

Full Length Research Paper

Effects and mechanism of action of immunomodulating agents against schistosomiasis-induced hepatic inflammation and fibrosis in mice

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While hepatic schistosomiasis results in morbidity from infection and complications of liver fibrosis, there are few medicines/means available to prevent, control, or treat this fibrosis. The aim of this study was to assess the potential beneficial effects of immunomodulating agents against hepatic schistosomal fibrosis. Swiss mice were infected with *Schistosoma mansoni* live cercariae and then left untreated or administered praziquantel (500 mg/kg/d), rosiglitazone (4 mg/kg/d), propolis (250 mg/kg/d), bisphenol A diglycidyl ether (BADGE) (30 mg/kg/d), or a combination of praziquantel + propolis, praziquantel + rosiglitazone, praziquantel + BADGE, or rosiglitazone + BADGE. Blood samples were collected at various time points post-infection to determine serum interleukin (IL)-2 and IL-10, and IgE and IgG levels, as well as those of alanine and aspartate aminotransferases (ALT and AST). Liver and intestine were recovered at 8 and 12 weeks post-infection, the liver was assessed for hepatic hydroxyproline content and, as with the colon samples, underwent immunohistochemical examination to assess tissue egg count. The results indicate that serum IL-2 levels were significantly increased in infected mice treated with praziquantel and praziquantel + propolis, praziquantel + rosiglitazone, or praziquantel + BADGE (each compared to infected-only hosts), but decreased in infected mice that received rosiglitazone alone. Serum IL-10 levels were significantly decreased in all drug-treated infected mice except those that received rosiglitazone, treatment with BADGE alone caused no change. Serum IgE, IgG, ALT, AST and hepatic hydroxyproline levels were significantly decreased in all drug-treated infected mice except for those that received BADGE alone (no change). From these results, we conclude that combination of chemotherapy plus immunomodulating agents modulate cellular and humoral immune responses and that this leads to significant reductions in hepatic hydroxyproline build-up/liver pathologies associated with schistosomiasis.

Key words: *Schistosoma mansoni*, hepatic fibrosis, praziquantel, rosiglitazone, propolis.

INTRODUCTION

Schistosomiasis is one of the most important public health problems affecting Egyptians, especially its rural inhabitants of the Nile Delta (Kloos and David, 2002). During an ongoing infection, eggs of *Schistosoma mansoni* embolize to the liver, where a granulomatous inflammatory response induces pre-sinusoidal lymphocytes have been implicated as playing a

regulatory role in schistosome granuloma formation (King, 2001). Hepatic fibrosis, a precursor of liver cirrhosis, is a major consequence of the severe liver damage that occurs in many patients with the chronic form of this disease. This pathology involves the abnormal accumulation of both extracellular matrix (ECM) components and hepatic stellate cells (HSC) (Yang et al., 2007).

HSC, key to the development of hepatic fibrogenesis, can undergo a process of activation wherein they trans-differentiate to a myofibroblast-like phenotype. The latter

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form is characterized by an increase in cell proliferation, loss of Vitamin A storing capability, expression of α -smooth muscle actin (α SMA), and over-production of ECM components, especially Type I collagen (Friedman, 2004).

Praziquantel has become the drug of choice against schistosomiasis. Indeed, it has effectively become the only anti-schistosomal drug that is commercially available. Praziquantel has activity against all schistosome species with minimal adverse effects (albeit that there have been reports of transient urticaria, rash, pruritus, and eosinophilia), it is also effective against other trematode and cestode infections (Hagan et al., 2004). This drug is effective mainly against mature schistosomes, although the precise mode of action is not clear, there is evidence that praziquantel increases the permeability of schistosome membranes to calcium (thereby inducing paralysis in a contracted state). The dying parasites are then dislodged in the host and destroyed by local/circulating immune cells (Muñoz et al., 2010).

Though, as noted previously, praziquantel induces short-lived inflammation-related effects in a host, it is not characterized as an inflammatory or an anti-inflammatory agent. In contrast, propolis exhibits anti-inflammatory effects against acute and chronic models of inflammation (for example, formaldehyde- and adjuvant-induced arthritis, carrageenan- and prostaglandin (PGE)-induced paw edema, cotton pallet granuloma). The exact mechanisms of these anti-inflammatory actions are still unclear (Castaldo and Capasso, 2002). Among other compounds tested, only caffeic acid phenethyl ester (CAPE) and galangin modified the anti-inflammatory activity of propolis (albeit with the CAPE contribution being greater). Propolis also exhibits immunostimulatory and immunomodulatory effects *in vitro* on macrophages; *in vivo* it increases the ratio of CD4:CD8 cells in mice (Claus et al., 2000).

Peroxisome proliferator activated receptor- γ (PPAR- γ) activation has been found to affect diverse physiological and pathophysiological events, including regulation of inflammation and induction of apoptosis (Brasier, 2006). Liver fibrosis is a process in which synthesis of ECM exceeds their decomposition, it is also associated with excessive bouts of inflammation (Zhao et al., 2004). As such, the potential benefit from use of a drug like rosiglitazone, a PPAR- γ modulating agent, in the fight against schistosomiasis and its subsequently induced hepatic fibrosis remains and associated inflammatory responses remains to be determined.

The aim of this study was to assess the possible disturbance in the immune system and mechanism through which immunomodulating agents like praziquantel and propolis, along with two other potentially beneficial drugs - rosiglitazone and bisphenol A diglycidyl ether (BADGE) - can produce beneficial effects against the onset/progression of schistosomiasis-induced hepatic inflammation and fibrosis.

MATERIALS AND METHODS

Experimental animals

Male Swiss albino mice (CD strain, 8 weeks of age, 18 to 20 gm) obtained from the Schistosome Biological Supply Center at the Theodor Bilharz Research Institute (TBRI, Imbaba, Giza, Egypt) were used in this study. All mice were housed under specific pathogen-free conditions with a 12 h light/12 h dark cycle, constant temperature ($25 \pm 2^\circ\text{C}$) and relative humidity of $55 \pm 5\%$, and had access to food and water *ad libitum*. All animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Host infection

For these studies, mice were infected with *Schistosoma mansoni* live cercariae (John Bruce Egyptian strain) obtained from *Biomphalaria alexandrina* snails (TBRI) or were left uninfected. All infections were via insertion of the animal tail into a vial containing 80 cercariae in 2 ml de-chlorinated tap water. Mice were left in contact with the infective cercariae for 1 h, after this period, their tails were allowed to air-dry.

The studies here required the use of eight separate exposure groups: Group I (Control group, n=8) mice were not infected or immunomodulated and served as a control, Group II (control: infected untreated group, n=8) mice were infected with the *S. mansoni* cercariae only; Group III (Praziquantel group, n=8) mice were infected and then treated with praziquantel in water (500 mg/kg/d for 2 d beginning 4 weeks post-infection) (Botros et al., 2007); Group IV (Rosiglitazone group, n=8) mice were infected and then treated with the PPAR- γ -modulating agent rosiglitazone (4 mg/kg/d during 5th to 12th weeks post-infection) (Chen et al., 2008); Group V (Propolis group, n=8) mice were infected and then treated with propolis (250 mg/kg/day during 5th to 12th week post-infection) (Issa, 2007); Group VI (Praziquantel + Propolis group, n=8) mice were infected and then treated with a combination of praziquantel (500 mg/kg/d for 2 d beginning 4 weeks post-infection) and propolis (250 mg/kg/d during 5th to 12th weeks post-infection); and, Group VII (Praziquantel + Rosiglitazone group, n=8) mice were infected and then treated with praziquantel (500 mg/kg/d for 2 d beginning 4 weeks post-infection) and rosiglitazone (4 mg/kg/d during 5th to 12th weeks post-infection). All drug treatments were via intragastric administration without the use of anesthesia.

At 8, 9, 10, 11, and 12 weeks post-infection, samples of blood were collected from the tail vein of mice in each group. The blood was then allowed to clot at room temperature and the serum isolated and stored at -80°C for later analyses of the various endpoints outlined subsequently.

At 8 and 12 weeks post-infection, 4 mice per group were euthanized by cervical dislocation. The liver and intestine (that is, colon) of each host was then removed, blot dried, and weighed. Thereafter, sub-samples of the liver were isolated for use in hydroxyproline content determination; the remaining tissue and the colon samples were used in analyses of total egg burden.

