The Antischistosomal Activity of *Fasciola gigantica* and *Schistosoma mansoni* Eggs is Influenced by Saponin Extracted from *Atriplex nummularia*

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Abstract: The objective of the present study was to evaluate the antischistosomal, biochemical and humoral immune response of *Fasciola gigantica* and *Schistosoma mansoni* eggs homogenate influenced with or without saponin extracted from *Atriplex nummularia*. The work was extended to study the histopathological picture of the liver before and after challenge. Total worms reduction recorded 57.14, 80.95 and 42.85% in immunized mice with *Fasciola* egg homogenate (50µg/100µl PBS/mouse), *Fasciola* egg homogenate influenced by saponin (50µg/100µl PBS/mouse) and saponin alone (50µg/100µl PBS/mouse), respectively. Immunized groups with *Schistosoma* egg antigen and *Schistosoma* egg antigen influenced by saponin showed reduction in total worms by 47.61, 52.38%, respectively. In conclusion, immunization with *Fasciola gigantica* egg homogenate possesses promising antischistosomal properties with an immunomodulatory response to saponin. Heterologous homogenate had antischistosomal activity more than homologous homogenate. In addition, heterologous homogenate influenced with saponin had more antischistosomal activity than its homologous homogenate. Moreover, *Fasciola gigantica* egg homogenate had an immunoprophylactic effects by increasing the IgM and IgG levels against *Schistosoma* egg antigen. [Journal of American Science. 2011;7(1):87-100]. (ISSN: 1545-1003).

Keywords: *Fasciola gigantica*- *Atriplex nummularia*- saponin- *Schistosoma mansoni*- egg antigen- immunoprophylactic

1. Introduction

Vaccine strategies represent an essential component for the future control of schistosomiasis, as an adjunct to chemotherapy (McManus and Loukas, 2008). Immunization of *Schistosoma mansoni* (*S. mansoni*) infected mice by *Fasciola* egg antigens (SEA) showed cross-reactivity that protecte against infection by reduction fluke burden and egg production (Hassan *et al.*, 2008a). Hassan *et al.* (2008b) demonstrated a structural homology in *Fasciola gigantica* excretory-secretory (E\S) and egg antigens. This homology was resided in the components of the similar molecular weights between both antigens. *Fasciola / Schistosoma* defined a cross-reactive antigen from *F. hepatica* worms that designated as FhSmIII. An antiserum of this antigen was developed and used as a probe to detect its presence (or its determinants) in different extracts of parasitic trematodes. In this manner, it was possible to demonstrate that FhSmIII was found in *S. mansoni, S. bovis* and *Paragonimus westermani* (Hillyer, 1984).

S. mansoni egg antigen (SEA) was found to contain numerous protein, polysaccharide, glycoprotein (Hassanein *et al.*, 1999) and enhances microsomal and mitochondrial enzymes including urea cycle enzymes (Kamel *et al.*, 1998). Vaccination of mice with egg antigen showed delayed ovulation of ova in the liver and stools (Dunn *et al.*, 1988). In addition, Hillyer *et al.*, (1988) noticed that SEA appears to be good candidate in developing a screening assay for the immunodiagnosis of schistosomiasis. Infection of mice with a multiple doses of SEA down regulated egg deposition before *S. mansoni* infection (Botros *et al.*, 1999).

Saponin was constituted as one of the most distributed classes of secondary metabolite from plant or animal domains (Al-Habori and Rahman, 1998). They have broad spectrum of biological activity, cytotoxicy, antitumor, antioxidant and anti-inflammatory (Yanamandra *et al.*, 2003). Triterpenoid and steroid saponin have been found to be immunomodulator and detrimental to several infectious protozoan such as *Plasmodium* and *Leishmanaia spp.* (Plock *et al.*, 2001). The addition of a new immunomodulator to the adjuvant-adaptation (ADAD) system with fatty acid binding proteins increased the protection against *F. hepatica* (Lopez-Aban *et al.*, 2008).

Saponin had a depressive effect against cancer cells by imitated specific cytokine inhibitors (Francis *et al.*, 2001) and detected specific toxic activity against macrophage (Kuroda *et al.*, 2001). Previous investigation on saponin revealed its role as

immunomodulator against *S. mansoni* infection (Maghraby *et al*., 2007).

The objective of this study was to evaluate the influence of saponin on the anti-schistosomal activity of *F. gigantica* and *S. mansoni* egg antigens before and post challenge with 100 cercariae of *S. mansoni* through determination of certain biochemical and humoral immune responses. The histopathological analysis of the liver was also done to support our findings.

2. Materials and Methods

Female Swiss albino mice CD-1 strain (18 – 22g) were obtained from the Animal House, National Research Centre, Cairo, Egypt. They lived in controlled environment of constant temperature and humidity with freely access of water and food. *Atriplex nummularia* (family: *Chenopodaceae*) collected from Marsa Matroh Desert, Cairo, Egypt. Extraction of plant saponin was carried out according to Maghraby *et al.* (2007).

Antigens preparation

Cercariae, worm and egg antigens of *S. mansoni* as well as *F. gigantica* eggs used for enzyme linked immunosorbent assay (ELISA) technique were obtained from Theodore Bilharz Research Institute (Giza, Egypt). Immunization protocol was done according to Maghraby *et al.* (2007).

Ethics

Anesthetic procedures complied with the ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA were approved by the Medical Ethical Committee of the National Research Centre in Egypt.

