

## Identification and characterization of a novel tegumental cathepsin B antigen from adult *Schistosoma mansoni* and assessment of its role in modulation of murine schistosomiasis.

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**Abstract:** Cysteine proteases are important virulence factors for parasites. Cathepsin B (SmCB) proteases are abundant in different stages of *Schistosoma* and it has 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 (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 five groups of 10 mice each; four batches of them were infected with a 100 *S. mansoni* cercariae as follows: One batch served as infected control, another one was immunized prior to infection, the third was pre-immunized and post-PZQ treated and finally the fourth one was post-treated with PZQ only. The fifth group was mice immunized with cathepsin B only. All groups were sacrificed 8 and 12 weeks post infection. 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*.

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### 1. 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. The systematic identification of immunogenic egg components is important to understand the specific basis of egg-induced immuno-pathology in schistosomiasis. In recent years, a new category of CD4+ CD25+ T regulatory lymphocytes have been

identified that maintain immune tolerance to self-antigens and they are involved in 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 schistosome 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 injection of purified SmCB antigen of *S. mansoni* adult worms into mice prior to infection with *S. mansoni* cercariae as an experimental trial to decreasing or modulating severe hepatic morbidity.

## 2. Material 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. Outbred 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

### 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 intraperitoneally with 100µg/ml of (Sm CB) antigen emulsified with complete Freund's adjuvant. Then, the animals were boosted two weeks later with 50µg/ml of SmCB emulsified with incomplete Freund's adjuvant and boosted again one week apart. However, groups 4 and 5 were additionally infected subcutaneously with 100 *S. mansoni* cercariae, one week after last immunization and only group 5 was further treated by PZQ at the 5<sup>th</sup> week post-infection (p.i.). Group 6 of mice was treated only with PZQ at the week 5<sup>th</sup> post-infection. Ten mice of each groups 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  $\pm$  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  $\times$  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 $\times$ 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  $\times$  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,  $\approx 100$  mg of liver tissue was incubated overnight at  $37^{\circ}\text{C}$  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  $\mu\text{l}$  of tissue sample suspension) were then removed/ sampled. To each sample, 160  $\mu\text{l}$  of 10 N NaOH and 40  $\mu\text{l}$  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 250  $\mu\text{l}$  aliquots of each sub-sample/liver were then removed and supplemented with 250  $\mu\text{l}$  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, 500  $\mu\text{l}$  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  $65^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$  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) of 46.2 % 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 tissue (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 reduction in 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).

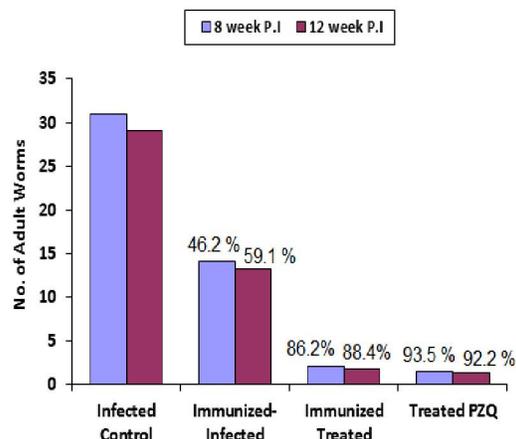


Fig.1 Effect of immunization on worm burden at 8 & 12 weeks post-infection in animal groups

#### 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 (Sm CB) 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).

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

Animal groups	Oogram pattern		
	Immature stage	Mature stage	Dead ova
Infected Control Group			
Week 8	45.2±0.22	44.36±11	10.44±0.2
Week 12	41.9±0.42	48.3±0.3	09.8±0.1
SmCB-Immunized Infected Group			
Week 8	37.1±0.33	44.7±0.53	18.2±1.0*
Week 12	32.9±0.28	40.7±0.54	26.4±5.1**
SmCB-Immunized& Treated Group			
Week 8	20.1±0.39 **	7.4±0.53**	72.5±0.53***
Week 12	16.5±0.73**	8.1±0.33**	75.4±0.53***
Infected Treated Group			
Week 8	22.4±0.19**	6.8±0.13***	70.8±0.23***
Week 12	20.5±0.14**	7.2±0.21***	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*.

Animal groups	8 week		12 week	
	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.

