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Antinociceptive activity of some *Scorzonera* L. species

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Aim: *Scorzonera* species are mainly used to relieve pain in Turkish traditional medicine. The roots of *Scorzonera latifolia* (Fisch. & Mey) DC., *S. tomentosa* L., *S. suberosa* C.Koch subsp. *suberosa*, and *S. mollis* M.Bieb. subsp. *szowitzii* collected from different regions of Anatolia, and *yakı sakızı*, which is prepared by drying the latex obtained from the roots of *Scorzonera latifolia*, were evaluated for their antinociceptive activities.

Materials and methods: For antinociceptive evaluation, *Scorzonera latifolia* (Fisch. & Mey) DC., *S. tomentosa* L., *S. suberosa* C.Koch subsp. *suberosa*, *S. mollis* M.Bieb. subsp. *szowitzii*, and *yakı sakızı* were tested on mice, and acetic acid-induced writhing and tail-flick tests were used.

Results: Extracts prepared from the roots of *Scorzonera latifolia*, *S. tomentosa*, *S. suberosa* subsp. *suberosa*, *S. mollis* subsp. *szowitzii*, and *yakı sakızı* showed significant inhibitory effects in an acetic acid-induced abdominal stretching test at a dose of 100 mg/kg. The extracts of *S. latifolia* and *S. tomentosa* produced higher inhibition in abdominal constriction numbers when compared to other extracts. However, a remarkable increase in tail-flick latency time was observed only with *S. latifolia* treatment at doses of 25 and 50 mg/kg at all of the time points and at a dose of 100 mg/kg dose at 150 min. *S. mollis*, *S. tomentosa*, and *yakı sakızı* exhibited only moderate activity, while *S. suberosa* did not show any remarkable activity in the tail-flick test.

Conclusion: The current study confirms the analgesic activity of *S. latifolia* in Turkish folk medicine. Furthermore, experimental results revealed that methanol-water extracts from the roots of *Scorzonera latifolia* had better antinociceptive activity compared to the other extracts and control groups.

Key words: Antinociceptive activity, Asteraceae, *Scorzonera*, tail-flick test, writhing test

Introduction

The genus *Scorzonera* L. (Asteraceae) is a large genus with more than 175 species. It is widely spread in arid regions of Eurasia and Africa (1,2). In Europe, 28 members of *Scorzonera* are distributed from northern Russia to Spain and Crete (2). Forty-nine species of *Scorzonera* have been found in different regions of Turkey with the addition of new species (3,4).

Scorzonera species are mainly used as a vegetable in Europe as well as in Turkey. *Scorzonera hispanica* L. is the most popular species that grows naturally and widely in Europe, and it has been cultivated since the 17th century as a food. The young leaves of the plant are used in salads and the roots are consumed as a cooked vegetable in European cuisine (5). *Scorzonera* species are used against pulmonary diseases and colds, for the treatment of wounds and

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gastrointestinal disorders, and for their stomachic, diuretic, galactogogue, antipyretic, and appetizing effects in European traditional medicine. They are also used for the treatment of diarrhea, lung edema, parasitic diseases, and fever caused by bacterial and viral infections in Chinese and Mongolian traditional medicine (6-8).

In Turkey, some species of the *Scorzonera* have also been used as vegetables, such as *S. mollis* M.Bieb. ("goftigoda"), *S. suberosa* C.Koch ("yabani havoc"), *S. cana* ("karakök," "tekesakalı"), and *S. latifolia* (Fisch. and Mey) DC. ("geniş yapraklı karakök" or "mesdek"). The roots and green buds of these plants are consumed fresh or after being cooked. Moreover, the plants of the genus *Scorzonera* have importance in Turkish folk medicine due to their treatment of a variety of illnesses including arteriosclerosis, kidney diseases, hypertension, diabetes mellitus, and rheumatism, as well as for pain relief and wound healing (9). *S. latifolia* is the most recognized species that has medicinal usage in Turkish folk medicine. A mastic called "*yakı sakızı*" is obtained from the root latex of *S. latifolia* and used mainly in the treatment of pain, as well as infertility, and for its anthelmintic activity (9,10). *S. tomentosa*, another species from the genus *Scorzonera*, is also used in Turkish folk medicine for its wound healing activity (11).

