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Protective Role of Purified Somatic, Excretory and Secretory Antigens from Adult *Echinostoma liei* Against Infection of *Schistosoma mansoni*

[1]Ibrahim Aly, [2]Elsayed M. Bayoumy, [1]Gehan El Enain and [3]Abeer Mahgoub

[1]Department of Parasitology, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt (TBRI)
[2]Hydrobiology Researches Department National Research, Center Giza, Egypt
[3]Department of Parasitology, Faculty of Medicine Cairo University, Cairo, Egypt

Abstract: The development of a vaccine against schistosomiasis and also the availability of a more sensitive diagnosis test are important tools to help chemotherapy in controlling disease transmission. In this study, developing a novel immunization protocol against *Schistosoma mansoni* by using *Echinostoma liei* somatic -antigen (S-antigen) and excretory/secretory (E/S) products from the adult worms was evaluated on the basis of parasitological, histopathological and serological parameters. CFW1 SPE albino mice were deployed into four groups. The first group of mice was immunized with S-antigen, the initial dose was 100 µg /ml, followed two weeks later by 50 µg /ml dose and after one more week, they received another dose of 50 µg /ml. The second group of mice followed the same treatment schedule and doses but received S/E product instead of the S-antigen. Three days later, after the last dose administration, both groups of mice and the third one were all infected with 100 *S. mansoni* and sacrificed 8wk post-infection along with the normal non-infected group of mice. The data revealed a remarkably ($p<0.001$) reduction of the worm burden, egg loads and granuloma diameter in the tissue of the immunized groups of mice compared to their counterparts in the infected controls. However, the recorded significant change of worm burden, egg load and granuloma size was greater in the tissues of the mice immunized with purified excretory/secretory products than those of mice treated with S-antigen. On the other hand, detection of anti-SEA serum specific immunoglobulin G and M levels revealed a considerable elevation ($p>0.05$) compared to the IgG and IgM levels in the infected control mice. Likewise, a noteworthy surge ($p<0.001$) of anti-SEA specific immunoglobulin isotype IgG1 in in the sera of all immunized mice was demonstrated when compared to the IgG1 levels in the sera of the infected controls. To sum up, the multiple intraperitoneal immunization with S antigen or S/E products have been shown to engender protective and modulatory effects on murine schistosomiasis 8wks post-infection.

Key words: *Schistosoma Mansoni · Echinostoma liei · Somatic Extract · Secertory/Execretory Products · Immunization*

INTRODUCTION

Schistosomiasis is one of the prominent public health problems brought about infectious flukes (trematodes) of the genus Schistosoma. Three species produce the most frequent clinical diseases: *Schistosoma haematobium* (endemic in Africa and the Middle East), *S. mansoni* (in Egypt, northern and southern Africa, some West Indies islands, northern 2/3 of South America) and *S. japonicum* (in Japan, China, the Philippines, Celebes, Thailand, Laos) [1]. Merck Manual, 15th Ed). Schistosomiasis causes significant morbidity and mortality in the developing world with recent studies indicating that the geographic extent and burden of the disease exceeds official estimates [1-3]. Praziquantel-based chemotherapy has achieved some success in controlling the disease but is not an optimal strategy due to its inadequate impact on reducing long-term transmission [4], re-infection and prevalence [5].

Corresponding Author: Elsayed M. Bayoumy, Hydrobiology Researches Department, National Resarsh. Center Giza, Egypt.

Egypt, the MENA (Middle East North Africa) country, has more than 10 million persons infected with schistosome [5-7] and experiences the highest prevalence (~9% of the total population) of schistosomiasis [8] in MENA, in spite of using Praziquantel [9]. Consequently, there remains a critical need for the development of alternate approaches to control this crippling disease [9&10]. A schistosomiasis vaccine might effectively help encounter the disease, particularly, if it provides a potent, long-lasting immunity to the disease [2]. Homologous antigens have been used extensively in murine schistosomiasis to induce immunization, whereas, the heterologous antigens received less attention [11]. Antigens-induced immunization against schistosomiasis and fascioliasis was a promising approach as reflected by diminishing of the morbidity, mortality and transmission. In addition to protective effects with significant worm burden reductions, some vaccine candidates also have anti-fecundity, anti-pathology and anti-embryonation effects [11, 12].

