneously with 65 ± 5 cercariae (Peters and Warren 1969).

Thirty mice (classified into three groups: ten mice/group) were used as preliminary study to determine bee venom sting dose and remain mice classified into 8 groups (seven mice in each group) as the following: control (untreated) group; BV sting (single bee sting weekly for two weeks); BV injection (0.1 mg/kg body weight, were dissolved in distilled water) and injection subcutaneous one dose weekly for two weeks; propolis treated (200 mg/kg orally for three consecutive days); *S. mansoni* infected group (9 weeks post infection (pi)); infected group (7 weeks pi) treated with BV sting (single bee sting weekly for two weeks); infected group (7 weeks pi) treated with BV injection (one dose weekly for two weeks) and infected group (7 weeks pi) treated with propolis (200 mg/kg orally for three consecutive days).

Determination of parasitological parameters

Worm burden estimation Hepatic and portomesenteric vessels were perfused (Duvall and De Witt 1967) to

recover worms. The recovered adult worms from each animal group were sexed and counted. The collected worms were stored at -20°C for flow cytometry analysis.

Oogram pattern Three fragments of the small intestine (1 cm length) and liver were cut and each placed between a slide and cover slip. Percentage of the different egg developmental stages (immature; mature and dead eggs) was examined (Pellegrino et al. 1962).

Ova count Apiece (0.5 g) of liver and intestine tissue was taken from each mouse and placed in a glass tube containing 5 ml of 5 % potassium hydroxide (KOH) solution and incubated at 37°C for 24 h until tissue completely hydrolyses. The digest tissue was well shaken and three samples (0.1 ml each) were pipette out from each tube and placed on counting slides. The number of ova per gram liver or intestine tissues was counted (Cheever and Anderson 1971).

Measurement of hepatic granuloma diameter and liver histopathology

For liver histological preparations, portion of liver tissues were fixed in 10 % natural formalin solution and embedded in paraffin wax. Five micrometer thick sections were prepared and stained with Ehrlich's haematoxylin and eosin (H&E) (Romeis 1989). The granuloma diameter was measured using an ocular micrometer (Von Lichtenberg 1962). Micrographs and measurements were taken under microscope (Olympus BX41, model BX 41TF, Japan) provided with digital camera (Olympus Imaging Crop, Model No. E420 DC 7.4 V).

Molecular studies

Detection of apoptosis and RNA of *Schistosoma mansoni* worms Flow cytometric analysis was carried out for detecting apoptosis and RNA of worms exerted from each groups.

Apoptosis. Single cell suspensions of *S. mansoni* worms were used for determination of apoptosis. The cell viability was determined by flow cytometry and apoptosis was measured by using the sub G1 peak staining with propidium Iodide (Cohen and Al-Rubeai 1995). The flow cytometer used is FACS caliber flow cytometer (Becton–Dickinson, Sunnyvale, CA, USA). The average number of evaluated nuclei per specimen 20,000 and the number of nuclei scanned was 120 per second. DNA histogram derived from flow cytometry was obtained with a computer program according to Dean and Jett mathematical analysis (Dean and Jett 1974). Data analysis was conducted using DNA analysis program MODFIT (verity software house,

Inc. Po Box 247, Topsham, ME 04086 USA, version: 2.0, power Mac with 131072 KB Registration No.: 42000960827-16193213).

RNA content. Worms cell suspension of 1×10^6 cells/ml was prepared in PBS and 2 mM MgCl_2 . Working solution was prepared daily by adding solution A (50 ml (10 Mm) EDTA + 75 ml (1 M) NaCl + 157.5 ml (0.4 M) Na_2HPO_4 + 92.5 ml (0.2 M) citric acid was completed to 120 ml with dist. H_2O) to Acridine orange (AO) (50 mg AO was dissolved in 50 ml dist. H_2O) on ice. 400 μl of 1 N NaOH or 1 N HCl 'PH 1.2' (1 m triton $10\times$ + 8 m (1 N)HCl + 15 ml (1 M) NaCl + 76 ml of dist. H_2O) was added to 200 μl of the cell suspension and incubated for 45 s. on ice. 1.2 ml of working solution was added and analyzed immediately by flow cytometer and percentage of RNA was estimated from resulted figures (Hamurcu et al. 2005).