Previously, a number of compounds such as dihydroisocoumarins (2), bibenzyl derivatives (8,12,13), flavonoids (14,15), lignans (8,16,17), phenolics (6,18), sesquiterpenes (7,12), and triterpenes (19) have been isolated from the *Scorzonera* species. Earlier investigations on the biological activity of *Scorzonera* species revealed that tyrolobibenzyls, which were isolated from *S. humilis*, showed no cytotoxic activity against P-388 cells and neither antibacterial activity against *Bacillus subtilis* nor antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (12,13). Moreover, some phenolic compounds were isolated from medicinal Mongolian plants *S. divaricata*, *S. pseudodivaricata*, and *S. radiata* which were found to be responsible for antioxidant activity (6,20). Khobrakova et al. (17) reported that the syringaresinol glucoside I isolated from *S. hispanica* possessed pronounced immunomodulating properties with respect to both cellular and humoral immunity response in the experimental model of azathioprine-induced immunosuppression. Additionally, an acetylated derivative of biguaiascorzolides A, isolated from *S. austriaca*, was found to be moderately active against the K562/ADM cell line (IC_{50}: 39.8 μm) and inactive against the MGC-803 cell line in a recent study (7).

In the present study, we aimed to evaluate the antinociceptive activity of *S. latifolia* (SL), *S. tomentosa* (ST), *S. suberosa* (SS), *S. mollis* (SM), and a mastic of *S. latifolia* [*yakı sakızı* (YS)] by using acetic acid-induced writhing and tail-flick tests to justify their traditional usages in Turkish folk medicine.

Materials and methods

Plant material

Scorzonera species were collected from different parts of Turkey and are listed in Table 1. Taxonomic identification of the plants was confirmed by H. Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University, and A.M. Gençler Özkan, Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey. Voucher specimens were kept in the herbarium of Ankara University's Faculty of Pharmacy (AEF).

Extraction of plant materials

Twenty grams each of the air-dried and powdered roots of the plant were extracted with 20% aqueous methanol (100 mL), separately, with continuous stirring at room temperature for 3 h. Each extract was filtered and concentrated to dryness under reduced pressure and low temperature (40-50 °C) on a rotary evaporator to give crude extracts. The yields of the plant materials were as follows (w/w): SL 23.7%, ST 16.2%, SM 20.4%, and SS 17.4%.

Drugs and chemicals

For the assessment of antinociceptive activity, test samples were dissolved in normal saline solution (0.9% NaCl, w/v). The acetyl salicylic acid was obtained from Bayer (İstanbul, Turkey).

Table 1. Locality of the plant samples.

Plant species	AEF No.	Locality	Date
S. latifolia (Fisch. & Mey) DC.	AEF 23830	Kars, Arpaçay	August 2005
S. mollis M.Bieb. subsp. *szowitzii*	AEF 23844	Ankara, Kızılcahamam	May 2006
S. suberosa C.Koch subsp. *suberosa*	AEF 23843	Kayseri, Pınarbaşı	May 2006
S. tomentosa L.	AEF 23841	Yozgat, Akdağmadeni	June 2005
Yakı sakızı		Van, local market	June 2006

Animals

Male BALB/C mice (20-24 g), 14 to 16 weeks old, were purchased from the Animal Housing Facility of Yüzüncü Yıl University. Divided into 21 groups were 126 mice, with each group consisting of 6 mice. All of the animals were housed in standard cages (48 × 35 × 22 cm) at room temperature (22 ± 2 °C) with artificial light from 7.00 am to 7.00 pm hours, and they were provided with pelleted food (Van Animal Food Factory, Van, Turkey) and water *ad libitum*. The protocol for the study was approved by the Ethical Committee of Yüzüncü Yıl University's Faculty of Medicine Animal Breeding and Research.

