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Evaluation of the Impact of Marjoram Herb Extract on Helicobacter Pylori (Agent of Gastric Ulcer)

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Abstract

Background and objective: Helicobacter pylori is considered as a gram-negative, spiral-shaped and microaerophilic bacterium, living in human stomach. This bacterium is often found in patients, who are suffering from chronic gastritis or gastric ulcer. Methodology: marjoram herb was selected for this experimental study. After collecting and preparing the herbs and botanical and pharmacological examinations, the hydro-alcoholic extract of the herbs was prepared using the percolation method and the antimicrobial impacts of this herb on helicobacter pylori was evaluated using disc diffusion method and by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and by using macro dilution method. Results: research results revealed that hydro-alcoholic extract of marjoram herb had antibacterial impacts on Helicobacter pylori in both methods of macro dilution method and disk diffusion method. The minimum inhibitory concentration of marjoram extract on Helicobacter pylori was found to be 120.25 µg / ml and the minimum bactericidal concentration of this extract was found to be 1100 µg / ml. Conclusion: Marjoram extract can be considered as anti-helicobacter pylori compound. The use of essential oil of this herb at higher concentrations and other methods of essential oil extraction would calrify the anti-Helicobacter pylori impacts of marjoram.

Keywords: Marjoram, Helicobacter Pylori, Antibacterial, Gastric Ulcer.

Introduction

Helicobacter pylori is considered as a gram-negative, spiral-shaped and microaerophilic bacterium, living in human stomach. This bacterium is often found in patients, who are suffering from chronic gastritis or gastric ulcer. The most common diseases caused by helicobacter pylori are seen in the stomach and the most common of these diseases is gastric ulcer, which destructs the stomach epithelium and causes a change in production of gastric acid, leading to stomach cancer in many cases. Research indicated that 70 to 100% of patients with gastroenteritis, gastric ulcers and duodenal ulcers have been infected with helicobacter pylori. The current research examines the antimicrobial effect of marjoram herb extract on helicobacter pylori. (Leodolter et al., 2001;. Dunn et al., 1997)

It is a perennial herb with a height of 30 to 60 cm and an aromatic odor. It grows in the dry, coastal, and mountainous areas as well as forests of various regions of Europe and the Southwest and Central Asia. It is also planted for therapeutic goals. Its stem is straight and fluffy and branched with reddish green color. Its leaves are elliptical with dark green color and covered with fluff on the lower surface and its free margins. Its flowers emerge since June to August in white color. Each of its flowers has a calyx leading to 5 equal indentations and a cup larger than calyx. There are four stamens (two large stamens and two small stamens) inside the flower cup. Its fruit is tetrakene enclosed in the remains of flower calyx. Two flowers are often seen among the numerous bases of this herb. In the past medicine, this herb was used to relieve various pains, especially headaches and low back pain. Eucalyptus incense is one of the products of this herb in the market. One of species of marjoram is considered as its constituent parts. This herb has various antimicrobial and antifungal properties. In research performed by Farzad Khademi et al., biopsy samples were taken from the gastric antrum part of 130 patients with gastrointestinal symptoms including gastritis, gastric ulcer, duodenal ulcer and stomach cancer, admitted to the endoscopy unit of the hospitals in Isfahan city between October 2011 and May 2012. Sampling was performed in this way that one sample was taken quickly for urease test, and if this test result was positive, another sample of the same patient was taken to transfer it to microbiology lab of Isfahan University of Medical Sciences in the transfer medium of BHI broth +2% glucose. (Agha-Amiri et al.,2001; Dulciene et al.,1999)

Antibiotic sensitivity test was determined for 30 isolates of helicobacter pylori against different antibiotics by disk diffusion and E-test methods. The number of helicobacter pylori strains resistant to the three antibiotics of metronidazole, clarithromycin, and amoxicillin

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was equal in both of the MDDM and E-test methods. (Anthony,2007; Czajkowsky et al.,2005)

Clarithromycin-resistant Helicobacter pylori isolates had MIC: 4, 2, 2, 1.5 and metronidazole-resistant strains had MIC: 128, 64, 32, 24, 12, 8, 6 and amoxicillin-resistant strains had MIC 4 and 2 µg / ml. Given the prevalence of antibiotic resistances of Helicobacter pylori, much attention has been paid to antimicrobial properties of herbal extracts in recent years. (Ashour et al.,2001; Goossens et al.,1992, Telford et al.,2003)

Methodology

The dried herb of marjoram was grinded after purchasing it from market and being approved in Herbarium Department of the Pharmaceutical Sciences University at the Pharmacognosy Laboratory. Extraction was performed by methanol solvent (Merck) by using the maceration method. Accordingly, the herb powder was first mixed with 800 ml pure methanol after weighing (150 g). Then, it was poured into glass decanters and extraction was performed in three stages (800 ml). The solutions derived from extraction, which included dissolved compounds, were placed in rotary apparatus at temperature of 40 ° C to preserve possible volatile substances and they were concentrated. The extract was prepared in concentrated brown juice and it was kept in darkness and temperature of 4 ° C until the experiments. Consecutive concentrates were prepared from methanol solvent extracts (1000, 500, 250, 125, 62.5 mg / ml) and stored at 4 ° C for well diffusion method. (Ghalem et al.,2009; Goossens et al.,1992)