Immunization with *Schistosoma mansoni* eggs antigen

Mice were divided into eleven groups (5 mice/ group). Group 1, served as normal control group subcutaneously injected with 100µl of phosphate buffer saline (PBS). Groups 2-4 received 50 µg protein/ 100µl PBS of *S. mansoni* egg homogenate (SEA), saponin (SAP), SEA mixed with saponin (SEA+SAP), respectively and sacrificed 15 days post the 1st immunization. Groups 5-7 immunized with 50 µg protein/ 100µl PBS of SEA, SAP and SEA+SAP. At 15th day post the 1st immunization each group was booster by 2nd immunization with 50 µg / 100µl PBS of SEA, SAP, SEA+SAP, respectively and sacrificed 15 days later. Group 8 served as positive control infected mice with 100 *S. mansoni* cercariae by tail immersion technique (Oliver and Stirewalt, 1952). Groups 9-11 immunized at 0 time with 50 µg / 100µl PBS of SEA, SAP and SEA+SAP, respectively. At 15 days post 2nd immunization mice were challenged with 100 *S. mansoni* cercariae and sacrificed after two months of infection.

Immunization with *Fasciola gigantica* eggs antigen

Vaccination regimens and animal grouping was carried out as described above, whereas *Fasciola* egg homogenate (FEA) and *Fasciola* egg homogenate influenced with saponin (FEA+SAP) were replaced all SEA groups.

Parasitological studies

Worms were recovered by liver perfusion as described by Smithers and Terry (1965). The percent of reduction in worm burden was calculated by the method of Tendler et al. (1968) as follows P = (C - V) / C x 100

Where, P was the percentage of protection; C was the mean number of parasite recovered from infected mice; and V was the mean number of parasite recovered from vaccinated mice. The relative sex ratio (RSR) was calculated by the method of Fallon *et al.* (1994) according to the formula:
RSR= [Male/ female ratio in treated group] / [Male / female ratio in untreated group]
The ratio of untreated groups was standardized as 1.

Enzyme linked Immunosorbent Assay (ELISA)

The assay was performed according to Hillyer *et al*. (1979). This assay was used for determination of IgM and IgG levels in immunized mice sera before and after infection with *S. mansoni* cercariae. Plates were coated with cercarial antigen preparation (CAP), soluble worm antigen preparation (SWAP), soluble egg antigens (SEA) and incubated at room temperature overnight. Orthophenylene diamin dihydrochloride (OPD) was used as a substrate. The reaction was read at 490 nm using Micro Well Plate Reader.

Liver tissue homogenate and biochemical analysis

The liver was removed, blotted, weight and homogenized in 4.5 volume of 0.1% hecadecyle trimethyle ammonium bromide (CTB) for urea cycle enzymes determination. For estimation of succinate and lactate dehydrogenase and antioxidant

parameters, liver tissue was homogenized in 0.9 N NaCl (1:10 w/v).

Protein was estimated by the method of Bradford (1976). Lipid peroxides was determined as malondialdehyde. Its concentration was calculated using the extinction coefficient value $1.56 < 10^5 M^{-1}$ cm^{-1} and read at 535 nm by the method of Buege and Aust (1978). Glutathione was estimated by the method of Moron *et al.* (1979) using dithiobis-2-nitrobenzoic acid (DTNB) in PBS. Catalase activity was assayed spectrophotometrically according to Nelson and Kiesow (1972). Superoxide dismutase (SOD) was estimated by the method of Nishikimi *et al*. (1972). Urea cycle enzymes; ornthine aminotransferase (OAT), argininosuccinate synthetase (ASS), argininosuccinate layase (ASL) and arginase (Arg.) were employed according to the method of Linton and Campell (1962). Estimation of lactate dehydroginase (LDH) was carried out according to the method of Babson and Babson (1973). Succinate dehydrogenase (SD) was measured colorimetrically at 490 nm (Shelton and Rice, 1957).

Liver histopathology

Sections of liver tissue from each experimental group were fixed in 10% buffered formalin solution for histopathological studies. Paraffine embedded sections (5 µm thick) were taken after fixation. Slides were stained using haemoatoxylin and eosin (H&E) according to the method by Hirsch *et al*. (1997).

Statistical analysis

Statistics is carried out by one way analysis of variance (ANOVA), Costat Computer Program accompanied by least significance difference between groups at $P<0.05$.

Antishistosomal activity and immunological determination will be carried out by independent *t*-test (Ronald *et al*., 1983) and Graph pad instat software.

3. Results

Immunized groups with *Fasciola* egg homogenate (FEA) recorded reduction in total, male and female worms by 57.14, 42.10 and 46.15%, respectively with a relative sex ratio of 1.07. *Fasciola* egg homogenate influenced by saponin (FEA+SAP) resulted reduction by 80.95, 73.68 and 76.92% as compared to infected group with relative sex ratio of 1.14. On the other hand, mice immunized by SAP showed diminution in total, male and female worms by 42.85, 31.57 and 15.38 %, respectively. Immunized groups with *Schistosoma* egg antigen (SEA) recorded reduction by 47.61, 26.31 and 38.46% in total, male and female worms, respectively. Attenuation in total, male and female worms post immunization with SEA+SAP was 52.38, 36.84 and 38.46 %. The relative sex ratio recorded 0.80, 1.19 and 1.02 for the last three antigens, respectively (Table 1).

Immunoprophylactic effect of *Fasciola gigantica* egg homogenate and *Schistosoma* egg antigen

Post 2nd immunization, the IgM level against SEA showed significant elevation in immunized group with FEA+SAP (Fig.1). Post 1st and 2nd immunization with SEA+SAP, the level of IgM against CAP showed a significant stimulation. SEA and SEA+SAP showed significant stimulation against SWAP. After challenge with *S.mansoni* cercariae, the level of IgM in immunized mice by SEA against SWAP showed a significant elevation, but insignificant stimulation was recorded with SEA+SAP as compared to the infected group (Fig.1).

Post first and second immunization with FEA and FEA+SAP as well as SEA, the levels of IgG showed significant elevation in immunized mice sera against CAP and SWAP. Post 2nd immunization with SEA+SAP, IgG level against CAP was significantly elevated. IgG level in immunized mice sera of SEA+SAP against SEA showed a significant elevation as compared to the infected group (Fig.2).

