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Immunoprophylactic effect of single and mixed schistosomal antigens on *Schistosoma mansoni* infected mice

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Abstract

B This study aimed to detect the cellular, humoral immune responses and protection against schistosomes induced by cercarial (CAP), soluble worm (SWAP), soluble egg (SEA), and mixed (SEA + CAP), (SEA + SWAP) and (CAP + SWAP) antigens to *Schistosoma mansoni* infection in mice, and the improvement in the liver enzyme activities before and after challenge with *S. mansoni*. Each mouse was sensitized with an initial immunization of 0.6 ml of the extracted antigen (30µg protein/ml). After one week, a second injection of 0.4 ml was given (20µg protein/ml). Then, each mouse was exposed to 80 cercariae. Six weeks post challenge the protection percentage was 42.5, 58.33, 53.33, 60.91, 78.16 and 64.77% for CAP, SEA, SWAP, (SEA +CAP), (SEA+SWAP) and (CAP + SWAP), respectively. The results revealed a high significant interrelation between number of lymph node cells (MLN) ($P \leq 0.001$), splenocytes ($P \leq 0.04$) and thymocytes ($P \leq 0.001$) that obtained with each immunized group compared to controls. A high significant difference ($P \leq 0.001$) between levels of IgG obtained between different antigen groups and the control before and after challenge. The immunization with previous antigens resulted in a remarkable improvement in the liver enzyme activities, which were disturbed after infection. Thus, vaccination of mice with the previous different antigens has an immunoprophylactic effect and may protect liver against infection through reduction in worm burden leading to the improvement of many liver enzymes.

Key words: Liver, Mesenteric lymph nodes, Schistosoma mansoni; Splenocytes, Thymocytes.

Introduction

Schistosomiasis is the second major parasitic disease in the world after malaria, affects 200 million people. Vaccine strategies represent an essential component of the control of this chronic debilitating disease where the deposition of millions of eggs in the tissues is the main cause of pathology. While there are many challenges in vaccine development, none is greater than that of developing vaccines against large metazoan parasites such as schistosomes, the parasitic worms that are responsible for schistosemiasis (Capron, 2003).

Research developed over the last 20 years has led to the identification of novel effector mechanisms, pointing for the first time to the protective role of the Th2 responses and of IgE antibodies now supported by seven studies in human populations. Despite the remarkable progress made in identifying and producing protective antigens, at present there are no generally accepted vaccines against parasitic disease because schistosomes are a multicellular parasites with differentiated tissues, and therefore antigenitically complex. The approach most favoured by investigators is to select a subset of total worm antigens and examine their reactivity with serum from infected humans or laboratory hosts. It is an implicit assumption that the subset of antigens chosen for investigation contains the targets of the protective immune response.

Immunoprecipitated 125I-labeled antigens solublized from 3 hrs-old

mechanically trans-formed schistosomula using serum from infected patients of St-lucian or African origin. They detected antigens of molecular mass of 38-32 and 20 KDa; the resistant status of their patients was unknown (Simpson and Smithers 1985; Simpson *et al*., 1986; Abath, 2000).

Immunization of New Zealand rabbit with an adult worm antigen extract is capable of inducing a response that results in a significant reduction of the mean worm burden of the primary infection earlier than did homologous infection, as compared to worm reduction due to a second infection (Tendler *et al*., 1991). Vaccination with SMW 68, and Mr 68,000 glycoprotein of *S. mansoni* induces a significant protection in mice against challenge schistosome infection. The protective protein or epitope was localized in three developmental stages of the parasite by immunoelectron microscopy using McAb31-3B6 and polyclonal antisera raised against purified SMW 68. In cercariae and schistosomula, McAb 31-3B6 bound electron granules within the head gland and similar granules in the preacetabular gland. In adult worms, SMW 68 or related antigens were found to be widely distributed in tissues (Blanton *et al*., 1991).

The identification and molecular cloning of target antigen, a glutathione S-transferase (GST), has made it possible to demonstrate its vaccine potential in several animal species (rodents, cattle, primates) and to establish consistently the capacity of vaccination to reduce female worm fecundity and egg viability through the production of neutralizing antibodies (IgA and IgG), with these results we are on the way towards a feasible approach of vaccine development against a major human parasitic disease (Capron *et al*., 2001).

With the schistosome genome projects at an advanced stage plus the power of the proteomics, perhaps it is still too early to call time on schistosome vaccine development (Hagan and Sharaf.,2003).

So this study is planned to detect the cellular, humoral immune responses and protection against schistosomes induced by cercarial (CAP), soluble worm (SWAP), soluble egg (SEA), and mixed (SEA + CAP), (SEA + SWAP) and (CAP + SWAP) antigens to *S. mansoni* infection in mice, and the improvement in the liver enzyme activities before and after challenge with *S. mansoni* effects as a step in schistosomal vaccination way.

Materials and methods

Animals

Female albino mice (strain CDI) obtained from Theodor Bilharz Institute, ranging in weight from 20-22 grams. They were fed on a standard protein rich pellet diet obtained from El-Kahira Company for Oil and Soap.

Experimental design

Mice were divided as the following groups, normal group and six immunized groups by different antigens, cercarial antigen (CAP), soluble worm antigen (SWAP), soluble egg antigen (SEA) and mixed antigens as soluble egg antigen mixed with cercarial antigen (SEA+CAP), soluble egg antigen + Soluble worm antigen (SEA+SWAP) and cercarial antigen + soluble worm antigen (CAP+SWAP) respectively. In each group eight mice were used for immunological and biochemical determination. In the 2nd part of this work mice were divided into 7 groups, which are control infected and challenge immunized mice by the *S. mansoni* for determination of enzymes AST, ALT, LDH, ALP and acid phosphatase. Animals were sacrificed 2 weeks post immunization to study the direct effect of these antigens on immune response and liver enzyme activities and 6 weeks post infection to study the improvement by vaccination.