Schistosomes and -Echinostomes are two groups of digenean trematodes that co-exist in snail populations [13, 14]. Despite the two parasites temporarily inhabit the snail intermediate host, schistosome complete their life cycle in the blood vessels of the definitive host (either birds or mammals) and echinostome requires one more snail intermediate host before infecting the definitive host (birds or mammals). It is worth noting that when a mutual snail host harbors the two parasites, echinostome typically dominates the interaction due to its larval attributes that are missed in the schistosomes [15-17]. The direct antagonism between the larval stages of these parasites has been well-established within the snail intermediate host [18-20], yet the effects of this conflict on other aspects of the interaction have received far less attention.

This research was designed to investigate the possible effect of vaccine-induced immunization against *S. mansoni* infection with purified somatic -antigen and excretory/secretory products from adult worms of *Echinostoma liei*.

MATERIALS AND METHODS

Schistosoma Mansoni Cercariae: *S. mansoni* cercariae were obtained from the Schistosome Biological Supply Program (SBSP) at TBRI; this strain has been passages through outbreed mice and *Biomphalaria alexandrina* snails, cared for and maintained at SBSP/TBRI.

Somatic Antigen Preparation: Adult worms were collected from the intestines of rats four weeks post-infection (wpi) with 100 metacercariae of *E. liei*. After thorough washings with phosphate buffered saline (PBS, pH 7.4), the worms were homogenized in culture medium of PBS containing 0.8 mM phenylmethylsulfonyl fluoride (Sigma), 100U penicillin (Sigma) and 100 µg/ml streptomycin (Sigma). After initial centrifugation at low speed to remove larger particles, the supernatant was centrifuged at 15000 g for 30 min at 4°C. The protein content was measured by the Bio-Rad protein assay and adjusted to 1 mg/ml [11].

Excretory/Secretory Products: Adult worms were collected as described above, yet the adult worms were maintained in the culture medium at concentration of 10worm/ml for 12 hrs at 37°C. The medium was collected and centrifuged as before and the supernatant was collected and concentrated to 1 mg/ml using an ultra filtration membrane (YM-3, Amicon). All prepared antigens were stored at –20°C for use [12].

Antigen Purification: The prepared S antigen and E/S products of *E. liei* were purified using cyanobromide-(CNBr-) activated sepharose column according to Axen *et al*. [21].

Animals: CFW1 SPE albino mice 6 weeks old, 18 – 20 g each, were bred at Theodor Bilharz Research Institute (TBRI), Cairo, Egypt and maintained under conventional conditions. Mice were deployed into four groups (10 in each). The first group of mice was primarily immunized with intraperitoneal dose (0.1 ml; 100µg /ml/mouse) of purified S- antigen of *E. liei* emulsified in a complete Freund's adjuvant. This immunization was boosted twice with 50 µg/ml of S-antigen in incomplete Freund's adjuvant such that the first boosting was two weeks after the initial administration and one week before the last boosting. The second group of mice was stimulated by the E/S products following the same schedule of time, dose and emulsification in the adjuvant. Three days later, these groups along with healthy mice were infected with 100 *S. mansoni* cercariae via tail immersion. A fourth group of healthy mice was maintained neither infected nor immunized and under the same laboratory conditions. All mice were sacrificed 8-weeks post infection for the parasitological, serological and histo-pathological studies.

Parasitological Criteria

Worm Burden: Hepatic and portomesenteric vessels were perfused [22] recover worms for subsequent counting.

Tissue Egg Load: The number of ova/gm hepatic or intestinal tissue was counted after digestion overnight in 5% KOH [23, 24].

Percentage Egg Developmental Stages "Oogram Pattern": The percentages of eggs at the developmental stages were examined in 3 samples/ mouse and the mean of each stage/animal was obtained [25].

