Global Veterinaria 14 (3): 329-338, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.03.9356

Role of *Schistosoma mansoni* Tegumental Cathepsin-B Antigen in Modulation of Murine Shistosomiasis

[1]Gehan El-Enain, [1]Ibrahim Rabia, [2]Abeer Mahgoub,
[3]Ismail M. Moharm, [4]Mohammed S. Hedaya, [5]Faten Nagy,
[1]Azza Fahmy, [6]Karima M. Metwalley and [1]Wafaa EL-Komy

[1]Department of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt
[2]Department of Medical Parasitology, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Department of Medical Parasitology, Faculty of Medicine, Menoufia University, Egypt
[4]Department of General Surgery, Theodor Bilharz Research Institute, Giza, Egypt
[5]Department of Immunology, Theodor Bilharz Research Institute, Giza, Egypt
[6]Department of Zoology, Faculty of Science, Al-Azher University, Cairo, Egypt

Abstract: Cysteine proteases are important virulence factors for parasites. Cathepsin B (SmCB) proteases are abundant in different stages of *Schistosoma* and they have been identified to induce a level of host-protective immune responses with amelioration of morbidity. In this study, the parasitological parameters, level of immunoglobulins, cytokines profile and hepatic granuloma were assessed to study the effect of immunization of mice with cathepsin B antigen with or without treatment using anti-helminthic drug praziquantel (PZQ). Multiple small doses of cathepsin B were intraperitoneally injected into naive mice; the first dose of a 100µg of purified (SmCB) was followed by two booster doses of 50µg each, at weekly intervals. The experimental design included six groups of 20 mice each;. Group 1 (normal control), Group 2 (infected control), Group 3, 4&5 were immunized intra-peritoneally with 100µg/ml of (SmCB) antigen emulsified with complete Freunds' adjuvant. Groups 4 and 5 were additionally infected subcutaneously with 100 *S. mansoni* cecariae, one week after last immunization and only group 5 was further treated by PZQ at the 5th week post-infection (p.i.). Group 6, mice were treated only with PZQ at the week 5th post-infection. Ten mice of each group were sacrificed at the end of week 8 and the other 10 were sacrificed at 12 weeks p.i. Parasitological, pathological and immunological parameters were evaluated. The histopathological and parasitological examinations revealed the highest remarkable increase in the percentage of degenerated ova (12%) within the diminished hepatic granulomas and the most significant decline percentage of the worm burden (46%), tissue egg loads (42.8% and 50% for hepatic and intestinal ova, respectively) were experienced by the infected pre-immunized and post-treated mice. The data collected from this research study might be useful in developing potential vaccine against *S. mansoni*.

Key words: Schistosomiasis · Cathepsin B · Immunization · Vaccine

INTRODUCTION

Schistosoma infections plague more than 240 million people worldwide. The most prevalent anthropophilic schistosome species globally is Schistosoma haematobium which accounts for nearly half of that number, primarily in sub-Saharan Africa and the Middle East [1]. Schistosomiasis is one of the most important public health problems affecting Egyptians, especially the rural inhabitants of the Nile Delta [2]. Eggs of *Schistosoma mansoni* embolize to liver, where the granulomatous inflammatory response induces presinusoidal inflammation and periportal fibrosis. T cells and B cells have also been implicated as playing a regulatory role in schistosome granuloma formation [3]. In infection with *S. mansoni*, hepatic granuloma formation is mediated by CD4+ T lymphocytes sensitized to egg antigens. New categories of CD4+ CD25+ T regulatory lymphocytes have been identified to maintain immune tolerance to self-antigens and they are involved in

Corresponding Author: Ibrahim Rabia, Departments ofParasitology, Theodor Bilharz Research Institute, Giza, Egypt.
E-mail: ibrahimshalash@yahoo.com.

immune regulation of various conditions, such as autoimmune diseases [4]. In infectious diseases, T regulatory lymphocytes may be induced in antigen-specific manner and may suppress the tissue destruction resulting from immune responses [5]. Several publications indicated that the CD4+ CD25+ T-regulatory subsets, which spontaneously arise in the thymus [6], can also be peripherally induced by antigen [7] and functions in the regulation of parasite-induced immunopathology [8-10]. Papain and other environmental allergens such as ficin, bromelain and Der p1 are members of the C-1 peptidases family and their proteolytic activity is believed to be necessary for the adjuvant-like induction of Th2-mediated responses [11-13]. Schistosomes express several members of the C-1 peptidases, including cathepsin B (SmCB) and cathepsin L (SmCL) that are known to play critical roles in the digestion of the host blood tissues and hemoglobin (SmCB1, SmCL1 and SmCL3), in reproduction (SmCL2) and surface tegument biogenesis (SmCB2) [7,14-19]. Praziquantel (PZQ) has become the drug of choice against Schistosomiasis. Indeed, it has effectively become the only anti-schistosomal drug that is commercially available. Despite the treatment with PZQ and other existing control methods, all have failed to eliminate the incidence of the disease, morbidity and mortality due to infection [15]. This is in part due to the inability of chemotherapy to prevent new infection and failure of previously infected individuals to develop an effective immune response against the parasite [16]. Therefore, a long-term disease control strategy implying compiling a mass chemotherapy with a protective vaccine is a mandatory. The development of an effective vaccine against schisto-some is a real challenge considering the trials conducted over two decades or so with a barely progress in producing a liable vaccine. One of the successful studies [17] in which animals were immunized with a combination of attenuated vaccines and recombinant-derived schistosome antigens, indicated a development of a feasible vaccine. The present study was designed to investigate different parasitological and immunological parameters in response to immunization of mice with purified SmCB antigen as an experimental trial to decrease or modulate severe hepatic morbidity of murine schistosomiasis.

