

Cross-Reactivity of *Schistosoma mansoni*-*Fasciola gigantica* Influenced by Saponins

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The aim of the present work was to investigate the *Schistosoma mansoni* and *Fasciola gigantica* cross-reactivity between adult worms and egg homogenates of the parasites. Immunoprophylactic effects of crude *Schistosoma mansoni* worms and egg antigens mixed with or without saponins extracted from *Atriplex nummularia* were studied followed by challenge with 80 cercariae of *Schistosoma mansoni*. Our results showed that post 1st immunization with schistosome egg antigens (SEA) there was a significant change ($P \approx 0.05$) in the IgM levels against *Fasciola* egg homogenate (FgEH) without saponins. Post 2nd immunization with SEA mixed with saponins the levels of IgM increased significantly ($P \approx 0.05$) against *Fasciola* worm homogenate (FgWH) as compared with a non-immunized group. Post 2nd immunization the level of IgG was significantly elevated ($P \approx 0.05$) by SEA mixed with saponins against FgWH. Post 2nd immunizations with SEA mixed with saponins showed a significant change ($P \approx 0.05$) in IgG levels against FgEH. These results clearly demonstrated that there is a cross-reactivity between *Schistosoma mansoni* eggs and *Fasciola gigantica* worms and eggs. Saponins were found to be immunostimulatory adjuvants in our study.

Key words: *Schistosoma mansoni*, *Fasciola gigantica*, *Atriplex nummularia*

Introduction

Several vaccine candidates have been identified in *Schistosoma mansoni* directed against the schistosomula as well as against other life cycle stages. Some of the promising antigens have now reached a more advanced stage of development including in the case of glutathione *S*-transferase, the stage of industrial manufacture and safety testing (Capron *et al.*, 1992; Bergquist *et al.*, 1994). Another approach studied closely related cross-reacting antigens from another trematode, *Fasciola hepatica* (Hillyer, 1995). Fascioliasis is an important trematode infection of herbivores worldwide with increasing evidence of prevalence as a disease of humans (Hillyer, 2005). Vaccines in schistosomiasis using homologous antigens have been studied extensively in experimentally infected mammalian hosts. A heterologous 12-kDa *F. hepatica* antigenic polypeptide reacted with *S. mansoni*. A cDNA has been cloned and se-

quenced, and the predicted amino acid sequence of the recombinant protein has been shown to have significant (44%) identity with a 14-kDa *S. mansoni* fatty acid binding protein. Thus in the parasitic trematodes fatty acid binding proteins may be potential vaccine candidates. Mice with intestinal schistosomiasis developed antibodies to both *F. hepatica* nFh12 and rFh15 after 6 weeks of infection. Both the *F. hepatica* and *S. mansoni* cross-reactive antigens may be cross-protective antigens with protection-inducing capability against both species. Immunodiagnosis of current fascioliasis in sheep naturally exposed to *F. hepatica* was performed by using a 2.9 kDa recombinant protein. Standardized diagnostic ELISA for fascioliasis bases on the detection of IgG responses to the 2.9-kDa *F. hepatica*-recombinant protein (FhrAPS) would be a valuable tool to diagnose early and current *F. hepatica* infections in sheep (Arias *et al.*, 2007). Excretory-secretory products of *Fasciola hepatica* (FhES) antigen suc-

ceeded to protect mice against schistosomiasis by a significant reduction in worm burden, ova count, granuloma size and number, and improvement in the histopathological architecture of the liver (Hamed, 2006). Immunization with *Fasciola gigantica* worm homogenate mixed with or without saponins against different schistosomal antigens showed an immunostimulation by increasing the mean number of splenocytes by vaccination against schistosomiasis (Maghraby *et al.*, 2007).

Saponins have an immunological adjuvant activity by inducing the IgG and IgM levels, and stimulate the splenocyte proliferation by increasing the mean number of splenocytes (Maghraby *et al.*, 2007). The most common sources of saponins are higher plants, but increasing numbers have been found in lower marine animals. They were isolated from the marine *Phyllum echinodermata* and particularly from species of the classes Holothuroidea (sea cucumber) and Asteroidea (starfish). The classical definition of saponins is based on their surface activity. Many saponins have detergent properties, give stable foam in water, show haemolytic activity, have a bitter taste, and are toxic to fish (Hostettmann, 1995). Saponins are conventionally defined on the basis of their molecular structure, namely as triterpene (Fig. 1a), steroid (Fig. 1b), or steroid alkaloid (Fig. 1c).