Acetic acid-induced writhing test

The method of Koster et al. (21) was used with slight modifications. The animals were kept in a temperature-controlled environment (22 ± 2 °C) with a 12 hlight-dark cycle. Food and water were freely available. Abdominal writhing was introduced by intraperitoneal (ip) injection of acetic acid (6%, 60 mg/kg). The animals were pretreated with the extract through ip administration 5 min before the acetic acid injection, and the test began 5 min after the acetic acid administration. For the acetic acid-induced writhing test, 66 animals were used, and the following treatment regimen was conducted. Control animals (Group I) received the same volume of isotonic saline solution (ISS) (0.2 mL). Acetyl salicylic acid at a dose of 300 mg/kg, which is the preferential dose in similar studies, was given orally to Group II and used as a reference drug for comparison (22). Groups III, IV, and V received SL root extract and groups VI, VII, and VIII received ST root extract at 25, 50, and 100 mg/kg intraperitoneally, respectively. SS and SM root extracts were given to Groups IX and X at 100 mg/kg intraperitoneally, respectively. Plant samples were given to the animals, after being dissolved in ISS and filtered through sterile Whatman 0.45 µm CA w/ GMF syringe filters, by ip injection. After the drug application, pairs of mice were placed in a glass cage measuring 44 × 44 × 25 cm. The number of stretches occurring for 15 min immediately after the acetic acid injection was recorded. Each group consisted of 6 mice. The animals were sacrificed immediately after each 15-min experiment. The results were evaluated by calculating the mean number of stretches per group and were represented as the percentage of stretching-movement inhibition compared to the control group.

Tail-flick test

Antinociceptive response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Letica, Barcelona, Spain) using a method initially described by D'Amour and Smith (23).

The animals were gently immobilized using a glove, and radiant heat was focused on a blackened spot 1-2 cm from the tip of the tail. Beam intensity was adjusted to give a tail-flick latency of 5-8 s in the control animals. Measuring was terminated if the latency exceeded the end of time (15 s) to avoid tissue damage. In all of the experiments, the mice were tested twice, 30 min before drug administration in the baseline latency and 30, 90, and 150 min after drug administration. For the tail-flick test, 72 animals were used. Aspirin (300 mg/kg orally) (Group I) and morphine hydrochloride (10 mg/kg subcutaneously) (Group II) were used as reference standards (24,25). Only ip ISS (0.2 mL) was given to the control group (Group III). Groups IV, V, and VI received ip SL root extract and Groups VII, VIII, and IX received i.p. ST

root extract at 25, 50, and 100 mg/kg, respectively. SS and SM root extract were given to Groups X and XI at 100 mg/kg, respectively, intraperitoneally. Plant samples were given to the animals, after being dissolved in ISS and filtered through sterile Whatman 0.45 µm CA w/GMF syringe filters, by i.p. injection.

Statistical analysis

Results are reported as mean ± standard error of the mean (SEM). The total variation was analyzed by performing one-way ANOVA. Tukey's honestly significant difference test was used for determining significance. $P < 0.05$ was considered significant.

Results

Acetic acid-induced writhing test

As reported in Table 2, all of the tested extracts significantly inhibited acetic acid-induced abdominal constrictions in mice. ST, SL, and SM extracts exhibited remarkable antinociceptive activities in the acetic acid-induced writhing test at 100 mg/kg with values of 96.61%, 88.70%, and 87.50%, respectively, compared to the control, while acetyl salicylic acid (300 mg/kg) displayed only abdominal constriction inhibition with a value of 44.49%. A dose-dependent inhibitory activity was observed on writhing response for the SL and ST extracts. Moreover, the SS and YS extracts exhibited notable antinociceptive activity (76.28% and 70.63%, respectively) in this model when compared to the control animals.

Tail-flick test

The effects of the tested extracts on tail-flick response in mice are presented in Table 3. The antinociceptive activity of acetyl salicylic acid started at 30. min; however, the highest activity was observed at 150. min. On the other hand, the morphine, as a reference drug, showed remarkable antinociceptive activity at 30. and 90. min, but only slight activity was retained at 150. min. The 100 mg/kg dose of SL extract displayed significant activity in the tail-flick test only at 150. min. The YS extract also showed similar activity to the SL extract. Administration of 25 and 50 mg/kg doses of SL resulted in a remarkable increase in tail-flick response latency time at all of the time points as compared to the control group. Moreover, a relatively significant alteration was observed in the reaction

Table 2. Results of *Scorzonera* extracts on the acetic acid-induced writhing test in mice.