MATERIALS AND METHODS

Mice and Parasites: Cercariae of an Egyptian strain of S. mansoni were obtained from the Schistosome Biological Materials Supply Program, Theodore Bilharz Research Institute (SBSP/TBRI), Giza, Egypt and used for infection immediately after shedding from Biomphalaria alexandrina snails. Out bred six-week-old female CD1 mice were raised at SBSP/TBRI and housed throughout experimentation in the Animal Facility of the Faculty of Science, Cairo University. Every effort was made to minimize animal suffering including a change of bedding thrice weekly, a clean, air-conditioned and quiet housing, a delicate handling on injection and exposure to infection, as well as euthanizing and no extension of experiments beyond 8 weeks after infection. All animal experiments were performed following the recommendations of the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Washington, DC. Studies on mice were approved by the Animal Care and Use Committee of the Theodore Bilharz Research Institute, Giza, Egypt.

Preparation of *S. mansoni* cathepsin B antigen (SmCB): Mature *S. mansoni* worms were incubated for 16 hrs at 37°C in RPMI 1640 (PH 7.3) (Sigma, St. Louis, MO). The suspension was centrifuged at 14,900 g for 30 min and the supernatant (ES) was collected and stored at -20°C as aliquots until used. SmCB antigen was purified by two steps DEAE sephadex G-50 ion exchange chromatography and gel filtration chromatography on sephadex-G-100 HR column [12].

Experimental Design: The mice were divided into 6 groups of 20 mice each. Group 1 (normal control), Group 2 (infected control), Group 3, 4&5 were immunized intra-peritoneally with 100µg/ml of (SmCB) antigen emulsified with complete Freunds' adjuvant. Then, the animals were boosted two weeks later with 50ug/ml of SmCB emulsified with incomplete Freund's adjuvant and boosted again one week apart. Groups 4 and 5 were additionally infected subcutaneously with 100 *S. mansoni* cecariae, one week after last immunization and only group 5 was further treated by PZQ at the 5th week post-infection (p.i.). Group 6 of mice was treated only with PZQ at the week 5th post-infection. Ten mice of each group were sacrificed at the end of week 8 and the other 10 were sacrificed at 12 weeks p.i. Effects of the used drug were evaluated concerning parasitological, pathological and immunological parameters.

Parasitological Parameters

Worm Burden: Perfusion of adult worms from the liver and porto-mesenteric system was performed 8 weeks after infection according to Duvall and Dewitt [20]. Mean values ± SD for each group were calculated. Percent

change was evaluated by the formula:% change = mean number in infected controls-mean number in infected, immunized mice/mean number in infected controls×100.

Tissue Egg Load: The number of eggs per gram tissue (liver and intestine) was studied according to the procedure by Cheever [21].

Oogram Pattern: The percentages of immature, mature and dead ova in the small intestines were computed from a total of 100 eggs per intestinal segment and classified according to the categories previously defined by Pellergrino *et al*. [22].

Granuloma Diameters: Livers of mice were fixed in 10% buffered formalin, processed into paraffin blocks, serially cut at 4 µm thickness and stained with hematoxylin and eosin. Hepatic granuloma measurements were done according to von Lichtenberg [23] using an ocular micrometer for those containing a central ovum only. Counting was carried out in 5 successive microscopic fields (10×10) in serial tissue sections of more than 250 µm apart. The percent reduction in granuloma diameter relative to infected control was calculated as follows:% reduction of granuloma diameter = mean diameter of controls mean diameter of test groups/mean diameter of control group x 100.

Determination of Hepatic Hydroxyproline Content: The total collagen present in the liver samples recovered from each host was determined by estimating hydroxyproline content. The latter value was obtained using base hydrolysis for the dissolution of tissues as described previously by Reddy and Enwemeka [24]. Briefly, ≈100 mg of liver tissue was incubated overnight at 37°C in 5% KOH (6 ml/100 mg tissue weight) in a test tube. The mixture was occasionally inverted to re-suspend the dissolved tissue. Duplicate sets (each contained 600 µl of tissue sample suspension) were then removed/ sampled. To each sample, 160 µl of 10 N NaOH and 40µl distilled water were added and all proteins present subjected to hydrolysis by placing the screw-capped tubes in an autoclave for 20 min. Normal tissues were used as the background for the standards. Duplicate 250µl aliquots of each sub-sample/liver were then removed and supplemented with 250µl chloramine-T solution (saturated in 50% n-propanol [in water]); Sigma, St. Louis, MO). All samples were then incubated at room temperature for 3 h. Thereafter, 500µl of freshly prepared Ehrlich's reagent (0.15 g/ml of n-propanol/ perchloric acid [2:1, v/v]) was added to each tube and the materials incubated at 65°C for 20 min. Each sample was then analyzed in a UV-VIS spectrophotometer at 550 nm.

Sample Preparation: Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum was stored at -20°C until used for biochemical assays.