In saponins one or more sugar chains are attached to the aglycone. Monodesmosidic saponins (Fig. 2a) have one single sugar chain, normally

attached at C-3. Bidesmosidic saponins (Fig. 2b) have two sugar chains, one bound through glycosidic linkage at C-3 and one attached through an ester linkage at C-28.

Saponins are a class of substances exhibiting immunological properties (Hostettmann, 1995). The term “immunomodulator” includes on the one hand immunostimulants, which boost the immune system, and on the other hand immunosuppressive factors, which block the immune system. Saponins fed orally enhance cell proliferation *in vivo* and induce helper T-cell activation as well as T-independent B-cell stimulation by LPS (lipopolysaccharides). Plants belonging to the genus *Atriplex* had been used in folk medicine as a wash against itches and rashes such as chicken pox. A poultice of the crushed leaves can be applied to reduce pain and swelling after ant bites. Our previous studies on *Atriplex semibaccata* revealed the presence of saponins (Kamel *et al.*, 2003) and flavonoids (Kamel and Mostafa, 2004). Saponins of *Atriplex semibaccata* are expected to have immunological effects because of their structure relationship to songarosaponin C which possesses the highest immunosuppressive activity *in vitro* (Hartleb and Seifert, 1995). The literature lacks information on the chemical constituents and bioactivities of *A. nummularia* which encouraged us to study the immunological activities of *A. nummularia* L. So, the aim of the present research was to study *S. mansoni* and *F. gigantica* cross reactivities between adult worms and egg homogenates of the parasites, using saponins extracted from *A. nummularia* as immunological adjuvant. Immunoprophylactic effects of a crude *S. mansoni* worm and egg antigens mixed with or without saponins followed by challenge with cercariae of *S. mansoni* were also studied.

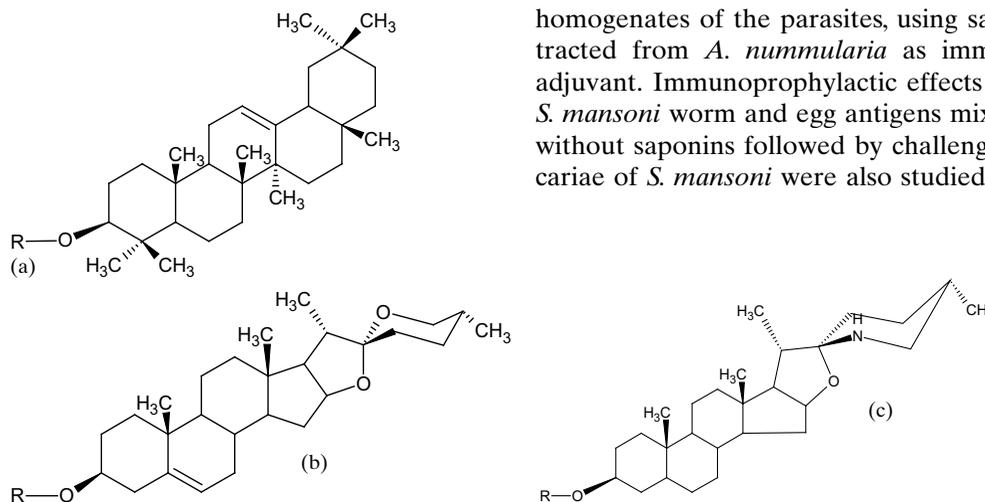


Fig. 1. Chemical structures of (a) triterpenoid, (b) steroid and (c) steroid alkaloid saponins, respectively.