Determination of total serum Interleukins (IL)-2 and -10, IgE, and IgG

Commercially available ELISA kits were used to measure serum IL-2 levels (Biosource International, Camarillo CA), IgE levels (BD OptEIA™ Set, San Diego, CA), and IgG levels (Life Diagnostics, Inc., Westchester, PA) in the isolated samples.

Determination of serum alanine (ALT) and aspartate aminotransferase (AST)

Serum alanine aminotransferase and aspartate aminotransferase levels were determined colorimetrically based on the method of Reitman and Frankel (1957), using a Diamond Diagnostics kit (Cairo, Egypt). In this kit, AST and ALT levels were estimated via measures of formation of oxaloacetate and pyruvate, respectively, using kit-provided enzyme precursors. After completion of the reaction, according to manufacturer's instructions, each sample was then analyzed in a double beam UV-VIS spectrophotometer (Model UV-1601 PC, Shimadzu, Kyoto, Japan) at 520 nm.

Determination of hepatic hydroxyproline content

The total collagen present in the liver samples recovered from each host was determined by estimating hydroxyproline content. The latter value was obtained using base hydrolysis for the dissolution of tissues as described previously by Reddy and Enwemeka (1996). Briefly, ≈ 100 mg of liver tissue was incubated overnight at 37°C in 5% KOH (6 ml/100 mg tissue weight) in a test tube. The mixture was occasionally inverted to re-suspend the dissolved tissue. Duplicate sets (each contained 600 μ l of tissue sample suspension) were then removed/ sampled. To each sample, 160 μ l of 10 N NaOH and 40 μ l distilled water were added, and all proteins present subjected to hydrolysis by placing the screw-capped tubes in an autoclave for 20 min. Normal tissues were used as the background for the standards. Duplicate 250 μ l aliquots of each sub-sample/liver were then removed and supplemented with 250 μ l chloramine-T solution (saturated in 50% n-propanol [in water]); Sigma, St. Louis, MO). All samples were then incubated at room temperature for 3 h. Thereafter, 500 μ l of freshly prepared Ehrlich's reagent (0.15 g/ml of n-propanol/ perchloric acid [2:1, v/v]) was added to each tube and the materials incubated at 65°C for 20 min. Each sample was then analyzed in a UV-VIS spectrophotometer at 550 nm.

Immunohistochemical examination

The procedure for immunostaining was conducted according to the method described by Taylor et al. (2002). Monoclonal antibodies directed against Type IV collagen [CD54/ICAM-1, Ab-4 (ClonePHM-12) MS375R7 (NeoMarkers, Lab Vision, Newmarket, UK) and polyclonal rabbit antibody against nm23/NDP Kinase Ab-1, RB-116-R1 (Lot. 116R606B, NeoMarkers) were utilized.

Briefly, at sacrifice, liver and colon tissues from each mouse were fixed in formalin and then processed to prepare sections (3 to 5 μ m thick) on glass slides. The slides were then deparafinized in xylene, re-hydrated in decreasing strengths of ethyl alcohol (followed by distilled water), and then rinsed in a 0.01 M phosphate-buffered saline (PBS; pH 7.3). Slides were then treated with 0.0025% protease solution (Sigma) and incubated at 37°C in a closed humidity chamber for 20 min.

The slides were then rinsed three times in PBS and incubated in normal goat serum for 30 min at room temperature to block non-specific binding events. Thereafter, the slides were placed in a container with the monoclonal/polyclonal antibodies (in permeabilization buffer solution) and left overnight at room temperature. Non-adherent primary antibodies were rinsed away by three washes in PBS; biotinylated secondary antibody was then added to the container and the slides incubated 1 h at room temperature. Following a wash with PBS, streptavidin-biotin horseradish peroxidase (Standard ABC Kit; Vector Laboratories, Burlingame, CA) was applied to each slide for 30 min at room temperature. After a wash in PBS, slides were coated with 3,3'-Diaminobenzidine (DAB) chromogen solution and was incubated 10

min at room temperature. After gentle rinsing, each slide was counterstained with Mayer's hematoxylin (Bancroft and Gamble, 2002) for 1 min. The slides were then dehydrated in alcohol and cover-slipped using DPX mountant (Merck-BDH, Lutterworth, England). Ultimately, each stained tissue section was examined using a light microscope (Olympus BX 51, Olympus America, Melville, NY) and photographed with a digital camera (Olympus DP11) connected to the microscope.

Tissue egg count (in liver and colon)

The number of *S. mansoni* eggs in the liver and intestine (from duodenum to rectum) samples isolated from each mouse was estimated after alkali digestion (Cheever, 1968). Briefly, 0.5 g of each liver and colon sample was placed in dedicated glass bottles each containing 2 ml of 4% KOH and incubated at 37°C overnight. The following day, each sample was placed in a 60°C incubator for 1 h. The bottles were then vigorously shaken and 0.1 ml aliquots were removed and placed on glass slides. The slides were then cover-slipped and examined under a microscope for *S. mansoni* ova. The average number of ova/0.1 ml aliquot was determined and the number of ova/gram tissue then calculated.

Statistical analysis

Results were expressed as the mean \pm SD. Regression analysis and correlation coefficient were done for standard curves. Comparison between different groups was carried out by one way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$. The statistical package for social sciences (SPSS) computer software was used to carry out the statistical analysis.

RESULTS

Effects on serum IL-2

Mice infected with *Schistosoma mansoni* showed a significant time-dependent decrease in serum IL-2 levels (Table 1). The greatest decrease in IL-2 was noted at 12 weeks post-infection. The results in Table 1 also illustrate that treatment of infected mice with propolis alone, BADGE alone, or rosiglitazone + BADGE displayed no significant difference in serum IL-2 at any of the time points analyzed (up to 12 weeks post-infection) as compared to the levels noted in their untreated infected counterparts, that is, serum IL-2 levels still declined over time. In contrast, treatment of infected mice with praziquantel alone or in combination with propolis, or with rosiglitazone + BADGE showed a significant time-dependant increase in serum IL-2 relative to the values in the untreated infected mice. Of all the regimens, only treatment with rosiglitazone alone resulted in a significant time-dependent decrease in the serum IL-2 that was greater than that in the untreated infected mice.

Effects on serum IL-10

Mice infected with *S. mansoni* displayed a significant

Table 1. Effect of immunomodulating agents and chemotherapy on serum IL-2 (pg/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 28.43 ± 0.36 | 28.50 ± 0.36 | 28.45 ± 0.35 | 28.06 ± 0.25 | 28.28 ± 0.40 |
| DMSO | 26.25 ± 2.96* | 26.38 ± 3.25* | 26.00 ± 3.96* | 27.00 ± 2.83* | 27.13 ± 2.90* |
| Infected | 22.25 ± 1.67* | 19.25 ± 1.39* | 17.88 ± 1.46* | 16.75 ± 3.92* | 15.25 ± 2.49* |
| Praziquantel | 22.50 ± 2.88* | 24.50 ± 2.88 ^{ab} | 25.63 ± 2.13 ^{ab} | 26.00 ± 3.21 ^{ab} | 27.50 ± 2.78 ^{ab} |
| Propolis | 19.88 ± 2.80* | 18.83 ± 2.26* | 16.38 ± 3.02* | 15.38 ± 2.45* | 13.75 ± 2.33* |
| Rosiglitazone | 18.50 ± 2.56* | 17.75 ± 2.49* | 14.50 ± 2.39 ^{ab} | 14.00 ± 1.85* | 11.75 ± 1.67 ^{ab} |
| BADGE | 21.50 ± 2.83* | 19.63 ± 1.69* | 17.88 ± 1.73* | 17.25 ± 2.38* | 15.25 ± 2.31* |
| Rosiglitazone plus BADGE | 21.00 ± 3.12* | 18.38 ± 2.62* | 16.25 ± 3.24* | 15.88 ± 3.04* | 13.75 ± 3.11* |
| Praziquantel plus Propolis | 21.13 ± 3.44* | 24.38 ± 4.17 ^{ab} | 23.88 ± 3.00 ^{ab} | 25.38 ± 3.38 ^{ab} | 25.38 ± 4.69 ^{ab} |
| Praziquantel plus Rosiglitazone | 22.88 ± 4.45* | 22.75 ± 4.33 ^{ab} | 23.88 ± 3.88 ^{ab} | 22.75 ± 2.55 ^{ab} | 25.25 ± 3.37 ^{ab} |
| Praziquantel plus BADGE | 22.25 ± 4.13* | 22.88 ± 4.52* | 23.63 ± 2.97 ^{ab} | 26.38 ± 4.27 ^{ab} | 25.88 ± 3.04 ^{ab} |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

