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The effect of peppermint essential oil on *Shigella dysenteriae* in Wistar rats with regard to the health of working people

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Abstract

Introduction: *Shigella dysenteriae* is a gram-negative bacterium that causes bacillary dysentery or shigellosis. Dysentery, caused by *Shigella dysenteriae*, is a disease in poor and crowded communities and as an occupational disease can be contracted in workplaces such as agriculture, animal husbandry, tanning, hospital staff and patients. Mint with the scientific name of Mentha, in addition to medicinal use, it is used as a flavoring in the production of various food products.

Methods: In this study, after collecting and drying the plant leaves, the essential oil was extracted by water distillation (Hydro distillation) by Clevenger apparatus and the essential oil compounds were identified by GC-MS. Took place. The antimicrobial effects of essential oil were determined by MIC (minimum inhibitory concentration) & MBC (minimum bactericidal concentration). Then, 0.1 cc of the effective concentration of essential oil was injected, with insulin syringe, to the infected rats with shigellosis for 7 days.

Results: In the disk diffusion method MIC, at 0.6 % concentration of essential oil, the diameter of the growth inhibition zone was 15 mm. The minimum bactericidal concentration was observed at 0.6 % concentration of essential oil. In an animal test, after 7 days of intraperitoneal injection in rats, the infection of *S. dysenteriae* was almost zero.

Discussion: Considering the degree of *S. dysenteriae* infection and the results obtained in this study, 0.6 % concentration of essential oil concentration can be considered as an herbal nano supplement for the treatment of gastrointestinal infections at this level.

1. Introduction

Shigellae are gram-negative, facultatively anaerobic, non-spore-forming rods and non-motile bacilli belonging to the Enterobacteriaceae family. Based on combination of biochemicaland serological characteristic, the genus is divided into four serogroups with multiple serotypes: A (S. dysenteriae, 12 serotypes); B (S. flexneri, 6 serotypes); C (S. boydii, 18 serotypes); and D (S. sonnei, 1 serotype) (Hale, 1991). Transmission occurs by direct contact from person-to-person or contaminated water and food

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and are considered highly infectious, as infection can result from ingestion of 10-200 organisms (Mégraud, Musso, Drancourt, & Lehours, 2017). S. dysenteriae can cause the most severe disease with a 30% mortality rate (Baker & The, 2018; Mattock & Blocker, 2017). Shigella is estimated to cause at least 80 million cases of dysentery and 700,000 deaths per year. 99% of Shigella infections occur in developed countries and the majority of cases (70%) and mortality (60%) are among children. It occurs in less than 5 years. Perhaps less than 1% of cases are treated in hospitals (Khalil et al., 2018; Weill et al., 2017).

The role of peppermint in the treatment of disease:

Infectious diseases are one of the great challenges of medical science in the 21st century, and naturally, the production of new antibiotics is increasing day by day. At the same time, the growing spread of bacterial resistance to antibiotics has made treatment of infectious diseases difficult and costly; therefore, today, researchers have turned to herbal alternatives that, while having antibacterial effects, have no side effects from the use of chemical drugs (González-Bello, 2017; Tariq et al., 2019). Today, the proportion of official herbal medicines used to treat diseases is increasing compared to the total number of official medicines in the world (Watanabe et al., 2019). This ratio is highest in countries such as China and India with more than 70% and in countries such as the United States with about 20% is low (Enioutina et al., 2017; Shahrajabian, Sun, & Cheng, 2019; Watanabe et al., 2019). Unfortunately, in Iran, this ratio is low; it is currently about 5% (Sienkiewicz et al., 2017; Zhaleh et al., 2018). Some plant essential oils have been reported as important natural antimicrobial agents. Essential oils are aromatic oily liquids that come from various parts of the plant such as seeds, roots, buds, bark, branches, Leaves and flowers are obtained. Mainly phenolic compounds are responsible for the antimicrobial properties of essential oils. Essential oils can have up to 60 types of compounds and the main compounds may make up 85% of the essential oil. The results of some studies indicate that the antibacterial effects of essential oils are completely greater than the effects of individual components (Chouhan, Sharma, & Guleria, 2017; Leyva-López, Gutiérrez-Grijalva, Vazquez-Olivo, & Heredia, 2017; Valdivieso-Ugarte, Gomez-Llorente, Plaza-Díaz, & Gil, 2019). Essential oils, due to their hydrophobic properties, penetrate the membrane fat of the bacterial cell and consequently lead to releasing ions and finally causes cell death (Pandey, Kumar, Singh, Tripathi, & Bajpai, 2017). Mint is one of the most common plants that has been used since ancient times because of its healing and aromatic properties. There are 4,000 species of peppermint in 200 genera. Mentha spicata L from the genus Lamiaceae is one of the most important and famous species of mint that is distributed in different parts of the world. In terms of essential oil composition, this species has differences with other types of mint, the main of which are the absence of menthol and the formation of a compound called carvon, which contains a high percentage (73%) of essential oil (Badea et al., 2019; Desam et al., 2019; Marwa, Fikri-Benbrahim, Ou-Yahia, & Farah, 2017)