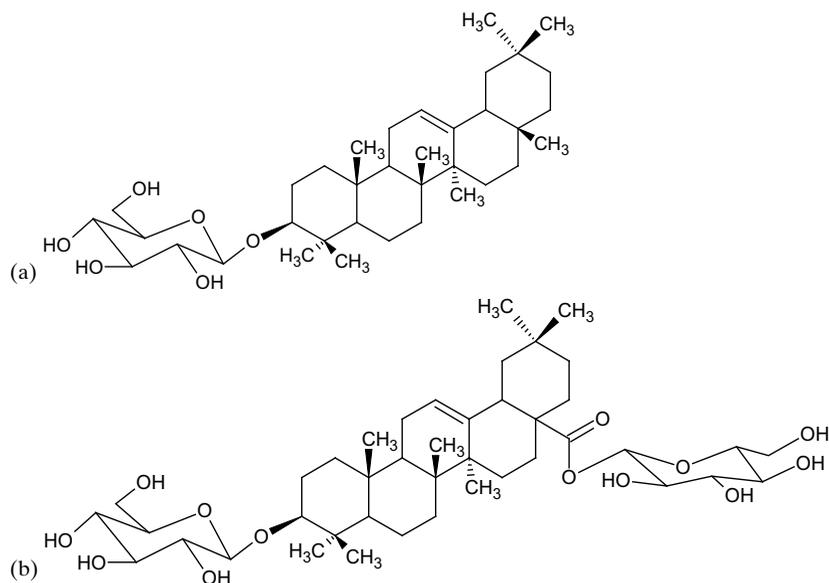


Fig. 2. Chemical structures of (a) monodesmosidic and (b) bidesmosidic saponins, respectively.

Materials and Methods

Plant

Samples from the whole plant of *A. nummularia* were collected at Marsa Matroh in August 2002. The plant material was identified by Prof. Ibrahim El-Garf, Faculty of Science, Department of Botany, Cairo University, Egypt. A voucher specimen of the plant is deposited at the herbarium of the National Research Center, Cairo, Egypt. The plant (herbs or shrubs) belongs to the family Chenopodiaceae which consists of about 1500 species. The plant grows up to 5 cm, with long and often broad and frequently sharply dentate leaves (Tackholm, 1974).

Extraction of saponin from *Atriplex nummularia*

The dried powder of the whole plant was exhaustively extracted with 80% ethanol. The ethanol extract was concentrated under reduced pressure at 40 °C and partitioned between water/chloroform and *n*-butanol. The butanol extract contained crude saponins (the content of saponins was 75% per dry plant).

Screening experiments

A screening test for the crude saponins was carried out according to Faten *et al.* (1994), in

addition to haemolytic activity and Libermann-Burchardt tests (Wall *et al.*, 1952).

Antigen preparation

Schistosoma mansoni antigens: Cercariae, worms and eggs were obtained from Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.

Fasciola gigantica antigens: A *Fasciola gigantica* worm antigens preparation (FWAP) was prepared as described by Hillyer and Santiago de Weil (1977). *Fasciola gigantica* egg antigens were obtained from Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.

Experimental groups and immunization design

Thirty female Swiss albino mice, weighing 18 \approx 20 g, were obtained from the animal house of National Research Center, Dokki, Cairo, Egypt. Mice were divided into six groups (5 animals/group). Mice of the first group were immunized with worm antigens subcutaneously (50 μ g/100 μ l PBS). Mice of the second group were immunized with worm antigens subcutaneously (50 μ g/100 μ l PBS) mixed with saponins (50 μ g/mouse). Mice of the third group were immunized with schistosome egg antigens (50 μ g/100 μ l PBS). Mice of the fourth group were immunized with schistosome egg antigens (50 μ g/100 μ l PBS) mixed with saponins. The

fifth group was immunized with saponins (50 $\mu\text{g}/\text{mouse}$). The sixth group was injected with PBS (100 $\mu\text{l}/\text{mouse}$) and used as control. The levels of both IgM and IgG in sera from immunized mice with different schistosomal antigens mixed with or without saponins were detected against *F. gigantica* worm and egg homogenate using an enzyme-linked immunosorbent assay (ELISA).

Infection with cercariae of *Schistosoma mansoni*

Post 2nd immunization mice were infected by tail immersion with 80 *Schistosoma mansoni* cercariae per mouse (Oliver and Stirewalt, 1952).

Enzyme-linked immunosorbent assay (ELISA)

The assay was performed according to (Hillyer *et al.*, 1979) with some modifications. This assay was used for the determination of the levels of IgG and IgM in sera of the experimental groups. Plates were coated with 50 and 10 $\mu\text{g}/\text{ml}$ of *F. gigantica* worm and egg homogenate, respectively. Plates were incubated at room temperature over

night. They were washed using PBS and 0.05% T20. Plates were blocked for sites free of antigen using blocking buffer (1% BSA, PBS, 0.05% T20). Then sera at the dilution of 1:100 were added and incubated at 37 °C for 2 h. Antimouse IgG and IgM peroxidase conjugates were added at a dilution of 1:5000 and 1:10000 in blocking buffer and incubated for 1 h at 37 °C. *ortho*-Phenylene-diamin dihydrochloride (OPD) was used as substrate. The reaction was followed at 490 nm using an ELISA reader.