Cercariae and infection

The used cercariae were the infective stage of an Egyptian strain *S. mansoni* Cercariae were collected from infected snails which had been induced to shed by exposure to light at least 10 infected snails were used to ensure bisexual infection (Awadalla *et al*., 1975). They were obtained from Theodor Bilharz Institute. Each mouse was exposed to 80 cercariae by using tail immersion method for 90 min (Oliver and

Stirewalt, 1952). Mice were housed in well-ventilated rooms kept between 22 and 23˚C.

Immunological analysis

Antigen Preparations

Cercariae, Soluble worm, Soluble egg antigens were prepared by the methods of Carter and Colley (1978), Salih et al. (1978) and Boros and Warren (1970), respectively.

Antigen administration regimes

The total protein content of each antigen preparation was determined by the method of Bradford (1976) and the final concentration adjusted to 50 µg/ml. The immunization schedule was performed according to the method of Nabih and Soliman (1986). Each mouse was sensitized with an initial intraperitoneal injection of 0.6 ml of the extracted antigen with total antigen concentration contained 30µg protein. After one week, a second intraperitoneal injection of 0.4 ml of the same antigen was given containing 20 µg/ml protein, hence each mouse received 50 µg/ml protein.

Preparation of splenocytes, thymocytes and mesentric lymph node cells

Splenocytes, thymocytes and mesenteric lymph nodes cells (MLN) suspension were prepared by teasing the spleen, thymus, MLN through a stainless steel mesh and were further disrupted by aspiration several times in a Pasteur pipette and then transferred to a plastic test tube. The remaining clumps of tissues were allowed to settle for 5 minutes (the same for thymocytes and mesentric lymph node cells). The supernatant was aspirated and centrifuged at 200 G, 5 minutes at room temperature. The cell pellet was resuspended in culture medium. Red blood cells (in case of spleen) were lysed with lysis buffer for 3 minutes at room temperature. The spleen cells were washed twice by centrifugation (200 G, 5 minutes) in culture medium. Assessing viability was performed by trypan blue dye, exclusion method in which only viable lymphocytes exclude the dye while dead cells appear blue. Equal volume (0.1 ml) of whole suspension and trypan blue were mixed and examined under LEITZ microscope using Neubaur haemocytometer. Viable lymphocytes were counted and viability was calculated.

Determination of immunoglobulins titre using enzyme linked immunosorbent assay (ELISA)

The developed sandwich enzyme linked immunosorbent assay (ELISA) was carried out according to Erhard et al. (1992) as follows: The ELISA plate was coated overnight with anti-IgG (2 µg/ml in PBS, pH 7.2), by putting 200 µl per well at 4˚C and blocked with 0.5% gelatin in PBS by putting 200 µl per well for 60 minutes at 37^{o}C. As a sample, standard serum was prediluted 1:5,000 and incubated at 37^{o}C with 100 µl per well for 90 minutes. The second, peroxidase marked antibody IgG dilute to 1:4000, then 100 µl per well at 37˚C for 90 minutes was applied as conjugate. The enzyme reaction was made visible again with ABTS buffer (substrate buffer), 100µl per well 2 mol/L HCl where the reaction was stopped with 5 µl per well 2 mol/L HCl and was measured at 492 nm with an ELISA reader (Dynatec MR 7000).

Assessment of worm burden

Mice were exposed to 80 S. mansoni cercariae post 2nd immunization and assessed for worm burden 6 weeks post infection by liver perfusion. Three separate experiments were performed. The perfusion methods described by Smithers and Terry (1965). The degree of protection was calculated as follows (Tendler *et al.*, 1986):
P = C - V / C (100).
Where: P = % protection, C = mean number of parasites recovered from infected mice and V= mean of parasites recovered from immunized mice.

Biochemical analysis

Measurement of alanine and aspartate aminotransferases (ALT & AST)

Alanine and aspartate aminotrans-ferases, Lactate dehydrogenase, Alkaline phosphatase, phosphate, Acid phosphatase were estimated in the homogenate of liver tissues by the method of Bergmeyer and Bernt (1974), Babson and Babson (1973),

Belfield and Goldberg (1971), Fiske and Subbarow (1925), Wattiaux and De Duve (1956) respectively.

Statistical analysis

Mean and standard Error of the obtained data from each different experimental group were calculated. One-way Analysis of Variance (ANOVA) was applied to the data versus the corresponding values of the control. Differences were higher than the theoretical one at $P \leq 0.05$

Histological analysis

Schistosomal livers of immunized mice were prepared for histological analysis according to Afifi (1986).

Results

Immunological responses

Cellular immune response

The statistical analysis of the observed data using one-way ANOVA test, revealed a high significant interrelation between values of the mean number of mesenteric lymph node cells (MLN), splenocytes and thymocytes that obtained with each injected group. As well, signif-icant difference was observed between the different injected groups (CAP, SWAP, SEA, (SEA+CAP), (SEA+SWAP) & (CAP+SWAP) and the control groups ($P\leq 0.001$) in (MLN), ($P\leq 0.04$) in splenoc-ytes and ($P\leq 0.001$) in thymocytes.

After challenge with 80 cercariae of S. mansoni, the statistical analysis of the observed data using one-way ANOVA test, revealed a high significant interrelation between values obtained with each immunized group, as well as, significant correlation was observed between the different immunized groups CAP, SWAP, SEA, (SEA+CAP), (SEA+SWAP), (CAP + SWAP), and control infected groups ($P\leq 0.001$) in lymph node cells, splenoc-ytes and thymocytes (Tables 1,2 and 3).

Humoral Immune Response

The statistical analysis of the observed data using one-way ANOVA test, revealed a high significant difference ($P\leq 0.001$) between values of the levels of IgG obtained from injected groups with different experimental antigens (CAP, SWAP, SEA, (SEA+CAP), (SEA+SWAP) & (CAP+SWAP) and the control group.

