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Mentha longifolia protects against acetic-acid induced colitis in rats

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ABSTRACT

Ethnopharmacological relevance: *Mentha longifolia* L (Wild Mint or Habak) (ML) is used in traditional medicine in treatment of many gastrointestinal disorders.
Aim of the study: This study aimed to evaluate potential protecting effect of ML and its major constituent, eucalyptol, against acetic acid-induced colitis in rats, a model of human inflammatory bowel disease (IBD).
Materials and methods: Rats were divided into ten groups (n=8) given orally for three days (mg/kg/day) the following: normal control, acetic acid-induced colitis (un-treated, positive control), vehicle (DMSO), sulfasalazine (500), ML extract (100, 500, 1000), and eucalyptol (100, 200, 400). After 24 h-fasting, two ML of acetic acid (3%) was administered intrarectally. On the fifth day, serum and colonic biochemical markers, and histopathological changes were evaluated.
Results: Colitis significantly increased colonic myeloperoxidase activity and malonaldehyde level, and serum tumor necrosis factor-α, interleukin-6, and malonaldehyde levels while significantly decreased colonic and serum glutathione levels. All treatments (except ML 100, ML 1000, and eucalyptol 100) significantly reversed these changes where eucalyptol (400) showed the highest activity in a dose-dependent manner. The colitis-induced histopathological changes were mild in sulfasalazine and eucalyptol 400 groups, moderate in ML 500 and eucalyptol 200 groups, and severe in ML 100, ML 1000, and eucalyptol 100 groups nearly similar to colitis-untreated rats.
Conclusion: ML (in moderate doses) and eucalyptol (dose-dependently) exerted protective effects against acetic acid-induced colitis in rats possibly through antioxidant and antiinflammatory properties suggesting a potential benefit in treatments of IBD. To our knowledge this is the first report addressing this point.

1. Introduction

Inflammatory bowel disease (IBD), an idiopathic chronic inflammation of the gastrointestinal tract, includes Crohn's disease (CD) and ulcerative colitis (UC) as its two major types. Being a worldwide disease, IBD is a main cause of economic burden and duty nonappearance. In CD, the inflammation is transmural and affects the whole length of the GI tract while in UC the inflammation is superficial and affects only the colon and rectum (Carty and Rampton, 2003). Multiple antiinflammatory and/or immunomodulatory drugs are used for treatment of IBD, including 5-aminosalicylic acid compounds (e.g., sulfasalazine), corticosteroids, methotrexate, and anti-tumor necrosis factor-α agents (e.g., infliximab). In addition, antimicrobials and probiotics help control infections, promote healing of abscesses and fistulas, restore the normality of gut microbiota, reset the altered immune mechanisms, and reduce inflammation in IBD (Affronti et al., 2015; Orel and Kamhi Trop, 2014). Normally there is a balance between mediators from the T-helper 1 (Th1) lymphocytes (e.g. interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ) and Th2 lymphocytes (e.g. IL-4, IL-5, IL-6, and IL-13) (Fig. 1). Shift of the balance to Th1 or Th2 side favors development of autoimmune or atopic diseases respectively (Chen and Blaser, 2007).

Traditional medicines, used for a number of diseases

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[1] He designed the study, conducted the pharmacological and biological tests, made statistics, and wrote the paper.
[2] He collected the plant, prepared the extract and the volatile oil, isolated eucalyptol, and wrote the paper.
[3] She conducted the histopathological work.

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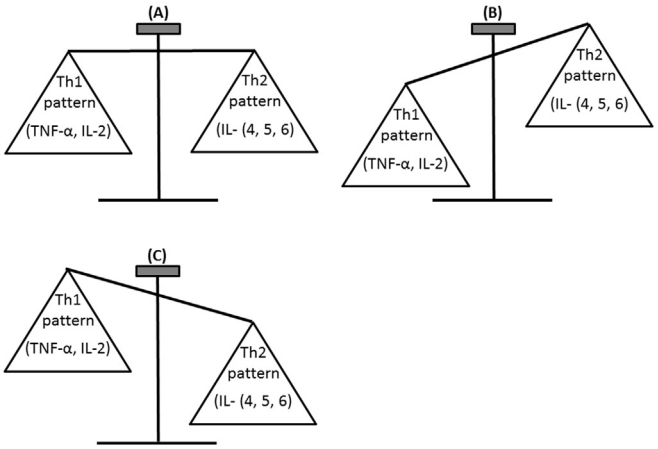

Fig. 1. A simplified T-helper 1/2 (Th1/Th2) immune pattern showing the: (A) normal balance, (B) imbalance in autoimmune diseases, and (C) imbalance in atopic diseases.

