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Original article

Immunostimulatory activity of schistosomula in mice

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Abstract: *Introduction* — Our previous study cleared that both immunization with Egyptian *Schistosoma mansoni* schistosomula and therapy with praziquantel were able to induce significant levels of protection against re-infection as compared with immunization or treatment only.
Aim — The current study aimed to evaluate the immunostimulatory activity of schistosomula in naïve mice post injection with schistosomula either alone or with praziquantel to answer the question: can schistosomula be used as immunoadjuvant?
Material and Methods — Ten naïve mice were administered 3 doses of phosphate buffered saline. Ten mice injected with 500 schistosomula at day 0 and at 14th day. Ten mice were administered praziquantel (PZQ) (250 mg/kg body weight).Ten mice injected with schistosomula at day 0 and at 14th day and received PZQ. The levels of IgM and IgG against soluble egg antigen (SEA) and soluble worm antigen preparation (SWAP) were detected by ELISA. Immunophenotyping of mesenteric lymph nodes (MLN) lymphocytes and thymocytes were carried out.
Results — IgM and IgG levels were significantly elevated in sera from mice injected with schistosomula alone or with PZQ or PZQ only. The mean percentage of MLN CD4$^+$, CD8$^+$ T and B lymphocytes were increased but considered not significant. The MLN CD4$^+$/CD8$^+$ T ratios were >1. The mean % of CD4$^+$ and CD8$^+$ thymocytes were increased and the CD4$^+$/CD8$^+$ thymocytes ratios were >1.
Conclusion — Immunostimulatory activity schistosomula was detected by enhancing Igs titers, stimulating the mean % of CD4$^+$, CD8$^+$ T, B-MLN cells and thymocytes CD4$^+$, CD8$^+$ T. CD4$^+$/CD8$^+$ T cells ratios were >1 in MLN and thymus gland.

Keywords: immunostimulatory, schistosomula, mesenteric lymph nodes, thymus, CD4+/CD8+ T lymphocytes ratio, B-lymphocytes.

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Introduction

Schistosomiasis, a debilitating disease caused by trematode blood flukes of the genus *Schistosoma*, is recognized as the most important human helminth infection in terms of morbidity and mortality. According to the world health organization, schistosomiasis affects about 250 million people worldwide [1].

Schistosoma mansoni infection occurs after direct contact with freshwater harboring free swimming cercariae which penetrate the skin of humans and enter capillaries and lymphatic vessels en route to the lungs. After several days, the schistosomula migrate to the portal venous system, where they mature and unite. Then migrate to superior mesenteric veins. Eggs are deposited in the vein lumen and pass into the host tissues, and then many pass through the intestinal mucosa and are shed in the feces [2].

Despite the existence of praziquantel (PZQ), schistosomiasis is spreading into new areas, PZQ chemotherapy does have limitations. In particular, mass treatment does not prevent re-infection. Furthermore, the prospect of relying on a single drug is of concern and the potential for drug resistance, particularly in areas of high transmission must be considered [3, 4].

Consequently, vaccine strategies represent an essential component for the future control of schistosomiasis as an adjunct to chemotherapy. These vaccines function mainly through inducing specific antibody responses or activating CD4$^+$-T cells against schistosomula or adult worms [5, 6]. However, the role of CD8$^+$-T cell responses has been considered as a potential aspect in the development of vaccine against schistosomiasis [7]. Evidence indicated that an Ag specific CD8$^+$-T cell response was induced in schistosome infected mice [8, 9].

Vaccines efficacy is directly related to adjuvant effect that prolong the immunological memory of vaccines and broaden the antibody repertoire. Adjuvants can drive the immune system to a Th1, Th2 or mixed Th1/Th2 response [10].

Lung schistosomula are more efficient stimulators of lymphocyte proliferation and secretion of Th1 cytokines than those from cercariae and skin-stage larvae [11, 12]. Immunization of mice with schistosomula tegument (Smteg) plus Freunds adjuvant, characterized by IFN-γ, IgG1 and IgG2c production, induced damage in parasite tegument [13, 14]. The objective of the present study is to determine the immunostimulatory activity of Egyptian *S. mansoni* schistosomula on cellular and humoral

immune responses. In addition, can schistosomula be used as immuno-adjuvant?