Electrophoretic pattern of nucleic acids (RNA) RNA pattern was detected by gel electrophoresis from tissue lysate (Hassab El-Nabi et al. 2001). A piece of 10 mg of hepatic, intestinal and splenic tissues were squeezed and lysed with 200 μl lysing buffer (50 mM NaCl, 1 mM Na_2EDTA , 0.5 % SDS, pH8.3). After 1 h, 20 μl was loaded on gel wells (gel was prepared using 1.8 % electrophoretic grade agarose. The agarose was boiled with tris borate EDTA buffer ($1 \times$ TBE buffer; 89 mM tris, 89 mM boric acid, 2 mM EDTA, PH8.3), and then 0.5 $\mu\text{g/ml}$ ethidium bromide was added to agarose mixture at 40°C . Gel was poured and allowed to solidify at room temperature for 1 h before sample were loaded to detect electrophoretic pattern of nucleic acids of lysate tissue then adding 5 μl loading buffer.

Electrophoresis was performed at 50 V where RNA were visualized using a 312 nm UV transilluminator. The intensity of RNA was measured by gel pro analyzer program as maximum optical density.

Data analysis of electrophoretic pattern of nucleic acid of liver, intestine and spleen tissues was analyzed according to Hassab El-Nabi and Elhassaneen (2008). Three main broad bands in gel were appeared from bottom to top; RNA area, nucleoprotein area and DNA area located near to the gel wells.

Statistical analysis

Data are presented as mean $\pm$ standard error ($M \pm \text{SER}$). Comparisons were made between the treated and untreated groups. Data were analyzed using Student's *t* test, for normally-distributed data (Mc-Clave and Dietrich 1991). The significance of differences was calculated between mean values of experimental and control groups in each experiment at a level of significance of $P \leq 0.05$.

Results

Bee venom (BV) sting dose

Results illustrated that mice exposed to one sting weekly recorded no mortality rate. While, mice exposed to two stings (one sting/day) for two consecutive days recorded 40 % mortality. Moreover, 100 % mortality was recorded with mice group received three stings for three consecutive days. So, one sting dose weekly was selected through the present study.

Parasitological parameters

Worm burden *Schistosoma mansoni* infected mice treated with bee venom (sting or injection) or proplis showed a significant decrease ($P \leq 0.05$) in mean total worm numbers (4.7 ± 0.29 ; 3.29 ± 0.29 and 3.57 ± 0.2 , respectively) comparing with infected mice (9.43 ± 0.2). In the same time, result illustrated significant decrease ($P \leq 0.05$) in mean couple worm numbers in *S. mansoni* infected mice treated with bee venom (sting or injection) with values 3.29 ± 0.18 and 1.43 ± 0.2 , respectively as compared with infected group (3.57 ± 0.2). Also, infected mice treated with bee venom or proplis showed decrease in mean number of male and female worms compared with infected mice as indicated in Table 1.

Ova count Ova count/g liver in infected group treated with bee venom (sting or injection) or proplis illustrated significant decrease ($P \leq 0.05$) in mean numbers (1004.5 ± 9.6 ; 863.2 ± 9.73 and 1308.57 ± 19.18 , respectively) when compared with infected mice (1478.386 ± 6.87). Also, *S. mansoni* infected mice treated with bee venom injection showed a decrease in mean value of ova count/g intestine with value 880.28 ± 17.64 as compared with infected group (994.66 ± 40.9). But, infected groups treated with bee venom sting or proplis showed an increase in mean numbers of ova count/g intestine (1775.4 ± 34.6 and 1200.68 ± 19.67 , respectively) when compared with infected group (994.66 ± 40.9) (Table 1).