Determination of Serum Alanine (ALT) and Aspartate Aminotransferase (AST): Serum alanine aminotransferase and aspartate aminotransferase levels were determined colorimetrically based on the method of Reitman and Frankel [30], using a Diamond Diagnostics kit (Cairo, Egypt). In this kit, AST and ALT levels were estimated via measures of formation of oxaloacetate and pyruvate, respectively, using kit-provided enzyme precursors. After completion of the reaction, according to manufacturer's instructions, each sample was then analyzed in a double beam UV-VIS spectrophotometer (Model UV-1601 PC, Shimadzu, Kyoto, Japan) at 520 nm.

Determination of Total Serum Interleukins (IL)-2 and (IL)-10, IgE and IgG: Determination of total serum Interleukins (IL)-2 and (IL)-10, IgE and IgG Commercially available ELISA kits were used to measure serum IL-2 and (IL)-10 levels (Biosource International, Camarillo CA), IgE levels (BD OptEIA™ Set, San Diego, CA) and IgG levels (Life Diagnostics, Inc., Westchester, PA) in the isolated samples.

Data Analysis and Statistics: All values were tested for normality. Student's unpaired 2-tailed t-test, Mann–Whitney and ANOVA tests were used to analyze the statistical significance of differences between experimental and control values and considered significant at $P<0.05$.

RESULTS

At both 8 and 12 week p.i., immunization of mice with (SmCB) antigen induced a significant reduction in the mean total number of worm burden on comparing the immunized infected group to the infected control group ($P<0.001$), with percent reduction (% PR) of46.2% and 59.1% for 8 and 12 weeks p.i respectively (Fig.1). Moreover, a highly significant reduction in the mean total number of worm burden was observed in immunized infected mice treated with PZQ at both 8 and 12 weeks p.i. Significant reduction in the mean number of ova/ gram

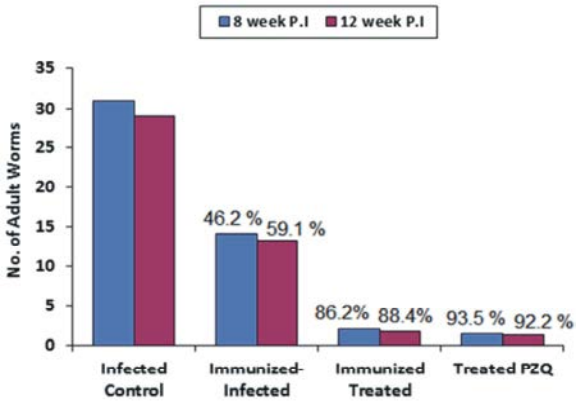

Fig. 1: Effect of immunization on worm burden at 8 &12 weeks post-infection in animal groups

Table 1: Oogram pattern detected at 8&12 weeks post-infection in animal groups.

	Oogram pattern		
Animal groups	Immature stage	Mature stage	Dead ova
Infected Control Group			
Week 8	45.2±0.22	44.36±11	48.3±0.3
Week 12	41.9±0.42	10.44±0.2	09.8±0.1
SmCB-Immunized Infected Group			
Week 8	37.1±0.33	44.7±0.53	40.7±0.54
Week 12	32.9±0.28	18.2±1.0*	26.4±5.1**
Infected Treated Group			
Week 8	22.4±0.19**	6.8±0.13***	7.2±0.21***
Week 12	20.5±0.14**	70.8±0.23***	72.3±0.16***

*p<0.05, **p< 0.01 and ***p<0.001 are significant differences from infected controls.

Table 2: Effect of immunization of SmCB and PZQ on serum ALT& AST (U/ml) in mice infected with *S. mansoni*

	8 week		12 week	
Animal groups	ALT	AST	ALT	AST
Normal Control Group	60.00±5.73	120.80±5.45	62.50±2.30	125.88±9.45
Infected Control Group	117.12±5.3	333.50±13.61	138.24±4.86	374.25±16.80
Immunized Control Group	58.12±2.2	118.41±4.14	60.43±3.20	122.16±5.22
SmCB-Immunized Infected Group	88.22±6.2	217.24±6.10*	77.12±4.70**	220.41±7.30*
SmCB-Immunized & Treated Group	77.42±8.3*	190.67±7.90**	82.29±8.20*	201.52±3.90**
Infected Treated Group	64.45±4.3**	177.72±6.92***	62.23±6.40**	196.26±2.10**

*p<0.05 and **p< 0.01 are significant differences from infected controls.

issue (liver and intestine) was detected in the group immunized with (SmCB) antigen compared to infected controls (P<0.01). The percent of immature ova was less in the immunized group than the infected one while the percent of dead ova was higher (18.2%&26.4%) in the immunized group than the infected control (P<0.05) for 8 and 12 weeks p.i. respectively. The highest significant presence of dead ova (72.5%& 75.4%) was observed in the immunized infected mice treated with PZQ for 8 and 12 weeks p.i. respectively (Table.1).

Effect on Serum ALT and AST: Infection with *S. mansoni* caused a significant increase in both ALT and AST levels at both 8 and 12 weeks p.i. Immunization of mice with (SmCB) antigen induced a significant (p<0.05) reduction in both ALT and AST levels at both 8 and 12 weeks p.i. Yet, the greatest reduction(p<0.001) observed in AST level was in favor of the group immunized, infected and treated with PZQ at 12 weeks p.i. (Table 2).