Urea cycle enzyme activities

After 1st immunization with saponin, FEA and FEA+SAP, significant increase in urea cycle enzymes; OAT and ASL were recorded. Significant decrease was observed in ASS and arginase as compared to normal group. After 2nd immunization with SAP, FEA and FEA+SAP, significant increase in OAT and significant decrease in ASS were noticed. On the contrary, ASS, ASL and arginase enzyme activities showed significant increase in all immunized infected groups (Table 2). Significant increase in all urea cycle enzyme activities after 1st and 2nd immunization with SEA+SAP was recorded. A significant decrease by 29.46% was observed in ASS after 1st immunization with SAP. Arginase enzyme activity showed significant decrease after 1st immunization with SAP and significant increase after 2nd immunization with SEA+SAP compared to normal mice. Meanwhile OAT exhibit significant increase in SAP vaccinated group and insignificant change in SEA or in SEA+SAP (Table 3).

Glycolytic enzyme activities

Significant decrease was noticed in glycolytic enzymes (SD and LDH) after 1st and 2nd immunization with SAP, FEA and FEA+SAP **(**Table 3). Total protein recorded significant increase after 1st

immunization with FEA while significant decrease was noticed with saponin. After 2nd immunization with SAP, FEA and FEA+SAP a significant increase in total protein was recorded. The glycolytic enzyme; SD showed significant reduction after 1st immunization with SAP, SEA+SAP. LDH showed a significant reduction after 1st immunization with SAP. In 2nd immunization, significant decrease was noticed.

Antioxidant parameters

S. mansoni infected mice and all immunized groups recorded significant increase in lipid peroxides and superoxide dismutase levels, while significant decrease in glutathione and catalase levels were observed. In post challenged groups, the antioxidant levels recorded significant improvement in mice immunized by saponin. In addition, FEA and FEA+SAP recorded more potent effect than SEA and SEA+SAP (Table 4).

Histopathological studies

Figures (3 and 4) showed normal histological profile, arrows indicated normal hepatocytes (A). Immunized mice liver showed normal hepatic lobular structure, central vein and portal spaces normally distributed and very mild lymphoid infiltration were observed in few portal spaces (C, D, E, F, I, J, K, L). Liver of infected mice showed the presence of foci with acute inflammation destroying hepatic trabecular structure, portal space with biliary duct hyperplasia and chronic inflammatory infiltrations (B). Post challenged mice revealed remarkably reduced of granuloma size and count with minimal hepatic infiltrations (G, H, M, N).

Table 1: Reduction of total, male and female *S. mansoni* worms and relative sex ratio in immunized groups post challenge infection.

Groups	Total worm (TW)	Male (M)	Female (F)	%R (TW)	%R (M)	%R (F)	RSR
Infected	42.00 ± 4.52	19.00 ± 1.26	13.00 ± 1.82	-	-	-	1
***Schistosoma* eggs**	22.00 ± 1.59	14.00 ± 1.41*	8.00 ± 1.16*	47.61	26.31	38.46	1.19
Saponin	24.00 ± 1.41*	13.00 ± 1.41*	11.00 ± 1.32*	42.85	31.57	15.38	0.80
***Schistosoma* eggs + saponin**	20.00 ± 1.41*	12.00 ± 1.62*	8.00 ± 1.67*	52.38	36.84	38.46	1.02
***Fasciola* eggs**	18.00 ± 1.85*	11.00 ± 1.41*	7.00 ± 1.72*	57.14	42.10	46.15	1.07
***Fascioa* eggs + saponin**	8.00 ± 1.62*	5.00 ± 1.72*	3.00 ± 1.11*	80.95	73.68	76.92	1.14

- Values represented mean ±SD of 5 mice in each group.
- (*) Level of significance as compared to infected group at P<0.01
- % R is the percentage of reduction as compared to infected group.
- RSR is the relative sex ratio of each group.
  where RSR = (male / female) ^{treated} / (male / female) ^{infected}

Table (2): Change in urea cycle enzyme activities in immunized groups with *F. gigantica* and *S. mansoni* eggs homogenate influenced with and without saponin before and after challenge with *S. mansoni*

Part A

Groups	Immunized groups with *F. gigantica* eggs homogenate				Immunized groups with *S. mansoni* eggs homogenate			
	OAT	ASS	ASL	Arg.	OAT	ASS	ASL	Arg.
F / S 1st	6.09 ± 0.37 c	1.11 ± 0.08 d	27.72 ± 0.48 a	43.93 ± 0.74 ef	7.23 ± 0.66 bc	1.750 ± 0.23 ab	29.35 ±0.90 b	47.43 ±2.08 bc
SAP + (F / S 1st)	6.70 ± 0.24 b	1.19 ± 0.01 bc	23.97 ± 0.82 c	45.22 ± 1.17 cd	8.58 ± 0.67a b	2.13 ± 0.26 a	31.24 ±1.78 ab	47.85 ±2.11 bc
F / S 2nd	7.31	1.19	23.91	44.40	8.98	1.83	30.15	50.33

	± 0.25 [a]	± 0.03 [cd]	± 0.46 [c]	± 1.42d [e]	± 0.69 [a]	± 0.36 [ab]	±1.83 [ab]	±0.59 [b]
SAP + (F / S [2nd])	4.90 ± 0.30 [e]	1.95 ± 0.05 [a]	26.75 ± 0.36 [b]	46.67 ± 61 [b]	9.60 ± 0.49 [a]	2.04 ±0.12 [a]	33.15 ±0.85 [a]	55.98 ±1.77 [a]
F / S + Infection	1.94 ± 0.103 [h]	0.79 ± 0.07 [f]	19.45 ± 0.98 [e]	22.43 ± 2.31 [h]	3.98 ± 0.89 [e]	1.06 ±0.29 [bc]	21.36 ±1.48 [e]	34.65 ±3.90 [e]
SAP + F / S + Infection	3.1 ± 0.188 [g]	0.77 ± 0.06 [g]	20.35 ± 1.23 [d]	31.10 ± 1.52 [g]	5.20 ± 1.82 [d]	1.17 ±0.11 [bc]	24.40 ±3.55 [cde]	44.58 ±3.80 [cd]