Oogram pattern Liver. Table 1 showed developmental stage of hepatic eggs (immature; mature and dead) in *S. mansoni* infected mice treated with bee venom or proplis. *S. mansoni* infected mice treated with bee venom (sting or injection) or proplis showed a significant decrease ($P \leq 0.05$) in mean numbers of immature eggs (8.17 ± 0.34 ; 5.33 ± 0.18 and 8.8 ± 0.1 , respectively) and dead eggs (7.76 ± 0.27 ; 9.43 ± 0.27 and 10.26 ± 0.39 , respectively) as compared with infected group (9.86 ± 0.43 and 15.53 ± 0.3 , respectively). While

Table 1 Effect of bee venom or proplis on worm burden; ova count; oogram pattern and granuloma diameter of *Schistosoma mansoni* infected mice

Groups	Worm burden		Ova count		Oogram pattern (liver)			Oogram pattern (intestine)			Granulomas diameter (nm)		
	Total (σ^2)	Couple (σ^2)	Male (σ)	Female (σ)	Liver	Intestine	Immature egg	Mature egg	Dead egg	Immature egg		Mature egg	Dead egg
Infected mice	9.43 ± 0.2	3.57 ± 0.2	5 ± 0.3	0.57 ± 0.2	1478.386 ± 6.87	994.66 ± 40.9	9.856 ± 0.43	74.614 ± 0.6	15.53 ± 0.3	12.78 ± 0.28	73.822 ± 0.32	13.398 ± 0.38	260.67 ± 1
Infected mice treated with bee venom sting	*4.7 ± 0.29	3.29 ± 0.18	*1.29 ± 0.29	0.43 ± 0.2	*1004.5 ± 9.6	*1775.4 ± 34.6	*8.17 ± 0.34	*84.07 ± 0.38	*7.76 ± 0.27	*8.739 ± 0.32	*64.4 ± 0.35	*26.861 ± 0.42	*272.78 ± 1.18
Infected mice treated with bee venom injection	*3.29 ± 0.29	*1.43 ± 0.2	*1.7 ± 0.18	0 ± 0	*863.2 ± 9.73	*880.28 ± 17.64	*5.33 ± 0.18	*85.24 ± 0.32	*9.43 ± 0.27	*1.9 ± 0.09	*46.1 ± 0.5	*52 ± 0.53	*266.9 ± 1.86
Infected mice treated with proplis	*3.57 ± 0.2	3.57 ± 0.2	0 ± 0	0 ± 0	*1308.57 ± 19.18	*1200.68 ± 19.67	*8.8 ± 0.1	*80.9 ± 0.45	*10.26 ± 0.39	*3.5 ± 0.19	*56.54 ± 0.36	*39.9 ± 0.35	

Data are expressed as mean $\pm$ standard error (SER)

* Significant difference compared to infected control group at ($P \leq 0.05$)

Plate 1 Photomicrograph of liver section of **1** normal mouse showing central vein (C.V.) surrounding by normal hepatocyte ($\times 400$), **2** *S. mansoni*-infected mouse (9 weeks post infection) showing Schistosoma granuloma (G) formed of *Schistosoma* ova (O) surrounding by lymphocytes and fibrocytic cells ($\times 200$), **3** *S. mansoni*-infected mouse (7 weeks post infection) and treated with bee venom sting showing granuloma (G) consist of lymphocytes and

fibrocytic cells surrounding by *Schistosoma* ova ($\times 200$), **4** *S. mansoni*-infected mouse (7 weeks post infection) and treated with bee venom injection showing granuloma (G) consist of *Schistosoma* ova surrounded by lymphocytes and fibrocytic cells ($\times 200$), **5** *S. mansoni*-infected mouse (7 weeks post infection) and treated with proplis showing leukocytic inflammatory cells (LI) and granuloma (G) ($\times 200$)

the mean numbers of mature eggs indicated a significant increase ($P \leq 0.05$) with values 84.07 ± 0.38 ; 85.24 ± 0.32 and 80.94 ± 0.45 , respectively in infected groups treated with bee venom (sting or injection) or proplis comparing with infected group (74.6 ± 0.6).