Material and Methods

Animals

A total of 40 female Swiss albino mice, 18-20 gram, were used. Animals were fed on standard chew, supplied with water and maintained at ambient temperature 25°C.

Schistosomula – Egyptian *S. mansoni* schistosomula were prepared from cercariae of *S. mansoni* strain according to James and Taylor [15].

Praziquantel – (Biltricide® - manufactured by Alexandria co. for Pharmaceuticals and Chemical. Ind., Alexandria, Egypt under license of BAYER Leverkusen, Germany) was suspended in 0.01 M phosphate buffered saline (PBS), pH 7.2.

Experimental groups

Group1: A total of 40 female Swiss albino mice were divided into ten naïve mice were orally administered 3 doses of phosphate buffered saline and maintained at the same condition to be used as normal group.

Group 2: Ten mice were subcutaneously injected with 500 schistosomula at day 0 and received the second dose at 14th day. Post 1st and 2nd injections, blood samples were collected from each individual mouse via optical vein and sera were separated.

Group 3: Ten mice were orally administered 3 doses of PZQ (250 mg/kg body weight). After each dose of PZQ, individual serum was separated.

Group 4: Ten mice were injected with schistosomula at day 0 and received the second dose at 14th day followed by administration of 3 doses of PZQ at the same time of the Group 2. After the last dose of PZQ individual blood samples were collected and sera were separated and frozen at -80°C till being used.

Determination of antibody titers in the mice sera by enzyme linked immune-sorbent assay (ELISA)

The levels of IgM and IgG in sera from animals were detected by ELISA according to Maghraby & Bahgat [16]. Plates were coated with (50 µl/well) of soluble worm antigen preparation (SWAP: 50 µg/ml) and soluble egg antigen (SEA: 50 µg/ml). Antigen free sites were blocked against non-specific binding using (100 µl/well) of phosphate buffered saline containing 0.05% Tween20-5% fetal calf serum (PBST-FCS) and incubated at 37ºC for 1 h. After thee washes with phosphate buffered saline containing 0.05% Tween-20 (PBS-T20), diluted sera (1:50) in PBST-FCS were applied (50 µl/well) and plates were incubated at 37ºC for 2 h. For total IgG detection, peroxidase conjugated anti-mouse IgG (H+L) was added (50 µl/well) at 1:500 dilution in PBST-FCS and plates were incubated at 37ºC for 1 h. For IgM Goat- anti mouse IgM (µ) conjugated with Horseradish peroxidase was added (50 µl/well) at 1:500 dilutions in PBST-FCS and plates were incubated at 37ºC for 1 h. For color development, a volume of (100 µl/well) of Orthophenylenediamine (OPD) (Sigma, St. Louis, Mo, USA) diluted in substrate buffer and left for 10 min at room temperature till color development. The enzymatic reaction was stopped using 50 µl of 2 M HCl and the changes in optical density (OD) were recorded at λ max 490 nm using a multi-well plate reader (139 Tecan; Sunrise,GmbH, Grödig, Austria).

Table 1. Determination of IgM level in sera from schistosomula or/and PZQ administered mice against SWAP

Experimental groups	*Mean ± SD*
Group 1	0.183 ± 0.038
Group 2	
- 1st dose of PZQ	0.406 ± 0.076*
- 2nd dose of PZQ	0.453 ± 0.086*
- 3rd dose of PZQ	0.586 ± 0.062**
Group 3	
- 1st injection	0.566 ± 0.157**
- 2nd injection	0.703 ± 0.054**
Group 4	0.635 ± 0.084**

*Significant values P<0.01; **Significant values P<0.001.
Group1: PBS administered naïve mice; used as negative control group.
Group 2: PZQ administered mice (1st, 2nd and 3rd dose).
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Immunophenotyping of different lymphocytes populations

Mesenteric lymph nodes (MLNs) and thymus were excised, gently teased in petri dishes containing PBST-FCS using glass slides. Cells from individual mouse were washed three times with PBST-FCS followed by centrifugation at 1500 g at 4°C for 10 min. CD4^{+}, CD8^{+} T-cell subsets were identified by labeling with fluorescence isothiocyanate (FITC) conjugated monoclonal anti-mouse CD4^{+}, CD8^{+} respectively (Biolegend San Diego, CA, USA) while, B-cells were labeled by FITC labeled anti-mouse IgG (H+L) chain (KPL). To calculate the mean percentage of CD4^{+}, CD8^{+} T- and B-lymphocytes, the green fluorescence stained lymphocytes were counted in a minimum of 100-200 viable cells using a fluorescence microscope (Zeiss Axioskop, Jena, Germany) according to Maghraby [17].