Histopathological Parameters: Livers of mice were fixed in 10% buffered formalin, processed into paraffin blocks, serially cut at 4µm thickness and stained with hematoxylin and eosin. Hepatic granuloma measurements were done according to Von Lichtenberg [26] using an ocular micrometer for those containing a central ovum only [26].

Serological Parameters

Determination of Serum-Specific Immunoglobulins: Anti-SEA IgG, IgM, IgG1 and polyvalent mouse immunoglobulins were measured using indirect ELISA [27] based on the method of Engvall and Perlman [28]. To measure polyvalent immunoglobulins; IgG, IgM and IgG1; ELISA microtitre plates were coated with 250µ/well of 30 µg /ml of SEA and sera were diluted 1:100 for measuring the candidate Igs. The peroxidase conjugate, polyvalent rabbit anti-mouse Igs (Sigma) and monoclonal sheep anti-mouse IgG, IgM and IgG_1 (Binding site, Birmingham, UK) were used at a dilution of 1/1000, 1/3000, 1/5000 & 1/500, respectively. The reactions were read at optical density (OD) values 492 nm using an ELISA reader (Bio-Rad Micoplate Reader, Richmond, CA, USA).

Statistical Analysis: The data are presented as mean ± standard error of mean (X ± SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

RESULTS

Parasitology: Immunization with S antigen against *S. mansoni* infected mice showed a remarkable decline (31.4 % reduction) in the worm burden concomitant with higher percentage of reduction in the deposited intestinal and hepatic *S. mansoni* ova (69.7 % and 67.1 %, respectively), compared to the data collected from the infected controls. On the other hand, the worm burden evaluated in mice immunized with E/S products was significantly ($p<0.001$; 38.9%) lower than those of the S-antigen immunized mice and the infected controls. Moreover, the *S. mansoni* ova count in the intestine and liver was significantly ($p<0.001$) decreased (74.8 % and 70.8%) compared to that of the infected mice (Table 1) and still the % reduction of all parasitological parameters in E/S immunized mice is higher than those recorded for mice immunized with SE (Fig. 1).

Histopathology: As depicted in Table 2, the intraperitoneal immunization with S-antigen against *S. mansoni* infection mice had significantly ($p<0.001$) decreased (55.4%) the granuloma diameter (142.2 µm), yet, the E/S products reduced it (119.2µm) more (62.6 %) when compared to the mean granuloma diameter (318.8µm) of the infected controls (Fig. 2).

Sera-Specific Immunoglobulins: Table (2) shows that anti-SEA specific immunoglobulins Ig G and IgM considerably ($p<0.05$) increased in S-antigen- and E/S-immunized mice compared to the infected control group. Compared to the levels of Ig G1, the levels of the latter were the highest in the sera of the mice immunized with S-antigen products against *S. mansoni* infection.

DISCUSSION

The helminthes genus *Echinostoma* has a large geographic distribution due to their ability to parasitize a variety of invertebrate and vertebrate hosts. Some species develop their larval stages in *B. alxendrina* snails, the most important intermediate host of *S. mansoni* [17, 27]. The interference effect of the larval stages of schistosoma and echinostome was studied intensively in the co-infected snails [15, 17, 27, 29, 30], however, a few investigations evaluated the mutual immune response and its outcome in murine models [31, 32]. The interactions between *E. caproni* and *S. mansoni* in the homologous or heterologous definitive hosts were investigated. Mice pre-infected by *E. caproni* showed an increase in its natural resistance concomitant with a decrease of the *S. mansoni* load [31, 33, 34]. Echinostomes have been proposed as potential bio-schistosomiasis-control agents due to the capability of *E. liei* to inhibit the infection of *B. alexandrina* by *Schistosoma* miracidia [17, 20, 31, 32]. These successfully promising approaches that explained the relationship between Echinostoma and Schistoaoma have initiated our present study to investigate the potential efficacy of purified antigens from *E. liei* against *S. mansoni* infection in a vaccine-induced immunization model system.

