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EVALUATION OF ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF HELICHRYSUM GAERTNER SPECIES (ASTERACEAE)

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Abstract

The aqueous extracts obtained from three Helichrysum species (Asteraceae) growing in Turkey, H. arenarium (L.) Moench. subsp. aucheri (Boiss.) Davis & Kupicha, H. noeanum Boiss. and H. pilicatum DC. subsp. pilicatum, were evaluated for their in vivo anti-inflammatory activity using carrageenan-induced hind paw edema and antinociceptive activity using p-benzoquinone-induced abdominal constriction test. Aqueous extracts from H. arenarium and H. pilicatum subsp. pilicatum showed 37.0 %, 30.4 % inhibition at 100 mg/kg doses and 31.2%, 28.3% at 200 mg/kg doses in p-benzoquinone-induced abdominal constriction test, respectively, without inducing any gastric damage. The aqueous extracts of all the plants didn't show any anti-inflammatory activity in carrageenan-induced hind paw edema model at 50, 100 and 200 mg/kg doses. During the acute toxicity evaluation, neither death nor gastric bleeding was observed for any of the plant extracts.

Keywords: *Anti-inflammatory activity, Antinociceptive activity, H. arenarium (L.) Moench. subsp. aucheri (Boiss.) Davis & Kupicha, H. noeanum Boiss., and H. pilicatum DC. subsp. pilicatum, Asteraceae*

Helichrysum Gaertner (Asteraceae) Türlerinin Anti enflamatuvar ve Antinosiseptif Aktivitelerinin Değerlendirilmesi

Türkiye'de yetişen üç Helichrysum türünden (H. arenarium (L.) Moench. subsp. aucheri (Boiss.) Davis & Kupicha, H. noeanum Boiss. and H. pilicatum DC. subsp. pilicatum) hazırlanan sulu ekstrelerin in vivo olarak anti-enflamatuar aktivitesi karragenin nedenli arka ayak ödemi testi ve antinosiseptif aktivitesi p-benzokinon nedenli kıvranma testi uygulanarak değerlendirilmiştir. H. arenarium ve H. pilicatum subsp. pilicatum sulu ekstreleri p-benzokinon nedenli kıvranma testinde 100 mg/kg dozda sırasıyla % 37.0 ve % 30.4 oranında ve 200 mg/kg dozda % 31.2 ve % 28.3oranında herhangi bir gastric lezyon oluşturmaksızın inhibisyon göstermiştir. Karrageni- nedenli arka ayak ödemi testinde 50, 100 ve 200 mg/kg dozlarda ekstrelerin herhangi bir antienflamatuvar aktivitesi gözlenmemiştir. Akut toksisitelerine bakıldığında, ekstrelerin hiçbirisi herhangi bir gastric lezyon ya da ölüme sebep olmamıştır.

Anahtar Kelimeler: *Anti-enflamatuvar, Antinosiseptif, H. arenarium (L.) Moench. subsp. aucheri (Boiss.) Davis & Kupicha, H. noeanum Boiss., and H. pilicatum DC. subsp. pilicatum, Asteraceae*

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INTRODUCTION

The genus *Helichrysum* Gaertner (Asteraceae), represented by approximately 1000 species in the world (1), consists of taxonomically complex group of plants used in folk medicine (2). In Flora of Turkey, the genus is composed of 18 taxa, of which 9 are endemic (3).

Some members of this genus are used in traditional medicine and known for their important activities.

In folk medicine, *Helichrysum* species have been used for gall bladder disorders as medicinal tea, because of their bile regulatory and diuretic effects (4). Some of them are used for anti-inflammatory and anti-allergic properties (1, 5). Moreover, this genus is traditionally used in the treatment of wounds, infections and respiratory conditions (6). In Turkey, several *Helichrysum* species are used in folk medicine as diuretic and cholagogue, and for removing the kidney stones. The medical properties of this genus are attributed principally to the presence of flavonoids (4, 7).

H. noeanum is an endemic species growing in Turkey, especially central Anatolia and adjacent east Anatolia. *Helichrysum arenarium* subsp. *aucherii* is also an endemic species and grows mainly inner Anatolia and adjacent north Anatolia. *H. pilicatum* DC. subsp. *pilicatum* is a wide spread species in Anatolia, except in the western part (3). There is no ethnobotanical report and phytochemical studies on these species. However, a few biological activity researches are performed previously. The antioxidant activity was examined on the methanol extract of these species in the present study (5). *H. pilicatum* showed antimicrobial activity against *S. aureus* strains (8).