2. Methods

Preparation of plants and essential oils: Mint plant was prepared by University Jihad Agricultural Research Center from the southern regions of Iran. It was used after the scientific approval of this center. The plant was washed twice with distilled water and dried in a dark room at 23 ± 2 ° C in traditional way. Then, by crushing machine, the plant was turned into powder and 30 grams of plant powder with 300 cc of distilled water, double sterile distillation, was transferred to a 1 liter clevenger apparatus and the essential oil extraction process was performed using a heater. The plant washed twice with ordinary water and dried in a dark place at 23 ± 2 ° C. So, powdered by crushing machine then, 30 g of plant powder add to 300 cc sterile double distilled water in Clevenger apparatus and the essential oil extraction process was performed.

Preparation of bacterial species:

Bacteria are obtained from the microbial ward of the Islamic Azad University, Arak Branch. The type of bacteria is identified after biochemical tests and the use of differential culture media.

Microbial susceptibility to essential oil - Minimum bactericidal concentration (MBC)

Microbial susceptibility test to essential oil by disk diffusion method (Kirby Bauer)

Microbial susceptibility to essential oil was confirmed by disk diffusion test on Muller-Hinton medium (Kirby Bauer), performed according to the Clinical Laboratory Standard Institute (CLSI) guidelines (Sahu, Jain, Mishra, & Prasad, 2018).

For this purpose, microbial suspension was spread on a Muller Hinton agar medium with a sterile swab after preparation of suspension in a half McFarland concentration (1.5×10^8 bacteria per milliliter Liter) and the blank disk was impregnated with 20µl of different concentrations of peppermint essential oil and placed on the culture medium at a suitable distance from the plate wall. The plates were incubated at 37 ° C for 24 hours and then, the diameter of the bacterial growth inhibition zone was measured and recorded in millimeters based on CLSI guidelines (Sezgin & Alagoz, 2017).

Minimum Inhibitory concentration (MIC) assay

microplate dilution method: In order to determine the Minimum Inhibitory concentration of the essential oil, after preparing 0.5 McFarland of bacteria in Müller-Hinton Broth medium, 100 µl of this suspension was added to each of the 96-well plate wells. Then, 100 µl of peppermint essential oil was added to the first well and mixed well. Then 100 lµ of suspension was removed from this well and added to the next well .This was repeated until number 8 well and then in number 9 well, 100 lµ of solution was thrown away. In the tenth well, bacterial suspension was added as a positive control. After 20-18 hours of incubation, the amount of turbidity was investigated using a spectrophotometer at 550 nm (Humphries et al., 2018; Snyder, Savitske, & Credille, 2020).

Perform experiments on rats: In this study, rats underwent general anesthesia with ketamine and xylazil. After preparation of 0.5 McFarland of bacteria, the suspension was injected into the tail of a mouse using an insulin syringe. Then, to ensure the infection of rats, rectometry and random blood culture were used. After wounding, rats are divided into three groups of 7. The treatment group was treated with essential oil, the control group was treated normally and the other group was considered as control and without treatment. Sampling of urine and feces of rats and control of clinical signs of infection were evaluated and continued until the condition of rats returned to normal.

0.1 cc of diluted peppermint essential oil was injected into the rat peritoneum twice a day with an insulin syringe. The treatment period for rats was 7 days. Dosage and volume of injection were determined experimentally following microplate dilution method.

Results

In this study, Mentha essential oil compounds were identified by GC-MS. The most important constituents of essential oil were: menthol (34.71%), menton (18.88%), neo-menthol (9.13%), methyl acetate (7.36%).

In the disk diffusion method, the diameter of the growth inhibition zone at this concentration of essential oil was 15 mm and the minimum bactericidal concentration in the microplate dilution method was in the fourth well.

