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Anti-Inflammatory Activities of Diethyl-Ether Extracts of *Helichrysum plicatum* DC. and *Tanacetum balsamita* L. in Rats


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Abstract: The aim of this study was to investigate anti-inflammatory activity of the diethyl ether extract of *Tanacetum balsamita* L. subsp. (TB) and *Helichrysum plicatum* DC. subsp. (HP) in carrageenan-induced inflammation in rats. Lambda carrageenan (0.05 mL) was injected into the subplantar region of the right hind paw to induce inflammation. Control group and the reference group were administered isotonic saline solution and indomethacin, respectively. TB extract was injected in doses of 25, 50 and 100 mg kg⁻¹ in the groups TB-25, TB-50 and TB-100, respectively. HP-25 HP-50 and HP-100 groups were injected HP extract in doses of 25, 50 and 100 mg kg⁻¹. Before the injections and 3 h after the injections the volume of right hind-paw of rats was measured using a plethysmometer. TB and HP had anti-inflammatory effects matching to that of the reference agent at all doses. It was found that reduction in the inflammation was 95.21% with indomethacin, 51.93% with TB-25, 52.55% with TB-50, 61.51% with TB-100, 70.73% with HP-25, 73.15% with HP-50 and 82.90% with HP-100. Median effective dose (ED₅₀) value of TB and HP were found to be 81.484 and 73.030 mg kg⁻¹, respectively. The results showed that *Tanacetum balsamita* L. subsp. and *Helichrysum plicatum* DC. subsp. had a significant anti-inflammatory activity.

Key words: *Tanacetum balsamita* L. subsp., *Helichrysum plicatum* DC. subsp., anti-inflammatory effect, rat

INTRODUCTION

*Tanacetum balsamita* L. (Eng. costmary), grown widely in the Northern Anatolian region, is a perennial herbal plant that can grow up to 80 cm. It is called subsp. *balsamoides* if it has tongue shaped white flowers on its capitulum and subsp. *balsamita* if not. Its branches with flowers are widely used as folk remedy in Turkish folk medicine as diuretic, lithagogue, anti bloating, appetizer, aphrodisiac, vermifuge, emmenagogue and for migraine (Baytop, 1999; Çubukcu et al., 2002a). There has been no study on the biological effects of *Tanacetum balsamita*, although there have been some studies with the other species of the genus *Tanacetum*. A parthenolide-depleted extract of *Tanacetum parthenium* was shown to protect skin from ultraviolet light (Martin et al., 2008). The chloroform extract of *Tanacetum vulgare* L. had cytotoxic effects on various human cancer cell lines.
(Ramirez-Brea et al., 2007) while acidic polysaccharides isolated from it had immunosuppressive effects (Xie et al., 2007). Aqueous extracts of *Tanacetum vulgare* L. also had vasodilating and strong diuretic effects (Labow et al., 2007, 2008). Volatile oils of *Tanacetum argentum* subsp. *albidifolium* had antibacterial activity (Tangos et al., 2007). The crude extract of *Tanacetum artemisioides* showed anti-inflammatory, analgesic and calcium channel blocking effects (Eskici et al., 2007). Some flavonoids extracted from *Tanacetum microphyllum* inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 (Güner et al., 2006).

*Helichrysum plicatum* DC (Eng everlasting flower) is a perennial herbal plant that can grow 10–40 cm and commonly found in Anatolia. Their leaves are flat and pubescent on both sides. The bracts around capitulum are yellow or yellowish white in color. It is used in Turkish folk medicine mainly as diuretic, lithagogue and for stomachache (Serin et al., 2001; Eyüboğlu, 1999; Çubukçu et al., 2002). Aqueous and ethanol extracts of *Helichrysum plicatum* sp. were shown to decrease blood sugar in streptozotocin-induced diabetes in rats and had antioxidant activity (Aslan et al., 2007) as well as antibacterial effects (Smirnov et al., 1982).

This study aimed to investigate anti-inflammatory effects of distilled ether extract of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC collected around Van and Miss districts in Turkey in carrageenan induced paw oedema in rats.