Effect on Hepatic Hydroxyproline Content: The measurements of hepatic hydroxyproline were presented in Table (3) showing a highly significant increase in the level of hepatic hydroxyproline in the infected group compared to the normal mice. Immunization of infected mice with SmCB significantly decreased (as compared to levels in infected mice) the hepatic hydroxyproline

Table 3: Effect of immunization of SmCB and PZQ on hepatic hydroxyproline(µg/g tissue) in mice infected with *S. mansoni*8-& 12-wks post-infection

	Hepatic Hydroxyproline Content	
Animal groups	8 week	12 week
Normal Control Group	150.50±7.43	143.70±5.83
Infected Control Group	386. 40±4.95	1088.80±7.60
Immunized Control Group	161.12±2.25*	147.22±2. 4**
SmCB-Immunized Infected Group	201.25±5.45*	776.34±6. 6**
SmCB-Immunized infected & Treated Group	188.00±7.58*	544.40±16.78*
Infected Treated Group	242.30±6.81*	864.35±10.82*

*p<0.05 and **p< 0.01 are significant differences from infected controls.

Table 4: Effect of immunization of (SmCB) antigen and PZQ on serum IL-10& IL-12

	8 week		12 week	
Animal groups	IL-10	IL-12	IL-10	IL-12
Normal Control Group	20.00±2.71	25.66±2.4	22.12±1.8	26.39±3.28
Infected Control Group	160.00±12.34	40.80±3.5	252.02±11.3	35.83±4.52
Immunized Control group	38.11±2.1***	27.21±1.2*	23.53±1.5***	24.15±1.2*
SmCB-Immunized Infected Group	98.72±7.4**	32.42±3.6	217.25±4.7	29.45±2.30
SmCB-Immunized infected & Treated Group	71.2±3.3**	29.71±2.9*	162.91±4.2*	28.59±1.90
Infected Treated Group	88.2±2.7*	33.15±1.5	199.15±2.8	30.92±2.60

*p<0.05 and **p< 0.01 are significant differences from infected controls.

Table 5: Effect of immunization of SmCB and PZQ on serum immunoglobulins IgE&IgG.

	8 week		12 week	
Animal groups	IgG	IgE	IgG	IgE
Normal Control	320.50±12.88	05.90±0.40	322.12±13.70	06.31±0.28
Infected Control Group	869.08±17.42	33.30±0.50	882.27±21.40	42.39±0.50
Immunized Control Group	590.81±19.4*	10.32±0.2*	580.7±11.5*	12.9±0.2*
SmCB-Immunized Infected Group	981.72±12.40*	40.26±0.60	917.55±14.20*	44.52±0.30
SmCB-Immunized infected & Treated Group	999.00±13.90*	37.91±0.90	982.10±15.21**	36.92±0.90
Infected Treated Group	779.22±10.44*	28.31±1.20	796.5±12.15	31.27±0.55*

*p<0.05 and **p< 0.01 are significant differences from infected controls.

content. Yet, the treatment of immunized infected mice with PZQ boosted this reduction to the greatest in favor of mice sacrificed at 12 weeks post-infection.

Effects on Serum IL-10 & IL-12: As depicted in Table (4), Th-1 related cytokine IL-10 showed significant increase in infected control (P<0.001) compared to normal control. On the other hand, it showed slightly significant (P<0.05) decrease in immunized infected controls compared to infected controls. The groups treated with PZQ showed a slightly decrease (P<0.05) in the level of IL-10 compared to immunized infected control. On the other hand, the Th-2- related cytokines IL-12 showed no significant difference in the infected controls compared to normal mice. Meanwhile, it showed a moderate decrease (P<0.01) in the immunized infected control compared to infected control. Yet the greatest reduction (P<0.001) was detected in the mice immunized, infected and treated.

Effects on Serum Immunoglobulins IgE&IgG: Table (5) showed that the level of sera IgG and IgE in samples of mice infected with *S. mansoni* was time-dependent with a highly significant increase at 8- and 12-weeks p.i compared to the normal group. The levels of sera IgG in immunized, infected mice or immunized treated ones showed a significant increase at both 8and 12 weeks p.i. compared to those of the untreated infected mice, maximum effects were again found at 8weeks p.i.

Granuloma Diameters: At 8 weeks p.i. the mean granuloma diameter in infected control group was 390.34±0.49 µm while in SmCB immunized infected group, it was 212.22±0.22µm and the reduction in granuloma diameter was 45.6%, whereas the mean granuloma diameter in SmCB immunized infected and treated group was 201.42±0.35 µm and the reduction in granuloma diameter was 48.4%, while in treated infected group the granuloma diameter was 322.30± 6.81µm as showing in

