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The Comparison of intravenous and oral administration of anise plant on BALB/c mice with listeriosis

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ABSTRACT

Background & Aim: Anise studies have demonstrated different properties such as anti-cancer, anti-fungal, antibacterial, antioxidant, strengthen the liver, gastrointestinal tract, wounds heal and strengthen the nerve cells. Outbreaks of listeriosis in the general population are 7.0 per 100,000 populations. Pregnant women, infants, the elderly and immunocompromised patients or those weaknesses comprise most cases. The aim of the present study was to investigate the effect of intravenous and oral administration of anise herb to BALB/c race of mice with listeriosis.
Experimental: 2 units of *listeria monocytogenes* were prepared in 0.5 Mc Farland concentrations and injected to BALB/c trace mouse intraperitoneal. Before and after treatment, the mice were examined for agglutination test.
Results: The results of this study indicated that the effect of oral treatment with aqueous extract is better than injection of essential oil of anise.
Recommended applications/industries: According to results of the present study, anise aqueous extract was recommended as a potential antibacterial agent against *listeria monocytogenes.*

1. Introduction

In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Arnous *et al.*, 2001; Huda-Faujan *et al.*, 2009). Plants are a large source of new bioactive molecules with therapeutic potentials. Only a small percentage of living plants on Earth have been phytochemically investigated. Plants are thus an enormous reservoir of pharmaceutically valuable molecules to be discovered (Hostettmann *et al.*, 1998; Balandrin *et al.*, 1985; Kosalec *et al.*, 2005).

Pimpinella anisum L. (Apiaceae) is an annual herb indigenous to the near east and widely cultivated in the Mediterranean regions as well as Mexico and Chile. It has been used as an aromatic herb and spice since Egyptian times (Kosalec *et al.*, 2005). The star anise and anise have both been widely used in Iranian traditional medicine for their antimicrobial effects (Amin, 2005). The antimicrobial properties of these species such as anise and star anise have been reviewed by several researchers (Kosalec *et al.*, 2005; Gulcın *et al.*, 2003; Sungog *et al.*, 2007; Yazdani *et al*. 2008).

Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine (Amin *et al.*, 2005). Aniseed has

warm and dry nature and can increase milk production, menstruation, urine, and sweat secretion and also making good complexion. It is also effective in polishing of teeth. In some traditional texts, anise is mentioned for melancholy, nightmare, and also in treatment of epilepsy and seizure (Mirheydar *et al.*, 2001; Aghili Khorasani *et al.*, 2001).

Aniseed contains 1.5–6.0% of a volatile oil consisting primarily of trans-anethole and also as much as 8–11% of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 % of carbohydrates, and 18 % of protein (Besharati-Seidani *et al.*, 2005). Other studies have demonstrated the presence of eugenol trans-anethole, methylchavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes as the major compounds of the essential oil of anise seed (Gulcin et al., 2003). Study of the essential oil of *Pimpinella anisum L*. fruits by GC and GC-MS showed the presence of trans-anethole (93.9%) and estragole (2.4%).

Other compounds that were found with concentration higher than 0.06% were (E)-methyleugenol, α-cuparene, α- himachalene, β-bisabolene, p-anisaldehyde, and cis-anethole (Ozcan and Chalchat, 2006). In another study for determination of the composition of essential oil of Pimpinella anisum L. fruits obtained from different geographical areas of Europe, in addition to the major components (trans-anethole (76.9–93.7%) and γ- himachalene (0.4–8.2%), some other compounds such as trans-pseudoisoeugenyl 2-methylbutyrate, p-anisaldehyde, and methylchavicol were also identified in essential oil (Orav *et al.*, 2008).

Essential oil and methanol extract of these plants exhibited antibacterial activity against most tested pathogens, and the maximum effect was observed against Staphylococcus aureus, Bacillus cereus, and Proteus vulgaris. However, combination of essential oil and methanol extracts of these plants showed an additive effect against most tested bacteria especially Pseudomonas aeruginosa (Al-Bayati, 2008). The antibacterial potential of aqueous decoctions of black pepper, bay leaf, aniseed, and coriander against 176 bacterial isolates belonging to 12 different genera were detected by the mean of disc diffusion technique. The findings showed that the aqueous decoction of black pepper was the most bacterial-toxic exhibited 75% antibacterial activity and decoction of aniseed exhibited 18.1% antibacterial activity (maximum antibacterial activities exhibited against Micrococcus roseus) (Chaudhry & Tariq, 2006). In addition to antibacterial activity, the essential oil of aniseed showed significant inhibitory activity against fungi, and the most active component of it was anethol (Shukla & Tripathi, 1987).

