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## **Immune Response on Mice Infected with *Schistosoma mansoni* and Treated with Myrrh**

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Myrrh is an oleoresin gum extracted from a tree known as *Commiphora molmol*. In the present study the effects of myrrh on mice infected with 100 cercariae of *Schistosoma mansoni* (*S. mansoni*) were studied. We found that serum antischistosomal antibodies of mice infected with *Schistosoma mansoni* was decreased significantly ( $p < 0.0001$ ) after three weeks of the treatment with myrrh (10 mg/kg body weight) as well as praziquantel (PZQ) (250 mg kg<sup>-1</sup> body weight) compared with the infected non-treated group (control group). The mean serum level of IL-2 was decreased significantly after treatment with PZQ and myrrh ( $p < 0.05$ ; 0.01, respectively). On the other hand, the level of gamma interferon (IFN- $\gamma$ ) did not affected by the treatment with PZQ but decreased non significantly after treatment with myrrh. In conclusion myrrh can improve the cellular immunity of schistosomiasis infected mice.

**Key words:** *Schistosoma mansoni*, granulomatous response, myrrh, antischistosomal, antibodies

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## INTRODUCTION

Schistosomiasis is a chronic disease and the latest estimates for Saharan Africa demonstrate that over 200 million people worldwide suffer from it and kills more than 20000 people every year (Utzinger and Keiser, 2004). A disease caused by infection with parasitic worm of the genus schistosoma (Butterworth *et al.*, 1994). The adult worm of *Schistosoma mansoni* resides in the inferior mesenteric vein where the females can lay eggs. Eggs can pass through the intestinal wall to be deposited with the feces or carried by the blood flow into the liver where they induce vigorous granulomatous response (Boros, 1989). Chemotherapy is the most widely methods to control schistosomiasis (Tsai *et al.*, 2000). Praziquantel is the current drug of choice and the cornerstone of schistosomiasis control over the last decades and well remain the strategy of choice in the near future (WHO, 1999). Indications of resistance came initially from laboratory, where the possibility of selecting schistosome parasites largely insensitive to praziquantel had been demonstrated in mice (Fallon and Doenhoff, 1994; Ismael *et al.*, 1994). Additional concern was raised in the field studies conducted in Egypt, where low susceptibility of *Schistosoma mansoni* were found in 1-2.4% of individuals (Ismail *et al.*, 1996) and in Senegal as mentioned by Gryseels *et al.* (1994); Guisse *et al.* (1997); Picquet *et al.* (1998) and De Clercq *et al.* (1999). According to these studies, an alternative safe and effective antischistosomal drug is urgently needed (Sheir *et al.*, 2001). Among herbal products, myrrh is an oleo-gum resin obtained from the stem of a plant *Commiphora molmol*. It is a safe, natural flavoring substance and has been approved by the United Stat Food and Drug Administration (Ford *et al.*, 1992). Recently, many reports have been shown that, myrrh is a potent cytotoxic drug against Ehrlich solid tumor cells with no clastogenic effects (Alharbi *et al.*, 1994) and also have antischistosomal effects (Massoud *et al.*, 1998; Sheir *et al.*, 2001).

The aim of the present study is to determine the level of the antischistosomal antibodies, level of cellular immune cytokines (Gamma interferon and interleukin-2).

## MATERIALS AND METHODS

**Infected animals:** Swiss albino infected (100 cercariae of *S. mansoni*) mice were purchased from Theodore Bilhars institute, Cairo Egypt.

**Mice treatment with praziquantel and myrrh:** Praziquantel was suspended in dist. water then mice were

treated orally using stomach tub with 250 mg kg<sup>-1</sup> body weight for 3 alternative days starting from the 42th day of infection.

Myrrh (commercially, Merazide, Pharco Co. Alex. Egypt) were suspended in corn oil and mice were treated for 3 successive days with a dose of 10 mg kg<sup>-1</sup> body weight using stomach tub after 6 week of infection. After 3 weeks of the last dose of treatment, mice from all groups were sacrificed and blood samples were collected.

### Determination of antischistosomal antibodies:

Polystyrene flat bottom microtiter plate was coated with 50 µL mL<sup>-1</sup> of 25 µg mL<sup>-1</sup> from schistosome antigenic preparation (SWAP) at room temperature over night. After blocking, pooled sera from treated and non treated mice diluted in PBS-T20 were added (50 µL mL<sup>-1</sup>) and incubated at 37°C for 2 h. After washing with PBS-T20, 50 µL mL<sup>-1</sup> of goat anti-mouse IgG Alkaline phosphatase conjugate (Sigma) diluted 1:500 in 0.2% (w/v) non-fat dry milk in PBS-T20 was added and incubated at 37°C for 1 h. After washing, 1 mg mL<sup>-1</sup> of P-nitrophenyl phosphate (Sigma) was used as a substrate and the absorbance was read at 405 nm using micro plate auto reader (Bio-Tek Instruments).

**Cytokine assay:** A polystyrene flat bottom microtiter plate was coated with 50 µL mL<sup>-1</sup> of treated and non treated sera diluted (1:100) in carbonate/bicarbonate buffer, (pH 9.6) overnight. After blocking, rat anti-mouse IFN - γ mAb and goat anti-mouse IL-2 mAb (Sigma) diluted 1:1000 in PBS, PH 7.2 were added at 37°C for 1 h. After washing, 50 µL mL<sup>-1</sup> of goat anti-rat IgG alkaline phosphatase conjugate (for IFN-γ) and rabbit anti goat IgG alkaline phosphatase conjugate for IL-2 diluted (Sigma) 1:600 in PBS-T 20 were added at room temperature for 2 h. One milligram per milliliter of P-nitro phenyle phosphate was used as Substrate and the absorbance was read at 405 nm using microtiter plate auto reader (Bio-Tek Instruments).