Statistical analysis

Statistical significance values between groups were determined by Student t-test according to Ronald *et al.* (1983). Graph Pad Software and Graph Pad InStat were also used.

Results

Detection of saponins

The crude saponins from the butanol fraction formed a stable foam when agitated with water,

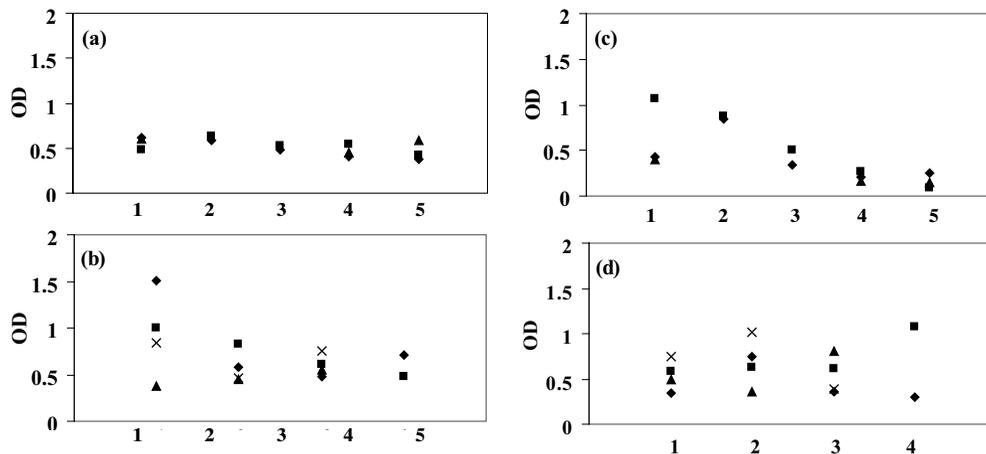


Fig. 3. (a) The IgM level in sera from mice immunized with SWAP mixed with or without saponins against FgWH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (b) The IgM level in sera from *Schistosoma mansoni*-infected mice immunized with SWAP mixed with or without saponins against FgWH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni* immunized with SWAP; group 3, *Schistosoma mansoni*-infected mice immunized with SWAP mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with SWAP mixed with saponins. (c) The IgM level in sera from mice immunized with SWAP mixed with or without saponins against FgEH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (d) The IgM level in sera from *Schistosoma mansoni*-infected mice immunized with SWAP mixed with or without saponins against FgEH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SWAP; group 3, *Schistosoma mansoni*-immunized group with SWAP mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins.

showed high haemolytic activity and gave a positive Libermann-Burchardt test for triterpenes.

IgM levels in sera from mice immunized with schistosomal worm antigens against Fasciola gigantica worm or egg homogenates before and after infection with Schistosoma mansoni using ELISA

The levels of IgM induced by schistosomal worm antigens (SWAP) against *Fasciola gigantica* worm homogenate (FgWH) with or without saponin were studied. Post 1st and 2nd immunizations there was no significant change in the IgM levels against FgWH (Fig. 3a). Challenge with cercariae of *S. mansoni* induced an elevation in the IgM level in SWAP but it was considered not significant against FgWH as compared with the infected non-immunized group (Fig. 3b).

The levels of IgM in sera from mice immunized with SWAP against *Fasciola gigantica* egg homogenate (FgEH) with or without saponins were also studied. 1st and 2nd immunizations produced an elevation in the IgM level which was

considered as non-significant with or without saponin against FgEH as compared with the non-immunized group (Fig. 3c). Post challenge with cercariae of *S. mansoni* there was an elevation in the IgM level of SWAP but it was considered not significant against FgEH as compared with the infected non-immunized group (Fig. 3d).

IgG levels in sera from mice immunized with schistosomal worm antigens against Fasciola gigantica worm or egg homogenates before and after infection with Schistosoma mansoni using ELISA

The levels of IgG induced by SWAP against FgWH with or without saponin were studied. Post 1st and 2nd immunizations there was an elevation in the IgG responses but it was not considered significant against FgWH (Fig. 4a). Post challenge with cercariae of *S. mansoni* there was an elevation in the IgG level in SWAP but it was considered not significant against FgWH as compared with the infected non-immunized group (Fig. 4b).