Material		Dose (mg/kg)	Abdominal stretching (mean ± SEM)	Inhibition of stretching (%)
Control		0.5 mL	17.71 ± 1.41	-
ASA		300	[a]9.83 ± 0.60	44.49
S. latifolia	SL-I	25	[a]4.17 ± 0.80	76.45
	SL-II	50	[a]4.00 ± 1.26	77.41
	SL-III	100	[ab]2.00 ± 0.78	88.70
S. mollis subsp. *szowitzii*	(SM)	100	[ab]2.20 ± 0.86	87.57
S. suberosa subsp. *suberosa*	(SS)	100	[a]4.20 ± 1.99	76.28
S. tomentosa	ST-I	25	[a]9.00 ± 2.62	49.18
	ST-II	50	[a]6.17 ± 2.24	65.16
	ST-III	100	[ab]0.60 ± 0.40	96.61
Yakı sakızı	(YK)	100	[a]5.20 ± 2.08	70.63
F-value			10.662	
P-value			0.000	

Values of abdominal stretching are mean ± SEM, n = 6; $P < 0.05$ is significant.
Post hoc least significant difference test .
[a]$P < 0.05$ in comparison with control group, [b]$P < 0.05$ in comparison with acetyl salicylic acid group.

Table 3. Results of *Scorzonera* extracts, acetyl salicylic acid, morphine, and control treatments on the tail-flick test.

Groups	0 min	0-30 min	0-90 min	0-150 min
Control	6.90 ± 0.29	7.03 ± 0.25	7.08 ± 0.28	6.90 ± 0.20
SL-I	7.00 ± 0.30	*10.57 ± 0.64	*10.58 ± 0.71	*10.78 ± 0.71
SL-II	7.70 ± 0.38	*11.72 ± 1.18	*12.27 ± 0.56	*12.14 ± 0.52
SL-III	8.00 ± 0.18	8.44 ± 0.43	7.23 ± 0.32	*9.20 ± 0.20
SM	7.00 ± 0.14	6.99 ± 0.68	8.01 ± 0.33	8.64 ± 0.26
SS	6.70 ± 0.60	8.00 ± 0.63	*8.01 ± 0.33	*8.64 ± 0.26
ST-I	6.60 ± 0.42	6.46 ± 0.34	6.85 ± 0.55	7.55 ± 0.46
ST-II	6.40 ± 0.40	6.36 ± 0.21	7.63 ± 0.40	7.74 ± 0.27
ST-III	6.10 ± 0.20	*7.04 ± 0.14	*7.20 ± 0.35	*7.36 ± 0.32
YK	7.50 ± 0.74	9.52 ± 0.34	9.50 ± 0.61	*10.65 ± 1.04
ASA	7.20 ± 0.28	*8.75 ± 0.47	*8.72 ± 0.42	*9.43 ± 0.44
Morphine	7.90 ± 0.46	*11.29 ± 0.67	*12.96 ± 0.49	7.82 ± 0.71

Values are expressed as mean ± SEM, n = 6.
*$P < 0.05$ is significant.

times with treatment by the SM and ST extracts at 100 mg/kg at 90. min, 150. min, and all time points, respectively.

Discussion

In the present study, the antinociceptive activity of four *Scorzonera* species and the mastic of SL were investigated to clarify their traditional usage in pain treatment using acetic acid-induced writhing and tail-flick tests. All of the tested extracts inhibited abdominal stretching significantly in the acetic acid-induced writhing test but no notable activity was found in the tail-flick test, except with the SL extract when the same dose was utilized. This may be explained by the fact that the acetic acid-induced writhing test is considered an inflammatory visceral pain model, enabling the detection of both strong and weak analgesic compounds (26). This method is reported to be useful for investigating the analgesic or antiinflammatory activities of new substances with good sensitivity and poor specificity (27). Administration of acetic acid by i.p. injection is considered to produce peritoneal inflammation, which causes the contraction of the abdominal muscles as a response. Arachidonic acid is produced by cyclooxygenase and prostaglandin biosynthesis, playing a role in the inflammation and the activity mechanism of the compound, which was found to be active in the acetic acid-induced writhing test and is probably related to the reduced synthesis of the inflammatory mediators (27-29). Unlike the writhing test, thermally induced pain models including the tail-flick test are very well known tests for discriminating morphine-like analgesics from nonopiate analgesics. The tail-flick test allows us to identify the central analgesic compounds (30). The experimental evidence obtained in this study suggested that the antinociceptive activity of the extracts was probably due to their antiinflammatory properties. However, the SL extract, which was also found to be active in the tail-flick test, is thought to show its antinociceptive activity in a peripheral as well as a central way.

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