

Comparison of the Effect of Subcutaneous Administration of Anise Essential Oil and Oral Administration of Anise Aqueous Extract in BALB/c Mice Contaminated with Listeriosis

Amin Hashempour ^{*1}, Mohammad Hosein Sadeghi Zali ², Saber Yousefi ³

1. Young Researchers and Elites Club, Urmia Branch, Islamic Azad University, Urmia, Iran

2. Department of Bacteriology, Urmia Branch, Islamic Azad University, Urmia, Iran

3. Department of Microbiology, Urmia University of Medical Sciences, Urmia, Iran

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***Corresponding author:**

Amin Hashempour. Young Researchers and Elites Club, Urmia Branch, Islamic Azad University, Urmia, Iran

Phone: +9843112201

Fax: +9843311172

Email: amin_hp1988@yahoo.com

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Abstract

Introduction: Studies have shown antibacterial activity of anise and on the other hand, *Listeria monocytogenes*, one Gram-positive pathogen is responsible for listeriosis. Thus, this study aimed to investigate the effect of subcutaneous administration of anise essential oil and oral administration of its aqueous extract and also comparison of their effects in BALB/c mice infected with listeriosis.

Methods: Two units, by using insulin syringe, of *Listeria monocytogenes* prepared in 0.5 McFarland standard was intraperitoneally administrated to mice and agglutination test was done to ensure before anise administration. BALB/c mice (n=50) infected with listeriosis received anise aqueous extract (AAE) (5 g/100 g drink water) and 1-unit anise essential oil (AEO) by using insulin syringe was subcutaneously administrated to other mice (n=50) for 1 week. Other mice (n=24) were considered as control.

Results: Results showed that AAE and AEO could improve mice contaminated with listeriosis, although mice treated with AAE were efficiently cured than those who received AEO (37 vs. 12).

Conclusion: On the basis of the results, it can be recommended that the use of AAE for treatment of listeriosis is tenable.

Keywords: Agglutination Test, Anise Aqueous Extract, Antibacterial Activity, Listeriosis

Introduction

Listeria monocytogenes, a Gram-positive pathogen with widespread distribution in nature, is responsible for listeriosis. Listeriosis is known as a dangerous and serious disease and causes high mortality rate (1). *L.monocytogenes* is usually capable to survive and grow under different conditions, i.e. low pH, refrigeration temperatures and high salt concentration (2). *L.monocytogenes* is easily transmitted by food products and can infect human. Patients with listeriosis usually show mild gastrointestinal symptoms and also inefficiencies in immune system. Individuals with immune deficiency are more prone to listeriosis. Listeria has been shown to have intracellular life cycle, it firstly infects epithelial cells and macrophages and then

transfers from cell to other cell (3). It has been shown that the efficiency of some antimicrobials such as sodium chloride and sodium nitrite inhibit the *L.monocytogenes* growth in foods as source for it (4). In addition, negative effects of these antimicrobials have been shown, such as resistance of this bacterium against environmental stressors by sodium chloride (5) and increase in cancer risk by sodium nitrite (6). In vivo studies have used different antibiotics for treatment of listeriosis, such as amoxicillin (7) and ampicillin with gentamicin (8), although antibiotics have limitations and side effects. Antibiotic-associated diarrhea (9) and disturbance in gut microbiota ecosystem (10) are examples of negative effects of antibiotics in human. Today, the use of

medicinal plants for treatment of some diseases has attracted interest. It seems medicinal plants and their derivatives safely treat diseases. Plants act as antibacterials due to some antibacterial compounds present in their structure, such as alkaloids, phenols and terpenes (11). The efficiency of plant extracts in suppression of *L. monocytogenes* growth has been reported (12). The used medicinal plant in the present study, anise (*Pimpinella anisum* L.), belongs to the family of *Apiaceae*, and is highly found in Mediterranean area. Anise is mostly applied in pharmaceuticals, perfumery, food and cosmetic industries (13). In the recent years, anise has attracted interest because of antimicrobial, antifungal and antioxidative properties in anise (14-15). Antibacterial activity of anise derivatives has been shown against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia* (16), *Micrococcus luteus* and *Mycobacterium smegmatis* (17). It is well known that essential oils and extracts have different compounds and on the other hand, these studies well show antibacterial activity of anise. Thus, we hypothesize anise can efficiently show antibacterial activity against *L. monocytogenes* and hence, the purpose of this study is to investigate the effect of subcutaneous administration of anise essential oil and oral administration of its aqueous extract and to compare their effects in BALB/c mice infected with listeriosis.

Methods

In the present study, 128 adult BALB/c mice were purchased from Research Center of Zist Faravard Pars (Rasht-Iran). Animals purchased were kept under lighting program 12h light: 12h dark and temperature $23\pm 1^{\circ}\text{C}$ in the Faculty of Veterinary, Islamic Azad University (Urmia, Iran). In the present study, BALB/c mice were used because of their sensitivity to listeriosis. All the ethical principles were in agreement with National Institutes of Health guide for the care and use

of Laboratory animals (NIH Publications No. 8023, revised 1978).