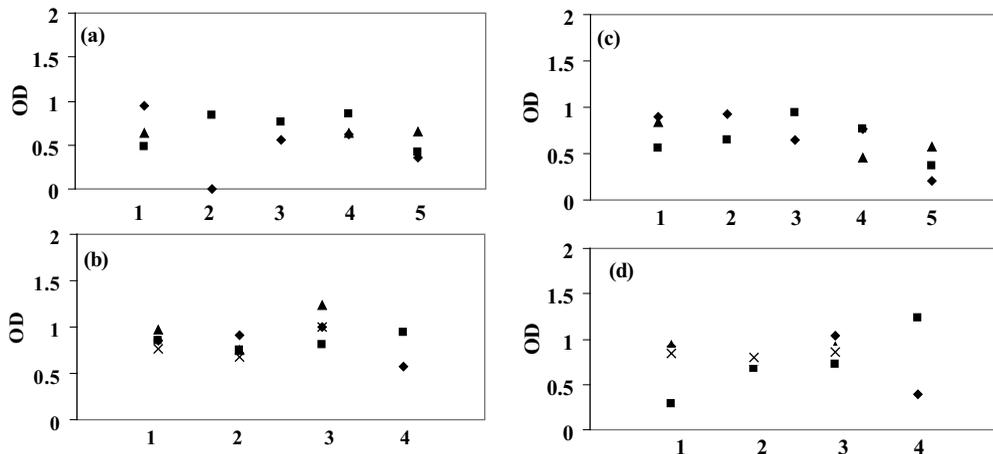


Fig. 4. (a) The IgG level in sera from mice immunized with SWAP mixed with or without saponins against FgWH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (b) The IgG level in sera from *Schistosoma mansoni*-infected mice immunized with SWAP mixed with or without saponins against FgWH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SWAP; group 3, *Schistosoma mansoni*-immunized group with SWAP mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins. (c) The IgG level in sera from mice immunized with SWAP mixed with or without saponins against FgEH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (d) The IgG level in sera from *Schistosoma mansoni*-infected mice immunized with SWAP mixed with or without saponins against FgEH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SWAP; group 3, *Schistosoma mansoni*-immunized group with SWAP mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins.

The levels of IgG in sera from mice immunized with SWAP against FgEH with or without saponins were also studied. Post 1st, 2nd immunizations and challenge with cercariae of *S. mansoni* there was an elevation in the IgG level in SWAP but it was considered not significant against FgEH as compared with the infected non-immunized group (Figs. 4c and d).

IgM levels in sera from mice immunized with schistosomal egg antigens against Fasciola gigantica worm or egg homogenates before and after infection with Schistosoma mansoni using ELISA

The levels of IgM induced by schistosomal egg antigens (SEA) against FgWH with or without saponins were studied. Post 1st immunization there was no significant change in the IgM levels against FgWH with or without saponins. Post 2nd immunization there was a significant increase ($P < 0.05$) in the IgM levels in the group immunized with SEA mixed with saponin against FgWH as compared with the non-immunized group (Fig. 5a).

Post challenge with cercariae of *S. mansoni* there was an elevation in the IgM level in SEA but it was considered not significant against FgWH as compared with the infected non-immunized group (Fig. 5b).

The levels of IgM induced by SEA against FgEH with or without saponins were studied. Post 1st immunization there was a significant change ($P < 0.05$) in the IgM levels against FgEH without saponins. Post 2nd immunization and challenge with cercariae of *S. mansoni* there was an elevation in the IgM level in SEA but it was considered not significant against FgEH as compared with the infected non-immunized group (Figs. 5c and d).

IgG levels in sera from mice immunized with schistosomal egg antigens against Fasciola gigantica worm or egg homogenates before and after infection with Schistosoma mansoni using ELISA

The levels of IgG induced by SEA against FgWH with or without saponins were studied. Post 1st immunization there was an elevation

