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Anti-inflammatory activity of *Anthemis aciphylla* var. *aciphylla* Boiss.

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Abstract: Ethanolic (EEAA) and sesquiterpene lactone (SEAA) extracts of the aerial parts of *Anthemis aciphylla* var. *aciphylla* Boiss. (Asteraceae) were evaluated for anti-inflammatory activity on carrageenin-induced paw edema (acute model) and cotton pellet-induced granuloma (chronic model) in rats. In carrageenin-induced paw edema, EEAA and SEAA at intraperitoneal doses of 50, 100, and 200 mg/kg dose-dependently inhibited the paw edema. In cotton pellet-induced granuloma, the oral administration of EEAA and SEAA at 50, 100, and 200 mg/kg dosages was also found to significantly inhibit granuloma tissue formation.

Key words: *Anthemis aciphylla*, anti-inflammatory activity, carrageenin, paw edema, cotton pellet

Anthemis aciphylla var. *aciphylla* Boiss.'in antienflamatuvar aktivitesi

Özet: *Anthemis aciphylla* var. *aciphylla* Boiss. (Asteraceae) bitkisinin topraküstü kısımlarından hazırlanan etanol (EEAA) ve seskiterpen lakton (SEAA) ekstrelerinin antienflamatuvar aktivitesi, sıçanlarda karragen ile indüklenen pençe ödemi yöntemi (akut model) ve koton pelet ile indüklenen granülom teşekkülü (kronik model) yöntemiyle değerlendirilmiştir. Karragen ile indüklenen pençe ödemi yönteminde, EEAA ve SEAA ekstrelerinin, 50, 100, ve 200 mg/kg dozlarda intraperitonal uygulanmasıyla, doza bağlı pençe ödemini önlediği gözlendi. Koton pelet ile indüklenen granülom teşekkülü yönteminde EEAA ve SEAA ekstrelerinin ayni dozlarda oral olarak verildiğinde önemli derecede granülom doku oluşumunu önlediği saptandı.

Anahtar sözcükler: *Anthemis aciphylla*, anti-enflamatuvar aktivite, karagenin, pençe ödemi, koton pelet

Introduction

The genus *Anthemis* L. (Asteraceae) is represented in the Flora of Turkey by 81 taxa belonging to 51 species, 29 of which are endemic to Turkey (1). *Anthemis aciphylla* var. *aciphylla* Boiss. is one of these endemic species (1,2). *Anthemis* species, known locally as papatya and yavşan, are extensively used in Turkish folk medicine for the treatment of inflammatory disorders, menstrual pain, hepatic diseases, gastrointestinal disorders, hemorrhoid, stomachache, abdominal pain, and kidney stones (3-8). An infusion of the plant is taken for intestinal and abdominal colic and a decoction can be applied topically to sun-burned skin or skin affected by various types of inflammation (6,9).

Sesquiterpene lactones, flavonoids, and polyacetylenes are the 3 main classes of secondary metabolites of the genus (10-12). Although several

plants belonging to this genus have been shown to possess important anti-inflammatory (13), anti-*Helicobacter pylori* (10), antiprotozoal (14), and antimicrobial properties (15), there have been no reports on the biological activity of *A. aciphylla* var. *aciphylla* Boiss to our knowledge. The present study was therefore undertaken to evaluate the anti-inflammatory activity of the ethanolic and sesquiterpene lactone extracts of the aerial parts of *A. aciphylla* var. *aciphylla* using 2 experimental models: carrageenin-induced paw edema and cotton pellet-induced granuloma in rats.

Materials and methods

Plant material

In May 2007, the aerial parts of *Anthemis aciphylla* var. *aciphylla* (Asteraceae) were collected from İzmir-Bayındır (Alankıyı village) in western Anatolia. These samples were identified by Ö. Seçmen from the Department of Biology, Botany Section, of Ege University. A voucher specimen (No: 1365) was deposited in the Herbarium of the Faculty of Pharmacy at Ege University in İzmir.

Chemicals

The chemicals used in this study were carrageenin (Sigma), indomethacin (Deva), and thiopental sodium (Abbott).

Preparation of ethanolic extract of *Anthemis aciphylla* var. *aciphylla* (EEAA)

The air-dried and powdered samples of *Anthemis aciphylla* var. *aciphylla* (500 g) were twice extracted with ethanol (3000 mL, first for 5 h and then for 8 h) under stirring. The combined organic phases were filtered and distilled in vacuo (yield 11%).