Part B

Common groups	OAT	ASS	ASL	Arg.
SAP [1St]	6.65 ± 0.41 [bc]	0.91 ± 0.08 [e]	26.53 ± 0.39 [b]	42.93 ± 1.05 [f]
SAP [2nd]	5.87 ± 0.19 [d]	1.20 ± 0.03 [bcd]	24.30 ± 1.02 [c]	46.75 ± 1.69 [bc]
Infection	3.5 ± 0.14 [g]	0.57 ± 0.10 [h]	2.04 ± 0.25 [g]	4.79 ± 0.71 [i]
SAP + Infection	7.48 ± 0.31 [a]	0.72 ± 0.02 [g]	16.61 ± 1.37 [f]	30.61 ± 1.73 [g]
Control	4.60 ± 0.26 [f]	1.29 ± 0.04 [b]	24.73 ± 0.26 [b]	49.39 ± 1.05 [a]

- Data are mean ± SD of five mice in each group.
- Values are expressed as: nmol/mg protein for lipid peroxide and catalase, µmol/g protein for superoxide dismutase, µg/mg protein for glutathione.
- OAT: ornthine aminotransferase, ASS: argininosuccinate synthetase, ASL: argininosuccinate layase, Arg: arginase, SAP: saponin, *F*: *Fasciola gigantica*, S: *Schistosoma mansoni*, 1[st]: first immunization, 2[nd]: second immunization.
- Unshared letters between groups are the significance values at p< 0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA), Costat Computer Program.

Table 3: Change in glycolytic enzyme activities and total protein content in immunized groups with *F. gigantica* and *S. mansoni* eggs homogenate influenced with and without saponin before and after challenge with S. *mansoni* infection.

Part A.

Groups	Immunized groups with *F. gigantica* eggs homogenate			Immunized groups with *S. mansoni* eggs homogenate		
	SD	LDH	Total protein	SD	LDH	Total protein
FEA / SEA [1st]	0.29 ± 0.01[e]	96.27 ± 3.92 [d]	44.25 ± 0.95 [e]	1.17 ± 0.11 [ab]	145.25 ± 2.22 [b]	40.18 ± 0.78 [ef]
SAP + (FEA / SEA [1st])	0.32 ± 0.02 [d]	113.43 ± 2.33 [b]	38.25 ± 0.95 [g]	0.97 ± 0.05 [ab]	148.150 ± 1.39 [ab]	40.95 ± 0.98 [e]
FEA / SEA [2nd]	0.32 ± 0.02[d]	94.83 ±2.88 [j]	70.50 ± 1.30 [c]	1.25 ± 0.09 [ab]	150.25 ± 6.65 [ab]	47.60 ± 2.28 [d]
SAP + (FEA / SEA [2nd])	0.24 ± 0.01[e]	102.75 ± 2.22 [c]	80.25 ± 1.25 [b]	1.39 ± 0.06 [a]	155.40 ± 5.49 [a]	59.15 ± 3.00 [b]
FEA / SEA + Infection	1.65 ± 0.01[a]	79.37 ± 3.00 [h]	29.75 ± 0.50 [j]	1.42 ± 0.07[a]	91.41 ± 5.40 [d]	35.88 ± 4.09 [f]
SAP + FEA / SEA+ Infection	0.75 ± 0.01 [c]	84.58 ± 4.09 [f]	38.00 ± 0.82 [g]	1.39 ± 0.13 [a]	123.70 ± 9.96 [c]	45.01 ± 4.5[d]

Part B.

Common groups	SD	LDH	Total protein
SAP 1St	0.27 ± 0.01 e	92.62 ±1.36 e	36.75 ± 2.36 h
SAP 2nd	0.29 ± 0.01 e	93.92 ± 3.87 e	54.50 ± 1.29 d
Infection	0.10 ± 0.02 f	60.75 ± 0.47 i	83.25 ± 4.28 a
SAP + Infection	1.03 ± 0.06 b	83.08 ± 1.47 g	35.75 ± 0.95 i
Control	1.66 ± 0.04 a	143.93 ± 3.45 a	39.75 ± 1.71 f

- Data are mean ± SD of five mice in each group.
- Values are expressed as µl mol/mg protein / min and total protein in mg protein /ml.
- SD: succinate dehydrogenase, LDH: lactate dehydrogenase, SAP: saponin, *F*: *Fasciola gigantica*, S: *Schistosoma mansoni*, 1st: first immunization, 2nd: second immunization.
- Unshared letters between groups are the significance values at p< 0.0001
- Statistical analysis is carried out using one way analysis of variance (ANOVA), Costat Computer Program.

Table 4: Antioxidant parameters in immunized groups with *F. gigantica* and *S. mansoni* eggs homogenate influenced with and without saponin before and after challenge with *S. mansoni* infection.

Part A.

