

ajava

Asian Journal of Animal and Veterinary Advances



Academic
Journals Inc.

www.academicjournals.com

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

¹M. Karaca, ²H. Özbek, ¹H.A. Akkan, ³M. Tütüncü, ⁴F. Özgökce, ⁵A. Him and ⁶B. Bakir

¹Department of Internal Medicine, Faculty of Veterinary Medicine,
Yüzüncü Yıl University, Van-Turkey, Turkey

²Directorate of Drug and Pharmacy, Ministry of Health, Ankara, Turkey

³Gevas Vocational College, Van-Turkey, Turkey

⁴Department of Biology, Faculty of Science and Literature, Van-Turkey, Turkey

⁵Department of Physiology, Faculty of Medicine, Van-Turkey, Turkey

⁶Department of Surgery, Faculty of Veterinary Medicine,
Yüzüncü Yıl University, Van-Turkey, Turkey

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. *balsamitoides* 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; Cubukcu *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

Corresponding Author: Dr. Mehmet Karaca, Department of Internal Medicine, Faculty of Veterinary,
Yüzüncü Yıl University, Hayvan Hastanesi, Campus, 65080-VAN, Turkey
Tel: +90432 2251026 Fax: +90432 2251127

(Ramirez-Erosa *et al.*, 2007) while acidic polysaccharides isolated from it had immunotherapeutic effects (Xie *et al.*, 2007). Aqueous extracts of *Tanacetum vulgare* L. also had vasorelaxing and strong diuretic effects (Lahlou *et al.*, 2007, 2008). Volatile oil of *Tanacetum argenteum* subsp. *flabellifolium* had antibacterial activity (Tabanca *et al.*, 2007). The crude extract of *Tanacetum artemisioides* showed anti-inflammatory, analgesic and calcium channel blocking effects (Bukhari *et al.*, 2007). Some flavonoids extracted from *Tanacetum microphyllum* inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 (Guerra *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 (Sezik *et al.*, 2001; Baytop, 1999; Cubukcu *et al.*, 2002b). 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 diethylether extract of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC collected around Van and Mus districts in Turkey in carrageenan induced paw oedema in rats.

MATERIALS AND METHODS

Plant Material

Helichrysum plicatum DC. subsp. *plicatum* was collected around Yukariköy Village (1975 m) (Gevas, Turkey) in July 2007 (Fig 1). *Tanacetum balsamita* L. subsp. *balsamitoides* was collected from Laladağ Mountain (Malazgirt, 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, Yüzüncü Yıl University (F: 13192, F: 13194).

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 (Ildam, 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 7.50% (w/w).



Fig. 1: *Helichrysum plicatum* DC. subsp. *plicatum* was collected around Yukariköy Village (1975 m) (Gevas, Turkey) in July 2007



Fig. 2: *Tanacetum balsamita* L. subsp. *balsamitoides* was collected from Laladağ Mountain (Malazgirt, Turkey) in August 2007

Animals

Male and female Sprague-Dawley rats weighing 110-220 g, purchased from the Animal House of the Medical School, Yüzüncü Yıl University (Van, Turkey) were used in the present study. The animals were housed at room temperature ($20 \pm 2^\circ\text{C}$) 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), CCl_4 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 diethyl ether extracts of *Helichrysum plicatum* 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 Aslan *et al.* (2007) and Bukhari *et al.* (2007). The dose of indomethacin was chosen according to Rimbau *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 oedematous response. 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):

$$I \% = [(1-(dt/dc)] \times 100$$

where, dt is the difference in paw volume in the drug-treated group and dc 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 (ED₅₀) value was calculated by non-linear regression analysis (SigmaPlot for Windows Version 9.0).

RESULTS AND DISCUSSION

The effects of diethyl 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 (ED₅₀) values of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC. were found to be 81.484 and 73.030 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, ermanin, centaureidin and 5,3'-dihydroxy-4'-methoxy-

Table 1: The effects of diethyl ether extract of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC. on carrageenan-induced hind paw oedema in rats

Groups	Paw oedema (mL %)	Inhibition (%)
ISS	0.923±0.065	-
Indomethacin	0.043±0.015 ^a	95.21
TB-25	0.434±0.045 ^{ab}	51.93
TB-50	0.428±0.054 ^{ab}	52.55
TB-100	0.347±0.116 ^a	61.51
HP-25	0.264±0.090 ^a	70.73
HP-50	0.242±0.096 ^a	73.15
HP-100	0.154±0.190 ^a	82.90

F-p values = 16.229-0.000

The values represent the Mean±SEM (n = 6); ED₅₀ for *Tanacetum balsamita* L.: 81.484 mg kg⁻¹; ED₅₀ for *Helichrysum plicatum* DC. : 73.030 mg kg⁻¹. Post-hoc Tukey's HSD test: ^ap<0.05 with respect to the ISS group; ^bp<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 Schinella *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 (gnaphaliin, 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.

ACKNOWLEDGMENTS

This study was supported by a grant (2007-VF-B008) from Yüzüncü Yil University, The Directorate of Scientific Research Projects.

