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Efficacy of *Mentha pulegium* extract in the treatment of functional dyspepsia: A randomized double-blind placebo-controlled clinical trial

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ABSTRACT

Ethnopharmacological evidence: *Mentha pulegium* L. leaves are used in the Iranian traditional medicine for the treatment of functional dyspepsia. *Aim of study:* To study the efficacy and safety of *M. pulegium* in the treatment of functional dyspepsia patients fulfilling the Rome III criteria.

Materials and methods: The efficacy and safety of a standardized *Mentha pulegium* leaf extract (drug extract ratio: 15.9:1, extraction solvent: 70% v/v aqueous ethanol) (330 mg three times daily taken for 2 months) as add-on to one famotidine 40 mg tablet per day in the treatment of 50 functional dyspepsia patients were compared with those of a parallel placebo group (n =50).

Results: The extract significantly decreased the total dyspepsia score measured by the Hong Kong dyspepsia index compared to the placebo and baseline (P=0.011 and $P < 0.001$ respectively). The stomach pain, upper abdominal bloating, upper abdominal dull ache, belching and total dyspepsia scores were decreased from baseline in the extract group significantly compared to the placebo ($P < 0.001$, $P < 0.001$, P=0.003, $P < 0.001$ and $P < 0.001$ respectively). However, the decreases of other dyspepsia symptoms scores from baseline in the extract group were not significant compared to the placebo ($P > 0.05$). The extract improved the quality of life measured by the SF-36 questionnaire significantly compared to the placebo and baseline (P=0.003 and $P < 0.001$ respectively). Moreover, the extract lowered the rate of *H. pylori* infection determined by the urease test significantly compared to the placebo and baseline (P=0.001 and $P < 0.001$ respectively). The extract did not significantly affect the complete blood count and liver and kidney function tests ($P > 0.05$). The patients did not experience any adverse drug effect.

Conclusions: *M. pulegium* extract (genuine drug extract ratio: 19.4:1; extraction solvent: 70% v/v aqueous ethanol) 270 mg three times daily taken for 2 months as adjunct to one famotidine 40 mg tablet per day seems safe, improves dyspeptic symptoms and quality of life and eradicates *H. pylori* in functional dyspepsia patients.

1. Introduction

Functional dyspepsia is a common disorder of the upper gastrointestinal system affecting up to 40% of the population (Mahadeva and Ford, 2016). Rome III criteria define functional dyspepsia as the presence of symptoms thought to originate in the gastroduodenal region (early satiation, post-prandial fullness, epigastric pain or burning) in the absence of any organic, systemic or metabolic disease likely to explain the symptoms. Although, functional dyspepsia is a non-life-threatening disorder, it markedly reduces patients' quality of life. Economic costs of functional dyspepsia are considerable and caused by lost productivity and cost of diagnosis and treatment (Talley et al., 2016). The treatment of functional dyspepsia can be confusing to the healthcare practitioners because no agent is approved for the treatment. Effective therapies for functional dyspepsia are limited although H_2 blockers, proton pump inhibitors, prokinetics, tricyclic anti-depressants and mirtazapine may provide some symptom relief in selected patients. The treatment of functional dyspepsia remains unsatisfactory for many patients. Development of more effective and specific treatments for functional dyspepsia is warranted (Talley, 2016). Phytotherapeutic agents have a long history of use in the treatment of dyspeptic symptoms, possibly due to constituents such as essential oils having spasmolytic, carminative and local anesthetic actions (Saller et al., 2001). Alternative and innovative

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functional dyspepsia therapies which have desirable efficacy and safety may be developed from medicinal plants (Holtmann and Talley, 2015).

Mentha pulegium L. (*M. pulegium*) (pennyroyal) (Labiatae) leaves are used in the Iranian traditional medicine to treat gastrointestinal disorders including dyspepsia, nausea, vomiting, bloating, stomachache, infections and diarrhea. The dosage of the plant dry leaves is 1–4 g as decoction up to three times daily (Zargari, 1996). Smooth muscle relaxant (Estrada-Soto et al., 2010; Soares et al., 2005), smooth muscle spasmolytic (Estrada-Soto et al., 2010), calcium antagonist (Estrada-Soto et al., 2010), anti-inflammatory (Kogiannou et al., 2013; Moussaid et al., 2011), anti-oxidative (Kogiannou et al., 2013; Moussaid et al., 2011), antigenotoxic (Romero-Jiménez et al., 2005), antibacterial (Ibrahim, 2013; Mahboubi and Haghi, 2008), neuroprotective (López et al., 2010), acaricidal (Rim and Jee, 2006), larvicidal (Cetin et al., 2006) and nematicidal (Ntalli et al., 2010) effects of *M. pulegium* have been demonstrated. There has been no study evaluating the effects of *M. pulegium* in the treatment of functional dyspepsia.

Considering the above data, this study was conducted to evaluate the efficacy and safety of *M. pulegium* in the treatment of patients with functional dyspepsia. Moreover, H_2 blocker is a routine therapy for functional dyspepsia (Talley, 2016), so for ethical reasons, the effects of *M. pulegium* combined with famotidine were examined in the present trial.