Statistical analysis

All obtained data were analyzed by the Students't-test using the Graph Pad InStat Software. The data were expressed as mean with standard deviation – Mean±SD. Data were considered significant when P-values were <0.05. The non significanct data were represented as: N.S.

Results

Determination of IgM level in sera from schistosomula or/and PZQ administered mice against SWAP

Post 1st and 2nd injections with schistosomula, IgM level in sera from schistosomula injected mice was significantly increased (P<0.001) as compared with negative control level. Although the 2nd dose (0.70±0.05) showed higher IgM level than 1st dose (0.57±0.16; fold=1.24), the difference was not considered significant (P>0.05) (*Table* 1).

The IgM level was significantly increased (P<0.01, 0.01, 0.001) in sera from mice post 1st, 2nd and 3rd doses of PZQ respectively as compared with normal level. Although the 3rd dose showed higher IgM response (0.59±0.06) than the 1st dose (0.41±0.08; fold=1.44) and the 2nd dose (0.45±0.09; fold=1.29), the differences among the 3 doses were not significant (P>0.05) (*Table* 1).

The IgM level in sera from schistosomula-PZQ administered mice showed significant increase (P<0.001) as compared with normal level. The IgM level in sera from this group (0.64±0.08) showed significant increase (P<0.05) as compared with that from

PZQ administered mice after 1st dose of PZQ (0.41±0.08). However, it showed non-significant increase (P>0.05) when compared with IgM level in sera from mice administered the 2nd (0.45±0.09; fold=1.4) or the 3rd (0.59±0.06; fold=1.08) doses of PZQ. It was also observed that the IgM level in sera from schistosomula-PZQ administered mice showed non-significant changes (P>0.05) as compared with schistosomula injected mice (*Table* 1).

Determination of IgG level in sera from schistosomula or/and PZQ administered mice against SWAP

Post 1st and 2nd injections with schistosomula, IgG level in sera from injected mice was significantly increased (P<0.001) as compared with normal level. Although the 2nd dose (0.63±0.07) showed higher IgG level than 1st dose (0.47± 0.07; fold=1.33), the difference was not considered significant (P>0.05) (*Table* 2).

The IgG level was significantly increased (P<0.001) in sera from PZQ administered mice post 1st, 2nd and 3rd dose as compared with normal level. Although the 3rd dose showed higher IgG response (0.54±0.03) than the 1st dose (0.46±0.08; fold=1.19) and the 2nd dose (0.47±0.14; fold=1.16), the differences among the 3 doses were not significant (P>0.05) (*Table* 2).

The IgG level in sera from schistosomula-PZQ administered mice showed a significant increase (P<0.001) as compared with normal level. However, the IgG level in sera from this group showed non-significant increase (P>0.05) when compared with IgG level in sera from PZQ administered mice after the 1st (0.46±0.08; fold= 1.3), 2nd (0.47±0.14; fold=1.27) and 3rd (0.54±0.03; fold= 1.09) doses of PZQ. It was also observed that the IgG level in sera from schistosomula-PZQ administered mice showed non-significant changes (P>0.05) as compared with schistosomula injected mice (*Table* 2).

Determination of IgM level in sera from schistosomula injected or/and PZQ administered mice against SEA

Post 1st and 2nd injections with schistosomula, IgM level in sera from injected mice was significantly increased (P<0.001) as compared with normal level. Although the 2nd dose (0.79±0.10) showed higher IgM level than 1st dose (0.68±0.13; fold=1.16), the difference was not significant (P>0.05) (*Table* 3). The IgM level was significantly increased (P <0.01, 0.001, 0.001) in sera from mice post 1st, 2nd and 3rd doses of PZQ respectively as compared with normal level. Although the 3rd dose showed higher IgM response (0.58±0.04) than the 1st dose (0.39±0.09; fold=1.49) and the 2nd dose (0.52±0.14; fold=1.13), the differences among the 3 doses were not significant (P>0.05) (*Table* 3).