Table 2. Effect of immunomodulating agents and chemotherapy on serum IL-10 (pg/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 23.13 ± 2.64 | 22.50 ± 2.51 | 23.63 ± 2.33 | 23.38 ± 2.39 | 23.00 ± 1.93 |
| DMSO | 22.88 ± 2.03 | 23.75 ± 2.71 | 22.75 ± 1.98 | 22.88 ± 2.17 | 22.88 ± 2.23 |
| Infected | 154.13 ± 16.44* | 165.00 ± 15.56* | 193.38 ± 11.02* | 212.88 ± 19.56* | 234.13 ± 36.53* |
| Praziquantel | 98.50 ± 11.21 ^{ab} | 89.63 ± 16.00 ^{ab} | 74.38 ± 15.31 ^{ab} | 73.75 ± 9.97 ^{ab} | 73.13 ± 12.11 ^{ab} |
| Propolis | 88.88 ± 9.52 ^{ab} | 85.50 ± 6.46 ^{ab} | 82.38 ± 5.01 ^{ab} | 76.63 ± 2.83 ^{ab} | 69.25 ± 5.90 ^{ab} |
| Rosiglitazone | 149.63 ± 10.82* | 173.63 ± 4.93* | 190.50 ± 8.16* | 206.30 ± 7.90* | 230.25 ± 6.88* |
| BADGE | 151.25 ± 11.49* | 168.88 ± 13.02* | 198.38 ± 9.29* | 212.75 ± 11.68* | 237.38 ± 28.78* |
| Rosiglitazone plus BADGE | 148.63 ± 10.57* | 174.80 ± 11.80* | 191.63 ± 14.90* | 213.63 ± 12.70* | 242.38 ± 23.42* |
| Praziquantel plus Propolis | 86.63 ± 6.57 ^{ab} | 85.38 ± 6.91 ^{ab} | 77.13 ± 4.61 ^{ab} | 77.13 ± 4.61 ^{ab} | 50.13 ± 14.72 ^{ab} |
| Praziquantel plus Rosiglitazone | 72.88 ± 2.47 ^{ab} | 64.25 ± 3.77 ^{ab} | 56.75 ± 4.03 ^{ab} | 56.75 ± 4.03 ^{ab} | 27.88 ± 5.44 ^{ab} |
| Praziquantel plus BADGE | 99.13 ± 7.57 ^{ab} | 91.25 ± 12.93 ^{ab} | 75.00 ± 13.98 ^{ab} | 75.00 ± 13.98 ^{ab} | 71.88 ± 15.20 ^{ab} |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

time-dependent increase in serum IL-10 levels (Table 2), with the greatest increase being seen at 12 weeks post-infection. Treatment of infected mice with praziquantel or propolis alone, or with praziquantel + propolis, praziquantel + rosiglitazone, and praziquantel + BADGE resulted in significant decreases in serum IL-10 at all time points (up to 12 weeks post-infection) as compared to levels in untreated infected counterparts. Again, the maximum effects were at 12 weeks post-infection. In contrast, treatment of infected mice with rosiglitazone or BADGE alone or a combination of rosiglitazone + BADGE caused no significant change (with respect to the infected untreated control group) in IL-10 at any of the time points examined.

Effects on serum IgE

Mice infected with *S. mansoni* evinced a significant time-

dependent increase in serum IgE levels (Table 3), with the greatest increase being seen at 12 weeks post-infection. Treatment of infected mice with praziquantel, propolis, or rosiglitazone alone, or with praziquantel + propolis, praziquantel + rosiglitazone, or praziquantel + BADGE gave rise to significant decreases in the IgE at all time points as compared to levels in untreated infected counterparts, maximum effects were again found at 12 weeks post-infection. However, treatment of infected mice with BADGE alone or rosiglitazone + BADGE resulted in no significant change (with respect to the infected untreated control group) in serum IgE at any of the chosen timepoints.

Effects on serum IgG

Infection with *S. mansoni* caused a significant time-dependent increase in serum IgG levels (Table 4), with

Table 3. Effect of immunomodulating agents and chemotherapy on serum IgE (ng/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 4.95 ± 0.24 | 4.81 ± 0.39 | 5.04 ± 0.39 | 4.95 ± 0.42 | 4.83 ± 0.41 |
| DMSO | 4.80 ± 1.01 | 5.45 ± 0.58 | 4.88 ± 1.07 | 5.10 ± 0.85 | 4.93 ± 0.68 |
| Infected | 62.63 ± 1.92* | 61.63 ± 4.66* | 63.00 ± 2.51* | 63.75 ± 3.01* | 65.13 ± 3.27* |
| Praziquantel | 37.00 ± 1.51 ^{ab} | 34.38 ± 1.69 ^{ab} | 32.25 ± 1.67 ^{ab} | 30.50 ± 2.45 ^{ab} | 30.50 ± 4.22 ^{ab} |
| Propolis | 32.88 ± 2.36 ^{ab} | 32.38 ± 3.11 ^{ab} | 30.00 ± 2.00 ^{ab} | 24.50 ± 2.45 ^{ab} | 20.13 ± 2.03 ^{ab} |
| Rosiglitazone | 32.50 ± 1.49 ^{ab} | 29.88 ± 1.73 ^{ab} | 25.38 ± 1.41 ^{ab} | 20.63 ± 2.67 ^{ab} | 14.38 ± 2.39 ^{ab} |
| BADGE | 61.88 ± 3.44* | 62.75 ± 3.49* | 62.13 ± 3.14* | 62.38 ± 4.24* | 62.13 ± 4.22* |
| Rosiglitazone plus BADGE | 58.75 ± 5.26* | 58.38 ± 6.99* | 59.38 ± 4.87* | 61.88 ± 4.19* | 62.13 ± 7.12* |
| Praziquantel plus Propolis | 28.38 ± 1.69 ^{ab} | 26.75 ± 1.49 ^{ab} | 20.63 ± 2.62 ^{ab} | 17.25 ± 2.12 ^{ab} | 13.25 ± 2.60 ^{ab} |
| Praziquantel plus Rosiglitazone | 30.50 ± 1.60 ^{ab} | 26.38 ± 1.69 ^{ab} | 23.25 ± 1.28 ^{ab} | 18.00 ± 2.07 ^{ab} | 13.13 ± 2.47 ^{ab} |
| Praziquantel plus BADGE | 33.38 ± 3.50 ^{ab} | 31.38 ± 6.93 ^{ab} | 31.50 ± 6.14 ^{ab} | 29.13 ± 5.33 ^{ab} | 26.13 ± 7.10 ^{ab} |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

Table 4. Effect of immunomodulating agents and chemotherapy on serum IgG (ng/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 367.63 ± 8.53 | 367.00 ± 7.82 | 366.00 ± 8.50 | 366.38 ± 8.28 | 372.25 ± 4.77 |
| DMSO | 365.38 ± 12.36 | 363.50 ± 10.27 | 365.75 ± 9.61 | 363.38 ± 10.86 | 365.38 ± 11.15 |
| Infected | 608.75 ± 7.83* | 614.88 ± 4.94* | 615.00 ± 4.69* | 615.63 ± 8.37* | 616.00 ± 7.03* |
| Praziquantel | 502.00 ± 3.30 ^{ab} | 485.13 ± 3.44 ^{ab} | 474.25 ± 3.62 ^{ab} | 464.13 ± 4.09 ^{ab} | 452.25 ± 1.67 ^{ab} |
| Propolis | 449.50 ± 2.07 ^{ab} | 438.00 ± 3.12 ^{ab} | 430.50 ± 2.88 ^{ab} | 422.88 ± 2.90 ^{ab} | 414.25 ± 3.73 ^{ab} |
| Rosiglitazone | 502.00 ± 2.00 ^{ab} | 480.38 ± 2.26 ^{ab} | 474.38 ± 2.83 ^{ab} | 463.75 ± 3.45 ^{ab} | 454.63 ± 3.29 ^{ab} |
| BADGE | 612.50 ± 5.13* | 612.75 ± 6.39* | 612.63 ± 6.16* | 612.25 ± 3.85* | 614.50 ± 4.50* |
| Rosiglitazone plus BADGE | 598.75 ± 14.67* | 612.25 ± 21.52* | 607.75 ± 11.80* | 606.88 ± 12.25* | 609.75 ± 7.75* |
| Praziquantel plus Propolis | 502.13 ± 5.82 ^{ab} | 461.50 ± 4.44 ^{ab} | 442.00 ± 4.84 ^{ab} | 419.13 ± 2.90 ^{ab} | 404.75 ± 3.85 ^{ab} |
| Praziquantel plus Rosiglitazone | 501.88 ± 3.04 ^{ab} | 486.25 ± 2.82 ^{ab} | 477.13 ± 2.36 ^{ab} | 466.63 ± 2.26 ^{ab} | 455.13 ± 2.47 ^{ab} |
| Praziquantel plus BADGE | 491.13 ± 7.40 ^{ab} | 486.00 ± 4.63 ^{ab} | 462.38 ± 4.14 ^{ab} | 445.13 ± 4.52 ^{ab} | 434.88 ± 3.36 ^{ab} |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