The studied organism: Helicobacter pylori was prepared from Tehran Taleghani Hospital for a 3-day planting and kept at completely sterile condition in anaerobic jar and away from sunlight to transfer it to Tarbiat Modarres University and Pharmaceutical Sciences University. (Dunn et al.,1997; Dulciene et al.,1999; Tong et al.,2007)

Evaluation of the strains sensitivity

Evaluation of the antibacterial impacts of extracts

Antimicrobial activity of the extracts was evaluated by agar (well) diffusion. Accordingly, suspension with concentration of 4 McFarland was first prepared from 72-hour isolate of Helicobacter pylori. Bacterial suspensions were cultured in completely sterile conditions with swab on the Brucella *Agar* medium (containing 10% sheep blood, FBS, vancomycin, trichytoprine, amphotericin B and polymyxin). Accordingly, wells with a pasteurized pipette of approximately 6 mm in diameter were drilled in plates at regular intervals and appropriate spacing from the plate wall. To examine the extract of each herb in separate plates, 25 µl of dilutions (250, 500, 1000, 5, 62.5, 125 mg / ml) were poured in each well. In addition, methanol was poured to a separate plate as control. Plates containing bacterial culture and extract were incubated in an anaerobic jar and incubator at 37 ° C for 5 to 7 days under same conditions. (Marshall et al.,1994)

Evaluating the impact of antibiotics (amoxicillin, clarithromycin and metronidazole)

Evaluating the antimicrobial impacts of three antibiotics of metronidazole, amoxicillin and clarithromycin were performed using the disk diffusion method. Accordingly, suspension with concentration of 4 McFarland was first prepared from 72-hour isolate of helicobacter pylori. Bacterial suspensions were cultured in completely sterile conditions with swab on the Brucella *Agar* medium (containing 10% sheep blood, FBS, vancomycin, trichytoprine, amphotericin B and polymyxin). In the well diffusion method, antibiotic disks of metronidazole, clarithromycin and amoxicillin were placed on the medium in a completely sterile condition beside the flame with medicine antibody mark. After storage for 96 hours, it was incubated at the temperature of 37 ° C and under microaerophilic conditions and in anaerobic jar. (Leodolter et al.,2001; Sadjadi et al.,2005)

Evaluation of the impact of marjoram extract on helicobacter pylori:

In this method, relative concentrations of the marjoram extract at ratios of the 1.9, 2.8, 3.7, 4.6, 5.5, 6.4, 7.3, 8.2, and 9.1 were prepared, which had the maximum antibacterial impact on helicobacter pylori. For this purpose, suspension with concentration of 4 McFarland was first prepared from 72-hours isolate of Helicobacter pylori. Bacterial suspensions were cultured in completely sterile conditions with swab on the Brucella *Agar* medium (containing 10% sheep blood, FBS, vancomycin, trichytoprine, amphotericin B and polymyxin). (Kikuchi et al.,1999; Sütö et al.,2000)

Accordingly, wells with a pasteurized pipette of approximately 6 mm in diameter were drilled in plates at regular intervals and appropriate spacing from the plate wall. The mixtures of the extract were poured in separate plates and 25 µl of extract of two herbs at the ratio of 1.9, 2.8, 3.7, 4.6, 5.5, 6.4, 7.3, 8.2, 9.1, and 800 mg / ml were poured to each well in completely sterile condition and inside

the hood and beside the flame using the sampler. Plates containing bacterial culture and extract were incubated inside the incubator and within the anaerobic jar at temperature of 37 ° C for 5 to 7 days in same conditions. (Horrocks et al.,1978; Goossens et al.,1992)

Results

The mean diameter of the produced zone of antibiotics and the extract of Marjoram herbs with different concentrations were measured and determined in two methods of disc diffusion and well plate. The mean difference between the diameter of the non-growth zone produced by the selected antibiotics and the extract of Marjoram herb and "epicarp a" was calculated by the ruler. The results are presented in the following table. In case of each herb extract, the diameter of the non-growth zones increased with increasing concentrations of the extracts, so that the diameter of the non-growth zone of marjoram extract reached 21 mm at concentration of 800 mg / ml. Hence, the antibacterial impact of marjoram extract was proven. (Nagayato,1986; McColl,2010)

Next table would present the non-growth zones obtained from each of the concentrations of the marjoram herb extract.