The IgM level in sera from schistosomula-PZQ administered mice showed significant increase (P<0.001) as compared with normal level. The IgM level in sera from this group showed significant increase (P<0.01) as compared with that from mice administered the 1st dose of PZQ (0.39±0.09). However, it showed non-significant increase (P>0.05) when compared with IgM level in sera from PZQ administered mice after the 2nd (0.52±0.14; fold= 1.22) and the 3rd (0.58±0.04; fold=1.08) doses of PZQ. It was also observed that the IgM level in sera from schistosomula-PZQ administered mice showed non-significant changes (P>0.05) as compared with schistosomula injected mice (*Table* 3).

Table 2. Determination of IgG level in sera from schistosomula -or/and PZQ administered mice against SWAP

Experimental groups	*Mean ± SD*
Group 1	0.178 ± 0.032
Group 2	
- 1st dose of PZQ	0.458 ± 0.079**
- 2nd dose of PZQ	0.467 ± 0.014**
- 3rd dose of PZQ	0.544 ± 0.029**
Group 3	
- 1st injection	0.472 ± 0.073**
- 2nd injection	0.630 ± 0.066**
Group 4	0.595 ± 0.069**

**Significant values at *P*<0.001.
Group1: PBS administered mice; used as negative control.
Group 2: PZQ administered mice (1st, 2nd and 3rd dose).
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Table 3. Determination of IgM level in sera from schistosomula injected or/and PZQ administered mice against SEA

Experimental groups	*Mean ± SD*
Group 1	0.192 ± 0.039
Group 2	
- 1st dose of PZQ	0.393 ± 0.086*
- 2nd dose of PZQ	0.516 ± 0.135**
- 3rd dose of PZQ	0.584 ± 0.044**
Group 3	
- 1st injection	0.677 ± 0.126**
- 2nd injection	0.788 ± 0.102**
Group 4	0.628 ± 0.070**

*Significant values P<0.01; **Significant values P<0.001.
Group1: PBS administered naïve mice; used as negative control group.
Group 2: PZQ administered mice (1st, 2nd and 3rd dose).
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Table 4. Determination of IgG level in sera from schistosomula or/and PZQ administered mice against SEA

Experimental groups	*Mean ± SD*
Group 1	0.157 ± 0.038
Group 2	
- 1st dose of PZQ	0.439 ± 0.036**
- 2nd dose of PZQ	0.429 ± 0.046**
- 3rd dose of PZQ	0.592 ± 0.074**
Group 3	
- 1st injection	0.407 ± 0.061**
- 2nd injection	0.428 ± 0.015**
Group 4	0.452 ± 0.035**

**Significant values at *P*<0.001.
Group1: PBS administered mice; used as negative control.
Group 2: PZQ administered mice (1st, 2nd and 3rd dose).
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Determination of IgG level in sera from schistosomula or/and PZQ administered mice against SEA

Post 1st and 2nd injections with schistosomula, IgG level in sera from immunized mice was significantly increased (P<0.001) as compared with normal level. Although the 2nd dose (0.43±0.02) showed higher IgG level than 1st dose (0.41±0.06; fold=1.05), the difference was not significant (P>0.05) (*Table* 4). The IgG level was significantly increased (P<0.001) in sera from PZQ administered mice post 1st, 2nd and 3rd doses as compared with normal level. Although the 3rd dose showed higher IgG response (0.59±0.07) than the 1st dose

(0.44±0.04; fold=1.35) and the 2nd dose (0.43±0.05; fold=1.38), the differences among the 3 doses were not significant (P>0.05) (*Table* 4).

The IgG level in sera from schistosomula-PZQ administered mice showed significant increase (P<0.001) as compared with normal level. There were non-significant changes (P>0.05) in IgG levels in sera among schistosomula-PZQ administered mice (0.45±0.04) and either the schistosomula injected mice or the PZQ administered mice (*Table* 4).

Mean percentage of thymocytes from schistosomula or/and PZQ administered mice

Post 1st and 2nd injections with schistosomula, the mean percentage (%) of $CD4^+$ or $CD8^+$ thymocytes showed non-significant changes (P>0.05) as compared with normal thymocytes %. Although 2nd schistosomula injected mice showed higher mean % of $CD4^+$ or $CD8^+$ thymocytes (43.2±1.3, 35.0±1.4 respectively) than 1st schistosomula injected mice 42.0±2.0 (fold=1.03), 33.1±2.1 (fold=1.06) respectively, these increments were not considered significant (P>0.05) (*Table* 5).