Groups	Immunized groups with *F. gigantica* eggs homogenate				Immunized groups with *S. mansoni* eggs homogenate			
	LPO	GSH	Cat	SOD	LPO	GSH	Cat	SOD
FEA / SEA 1st	0.56 ± 0.01 fg	125.83 ± 3.11 b	22.82 ± 0.50 c	469.96 ± 2.72 h	0.56 ± 0.01 b	125.83 ± 3.11 b	22.82 ± 0.51 c	469.96 ± 2.72 h
SAP + (FEA / SEA 1st)	0.78 ± 0.05 c	117.67 ± 2.06	3.31 ± 0.03 c	10.66 ± 0.26 e	0.80 ± 0.02 e	113.04 ± 1.58	17.73 ± 0.98 i	481.78 ± 1.91 e
FEA / SEA 2nd	0.58 ± 0.02 e	120.51 ± 1.36 d	22.99 ± 0.59 c	474.02 ± 1.64 g	0.61 ± 0.02 d	117.82 ± 1.65 e	22.50 ± 0.03 d	476.75 ± 2.62 f
SAP + (FEA / SEA 2nd)	0.51 ± 0.02 g	125.66 ± 2.26 b	23.41 ± 0.56 b	466.68 ± 1.56 i	0.55 ± 0.02 f	122.20 ± 1.14 c	23.19 ± 0.02 b	470.46 ± 1.52 gh
FEA / SEA + Infection	0.75 ± 0.02 c	113.20 ± 2.32 f	20.21 ± 0.79 f	571.15 ± 6.08 c	0.78 ± 0.01 c	94.59 ± 1.43 j	20.05 ± 0.08 g	579.79 ± 2.33 c
SAP + FEA / SEA + Infection	0.56 ± 0.01 fg	125.83 ± 3.11 b	22.82 ± 0.50 c	469.96 ± 2.72 h	0.56 ± 0.01 b	125.83 ± 3.11 b	22.82 ± 0.51 c	469.96 ± 2.72 h

Part B.

Common groups	LPO	GSH	Cat	SOD
SAP 1St	0.65 ± 0.01 d	116.41 ± 3.48 f	21.63 ± 0.44 e	481.56 ± 4.10 e
SAP 2nd	0.54 ± 0.02 g	123.73 ± 1.86 c	23.47 ± 0.53 b	468.88 ± 2.76 h
Infection	0.93 ± 0.01a	72.06 ± 1.55 i	15.81 ± 0.27 h	710.18 ± 6.71 a
SAP + Infection	0.85 ± 0.02 b	97.87 ± 1.54 h	17.98 ± 0.33 g	620.32 ± 6.86 b
Control	0.49 ± 0.02 h	129.18 ± 2.20 a	23.90 ± 0.18 a	463.25 ± 2.35 j

- Data are mean ± SD of five mice in each group.
- Values are expressed as: nmol/mg protein for lipid peroxide and catalase, µmol/g protein for superoxide dismutase, µg/mg protein for glutathione
- SOD: superoxide dismutase, GSH: glutathione, LPO: lipid peroxides, Cat: catalase, SAP: saponin, *F*: *Fasciola gigantica*, S: *Schistosoma mansoni*, 1st: first immunization, 2nd: second immunization.
- Unshared letters between groups are the significance values at p< 0.0001
- Statistical analysis is carried out using one way analysis of variance (ANOVA), Costat Computer Program.

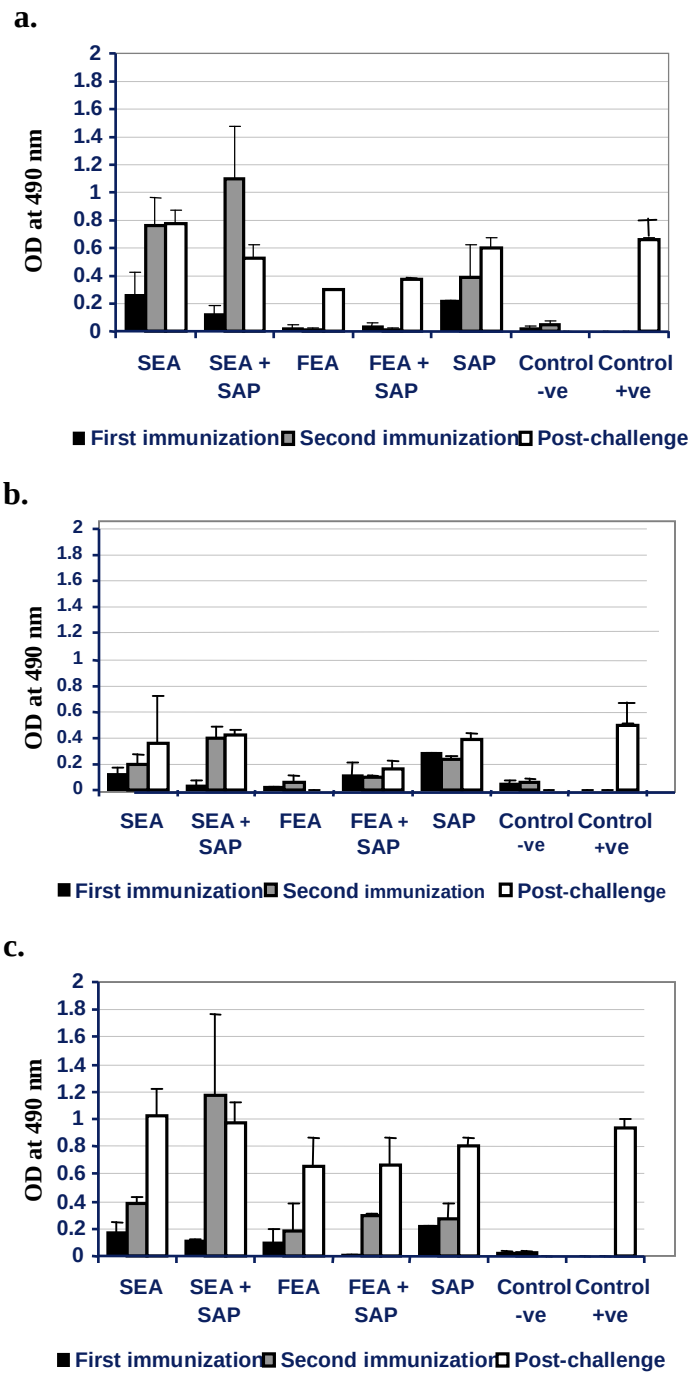

Fig. 1: Detection of IgM by ELISA in mice sera post the first immunization (black bars), second immunization (grey bars) with *S. mansoni* or *F. gigantica* homogenates influenced with or without saponin and post challenge (white bars) with 100 *S. mansoni* cercariaea. Plates were coated with CAP (a), SWAP (b), or SEA (c).

