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ScienceDirect
Experimental Parasitology 115 (2007) 25–31
Experimental Parasitology
www.elsevier.com/locate/yexpr

Giardia lamblia: The effects of extracts and fractions from *Mentha x piperita* Lin. (Lamiaceae) on trophozoites

F. Vidal [a], J.C. Vidal [a], A.P.R. Gadelha [a], C.S. Lopes [b], M.G.P. Coelho [b], L.H. Monteiro-Leal [a,*]

[a] *Department of Histology and Embryology, Laboratory of Microscopy and Image Processing, State University of Rio de Janeiro, Av. Prof. Manoel de Abreu, 444, 3° andar, Maracanã, Rio de Janeiro, RJ 20550-170, Brazil*

[b] *Department of Biochemistry, State University of Rio de Janeiro, Av. Prof. Manoel de Abreu, 444, 4° andar, Maracanã, Rio de Janeiro, RJ 20550-170, Brazil*

Received 7 December 2005; received in revised form 8 May 2006; accepted 9 May 2006
Available online 14 July 2006

Abstract

Giardia lamblia is a parasite that causes giardiasis in humans and other mammals. The common treatment includes different classes of drugs, which were described to produce unpleasant side effects. *Mentha x piperita*, popularly known as peppermint, is a plant that is frequently used in the popular medicine to treat gastrointestinal symptoms. We examined the effects of crude extracts and fractions from peppermint against *G. lamblia* (ATCC 30888) on the basis of trophozoite growth, morphology and adherence studies. The methanolic, dichloromethane and hexanic extracts presented IC_{50} values of 0.8, 2.5 and 9.0 μg/ml after 48 h of incubation, respectively. The aqueous extract showed no effect against the trophozoites with an $IC_{50} > 100$ μg/ml. The aqueous fraction presented a moderate activity with an IC_{50} of 45.5 μg/ml. The dichloromethane fraction showed the best antigiardial activity, with an IC_{50} of 0.75 μg/ml after 48 h of incubation. The morphological and adhesion assays showed that this fraction caused several alterations on plasma membrane surface of the parasite and inhibited the adhesion of *G. lamblia* trophozoites. Cytotoxic assays showed that *Mentha x piperita* presented no toxic effects on the intestinal cell line IEC-6. Our results demonstrated antigiardial activity of *Mentha x piperita*, indicating its potential value as therapeutic agent against *G. lamblia* infections.
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Index Descriptors: Protozoan; *Giardia lamblia*; *Mentha x piperita*; Giardiasis; Treatment

1. Introduction

Giardia lamblia is a parasite that colonizes the duodenum and upper jejunum of humans causing a disease known as giardiasis. The common treatment for this illness includes different drugs such as metronidazole, furazolidone and the class of benzimidazoles. In spite of their large use, these drugs can produce many side effects on patients including headache, vertigo, nausea, gastrointestinal disturbance, anorexia and dizziness (Gardner and Hill, 2001). Strains of *G. lamblia* have also developed resistance to some of these remedies (Kollaritsch et al., 1993; Liu et al., 2000).

Due to the factors presented above, the search for new agents to treat giardiasis has increased. A great number of papers is attempting to prove the efficacy of plant extracts against *G. lamblia* trophozoites (Cowan, 1999; Harris et al., 2000; Khan et al., 2000; Arrieta et al., 2001; McAllister et al., 2001; Ankli et al., 2002; Calzada et al., 2003, 2005; Gadelha et al., 2005; Sawangjaroen et al., 2005).

Mentha x piperita Lin. (Lamiaceae), commonly called peppermint, is a well-known herbal remedy used for a variety of symptoms and diseases (Guedón and Pasquier, 1994). In the popular medicine, it is used to treat nausea, flatulence, vomiting, indigestion, stomach cramps, menstrual cramps and parasitosis (Fonseka-Kruel and Fernandes, 2003). It is also recognized for its carminative, stimulant, antispasmodic, antiseptic, anti-inflammatory, antibacterial and antifungal activities (Guedón and Pasquier, 1994; Gershenzon et al., 2000; Inoue et al., 2002; Samarth and Kumar, 2003; Ruiz del Castillo et al., 2004; Duarte et al., 2005). As usual for a member of the plant group, *Mentha x*

* Corresponding author. Fax: +55 21 2587 6511.
E-mail address: mleal@uerj.br (L.H. Monteiro-Leal).

0014-4894/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2006.05.001

piperita produces a variety of metabolites such as terpenes, tannins, flavonoids and phenolic acids (Guedón and Pasquier, 1994). Among the identified compounds some had already been reported as having antimicrobial activity, including 1,8-cineole, limonene, linalool and menthol (Mazzanti et al., 1998; Iscan et al., 2002).

