Immunological and parasitological parameters in Schistosoma mansoni–infected mice treated with crude extract from the leaves of Mentha x piperita L.

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A B S T R A C T

Schistosomiasis is a chronic disease caused by an intravascular trematode of the genus Schistosoma. Praziquantel is the drug used for treatment of schistosomiasis; nevertheless failure of treatment has been reported. Consequently, the identification of new effective schistosomicidal compounds is essential to ensure the effective control of schistosomiasis in the future. In this work we investigated the immunomodulatory and antiparasitic effects of the crude leaves extract of Mentha x piperita L. (pepperment) on murine Schistosomiasis mansoni. Female Balb/c mice were infected each with 50 S. mansoni cercariae and divided into three experimental groups: (I) untreated; (II) treated daily with M. x piperita L. (100 mg/kg) and III) treated on 1/42/43 days post-infection with Praziquantel (500 mg/kg). Another group with untreated and untreated mice was used as a control. Subsequently, seven weeks post-infection, S. mansoni eggs were counted in the feces, liver and intestine. Worms were recovered by perfusion of the hepatic portal system and counted. Sera levels of IL-10, IL-5, IFN-γ, IgG1, IgE and IgG2a were assayed by ELISA. Animals treated with a daily dose of M. × piperita L. showed increased sera levels of IL-10, IFN-γ, IgG2a and IgE. Besides, M. × piperita L. treatment promoted reduction in parasite burden by 35.2% and significant decrease in egg counts in the feces and intestine.

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Introduction

Schistosomiasis is a chronic disease caused by an intravascular trematode of the genus Schistosoma. Schistosoma mansoni infection is characterized by the presence of adult worms living in the mesenteric venous system, depositing eggs in small veins in the submucosa of the intestine. The eggs are carried through the bloodstream to the liver promoting a granulomatous inflammatory reaction (El-Cheikh et al., 1994; Oliveira et al., 2011). The host–parasite relationship may lead to increased morbidity because the disease results in hepatosplenomegaly, hepatic fibrosis and ascites. The granulomatous process associated with schistosomiasis is dependent on CD4+ T cells and requires recruitment and accumulation of inflammatory cells at the egg deposition site. Soluble egg antigens (SEA) and soluble antigens of adult worms (SWAP) induce different cytokine responses. Studies in the murine model demonstrated that granuloma formation was induced by SEA released from schistosomal eggs (Boros, 1989; Langley and Boros, 1995). The granuloma is composed primarily of macrophages, eosinophils and lymphocytes (Cheever et al., 2002). The cells can trigger a Th1 response, with production of IL-2, TNF-α

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and IFN-γ or a Th2 response, with secretion of IL-4, IL-5 and IL-13 (Abath et al., 2006; Boros and Whitfield, 1999; Hoffmann et al., 2002; Pearce and MacDonald, 2002).

Schistosomiasis is a neglected disease related to poverty, given that the majority of the infected people live in poor communities without access to treated water and adequate sanitation. It is estimated that over 250 million people are infected worldwide (Hu et al., 2004), and more than 130,000 die each year from hyper-tension of the portal system caused by schistosomiasis in Africa (Van der Werf et al., 2003). However, after 30 years of Praziquan-tel (PZQ) usage, a decreased susceptibility to the drug and PZQ resistant schistosome strain has been observed in several countries (Botros and Bennett, 2007; Ismail et al., 1999; King et al., 2000; Nahed et al., 2009). Consequently, the identification of new effec-tive schistosomicidal compounds is essential to ensure the effective control of schistosomiasis.

*Mentha x piperita* L., known as peppermint, is popularly used for various therapeutic purposes. Originated from Europe and the Middle East, *M. x piperita* L. is widespread in Brazilian culture and can grow in any area of the country. Several descriptions of therapeutic activities of *M. x piperita* L. have been described in the literature. For example, *M. piperita* L. ethanol extract reduced and altered the morphology and viability of *Giardia lam-bila in vitro* (Vidal et al., 2007). Essential oils of *M. aquatic* L., *M. longifolia* L. and *M. x piperita* L. showed strong bactericidal activity, particularly against *Escherichia coli*. These oils also showed sig-nificant fungicidal activity (Neda et al., 2003). The use of inhaled *M. x piperita* L. oil concomitantly with multiple drugs therapies in patients with pulmonary tuberculosis led to a decrease in the number of bacilli (Shkurupij et al., 2002). However, there are no studies that evaluated the effect of *M. x piperita* L. against *S. mansoni*. This work was carried out to evaluate the immunomodulatory and anti-parasitic effects of *M. x piperita* L. on murine *S. mansoni* model.