PZQ administered mice showed non-significant change (P>0.05) in the mean % of $CD4^+$ or $CD8^+$ thymocytes as compared with naïve thymocytes mean % (*Table* 5).

Schistosomula-PZQ administered mice showed non-significant changes (P>0.05) in the mean percentage of $CD4^+$ or $CD8^+$ thymocytes as compared with the mean normal thymocytes %. They showed non-significant increments (P>0.05) in CD4+ thymocytes when compared with schistosomula 1st injected mice (42.0±2.0; fold=1.04) or 2nd injected mice (43.2±1.3; fold=1.009) or PZQ administered mice (42.3±3.3; fold=1.03). It was also observed that the mean % of $CD8^+$ thymocytes from schistosomula-PZQ administered mice (33.3±1.3) showed non-significant changes (P>0.05) as compared with the mean percentage of $CD8^+$ thymocytes from 1st schistosomula injected mice (33.1±2.1; fold=1.00) or 2nd schistosomula injected mice (35.0±1.4) or PZQ administered mice (35.1±2.7) respectively (*Table* 5).

Mean percentage of mesenteric lymph nodes (MLN) lymphocytes from schistosomula injected or/and PZQ administered mice

Post 1st and 2nd injections with schistosomula, the mean percentage (%) of $CD4^+$T or $CD8^+$T or B cells showed non-significant changes (P>0.05) as compared with the mean % of naïve control MLN cells. Although schistosomula 2nd injected mice (38.0±2.0, 44.2±3.8) showed higher mean % of $CD4^+$T and B cells respectively than 1st injected mice (37.3±3.1; fold=1.02, 38.0±3.9; fold=1.16), these increments were not considered significant (P>0.05). It was observed that schistosomula 2nd injected mice (28.7±0.6) showed significant increase (P<0.05) in the mean % of $CD8^+$T cells as compared with 1st injected mice (22.3±2.0) (*Table* 5).

PZQ administered mice showed non-significant change (P>0.05) in the mean % of $CD4^+$T or $CD8^+$T or B cells as compared with the mean % of naïve MLN cells.

Schistosomula-PZQ administered mice showed unsignificant increments (P>0.05) in the mean % of $CD4^+$T or $CD8^+$T or B cells as compared with the mean % of MLN lymphoctes. They also showed non-significant increments (P>0.05) in the mean % of $CD4^+$T cells as compared with the mean % of $CD4^+$T cells from schistosomula 1st injected mice (37.3±3.1; fold=1.11) or 2nd injected mice (38.0±2.0; fold=1.09) or PZQ administered mice (39.5±2.9; fold=1.05) respectively. It was also observed that the mean % of $CD8^+$T cells from schistosomula-PZQ administered mice showed significant increase (P<0.001) as compared with mean % of $CD8^+$T cells from 1st injected mice (22.3±2.0) or PZQ administered mice (24.8±1.8; fold=1.25) respectively. However, it showed non-significant increase (P>0.05) in $CD8^+$T cells mean % as compared with 2nd injected mice (28.7±0.6; fold=1.08). They also showed non-significant increments (P>0.05) when comparing the mean % of B-MLN cells with the mean % of B cells from 1st injected mice (38.0±3.9; fold=1.18) or 2nd injected mice (44.2±3.8; fold=1.01) or PZQ administered mice (38.3±5.2; fold=1.17) (*Table* 6).

Discussion

Our previous studies demonstrated that immunization with schistosomula associated with therapy were able to induce partial reduction in worm burden (68.5%) against *Schistosoma mansoni* re-infection and modulate the immunocellular response [18]. We observed that injection with schistosomula stimulated the humoral immune response in naïve mice whereas, IgM and IgG levels were elevated in mice post injection with schistosomula alone or with PZQ or PZQ. De Melo et al. [19] detected significant levels of anti-schistosomula tegument (Smteg) IgG antibodies in the sera of mice injected with Smteg compared to control group. This is also is in accordance with the results of de Melo et al. [20] who transferred sera from mice immunized with Smteg or Smp-80 to a naïve recipient and that was able to induce partial protection against challenge infection. Our data allowed us to speculate that, PZQ administration enhanced the humoral response in naïve mice. The IgM and IgG levels elevation observed in PZQ administered mice may reflect the sensitization of more lymphocytes by specific PZQ action, which is in accordance with the low toxicity and good tolerance of the drug in animals and healthy human volunteers [21].