**MATERIALS AND METHODS**

**Plant Material**

*Helichrysum plicatum* DC. subsp. *plicatum* was collected around Yukarıkıköy Village (1975 m) (Gevre, Turkey) in July 2007 (Fig. 1). *Tanacetum balsamita* L. subsp. *balsamitoides* was collected from Laladağ Mountain (Malatya, Turkey) in August 2007 (Fig. 2). The plant was collected and identified by Dr. F. Özgökçe and deposited at the herbarium of the Biology Department, Akdeniz University (F: 13192, F: 13193).

**Extraction of Plant Material**

The above-ground parts of the plants were grounded in an electric grinder and macerated in diethyl ether for 2 h using a soxhlet apparatus (Üden, Turkey). The extract was separated from the solvent by evaporation under vacuum using a rotary evaporator (IKA-WERKE, Germany). The yield for *Helichrysum plicatum* DC. was 3.39% (w/w) and for *Tanacetum balsamita* L. was 7.33% (w/w).

![Fig 1: Helichrysum plicatum DC. subsp. plicatum was collected around Yukarıkıköy Village (1975 m) (Gevre, Turkey) in July 2007](image-url)
Animals

Male and female Sprague-Dawley rats weighing 110-220 g, purchased from the Animal House of the Medical School, Yuzuncu Yıl University (Van, Turkey) were used in the present study. The animals were housed at room temperature (20±2°C) in standard cages with standard pellet food and water ad libitum. The approval of Medical School Ethics Committee was obtained.

Chemicals

Lambda-carrageenan and indomethacin were obtained from Sigma (Germany), CCl₄ and dimethyl sulfoxide (DMSO) from Merck (Germany). Lambda-carrageenan was prepared in distilled water (1%, w/v). Indomethacin was dissolved in ethyl alcohol (w/v) and dimethyl ether extracts of Helichrysum pilosum DC. (HP) and Tanacetum balsamita L. (TB) in DMSO (w/v).

Carrageenan-Induced Rat Paw Oedema

The method of Winter et al. (1962) was used with slight modification to induce inflammation in rats. Inflammation of the hind paw was induced by injecting 0.05 mL fresh lambda carrageenan into the subplantar surface of the right hind paw. Forty-eight rats were divided into eight groups of six animals each. The control group was given 0.1 mL of Isotonic Saline Solution (ISS). The reference group received indomethacin (3 mg kg⁻¹, i.p.), an anti-inflammatory agent. TB groups (TB-25, TB-50 and TB-100) received TB at the doses of 25, 50 and 100 mg kg⁻¹. HP groups (HP-25, HP-50 and HP-100) received HP at the doses of 25, 50 and 100 mg kg⁻¹. These drugs were injected into rats immediately before the injection of lambda carrageenan. All injections were made intraperitoneally using a Hamilton injector. The doses of TB and HP were chosen according to Awan et al. (2007) and Bakhtari et al. (2007). The dose of indomethacin was chosen according to Rimba et al. (1999). The rats were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in the oedema alone responses. The degree of oedema was measured 30 min before and 3 h after the injection of carrageenan. The difference between the volume of the paw before and after the injection of indomethacin indicated the severity of oedema. Volumes of right hind paw of the animals were measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The percentage inhibition of the inflammatory reaction was determined for each animal by comparing with controls and calculated by the following formula (Kouadio et al., 2000):
\[
I\ % = \left[\frac{d_{(t-d)}}{d_{(c-d)}}\right] \times 100
\]
where, \(d_t\) is the difference in paw volume in the drug-treated group and \(d_c\) the difference in paw volume in the control group.

**Statistical Analysis**

All data were represented as Mean±SE of mean (SEM) or as percentages. The Analysis of Variance (ANOVA) was used for the statistical analysis of data. LSD test (Least significant difference test) was used for determining significance. Probability levels of less than 0.05 were considered significant. The median effective dose (ED\(_{50}\)) value was calculated by non-linear regression analysis (SigmaPlot for Windows Version 9.0).