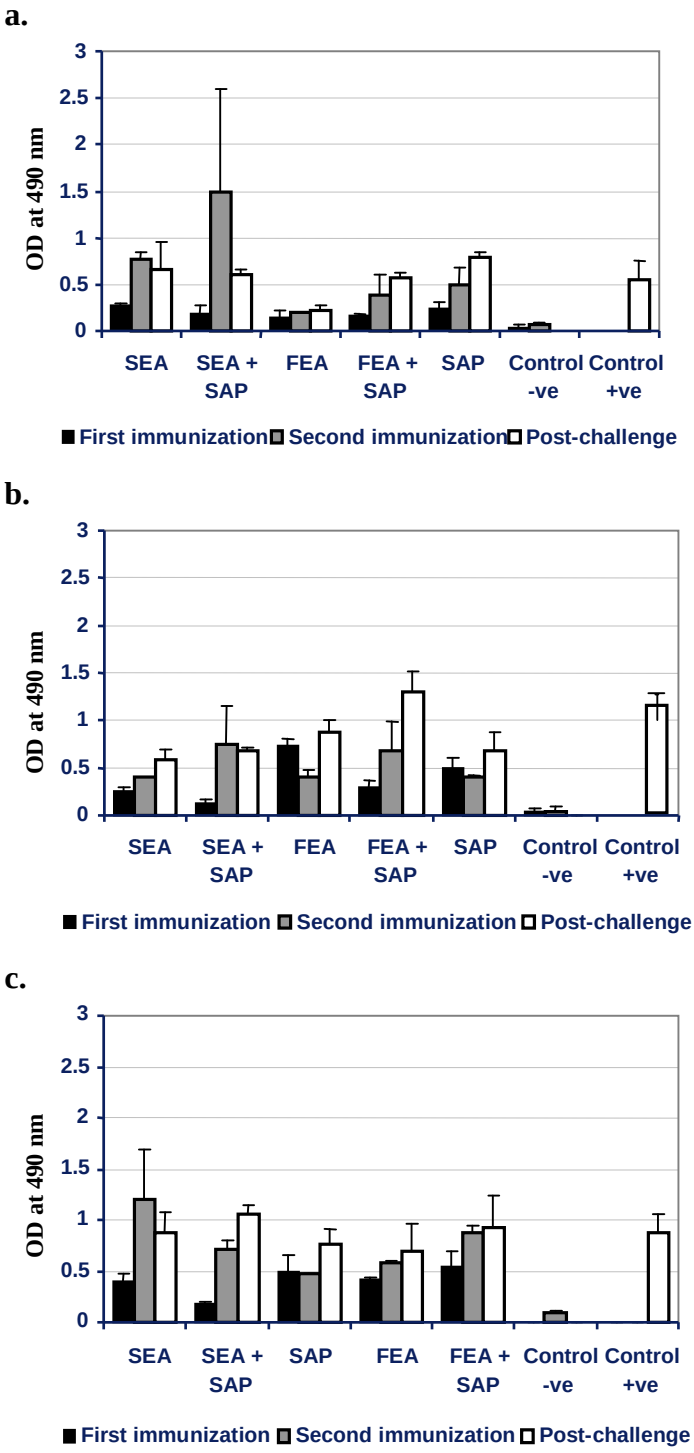

Fig. 2: Detection of IgG by ELISA in mice sera post the first immunization (black bars), second immunization (grey bars) with *S. mansoni* or *F. gigantica* homogenates influenced with or without saponin and post challenge (white bars) with 100 *S. mansoni* cercariaea. Plates were coated with CAP (a), SWAP (b), or SEA (c).