the greatest increase being found at 12 weeks post-infection. Treatment of infected mice with praziquantel, propolis, or rosiglitazone alone, or with either a combination of praziquantel + propolis, praziquantel + rosiglitazone, or praziquantel + BADGE causing a significant decrease in serum IgG (as compared to levels in untreated infected counterparts) at all time points up to 12 weeks post-infection; maximum effects were again noted at 12 weeks post-infection. Notably, treatment of infected mice with BADGE alone and rosiglitazone + BADGE caused no significant changes in (with respect to the infected untreated control group) in serum IgG at any of the time points examined.

Effect on serum ALT and AST

Infection with *S. mansoni* caused a significant increase in serum ALT levels at all time points beginning at 8 weeks

post-infection (Table 5), the greatest increase was seen at 12 weeks post-infection. Treatment of the infected mice with praziquantel, propolis, or rosiglitazone alone, or a combination of praziquantel + propolis, praziquantel + rosiglitazone, or praziquantel + BADGE resulted in a significant decrease in serum ALT at all time points up to 12 weeks post-infection as compared to levels in their respective untreated infected counterparts.

As with the other endpoints noted previously, the maximum effects (that is, decreases) were noted at 12 weeks post-infection. In contrast, treatment of infected mice with BADGE alone or rosiglitazone + BADGE caused no significant change in ALT levels at any of the timepoints. The *S. mansoni* infection also caused a significant increase in serum AST levels at each of the time points analyzed; the greatest increase was seen at 12 weeks post-infection (Table 6). Treatment of the infected mice with praziquantel, propolis, rosiglitazone alone, or a praziquantel + propolis, praziquantel +

Table 5. Effect of immunomodulating agents and chemotherapy on serum ALT (U/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 69.00 ± 5.73 | 69.88 ± 9.20 | 68.00 ± 11.15 | 69.50 ± 9.65 | 68.75 ± 10.33 |
| DMSO | 67.13±8.57 | 66.50±9.65 | 71.25±9.30 | 67.87±10.28 | 67.75±11.89 |
| Infected | 108.12 ± 9.83* | 106.12±15.46* | 104.25 ±9.15* | 101.13±11.96* | 103.88 ±9.86* |
| Praziquantel | 83.88±5.08 ^{*,a} | 82.00±5.18 ^{*,a} | 77.25±4.50 ^a | 75.13±5.46 ^a | 74.88±4.67 ^a |
| Propolis | 88.00±11.35 ^{*,a} | 87.75±12.73 ^{*,a} | 86.87 ±10.37 ^{*,a} | 85.00±11.03 ^{*,a} | 80.63±12.06 ^a |
| Rosiglitazone | 87.38±10.74 ^{*,a} | 87.00±11.51 ^{*,a} | 84.00±7.56 ^{*,a} | 83.75±15.66 ^{*,a} | 77.38±12.27 ^a |
| BADGE | 101.50±11.65* | 105.12±12.29* | 101.75±9.07* | 102.88±9.89* | 105.88±10.29* |
| Rosiglitazone plus BADGE | 100.75±13.95* | 98.50±7.27* | 94.50±10.46* | 97.50 ±10.77* | 96.88 ± 11.96* |
| Praziquantel plus Propolis | 79.38±9.10 ^a | 73.75±10.26 ^a | 73.63±10.43 ^a | 72.25 ±9.60 ^a | 69.25±8.81 ^a |
| Praziquantel plus Rosiglitazone | 77.25±8.63 ^a | 72.01±8.03 ^a | 72.25±5.31 ^a | 69.13±11.06 ^a | 67.75 ±7.94 ^a |
| Praziquantel plus BADGE | 69.00 ± 5.73 | 84.38±7.05 ^{*,a} | 68.00 ± 11.15 | 69.50 ± 9.65 | 74.63±11.94 ^a |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

Table 6. Effect of immunomodulating agents and chemotherapy on serum AST (U/ml) in mice infected with *S. mansoni*.

| Groups | 8 th week [#] | 9 th week [#] | 10 th week [#] | 11 th week [#] | 12 th week [#] |
|---------------------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 137.88± 9.45 | 139.25± 9.05 | 136.38± 9.13 | 137.75± 9.50 | 134.13 ± 5.22 |
| DMSO | 133.87±13.05 | 135.37±12.28 | 136.50±10.20 | 134.50±10.93 | 135.50±11.19 |
| Infected | 397.25± 12.81* | 399.25±13.17* | 398.62 ± 13.28* | 398.50±13.17* | 400.00 ± 10.38* |
| Praziquantel | 255.55±15.41 ^{*,a} | 233.38±4.75 ^{*,a} | 235.88 ±31.48 ^{*,a} | 210.88 ±9.48 ^{*,a} | 212.25 ±6.20 ^{*,a} |
| Propolis | 311.00±13.16 ^{*,a} | 295.38± 10.07 ^{*,a} | 284.75±7.72 ^{*,a} | 278.25 ±8.50 ^{*,a} | 266.00 ± 7.73 ^{*,a} |
| Rosiglitazone | 253.88±12.68 ^{*,a} | 236.38±4.06 ^{*,a} | 223.38±9.04 ^{*,a} | 215.50 ±9.29 ^{*,a} | 206.75±6.61 ^{*,a} |
| BADGE | 398.62±8.63 * | 397.38±12.66 * | 402.62 ±12.02* | 400.50±12.34* | 398.25±13.34* |
| Rosiglitazone plus BADGE | 394.12± 9.51* | 399.75±10.14* | 392.88 ±14.08* | 402.13±13.79* | 402.13±9.37 * |
| Praziquantel plus Propolis | 259.75±13.53 ^{*,a} | 237.75±6.84 ^{*,a} | 226.38 ±9.77 ^{*,a} | 210.13±7.90 ^{*,a} | 208.75 ± 8.60 ^{*,a} |
| Praziquantel plus Rosiglitazone | 233.5±5.21 ^{*,a} | 217.75 ±7.19 ^{*,a} | 201.13±8.29 ^{*,a} | 180.00±8.43 ^{*,a} | 134.13 ± 5.22 |
| Praziquantel plus BADGE | 265.00±12.80 ^{*,a} | 237.75±9.98 ^{*,a} | 228.38±7.73 ^{*,a} | 210.13±9.55 ^{*,a} | 135.50±11.19 |

[#]Time post-infection. At p < 0.05. *value is significantly different compared to control and/or ^asignificant compared to infected-only group. All values shown are Mean ± SD; n=8 for each group.

rosiglitazone, or praziquantel + BADGE resulted in significant decreases (as compared to levels in untreated infected mice) in serum AST levels at all of the timepoints. As with ALT, treatment of infected mice with BADGE alone or rosiglitazone + BADGE caused no significant change in AST levels at any of the timepoints.

Effect on hepatic hydroxyproline content

Infection with *S. mansoni* caused a significant increase in the hepatic hydroxyproline at each of the time points analyzed, the greatest increase was seen at 12 weeks post-infection (Table 7). Treatment of infected mice with praziquantel, propolis, rosiglitazone alone, or a praziquantel + propolis, praziquantel + Rosiglitazone or praziquantel + BADGE resulted in significant decreases

(as compared to levels in untreated infected mice) in the hepatic hydroxyproline content at all of the time points, the greatest decreases were seen at 12 weeks post-infection. On the contrary treatment of infected mice with BADGE and Rosiglitazone plus BADGE caused no significant change in the hepatic hydroxyproline content at any of the time points.