### 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 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 8 and 12 weeks p.i. compared to those of the untreated infected mice, maximum effects were again found at 8 weeks p.i.

**Table 3.** Effect of immunization of SmCB and PZQ on hepatic hydroxyproline ( $\mu\text{g/g}$  tissue) in mice infected with *S. mansoni* 8- & 12-wks post-infection.

Animal groups	Hepatic Hydroxyproline Content	
	8 week	12 week
Normal Control Group	150.50 $\pm$ 7.43	143.70 $\pm$ 5.83
Infected Control Group	386.40 $\pm$ 4.95	1088.80 $\pm$ 7.60
Immunized Control Group	161.12 $\pm$ 2.25*	147.22 $\pm$ 2.4**
SmCB-Immunized Infected Group	201.25 $\pm$ 5.45*	776.34 $\pm$ 6.6**
SmCB-Immunized Treated Group	188.00 $\pm$ 7.58*	544.40 $\pm$ 16.78*
Infected Treated Group	242.30 $\pm$ 6.81*	864.35 $\pm$ 10.82*

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

**Table 4:** Effect of immunization of (Sm CB) antigen and PZQ on serum IL-10& IL-12.

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

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

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

#### Granuloma diameters:

At 8 weeks p.i. the mean granuloma diameter in infected control group was 390.34  $\pm$  0.49  $\mu\text{m}$  while in SmCB immunized infected group, it was 212.22  $\pm$  0.22  $\mu\text{m}$ , and the reduction in granuloma diameter was 45.6%, whereas the mean granuloma diameter in SmCB immunized infected and treated group was 201.42  $\pm$  0.35  $\mu\text{m}$  and the reduction in granuloma diameter was 48.4%, while in treated infected group the granuloma diameter was 322.30  $\pm$  6.81  $\mu\text{m}$  as

showing in Table 6. At 12 weeks post-infection the mean granuloma diameter in infected control group was 280.41  $\pm$  0.92  $\mu\text{m}$  while in SmCB immunized infected group, it was 176.34  $\pm$  3.9  $\mu\text{m}$ , and the reduction in granuloma diameter was 45.6%, whereas the mean granuloma diameter in SmCB immunized infected and treated group was 144.40  $\pm$  6.8  $\mu\text{m}$  and the reduction in granuloma diameter was 48.5% while in treated infected group the granuloma diameter was 224.35  $\pm$  7.2  $\mu\text{m}$  as shown in Table 6.

**Table 6.** Effect of immunization of SmCB and PZQ in granuloma diameters ( $\mu\text{m}$ )

Animal groups	Granuloma diameters			
	8 week	% R	12 week	%R
Normal Control Group	-	-	-	-
infected Control Group	390.34 $\pm$ 0.49	-	280.41 $\pm$ 0.92	-
Immunized Control Group	-	-	-	-
SmCB-immunized Infected Group	212.22 $\pm$ 0.22*	45.6%	176.34 $\pm$ 3.9**	37.1%
SmCB-immunized treated Group	201.42 $\pm$ 0.35 *	48.4%	144.40 $\pm$ 6.8*	48.5%
Infected Treated Group	322.30 $\pm$ 6.81*	17.4%	224.35 $\pm$ 7.2*	20.0%

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

## 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 [14]. Previous researches already showed that *S. mansoni* penetrates the skin mainly depend on cercarial elastase secreted by cercariae while *S. japonicum* penetrates the skin chiefly by cathepsin B2. The illustration of molecular mechanism of schistosome cercariae 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 cathepsinB 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 of Cathepsin B resulted in a remarkably high levels 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 Bin 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 CathepsinB 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-preimmunized 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]. SmCB1 could act as an adjuvant to elicit an antigen-specific Th2 immune response to co-injected parasite molecules. SmCB1 also proposed that as immunization with SmCB1 would 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 (Sm CB) 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, both Th1 and Th2 responses directed against egg antigen and produce IFN gamma, IL-4, IL-5 and IL-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 downmodulation of the granulomatous response due to immunization [40]. Groups immunized with 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 pivotal role in suppressing Th1 cell development as well as limiting the magnitude of Th2 response directed against egg antigen by a process dependent upon IL-10 [39 & 41, 42]. The increasing level of IL-10 is probably implicated in the downregulation of granuloma formation as it reduces the intrahepatic 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 antifibrogenic role and slowdown 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.

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