Post immunization mice were challenged by 80 S. mansoni cercariae. The statistical analysis of the observed data using one-way ANOVA test, revealed a high significant interrelation ($P\leq 0.001$) between the level of IgG in infected control group and CAP, SWAP, SEA, and (SEA+CAP), (SEA+SWAP) and (CAP+ SWAP) vaccinated groups. As well as significant difference ($P\leq 0.00001$) was observed between the level of IgG of different immunized groups as compared with each other. The calculation of the LSD revealed significant difference between the IgG titer of infected group and CAP, SWAP, SEA and mixed groups (SEA+CAP), (SEA+SWAP) and (CAP+SWAP) (Figures, 2 and 3).

Effect of different antigens on the mean number of worm burden after challenge with 80 cercariae of S. mansoni

In each six separate experiments, which were immunized by different antigen (CAP,SWAP,SEA, (SEA+CAP), (SEA+ SWAP) and (CAP+SWAP), mice were challenged by 80 live *S. mansoni* cercariae. Liver worm burdens were assessed 6 wk later by liver portal perfusion. In each group of representative experiments showed that a mean number of worms in different immunized groups which were significantly higher ($P\leq 0.001$) as compared to positive control. The percentage of protection were 42.5, 58.33, 53.33, 60.91, 78.16 and 64.77% for CAP, SEA, SWAP, (SEA +CAP), (SEA+SWAP) and (CAP + SWAP), respectivlty (Figure 1).

Histopathological effect of different antigens on liver after challenge by 80 cercariae S. mansoni

Lymphocyte infilterations and lymph-atic aggregations further increased in size and number in infected control mice as compared to immunized groups by different antigens (Table 4). There was a significant

decrease($P \leq 0.01$) in lymphatic infilteration and lymphatic aggregation in all immunized groups by single or mixed antigens as compared to infected non-immunized group. There was a significant decrease in granular number ($P \leq 0.01$) in CAP, SWAP, SEA, (SEA+CAP), (SEA+SWAP) and (CAP + SWAP) vaccinated groups as compared to infected non-vaccinated group (Table 4).

Biochemical analysis

Table (5) demonstrated the effect of single antigens on different liver enzyme activities.The statistical analysis of the observed data using One-way ANOVA test, revealed non-significant correlation between control and different vaccinated groups with single antigens. All enzymes recorded a significant decrease as compared to control group. For AST and ALT, they reached 13.11, 23.77, 10.65, 7.52, 18.28 and 8.60% after vaccination with CAP, SWAP and SEA, respectively. In case of LDH and ALP enzymes activities, both of them recorded 19.76, 19.12, 23.12, 8.13, 2.19 and 6.50% after injection with CAP, SWAP and SEA for LDH and ALP, respectively. Acid phosphatase enzyme activity recorded 5.71, 10.47 and 5.71% after vaccination with CAP, SWAP and SEA, respectively. The calculation of the least significant difference (LSD) revealed significant difference in LDH enzyme activity between control group and SEA group ($P < 0.001$).

Results presented in Table (6) revealed the effect of mixed antigens on the different liver enzyme activities. There was non-significant correlation between mixed vaccinated groups and the control group. For AST and ALT, enzymes activities, they recorded an insignificant decrease after vaccination with SEA+CAP, SEA+SWAP and CAP+SWAP amounting 18.88, 6.29, 6.99, 25.24, 4.85 and 10.67%, respectively. LDH and ALP recorded insignificant decrease reaching 3.57, 5.57, 0.57, 7.82, 1.68 and 3.86% after immunization with SEA+CAP, SEA+SWAP and CAP+SWAP, respectively. In case of acid phosphatase enzyme activity, it recorded an insignificant change amounting 9.27, 22.68 and 6.18%. Post vaccination with the same antigens, respectively. LSD revealed significant difference in AST enzyme activity between control and SEA+CAP group. Also, LSD revealed significant difference of SEA+ CAP and SEA+SWAP with each other. There was a significant difference by using LSD in case of ALT enzyme activity between SEA+SWAP and the control group ($P \leq 0.001$).

After challenge, AST and ALT showed a significant increase in vaccinated group of CAP, SWAP and SEA amounting 13.68, 26.31 and 20.00% for AST and 31.08, 13.51 and 36.48% for ALT, respectively. A significant increase in LDH and ALP was also recorded for CAP, SWAP and SEA amounting 4.93, 8.50 and 10.37 for LDH and 11.58, 21.03 and 15.09% for ALP, respectively. In case of acid phosphatase a significant decrease was obtained in vaccinated group with CAP, SWAP and SEA reached 32.27, 40.21 and 34.92%, respectively. LSD revealed significant difference ($P \leq 0.001$) in ALT enzyme activity between infected group and CAP, SWAP groups. The LSD calculation decleared significance difference in ALP enzyme activity between infected group and CAP, SEA groups and also between SWAP and infected, CAP groups. In Case of AP, there was a significant difference by using LSD between infected group and CAP, SWAP, SEA, control. As well as between CAP, SWAP and control group ($P \leq 0.001$) (Table 7).

AST and ALT enzymes activities showed a significant increase after immuneization with SEA+CAP, SEA+SWAP and CAP+SWAP reaching 12.5, 4.54, 27.27, 13.69, 15.06 and 9.58%, respectively. LDH and ALP enzymes activities showed also significant increase reaching 13.83, 6.12, 6.08, 38.26, 29.26 and 16.31% post vaccination with SEA+CAP, SEA+SWAP and CAP+SWAP for LDH and ALP, respectively. In case of AP enzyme activity, it reached significant decrease after vaccination with SEA+CAP, SEA+SWAP and CAP+SWAP amounting 42.58, 40.21 and 37.03, respectively. LSD revealed a significant difference ($P < 0.001$) between different groups of mixed antigens with different enzymes as shown in Table (8).

Table 1: Mean number of lymph node cells before and after challenge with 80 cercariae of *S. mansoni*.