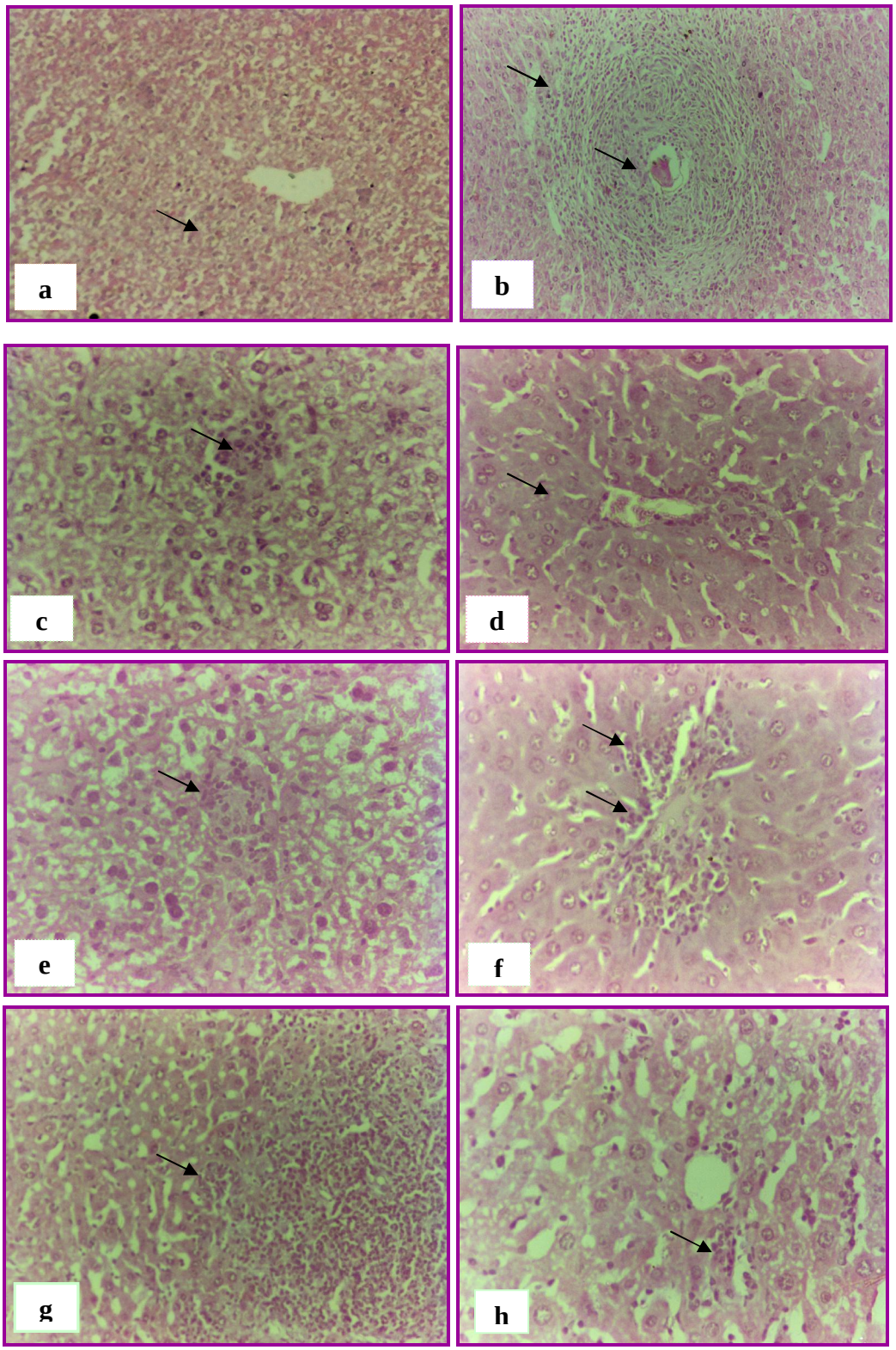

Fig. 3: Representative histological analysis photos for liver sections of different mice groups including: a. Normal structure of the liver control mice [100x]. b. infected group (positive control) [100x]. c. FEA immunized group after 1st immunization [200x]. d. FEA immunized group after 2nd immunization [200x]. e. FEA and SAP immunized group after 1st immunization [200x]. f. FEA +SAP immunized group after 2nd immunization [200x]. g. FEA infected group [100x]. h. infected FEA +SAP group [200x].