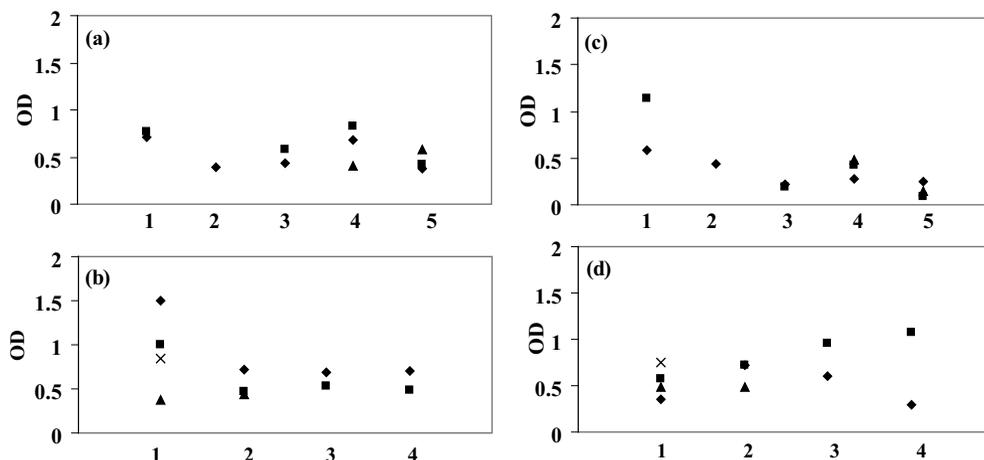


Fig. 5. (a) The IgM level in sera from mice immunized with SEA mixed with or without saponins against FgWH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (b) The IgM level in sera from *Schistosoma mansoni*-infected mice immunized with SEA mixed with or without saponins against FgWH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SEA; group 3, *Schistosoma mansoni*-immunized group with SEA mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins. (c) The IgM level in sera from mice immunized with SEA mixed with or without saponins against FgEH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (d) The IgM level in sera from *Schistosoma mansoni*-infected mice immunized with SEA mixed with or without saponins against FgEH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SEA; group 3, *Schistosoma mansoni*-immunized group with SEA mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins.

in the IgG responses but it was not considered significant against FgWH. Post 2nd immunization there was a significant change ($P < 0.05$) in the IgG levels in sera from mice immunized with SEA and mixed with saponins against FgWH (Fig. 6a). Post challenge with cercariae of *S. mansoni* there was an elevation in the IgG level in SEA but it was considered not significant against FgWH as compared with the infected non-immunized group (Fig. 6b).

The levels of IgG induced by SEA against FgEH with or without saponins were also studied. 1st immunization induced an elevation in the IgG responses but it was not considered significant against FgEH. Post 2nd immunization there was a significant change ($P < 0.05$) in the IgG levels in sera from mice immunized with SEA and mixed with saponin against FgEH (Fig. 6c). Post challenge with cercariae of *S. mansoni* there was an elevation in the IgG level in SEA but it was considered not significant against FgEH as compared with the infected non-immunized group (Fig. 6d).

Discussion

Cross-reactivity between *Fasciola hepatica* and *Schistosoma mansoni* is an important feature. In the present study, we used saponins extracted from *Atriplex nummularia* as immunological adjuvant mixed with immunized *S. mansoni* worm or egg homogenates and detected the cross-reactivity of immunized sera against *F. gigantica* worm or egg homogenates, respectively. Our results showed that there was a cross-reactivity between *F. gigantica* worm or egg homogenates and *S. mansoni* worm or egg homogenates with or without saponins by detecting the humoral immune responses, whereas the levels of IgM and IgG were elevated in sera from mice immunized by SWAP against *F. gigantica* worm homogenate (FgWH) or *F. gigantica* egg homogenate (FgEH) with or without saponins before and after infection with cercariae of *S. mansoni*.

The IgM level was increased in the SEA immunized group mixed with saponins against FgWH before and after challenge. The levels of IgG in-