REFERENCES

- Abad, M.J., P. Bermejo, M. Alvarez, J.A. Guerra, A.M. Silvan and A.M. Villar, 2004. Flavonoids and a sesquiterpene lactone from *Tanacetum microphyllum* inhibit anti-inflammatory mediators in LPS-stimulated mouse peritoneal macrophages. *Planta Med.*, 70: 34-38.
- Appendino, G., M. Ottino, N. Marquez, F. Bianchi, A. Giana and M. Ballero *et al.*, 2007. Arzanol, an anti-inflammatory and anti-HIV-1 phloroglucinol alpha-Pyrone from *Helichrysum italicum* ssp. *microphyllum*. *J. Nat. Prod.*, 70: 608-612.
- Aslan, M., D.D. Orhan, N. Orhan, E. Sezik and E. Yesilada, 2007. *In vivo* antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* capitulum in streptozotocin-induced-diabetic rats. *J. Ethnopharmacol.*, 109: 54-59.
- Baytop, T., 1999. *Therapy with Medicinal Plants in Turkey*. Nobel, Istanbul, ISBN: 975-420-021-1, pp: 309-310, 311.
- Bukhari, I.A., R.A. Khan, A.U. Gilani, A.J. Shah, J. Hussain and V.U. Ahmad, 2007. The analgesic, anti-inflammatory and calcium antagonist potential of *Tanacetum artemisioides*. *Arch Pharm. Res.*, 30: 303-312.
- Cubukcu, B., A.H. Mericli, A. Mat, G. Sariyar, N. Sütlüpinar and F. Mericli, 2002a. *Phytotherapy*. Istanbul University Pharmacy School Publications, Istanbul ISBN: 975-404-647-6, pp: 149.
- Cubukcu, B., A.H. Mericli, A. Mat, G. Sariyar, N. Sütlüpinar and F. Mericli, 2002b. *Phytotherapy*. Istanbul University Pharmacy School Publications, Istanbul ISBN: 975-404-647-6, pp: 82.
- Guerra, J.A., M.F. Molina, M.J. Abad, A.M. Villar and B. Paulina, 2006. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids isolated from *Tanacetum microphyllum*. *Int. Immunopharmacol.*, 6: 1723-1728.
- Kouadio, F., C. Kanko, M. Juge, N. Grimaud and A. Jean *et al.*, 2000. Analgesic and antiinflammatory activities of an extract from *Parkia biglobosa* used in traditional medicine in the ivory coast. *Phytother. Res.*, 14: 635-637.
- Lahlou, S., A. Tahraoui, Z. Israili and B. Lyoussi, 2007. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. *J. Ethnopharmacol.*, 110: 458-463.
- Lahlou, S., K.C. Tangi, B. Lyoussi and N. Morel, 2008. Vascular effects of *Tanacetum vulgare* L. leaf extract: *in vitro* pharmacological study. *J. Ethnopharmacol.*, 120: 98-102.

- Martin, K., R. Sur, F. Liebel, N. Tierney and P. Lyte *et al.*, 2008. Parthenolide-depleted Feverfew (*Tanacetum parthenium*) protects skin from UV irradiation and external aggression. *Arch Dermatol Res.*, 300: 69-80.
- Ramirez-Erosa, I., Y. Huang, R.A. Hickie, R.G. Sutherland and B. Barl, 2007. Xanthatin and xanthinosin from the burs of *Xanthium strumarium* L. as potential anticancer agents. *Can. J. Physiol. Pharmacol.*, 85: 1160-1172.
- Rimbau, V., C. Cerdan, R. Vila and J. Iglesias, 1999. Antiinflammatory activity of some extracts from plants used in the traditional medicine of north-African countries. *Phytother. Res.*, 13: 128-132.
- Sala, A., M. Recio, R.M. Giner, S. Manez, H. Tournier, G. Schinella and J.L. Rios, 2002. Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. *J. Pharm. Pharmacol.*, 54: 365-371.
- Sala, A., M.C. Recio, G.R. Schinella, S. Manez and R.M. Giner *et al.*, 2003. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. *Eur. J. Pharmacol.*, 461: 53-61.
- Schinella, G.R., H.A. Tournier, J.M. Prieto, P. Mordujovich de Buschiazso and J.L. Rios, 2002. Antioxidant activity of anti-inflammatory plant extracts. *Life Sci.*, 70: 1023-1033.
- Sezik, E., E. Yesilada, G. Honda, Y. Takaishi, Y. Takeda and T. Tanaka, 2001. Traditional medicine in Turkey X. Folk medicine in central anatolia. *J. Ethnopharmacol.*, 75: 95-115.
- Smirnov, V.V., N.E. Preobrazhenskaia and I.D. Kalashnikov, 1982. [Antibacterial properties of *Helichrysum plicatum* DC]. *Mikrobiol Zh*, 44: 71-72.
- Tabanca, N., F. Demirci, B. Demirci, D.E. Wedge and K.H. Baser, 2007. Composition, enantiomeric distribution and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil. *J. Pharm. Biomed. Anal.*, 45: 714-719.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111: 544-547.
- Xie, G., I.A. Schepetkin and M.T. Quinn, 2007. Immunomodulatory activity of acidic polysaccharides isolated from *Tanacetum vulgare* L. *Int. Immunopharmacol.*, 7: 1639-1650.