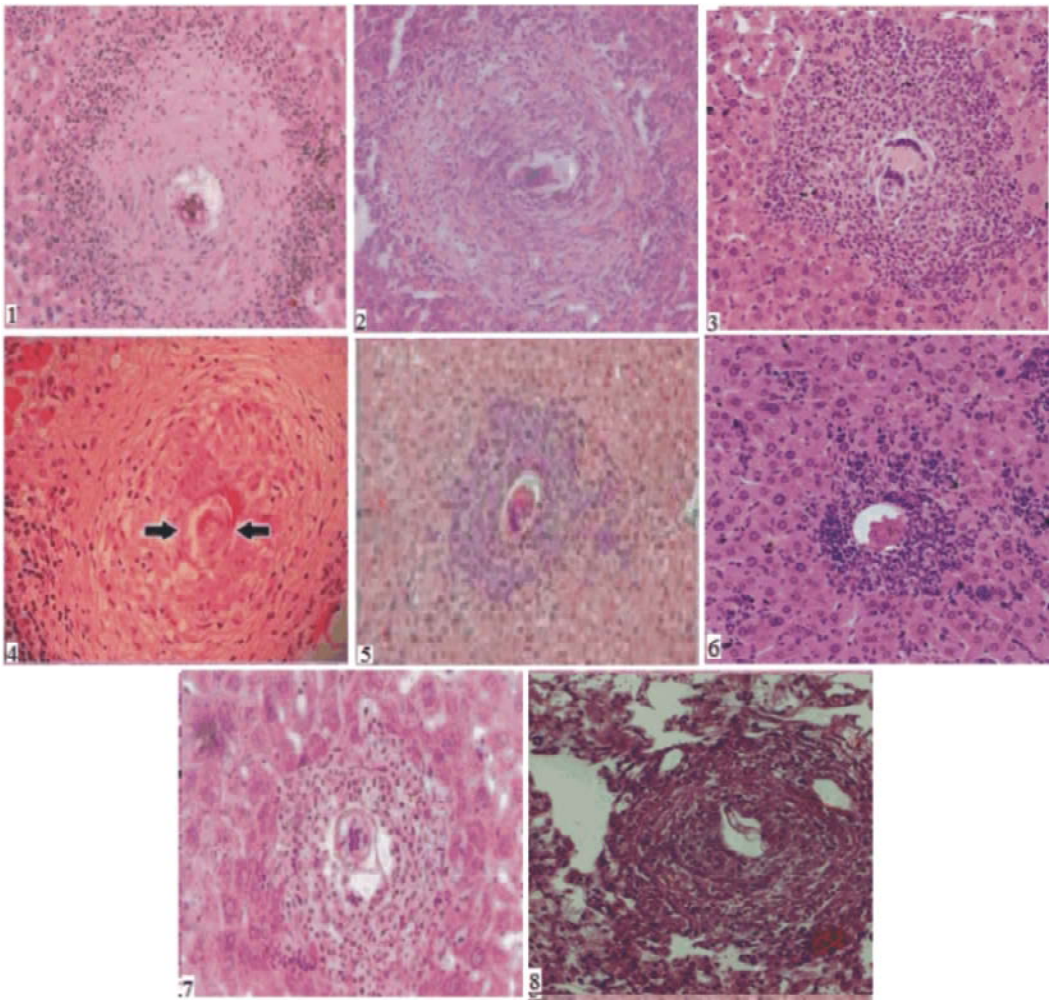

Fig. 2: Photomicrograph of liver granulomas of *S. mansoni* infected groups. (1): Infected control at 8 weeks; group, (2): Infected control at 12 weeks; (3) Infected immunized at 8 weeks; (4) Infected immunized at 12 weeks; (5) Infected immunized and treated at 8 weeks; (6) Infected immunized and treated at 12 weeks; (7) Infected and treated with PZQ at 8 weeks (8) Infected and treated with PZQ at 12 weeks (Haematoxylin and Eosin stain, X 200)

Table 6: Effect of immunization of SmCB and PZQ on granuloma diameters (µm)

	Granuloma diameters	
Animal groups	8 week% R	12 week%R
Normal Control Group	-	-
infected Control Group	390.34±0.49	280.41±0.92
Immunized Control Group	-	-
SmCB-immunized Infected Group	212.22±0.22* 45.6%	176.34±3. 9** 37.1%
SmCB-Immunized infected & Treated Group	201.42±0.35 * 48.4%	144.40±6.8* 48.5%
Infected Treated (PZQ) Group	322.30±6.81* 17.4%	224.35±7.2* 20.0%

Table 6. At 12 weeks post-infection the mean granuloma diameter in infected control group was 280.41±0.92µmwhile in SmCB immunized infected group, it was 176.34±3.9µm and the reduction in granuloma diameter was 45.6%, whereas the mean granuloma diameter in SmCB immunized infected and treated group was 144.40±6.8 µm and the reduction in granuloma diameter was 48.5% while in treated infected group the granuloma diameter was 224.35±7.2µmas shown in Table 6 and Fig. 2.

DISCUSSION

Schistosome cercariae must penetrate skin as an initial step to successfully infect the final host. Proteolytic enzymes secreted from the acetabular glands of cercariae contribute significantly to the invasion process. Nowadays, the researches of molecular mechanism of schistosome infection mainly focus on the cercarial secretions including serine protease and cysteine protease [24]. Previous researches already showed that *S. mansoni* penetrates the skin mainly depend on cercarial elastease secreted by cercariae while *S. japonicum* penetrates the skin chiefly by cathepsin B2. The illustration of molecular mechanism of schistosome cecariae infection will accelerate the identification of novel vaccines and drug targets [25]. Assuming that schistosome peptidases, besides, being likely vaccine targets may possess inbuilt adjuvant properties that could enhance their efficacy because of their intrinsic proteolytic activity. Consequently, this study aimed to detect the potential effect of cathepsin B alone and in combination with PZQ in two phases of the acute and chronic phases of the disease.