Table 1: Worm burden and tissue egg load in the experimental groups

Assessment	Worm Burden		Ova Count			
Group	X ± SD	% Reduction	Intestine X ± SD	% Reduction	Liver X ± SD	% Reduction
Infected Control	29.6 ± 0.26	_____	13599±154	____	2967±425	____
S-antigen-immunized mice	20.3 ± 0.31**	31.4 %	3969± 295***	69.7%	978 ± 289***	67.03 %
E/S-immunized mice	18.1 ± 0.24***	38.9 %	3292 ± 254***	74.86 %	864 ± 278***	70.87 %

** Statistically significant difference at p< 0.01 compared to infected control group

*** Statistically significant difference at p< 0.001 compared to infected control group

Table 2: Effect of immunization with somatic -antigen and excretory/secretory products from adult worms antigen on hepatic granuloma diameter of mice infected with *S. mansoni*

Group Name	$\bar{x}$ GD ± SE	% Reduction
Infected Control	318.8 µm ± 26.3	-
Group I	142.2 µm ± 25.1	***
Group II	119.2 µm ± 29.5	55.4 % *** 62.6%

*** Statistically significant difference at p< 0.001, compared to the controls

Table 3: Determination of serum-specific immunoglobulins

Animal group	$\bar{x}$ O.D± SE Poly.Igs	$\bar{x}$ O.D± SE IgG	$\bar{x}$ O.D± SE IgG_1	$\bar{x}$ O.D± SE IgM
Control	0.309±0.12***	0.289±0.23***	0.200±0.178***	0.259±0.217
Infected control	1.19±0.126	0.598±0.211*	0.377±0.123**	0.409±0.190*
Group I	1.42±0.222	0.908±0.188	0.877±0.219	0.601±0.198
Group II	1.66±0.143*	1.28±0.222**	0.903±0.190**	0.877±0.232***

$\bar{x}$ O.D: Mean readings were conducted at 492 nm.

SE: Standard Error.

* Statistically significant difference at p< 0.01 compared to infected control group

** Statistically significant difference at p< 0.05 compared to infected control group

*** Statistically significant difference at p< 0.001 compared to infected control group

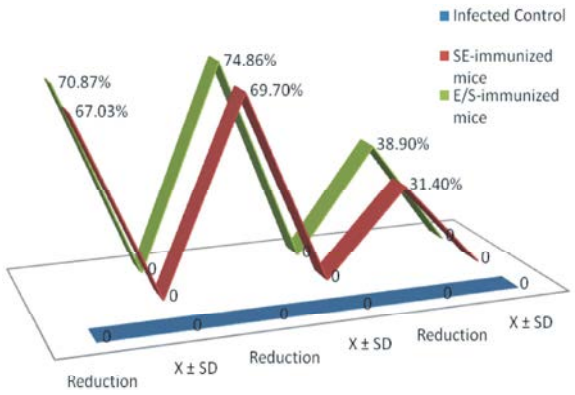

Fig. 1: Shows the efficay of vaccine-induced immunization on the worm burden and egg count int the experimental groups

Somatic -antigen and execretory/secretory -antigen of the adult worms of *E. liei* were the candidate antigens used in this experiment in combination with Freund's adjuvant to induce immunization against the *S. mansoni* infection in mice. The intraperitoneal injection of S-antigen and E/S products from *E. liei* in the infected mice yielded remarkable reduction in the worm burden, ova count and hepatic granuloma diameter when compared to the infected controls and the impact was more potent in the mice immunized with E/S. Likewise, using complete Freund's adjuvant and BCG with *S. mansoni* antigens reduced the number and size of the granulomas [33-36], which might boost the immunization effect in the both models. It's noteworthy that the prominent mechanism of BCG is to modulate the response of the T cells and the parasite damage by the lymphokine-activated macrophages [37, 39]. This kind of interaction upon exposure to the joint *S. mansoni* antigen and BCG could be also the same for *E. liei* S-antigen or E/S in combination with the Freund's adjuvant.