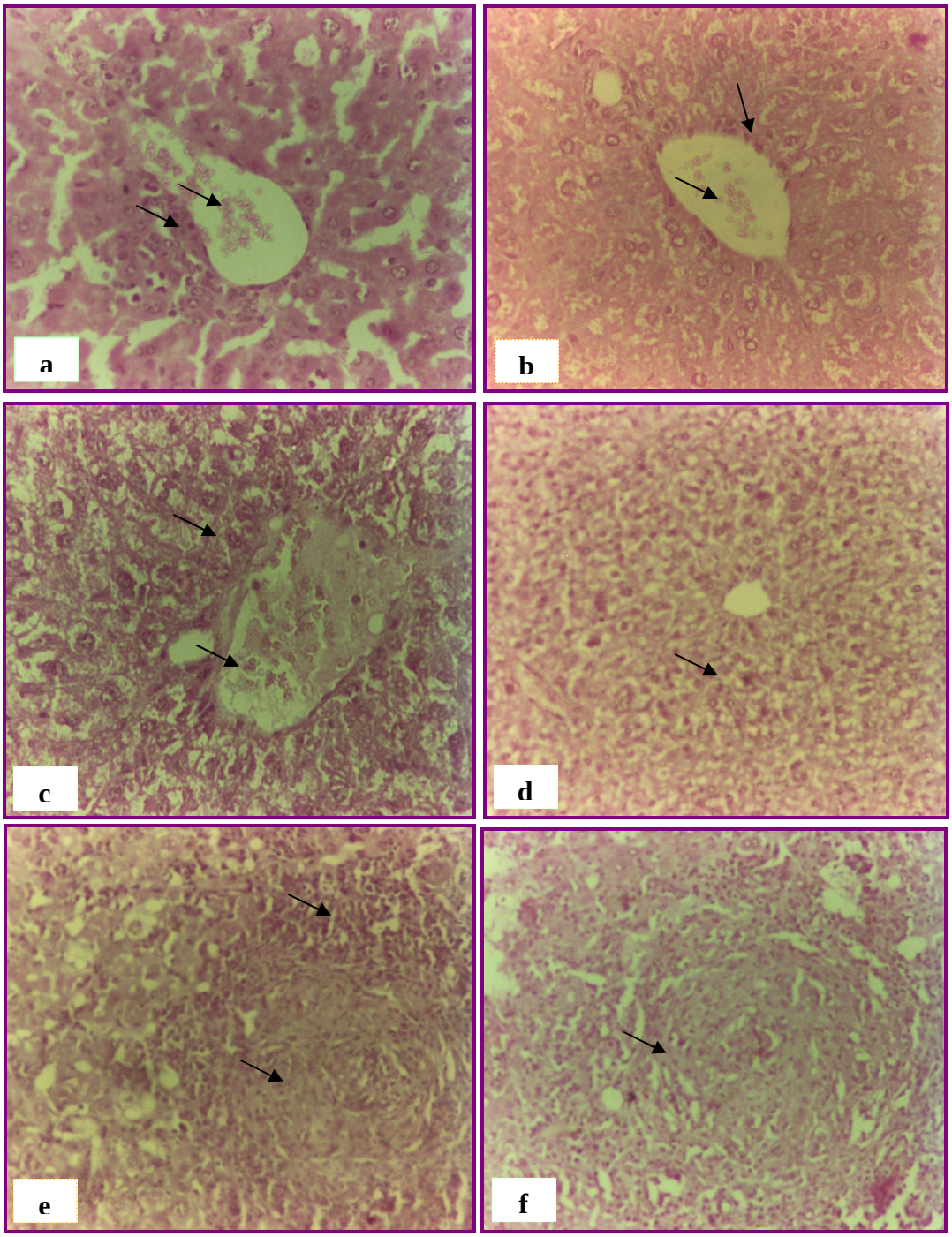

Fig. 4: Representative histological analysis photos for liver sections of different mice groups including: a. SEA group after 1st immunization [200x]. b. SEA group after 2nd immunization [100x]. c. SEA +SAP group after 1st immunization [200x]. d. SEA +SAP group after 2nd immunization [100x]. e. SEA-infected group [100x]. f. SEA - infected group [100x].

4. Discussion

Recent studies revealed that FEA enhances antibodies production supporting the hypothesis of immunomodulatory effect caused by vaccines (Schuster *et al*., 2007). *Fasciola* derived 12kd antigen developed antibodies that protected against *S. mansoni*, proofing its cross- reactivity (Cervi *et al*., 2004). Almeida *et al.* (2003) found *S. mansoni* (Sm14) induced high levels of protection against both *S. mansoni* and *F. hepatica.* Hillyer (2005) demonstrated that some vaccine candidates have anti-fecundity, anti-pathology, and anti-embryonation effects. El- Sayed *et al*. (2000); Noureldin *et al.* (2000) and Ruangittichai *et al.* (2006) reported that all cross-reactivity is ascertained by a significant protection of 57% against challenge. Our results recorded significant reductions ranged from 47.61: 80.95% in total worm of immunized mice with *Schistosoma*, *Fasciola* antigens and their combination with saponin. Yabe *et al.* (2008) and Maghraby *et al*. (2009) investigated interactions among *F. gigantica, Schistosoma spp.* and Barduagni *et al*. (2008) found patients co-infected by *Fasciola* spp. and *S. mansoni*. Abane *et al.* (2000) found that *F. hepatica* fatty acid binding protein (FABP) has been used in vaccination against *S. bovis* and López-Abán *et al*. (2008) reported that addition of a new immunomodulator to FABPs increase the protection against *F. hepatica*.

Our results showed that immunized mice with FEA+SAP and FEA caused a significant improvement in urea cycle enzymes, SD, LDH and total protein content. After challenged with *S. mansoni* infection, a significant reduction in urea cycle enzymes was observed. These results could be correlated to Mousa *et al.* (1975) and Senft (1976) who reported that bilharzial infection resulted in defect in protein metabolism through a defect in absorption of amino acid or defect in enzymes synthesis and hence derangement of many metabolic pathways which may involve detoxification mechanism in urea cycle regulation. Immunization with SEA or SEA+SAP showed a significant increase in OAT, ASS, ASL and an insignificant change was noticed in arginase, LDH and total protein content, while significant reduction was observed in SD enzyme activity. This can be explained on the basis that these antigen or metabolic product act on gene expression as a signal, so the transcription of DNA specific sequence into messenger RNA either be repressed or activated. Gene repression or activation is an effective way of changing enzyme activity (Hoek *et al.,* 1997). Saponin showed a definite reactive immunomodulatory action and has a diuretic action causing increasing in OAT (Zhang *et al*., 1990; Plohmonn *et al*., 1997; Yamanandra *et al*., 2003). Al-Habori and Raman (1998); Bogoiavlenskii *et al.* (1999) and Francis *et al.* (2001) showed that saponin modulate serum glycoprotein.

Pascal *et al*. (2000) and Hamed (2006) reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxides. In addition, Hamed (2006) showed that liver GSH was drastically depleted in *S. mansoni* infected mice and Gharib *et al.* (1999) attributed this decrease to the increased cytotoxicity with H_2O_2 produced as a result of inhibition of glutathione reductase that keeps glutathione in the reduced state. *S. mansoni* infection impairs the antioxidant system since the level of GSH depletion was used as an index of oxidative stress and a sign that hepatic cells are utilizing more antioxidant defenses (Ip, 2000). Therefore, all antigens used improved the level of antioxidants due to the reduction of schistosomal toxins evolved by worms which was confirmed through the observed reduction in worm burden. Catalase activity in our results recorded significant decrease after infection. Hahn *et al*. (2001) attributed this decrease to the oxidative stress which leads to peroxide radicals that detoxified by catalase and thus results in decline in its activity. Our results were in accordance with Son *et al.* (2007) who attributed the increase in superoxide dismutase to the enhancement of its mRNA expression as a result of exposure to superoxide and hydroxil radicals.

Histopathological examination revealed that the hepatic cells, central vein and portal triad were normal in immunized groups. Fibrous perihepatitis and hepatic parenchyma showed severe fibrosis and severe diffuse inflammatory infiltration, causing the distortion of hepatic lobules (Ali and Hamed 2006; EL-Banhawy *et al*., 2007). The current study showed that *S. mansoni* egg granuloma sizes were reduced after immunization with all antigens used. Saponin was maintaining liver architecture that in turn preventing the development of malignancies (Haridas *et al.*, 2001).

In conclusion, *Fasciola* eggs, as a heterologous antigen, exerted more antischistosomal activity than the homologus *Schistosoma* one. In addition, combination of saponin enhanced the protective immunity of *Fasciola* eggs homogenate with more potent effect than *Schistosoma* eggs antigen.

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