

Anti-inflammatory effects of different extracts from three *Salvia* species

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Abstract: *Salvia* L. species have been used for the treatment of various inflammatory ailments in traditional medicine. In order to evaluate this ethnobotanical information, water, methanol, n-butanol, acetone, and chloroform extracts from 3 *Salvia* species (*S. fruticosa*, *S. verticillata*, and *S. trichoclada*) were screened for their anti-inflammatory activity using in vivo experimental models in rats. For this purpose a carrageenan-induced inflammatory paw edema model was used. All extracts demonstrated anti-inflammatory activities; however, n-butanol extract of *Salvia fruticosa* (syn. *S. triloba*), which is known as Turkish sage, was found to be the most active. It can be expected that the active flavonoids, phenolic acids, and terpenoids may be responsible for the anti-inflammatory activity of these plants.

Key words: *Salvia*, Lamiaceae, anti-inflammatory activity

Salvia türünün farklı ekstrelerinin antienflamatuvar etkileri

Özet: *Salvia* L. türleri geleneksel tıpta birçok enflamatuvar hastalığın tedavisi amacıyla kullanılmaktadır. Bu etnobotanik bilgileri değerlendirmek amacıyla, 3 *Salvia* türünün (*S. fruticosa*, *S. verticillata* ve *S. trichoclada*) su, metanol, n-butanol, aseton ve kloroform ekstreleri *in vivo* deneysel sıçan modelleri kullanılarak antienflamatuvar etki açısından incelendi. Bu amaçla karrageninle indüklenen enflamatuvar pençe ödemi modeli kullanıldı. Tüm ekstreler antienflamatuvar aktivite göstermesine rağmen, en etkili ekstre Türk Ada Çayı olarak da bilinen *Salvia fruticosa* (syn. *S. triloba*)'nın n-butanol ekstresi olarak bulundu. Bitkilerin antienflamatuvar etkilerinden içeriklerindeki aktif flavonoidler, fenolik asitler ve terpenoidler sorumlu olabilirler.

Anahtar sözcükler: *Salvia*, Lamiaceae, antienflamatuvar aktivite

Introduction

The genus *Salvia* L. (Lamiaceae), with about 900 species, is one of the most widespread members of the family Lamiaceae. It is represented in Turkey by 94 taxa belonging to 89 species with 50% endemism (1,2). A large number of beneficial secondary metabolites, belonging to various chemical groups, such as essential oils, and terpenoid and phenolic compounds, have been isolated from this genus, which features prominently in the pharmacopoeias of many countries throughout the world (3-8). Ethnobotanical, phytochemical, and pharmacological studies showed that *Salvia* species are considered "cure-all" type plants (9,10). They possess a number of biological activities including antiseptic, antibacterial (11), astringent, antiinflammatory (12,13), antiviral (14), cytotoxic (15), spasmolytic, anticonvulsant (16), antimycobacterial (17), and carminative activities. Moreover, *Salvia* species have been used for the treatment of various inflammatory ailments in traditional medicine (18).

Previously, aethiopinone, an *o*-naphthoquinone diterpene from *Salvia aethiopsis* L. roots, and 2 hemisynthetic derivatives have been evaluated for anti-inflammatory activity and they showed a pharmacological profile similar to that of other NSAID substances on reducing the edema induced by carrageenan and contractions induced by phenyl-*p*-quinone. In the TPA-induced ear inflammation model, 3 compounds showed a moderate reduction of edema, and aethiopinone produced a significant increase in the reaction time against thermal painful stimuli in the tail immersion test (19). *S. miltiorrhiza* has been used in inflammatory diseases in East Asia. In another work, tanshinone derivatives isolated from *S. miltiorrhiza* were found to possess *in vivo* anti-inflammatory activity in rat carrageenan-induced paw edema and adjuvant-induced arthritis (20). *Salvia officinalis* L. leaves were investigated for their topical anti-inflammatory properties. The *n*-hexane and the chloroform extracts dose-dependently inhibited the Croton oil-induced ear edema in mice, the chloroform extracts being the most active. Chemical and pharmacological investigation of the chloroform extract revealed ursolic acid as the main component involved in its anti-inflammatory activity. The anti-inflammatory effect of ursolic acid was more

potent than that of indomethacin, which was used as a reference for non-steroidal anti-inflammatory drugs (21).