**RESULTS AND DISCUSSION**

The effects of ethyl ether extracts of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC. on the carrageenan induced oedema are presented in Table 1. Indomethacin, a commonly used anti-inflammatory drug, produced a significant inhibition of carrageenan induced inflammation (95.21\%, \(p<0.05\)). TB and HP at all doses studied caused significant decreases in inflammation \((p<0.05)\). Although the reduction in inflammation caused by 25 and 50 mg kg\(^{-1}\) TB was significant (51.93 \% and 52.55\%), it was not as great as that of indomethacin \((p<0.05)\). Anti-inflammatory effect of 100 mg kg\(^{-1}\) TB (61.51\%) was similar to that of indomethacin \((p<0.05)\). There were no significant differences between the effects of indomethacin and 25, 50 and 100 mg kg\(^{-1}\) HP \((70.73, 73.15\) and 82.90\%, respectively \((p>0.05)\), showing that they have anti-inflammatory effects similar to indomethacin. The median effective dose (ED\(_{50}\)) values of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC. were found to be 81.48 and 73.03 mg kg\(^{-1}\), respectively.

This study showed that the ethyl ether extracts of both *Tanacetum balsamita* L. and *Helichrysum plicatum* DC. had anti-inflammatory effect on carrageenan-induced hind paw oedema in rats. Their effects were dose dependant and were similar to that of indomethacin, a well known anti-inflammatory agent. Although some other species of the genus *Tanacetum* were reported to have anti-inflammatory activities this is the first study showing that *Tanacetum balsamita* L. reduced inflammation in an animal model. *Tanacetum* species may have in general some anti-inflammatory effects. Bukhari *et al.* (2007) reported that the crude extract of *Tanacetum artemisioides* showed anti-inflammatory effects in carrageenan induced rat paw edema, which was attributed to the flavonoid compounds of the plant. The anti-inflammatory effects of the flavonoids may be due to their inhibitory action on the expression of inducible nitric oxide synthase and cyclooxygenase-2 (Guerra *et al.*, 2006). Abad *et al.* (2004) showed that santin, eremarin, centaureidin and 5,3'-dihydroxy-4'-methoxy-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw edema (ml, %)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS</td>
<td>0.92±0.005</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.043±0.015(^a)</td>
<td>95.21</td>
</tr>
<tr>
<td>TB-25</td>
<td>0.43±0.015(^a)</td>
<td>51.93</td>
</tr>
<tr>
<td>TB-50</td>
<td>0.42±0.015(^a)</td>
<td>52.55</td>
</tr>
<tr>
<td>TB-100</td>
<td>0.34±0.110(^a)</td>
<td>61.51</td>
</tr>
<tr>
<td>HP-25</td>
<td>0.264±0.090(^a)</td>
<td>70.73</td>
</tr>
<tr>
<td>HP-50</td>
<td>0.242±0.096(^a)</td>
<td>73.15</td>
</tr>
<tr>
<td>HP-100</td>
<td>0.154±0.190(^a)</td>
<td>82.90</td>
</tr>
</tbody>
</table>

F-p values = 16.229-0.000

The values represent the Mean±SEM \((n=6)\). ED\(_{50}\) for *Tanacetum balsamita* L.: 81.484 mg kg\(^{-1}\); ED\(_{50}\) for *Helichrysum plicatum* DC.: 73.030 mg kg\(^{-1}\). Post-hoc Tukey's HSD test: \(p<0.05\) with respect to the ISS group; \(p<0.05\) with respect to the indomethacin group.
7-methoxycarbonylflavonol, naturally occurring flavonoids in the *Tanacetum* species, inhibited lipopolysaccharide-induced nitric oxide and prostaglandin E2. Moreover, the findings support the traditional reputation of the genus *Tanacetum* for its therapeutic benefits in inflammatory conditions.

Although, *Helichrysum plicatum* DC. is mainly used as diuretic, lithagogue, and for stomachache in Turkish folk medicine, here we report its anti-inflammatory effect first time. Some of the other members of the genus *Helichrysum* were shown to have similar effects. Sala et al. (2002, 2003) and Schmelka et al. (2002) reported that the above-ground parts of the *Helichrysum italicum* had anti-inflammatory effect which may be due to its flavonoid compounds (gumphanin, pinocembrin and tiliroside). Acetone extract of *Helichrysum italicum* sp. *Microphyllum* also decreased the release of pro-inflammatory cytokines from activated monocytes (Appendino et al., 2007). The results of the present study showed that, similar to the other *Helichrysum* species, *Helichrysum plicatum* DC had anti-inflammatory effect.

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**REFERENCES**


