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 protect 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, cytotoxicity, 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 Leishmania 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% hechedyle trimethyl 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; ornithine 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 dehydrogenase (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 haematoxylin 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total worm (TW)</th>
<th>Male (M)</th>
<th>Female (F)</th>
<th>% R (TW)</th>
<th>% R (M)</th>
<th>% R (F)</th>
<th>RSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>42.00</td>
<td>19.00</td>
<td>13.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>± 4.52</td>
<td>± 1.26</td>
<td>± 1.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosoma eggs</td>
<td>± 1.59</td>
<td>± 1.41</td>
<td>± 1.16</td>
<td>47.61</td>
<td>26.31</td>
<td>38.46</td>
<td>1.19</td>
</tr>
<tr>
<td>± 2.00</td>
<td>± 1.00</td>
<td>± 1.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>± 1.41*</td>
<td>± 1.41</td>
<td>± 1.32</td>
<td>42.85</td>
<td>35.17</td>
<td>15.38</td>
<td>0.80</td>
</tr>
<tr>
<td>Schistosoma eggs + saponin</td>
<td>± 1.41*</td>
<td>± 1.62</td>
<td>± 1.67</td>
<td>52.38</td>
<td>36.84</td>
<td>38.46</td>
<td>1.02</td>
</tr>
<tr>
<td>± 2.00</td>
<td>± 1.00</td>
<td>± 1.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciola eggs</td>
<td>± 1.85*</td>
<td>± 1.41</td>
<td>± 1.72</td>
<td>57.14</td>
<td>42.10</td>
<td>46.15</td>
<td>1.07</td>
</tr>
<tr>
<td>± 1.00</td>
<td>± 1.00</td>
<td>± 1.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fascia eggs + saponin</td>
<td>± 1.62*</td>
<td>± 1.72</td>
<td>± 1.11</td>
<td>80.95</td>
<td>73.68</td>
<td>76.92</td>
<td>1.14</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Part A</th>
<th>Groups</th>
<th>Immunized groups with F. gigantica eggs homogenate</th>
<th>Immunized groups with S. mansoni eggs homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OAT</td>
<td>ASS</td>
<td>ASL</td>
</tr>
<tr>
<td>F / S 1st</td>
<td>6.09</td>
<td>1.11</td>
<td>27.72</td>
</tr>
<tr>
<td>± 0.37 c</td>
<td>± 0.08 d</td>
<td>± 0.48 a</td>
<td>± 0.74 cd</td>
</tr>
<tr>
<td>SAP + (F / S 1st)</td>
<td>6.70</td>
<td>1.19</td>
<td>23.97</td>
</tr>
<tr>
<td>± 0.24 b</td>
<td>± 0.01 bc</td>
<td>± 0.82 c</td>
<td>± 1.17 cd</td>
</tr>
<tr>
<td>F / S 2nd</td>
<td>7.31</td>
<td>1.19</td>
<td>23.91</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Immunized groups with F. gigantica eggs homogenate</th>
<th>Immunized groups with S. mansoni eggs homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>LDH</td>
</tr>
<tr>
<td>FEA / SEA 1st</td>
<td>0.29 ± 0.02</td>
<td>96.27</td>
</tr>
<tr>
<td>SAP + (FEA / SEA 1st)</td>
<td>0.32 ± 0.02</td>
<td>113.43</td>
</tr>
<tr>
<td>FEA / SEA 2nd</td>
<td>0.32 ± 0.02</td>
<td>94.83</td>
</tr>
<tr>
<td>SAP + (FEA / SEA 2nd)</td>
<td>0.24 ± 0.02</td>
<td>102.75</td>
</tr>
<tr>
<td>FEA / SEA + Infection</td>
<td>1.65 ± 0.01</td>
<td>79.37</td>
</tr>
<tr>
<td>SAP + FEA / SEA + Infection</td>
<td>0.75 ± 0.01</td>
<td>84.58</td>
</tr>
</tbody>
</table>

### Part B.

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

- 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.
- 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.
• 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.
• 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.

<table>
<thead>
<tr>
<th>Part A.</th>
<th>Groups</th>
<th>Immunized groups with F. gigantica eggs homogenate</th>
<th>Immunized groups with S. mansoni eggs homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPO</td>
<td>GSH</td>
<td>Cat</td>
</tr>
<tr>
<td>FEA / SEA 1st</td>
<td>0.56 ± 0.01</td>
<td>125.83 ± 3.11</td>
<td>22.82 ± 0.50</td>
</tr>
<tr>
<td>SAP + FEA / SEA 1st</td>
<td>0.78 ± 0.05</td>
<td>117.67 ± 2.06</td>
<td>3.31 ± 0.03</td>
</tr>
<tr>
<td>FEA / SEA 2nd</td>
<td>0.58 ± 0.02</td>
<td>120.51 ± 1.36</td>
<td>22.99 ± 0.59</td>
</tr>
<tr>
<td>SAP + FEA / SEA 2nd</td>
<td>0.51 ± 0.02</td>
<td>125.66 ± 2.26</td>
<td>23.41 ± 0.56</td>
</tr>
<tr>
<td>FEA / SEA + Infection</td>
<td>0.75 ± 0.02</td>
<td>113.20 ± 2.32</td>
<td>20.21 ± 0.79</td>
</tr>
<tr>
<td>SAP + FEA / SEA + Infection</td>
<td>0.56 ± 0.01</td>
<td>125.83 ± 3.11</td>
<td>22.82 ± 0.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Part B.</th>
<th>Common groups</th>
<th>LPO</th>
<th>GSH</th>
<th>Cat</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP 1st</td>
<td>0.65 ± 0.01</td>
<td>116.41 ± 3.48</td>
<td>21.63 ± 0.44</td>
<td>481.56 ± 4.10</td>
<td></td>
</tr>
<tr>
<td>SAP 2nd</td>
<td>0.54 ± 0.02</td>
<td>123.73 ± 1.86</td>
<td>23.47 ± 0.53</td>
<td>468.88 ± 2.76</td>
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<tr>
<td>Infection</td>
<td>0.93 ± 0.01</td>
<td>72.06 ± 1.55</td>
<td>15.81 ± 0.27</td>
<td>710.18 ± 6.71</td>
<td></td>
</tr>
<tr>
<td>SAP + Infection</td>
<td>0.85 ± 0.02</td>
<td>97.87 ± 1.54</td>
<td>17.98 ± 0.33</td>
<td>620.32 ± 6.86</td>
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</tr>
<tr>
<td>Control</td>
<td>0.49 ± 0.02</td>
<td>129.18 ± 2.20</td>
<td>23.90 ± 0.18</td>
<td>463.25 ± 2.35</td>
<td></td>
</tr>
</tbody>
</table>

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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* cercariae. 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 influenched with or without saponin and post challenge (white bars) with 100 S. mansoni cercariae. 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, proving 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 antifecundity, 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 recorded significant reductions ranged from 47.61: 80.95% in total worm of immunized mice with S. mansoni and S. bovis infection. Our results showed that immunized mice with FEA+SAP and FEA caused a significant increase 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; Plohmann et al., 1997; Yamananda et al., 2003). Al-Habori and Raman (1998); Bogoiaivenskii 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 H2O2 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 hydroxyl 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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5. References