In order to evaluate ethnobotanical and scientific information, various extracts from 3 *Salvia* species (*S. fruticosa*, *S. verticillata*, and *S. trichoclada*) were screened for their anti-inflammatory activity using *in vivo* experimental models in rats.

Materials and methods

Plant material

The aerial parts of *Salvia trichoclada* Benth. were collected from Yüksekova, Hakkari Province, in June 2002. The voucher specimen is stored in the Herbarium at the Faculty of Science, Hacettepe University (AAD 11036). *S. fruticosa* Mill. (syn. *S. triloba*) were collected from Kemer, Antalya Province, in April 2005, *S. verticillata* L. were collected from Çubuk-Karagöl Ankara (alt. 960 m), in June 2005. Voucher specimens are stored in the Herbarium at the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 08014 and 08011).

Extraction and preparation of the test samples

Thirty grams of shade dried and powdered aerial parts of each plant were extracted separately under reflux in a water bath with 150 mL of acetone at 40 °C for 8 h. After filtration, combined extracts were evaporated under vacuum until dry (acetone extracts: 0.6%). The residues were extracted with 150 mL of methanol and filtered. The filtrate was evaporated under vacuum until dry (methanol extracts: 1.8%). Crude methanol extracts were dissolved in 150 mL of water and extracted with 150 mL of chloroform and 150 mL of *n*-butanol, respectively. All extracts were evaporated separately under vacuum at 40 °C until dry. The aqueous phases were further lyophilized (water extracts: 0.5%; chloroform extracts: 0.15%; *n*-butanol extract: 0.2%)

Animals

The animals used in this study were 96 adult male Wistar rats weighing between 200 and 220 g. They were maintained at the Medical Experimental Research Center, Atatürk University, Erzurum. They were kept in rat cages and fed under normal conditions (22 °C) in separate groups. All experimental protocols

were in compliance with the Atatürk University Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Chemicals

Carrageenan was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The standard drug used was indomethacin from Deva Holding, İstanbul, Turkey. All chemicals used were of analytical grade and purchased from Merck.

Inflammatory Paw Edema Test in Rats

In this part of the experiment, the anti-inflammatory activities of 5 different polarities having extracts of 3 *Salvia* species were investigated on carrageenan-induced inflammatory paw edema (22). All extracts were dissolved and dispersed in distilled water and administered by oral gavage into groups of rats at 50 and 100 mg/kg dosages. Distilled water was given to the control group at the same volume as vehicle. One hour after administration, 0.1 mL of 1% carrageenan solution was injected into the footpad of the hind paws of each rat in all groups. Prior to carrageenan injection, the rats' paw volume until the knee joint was measured with a plethysmometer. Increasing of carrageenan induced inflammatory paw volume was measured at 1 h intervals over the next 4 h. The anti-inflammatory activity of *Salvia* extracts was compared with that of 25 mg/kg indomethacin.

The percentage inhibition of the inflammation was calculated from the formula: $\text{inhibition\%} = (D_0 - D_1)/D_0 \times 100$, where D_0 is the average inflammation (hind paw edema) of the control group of rats at a given time; and D_1 is the average inflammation of the drug treated (i.e. extracts or reference indomethacin) rats at the same time.

Acute toxicity

Extracts in 2000 mg/kg doses were administered to the rats orally (p.o.). All animals were observed for 24 h after drug administration. No mortality was recorded.

Statistical analyses

To evaluate the significance of the observed differences, the least significant difference (LSD) test was used. The observations were expressed as mean \pm S.D. The difference in response to test drugs was

determined as a percentage. $P < 0.05$ was considered significant when compared to the control group. All statistical calculations were performed using SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL, USA).

Results and discussion

In this study, anti-inflammatory activities of 3 *Salvia* spp. were evaluated using carrageenan-induced paw edema in rats with 2 doses for each extract. Carrageenan-induced inflammation peaked at 3 h after the injection and the highest anti-inflammatory effects for the extracts were recorded at this hour. Therefore, we emphasized the results at 3 h in Table 1. Moreover, we listed the results at other hours (1, 2, and 4 h) in Table 2. Both of the tables demonstrate the results of extracts that were active. The extracts that are not present in the tables were not active.