Antigens	Before challenge (M ± SD)	After infection (M ± SD)	P. Value
Control	29.38 ± 6.08	33.31 ± 3.30	N. S.
CAP	30.12 ± 1.62	39.07 ± 2.07	0.01*
SWAP	31.48 ± 3.92	40.35 ± 5.28	0.03*
SEA	30.61 ± 3.42	38.90 ± 4.29	N. S.
Control Mixed	29.52 ± 9.14	49.84 ± 1.19	0.001*
CAP + SEA	50.52 ± 1.60	56.19 ± 3.83	0.01*
SEA + SWAP	45.60 ± 1.26	67.08 ± 5.51	0.001*
CAP + SWAP	36.09 ± 4.71	70.91 ± 1.15	0.001*

- All values are means ± SD of each group.
- Eight mice in each group were injected with total antigen dose 50 µg protein/mouse
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigens, respectively.
- N.S. Non-significant values as compared to control before and after challenge.

* Significant values as compared to control before and after challenge at P≤0.03, 0.01 and 0.001.

Table 2: Mean number of splenocytes before and after challenge with 80 cercariae of *S. mansoni*.

Antigen	Before challenge (M ± SD)	After infection (M ± SD)	P. Value
Control	68.82 ± 2.05	134.70 ± 4.68	0.001*
CAP	76.02 ± 1.86	141.11 ± 2.27	0.001*
SWAP	74.98 ± 3.57	143.99 ± 1.50	0.001*
SEA	76.93 ± 1.88	148.16 ± 1.75	0.001*
Control Mixed	73.05 ± 3.50	153.62 ± 2.34	0.001*
CAP + SEA	75.19 ± 3.01	158.98 ± 2.24	0.001*
SEA + SWAP	74.13 ± 2.41	166.65 ± 2.42	0.001*
CAP + SWAP	77.81 ± 2.63	169.00 ± 4.57	0.001*

- All values are means ± SD of each group.
- Eight mice in each group injected with total antigen dose 50 µg protein/mouse
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigens, respectively.

* Significant values as compared before and after challenge at P≤ 0.001.

groups (P≤ 0.001) in thymocytes.

Table 3: Mean number of thymocytes cells before and after challenge with 80 cercariae of *S. mansoni*.

Antigens	Before challenge (M ± SD)	After infection (M ± SD)	P. Value
Control	38.36 ± 2.29	34.99 ± 3.82	0.02*
CAP	50.82 ± 0.79	41.40 ± 2.08	0.001*
SWAP	57.46 ± 6.85	47.41 ± 4.70	0.01*
SEA	58.01 ± 0.52	48.73 ± 4.56	0.04*
Control Mixed	42.47 ± 0.57	34.22 ± 0.90	0.0001*
CAP + SEA	56.80 ± 0.30	37.60 ± 4.65	0.001*
SEA + SWAP	58.02 ± 1.09	35.95 ± 3.98	0.001*
CAP + SWAP	44.60 ± 1.64	38.16 ± 1.98	0.001*

- All values are means ± SD of each group.
- Eight mice in each group injected with total antigen dose 50 µg protein/mouse
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigens, respectively.

Significant values as compared to control before and after challenge at P≤ 0.01, 0.02, 0.04, 0.001.

Table 4 : Mean number of lymphocyte infiltrations, lymphocytes aggregations and granuloma.

Test Groups	L.I (M± SD)	P ≤	L.A. (M± SD)	P ≤	Granuloma (M± SD)	P ≤
Infected	8.0 ± 0.70	-	9.75 ± 1.47	-	10.75± 1.92	-
CAP	2.87 ± 1.05	h.s.	3.08 ± 1.10	h.s.	7.0 ± 1.87	s.
SWAP	5.75 ± 2.16	h.s.	3.36 ± 2.11	h.s.	6.5 ± 2.69	s.
SEA	2.75 ± 1.47	h.s.	3.4 ± 2.04	h.s.	7.75 ± 2.70	s.
SEA + CAP	3.37 ± 1.70	h.s.	3.91 ± 2.13	h.s.	6.25 ± 2.16	s.
SEA + SWAP	2.87 ± 1.76	h.s.	4.36 ± 2.58	h.s.	6.5 ± 2.29	s.
CAP + SWAP	2.95 ± 1.70	h.s.	4.26 ± 2.49	h.s.	7.5 ± 3.20	s.
(LI) Lymphocytes infiltration			(L. A.) Lymphocytes aggregation			

- All values are means ± SD of each group.
- Eight mice in each group injected with total antigen dose 50 µg protein/mouse
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigens, respectively.
- S. significant values as compared to control group.

h.s. high significant values as compared to control group.

Table 5: Effect of single antigens on enzymes activities after 2 wks of injection.

Enzymes Groups		AST	ALT	LDH	ALP	AP
Control	Mean ± SD LSD 1	1.22 ±0.36 -	0.93 ±0.09 -	7.74 ±0.84 (4)	12.3 ±0.53	1.05 ±0.17
CAP	Mean ± SD LSD 2	1.06 ±0.18 -	0.86 ±0.08 -	6.21 ±0.73 -	11.3 ±0.35 -	0.99 ±0.12 -
SWAP	Mean ± SD LSD 3	0.93 ± 0.11 -	0.76 ±0.06 -	6.26 ±0.64 -	12.03 ±0.45 -	0.94 ±0.07 -
SEA	Mean ± SD LSD 4	1.09 ± 0.26 -	0.85 ± 0.09 -	5.95 ± 0.29 1	11.5 ±0.56 -	0.99 ±0.14 -
	ANOVA	N.S.	N.S.	N.S.	N.S.	N.S.

- All values are means ± S.E. of each group and expressed as µ mol/min/mg protein.
- Eight mice in each group injected with total antigen dose 50 µ g protein / mouse.
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigen respectively.
- N.S.: Non-significant values as compared to control group.
- LSDno.: Least significant difference for various groups, control, CAP, SWAP, SEA (1,2,3,4), respectively.

Table 6: Effect of mixed antigens on enzymes activities after 2 wks of injection.