Effects on hepatic and intestinal egg count

Treatment of infected mice with BADGE, Rosiglitazone and Rosiglitazone + BADGE caused no significant change in the hepatic egg count at 12 weeks post-infection as compared with their respective infected groups (Table 8). However in Praziquantel, Propolis alone or a Praziquantel + Propolis, Praziquantel + Rosiglitazone and Praziquantel + BADGE groups, the mean number of *Schistosoma mansoni* ova per gram

Table 7. Effect of immunomodulating agents and chemotherapy on hepatic hydroxyproline ($\mu\text{g/g}$ tissue) in mice infected with *S. mansoni*

| Groups | 8 th week [#] | 12 th week [#] |
|---------------------------------|--------------------------------------|------------------------------------|
| Control | 163.75 \pm 6.50 | 163.50 \pm 7.43 |
| DMSO | 162.25 \pm 6.45 | 166.00 \pm 12.75 |
| Infected | 464.50 \pm 6.95* | 1141.38 \pm 8.40* |
| Praziquantel | 367.25 \pm 7.74 ^a | 862.13 \pm 5.96 ^a |
| Propolis | 319.75 \pm 14.45 ^a | 697.75 \pm 8.08 ^a |
| Rosiglitazone | 353.50 \pm 6.46 ^a | 798.75 \pm 7.76 ^a |
| BADGE | 470.00 \pm 7.93* | 1139.50 \pm 6.52* |
| Rosiglitazone plus BADGE | 465.00 \pm 7.87* | 1141.63 \pm 20.30* |
| Praziquantel plus Propolis | 211.00 \pm 7.58 ^a | 579.00 \pm 16.78 ^a |
| Praziquantel plus Rosiglitazone | 345.88 \pm 5.62 ^a | 776.00 \pm 5.32 ^a |
| Praziquantel plus BADGE | 366.75 \pm 4.77 ^a | 859.75 \pm 3.41 ^a |

[#]Time post-infection. At $p < 0.05$. *value is significantly different compared to control and/or ^asignificant compared to infected-only group.

All values shown are Mean \pm SD; n=8 for each group.

Table 8. Effect of immunomodulating agents and chemotherapy on number of ova in intestine/liver of mice infected with *S. mansoni*.

| Groups | Number of ova in intestine (ova/gm intestine) | Number of ova in liver (ova/gm liver) |
|---------------------------------|--|--|
| Control | Nil | Nil |
| DMSO | Nil | Nil |
| Infected | 4841.00 \pm 311.06 | 4447.38 \pm 376.24 |
| Praziquantel | 487.5 \pm 84.72* | 380.63 \pm 13.44* |
| Propolis | 2420.75 \pm 155.89* | 2285.88 \pm 79.43* |
| Rosiglitazone | 4735.50 \pm 417.81 | 4243.38 \pm 444.04 |
| BADGE | 4655.88 \pm 554.84 | 4567.88 \pm 160.43 |
| Rosiglitazone plus BADGE | 4695.25 \pm 454.47 | 4142.00 \pm 299.53 |
| Praziquantel plus Propolis | 440.63 \pm 45.31* | 220.75 \pm 15.14* |
| Praziquantel plus Rosiglitazone | 518.38 \pm 33.34* | 355.50 \pm 57.24* |
| Praziquantel plus BADGE | 556.00 \pm 67.80* | 298.88 \pm 90.75* |

At $p < 0.05$. *value is significantly different compared to control.

All values shown are Mean \pm SD; n=8 for each group.

liver was significantly decreased as compared with their respective infected groups.

The mean number of *Schistosoma mansoni* ova per gram of intestine in the infected group was 4841.00 \pm 311.06. Treatment of infected mice with BADGE, Rosiglitazone alone or Rosiglitazone + BADGE caused no significant change in the intestinal egg count at 12 weeks post-infection as compared with their respective infected groups. While treatment of infected mice with Praziquantel, Propolis alone or a Praziquantel + Propolis, Praziquantel + Rosiglitazone, and Praziquantel + BADGE caused significant decreases in the mean number of *Schistosoma mansoni* ova per gram of intestine as compared with their respective infected groups (Table 8).

Effects on hepatic histology

Under the light microscope, the liver from animals infected with *S. mansoni* scarified after 12 weeks caused strong in deposition of positive collagen fibre (Figure 1) and minimal or absent collagen type IV reaction (Figure 2). Treatment of infected mice with Praziquantel caused significant increase in deposition of positive collagen cells in the mouse liver section in comparison with normal control group (Figure 3). Similarly, treatment of infected mice with Rosiglitazone caused dense deposition of positive collagen type IV in the mouse liver sections in comparison with infected control group (Figure 4). However, animals treated with the combination of

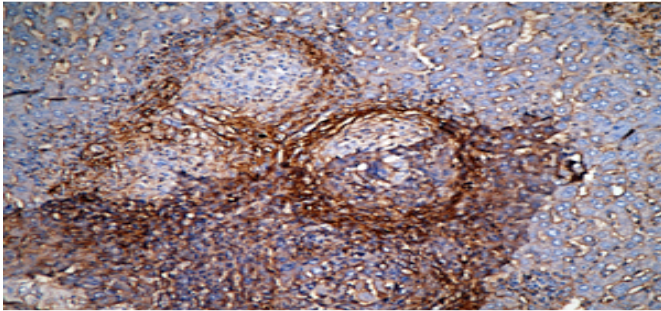


Figure 1. Liver section from fibrotic mice (infected group) showing dense positive collagen Type IV bundles around granuloma (PAP, 250X).

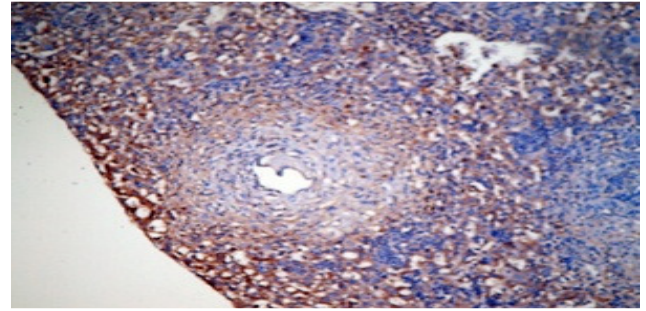


Figure 5. Liver section from infected group treated with praziquantel + rosiglitazone showing minimal fibrous collagen deposits around bilharzial ova (PAP, 250X).

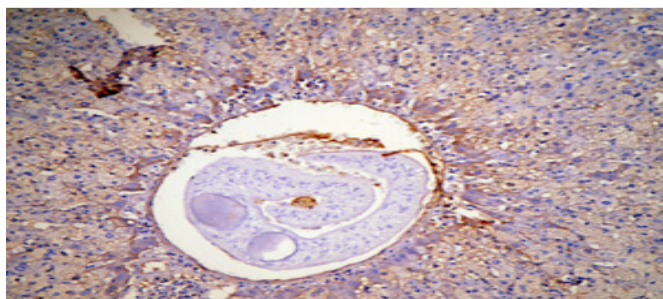


Figure 2. Liver section from fibrotic mice (infected group) showing bilharzial worm inside the venules with minimal collagen Type IV reaction (PAP, 400X).

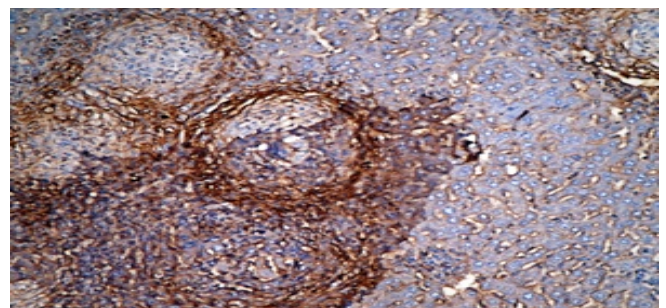


Figure 6. Liver section from infected group treated with BADGE showing large number of positive collagen deposits in the slice of mouse liver (PAP, 250X).

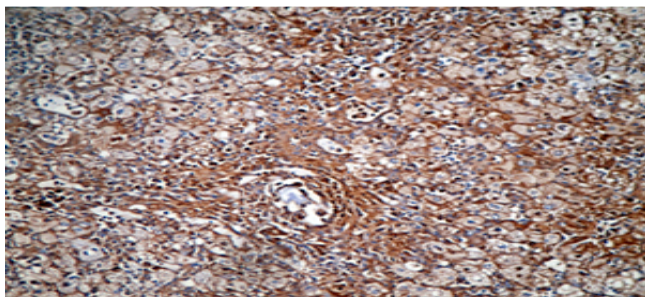


Figure 3. Liver section from infected group treated with praziquantel alone showing large number of positive collagen deposits in the slice of mouse liver (PAP, 250X).