The decline of the parasites burden was concurrently associated with changes in the dynamics of specific immunoglobulins; the levels of all specific immunoglobulins increased in the infected mice sera compared to those of the uninfected controls, however, the significant increase was in favor of the total specific polyvalent Igs which may be attributed to the increase of other Igs as well as IgE. It had been reported that

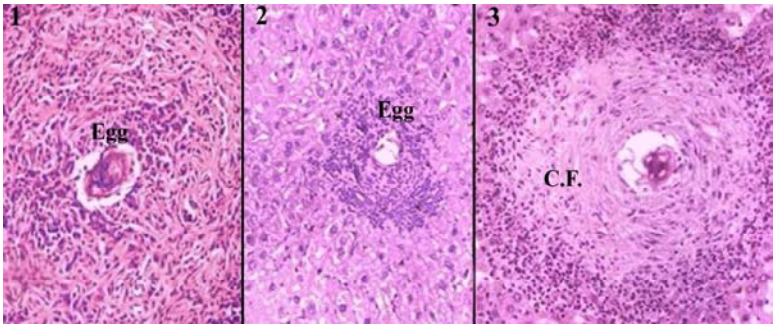

Fig. 2: Photomicrograph of liver granulomas of *S. mansoni* infected groups. (1): Infected somatic extract-immunized group, (2): Infected excretory/secretory products -immunized group, (3): Infected control group, (Haematoxylin and Eosin stain, X 200)

antibodies IgM, IgG and IgA classes are produced in response to worm antigens, even though the most significant immunoglobulin class involved in resistance to parasite worms is IgE [39]. The recorded surge of specific antibodies IgM, IgG and IgG_1 was associated with a great drop in ova count and worm burden. Likely, the B cell response was brought about in immunization models used *S. mansoni* soluble egg antigen [41, 42].

In essence, the current paper research might shed some of the light on the capability of *E. liei* antigens to antagonize the immune response to *S. mansoni* infection. Yet, we still need to investigate the T cell response and the cytokines secreted due to the immunization with S-antigen and E/S extracts from *E. liei* against *S. mansoni* infection.

REFERENCES

1. MERCK Manual 15th Ed. An encyclopedia of chemicals, drugs and biological. RSC publishing.
2. Patrick, D., D.L. Doolan, A. Loukas, P.L. Felgner and D. P. McManus, 2010. Schistosomiasis vaccine discovery using immunomics. Parasites and Vectors, 3(4): 1-5.
3. Rezende, C.M., M.R. Silva, I.G. Santos, G.A. Silva, D.A. Gomes and A.M. Goes, 2011. Immunization with rP22 induces protective immunity against *Schistosoma mansoni*: effects on granuloma down-modulation and cytokine production. Immunol. Lett., 141: 123-33.
4. Bergquist R., J. Utzinger and D.P. McManus 2008. Trick or treat: the role of vaccines in integrated schistosomiasis control. PloS. Negl. Trop. Dis., 2: e244.
5. King, C.H., 2010. Parasites and poverty: the case of schistosomiasis. Acta. Trop., 113: 95-104.
6. Hotez, P.J., 2009. The neglected tropical diseases and their devastating health and economic impact on the member nations of the organisation of the Islamic conference PLoS. Negl. Trop. Dis., 3: e539.
7. Hotez, P.J., L. Savioli and A. Fenwick, 2012. Neglected tropical diseases of the Middle East and north Africa: review of their prevalence, distribution and opportunities for control. PLoS. Negl. Trop. Dis., 6: e1475.
8. Utzinger, J., G. Raso, S. Brooker, D. De Savigny, M. Tanner and N. Ornbjerg, 2009. Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitolgy, 136(13): 1859-1874.
9. El Ridi, R.A.F. and H.A.M. Tallima, 2013. Novel Therapeutic and Prevention Approaches for Schistosomiasis: Review. J. Adv. Res., 4: 467-478.
10. Bennett, J.L., T. Day, F.T. Liang, M. Ismail and A. Farghaly, 1997. The development of resistance to anthelmintics: a perspective with an emphasis on the antischistosomal drug praziquantel. Exp. Parasitol., 87: 260-267.
11. Bashtar, A., S.A. Ahmed, A.M. Soliman and M.A. Hamed, 2006. Biochemical Studies on hepatocytes after immunization of mice with schistosomal worm and egg antigens. Asian J. Bioch., 1(3): 224-235.
12. El Ridi, R.A.F. and H.A.M. Tallima, 2013. Vaccine induced protection against murine *schistosomiasis mansoni* with larval excretory- secretory antigens and papain or type 2-cytokines. J. Parasitol., 99: 194-202.
13. Moravec, F and V. Barus, 1974. Antagonism of *Echinoparyphium recurvatum* against *Schistosoma haematobium* in the snail *Bulinus truncatus*. Folia Parasitol., 21: 127-41.