Many studies approached a possible way to amend the disease severity or morbidity by inhibiting the host reaction around *S. mansoni* eggs. The present study was designed to assess the possible disturbance of fibrosis deposition in *S. mansoni* egg-induced hepatic granuloma and evaluate the potentiality of a biological molecule such as cathepsin B to ameliorate the consequences of *S. mansoni* infection. Thus, the cellular immunity (Serum IL-2 and IL-10), humoral immunity (IgG and IgE), fibrotic markers (ALT, AST, hepatic hydroxyproline content) and egg count in tissues (liver and intestine) as well as hepatic histopathology were determined in this research paper. This current study demonstrated that immunization with Cathepsin B resulted in a remarkably high level of protection when challenged with *S. mansoni* infection, reduction in the worm load, hepatic and intestinal ova together with a change in oogram pattern and a decrease in the deposited hepatic eggs at both 8 and 12 weeks post-infection. This could be due to enhancement of immune response or would be acting as assort of primary infection that somewhat hinders the challenge one. The treatment of PZQ alone or combined with Cathepsin-B in immunized infected animals caused almost similar high percentage of eradication of worms and tissue egg load; results in agreement with previous studies [26, 27]. The death of the worms due to the treatment with antischistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of the treated worms [28]. At the same time, percent reduction in the egg count in both immunized infected and treated groups was found to be higher in the intestinal tissue than in hepatic tissue at both 8 and 12 weeks post-infection. This variation could be attributed to the excretion of some ova from the intestine prior to digestion and to hepatic shift of worms after treatment [28, 29]. We have found that pre-immunization with Cathepsin-B induced a protective effect which was manifested by increased levels of specific immunoglobulins, as well. Pre-immunization led to increasing the levels of IgG and IgE levels in the sera of mice, 8 and 12 weeks post infection. All treated groups had increased levels of IgG and IgE, but slight increase in the level of IgG was observed in PZQ-treated mice or Cathepsin B-pre-immunized and PZQ-treated infected animals at 8 than at 12 weeks post-infection. This increase in the production of immunoglobulins have an important role in the improvement of the pathology and the reduction in the ova count and worm burden [30, 31]. SmCB1could act as an adjuvant to elicit an antigen-specificTh2 immune response to co-injected parasite molecules. SmCB1 also proposed that as immunization with SmCB1would result in the production of cathepsin B-specific antibodies, it would likely elicit a higher level of protection [26]. Through analysis of the experiment carried out by de Oliveira *et al*. [32] show that the *S. mansoni* cysteine protease SmCB1 is rapidly targeted by an antigen-specific IgE response. The induction of this response is independent of schistosome eggs as infection with male or female worms alone also induced SmCB1-specific IgE and the SmCB1-specific IgE response is dependent on cognate CD4+ T cell help and IL-4, suggesting that prepatent Th2 responses provide T cell help for the SmCB1-specific IgE response.

Mice infected with *S.mansoni* showed a time-dependent increase in serum ALT and AST levels compared with their control group. The highest increase in the serum ALT and AST were found after 12 weeks. The elevation of serum ALT and AST of infected mice seems to be a consequence of the damage of hepatic cells and/or impaired permeability of cell membranes, or may be due to heavy schistosome egg deposition. Immunization of infected mice with (Sm CB) alone or combined with praziquantel revealed significant decrease in the serum ALT and AST when compared with their respective infected group. The maximum decreases were found after 12 weeks. These results are in accordance with others [33, 34] who found that infection of mice with

Schistosoma intact cercariae elevated serum ALT and AST levels compared with the normal control group, whereas post infected-treated Praziquantel mice revealed a decline in the serum ALT and AST toward the normal values at week 16 post infection. In addition, the total collagen contents of granulomatous livers (hydroxyproline content) estimated in this study showed that pre-immunization of infected mice with (SmCB) significantly decreased the hepatic hydroxyproline content. The maximum decrease was found after 12 weeks. It was interesting to find that Cathe-B affected the level of hepatic hydroxyproline content and had a potent effect on histopathological changes in the liver section. This finding was confirmed histopathologically and immunohistochemically in the H&E-stained liver sections of immunized infected mice that preserved significant changes in the fibroplasia, hepatocellular necrosis and inflammatory cell infiltration in the liver when compared with infected fibrotic group. In agreement with this result [35] it was found that, treatment of mice with praziquantel alone did not show any significant changes in the liver sections compared with infected non-treated mice while immunized mice modulate the liver pathology.

Cytokines which act on lymphocytes are of special interest because of their role in regulating cells of the immune response [36]. During schistosomal infection, bothTh1and Th2 responses directed against egg antigen and produce IFN gamma, IL-4, IL-5 andIL-13 [37-39]. In this study, the diminished production of IL-10 (Th1 cytokine) and IL-12 (Th2 cytokine) in the immunized group may be implicated in the down modulation of the granulomatous response due to immunization [40].Groups immunized Cathepsin-B alone or treatment of PZQ showed significant decrease in IL-10 and non-significant decrease in IL-12 at 8 weeks pi. On the other hand, groups immunized with Cathepsin B alone or treatment with PZQ showed non-significant increase in both IL-10 and IL-12 at 12 weeks p.i. Recent studies suggest that Treg cells play a pivotalroleinsuppressingTh1celldevelopmentas well as limiting them magnitude of Th2 response directed against egg antigen by a process dependent uponIL-10 [39, 41, 42]. TheincreasinglevelofIL-10 is probably implicated in the down regulation of granuloma formation as it reduces the intra hepatic inflammatory response and hence it has an anti-fibrotic effect [43, 44]. These results indicate the importance of immunization with Cathepsin B as it has a potent anti fibrogenic role and slow down the progression of liver fibrosis [45, 46]. Recent studies recommended the immunization with protective antigen like Cathepsin-B in early stage of infection and in a long-term treatment [12, 13]. In conclusion, immunization of infected mice with (SmCB) antigen resulted in significant reduction of parasitological parameters and rise of specific Igs in addition immunization with (SmCB) potentiated an anti-pathology effects which minimized and ameliorated liver fibrosis. The data collected from this research study might be useful in developing potential vaccine against *S. mansoni* using Sm CB.