Intestine. The mean values of immature and mature egg numbers showed a significant decrease ($P \leq 0.05$) in infected mice treated with bee venom (sting or injection) or proplis as compared with infected group (12.78 ± 0.28 and 73.822 ± 0.32 , respectively). On contrast, infected mice treated with bee venom (sting or injection) or proplis illustrated a significant increased in mean value of dead eggs with values 26.861 ± 0.42 ; 52 ± 0.53 and 39.9 ± 0.35 , respectively when compared by infected mice (13.398 ± 0.38), as indicated in Table 1.

Histological observations and hepatic granulomas measurement

Examination of liver sections of bee venom or proplis treated groups were showed in Plate 1. Normal control mice showed central vein surrounded by cords of normal hepatocytes. Hepatic cell has a vascular nucleus.

Typical liver granuloma was formed in *S. mansoni* infected group. Granuloma consists of *Schistosoma* ova surrounding by leukocytic inflammatory cells and fibrocytic cells. Sections showed also abnormal hepatocyte.

Also, *S. mansoni* infected mice treated with bee venom (sting or injection) or proplis illustrated granuloma which consists of ova surrounding with leukocytic inflammatory cells and fibrocytic cells. Abnormal hepatocytes which lost

Fig. 1 Flow cytometric analysis of *S. mansoni* RNA worms collected from treated mice with bee venom (sting or injection) or propolis

their chromatin material were observed in infected mice treated with bee venom. Infected group treated with propolis hepatic cell damage little compared with infected mice.

Result illustrated an increase in mean numbers of granuloma diameter in infected mice (7 weeks post infection) and treated with bee venom (sting and injection) with mean values 272.78 and 266.9, respectively as compared with infected group (9 weeks post infection) (260.67). But, *Schistosoma mansoni* infected mice (7 weeks post infection) and treated with propolis showed a significant decrease in mean granuloma diameter (229.35 ± 1.06) as compared with infected mice. As shown in Table 1 and Plate 1.

Molecular study

Percentage of apoptosis and RNA of *Schistosoma mansoni* worms By using flow cytometry analysis; results showed a decrease in RNA percentage of worms exerted from *S. mansoni* infected mice and treated with bee venom (sting or injection) or propolis with values 19, 20.5 and 35 %; respectively comparing with infected group (37.87 %). While, an increment in the percentage of apoptosis worms in worms exerted from *S. mansoni* infected mice and treated with bee venom (sting or injection) groups was recovered (49 and

51.17 %; respectively) comparing with infected group (9 weeks post infection) (39.21 %) (Figs. 1, 2, 3).

Electrophoretic pattern of nucleic acid (RNA) Result indicated an increase in mean values of maximal optical density of RNA in liver, Intestine and spleen of normal mice treated with bee venom (sting or injection) or propolis with values 60.28 ± 0.6 , 61.14 ± 1.13 , 65.57 ± 1.12 ; 33.2 ± 0.84 , 35.1 ± 0.9 , 43.4 ± 0.9 and 39.3 ± 1.2 , 38 ± 1.1 , 40.1 ± 0.76 , respectively when compared with normal control of liver, intestine and spleen (42.4 ± 1 , 32.1 ± 0.8 and 35.4 ± 1.3 ; respectively). On the hand, Liver and spleen of *S. mansoni* infected mice (9 weeks post infection) showed significant decrease ($P \leq 0.05$) in mean number of maximal optical density with values 35 ± 0.9 and 27.9 ± 0.7 ; respectively compared to normal control. Also, *S. mansoni* infected mice treated with bee venom (sting or injection) illustrated a decrease in mean of maximal optical density in liver and intestine (33.1 ± 0.65 , 30.6 ± 0.88 and 27.4 ± 0.87 , 30.6 ± 0.66 ; respectively) as compared with infected group. While, mean of maximal optical density in spleen of *S. mansoni* infected mice treated with bee venom (sting or injection) or propolis indicated significant increase ($P \leq 0.05$) with values