Table 5. Mean percentage of thymocytes from schistosomula or/and PZQ administered mice

Experimental group	*$CD4^+$T* (Mean ± SD)	*$CD8^+$T* (Mean ± SD)	*$CD4^+/CD8^+$*
Group 1	42.0 ± 2.3	35.7 ± 1.5	1.178
Group 2	42.3 ± 3.3	35.1 ± 2.7	1.200
Group 3			
- 1st injection	42.0 ± 2.0	33.1 ± 2.1	1.269
- 2nd injection	43.2 ± 1.3	35.0 ± 1.4	1.234
Group 4	43.6 ± 1.9	33.3 ± 1.3	1.309

Group1: PBS administered mice; used as negative control.
Group 2: PZQ administered mice.
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Table 6. Mean percentage of mesenteric lymph nodes lymphocytes from schistosemula or/and PZQ administered mice

Experimental group	*$CD4^+$T-MLN* (Mean±SD)	*$CD8^+$T-MLN* (Mean±SD)	*$CD4^+/CD8^+$ T-ratio*	*B-MLN* (Mean±SD)
Group 1	38.1 ± 2.4	25.3 ± 4.3	1.51	36.4 ± 3.4
Group 2	39.5 ± 2.9	24.8 ± 1.8	1.59	38.3 ± 5.2
Group 3				
- 1st injection	37.3 ± 3.1	22.3 ± 2.0	1.67	38.0 ± 3.9
- 2nd injecttion	38.0 ± 2.0	28.7 ± 0.6	1.33	44.2 ± 3.8
Group 4	41.6 ± 4.1	31.1 ± 0.9	1.34	44.7 ± 2.5

Group1: PBS administered mice; used as negative control.
Group 2: PZQ administered mice.
Group 3: Schistosomula injected mice (1st and 2nd dose).
Group 4: Schistosomula-PZQ administered mice.

Cellular immune responses are also important in parasite elimination. Recent studies showed that protective immunity associated Th2 profile was observed in out-bred mice immunized with glyceraldehyde3-phosphate dehydrogenase (SG3PDH) and peroxiredoxin (TPX) [22]. Blocking IL-10 with neutralizing antibodies enables protection against challenge infection in mice previously infected with *S. mansoni* and treated with praziquantel [23]. In *S. japonicum* infection, blocking IL-17 with neutralizing antibodies enhances antibody production and protection in infected mice [24].

Although $CD8^+$T cells are classically related to immune responses against intracellular pathogens, its role in schistosome elimination has been recently described. Immunization of mice with the *S. japonicum* 22.6/26GST coupled to sepharose 4B bead induced a significant reduction in parasite burden that was associated with an increase in the number of activated $CD8^+$T cells. These activated $CD8^+$T cells were able to promote death of parasite carrying host the major histocompatibility 1 (MHCI) molecules in its surface [9]. *S. japonicum* calreticulin is one of the immunostimulatory molecules released from radiation-attenuated schistosomula cells, might play a crucial role in conferring a Th1-polarized immune response induced by radiation-attenuated cercariae/schistosomula in mice [25].

Conclusion

In the current our study, the schistosomula may be considered as immunostimulatory adjuvant by enhancing the humoral immunostimulating response of IgM and IgG levels. Furthermore, schistosomula stimulated the cellular immune response of thymus and mesenteric lymph nodes organs whereas the ratio of thymocytes and MLN-$CD4^+$/$CD8^+$ T-lymphocytes >1. Also, and MLN-B cells were evoked. Of particular interest to this study, the schistosomula-PZQ administration was able also to stimulate the humoral and cellular immune responses also, the ratio of MLN-$CD4^+$/$CD8^+$ T-lymphocytes >1 and the mean percentage of MLN B-lymphocytes was stimulated.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

Anesthesia procedures complied with ethical guidelines of the National Institutes of Health in the USA and were approved by the Medial Ethical Committee of the National Research Centre in Egypt with a registration's number 10135.

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