**Materials and methods**

**Mice**

Female inbred Balb/c mice, weighing 18–20 g, were obtained from the animal facilities of the University of São Paulo at Ribeirão Preto School of Pharmaceutical Sciences, Brazil (FMRP-USP) and were maintained under standard laboratory conditions throughout the experimental procedures. This project was approved and con ducted in accordance with the guidelines established by Federal University of São Carlos Animal Care Committee.

**Parasite and infection**

The LE (Luiz Evangelista) strain of *S. mansoni* (Pellegrino and Katz, 1968) is routinely maintained by serial passages through *Biomphalaria glabrata* snails and Balb/c mice in the Prof. Dr. Van derlei Rodrigues’s laboratory at University of São Paulo, Ribeirão Preto, Brazil.

Infected snails were induced to shed cercariae under light exposure for 2 h; cercariae were recovered by sedimentation on ice. The number of cercariae in suspension was determined and ice-cold were percutaneously infected with 50 cercariae/mouse using a procedure adapted from the ring method (Smithers and Terry, 1965).

*M. x piperita* L. crude leaves extract

*Mentha x piperita* L. (peppermint) were collected at Medicinal and Toxic Plant Garden, in Araraquara, São Paulo, Brazil (21°48′51.4″S and 48°12′05.1″W) and authenticated in loco by Professor Dr. Luis V.S. Sacramento and confirmed in the SJRP Herbarium through the voucher sample n° 24229. The leaves were air dried at room temperature. A total of (100 g) dried leaves were extracted by maceration (100 g of dried leaf in 1000 mL of a 3:7 (v/v) mixture of water/EtOH) for 72 h. The solution were filtered and evaporated to dryness (under vacuum and 55–65 °C). After drying, a total of 100 mg of dry extract was harvested (10% yield) and the dried crude leaves extract was kept at −20 °C until use.

**Experimental design and treatment protocol**

The *S. mansoni*-infected mice were divided into three experi-mental groups: (I) untreated group; (II) *M. x piperita* L. treated group. Each mouse in this group was orally treated with *M. x piperita* L. crude leaves extract. The extract was dissolved in drinking water and given daily to each mouse by gavage at a dose of 100 mg/kg body weight during 7 weeks, the treatment started on infection day; (III) PZQ treated group. Each mouse in this group was treated with Praziquan-tel (PZQ). PZQ (FIOCRUZ), was dissolved in 2% Cre-mophor EL, and was given orally to each mouse by gavage at a dose of 500 mg/kg body weight on infection day and on 42 and 43 days post-infection (Helmy et al., 2009).

Another group with uninfected and untreated mice was used as a control (Control group).

Each group was composed by seven mice and all experiments were performed twice, the total number of mice used was 56. Seven weeks post- *S. mansoni* infection animals were euthanized according to laboratory protocols, using a CO2 chamber, to be performed the parasitological and immunological analyses.

**Parasitological parameters**

Worm counts were performed at seven weeks post-infection. Worm recovery was made by perfusion of hepatic and port mesen-teric vessels (Duvall and DeWitt, 1967). The number of eggs per gram intestinal or hepatic tissue was determined after overnight digestion with 5% KOH (Cheever, 1968; Kamel et al., 1977). Egg counts in feces were performed using Kato-Katz technique (HelmTest BioManguinhos FIOCRUZ) and the number of eggs/g feces was determined.

**Immunological parameters**

Seven weeks post-infection, blood samples were collected by cardiac puncture and sera samples were obtained from blood after centrifugation at 400 × g for 15 min at 4 °C, then divided into aliquots and stored at −70 °C until used. Sera levels of antibodies and cytokines were determined using ELISAs. The ELISAs for cytokines detection were performed according to the manufacturer’s instructions using commercial BD kits for IL-5 (15.6–1000 pg/ml, assay range), IL-10 (31.3–2000 pg/ml), and R&D kits for IL-13 (7.8–500 pg/ml) and IFN-γ (9.4–600 pg/ml). To mea-sure IgG1, IgG2a and IgE levels against *S. mansoni* in the serum sample, the antigen dilution to IgG1 was 5 mg/ml of protein anti-gen to 1:64 dilution of serum sample, and to IgG2a and to IgE were 10 mg/ml of protein antigen to 1:4 dilution of serum sample. Anti-bodies in the serum sample were detected using coated plate with total protein antigen of *S. mansoni* (SWAP) (Webster et al., 1997). Secondary Ab-biotin conjugated was used at a concentration of 0.125 mg/ml. Sera giving absorbance values higher than the cut-off value (=mean absorbance of serum from uninfected/untreated control mice) were considered positive. In the dosage of antibod-ies (Fig. 1) the background absorbance was discounted using the cut-off value.