Enzymes Groups		AST	ALT	LDH	ALP	AP
Control	Mean ± SD LSD 9	1.43 ±0.18 (6)	1.03 ±0.24 -	7.0 ±0.85 -	10.16 ±1.12 -	0.97 ±0.26 -
SEA+CAP	Mean ± SD LSD 6	1.16 ±0.20 (7.9)	0.77 ±0.13 -	6.75 ±0.19 -	9.31 ±0.39 -	1.06 ±0.35 -
SEA+SWAP	Mean ± SD LSD 7	1.34 ±0.12 (6)	0.98 ±0.22 (9)	7.39 ±0.89 -	9.93 ±0.87 -	1.19 ±0.16 -
CAP+SWAP	Mean ± SD LSD 8	1.33 ±0.12 -	0.92 ±0.17 -	7.04 ±1.04 -	9.71 ±0.91 -	1.03 ±0.14 -
	ANOVA	N.S.	N.S.	N.S.	N.S.	N.S.

- All values are means ± S.E. of each group and expressed as µ mol/min/mg protein.
- Eight mice in each group injected with total antigen dose 50 µ g protein / mouse.
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigen respectively.
- N.S. Non-significant values as compared to control group.
- LSDno.: Least significant difference for various groups, (SEA+CAP), (SEA+SWAP), (CAP+SWAP) and control (6,7,8,9), respectiv

Table 7: Effect of different single antigens on enzymes activities after challenge with 80 cercariae of *S. mansoni*.

Enzymes Groups		AST	ALT	LDH	ALP	AP
Control	Mean ± SD LSD 9	1.43 ±0.18 (6)	1.03 ±0.24	7.0 ±0.85	10.16 ±1.12	0.97 ±0.26
Infected	Mean ± SD LSD 5	0.95 ±0.05 -	0.74 ±0.04 (6.7)	5.88 ±0.28 -	6.56 ±0.77 (6.8)	1.89 ±0.49 (6,7,8,9)
CAP	Mean ± SD LSD 6	1.08 ±0.08	0.97 ±0.12 (5)	6.17 ±0.37	7.32 ±0.47 (5,8)	1.28 ±0.22 (9)
SWAP	Mean ±SD LSD 7	1.20 ±0.03	0.84 ±0.08	6.38 ±0.19	7.94 ±0.59 (5,6)	1.13] ±0.19 (9)
SEA	Mean ± SD LSD 8	1.14 ±0.09 -	1.01 ±0.18 -	6.49 ±0.28 -	7.55 ±0.43 -	1.23 ±0.09 -
	ANOVA	S.	S.	S.	S.	S.

- All values are means ± S.E. of each group and expressed as µ mol/min/mg protein.
- Eight mice in each group injected with total antigen dose 50 µ g protein / mouse.
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigen respectively.
- S. significant values as compared to control group.
- P: level of significance is 0.0001 between different groups.

LSDno.: Least significant difference for various groups, infected, CAP, SWAP, SEA and control (5,6,7,8,9), respectively.

Table 8: Effect of different mixed antigens on enzymes activities after 6 wks of immunization.

Enzymes Groups		AST	ALT	LDH	ALP	AP
Control	Mean ± SD LSD 9	1.43 ±0.18 (5,7,8)	1.03 ±0.24 (5,6,7,8)	7.0 ±0.85 (5,7)	10.16 ±1.12 (5,6,7,8)	0.97 ±0.26 (5,7)
Infected	Mean ± SD LSD 5	0.88 ±0.14 (8,9)	0.73 ±0.269 9	5.71 ±1.04 (8,9)	6.56 ±0.77 (6,8,9)	1.89 ±0.46 (5,6,8,9)
SEA+CAP	Mean ± SD LSD 6	0.99 ±0.06 (9)	0.83 ±0.12 (9)	6.50 ±0.51	9.07 ±0.61 (5,7,8,9)	1.08 ±0.20 (5,7)
SEA+SWAP	Mean ± SD LSD 7	0.92 ±0.13 (8,9)	0.84 ±0.14 (9)	6.06 ±0.96 (8,9)	8.48 ±0.56 (6,8,9)	1.13 ±0.23 (6,8,9)
CAP+SWAP	Mean ± SD LSD 8	1.12 ±0.34 (5,7,9)	0.80 ±0.10 (9)	6.08 ±0.29 (5,7)	7.63 ±0.53 (5,6,7,9)	1.19 ±0.08 (5,6)
	ANOVA	S.	S.	S.	S.	S.

- All values are means ± S.E. of each group and expressed as µ mol/min/mg protein.
- Eight mice in each group injected with total antigen dose 50 µ g protein / mouse.
- CAP, SWAP and SEA are cercariae antigen preparation, soluble worm antigen preparation and soluble egg antigen respectively.
- S. significant values as compared to control group.
- P: level of significance is 0.0001 between different groups.
- LSDno.: Least significant difference for various groups, infected, (SEA+CAP), (SEA+SWAP), (CAP+SWAP) and control (5,6,7,8,9), respectively.

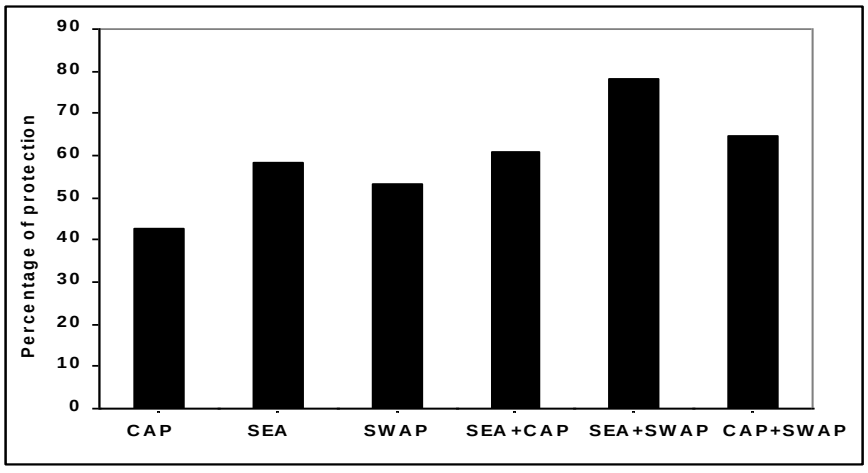

Figure 1: Effect of different antigens on the mean number of worm burden after challenge with 80 cercariae of S. mansoni. Expressed as mean percentage of protection (%).

