The Comparison of Intravenous and Oral Administration of Anise Plant on BALB/c Mice with Listeriosis

Amin Hashempour1*, Mohammad Hosein Sadeghi Zali2, Saber Yousefi3

1Young Researcher and Elites club, Urmia Branch, Islamic Azad University, Urmia, Iran;
2Department of Bacteriology, Veterinary Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran;
3Department of Microbiology, Virology and Immunology, Urmia University of Medical Sciences, Urmia, Iran;

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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; Gulec 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)-methylcyrene, 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>
<thead>
<tr>
<th>S. No</th>
<th>Name</th>
<th>Type</th>
<th>PTCC No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Listeria</td>
<td>Gram positive</td>
<td>PTCC1298</td>
</tr>
<tr>
<td></td>
<td>monocytogenes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 pharmacies, 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.

<table>
<thead>
<tr>
<th>Total samples</th>
<th>Treatment</th>
<th>Cured</th>
<th>not-cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Water extract (oral)</td>
<td>37</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>Essential oil</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td>(Subcutaneous)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>49</td>
<td>75</td>
</tr>
</tbody>
</table>

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*, *Escherichia 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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