REFERENCES

1. Fu, C.L., J.I. Odegaard, D.R. Herbert and M.H. Hsieh, 2012. A novel mouse model of Schistosoma haematobium egg-induced immunopathology. PLoS Pathog, 8(3): e1002605.
2. Kloos, H. and R. David, 2002. The Paleoepidemiology of Schistosomiasis in Ancient Egypt. Research in Human Ecology, 9(1): 14-25.
3. Gryseels, B., K. Polman, J. Clerinx and L. Kestens, 2006. Human schistosomiasis. Lancet, 368: 1106-1118.
4. Singh, K.P., H.C. Gerard, A.P. Hudson, T.R. Reddy and D.L. Boros, 2005. Retroviral Foxp3 gene transfer ameliorates liver granuloma pathology in *Schistosoma mansoni* infected mice. Immunology, 114(3): 410–417.
5. Watanabe, K., P.N. Mwinzi, L.B. Carla, M.O. Erick, M.S.K. Diana, W.E. Secor and D.C.T. Colley, 2007. Regulatory cell levels decrease in people infected with *Schistosoma mansoni* on effective treatment. Am J Trop Med Hyg., 77(4): 676-682.
6. Shevach, E.M., 2002. CD4+ CD25+ suppressor T cells: More questions than answers. Nat Rev Immunol, 2: 389-400.
7. Sakaguchi, S., 2000. Regulatory T cells: key controllers of immunologic self-tolerance. Cell, 101: 455-458.
8. El Ridi, R., H. Tallima, S. Selim, S. Donnelly, S. Cotton, B.G. Santana and J.P. Dalton, 2014. Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. PLoS ONE, 9(1): e85401.
9. Asahi, H., A. Osman, R.M. Cook, P.T. LoVerde and M.J. Stadecker, 2000. *Schistosoma mansoni* phosphoenol pyruvate carboxykinase, a novel egg antigen: Immunological properties of the recombinant protein and identification of a T-cell epitope. Infect Immun, 68: 3385-3393.
10. Stadecker, MJ., 1994. The shrinking schistosomal egg granuloma: How accessory cells controlT cell-mediated pathology. Exp Parasitol, 79: 198-201.

11. Hassanein, H., M. Akl, Z. Shaker, H. el-Baz, R. Sharmy and I. Rabie, 1997. Induction of hepatic egg granuloma hyporesponsiveness in murine schistosomiasis *mansoni* by intravenous injection of small doses of soluble egg antigen. APMIS, 105: 773-783.
12. El Ridi, R. and H. Tallima, 2013. Vaccine-induced protection against murine schisto somiasis *mansoni* with larval excretory-secretory antigens and papain or type-2 cytokines. J. Parasitol., 99: 194-202.
13. El Ridi, R., H. Tallima, J.P. Dalton and S. Donnelly, 2014. Induction of protective immune response sagainst schistosomiasis using functionally active cysteine peptidases. Evolutionary and Genomic Microbiology, 5(119): 1-7.
14. Chapman, M.D., S. Wu¨nschmann and A. Pome´s, 2007. Proteases as Th2 adjuvants. Curr Allergy Asthma Rep., 7: 363-367.
15. Tang, H., W. Cao, S.P. Kasturi, R. Ravindran and H.I. Nakaya, 2010. The T-helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. Nat Immunol; 11: 608-617.
16. Liang, G., T. Barker, Z. Xie, N. Charles and J. Rivera, 2012. Naive T cells sense the cysteine protease allergen papain through protease-activated receptor 2 and propel TH2 immunity. J Allergy Clin. Immunol., 129: 1377-1386.
17. Cunningham, P.T., C.E. Elliot, J.C. Lenzo, A.G. Jarnicki and A.N. Larcombe, 2012. Sensitizing and Th2 adjuvant activity of cysteine protease allergens. Int Arch Allergy Immunol., 158: 347-358.
18. Bos, D.H., C. Mayfield and D.J. Minchella, 2009. Analysis of regulatory protease sequences identified through bioinformatic data mining of the *Schistosoma mansoni* genome. BMC Genomics, 10: 488.
19. Maizels, R.M., J.P. Hewitson and K.A. Smith, 2012. Susceptibility and immunity to helminth parasites.Curr Opin Immunol, 24: 459-466.
20. Duvall, R.H. and W.B. DeWitt, 1967. An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J. Trop. Med. Hyg., 16: 483-486.
21. Cheever, A.W., 1968. Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma mansoni* eggs in tissues. Bull World Health Organ., 39: 328-331.
22. Pellegrino, J., C.A. Oliveira, J. Faria and A.S. Cunha, 1962. New approach to the screening of drugs in experimental Schistosomiasis *mansoni* in mice. Am. J. Trop. Med. Hyg., 11: 201-215.
23. von Lichtenberg, 1962. Host response to eggs of *S. mansoni*. I. Granuloma formation in the un sensitized laboratory mouse. Am. J. Pathol., 41: 711-731.
24. Reddy, G.K. and C.S. Enwemeka, 1996. A simplified method for the analysis of hydroxyproline in biological tissues. Clin. Biochem, 29: 225-229.
25. Wilson, R.A. and P.S. Coulson, 2006. Schistosome vaccines: a critical appraisal. Mem Inst Oswaldo Cruz, 101: S13-20.
26. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol, 28: 56-63.
27. Suleiman, M.I., E.I. Akarim, K.E. Ibrahi, A.M. Saad, A.E. Mohammed, B.M. Ahmed and S.M. Sulaiman, 2004. Antischistosomal effects of praziquantel, its alkaline hydrolysis and sun decomposed products on experimentally *S. mansoni* infected albino mice. (A) Efficacy assessment based on clinicopathological findings. J Egypt Soc Parasitol, 34: 131-42.
28. Doenhoff, M.J., J.R. Kusel, G.C. Coles and D. Cloll, 2002. Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? Trans R Soc Trop. Med. Hyg., 96: 465-469.
29. Abdel-Ghaffar, O. and A.A. Qurtam, 2001. Parastiological, hematological and biochemical assessment of the efficacy of Ro 15-5458 against the Egyptian strain of *Schistosoma mansoni* in mice. J. Egypt Ger Soc Zool., 36: 571-603.
30. Abdel-Ghaffar, O., 2004. Assessment of the efficacy of Ro 16-2308 against the Egyptian strain of *Schistosoma mansoni* in mice: Parasitological, hematological and biochemical criteria. Egypt J. Zool., 42: 173-203.
31. El- Ahwany, E.G., M.M. Nosseir and I.R. Aly, 2006. Immunompulmonary and hepatic granulomatous response in mice immunized with purified lung- stage schistosomulae antigen. J. Egypt Soc Parasitol., 36(1): 335-350.
32. de Oliveira, L.A., E.W. Lamb, E.C. Moreno, M. Chatterjee1, J. Dvoøák, M. Delcroix, M. Sajid, C.R. Caffrey and SJ Davies, 2010. Rapid induction of IgE responses to a worm cysteine protease during murine pre-patent schistosome infection.BMC Immunology, 11: 56.

