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EXPERIMENTAL STUDY

Protective effect of essential oil of Pistacia atlantica Desf. on peptic ulcer: role of α -pinene

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Abstract

OBJECTIVE: To evaluate the efficacy of Pistacia atlantica Desf. oleoresin essential oil on peptic ulcer (PU) and its antibacterial effect on metronidazole-resistant Helicobacter pylori, as well as chemical composition of the essential oil.

METHODS: The essential oil was standardized using gas chromatography mass spectrometry (GC/MS) analysis. Acute toxicity of the essential oil was assessed in animal model. *In vitro* anti-Helicobacter pylori activity was performed through disc diffusion and minimum inhibitory concentration method. For gastroprotective assay, rats received Pistacia atlantica Desf. essential oil (25, 50 and 100 mg/kg orally) 1 h before induction of ulcer by ethanol. Macroscopic (ulcer index and protection rate) and microscopic examination were performed.

RESULTS: The GC/MS analysis of the essential oil led to the identification of twenty constituents and α -pinene is predominant constituent. The essential oil was safe up to 2000 mg/kg. All Helicobacter pylori strains were susceptible to the essential oil and the MIC ranged from 275 to 1100 µg/mL. The ulcer index for treated groups was significantly reduced compared to control (*P* < 0.001) with EC₅₀ value of 12.32 mg/kg. In microscopic examination, Pistacia atlantica attenuated destruction and necrosis of gastric tissue.

CONCLUSION: Current study exhibited protective effect of standardized Pistacia atlantica essential oil against ethanol-induced gastric ulcer and its antibacterial activity on Helicobacter pylori. α -pinene might be the responsible agent.

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Key words: Peptic ulcer; Pistacia; Oils, volatile; Oleoresins; Gastroprotective; Helicobacter pylori; Medicine, traditional

INTRODUCTION

Peptic ulcer (PU), a highly prevalent gastrointestinal disorder, includes gastric and duodenal ulcers. It has been revealed that PU occurs when biological balance between noxious and defense factors in gastrointestinal tract is disturbed. Free radicals and oxidants, gastric acid and pepsin secretion as well as exogenous agents such as ethanol or NSAIDs are among noxious factors. In addition, gastric defensive agents including gastric mucus, bicarbonate, antioxidant enzymes, nitric oxide (NO) and prostaglandins protect from aggressive agents.¹⁻³ It has been proven that colonization of Helicobacter pylori, a common human pathogen with approximately 70% asymptomatic stomach colonization, within gastric epithelial surface via microaerophilic growth capacity and the production of numerous virulence factors is a major risk factor for the development of PU disease, gastric atrophy and gastric adenocarcinoma.4,5

Various chemical agents are used for the management of PU such as proton pump inhibitors and H_2 receptor antagonists and antibiotics for Helicobacter pylori eradication. These drugs possess adverse effects, cannot prevent recurrence of ulcer, and also show interaction with many other chemical agents.^{1,4} In addition, there is a notable prevalence of antimicrobial resistance in Helicobacter pylori infected patients. Development of Helicobacter pylori resistance to metronidazole is much higher than other antibiotics so that in some areas in developing countries almost all strains are resistant to metronidazole, in which the need for new Helicobacter pylori eradication strategy and PU treatment has been created.⁶

According to World Health Organization (WHO) focus on progression of native medicaments and ethnomedicines, there are so many researches on natural remedies in order to exploring alternative medicines and novel phytochemical agents for the management of diseases.^{2,4,5} Pistacia atlantica Desf subsp mutica (F & M) Rech. f, (Anacardiaceae alt. Pistaciaceae)-which grows wildly in the western, central and eastern areas of Iran-is an important plant for people in areas of natural forest for multiple traditional foods and medicine uses.^{7,8} The oleoresin of Pistacia atlantica is obtained as an exudate after hurting the trunk and branches used for different traditional medicinal indications such as wound healing, treatment of PU, gastrointestinal disorders and motion sickness and as an antiseptic, appetizer, phlegm purgative, astringent, laxative, emmenagogue, diuretic and carminative drug. In modern studies Pistacia atlantica has been reported to possess remarkable antioxidant, antibacterial, antifungal, antidiabetic, antitumor and anticholinesterase activity.9,10 To the best of our knowledge, there is no published research into Pistacia atlantica essential oil for its gastroprotective and anti-Helicobacter pylori activities. Therefore, this study was to evaluate its beneficial effects on ethanol-induced PU in animal model, antibacterial activity against metronidazole-resistant Helicobacter pylori *in vitro* as well as standardization of the essential oil using gas chromatography/mass spectrometry (GC/MS) analysis.

MATERIALS AND METHODS

Plant material

Oleoresin of P.atlantica was collected during May to July 2013 from Kermanshah province, west of Iran and authenticated by Dr. G. Amin (Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science), and a voucher specimen (No. PMP-818) deposited in the Herbarium of Faculty of Pharmacy.

Preparation of essential oil

Essential oil was obtained according to method described previously.¹¹ Briefly, oleoresin was subjected to hydrodistillation using a clevenger apparatus for 4 h. The obtained essential oils were dried using anhydrous sodium sulfate (Sigma-Aldrich, Steinheim, Germany) and stored at low temperature (+4 $^{\circ}$ C) in an amber vials for analysis.

The essential oil was analyzed using an Agilent 6890 gas chromatography (GC) with BPX5 column (30 m × 0.25 mm, ft 0.25 µm); carrier gas, Helium; flow rate, 0.5 mL/min, split ratio, 1:20 and using a flame ionization detector. Column temperature: the initial temperature was 50 °C, heated to 240 °C at 3 °C/min and then at 15 °C/min until 300 °C. Mass spectrometry was Agilent 5973 model with ionization voltage of 70 eV, using EI ionization and ionization source temperature was 220 °C. Retention indices were calculated using retention times of n-alkanes (purchased from Fluka). Electronic integration of the FID peak areas was used for determining quantitative data. Identifying the components of the essential oil was performed by comparison of the mass spectra and retention indices with those in GC/MS library databases (Wiley7n, Wiley 275 and NIST 98).12

Animals

Experiments were conducted using matured Wistar strain male albino rats (190-230 g). The animals were kept in standard controlled laboratory conditions [12 h light/dark photo-cycle, temperature (22 ± 2) °C] with ad libitum access to food and water. The rats were fasted 24 h prior to the start of experiments.

The study was conducted in compliance with the ethical recommendations principles about use of laboratory animal guidelines and the experimental protocol was approved by the Institutional Ethics Committee of Tehran University of medical sciences.

Acute toxicity

Twenty male Wistar rats were divided into four groups

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