worldwide, are getting importance in the modern medical practice. *Mentha longifolia* L. (Wild Mint or Habak) (ML) is an aromatic perennial herb which belongs to Lamiaceae. It is commonly known as wild mint, habak, or hasawy in Almadinah Almunawwarah region (western Saudi Arabia). The plant is widely used in traditional medicine for treatment of many gastrointestinal disorders such as abdominal pain, diarrhea, ulcers, and gut spasm (El-Badry et al., 2010; Shah et al., 2010) and respiratory disorders such as cough, cold, and influenza (Naghibi et al., 2009). In addition, ML is used as flavoring agent and it is reported as safe and effective therapy in diabetes, hypertension, inflammation, and secondary amenorrhea in human through different mechanisms (Mokaberinejad et al., 2012). The plant is rich in volatile oil which contains mainly menthone, pulegone, eucalyptol, and piperitone (Ibrahim et al., In Press; Oyedeji and Afolayan, 2006). The essential oil of ML showed decongestant, antispasmodic, antimicrobial, and antioxidant activities (Hutchings and van Staden, 1994; Iqbal et al., 2013). Pulegone, the major monoterpenoid constituent of the ML oil, is a strong hepatotoxic agent (Raza et al., 2015), while eucalyptol (1,8-cineole), the second monoterpenoid component is safely included in many brands used as mouthwashes and cough therapies (NIH, 2016). The fresh ML plant is used either as a flavoring agent especially with hot tea or as herbal drink by extraction in boiled water. The pattern of this traditional use of ML results in "much less amount of pulegone in foodstuffs and beverages than the allowed limit" set by the "Scientific Committee on Food, Health & Consumer Protection Directorate General, European Commission (EEC (Council Directive 88)/388/EEC,1988)" (Alam et al., 2016). Eucalyptol was found to reduce production of the mediators of inflammation subsequent to inflammatory stimuli (Juergens et al., 1998), thus prophylactic (but not curative) eucalyptol ameliorated the acute trinitrobenzene sulfonic acid-colitis in rats (Santos et al., 2004). Consequently, the pre-treatment (prophylactic) model was used to show that this traditional use could protect against CD.

Based on the increasing incidence and prevalence of IBD (Molodecky et al., 2012), availability of the ML plant, and the recently-introduced trend of encouraging searching evidences for the traditional use of herbs, the present study was designed to search for a support for the hypothesis that the traditional use of ML potentially protects against CD. A model of colitis was induced in rats by using acetic acid. This model shows a picture similar to that in human IBD including epithelial damage, local inflammation, and increased inflammatory mediators (Elson et al., 1995). To our knowledge, this is the first study addressing this issue.

2. Materials and methods

2.1. General conditions of the gas chromatography/mass spectrometer (GC/MS) analysis

The GC/MS analysis was performed with Shimadzu Model GC-17A gas chromatography interfaced with a Shimadzu model QP-5000 mass spectrometer (Japan). Volatiles were separated on DB5-MS column (30 m length, 0.5 mm inner diameter, and 0.25 μ) (J&W Scientific, Santa Clara, CA, USA). Injections were made in the splitless mode for 30 s. The gas chromatograph was operated under the following conditions: injector 220 °C, column oven 38 °C for 3 min, and then programmed at a rate of 12 °C min^{-1} to 220 °C for 2 min, and the carrier gas was set at 1 ML min^{-1}. The transfer line and ion-source temperatures were adjusted at 230 and 180 °C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at *m/z* 40-500. Volatile components were identified using the procedures described before (Ibrahim et al., In Press) and peaks were first deconvoluted using the Automated Mass Spectral Deconvolution and Identification System Automated Mass Spectral Deconvolution and Identification System software (AMDIS), (2003) and were identified by their retention indices (RI) relative to n-alkanes (C6-C20), mass spectra matching to NIST, WILEY library database (> 90% match), and with authentic standards when available.

2.2. Plant material

The fresh flowering aerial parts of *Mentha longifolia* L. were collected in March from Almadinah Almunawwarah region. Authentication of the plant sample was established morphologically by Dr. Emad Alsherif, Associate Professor of Plant Ecology, Dept. of Biology, Faculty of Science and Arts, Khulais, King Abdulaziz University, Saudi Arabia. A voucher specimen was kept at the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University (ML-1-2013).

2.3. Preparation of methanol extract

The air-dried plant material (500 g) was extracted with MeOH (1 L × 3) at room temperature using Ultraturrax until exhaustion and the combined extracts were evaporated under vacuum. The methanol extract (ML, 10 g) was suspended in DMSO for the biological study.

2.4. Preparation of volatile oil

Fresh plant material was subjected to hydrodistillation. The volatile oil obtained was dried over anhydrous sodium sulfate and kept in a refrigerator for GC/MS analysis and biological study.

2.5. Isolation of the major constituents from the oil sample

The collected essential oil from distillation was chromatographed on silica gel 60 column ($6 \times 150\ cm^2$) using hexane-ethyl acetate (9.7:0.3 v/v). Fractions (200 ML each) were collected and subjected to TLC on silica gel G plates against standard eucalyptol (Sigma–Aldrich, St. Louis, MO, USA).), using solvent system (Hexane-ethyl acetate, 9:1) as a developing solvent and vanillin/sulfuric acid (Wagner et al., 1984) as a spray reagent. Fractions rich in eucalyptol were rechromatographed on a silica gel 60 column ($2 \times 50\ cm^2$) using hexane-ethyl acetate (9.5:0.5 v/v) to isolate pure eucalyptol.