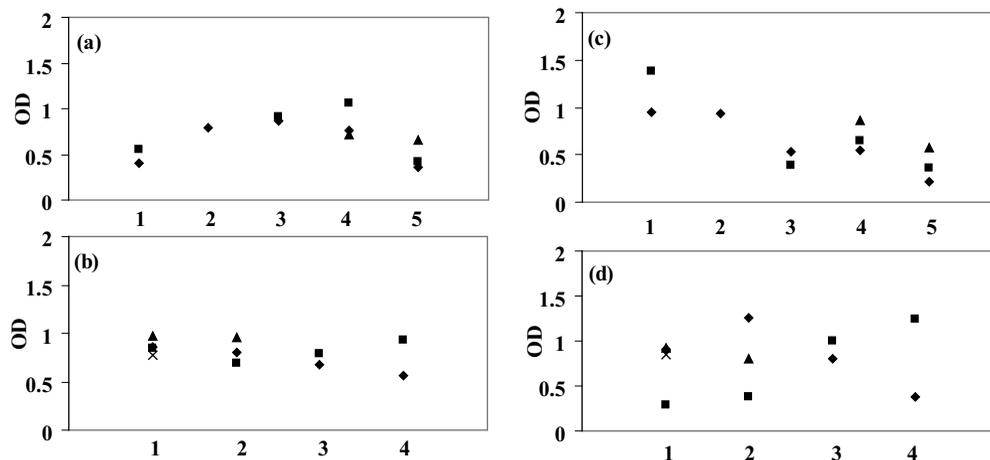


Fig. 6. (a) The IgG level in sera from mice immunized with SEA mixed with or without saponins against FgWH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (b) The IgG level in sera from *Schistosoma mansoni*-infected mice immunized with SEA mixed with or without saponins against FgWH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SEA; group 3, *Schistosoma mansoni*-immunized group with SEA mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins. (c) The IgG level in sera from mice immunized with SEA mixed with or without saponins against FgEH. Group 1, Non-immunized sera; group 2, 1st immunization with SWAP; group 3, 1st immunization with SWAP mixed with saponins; group 4, 2nd immunization with SWAP; group 5, 2nd immunization with SWAP mixed with saponins. (d) The IgG level in sera from *Schistosoma mansoni*-infected mice immunized with SEA mixed with or without saponins against FgEH. Group 1, *Schistosoma mansoni*-non-immunized group; group 2, *Schistosoma mansoni*-immunized group with SEA; group 3, *Schistosoma mansoni*-immunized group with SEA mixed with saponins; group 4, *Schistosoma mansoni*-immunized group with saponins.

duced by SEA against FgWH and FgEH with or without saponins were detected. Post 2nd immunizations there was an increase in the IgG level against FgWH mixed with saponins. Post challenge there was an elevation in the IgG level in schistosomal antigens against FgWH and FgEH. Our previous result (Maghraby *et al.*, 2007) showed that immunized sera from *F. gigantica* worm homogenates mixed with saponins have an immunocellular and humoral stimulation by increasing the mean number of splenocytes and the levels of IgM and IgG in vaccination against schistosomiasis. Our present data confirmed the observation of Hillyer (1984) who showed a cross-reactivity between *F. hepatica* and *S. mansoni* due to the occurrence of antigenic determinants common to both species. Thus *F. hepatica* and *S. mansoni* adults share certain antigenic determinants in the parenchymal antigen mosaics. The protective antigens were shown to cross-react with antibodies prepared against *S. mansoni* fractions. Sera from animals and humans infected with *S. mansoni* or another trematode, *F. hepatica*, cross-react in diagnostic assays that use either *Schistosoma* or *Fasciola* antigens. This antigenic cross-reactivity has provided a basis for suggesting that *F. hepatica* glycoprotein extracts may be useful reagents for the development of a subunit vaccine for *S.*

mansoni (Hillyer *et al.*, 1977). Also, our previous studies explained that saponins had an immunological adjuvant activity by inducing the cellular and humoral immune responses and elicited induction in the IgG and IgM levels (Maghraby *et al.*, 2007). In many studies saponins were used as immunological adjuvant for efficient saponin-based vaccines against *Apicomplexan babesia* divergens. For example, Oliveira-Freitas *et al.* (2006) used acylated and deacylated saponins of *Quillaja saponaria* as adjuvants for the vaccine against visceral leishmaniasis. (It has previously been shown that dogs can be vaccinated against heterologous *Babesia canis* infection using a vaccine containing soluble parasite antigens (SPA) from *in vitro* cultures of *B. canis* and *B. rossi* that are adjuvanted with saponins (Schetters *et al.*, 2006). The Riedel de Haen saponin extract (Oliveira-Freitas *et al.*, 2006) is capable of inducing a specific and strong immunoprotective response and a stronger *Leishmania*-specific splenocyte proliferation in vaccination against murine visceral leishmaniasis. Ginsenoside Rd, a saponin isolated from the roots of *Panax notoginseng*, had immunological adjuvant activity, and elicited a Th1 and Th2 immune response by regulating the production and gene expression of Th1 cytokines and Th2 cytokines (Yang *et al.*, 2007).

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