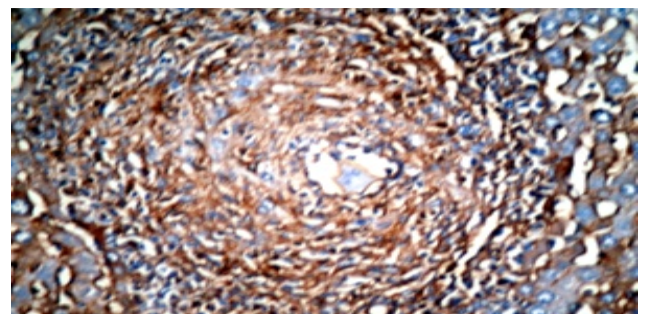


Figure 7. Liver section from infected group treated with BADGE + rosiglitazone showing dense positive collagen Type IV bundles (PAP, 250X).

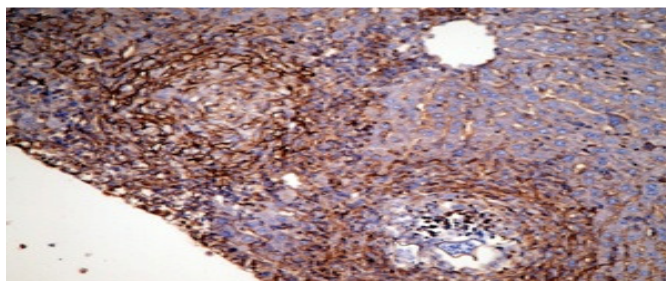


Figure 4. Liver section from infected group treated with rosiglitazone alone showing positive fibrous collagen deposits in the slice of mouse liver (PAP, 250X).

Praziquantel plus Rosiglitazone caused dramatic decrease in deposition of positive type IV collagen in comparison with infected liver sections (Figure 5). On the other hand, treatment of infected mice with BADGE alone or in combination with Rosiglitazone or Praziquantel caused dense deposition of positive collagen bundles (Figures 6, 7, and 8). In contrast, treatment of infected mice with Propolis caused very thin collagen within the granuloma (Figure 9). The combination of Praziquantel plus Propolis caused that most of granulomas in Praziquantel plus Propolis treated mice contained very

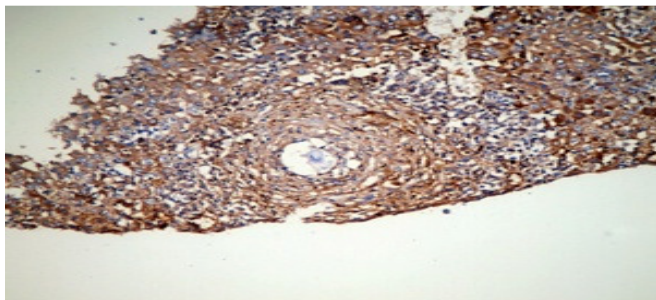


Figure 8. Liver section from infected group treated with BADGE + praziquantel showing positive fibrous collagen deposits in the slice of mouse liver (PAP, 250X).

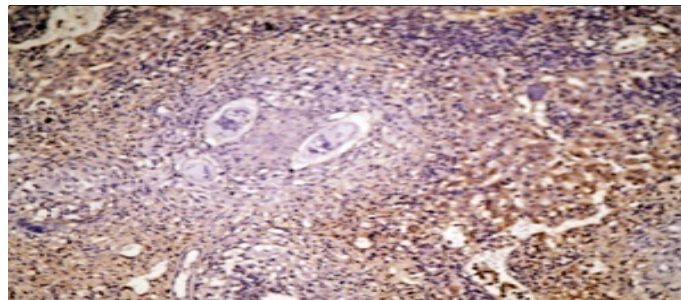


Figure 10. Liver section from infected group treated with propolis + praziquantel showing minimal or absent collagen Type IV inside or around granuloma in the slice of mouse liver (PAP, 250X).

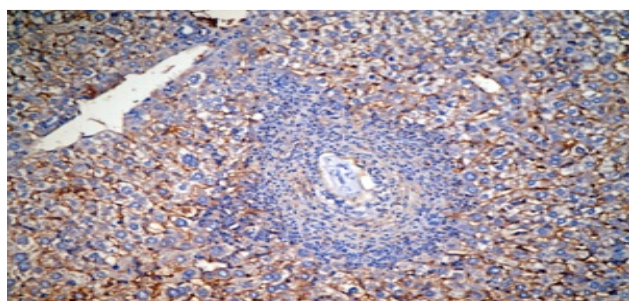


Figure 9. Liver section from infected group treated with propolis showing very thin collagen within the granuloma in the slice of mouse liver (PAP, 250X).

fine collagen fibres with a minimal interstitial and perivascular collagen deposition (Figure 10).

DISCUSSION

Schistosomiasis is the major public health problem in rural Egypt. The main agent of human schistosomiasis is *Schistosoma mansoni* (dos Santos et al., 2007). Previous study reported that the severity of the disease has been shown to be a consequence of immunopathological processes that lead to the development of fibrosis caused by delayed-type inflammatory granulomatous reaction around the schistosome eggs that are trapped in the small vessels of the liver. However, granulomas exert protective effects, since mice which fail to develop granulomas experience extensive hepatotoxic effects around the parasite eggs (Amiri et al., 1992).

The present study was designed to assess the possible disturbances in the immune system that is associated with Schistosomiasis-induced hepatic fibrosis and the possible mechanism through which the different immunomodulating agents can produce their beneficial effects in Schistosomiasis induced hepatic fibrosis. This was achieved by determination of cellular immunity (Serum IL-2 and IL-10) and humoral immunity (IgG and

IgE), ALT, AST, hepatic hydroxyproline content as a fibrotic markers and egg count in tissues (liver and intestine) as well as the histopathological and immunohistochemical examination of the liver.

The granulomatous reaction around schistosome eggs in the liver and the hepatic fibrosis critically dependent on IL-2 levels (Cheever et al., 1993). Infection of mice with *S. mansoni* caused significant decreases in all time points analyzed, the greatest increase having been seen at 12 weeks post-infection. Yamashita et al., 1987 observed an 80 to 90% reduction in the production of IL-2 produced by infected mice, which was not accounted for by a reduction in the number of IL-2 producing T-lymphocytes and suppressive macrophages. On the contrary, Khalil et al. (1995) revealed that IL-2 level was higher in all patients infected with schistosomiasis and the level was higher in lightly infected group than heavily infected ones with no respect to age. Praziquantel is an effective but safe non hepatotoxic schistosomicide drug (Khalil et al., 1995) that may reverse schistosomal-induced liver pathology and eliminate the parasitic worms from infected host. Also, it was able to reduce the number of ova in both the liver and intestine with complete absence of immature stages and increase of the number of dead ova (Mahmoud et al., 2002). After antischistosomal therapy with praziquantel, IL-2 became up to normal within 3 months (Khalil et al., 1995). These results are in accordance with the finding reported in this study since, treatment with praziquantel caused significant increase in serum IL-2 when compared to levels in untreated infected hosts, the greatest increase was found at 12 weeks post-infection. In this study, treatment of infected mice with Propolis alone caused no significant change in the serum IL-2 at all time points determined up to 12 weeks compared with their respective infected groups.

On the other hand, treatment of infected mice with praziquantel + propolis caused a time dependant increase in the serum IL-2. These results could be explained by the findings of Popova et al. (2004) who reported that several types of flavonols (contents of propolis) stimulate human peripheral blood leukocyte

proliferation. They significantly increase the activity of helper T-lymphocytes, cause increases in the levels of cytokines IL-2 and interferon [IFN]- γ , and help to activate macrophages and are thus useful in the treatment of several diseases caused by immune dysfunction (Kawakita et al., 2005). It is thus apparent that the immunostimulatory effect produced by the Propolis may be due to cell mediated and humoral antibody mediated immune response. So that, administration of propolis and its extract increase IL-2 (Havsteen, 2002).

In the present study, it was found that, treatment of infected mice with rosiglitazone alone revealed a time dependent decrease in the serum IL-2. Treatment of infected mice with praziquantel in combination with rosiglitazone caused a time-dependant increase in the serum IL-2 when compared with their respective infected groups (maximum increases were found at 12 weeks post-infection). These results in agreement with Clark et al. (2000) who suggested an immunoregulatory role of PPAR in macrophages and monocytes, but recently also on lymphocyte function. It has been shown that PPAR ligands inhibit T-helper cell responses in terms of inhibition of IL-2 production by T-cell clones, while not inhibiting proliferation of such clones. Similarly, data from murine splenocytes showed that PPAR γ and PPAR γ ligands decreased IL-2 and IFN α production in mitogen-activated cells, but had modest effects on proliferation (Cunard et al., 2002).