33. Mahmoud, M.A., M.A. El-Shafei, R. Mtta and A.A. Hany, 2002. Parasitological and immunological changes in murine hepatic schistosomiasis before and after praziquantel treatment. J. Egypt Soc Parasitol, 32: 551 -560.
34. Elghrabawy, R.M., 2011. Protection AgainstSchistosomiasis-Induced Hepatic Fibrosis By Modulating The Immune System. WebmedCentral PHARMACOLOG, 2(1):WMC001498
35. Chen, H., Y.W. He, W.Q. Liu and J.H. Zhang, 2008. Rosiglitazone prevents murine hepatic fibrosis induced by *Schistosoma japonicum*. World J. Gastroenterol, 14(18): 2905-11.
36. Kim, J.J., V. Aggvoo, M.L. Bagarazzi, M.A. CHattergoon, K. Dang, B. Wang, J.D. Boyer and D.B. Weiner, 1997. In vivo Engineering of a cellular immune response by co-administration of IL-12 expression vector with a DNA immunogn. J. Immunol, 158: 816-826.
37. Hoffmann, K.F., T.A. Wynn and D.W. Dunne, 2002. Cytokine mediated host responses duringschistosome infections; walking the fine line between immunological control and immunopathology. Adv. Parasitol, 52: 265-307.
38. Sadler, C.H., L.I. Rutitzky, M.J. Stadecker and R.A. Wilson, 2003. IL-10 is crucial for the transition from acute to chronic disease state during infection of mice with *Schistosoma mansoni*. Eur J. Immunol., 33: 880-888.
39. Stadecker, M.J., H. Ashai, E. Finger, H.J. Hernandez, Rutitzky and J. Sun, 2004. The immunobiology of Th1 polarizaiton in high-pathology Schistosoniasis. Immunol Rev, 201: 168-179.
40. Chensue, S.W., P.D. Terebuch, K.S. Warmington, S.D. Hershey, H.L. Evanoff and L. Kunkel, 1992. Role of IL-4 and IFN- γ in *S. mansoni* egg-induced hypersensitivity granuloma formation. Orchestration, relative contribution and relationship to macrophage function. J. Immunol., 148: 900-906.
41. Hori, S., T. Nomura and S. Sakaguchi, 2003. Control of regulatory T cell development by the transcription factor Foxp 3. Science, 299: 1057-1061.
42. Wynn, T.A., R.W. Thompson, A.W. Cheever and M.M. Mentink Kane, 2004. Immunopathogenesis of schistosomiasis. Immunol Rev., pp: 156-167.
43. Nelson, D.R., Tu 2 and C soldevila-Pico, 2003. long-term interleulin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology, 38: 859-868.
44. Thompson, K., J. Maltby and J. Fallowfied, 1998. Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. Hepatology, 28: 1597-1606.
45. Afdhal, N.H. and D. Nunes, 2004. Evaluation of liver fibrosis: a concise review. Am J. Gastroenterol., 99: 1160-1174.
46. Jhy-wen wu, Lie-chwen Liu and Tung-Hu Tsai, 2009. Drug- drug interactions of silymarin on the perspective of pharmacokinetics. J. Ethn Pharm., pp: 185-193.