Preparation of sesquiterpene lactone extract of *Anthemis aciphylla* var. *aciphylla* (SEAA)

The dried aerial parts of *Anthemis aciphylla* var. *aciphylla* (8.68 kg) were finely ground and extracted at room temperature with cyclohexane-Et_2O-MeOH (1:1:1). The extract was then washed with brine; the aqueous layer was subjected to extraction once again with EtOAc, and the organic layer was dried with Na_2SO_4 and concentrated under reduced pressure (yield 8%).

Phytochemical Analysis

Preliminary phytochemical screening was carried out on the EEAA and SEAA extracts using the standard screening method described by Trease and Evans (16).

Animals

Female Wistar rats (Laboratory Animal Science Department, Dokuz Eylül University, İzmir, Turkey) weighing 150-200 g were used. The animals were maintained under standard laboratory conditions of humidity, temperature (24 ± 2 °C), and light (12 h day:12 h night), and allowed free access to food and water ad libitum. All experimental designs and procedures received approval from the Local Animal Ethics Committee of Dokuz Eylül University.

Anti-inflammatory activity in vivo models

Carrageenin-induced paw edema in rats (acute model)

Anti-inflammatory activity was assessed by the method described by Winter et al (17).

The rats were divided into 5 groups of 6 animals each. Group I (control) was treated with a 1% aqueous solution of sodium carboxymethylcellulose (CMS) (5 mL/kg) as a vehicle. Groups II, III, and IV were treated with EEAA and SEAA extracts at dosages of 200, 100, and 50 mg/kg, respectively. Our final group, Group V, was treated with 4 mg/kg indomethacin as a reference. Edema was induced by a subplanter injection of 0.1 mL of 1% (w/v) carrageenin (Sigma) into the right hind paw of each rat. The volume of the injected paws was measured using a plethysmometer (Plethysmometer 7140, Ugo Basile) 1, 2, 3, 4, and 5 h after the induction of inflammation. The test groups received the extract (50-200 mg/kg), the reference group received indomethacin (4 mg/kg), and the control animals received the vehicle only. All the doses were given intraperitoneally. The EEAA and SEAA extracts (200, 100, or 50 mg/kg) were given intraperitoneally 1 h prior to the injection of carrageenin. The inhibitory activity was calculated according to the following formula (18):

$$\% \text{ Inhibition} = (Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}/(Ct - Co)\ \text{control} \times 100$$

where Ct = the paw circumference at time t, Co = the paw circumference before the carrageenin injection, and $Ct - Co$ = edema.

Cotton pellet-induced granuloma in rats (chronic model)

The rats were divided into 5 groups with 5 animals in each group. After being shaved, the animals were anaesthetized with an intraperitoneal injection of 25 mg/kg thiopental sodium. Through a single needle incision, sterile pre-weighed cotton pellets (50 mg) were implanted in the dorsal region of each rat. The extracts (200, 100, or 50 mg/kg), reference drug indomethacin (5 mg/kg), and control vehicle were administered orally to the respective group of animals for 7 consecutive days following the day of cotton-pellet implantation. On the eighth day, the animals were anaesthetized again and the cotton pellets were surgically removed and freed from extraneous tissues. The pellets were then incubated at 37 °C for 24 h and dried at 60 °C to constant weight. The increase in the dry weight of the pellets was taken as the measure of granuloma formation. The percentage of inhibition increase in the weight of the cotton pellet was calculated according to the following formula (19):

$$\% \text{ Inhibition} = Wc - Wd/Wc \times 100$$

where Wd = the difference in pellet weight of the drug treated group and Wc = the difference in pellet weight of the control group.

Data analysis

Values reported are expressed as mean ± S.E.M. The statistical comparison of data was made by means of one way ANOVA using a Dunnett's test, performed using Graph Pad InStat. For the purposes of the present study, $P < 0.001$ was regarded as significant.

Results and discussion

In the anti-inflammatory studies, the results show that pretreatments with the EEAA and SEAA extracts (200, 100, or 50 mg/kg) and indomethacin significantly ($P < 0.001$) inhibited both carrageenin-induced paw edema (acute model) (Tables 1 and 2) and cotton pellet-induced granuloma tissue formation (chronic model) (Tables 3 and 4).