The results showed that many extracts have significant anti-inflammatory effects ($P < 0.05-0.0001$) (Figure); 100 mg/kg doses of SF-4, ST-5, and ST-2 have inhibition results 48.21%, 33.93%, and 35.71% respectively. It was clearly seen that the activity shown by n-butanol extract of *Salvia fruticosa* (SF-4) was the highest. The anti-inflammatory effects of SF-4 (50 mg/kg), ST-3 (100 mg/kg), and SV-1 (100 mg/kg) at 3 h on carrageenan-induced arthritis in rats were significant. Paw reductions were 25%, 26.79%, and 26.79%, respectively. ST-5, ST-2, ST-3, SV-2, SV-2, SV-4, and SV-1 had insignificant effects. At the same time, indomethacin reduced carrageenan inflammation 66.07% at the dose of 25 mg/kg (Figure).

These findings indicate that the polar extract of *Salvia fruticosa* possesses anti-inflammatory properties and provide pharmacological support to folkloric, ethnomedical uses of Turkish sage in the treatment and management of anti-inflammatory conditions.

Acute inflammation, such as carrageenan-induced edema, involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leukotrienes, and serotonin, all of which also cause pain and fever (23). Inhibition reaching the injured side or exposure to the pharmacological

Table 1. The effect of some *Salvia* extracts and indomethacin on carrageenan-induced edema at 3 h.

<i>Salvia</i> Extracts	Dose (mg/kg)	Animal number	Paw volumes of rats (mL)		Difference volume of paw (mL)	Anti-inflammatory effect (%)	P
			Paw volume before inflammation	3 h from carrageenan injection			
SF4	50	6	0.92 ± 0.07	1.34 ± 0.06	0.42 ± 0.04	25.00	<0.005
SF4	100	6	0.93 ± 0.05	1.22 ± 0.06	0.29 ± 0.03	48.21	< 0.0001
SV1	50	6	1.10 ± 0.02	1.59 ± 0.04	0.49 ± 0.04	12.50	> 0.05
SV1	100	6	1.03 ± 0.04	1.44 ± 0.05	0.41 ± 0.03	26.79	< 0.005
SV2	50	6	0.90 ± 0.04	1.42 ± 0.02	0.52 ± 0.03	7.14	> 0.05
SV2	100	6	0.99 ± 0.03	1.47 ± 0.02	0.48 ± 0.02	14.29	> 0.05
SV4	50	6	0.96 ± 0.04	1.51 ± 0.05	0.55 ± 0.02	1.78	> 0.05
SV4	100	6	0.99 ± 0.04	1.53 ± 0.06	0.54 ± 0.03	3.57	> 0.05
ST2	50	6	0.94 ± 0.06	1.39 ± 0.06	0.45 ± 0.04	19.64	> 0.05
ST2	100	6	0.92 ± 0.04	1.28 ± 0.06	0.36 ± 0.03	35.71	< 0.0001
ST3	50	6	0.95 ± 0.05	1.42 ± 0.05	0.47 ± 0.05	16.07	> 0.05
ST3	100	6	1.03 ± 0.03	1.44 ± 0.05	0.41 ± 0.04	26.79	< 0.005
ST5	50	6	0.98 ± 0.03	1.44 ± 0.03	0.46 ± 0.02	17.90	> 0.05
ST5	100	6	0.96 ± 0.05	1.33 ± 0.05	0.37 ± 0.01	33.93	< 0.0001
Indomethacin	25	6	1.01 ± 0.03	1.20 ± 0.03	0.19 ± 0.001	66.07	< 0.0001
Control	-	6	0.96 ± 0.06	1.52 ± 0.05	0.56 ± 0.003	-	-

SF4: n-butanol extract of *Salvia fruticosa*. SV1: acetone; SV2: methanol and SV4: n-butanol extracts of *Salvia verticillata* ST2: methanol; ST3: chloroform and ST5: water extracts of *Salvia trichoclada*

Table 2. The effect of some *Salvia* extracts and indomethacin on carrageenan-induced edema during the experiment.