Fig. 2 Flow cytometric analysis of *S. mansoni* apoptotic worms collected from treated mice with bee venom (sting or injection) or propolis

Fig. 3 Percentage of RNA and apoptosis of worms exerted from *S. mansoni* infected mice and treated with bee venom (sting or injection) or propolis

32.1 ± 0.47 , 33.4 ± 0.47 and 40.1 ± 0.6 ; respectively compared to infected control mice (27.9 ± 0.7), as shown in Figs. 4 and 5.

Discussion

For the evaluation of the effect of anti-schistosomal agent on the infection, it is important to study some criteria related to the worm intensity, stages and distribution in the host tissue. Among these criteria, the worm burden, the

percentage of ova pattern and ova count in liver and intestine were considered (Abdel-Ghaffar 2004; Abdel-Ghaffar et al. 2005).

The present results illustrated a reduction in mean number of total; couple; male and female worms in *S. mansoni* treated with bee venom, comparing with *S. mansoni* infected mice. At the same time, the result indicated a decrease in mean number of total ova count/gram intestine and an increase in dead eggs in intestinal tissue in the same groups as compared with infected group. The obtained results may be attributed to the presence of one or more bioactive components in bee venom. Similarly, Son et al. (2007) mention that bee venom contains a number of bioactive compounds, including histamine, epinephrine, free amino acids, enzymes (e.g., phospholipase A₂; PLA₂), and a variety of peptides (e.g., melittin and apamin). Guillaume et al. (2006) reported effect of bee venom phospholipase A₂ as anti-plasmodium toxicity. They illustrated the capacity of secreted phospholipases A₂ to be active on biological targets and showed the potential role for oxidized lipoproteins and/or endogenous secreted phospholipases A₂ in the host defense against malaria. Phospholipase A₂ enzymes exhibit a variety of physiological activities in addition to intrinsic lipolytic action. These enzymes catalyze the hydrolysis of the sn-2 ester bond of glycerophospholipids, leading to the production of

Fig. 4 Electrophoretic pattern of nucleic acids in tissue lysate of *S. mansoni* -infected mice **a** liver; **b** intestine & **c** spleen treated with bee venom (sting or injection) or proplis. Lane 1 Normal control; Lane 2 Normal treated with bee venom sting; Lane 3 Normal treated

with bee venom injection; Lane 4 Normal treated with proplis; Lane 5 Infected control; Lane 6 Infected treated with bee venom sting; Lane 7 Infected treated with bee venom injection and Lane 8 Infected treated with proplis

Fig. 5 Mean of maximal optical density of total RNA in tissue lysate of *S. mansoni* infected mice liver; intestine and spleen treated with bee venom or proplis

lysophospholipids. Bee venom Phospholipase A₂ enzymes are potent inhibitors of the in vitro intra-erythrocytic development of *Plasmodium falciparum* (Deregnaucourt and Schrvel 2000; Guillaume et al. 2004). In addition, Eckey et al. (1997) analyzed incidence of lipoprotein oxidation on bee venom phospholipase A₂ particle binding and anti-plasmodium activity. They indicated that lipolyzed particles or lipid products are responsible for parasite killing. Moreover, Mulfinger (1990) studied in vitro the

anti bacterial synergistic effect between melittin and the antibiotic polymyxin B against *Escherichia coli*. Similar effect was also seen in studies on sublethal *E. coli* infections of mice.