Carrageenin-induced rat paw edema is widely used as an experimental animal model for the evaluation of anti-inflammatory potential in natural products (20). The present study establishes the anti-inflammatory activity of the EEAA and SEAA extracts in the models used. The pretreatment of animals with EEAA and SEAA extracts resulted in a significant and dose-related inhibition of carrageenin-evoked hind paw edema. The reference drug indomethacin also showed significant edema inhibition in all the phases. Among the EEAA and SEAA extracts, the SEAA extract (200 mg/kg) exhibited significant anti-inflammatory activity (75%, 71.42%, 69.81%, 77.04%) at 1 h, 2 h, 3 h, 4 h post-procedure, which was comparable to that of indomethacin (85.71%, 78.57%, 79.24%, 86.88%) (Table 2).

Table 1. The effects of the ethanolic extract of *A. aciphylla* var. *aciphylla* (EEAA) on carrageenin-induced paw edema in rats.

Treatment and dose (mg/kg, IP)	**Right paw volume (mL × 100)**					
	0 h	1 h	2 h	3 h	4 h	5 h
Control	0.90 ± 0.0081	1.15 ± 0.0088	1.21 ± 0.0137	1.25 ± 0.0157	1.28 ± 0.0163	1.26 ± 0.0160
EEAA (50 mg/kg)	0.90 ± 0,0105	1.08 ± 0.0094* **(28)**	1.11 ± 0.0091* **(32.25)**	1.15 ± 0.0113* **(28.57)**	1.17 ± 0.0094* **(28.94)**	1.15 ± 0.0102* **(30.55)**
EEAA (100 mg/kg)	0.90 ± 0,0088	1.05 ± 0.0196* **(40)**	1.08 ± 0.0070* **(41.93)**	1.10 ± 0.0083* **(42.85)**	1.12 ± 0.0076* **(42.10)**	1.10 ± 0.0067* **(44.44)**
EEAA (200 mg/kg)	0.90 ± 0.0068	0.98 ± 0.0061* **(68)**	1.01 ± 0.0070* **(64.51)**	1.03 ± 0.0055* **(62.85)**	1.06 ± 0.0080* **(57.89)**	1.05 ± 0.0076* **(58.33)**
Indomethacin (4 mg/kg)	0.90 ± 0.0070	0.93 ± 0.0060* **(88)**	0.97 ± 0.0066* **(77.41)**	1.00 ± 0.0172* **(71.42)**	0.98 ± 0.0057* **(78.94)**	0.97 ± 0.0071* **(80.55)**

* Significantly different from control, $P < 0.001$ (ANOVA followed by Dunnett's test). Values in parentheses indicate the percentage inhibition rate.

Table 2. The effects of the sesquiterpene lactone extract of *A. aciphylla* var. *aciphylla* (SEAA) on carrageenin-induced paw edema in rats.

Treatment and dose (mg/kg, IP)	Right paw volume (mL × 100)					
	0 h	1 h	2 h	3 h	4 h	5 h
Control	0.88 ± 0.0091	1.16 ± 0.0061	1.30 ± 0.0087	1.41 ± 0.0116	1.49 ± 0.0125	1.49 ± 0.0101
SE AA (50 mg/kg)	0.89 ± 0.0088	1.05 ± 0.0101* **(42.85)**	1.13 ± 0.0080* **(42.85)**	1.19 ± 0.0088* **(43.39)**	1.23 ± 0.0094* **(44.26)**	1.22 ± 0.0080* **(45.90)**
SEAA (100 mg/kg)	0.89 ± 0.0076	1.00 ± 0.0080* **(60.71)**	1.06 ± 0.0090* **(59.52)**	1.11 ± 0.0084* **(58.49)**	1.11 ± 0.0085* **(63.93)**	1.09 ± 0.0095* **(67.21)**
SEAA (200 mg/kg)	0.89 ± 0.0066	0.96 ± 0.0098* **(75)**	1.01 ± 0.0095* **(71.42)**	1.05 ± 0.0103* **(69.81)**	1.03 ± 0.0084* **(77.04)**	1.02 ± 0.0081* **(78.68)**
Indomethacin (4 mg/kg)	0.89 ± 0.0063	0.93 ± 0.0066* **(85.71)**	0.98 ± 0.0047* **(78.57)**	1.00 ± 0.0047* **(79.24)**	0.97 ± 0.0060* **(86.88)**	0.95 ± 0.0061* **(90.16)**

* Significantly different from control, P < 0.001 (ANOVA followed by Dunnett's test). Values in parentheses indicate the percentage inhibition rate.

Table 3. The effects of the ethanolic extract of *A. aciphylla* var. *aciphylla* (EEAA) on the weight of granuloma formation in rats.