2. Materials and methods

2.1. Plant material

M. pulegium was collected from the lands in the Alborz province of Iran in August and a staff botanist visually identified the plant. A voucher specimen of the plant (number 21092) was deposited in the Tehran University Central Herbarium. The leaves were separated from the plant, washed and dried in shade at room temperature. The dry leaves were ground into powder.

2.2. Extraction

The dry leaf powder (70 kg) was extracted with 70% v/v aqueous ethanol as the solvent in a percolator for 72 h, the solvent was completely removed from the extract by a rotary evaporator, toast powder as an excipient was added to and mixed with the extract and the mixture was ground to a powder. The quantity of the dry extract powder produced was 4.4 kg. The excipient constituted 18% of the final extract. Drug extract ratio (DER) and DER native were 15.9:1 and 19.4:1 respectively.

2.3. Preparation of the extract and placebo capsules

The extract powder as the drug and toast powder as the placebo were separately filled into oral gelatin capsules by a hand-operated capsule-filling machine (Scientific Instruments and Technology Corporation, USA). The *M. pulegium* capsules contained 330 mg of the extract powder. To make the placebo capsules smell like the *M. pulegium* capsules, 1 μL of *M. pulegium* essential oil was put in the placebo capsules containers. The *M. pulegium* and placebo capsules were identical in all respects. A sample of the extract and placebo capsules is kept in the Center of Professional Analysis and Processing of Medicinal Plants (Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran).

2.4. Phytochemical analyses of the extract

The antioxidant activity using the DPPH radical scavenging assay and the total flavonoid and phenolic contents were determined by spectrophotometry as described previously (Gutfinger, 1981; Han et al., 2008; Yoo et al., 2008). Moreover, rosmarinic acid, chlorogenic acid and quercetin were quantified in the extract by HPLC according to the methods reported previously (Liu et al., 2013; Verma and Trehan, 2013; Wen et al., 2012). The measurements were done in triplicate. The analyses were performed for standardization of the extract and process control in the Center of Professional Analysis and Processing of Medicinal Plants (Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran). The analytical methods are validated.

2.4.1. Determination of the antioxidant activity

Inhibition of diphenyl-2-picrylhydrazyl (DPPH) radicals by the extract was assayed. A mixture consisting of an extract solution at different concentrations (1.5 mL) and the methanolic solution of the DPPH reagent (1.5 mL) was mixed in a volumetric flask. The mixture was left to stand for 30 min in a dark place, and then the absorption was measured at 517 nm using a spectrophotometer (Human, USA). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: RSA (%) =(Ac-As/Ac) ×100.

Abbreviations used in the equation: RSA: radical scavenging activity; Ac: absorbance of the negative control; As: absorbance of the plant sample or ascorbic acid.

Ascorbic acid was used as reference standard. The assay results were expressed as IC50 denoting the antioxidant concentration which reduces the DPPH radicals about 50%.

2.4.2. Determination of the total flavonoid content

The extract (1 mg/mL) or standard was mixed with 4 mL of distilled water and 300 μL of a 5% sodium nitrite solution and after five minutes 300 μL of 10% aluminum chloride solution was added to the mixture. After six minutes, 2 mL of 1 M sodium hydroxide and 3 mL of distilled water were added to the mixture. The solution was properly mixed and absorbance was measured at 510 nm using a spectrophotometer (Human, USA). Rutin (100 μg/mL up to 1200 μg/mL) was used to construct the standard curve and the results were expressed as mg of routine equivalents per capsule.

2.4.3. Determination of the total phenolic content

The total phenolic contents were determined through the Folin-Ciocalteu colorimetric method. In brief, the plant extract solution (1 mL) was mixed with 500 μL of the Folin-Ciocalteu reagent and 5 mL distilled water in a volumetric flask. After five minutes, 1 mL of 15% sodium carbonate solution was added to the mixture and then kept in the dark for 30 min, after which the absorbance was determined at 725 nm using a spectrophotometer (Human, USA). Gallic acid was used to generate the standard curve, and the reduction of the Folin-Ciocalteu reagent by the samples was expressed as mg of gallic acid equivalents per capsule.

2.4.4. Determination of the rosmarinic acid content

A Knauer HPLC (Germany) was used with pump K1001 and UV detector K2501 (Germany). The analytical column was Phenomenex NX-C18 (diameter 4.6 mm, length 250 mm). Analysis was repeated with added standards in order to ensure the results. All reagents were of analytical reagent grade and purchased from Merck (Germany). The analysis of rosmarinic acid was carried out by HPLC. 330 nm was selected as the wavelength for UV detection. Elution was carried out at a flow rate of 1.0 mL/min at 25 °C. Two mobile phases, A and B were used. Mobile phase A was 0.1% (v/v) formic acid solution in water, while mobile phase B was acetonitrile. A ratio of 88% A and 12% B was applied in the first 30 min. After 30 min, a ratio of 80% A and 20% B was used for the next 15 min. Finally, 70% A and 30% B were used after 45 min for an additional 15 min.

2.4.5. Determination of the chlorogenic acid content

A Knauer HPLC (Germany) was used with pump K1001 and UV detector K2501 (Germany). The mobile phase was acetonitrile and 0.5% aqueous phosphoric acid (11.5:88.5 v/v); the flow rate of 1.0 mL/