A number of different components have been found such as flavonoids, coumarins, phtalides, α-pyron derivatives, terpenoids, essential oils, volatile and fatty acids (2).

To our knowledge, the constituents of the species used in this study, have never been reported before. However, only we know that flavonoids, phenolic constituents, phthalides and coumarins were isolated from H. arenarium (L.) Moench. (2, 9-11).

The aim of this study was to evaluate *in vivo* anti-inflammatory and antinociceptive activities, respectively; using carrageenan-induced hind paw edema and *p*-benzoquinone-induced abdominal constriction test on three *Helichrysum* species; *H. arenarium* (L.) Moench. subsp. *aucheri* (Boiss.) Davis & Kupicha, *H. noeanum* Boiss. and *H. pilicatum* DC. subsp. *pilicatum*, two of them are endemic for Turkey.

EXPERIMENTAL

Plant materials

The species were shown in (Table 1) with the collection sites. All of the species were identified by Hayri Duman from Department of Biology, Faculty of Science & Art, Gazi University. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy of Ankara University (AEF).

Table 1. The *Helichrysum* species , collection sites, herbarium numbers and percentage percentage yields of H_2O extracts

Species	**Collection site**	**AEF No:**	**H_2O extract (w/w, %)**	**Plant part**
H. arenarium (L.) Moench. subsp. *aucheri* (Boiss.) Davis & Kupicha	Sivas-Topcuyenikoy at an altitude of 1400 m in June, 2005	23690	3.0024	AE
H. noeanum Boiss.	Sivas-Topcuyenikoy at an altitude of 1400 m in June, 2005	23689	2.9280	AE
H. pilicatum DC. subsp. *pilicatum*	Kars-Merkez at an altitude of 1860 m in June, 2005.	23691	1.7018	AE

AE: Aerial part (in flowering season)

Preparation of extracts

Aqueous extracts (H2O) of dried and powdered aerial parts of the plants (100 g) were prepared under reflux. The extracts were filtered and evaporated up to dryness over the water bath (40 ^{0}C) and under air-flow. Amounts of the aqueous extracts obtained from the plants were given in (Table 1).

Animal

Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for two days for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum.*

Preparation of test samples for bioassay

All the plant materials were administered in 50, 100 and 200 mg/kg dose after suspending in 0.5 % sodium carboxymethyl cellulose (CMC) suspension in distilled water. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 mg/kg) in 0.5 % CMC was used as reference drug.

Antinociceptive activity

p-Benzoquinone-induced abdominal constriction test (12) was performed on mice for determination of antinociceptive activity. According to the method, 60 min after the oral administration of test samples, the mice were intraperitonally injected with 0.1 ml/10 g body weight of 2.5 % (w/v) p-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5^{th} min after the PBQ injection. The data represent average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Aspirin (ASA) at 100 mg/kg dose was used as the reference drug in this test.

Anti-inflammatory activity

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity (13). The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. 60 min after the oral administration of test sample or dosing vehicle, each mouse was injected with freshly prepared (0.5 mg/25 µl) suspension of carrageenan (Sigma, St.Louis, Missouri, USA) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µl saline solutions were injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 24 h and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

Gastric-ulcerogenic effect

After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings.

Statistical analysis

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p<0.05$ was considered to be significant [* $p<0.05$; ** $p<0.01$; *** $p<0.001$].

RESULTS AND DISCUSSION

The aqueous extracts of these plants were investigated for *in vivo* anti-inflammatory activity using carrageenan-induced hind paw edema model and for antinociceptive activity using *p*-benzoquinone induced abdominal contractions test in mice at a dose of 50, 100 and 200 mg/kg body weight. The experimental results were listed in (Table 2) and (Table 3).