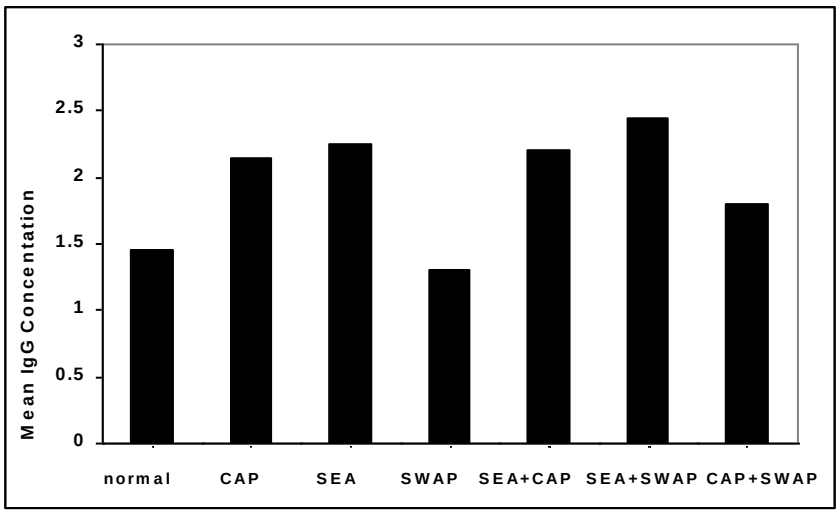

Figure 2: Detection of IgG by ELISA of uninfected mice sera immunized with different antigens.

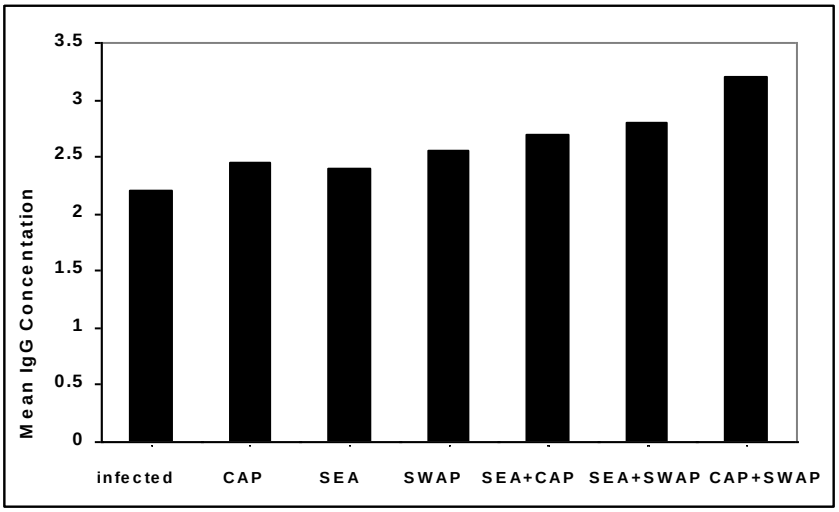

Figure 3: Detection of IgG by ELISA of infected sera immunized with different antigens.

Discussion

Uptill now, no method of vaccination has proved to be totally effective, since they gave partial and low levels of protection against *S. mansoni* infection (Gazzinelli *et al*., 1991, Cook, 1993, Gamal-Eddin *et al*., 1996). In order to develop an accurate immunization procedure, many different antigens have been prepared and tested by many authors in the world. The antigens tested up till now include adult worms antigens, egg antigens (Montesano *et al*., 1999) and irradiated cercariae antigen (Jankovic *et al*., 1999). Many ideas have been tried to find out a vaccine which can be taken by human (Attallah *et al.*, 1999). Development of a vaccine for schistosomiasis has been targeted as a priority by the World Health Orgnization (WHO 2000, Baras *et al*., 2000). Researchers demonstrated the ability of the humans to acquire natural immunity to schistosome infection, together with successful use of attenuated vaccines in animals both under laboratory and field conditions, suggest that the

development of a human vaccine is reasonable (Zhang *et al*., 1999). On the other hand, attenuated veccines for schistosomiasis are considered neither safe nor practicable for human use, and therefore, other approaches must be considered (Waine and McManus, 1999).

The present study is planned to detect the possible side-effects of different homologus antigens against schistosomiasis and to evaluate the protective effect of cercarial antigen (CAP), soluble worm antigen (SWAP), soluble egg antigen (SEA), and mixed antigen (SEA + CAP), (SEA + SWAP) and (CAP + SWAP). As well as to dectect their effect on cellular and humoral immune responses before and after challenge with *S. mansoni*.

Direct interaction between different antigens and normal immune response

The cellular part of the present study explores the interaction of injection of different antigens with the immune system of normal mice. No schistosome infection was attempted at this level of the study as we wished to examine the direct influence of injection on host immunologic competence. This is because there are serious tempts at schistosomiasis vaccinated, and it is reasonable to select antigens that do not interact negatively with the immune apparatus, and so impair its reactivity to putative invading parasites (Al-Sherbiny *et al*., 1995, Harrop *et al*., 1999). Therefore, mice were injected with an effective dose(50 µg) of antigens (SEA, CAP, SWAP and mixed antigen (SEA + CAP), (SEA + SWAP) & (CAP + SWAP). The finding in the present study revealed an elevation in spleen, mesenteric lymph node and thymus lymphocytes in all experimental groups at the 2nd injection. These findings are in accordance with Philips *et al*. (1975), who suggest that vaccination could be selected for schistosomiasis vaccination as it is not immunotoxic and appearantly it will not significantly interfere with the host ability to mount adequate immune responses against putative invading parasites (Abdel Wahab *et al*., 1975 , Nabih and Maghraby, 1999).