On the other hand in mice infected with *Schistosoma mansoni* live cercariae showed a time dependent increase in serum IL-10 level compared with the control group. The highest increase was found after 12 weeks. Treatment of infected mice with praziquantel revealed significant decrease in the serum IL-10 when compared with their respective infected group. The maximum decrease was found after 12 weeks. IL-10 involved in the regulation of T- lymphocyte responses in cases of human schistosomiasis, IL-10 undoubtedly plays a key role in controlling infection (Coutinho et al., 2010). Although, several publications reported the anti-inflammatory role of this cytokine (Cameron et al., 1997; Nicoletti et al., 1997; Amirshahrokhi et al., 2008), the present study may support the pre-described suggestion since serum IL-10 level was increased in the present infected mice in such condition. This controversy may be due to the evidence that IL-10 is a multifunctional cytokine with adverse effects on different immune cells (Moore et al., 2001). Martins-Leite et al. (2008) observed a significant reduction in the level of IL-10 following treatment with praziquantel in individuals with and without hepatic fibrosis. Previous research involving subjects with chronic schistosomiasis had suggested that the levels of IL-10 secretion are dependent on the intensity of infection, as determined by the number of eggs in the stool (Silveira et al., 2004).

In our study, treatment of infected mice with propolis and a combination of praziquantel + propolis resulted in a

significant decrease in serum IL-10 level (compared with values in the infected-only hosts) at all time points determined up to 12 weeks post-infection. The maximum decreases were found at 12 weeks. These results are in agreement with those of Sy et al. (2006) who found that administration of propolis and its extract strongly inhibit IL-10 secretion and exhibited anti-inflammatory effects.

On the other hand, treatment of infected mice with rosiglitazone alone showed no significant change in the serum IL-10 at all time points determined up to 12 weeks compared with their respective infected groups. Treatment of infected mice with praziquantel + rosiglitazone showed significant decrease in the serum IL-10 at all time points determined up to 12 weeks compared with their respective infected groups. The maximum decreases were found after 12 weeks. These results are in agreement with Clark et al. (2000) who suggested an immunoregulatory role of PPAR in macrophages and monocytes, but recently also on lymphocyte function. It has been shown that PPAR ligands inhibit T helper cell responses in terms of inhibition of interleukin (IL)-2 production by T cell clones, while not inhibiting proliferation of such clones. Similarly, data from murine splenocytes showed that PPAR- α and PPAR- γ ligands decreased IL-2 and interferon (IFN)- γ production in mitogen-activated cells, but had modest effects on proliferation (Cunard et al., 2002), while IL-10 production and leukocyte infiltration remained unchanged (Efrati et al., 2008).

Mice infected with *Schistosoma mansoni* live cercariae showed a time-dependent increase in serum IgE, IgG levels compared with the control group. The highest increase was found after 12 weeks. The present study showed that administration of praziquantel induced significant decrease in the serum IgE and IgG at all time points determined up to 12 weeks compared with their respective infected group, the maximum decreases were found at 12 weeks post-infection. Stevens et al. (1983) found that the concentration of total serum IgE and IgG were lowest in patients mono-infected with *Schistosoma haematobium* and highest in those with a mixed infection. *Schistosoma mansoni* may be considered to release more soluble antigenic material into circulation than *S. haematobium*, stimulating more intensively IgE- and IgG-producing B-lymphocytes. This should consequently lead to higher concentrations of IgE and IgG. The rapid reduction of serum IgE after specific chemotherapy has already been noted by Ito et al. (1972) and Kojima et al. (1972) who found that with *S. japonicum*, IgE concentrations reduced approximately by 50% 2 to 4 months after chemotherapy. The noted similar tendency of IgG to fall after efficient chemotherapy has been confirmed in a study of more heavily infected patients (Stevens et al., 1983).

In our study, treatment of infected mice with propolis and a combination of praziquantel + propolis resulted in a significant decrease in serum IgE, and IgG levels

(compared with values in the infected-only hosts) at all time points determined up to 12 weeks post-infection. The maximum decreases were found at 12 weeks. These results are in agreement with those of Sy et al. (2006) who found that administration of propolis and its extract strongly reduce serum levels of IgG and IgE, and exhibited anti-inflammatory effects.

Rühl et al. (2003) demonstrated that the PPAR γ ligand and, to a greater extent, the PPAR γ ligand inhibit IgE production and also the production of other isotypes (IgG and IgM) *in vitro* and *in vivo*. Co-culture experiments of B-lymphocytes and monocytes showed that the PPAR ligand-associated inhibition of IgE production is not directly mediated through activated B-lymphocytes, but rather primarily indirectly mediated via regulatory signal pathways of monocytes. The analysis of supernatants from peripheral blood monocyte (PBMC) in the presence of PPAR ligands revealed that inhibition of IgE synthesis is most likely related to the reduced secretion of several cytokines. In this study treatment of infected mice with rosiglitazone alone or praziquantel + rosiglitazone showed significant decrease in the serum IgE and IgG at all time points determined up to 12 weeks post-infection compared with levels in the respective untreated infected hosts (maximum decreases were found at 12 weeks post-infection).

In the present study, mice infected with *S. mansoni* showed a time-dependent increase in serum ALT and AST levels compared with their control group. The highest increase in the serum ALT and AST were found after 12 weeks. The elevation of serum ALT and AST of infected mice seems to be a consequence of the damage of hepatic cells and/or impaired permeability of cell membranes, or may be due to heavy schistosome egg deposition (Giboda et al., 1994).

Treatment of infected mice with praziquantel revealed significant decrease in the serum ALT and AST when compared with their respective infected group. The maximum decreases were found after 12 weeks. These results are in accordance with the findings of Mahmoud et al. (2002) who found that mice infected with *S. mansoni* live cercariae showed elevation of serum ALT and AST levels compared with the normal control group and treatment of infected mice with Praziquantel revealed reduction in the serum ALT and AST nearly to the normal values at 16 weeks post infection.

In the present study treatment with propolis produced an effective action against the hepatosplenic damaging effect, caused by *Schistosoma mansoni* infection, as shown by reducing the number of ova count in the liver and in the intestine and reduced the granuloma size. Eventually, the liver functions were improved as evidenced by a decrease of the elevated serum levels of ALT and AST when compared with their respective infected group. Similarly combination of praziquantel + propolis showed significant decrease in the serum ALT and AST at all time points determined up to 12 weeks

compared with their respective infected groups. The effect of propolis could, at least partly, be attributed to drug-induced modulation of the immune response to schistosome eggs trapped in the liver. In murine schistosomiasis, a variety of cytokines and lymphokines are implicated as mediators of the granulomatous inflammatory response (Issa, 2007). Accordingly, manipulation of cytokine levels can modify the intensity of the inflammatory response. Several studies point to the effect of propolis on the immune system. It was found to produce an increase in the ratio of helper to suppressor T cells and to enhance natural killer cell activity in normal volunteers (Sforzin, 2007).

Our experiments showed that treatment of infected mice with rosiglitazone, ameliorated hepatocyte degeneration, necrosis and infiltration of inflammatory cells and reduced the scores of necroinflammation significantly compared with model group. Liver functions (ALT, AST) were also improved apparently, treatment of infected mice with rosiglitazone alone or a combination of praziquantel + rosiglitazone revealed significant decrease in the serum ALT and AST when compared with their respective untreated infected hosts (maximum decreases were found at 12 weeks post-infection). These results demonstrated that PPAR γ agonists also had anti-inflammatory effects, and subsequently retarded the progression of hepatic fibrosis in mice. Currently, the U.S. Food and Drug Administration (FDA) have approved two thiazolidinediones (TZD) (that is, rosiglitazone and pioglitazone) for the treatment of Type 2 diabetes. A wealth of clinical studies indicated that rosiglitazone and pioglitazone have no hepatotoxicity (Yuan et al., 2004). Our experiments also showed that after administration with rosiglitazone for 5 weeks, no mice were observed to have hepatotoxicity; moreover, their liver functions (ALT, AST) were improved greatly compared with model mice. In conclusion, rosiglitazone, a PPAR γ ligand, greatly retards the progression of experimental hepatic fibrosis through inhibition of HSC activation and amelioration of hepatocyte necroinflammation in mice. Therefore, it is a potential new antifibrotic drug for clinical application.