14. Sandland, G.J., J.K. Rodgers and D.J. Minchella, 2007. Interspecific antagonism and virulence in hosts exposed to two parasite species. J. Inv. Pathol., 22: 344-349.
15. Lie, K.J., D. Heyneman and K.H. Jeong, 1967. Studies on resistance in snails evidence of interference with defense reaction in *Biomphalaria glabrata* by trematode larvae. J. Parasitol., 62: 608-615.
16. Melanie, M.L. and R. Poulin, 2011. In vitro culture of marine trematodes from their snail first intermediate host. Exp. Parasitol., 129: 101-106.
17. Loker, E.S. and C.M. Adema, 1995. *Schistosomes, echinostomes*: comparative immunobiology. Parasitol. Today, 11: 120-124.
18. Mounkassa, J.B. and J. Jourdane, 1990. Dynamics of the leukocytic response of *Biomphalaria glabrata* during the larval development of *Schistosoma mansoni* and *Echinostoma liei* J. Inv. Pathol., 55: 306-211.
19. Mohammad-Amir, A., 2010. Effects of Mediterranean dry year conditions on the survival of trematode-infected snails. Dried and Infected Snails, pp: 1-19.
20. Lim, H.K. and D. Heyneman, 1972. Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control. Adv. Parasitol., 10: 191-268.
21. Axen, R., J. Porath and S. Ernbach, 1967. Chemical coupling of peptides and protiens to polysaccharides by means of cyanogen halides. Nature, 214: 1302-1304.
22. Duvall, R. and W. DeWitt, 1967. An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am. J. Trop. Med. Hyg., 6: 483-486.
23. Cheever, A., 1968. Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma mansoni* are dependent on host antibody response. J. Immunol., 139: 215-20.
24. Kamel, I., A. Cheever, A. Eiwi, J. Mosimann and R. Danner, 1977. *Schistosoma mansoni* and *Schistosoma haematobium* infections in Egypt. Technique for recovery of worms at necropsy. Anim. J. Trop. Med. Hyg., 26: 696-701.
25. Pellegrino, J., F.F. Lima-Costa, M.A. Carlos and R.T. Mello, 1977. Experimental chemotherapy of *Schistosomiasis mansoni*. XIII. Activity of praziquantel, an isoquinoline-pyrazinoderivate, on mice, hamsters and Cebus monkeys. Z. Parasitol., 52: 151-168.
26. Von Lichtenberg, F.C., 1962. Host response to egg of *Schistosoma mansoni*. I. Granuloma formation in the unsensitized laboratory mouse. Anim. J. Pathol., 41: 75-93.
27. Ossama, M.S., M.H.M. Abu ElEinin and H. M. Abdel Twab, 2013. Inorganic elements alteration in *Biomophalaria alexandrina* snails naturally parasitized with *Echinostoma -liei* or *Schistosoma mansoni*. Sc. Int., 1: 139-143.
28. Engvall, E. and P. Perlmann, 1971. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry, 8: 871-4.
29. Hoda, A.T., M.S.M. Osama and A. Souad, 2004. Host-parasite relationship between *Schistosoma mansoni* and *Echinostoma liei* and their intermediate host *Biomophalaria alexandrina* using rapid PCR analysis. J. Egypt. Soc. Parasitol., 34: 577-588.
30. Juberlan, S.G., M.J. Arnaldo, J.B. Cláudio, D.R.C. Ligia, M.L. Reinalda and M.Z.C. Paulo, 2010. The effect of early infection with *Echinostoma paraensei* on the interaction of *Schistosoma mansoni* with *Biomphalaria glabrata* and Bi*omphalaria tenagophila* Mem. Inst. Oswaldo. Cruz., Rio de Janeiro, 105: 499-503.
31. Garcia, J.S., A.M. Junior, C.J. Bidau, C.L. Dos Rei, R.M. Lanfredi and P.M.Z. Coelho, 2010. The effect of early infection with *Echinoshistosoma paraensei* on the interaction of *Schistosoma mansoni* with *Biomophalaria glabrata* and *Biomphlaria tenagophila*. Mem. Inst. Oswaldo. Cruz., Rio de Janeiro, 105: 499-503.
32. El-Dafrawy, S.M., A.T. El Din, F.A. Bakry and A.M. Dosouky, 2001. Effect of double infection with *Schistosoma mansoni* and *Echinostoma liei* on some physiological parameters of *Biomphalaria alexandrina*. J. Egypt. Soc. Parasitol., 31: 433-47.
33. Hassan M.M., N.E. Moustafa, M.H. El-Motayam, M.A. El-Settawy and A.A. Taha, 2009. Evaluation of protective immunity of 28KD-GST as a vaccine against experimental *Schistosoma mansoni.* J. Egypt. Soc. Parasitol., 39: 403-12.
34. Christensen N.Ø., A.B. Odaibo and P.E. Simonsen, 1988. Echinostoma population regulation in experimental rodent definitive hosts. Parasitol. Res., 75: 83-87.
35. James, S.L., 1986. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. III. Correlation of resistance with induction of activated larvacidal macrophages. J. Immunol., 136: 2872-2879.