The aim of the present work is to analyze the activity of extracts and fractions from *Mentha x piperita* against trophozoites of *G. lamblia*. Using video-microscopy and transmission electron microscopy, we analyzed the effects on the morphology of the parasite. The IC_{50} value of different extracts and fractions derived from *Mentha x piperita* was also obtained. Adhesion assays were done to evaluate the activity of the plant on the adhesion capacity of *G. lamblia*. Cytotoxic assays were performed with the intestinal epithelial cell line IEC-6 using different concentrations of *Mentha x piperita*.

2. Materials and methods

2.1. Parasites culture

Giardia lamblia trophozoites, Portland-1 strain (ATCC 30888), were axenically cultivated in TYI-S-33 medium, enriched with 0.1% bovine bile and 10% fetal bovine serum, at 37 °C, for 48–72 h (Keister, 1983). Subcultures were done twice a week. Tubes containing cells at log phase were used in the experiments.

2.2. Intestinal epithelial cell line culture

Monolayers of IEC-6 (ATCC CRL-1592) were cultured at 37 °C in 25 cm^2 flasks and grown in Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Co., MO, USA) supplemented with 10% of fetal bovine serum and 100 U/ml of human regular insulin in an atmosphere of 5% CO_2 and 95% air (McCabe et al., 1991).

2.3. Plant material

The dry leaves of *Mentha x piperita* (Lin.) were obtained on a store specialized in medicinal plants in Niterói City, in the state of Rio de Janeiro, Brazil, in December of 2003. A voucher specimen (RB 412917) was deposited at the herbarium of Botanic Garden of Rio de Janeiro, Rio de Janeiro, Brazil.

2.4. Extraction

Dry leaves (5 g) were extracted with 100 ml of different solvents: methanol (Tedia, USA, HPLC grade), dichloromethane (EM-Science, USA, HPLC grade) and *n*-hexane (Tedia, USA, HPLC grade) for 15 days, at room temperature. The infusion was obtained by adding 100 ml of boiled water on 5 g of dry leaves of *Mentha x piperita*.

The extracts were filtered, then concentrated under vacuum conditions, and stored at 4 °C until further use. The infusion was lyophilized to obtain a dry extract.

2.5. Fractionation

The methanolic extract was dissolved in 100 ml of a solution of ethanol/water (3:7), and further fractionated by liquid–liquid extraction using 50 ml dichloromethane. The fractions were filtered and the solvents were removed under reduced pressure. Two fractions were obtained: (1) dichloromethane fraction (DCM) and (2) residual fraction. On the day of the experiments, the dry extracts and fractions were dissolved in DMSO (Merck, Germany) on a final concentration of 10 mg/ml.

For the sake of simplicity, the methanolic extract and the dichloromethane fraction will be designated, from now on, as follows: methanolic extract, MeOH extract; dichloromethane fraction, DCM fraction.

2.6. Growth inhibition assays

Trophozoites (5×10^4 cells/ml) were grown in 1.5 ml tubes in the presence of the extracts/fractions (Campanati et al., 2001). The concentrations of extracts and fractions employed were 1, 10, 50 and 100 μg/ml in culture medium, leading to a final concentration of DMSO (solvent used in stock solution) less than or equal to 1%. In our previous work, it was established that the above concentration range of DMSO did not induce alterations of the cell morphology or of any other cell parameter tested (Campanati et al., 2001). Trophozoites were exposed to the extracts and fractions for 2, 4, 6, 24 and 48 h. The total number of cells was obtained using a hemocytometer (Neubauer chamber). Dose–response graphics were elaborated. In addition, the concentration that causes 50% reduction on the number of thophozoites (IC_{50}) was calculated for each compound (Katiyar et al., 1994). The IC_{50} values of metronidazole and furazolidone were calculated in our previous work using the same methodology described above (Campanati and Monteiro-Leal, 2002).

These samples were also used to study the trophozoite morphology by video-microscopy and transmission electron microscopy.

2.7. Video-microscopy

An aliquot of cells was removed and mounted between slide and coverslip. Video-microscopy images were obtained using the Zeiss Axiophot microscope (Zeiss, Germany) equipped with a digital camera SIS CC-12 with the help of AnalySIS 3.1 (SIS-Soft Imaging System, Munster, Germany). The images were optimized by the use of the Paint Shop Pro program (JASC software, Inc., USA).

2.8. Transmission electron microscopy (TEM)

The cells were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde in PHEM buffer (Schliwa and van Blerkom, 1981), overnight at 4 °C. The samples were

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