Mice infected with *Schistosoma mansoni* live cercariae showed a time dependent increase in serum hepatic hydroxyproline levels compared with the control group. The highest increase was found after 12 weeks. Also, our results revealed that the mean number of *Schistosoma mansoni* ova per gram liver and intestine in the infected group were 4447.38 ± 376.24 and 4841.00 ± 311.06 , respectively. These results, supported by immunohistochemical examination of liver section, showed large number of positive collagen cells distributed not only in the central venule pericytes and the linkage region of lobules but also in fibrous proliferation and Disec cavities.

These results are in agreement with Cheever et al. (1994) who found that the magnitude of the granulomatous fibrosis and the severity of the fibrotic response appear to be dependent on the strain of host involved.

However, other investigators found that cytokines regulate the fibrotic process in both human (Henri et al., 2002) and murine infections (Fallon et al., 2000). Finally, the outcome of fibrosis is also influenced by the degradative matrix metalloproteinases (Nagase and Woessner, 1999) that break down the collagens produced, and by tissue inhibitors of these metalloproteinases which enhance fibrosis. Metalloproteinases have been reported to be located within the infected liver (Gomez et al., 1999).

The present study was designed to assay the total collagen contents of granulomatous liver, measured in terms of hydroxyproline content. Infected mice treated with praziquantel showed significant decrease in the hepatic hydroxyproline content. The maximum decrease was found after 12 weeks. It was interesting to find that, praziquantel affected the level of hepatic hydroxyproline content but did not affect histopathological changes in the liver section.

Also, treatment of infected mice with propolis alone or in combination with praziquantel in this study showed significant decrease in the hepatic hydroxyproline content after 12 weeks compared with their respective infected groups. The maximum decreases were found after 12 weeks. The combination of praziquantel + propolis was the best combination to decrease the value of hepatic hydroxyproline content. In agreement with these results, Chen et al. (2008) found that administration of propolis inhibited tTG activation and prevented the development of thioacetamide (TAA)-induced liver cirrhosis. Since propolis is safe for consumption by humans, it may have a beneficial role in chronic liver diseases caused by ongoing hepatic damage.

Treatment of infected mice with rosiglitazone and praziquantel + rosiglitazone showed significant decrease in the hepatic hydroxyproline content after 12 weeks, compared with their respective infected groups. Galli et al., (2002) found that rosiglitazone administration decreases the hepatic hydroxyproline content to the normal levels as controls. Intriguingly, rosiglitazone induces PPAR- γ activation to inhibit collagen and fibronectin synthesis in human HSCs initiated by transforming growth factor (TGF)- β_1 *in vitro*. These findings implicate PPAR- γ activation in HSC retarded fibrosis, suggesting the use of PPAR- γ ligands for the treatment of fibrosis following liver injury.

Also, praziquantel treatment led to highly significant decrease in eggs per gram tissue (liver and intestine). This reduction is manifested in egg deposition in the liver and large intestine, which results are in basic agreement with the finding of Issa (2007). The main possible explanations for these results are that praziquantel was able to reduce the *S. mansoni* worm and/or regulates the adhesion molecules playing a key role in egg trapping and increase the mobilization of eggs from liver and intestine to the exterior of the body in the feces. These data may suggest an anti-fibrotic effect of praziquantel in

the early stage of liver fibrosis. Praziquantel might be able to block liver fibrosis through killing parasites to alleviate liver inflammation (Mahmoud et al., 2002; Issa, 2007).

The effect of propolis could, at least partly, be attributed to drug-induced modulation of the immune response to schistosome eggs trapped in the liver since, in murine schistosomiasis, a variety of cytokines and lymphokines have been implicated as mediators of the granulomatous inflammatory response (Issa, 2007).

The present study demonstrated that propolis administration alone or in combination with praziquantel significantly decreased tissue load compared with infected untreated mice, a decrease that was also manifested in egg deposition in the liver and large intestine. This reduction was significantly different from that in infected untreated mice. These results could be explained by Ibrahim et al. (2000) who found that, the reduction in the number of eggs in the intestine and liver of mice infected with *Schistosoma mansoni*, after administration of propolis, is most probably secondary to that occurring in the worm load and its direct effects on the eggs, due to the enhancement of the immunological reactions. It has been recorded that when treatment was done with a daily dose of 250 mg/kg, starting from the first day of infection and with sacrifice at 16 weeks post-infection, there was a reduction in worm load compared to control, but the worms were not completely eradicated. Also, Issa (2007) found that administration of propolis to infected mice with *Schistosoma mansoni* produced direct effect on the eggs due to the enhancement of the immunological reactions. These findings are explained by reduction in worm load compared to the control group although the worms were not completely eradicated.

Combination of praziquantel + rosiglitazone led to a highly significant reduction in eggs per gram tissue (in liver and intestine). This reduction is manifested in egg deposition in liver (92%) and in the large intestine (89.2%) compared with their respective infected groups. On the other hand, treatment by rosiglitazone alone did not show any significant change in hepatic or intestinal egg count when compared with untreated infected group.

Immunohistochemical examination of liver section from praziquantel-treated mice preserved non-significant changes in the fibroplasias, hepatocellular necrosis and inflammatory cell infiltration in the liver sections when compared with infected fibrotic group. In agreement with this result, Chen et al. (2008) found that treatment of mice with praziquantel alone did not show any significant changes in the liver sections compared with infected non-treated mice.

Immunohistochemical examination of liver section from mice treated with propolis alone or in combination with praziquantel, showed little expression of Type IV collagen which was found only in the central venule pericytes and the linkage region of lobules from the mouse liver, and these results could be explained by Fitzpatrick et al.

(2001) who found that caffeic acid phenylethyl ester (CAPE) is an active component of propolis from honeybee hives that is widely known for its antiviral, anti-inflammatory, and immunomodulatory properties. Also, CAPE can suppress NF- β activation and thereby inhibits inflammatory responses and significantly reduces the level of pro-inflammatory cytokines (tumor necrosis factor [TNF] and IL-1) (Bose et al., 2009).

Immunohistochemical examination of liver sections from mice treated with rosiglitazone alone or in combination with praziquantel showed few collagens in the central venule pericytes and the linkage region of lobules from mouse liver. These results also suggest that PPAR γ ligand, rosiglitazone can impede liver fibrosis after *Schistosoma* infection. In agreement with these results, Chen et al. (2008) found that treatment of mice with rosiglitazone showed a significant reduction of liver fibrosis induced by *Schistosoma japonicum*. The effect of this compound on preventing liver fibrosis may be through down regulation of liver TGF- α_1 and collagens.

In order to elucidate whether the anti-inflammatory effect of rosiglitazone observed here is related to activation of the PPAR γ receptor, we also investigated the effect of a PPAR γ antagonist, bisphenol diglycidyl ether (BADGE), on the anti-inflammatory effects of rosiglitazone. The present study demonstrated that pre-treatment of hosts with BADGE attenuates the protective effects of rosiglitazone. Thus, we conclude that activation of PPAR γ (i) reduces development of inflammation, and (ii) contributes to the anti-inflammatory effects of rosiglitazone.

Treatment of infected mice with BADGE and rosiglitazone + BADGE showed no significant change in serum IL-2, IL-10, IgE, IgG, ALT, and AST levels, hepatic hydroxyproline content, and egg count (in liver and intestine) at all time points determined up to 12 weeks post-infection as compared with values associated with the infected-only hosts. Immunohistochemical examination also revealed no significant changes compared with fibrotic untreated mice.

Treatment of monocytes and macrophages with high concentrations of PPAR- γ agonists reduced secretion of inflammatory cytokines and inhibited macrophage activation. In particular, treatment of monocytes with TZD reduced release of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 (Cuzzocrea et al., 2004). In the present study, we found that the co-administration of BADGE and Rosiglitazone blocked these effects of the PPAR- γ agonist. These findings, therefore, confirm (i) that inflammation results in the activation and the subsequent expression of pro-inflammatory inflammatory cytokines, and suggest (ii) that rosiglitazone activates the PPAR- γ receptor resulting in the reduction of the release of these of inflammatory cytokines.

In conclusion, rosiglitazone and Praziquantel produce best beneficial immunomodulating effects against the onset/progression of schistosomiasis-induced hepatic

fibrosis. Additionally, the association of Propolis and Praziquantel increased the potency of the humoral and cellular responses. The use of Propolis extract as an adjuvant might contribute to the efficacy of Praziquantel.

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