In addition, proplis treatment (200 mg/kg orally) of *S. mansoni* infected mice showed an inhibitory effect in mean number of total; male and female worms comparing with infected control group. These findings were in agreement with Issa (2007) who studied the prophylactic and curative

effects of proplis (250 mg/kg/day) starting 5 days before infection and continued up to 45 days post infection in experimentally *S. mansoni* infected immunosuppressed mice and found significant reduction numbers of adult worms. Also, Hegazi et al. (2007a) recorded the highly effect of Siwa proplis on adult worms of *Fasciola gigantica* in vitro using scan electron microscope. They observed that proplis caused severe damage on the surface architecture of the *Fasciola gigantica* worm tegument. While, Orsi et al. (2010) illustrated that Bulgarian proplis preparations showed an important antibacterial action on *Salmonella Typhi* infection, as well as a synergistic effect with antibiotics action on the ribosome. David et al. (2010) studied proplis activity on *Giardia* trophozoites proliferation and revealed that anti-giardial activity of proplis.

The current study showed a reduction in percentage of immature and mature eggs and increase in percentage of dead eggs in intestinal tissue of *S. mansoni* infected mice treated with proplis compared with infected group. Also results indicated inhibited in total eggs count in hepatic tissue in the same group comparing with infected mice. These decrease in eggs count and immature and mature eggs after proplis treatment means a decrease in numbers of worms and led to decrease in relative liver body weight. Such results run in parallel with results of Issa (2007) who showed a reduction in total eggs count in stool and tissues in *Schistosoma mansoni* infected mice treated with proplis. Also, our findings are supported by the results of, Hegazi et al. (2007b) who studied effect of Egyptian proplis on *Fasciola gigantica* eggs and found evidence of inhibitory activity of proplis on the vitality and hatchability of immature eggs.

At the same time *S. mansoni* infected mice treated with bee venom (sting or injection) indicated an increase in granuloma diameter compared with infected control group. These findings were in agreement with those reported by Mukherjee et al. (1994) who recorded that phospholipase A₂ enzymes may be involved in cell proliferation and signal transduction as well as in the pathogenesis of disease processes such as inflammation. Human secretory phospholipase A₂ could induce the release of arachidonic acid and PGE₂ production in neutrophils, HL-60 granulocytes treated with calcium ionophore (Hara et al. 1991), mast cells (Fonteh et al. 1994) and mouse peritoneal macrophages (Miyake et al. 1994). While, *S. mansoni* infected mice treated with proplis illustrated significant decrease in granuloma diameter comparing with infected control mice. This finding runs in parallel with the results of Bhadauria et al. (2008) who indicated the potential of proplis as a hepatoprotective agent at doses 200 and 400 mg/kg against experimentally induced hepatorenal damage by CCl₄. Also, El-Khatib et al. (2002) showed that aqueous extract of

proplis has prophylactic hepatoprotective effect against CCl₄ induced injury.

At level of molecular studies, *S. mansoni* infected mice liver and intestine treated with bee venom showed reduction in optical density of RNA. While an increase in optical density of RNA of *S. mansoni* infected mice spleen treated with bee venom and proplis. Hassab El-Nabi et al. (2001) illustrated that pollutants or any stress on cells may activate some silent genes to transcript more RNA. The percentage of RNA of worms exerted from *S. mansoni* infected mice treated with bee venom showed decrease than non treated infected group by using flow cytometry analysis. On the other hand, the results indicated an increase in DNA damage of worms exerted from *S. mansoni* infected mice treated with bee venom in form of apoptosis. These may be due to DNA damage which induces cell death. This result confirmed with Adade et al. (2012) who indicated that *Apis mellifera* venom consists of many biologically active molecules and has been reported to exhibit remarkable anti-cancer effects. *Apis mellifera* venom can affect the growth, viability and ultrastructure of all *Trypanosoma cruzi* developmental forms by increasing apoptotic mechanism. They also observed abnormal nuclear chromatin condensation and DNA disorganization was observed in venom-treated trypomastigotes, suggesting cell death by an apoptotic mechanism.

Finally, it can be concluded that administration of bee venom is effective in case of *S. mansoni* infection although it caused at the same time an increase of granuloma diameter and this might be due to venom concentration and further studies are required to avoid such harmful effect. At the same time, proplis illustrated a beneficial effect as antiparasitic agent against *S. mansoni* infection by reducing worm burden, Ova count/liver and granuloma diameter.

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