Table 2. Effects of the aqueous extracts against carrageenan-induced paw edema in mice

Material	Dose mg/kg	Swelling thickness (x10^{-2}mm) ± SEM (% inhibition)			
		90 min	180 min	270 min	360 min
Control		49.7 ± 3.3	55.3 ± 3.9	59.7 ± 4.8	65.9 ± 4.1
H. arenarium subsp. *aucheri*	50	51.2 ± 4.1	57.8 ± 3.8	61.4 ± 3.1	69.7 ± 3.4
	100	45.4 ± 2.8 (8.7)	51.1 ± 2.6 (7.6)	52.6 ± 3.0 (11.9)	59.7 ± 2.1 (9.4)
	200	48.6 ± 2.0 (2.2)	52.8 ± 2.4 (4.5)	54.2 ± 2.7 (9.2)	60.5 ± 3.6 (8.2)
H. noeanum	50	55.1 ± 2.6	59.8 ± 3.0	62.7 ± 2.9	68.4 ± 3.1
	100	50.2 ± 3.4	59.5 ± 3.8	63.4 ± 3.1	69.7 ± 2.8
	200	53.1 ± 2.2	56.8 ± 2.4	61.1 ± 2.7	66.9 ± 2.3
H. pilicatum subsp. *pilicatum*	50	50.1 ± 3.3	54.8 ± 2.9	59.4 ± 3.0	67.1 ± 2.8
	100	47.2 ± 3.9 (5.0)	53.1 ± 3.8 (3.9)	56.5 ± 3.2 (5.4)	61.5 ± 3.7 (6.7)
	200	46.5 ± 3.1 (6.4)	52.1 ± 3.0 (5.8)	55.2 ± 2.8 (7.5)	60.1 ± 2.6 (8.8)
Indomethacin	10	31.5 ± 2.3 (36.6)**	37.6 ± 2.7 (32.0)**	38.2 ± 1.8 (36.0)***	40.6 ± 2.6 (38.4)***

*:$p<0.05$, **:$p<0.01$, ***:$p<0.001$

SEM: standard error mean

As shown in (Table 2), anti-inflammatory activity in carrageenan-induced hind paw edema was not observed on the aqueous extracts of none of the plants at 50, 100 and 200 mg/kg doses.

Table 3. Effects of the aqueous extracts against p-benzoquinone-induced writhings in mice

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		51.3 ± 4.4		0/6
H. arenarium subsp. *aucheri*	50	43.7 ± 3.4	14.8	0/6
	100	32.3 ± 3.1	**37. 0****	0/6
	200	35.3 ± 2.9	**31.2****	0/6
H. noeanum	50	55.3 ± 4.6	-	0/6
	100	45.3 ± 3.3	11.7	0/6
	200	43.2 ± 3.7	15.8	0/6
H. pilicatum subsp. *pilicatum*	50	41.2 ± 2.7	19.7	0/6
	100	35.7 ± 2.9	**30.4****	0/6
	200	36.8 ± 2.4	**28.3****	0/6
ASA	100	24.3 ± 2.2	**52.6*****	5/6

*:p<0.05, **:p<0.01, ***:p<0.001

SEM: standard error of the mean

The aqueous extracts of *H. arenarium* and *H. pilicatum* subsp. *pilicatum* showed 37.0 %, 30.4 % inhibition at 100 mg/kg and 31.2 %, 28.3 % inhibition at 200 mg/kg respectively, in *p*-benzoquinone-induced abdominal constriction test without inducing any gastric damage (Table 3). However, there was no activity observed at 50 mg/kg . During the acute toxicity evaluation, neither death nor gastric bleeding was observed for any of the plant extracts. While acetylsalicylic acid (ASA), the reference compound, showed 52.6 % inhibition at the dose of 100 mg/kg, moreover 5 out of 6 mice suffered from severe gastric damage.

The acute toxicity assessment has revealed that all extracts were safe in the administered doses.

Helichrysum species has a rich source of many compounds, including important bioactive ones. Even though large species of this genus have been extensively investigated, biological activity and phytochemical studies for many species have not been reported yet (6). The medicinal properties of this genus are mainly due to the presence of their flavonoids, but they may be also supported by other organic and inorganic component such as coumarins, phenolic constituents and some elements. In fact, flavonoids are one of the most numerous and widespread groups of phenolics in plants, exhibiting a range of biological and pharmacological effects (9, 14)

The aqueous extracts of *H. arenarium* subsp. *aucheri* and *H. pilicatum* subsp. *pilicatum* exhibit notable antinociceptive activity. The activity may be attributed to mainly flavonoids of this plant or a different class of components. It need some future research to be found active principles of this plants. However, further studies are necessary to assess the potential clinical use of *H. arenarium* subsp. *aucheri* and *H. pilicatum* subsp. *pilicatum*, or its extract or active principles, as analgesics.

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