Humoral immune response was determined by the detection of IgG titre using ELISA. Our data revealed a high significant difference ($P \leq 0.001$) between values of the levels of IgG obtained with injected groups with different experimental antigens CAP, SWAP, SEA, (SEA + CAP), (SEA + SWAP), (CAP + SWAP) and the control group. So the data recorded in the present study suggest that antigens used are not immunotoxic. It will significantly interfere with the host ability to mount adequate immune responses against putative invading parasites

The interaction between different antigens and parasite

Successful vaccine development for schistosomiasis has been hindered by a lack of consensus on the type of immune response that would provide maximum levels of protective immunity and in complete knowledge of the key antiparasite effecttor mechanisms (James, 1985, Gazzinelli *et al*., 1991, Cook, 1993, Gamal-Eddin *et al*., 1996, Wynn and Hoffman, 2000).

In each of the present six separate experiments, which were immunized by different antigen CAP, SWAP, SEA, (SEA + CAP), (SEA + SWAP) and (CAP + SWAP), mice were challenged percutaneously (P.C.) by 80 live unattenuated *S. mansoni* cercariae. Liver worm burdens were assessed 6 wks later by liver portal perfusion in each group of representative experiments showed that the percentage of protection in mice which were vaccinated by mixed antigen (SEA + SWAP) had the highly percentage of protection which equal to 78.16%. The following ones are (CAP + SWAP), (SEA + CAP), SEA, SWAP and CAP with percentage amounting 64.77, 60.91, 58.33, 53.33, 42.5%, respectively. Our results agree with Grzych et al., (1987), Gamal-Elddin *et al*. (1996) Dupre *et al*. (2001)and (Verity *et al*.,(2001), in which mean total worm burdens were significantly reduced in vaccinated mice by different antigens. Deborah *et al*. (2001) used the 14-3-3-protein as a vaccine and stated that the vaccine fed to protection ranging from 25-

46%, as determined by reduction of adult worm burden.

In our study the effect of different antigens after challenge with 80 cercariae of *S. mansoni* on cellular immune response were studied: A significant correlation was observed between the different immunized groups CAP, SWAP, SEA, (SEA + CAP), (SEA + SWAP), (CAP + SWAP) and control infected groups ($P \leq 0.001$) in lymph node cells, splenocytes and thymocytes, respectively. Whereas, an acceptable vaccine should activate cells for antigen presentation to overcome variation in host reactivity or parasite diversity, (Dunn *et al.*, 1995). . Mohamed and Khoder (1997) showed that schistosome antigens could possibly stimulate cellular immune response upon their use as a protective vaccine against schistosome infection Cellular immunity has also been demonstrated to play an important role in protection against disease (Andersons *et al*., 1998 and Matthais *et al.*, 2001).

Schistosome infection elicits a very intense humoral response among which the massive production of anaphylactic antibodies is striking (Rousseaux- prevost *et al*., 1978 and Ismail *et al*. 1986).

In the present study, the antibodies IgG against soluble worm antigen (SWAP) were detected using ELISA before and after vaccination in schistosomiasis mice. A serological study for the immunoglobulins level in the sera of schistosomiasis not immunized and immunized confirmed their presence in a high level as compared to the variations in the level of antibodies before and after vaccination. These results are in agreement with Al-sherbiny et al.,(2003) who showed a significant increase of IgG1 and IgM to recombinant Smp 17.7 *S. mansoni* antigen

Effect of antigens on schistosomal liver enzymes :

Immunization of mice with present antigens led to an outstanding alleviation of the disease symptoms, reduction in hepatic enlargement was observed at the first 2 weeks of immunization, and there was a significant reduction in the mature and immature worms after immunization with different antigens. Furthermore, histopathological study of the livers of immunized mice with Calpain showed that granulomas formed around eggs were diminshed in number in cell vaccinated groups these results agree with (Zhang *et al*., 2001).

In the present study changes in liver enzymatic activities in blood serum have suggested to be the result of involvement of the liver which is the main target organ in *S. mansoni* infection. In the present study, it was found that schistosoma infection affects all the enzymatic activities with significant reduction. It was concerned to study, transaminases enzyme activities which showed highly significant inhibition in both infected and as compared to control group. This inhibition in transaminases activities is in agreement with Ibrahim (1984) and Metwally *et al*. (1990) who recorded an increase in the serum AST and ALT of mice and rabbits infected with *S. mansoni*. They attributed the elevation of serum ALT and AST activities to hepatocellular damage resulted from egg deposition. In addition Sadun *et al*. (1969); Al-Aasar *et al*. (1989) and Hassan *et al*. (1991) attributed the dimineshion observed in transaminases activities in mice liver (which is accompanied with increase their levels in serum) to the reduction in hepatic cell population due to liver fibrosis. The present study recorded that immunized mice with CAP, SWAP and SEA, showed a significant increase in both ALT and AST activities as compared to infected groups. Enhancemnet in these enzyme activities were recorded for all antigen preparations used by 9.09, 17.48, 13.29, 22.33, 9.71 and 26.21% for CAP, SWAP and SEA and for AST and ALT respectively. This result was due to decrease in worm count and therefore their metabolic toxic products that affects on plasma membrane permeability and consequently lead to enzyme leakage to blood streem.

It can be deduced that treatment with mixed antigen CAP + SEA, SEA + SWAP and CAP + SWAP significantly activated AST and ALT enzymes in infected mice. Improvement in AST and ALT enzymes was noticed with mixed antigens CAP +

SEA, SEA + SWAP, CAP + SWAP recorded 11.5, 2.90, 17.39, 9.90, 10.87 and 6.99, respectively, although their values still recording significant reduction as compared to the normal control.