36. Heyneman, D., H.K. Lim and U. Jeyarasa, 1972. Antagonism of *Echinostoma liei* (Trematoda-Echinostomatidae) against treamtodes *Paryphostomum segregatum* and Schistosoma mansoni. Parasitology, 65: 223-233.
37. Christensen N.Ø., P. Nansesn, B.O. Fagbemi and J. Monrad, 1987. Heterologous antagonistic and synergistic interactions between helminths and between helminthes and protozoans in concurrent experimental infection of mammalian hosts. Parasitol. Res., 73: 387-410.
38. James, S.L. and E.J. Pearce, 1988. The influence of adjuvant on Induction of protective immunity against *Schistosoma mansoni* by non-living vaccine against schistosomiasis. J. Immunol., 140: 2753-2759.
39. Chernin, J., 2000. Vaccines In: Parasitology. M. Taylor and T. Francis, (Eds.), School of Biological Science, University of Portsmouth, UK, New Fetter Lane, London. UK, pp: 116-117.
40. Paul, W.E., 1993. Infection disease and immune system. Sci. Anim., 9(269): 91-97.
41. Botros, S., H. Hassanein, S. Hassan, M. Akl, S.S. Sakr, Z.G. Hafez, N. Ghorab and D. Dean, 1995. Immunoregulatory potential of exogenous *Schistosoma mansoni* soluble egg antigen in a model of experimental schistosomiasis. I-regulation of granuloma formation in vitro. Int. J. Immunopharmac, 17(4): 291-302.
42. Hassanein, H., M.Z. Akl H.A. Shaker, F. ELBaz, I. Rabia, R. Sharmy and B. Doughty, 1997. Induction of hepatic egg granuloma hyporesponsiveness in murine *schistosomiasis mansoni* by intravenous injection of small doses of soluble egg antigen. APMIS, 105: 77-83.