Fig. 1: Plate containing a well of marjoram extract

Table 1- mean non-growth zone diameter produced by methanol extract of marjoram herb

test	mean non-growth zone diameter (mm)
Marjoram extract 400 mg/ml	24
Marjoram extract 200 mg/ml	21
Marjoram extract 100 mg/ml	17
Marjoram extract 50 mg/ml	14
Marjoram extract 25 mg/ml	11

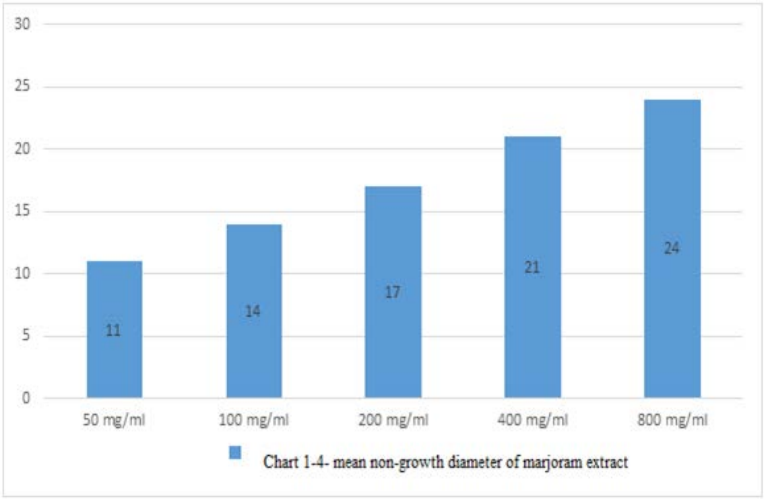

Fig. 2:

Among the antibiotics, the amoxicillin disk had greater non-growth zone compared to metronidazole and clarithromycin and it had the non-growth zone of 24.75. In fact, the impact of synthetic antibiotics (amoxicillin, metronidazole and clarithromycin) is higher than that of the studied extract of herbs extract given greater non-growth zone. The extract solvent (methanol), used as a control in the disk and well method, showed no antimicrobial activity on any of the bacteria studied.

Table 2- Mean zone diameter produced by antibiotics used

Test	mean non-growth zone diameter (mm)
metronidazole	22.25
amoxicillin	24.75
clarithromycin	23

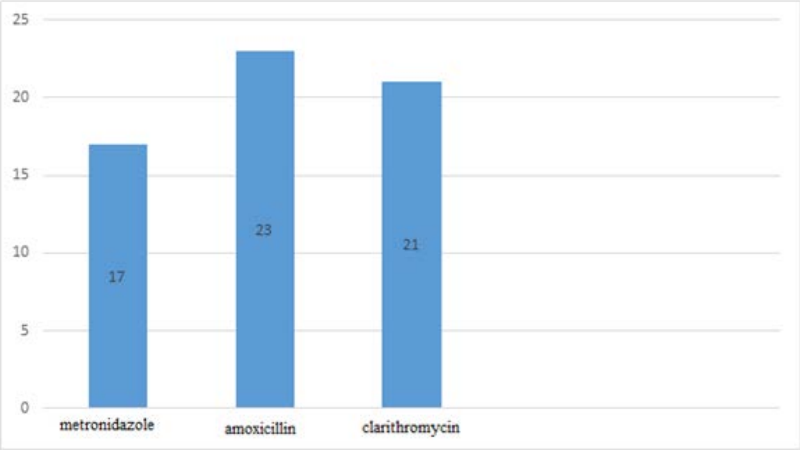

Fig. 3:

Discussion and Conclusion

The infection of helicobacter pylori, resulting in peptic ulcer and stomach cancer, is wide around the world. Re-infection and non-response to eradication therapy, increasing antibiotic resistance, and side effects are the main problems in the treatment process of these patients. Due to importance of medicinal herbs as the most important sources for treatment of diseases, many of the experts argue that the proper treatment is using the medicinal herbs. Owing to antimicrobial impacts of the herbs, they have been used in different nations for treatment of the diseases since old days. Most of the essential oils and extracts, as sources of antimicrobial compounds, are provided from specific and local herbs of a region. Hence, the present research evaluates the antibacterial impacts of marjoram, grown in Iran. (Peter et al., 2012; Perri et al.,2002; Nakamura,2001)

The research results suggest that marjoram extract had an impact on helicobacter pylori. The impact of marjoram alcoholic extract using the disk diffusion method and well diffusion method revealed that reduced concentrations of the extract was directly associated with reduced diameter of the non-growth zones in the bacteria studied. While the impact of 3 synthetic antibiotics of metronidazole, clarithromycin and amoxicillin was more than the antibacterial impact of these two herbs, the use of marjoram herb is cost-effective and it is associated with less side effects. In most of the studies conducted in the past, less antimicrobial effects of combination of two herbal extracts have been examined. In this research, as in the research carried out by Mitra Salehi et al., the antimicrobial activity of the extract increased as its concentration increased. However, unlike the mentioned research, the antibacterial effect of marjoram extract was significantly lower than that of other gram-negative bacteria in this research. Hence, it can be concluded that the effect of marjoram extract is more on Helicobacter pylori compared to gram-negative ones, such as Pseudomonas aeruginosa, Escherichia coli, and Proteus vulgaris. Ay Jun Hu et al identified a group of phenolic compounds in the extracts of marjoram species, grown in China. It can be concluded that the antibacterial compounds found in the marjoram herb extracts can be used in the future as an option along with antibiotic regimes. (Rajaei et al., 2011)

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