Tielens *et al*. (1994) reported that LDH inhibition could be attributed to the aerobic-anaerobic switch induced by the developing parasite. Concerning the data obtained on the effect of schistosoma infection on LDH activity it was found that the enzymatic activity was significantly reduced. This was in accordance to Metwally *et al*. (1990) when attributed the decrease in LDH activity in liver tissue to the changes occurred in the permeability of plasma membrane that lead to leakage of the enzyme from the cytoplasm of liver cells to the blood stream. Minai *et al*. (1982) confirmed the significant increase in LDH enzyme activities in serum of rabbits infected with *S. japonicum* and in calves infected with *S. bovis* (Mahmoud *et al.*, 1987). They suggested that this elevation may be due to tissue damage caused by larvae in the infected period that lead to discharge of the enzyme to the blood. Hock *et al*. (1997) reported that the reduction in enzyme activity in liver of infected mice may be due to the effect of egg and worm or their metabolic products that acts on gene products that in turn acts on gene expression as a signal, hence transcripition of DNA specific squence into mRNA is depressed. The obtained data showed that the immunized mice with different antigens used recorded significant decrease in LDH activity as compared to the control group. On the other hand, LDH activity showed a significant increase as compared to the infected group. It was noticed that LDH enzyme recorded improvement in its activity with the different antigen types CAP, SWAP and SEA by 4.14, 7.14 and 8.71%, respectively.

As concerned with the effect of mixed antigen (CAP+SEA), (SWAP+SEA) and CAP+SWAP) on the LDH enzyme activity, it showed significant increase as compared to the infected group. The percent of improvement of LDH enzyme after vaccination was recorded 11.29, 5.00 and 6.08%, respectively.

Concerning to acid and alkaline phosphatase activities, the present results showed that the immunization of infected mice with CAP, SWAP and SEA improved by 62.88, 78.35, 55.67, 7.48, 13.58 and 9.74% for CAP, SWAP and SEA and for AP and ALP, respectively. Our data showed non-significant change in acid and alkaline phosphatases levels with vaccines of all antigens used.

Immunized mice with CAP, SWAP and SEA, as compared to infected group, recorded significant inhibition in AP enzyme activity, while showing significant increase as compared to the normal control group, the reverse results for the ALP activity were recorded. This enhancement in AP enzyme activity resulted from decrease in worm and egg toxins that led to reduction in tissue catabolism which is confirmed by the reduction of worm burden due to vaccination with all antigen types. The elevation in acid phosphatase activity may be due to increased catabolism as a result of infection and from increased worm, egg and their metabolites, since all the lysosomal enzymes are activated in condition characterized by increased tissue catabolism (Salah *et al*., 1976). Our data were in accordance with Rizk (1997) who found a highly significant increase in acid phosphatase enzyme activity in hepatocytes of *S. mansoni* infected mice. Fredexiks and Marx (1988) stated that this elevation may be due to aberration of the lysosomes, where acid phosphatase is considered the lysosomal marker enzyme. This result was confirmed by Rodrigues (1988) who observed an important changes on the lipidic constitution of lysosomal membe-rane of *S. mansoni* infected mice, while El-Sharkawy *et al*. (1993) attributed the increase in enzyme activity to disturbance in metabolic function as a result of liver cell injury. On the other hand, Tanabe *et al* (1983) showed an insignificant decrease in enzyme activity in the liver cells of mice infected with *S. mansoni*. While Li *et al*. (1988) mentioned that there is a decrease in enzyme activity of rabbit liver infected with *S. japanicum* and attributed this decrease to the molecular and biological changes in

hepatic and granulomatous cells a results of infection.

The present study indicated the significant reduction in ALP activity in tissue haemogenate of infected mice as compared to the normal control group. This is in agreement with Metwally *et al*. (1990) who noticed that ALP activity was increased in serum of infected S. mansoni mice. Also, Tanabe *et al*. (1997) stated that serum ALP activity was elevated in patients infected with S. mansoni that is indicated by its low level in the liver. Moreover, it can be noticed that immunization of infected mice with mixed antigen (SEA+CAP), (SEA+SWAP) and (CAP + SWAP) induced significant decrease in ALP activity, while that of AP recorded a significant increase as compared to the normal control group. Improvement in enzymes activities was noticed in all immunized groups. The percent of improvement was recorded 24.7, 14.17, 10.53, 83.51, 78.35 and 72.16% for ALP and AP and for the same mixed antigen respectively.

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التأثير المناعى الوقائى للانتيجينات المفردة والمختلطة من طفيل البلهارسيا على الفيران المعدية بالبهارسيا المعوية.

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تهدف هذه الدراسة الى تقدير الاثار المناعية الوقائية للانتيجينات المستخلصة من السركاريا ، الديدان الناضجة والبويضات سواء كانت هذه اللقاحات مفردة أو مختلطة معاً.
CAP,SWAP,SEA,(CAP+ SWAP), (CAP+SEA),(SWAP+SEA).
وذلك قبل وبعد عدوى الفئران بالبلهارسيا المعوية . وقد تمت هذه الدراسة من خلال دراسة التأثير الخلوي لخلايا الليمفاوية من كل من العقد الليمفاوية الموجود بجدار الأمعاء الدقيقة – الطحال والغدة الثيموسية .وأيضا من خلال التأثير المصلى باستخدام اختيار ELISA .

وقد أوضحت الدراسة الإحصائية ان هناك ارتفاع معنوى فى عدد الخلايا الليمفاوية المفصولة من الغدد الليمفاوية – الطحال والغدة الثيموثية فى الفئران المحقونة بالانتيجينات السابق ذكرها اذا قورنت بالفئران الغير محقونة وذلك قبل وبعد العدوى. وقد لوحظ ارتفاع معدل IgG فى الفئران المحقونة بالانتيجينات بعد العدوى اذا قورنت بالفئران الغير محقونة .كما أوضحت الدراسة وجود تحسن ملحوظ فى أنزيمات الكبد فى الفئران المعدية والمحصنة اذا قورنت بالفئران المعدية فقد .

ونستنتج من هذه الدراسة ان حقن الفئران بالانتيجينات المستخلصة من المراحل المختلفة لطفيل البلهارسيا سواء كانت منفردة او مختلطة لها تأثير مناعي وقائي سواء كان خلوي أو مصلى ضد الإصابة بمرض البلهارسيا المعوية .