<i>Salvia</i> Extracts	Dose (mg/kg)	Anti-inflammatory effect (%)			
		1 h	2 h	3 h	4 h
SF4	50	8.3	17.9	25.00	20.4
SF4	100	20.8	33.3	48.21	40.8
SV1	50	4.9	5.1	12.50	7.4
SV1	100	5.1	12	26.79	18.3
SV2	50	4.1	5.1	7.14	4.08
SV2	100	5.6	8.2	14.29	7.1
SV4	50	1.4	-	1.78	2.7
SV4	100	-	3.8	3.57	4.0
ST2	50	8.3	12.8	19.64	12.3
ST2	100	16.7	23.1	35.71	28.6
ST3	50	5.6	7.6	16.07	9.5
ST3	100	7.7	12.5	26.79	18.4
ST5	50	4.1	12.8	17.90	14.3
ST5	100	16.6	25.6	33.93	19.4
Indomethacin	25	25	36.6	66.07	56.3

SF4: n-butanol extract of *Salvia fruticosa*. SV1: acetone; SV2: methanol and SV4: n-butanol extracts of *Salvia verticillata*. ST2: methanol; ST3: chloroform and ST5: water extracts of *Salvia trichoclada*

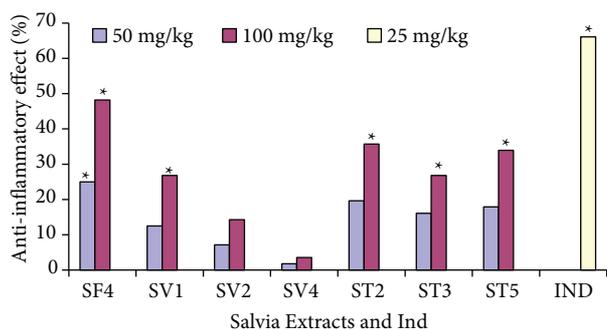


Figure. Anti-inflammatory effects of the *Salvia* extracts at 3 h of carrageenan inflammation.

* the effect was significant when compared to the non-treated control group.

effects of these mediators will normally ameliorate the inflammation and other symptoms. Carrageenan-induced rat paw is a suitable experimental animal model for evaluating the anti-edematous effect of natural products and is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (24). The 1st phase (1 h) involves the release of serotonin and histamine; the 2nd phase (over 1 h) is mediated by prostaglandins, the cyclooxygenase products; and the continuity between the 2 phases is provided by kinins (25).

The present study showed that n-butanol extract of *Salvia fruticosa*; water, methanol, and chloroform extracts of *Salvia trichoclada*; and acetone extract of *Salvia verticillata* had an antiedematogenic effect on paw edema induced by carrageenan.

It should be noted that the anti-inflammatory activities of many plants have been attributed to their triterpene (26) or flavonoid contents (27,28). It has been also demonstrated that various flavonoids (such as rutin, quercetin, and luteolin), biflavonoids, and triterpenoids (such as ursolic acid) produced significant antinociceptive and/or anti-inflammatory activities (29-32). *Salvia* spp. are rich in phenolic

compounds, such as phenolic acids and flavonoids, in addition to terpenoids (3-8,10,29). In our previous study, we isolated phenolic acids and flavonoids from n-butanol extract, and triterpene aglycones from acetone extract of *Salvia trichoclada* and *S. verticillata* (33). Therefore, the presence of anti-inflammatory activity may be due to phenol and terpenoid contents in these plants.

Non-steroidal anti-inflammatory drugs (NSAID) such as indomethacin act by the reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site (34). The observed anti-inflammatory activities of these extracts may be attributed to the overall effects of the plant constituents or the compounds having actions similar to NSAID. Although the active doses of the plant extracts were higher than those of the reference drug, it should be noted that the extracts have different compositions of several substances.

In conclusion, it can be expected that crude plant material with active flavonoids, phenolic acids, and terpenoids contents will be effective in therapy. This work demonstrates that *Salvia* spp. exhibit anti-inflammatory activity even when used orally, e.g., sage, and justify their medicinal uses in traditional conditions. The detailed anti-inflammatory mechanism studies on active extracts and compounds of *Salvia* species should be continued.

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