Treatment and dose (mg/kg, PO)	Weight of granuloma (mg)	Percentage inhibition
Control	157.72 ± 0.2297	**0**
EEAA (50 mg/kg)	98.81 ± 0.2369*	**37.35**
EEAA (100 mg/kg)	86.92 ± 0.2845*	**44.88**
EEAA (200 mg/kg)	71.18 ± 0.1928*	**54.86**
Indomethacin (5 mg/kg)	60.44 ± 0.3219*	**61.67**

*Significantly different from control, P < 0.001 (ANOVA followed by Dunnett's test).

Table 4. The effects of the sesquiterpene lactone extract of *A. aciphylla* var. *aciphylla* (SEAA) on the weight of granuloma formation in rats.

Treatment and dose (mg/kg, PO)	Weight of granuloma (mg)	Percentage inhibition
Control	135.23 ± 0.4122	**0**
SEAA (50 mg/kg)	82.19 ± 0.5227*	**39.22**
SEAA(100 mg/kg)	43.01 ± 0.4528*	**68.19**
SEAA (200 mg/kg)	31.86 ± 0.4103*	**76.44**
Indomethacin (5 mg/kg)	25.53 ± 0.3535*	**81.12**

*Significantly different from control, P < 0.001 (ANOVA followed by Dunnett's test).

Similarly, in the cotton pellet granuloma model of inflammation, the EEAA and SEAA extracts (200, 100, and 50 mg/kg) inhibited the granuloma formation significantly ($P < 0.001$), indicating that the extracts can also inhibit the chronic inflammatory process typified by the cotton pellet method (21). Among the EEAA and SEAA extracts, the SEAA extract (200 mg/kg) exhibited significant anti-inflammatory activity (76.44%) comparable to that of indomethacin (81.12%) (Table 4).

A phytochemical screening of the EEAA extract indicated the presence of the following secondary metabolites: flavonoids and saponins in high concentrations, as well as tannins in moderate concentration. The EEAA extract was, however, devoid of alkaloids and sesquiterpene lactones. Secondary metabolites in the SEAA extract included sesquiterpene lactones, flavonoids, and saponins in high concentrations and tannins in a low concentration. As with the EEAA extract, the SEAA extract was devoid of alkaloids.

In the present study, the anti-inflammatory activity of EEAA and SEAA extracts were evaluated in both acute and chronic in vivo models.

The accumulation of edema fluid as a function of time after the subplantar injection of the irritant (carrageenin) in rats is biphasic (22). Histamine and serotonin are usually responsible for eliciting the immediate response of inflammation in rats (first phase), whereas kinins and prostaglandins (PG) mediate the more prolonged delayed-onset responses (second phase) (23). From the results obtained, it can be inferred that the inhibitory effects of the EEAA and SEAA extracts on carrageenin-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase, which in turn leads to the inhibition of PG synthesis. The decrease in edema inhibition at 5.5 h may be a result of the increased generation of leukotrienes at that stage caused by the inhibition of PG synthesis in the second phase, since inhibition of PG synthesis diverts the reaction toward an increase in leukotriene synthesis (24).

The inflammatory response to a subcutaneously implanted cotton pellet in rats has been described in 3 phases: (1) a transudative phase, defined as the increase in wet weight of the pellet occurring during the first 3 h; (2) an exudative phase, occurring between 3 and 72 h after the pellet is implanted; and (3) a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation (25). The results indicate that both the EEAA and SEAA extracts exhibited significant ($P < 0.001$) antitransudative and antiproliferative effects by inhibiting both the wet and dry weights of cotton pellets when compared with the control. This finding reflects its efficacy in inhibiting the increase in the number of fibroblasts and the synthesis of collagen and mucopolysaccharide, which are natural proliferative events during granulation tissue formation (26). The effect of the extracts on the inflammation process induced by stimulus injection indicates that these agents act by affecting a time-delayed system in a similar fashion to glucocorticoids.

Preliminary phytochemical analysis of the EEAA and SEAA extracts shows the presence of flavonoids, saponins, and tannins. Furthermore, sesquiterpene lactones were also detected in the SEAA extract. These bioactive agents (flavonoids, saponins, and sesquiterpene lactones) have the ability to inhibit pain perception and can also serve as anti-inflammatory agents (27,28). The EEAA and SEAA extracts exhibited significant anti-inflammatory potential at the dose levels examined in this study. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

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