The results of blood sampling and blood culture of rats and gram staining in terms of the presence of bacteria were as follows: Antibiotic-resistant Shigella dysentery was isolated from the blood culture

of the control group, but no bacteria were isolated from the blood culture of the treatment group after recovery.

Gram staining of rat wounds showed that gram-negative bacteria had grown on the culture medium and after identification, it was determined that the bacterium was the same antibiotic-resistant S. dysentery.

Also, gram staining slides from the urine, feces and blood of rats, at the beginning, during and the end of treatment process, confirmed the presence of a large number of S. dysentery at the beginning of treatment and the absence of this bacteria at the end of treatment.

Figure 1: gram stained slide of rat Feces

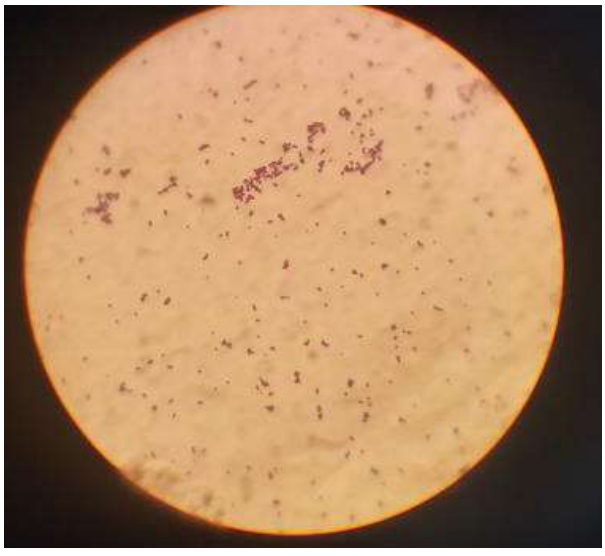

Figure 2: gram stained slide of rat blood

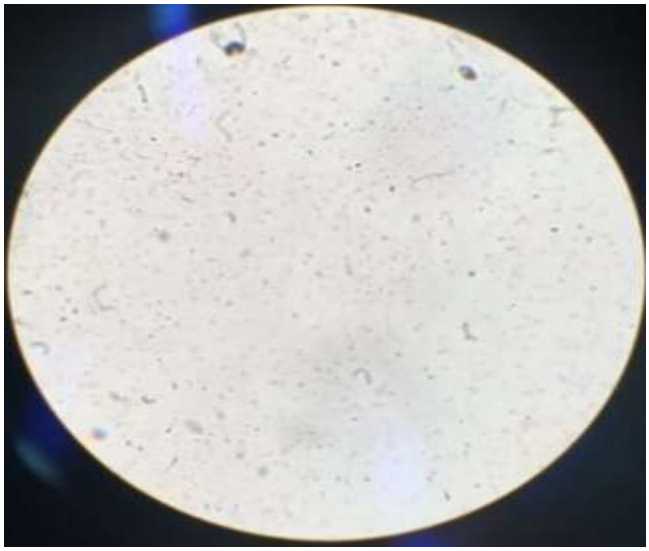

Figure 3: gram stained slide of rat Urine

4. Conclusion

Herbal products with antibacterial properties are considered as an alternative to synthetic antibiotics due to their cheap availability and low side effects. One of these plants is the mint family, which due to the diversity of species and the presence of various compounds of terpenoids, essential oils and phenolic compounds, especially flavonoids, can be studied for antimicrobial effects (KAZEM, Sharifan, & AGHAZADEH, 2011). The aim of this study was to evaluate the antibacterial effect of Mentha essential oil on *Shigella dysentery* in vivo and in vitro. The results of this study showed that the antibacterial effects of peppermint are significant. This finding is similar to reports from a few studies in this field (Vasavada, 2020). Sabaht et al. Showed that the peppermint essential oil has an effective antibacterial effect on *Shigella dysentery* with 12.5 mm growth inhibition zone (Saeed, Naim, & Tariq, 2006).

The antibacterial effect of the essential oil of this plant may depend on the carvone, limonene and menton of chemical composition in this plant. *(Moro et al., 2017)*. Phenolic metabolites in plants such as mint have the ability to release hydrogen from the hydroxyl group in their aromatic ring and causes the free radical oxidation of fats and other cell membrane biomolecules and eventually destroy it (Kang, Liu, Liu, & Wang, 2020).

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