**Statistical analysis:** Student t-test was performed using the statistical program package, instate software, version 2.03 (Graphpad, USA) and on IBM PCIAT compatible computer. The degree of significance, p<0.05 significant, p<0.01 highly significant and p<0.001 extremely significant.

## RESULTS

**Serum levels of anti-schistosomal antibodies:** From Table 1, the level of anti-schistosomal antibodies was decreased significantly (p<0.0001) in mice infected with 100 cercariae of *S. mansoni* and treated with PZQ as well

Table 1: Antischistosomal antibody titer in mice infected with *S. mansoni* and treated with myrrh and praziquantel compared with the infected non-treated control group

Mice groups	Schistosomal antibodies
Infected control (n=10)	0.97±0.2
Infected and treated With PZQ (n=10)	0.31±0.12 p1<0.0001***
Infected and treated With myrrh (n=10)	0.28±0.08 p1 = 0.0002*** p2 = 0.0955

p, probability; t, student t-test

Table 2: Serum level of gamma interferon and interleukin-2 in mice infected with *S. mansoni* and treated with myrrh and praziquantel compared with the infected non-treated control group

Mice groups	IFN- $\gamma$	IL-2
Infected control (n=10)	0.434±0.16	0.45±0.129
Infected and treated With PZQ (n=10)	0.451±0.17 p1 = 0.8664	0.29±0.14 p1 = 0.0137*
Infected and treated With myrrh (n=10)	0.393±0.125 p1 = 0.4691 P2 = 0.1420	0.384±0.05 p1 = 0.001** p2 = 0.353

p, probability; t, student t-test

as mice infected and treated with myrrh (p<0.0001). This result this result was confirmed by the result that, there no difference between PZQ and myrrh (p<0.05). The serum level of anti-schistosomal antibodies was decreased also after combined treatment with PZQ and myrrh (p<0.05).

**Serum levels of IFN- $\gamma$ :** Table 2 shows that, the level of IFN- $\gamma$  was decreased nonsignificantly after treatment with myrrh while decreased significantly after combined treatment with PZQ and myrrh (p = 0.05). No difference in the mean level was observed after treatment with PZQ (p>0.05).

**Serum levels of IL-2:** Unlike IFN- $\gamma$ , the level of IL-2 was decreased in mice infected with *S. mansoni* and treated with PZQ (p<0.05) and myrrh (p<0.01) and combined treatment with PZQ and myrrh (p = 0.05).

## DISCUSSION

Schistosomiasis is a widespread helminthic disease and infects one in 30 people (Elliott, 1996). A significant advance in the control of schistosomiasis chemotherapy was the introduction of praziquantel (Shekhar, 1991) but resistance to praziquantel is an emerging problem (Ismael *et al.*, 1994, 1996; Stelma *et al.*, 1995). Several possibilities were discussed that might explain the low cure rates of praziquantel, first it act in synergy with the immune system (Gryseeles *et al.*, 1994), second in mice drug resistant schistosomiasis is drug specific (Fallon and Doenhoff, 1994), third praziquantel is less active against

the juvenile stages of *Schistosoma mansoni* than in adult (Xiao *et al.*, 1985; Sabah *et al.*, 1986b) and fourth, it has been suggested that cure rates depend on infection intensities with higher cure rates in lower infection intensities (Andrews, 1981). In the present study, mice infected with *S. mansoni* were treated with PZQ in a dose of 250 mg/kg/day for three alternative days or with myrrh in a dose of 10 mg/kg/day for three days. Anti-schistosomal antibodies were used to evaluate the reduction of the infection and for the humoral immune response. The most important finding was that antischistosomal antibodies were decreased significantly after treatment with myrrh and PZQ separately (p<0.0001). Myrrh was found to an putative herbal antischistosomal drug. Sheir *et al.* (2001), found that treatment with myrrh in a dose of 10 mg kg<sup>-1</sup> of body weight for three days can induce cure rates of 91.7%. The respective roles of Th1 and Th2 cells in the immune response to *S. mansoni* infection has been the focus of intensive research (Kaplan *et al.*, 1998). Previous evidence suggested a role for both subsets, since the early stage of granuloma formation has the characteristic of Th1 (IFN- $\gamma$  and IL-2) cell response (Henderson *et al.*, 1992; Cook *et al.*, 1993). The results of our study revealed that, treatment of mice infected with *S. mansoni* with myrrh can improve the levels of both gamma interferons and interleukin-2. Otherwise treatment with PZQ can improve interleukin -2 only. From these result one can conclude that myrrh can act via in synergy with IFN- $\gamma$  and interleukin-2 (Th1 response), this can explain why myrrh give higher cure rates than PZQ as discussed by Sheir *et al.* (2001). Also we selected these cytokines because, at the time of dissecting their levels were dominant (Boros *et al.*, 1989) to give a clear difference after treatment. In the murine model of *S. mansoni* infection, the parasite ova settle in the liver, leading to the recruitment of various inflammatory cell types, primarily esinophils and to a lesser extent lymphocytes and macrophages to form granuloma which its function is to protect liver from the dissemination of toxic egg products (Weinstock *et al.*, 1992). In conclusion, myrrh is a new effective and save anti-schistosomal drug that can eliminate schistosomal infection compared with the already known praziquantel. The mechanism of action of may be take place via the improvement of cellular immune response.

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