For preparation of *Pimpinella anisum*, Fresh aerial parts of anise were collected from Urmia Mountain in Summer 2012 and immediately transferred to medicinal plants lab for identification by expert. The prepared samples were dried by using an oven at a temperature of 38°C , and then fined to moderate powders (290-340 μm) by mill. For preparation of aqueous extract, powder was dropped at distilled warm water (about 50°C) at 1:100 ratios for 24 h. The extract was filtered with Whatman No.2 filter paper. The mixture was concentrated under lowered pressure at 40°C by a rota evaporator. The preparation of essential oils was performed by using Clevenger apparatus for 85 min, then the density of essential oil was measured using the gravimetric procedure.

For acute toxicity test, After 24 h fasting, seventy-two BALB/c mice were divided to 12 groups (n=6). Six groups were allocated to anise aqueous extract (AAE) and 6 groups assigned to anise essential oil (AEO). In extract group, animals received 0, 3, 5, 8, 10 and 15 g AAE /100 g drink water and mice at AEO group subcutaneous received 0, 0.5, 1, 1.5, 2 and 3 units of AEO by insulin syringe. No mortality, behavioral and toxicity signs were observed during 48 h after administration. *L. monocytogenes* strain with PTCC 1298 was prepared from Center of Scientific and Industrial Researchs, Iran, and was purified after several subcultures at Blade agar medium culture. Medium culture was sterilized at 121°C and 20 minutes and then sheep blood was added to it.

For preparation of the 0.5 McFarland standard, The most common standard used in the microbiology laboratory is the 0.5 McFarland standard, which is prescribed for antimicrobial susceptibility testing and culture media performance testing. For preparation of the 0.5 McFarland standard, 0.5 ml of 0.048 M BaCl_2 (1.17% w/v $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) were added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) and then stirred

until preparation the homogenous suspension. The standard was disturbed into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. The tubes tightly sealed and maintained from evaporation and light at room temperature. The turbidity standard strongly agitated before use (18).

After preparation of 0.5 McFarland standard, 2 units of *L.monocytogenes*, were intraperitoneally administrated to BALB/c mice, using insulin syringe. The mice were blood taken 3days after administration of bacteria, through vein tail. On the same day same day, agglutination test was done by using kits prepared from Research Center of Zist Faravard Pars (Rasht-Iran).

Mice contaminated with listeriosis, confirmed by agglutination test, received AAE (5 g/100 g drink water) and also, 1-unit anise essential oil AEO was subcutaneous subcutaneously administrated to other mice by using insulin

syringe for 1 week. Other mice were considered as control and did not receive any AAE and AEO. Agglutination test was performed 1 week after treatment with AAE and AEO.

Results

Agglutination test before treatment showed that only 4 mice were not infected with listeriosis and other animals (n=124) were contaminated with listeriosis. Of 124 infected mice, 50 mice were assigned to AAE and 50 mice to AEO, and also 24 mice served as control. After administration of AAE and AEO, agglutination results showed that AAE recovered 37 mice (74%) and AEO cured 12 mice (24%), although both improved listeriosis, AAE was more efficient compared with AEO (74% vs 24%) (Table 1).

Table 1. Effects of anise aqueous extract (AAE) and anise essential oil (AEO) for 7 days on mice infected with listeriosis

Groups	Improved	Unimproved	Improved (%)
AAE (n=50)	37	13	74
AEO (n=50)	12	38	24
Control (n=24)	0	24	0

Discussion

Our results showed that the both, AAE and AEO, could improve mice infected with listeriosis, although the mice receiving AAE efficiently were improved. Antibacterial effect of AAE and AEO can be related to their compounds. Studies have been shown compounds for anise derivatives. Rodrigues *et al.* (19) reported anethole (90%), γ -himachalene (2- 4%), *p*-anisaldehyde (1%), methylchavicol (0.9-1.5%), *cispseudoisoeugenyl*-2-methylbutyrate (3%) and *t*-pseudoisoeugenyl-2-methylbutyrate (1.3%) as the major compounds present in anise oil. In another study, Tabanca *et al.* (20)

showed mono- sesqui and trinorsesquiterpenoids, propenylphenols, pseudoisoeugenols, trinorsesquiterpenoids and phenylpropanoid as the main compounds in *Pimpinella* species by using GC-MS. In another study, anethole has been found as the main constituent of AEO (21). However, studies have shown antibacterial activity of anise derivatives against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherchia coli*, *Klebsiella pneumonia* (16), *Micrococcus luteus* and *Mycobacterium smegmatis* (17). In addition, antibacterial activity of AAE and anise ethanolic extract against *Staphylococcus aureus*, *E. coli* and *pseudomonas aeruginosa* has been reported (22). *L.monocytogenes* is

one Gram-positive pathogen and it is well known that Gram-positive bacteria have simpler structure compared to Gram-negative bacteria and it seems that anise could efficiently show antibacterial activity against *L.monocytogenes*. However, antibacterial activity of methanol extract of anise seed against Gram-negative has been previously demonstrated (23). The differences between AAE and AEO may be attributed to the used dose, administration procedure and their compounds. It seems that antibacterial compounds in AAE are more soluble in water and the use of it in drink water efficiently can upgrade antibacterial activity. We believed antibacterial compounds present in AAE in oral form efficiently interact with *L.monocytogenes* and destroy it; resulting in improvement in listeriosis.

Conclusion

In conclusion, aqueous extract and essential oil of anise could improve mice with listeriosis, although antibacterial activity of aqueous extract against *L.monocytogenes* was more efficient compared with essential oil. Thus, the use of anise aqueous extract along with commercial drugs for treatment of patients with listeriosis can be suggested.

Ethical issues

No applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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