2. Materials and Methods

2.1. Preparation of mice

128 mice were prepared from research center of Zist Faravarde Pars in Rasht city, Guilan. Mice were transferred to animal houses of Islamic Azad University, Urmia branch.

2.2. Bacteria injection

After sub culture, bacteria transferred to peptone water media. After 24h incubation L.P was injected to mice**.**

2.3. Agglutination test after bacteria injection

Listeria Agglutination kits were prepared from research center of Zist Faravarde Pars in Rasht city, Guilan. Agglutination test was done 3 days after injection to ensure of listeriosis in mice. Blood samples were taken from tail vein.

Table 1. Characteristics of bacteria

S. No	Name	Type	PTCC No
1	*Listeria monocytogenes*	Gram positive bacteria	PTCC1298

2.4. Treatment by injection

After ensuring prevalence of listeriosis in mice subcutaneous injection were done daily at a rate of 1 unit by insulin syringes.

2.5. Treatment with anise seed extract in oral way

The extract was fed to mice by 5g per 100ml instead of drinking water.

2.6. Agglutination test after treatment

After one week of injection of anise essential oil, agglutination test was done to ensure that the treatment is completed.

3. Results and discussion

Anise (*Pimpinella anisum* L), is an annual important spice and medicinal plant belonging to the family of *Apiaceae*, and native to Mediterranean region. Today, anise seeds are important natural raw materials which are used in pharmaceutics, perfumery, food and cosmetic industries (Ross, 2001). In this study the effect of aqueous extracts and essential oils were compared and it was found that the effect of aqueous extract applied orally was more than injection of essential oil.

3.1. Agglutination test before treatment

Agglutination test was performed on 128 rats and almost the incidence of listeriosis was 100% in mice. Only four rats were not infected and therefore kept separately.

3.2. Treatment

According to table 2, treatment by injection with essential oils and orally water extracts was done on 50 mice separately for a week. According to results, antibacterial effect by injection of essential oils and orally water extracts were 24% and 74%, respectively.

Table 2. Number of cured and not-cured mice treated with anise plant essential oil and extract in 7 days.

Total samples	Treatment	Cured	not-cured
50	Water extract (oral)	37	13
50	Essential oil (Subcutaneous)	12	38
24	Control	0	24
	Total	49	75
		124	

In many studies antibacterial effect of anise seed was reported. For example, Al-Bayati (2008) was shown antibacterial activity of *Pimpinella anisum*. In another research conducted by Yazdi *et al.* (2014) antibacterial effect and strengthening immune responses of *Pimpinella anisum* was confirmed in broiler chicks (Yazdi *et al*., 2014).

Antimicrobial effects of water and ethanolic extracts of anise seeds against 10 bacterial species and *Candida albicans* were studied by Gulcin *et al*. (2003) with disc diffusion method. They reported that ethanolic extract showed significant inhibitory activity against all tested bacteria but was not effective on *Candida albicans*. However, the antimicrobial effect of water extract was not detected against gram-negative bacteria, *Pseudomonas aeruginosa*, and *Escherichia coli*, but it was effective against *Candida albicans* (Gulcin *et al*., 2003).

Also, alcoholic extracts of *Pimpinella anisum* seeds showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatis* (Ates & Erdogrul, 2003).

The antibacterial activities of the aqueous, methanol, acetone and petroleum ether extracts of *Pimpinella anisum* L. fruits were tested against 4 pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherchia coli*, and *Klebsiella pneumoniae*) by disc diffusion method. The results showed that only aqueous and methanol extracts exhibited fair antibacterial activity against all of the tested bacteria and the aqueous extract was found to be more effective than methanolic extract, whereas acetone and petroleum ether extracts did not show inhibitory effect on growth of the pathogenic tested bacteria (Akhtar *et al*., 2008).

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