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Original Article

Analgesic and anti-inflammatory properties of the hydroalcoholic, polyphenolic and boiled extracts of *Stachys lavandulifolia*

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Abstract

Extracts obtained from the aerial parts of *Stachys lavandulifolia* (Lamiaceae) are used in Iranian folk medicine as remedies for the treatment of various painful and inflammatory diseases. For evaluation of its probable analgesic and anti-inflammatory effects, hydroalcoholic, polyphenolic and boiled extracts of the aerial parts of the herb were prepared and their analgesic effects were studied in mice using formalin, acetic acid-induced writhing and light tail flick tests. Carrageenan test in rats was used for assessment of anti-inflammatory activity of the extracts. Results showed that all the tested extracts were able to reduce the abdominal constrictions in acetic acid-induced writhing test. These extracts also significantly (P<0.001) suppressed both phases of formalin test. In light tail flick test, none of the extracts showed analgesic activity. Only polyphenolic extract at a dose of 2 g/kg when given p.o. or i.p. significantly (P<0.05) inhibited carrageenan-induced paw oedema. Results of the present study confirm the traditional use of *Stachys lavandulifolia* for the treatment of painful and inflammatory conditions and calls for further investigations to determine the active chemical constituent(s).

Keywords: *Stachys lavandulifolia*; Lamiaceae; Analgesic; Anti-inflammatory

INTRODUCTION

Natural products of plant origin are still a major part of traditional medical systems in developing countries. There has also been a resurgence of interest in herbal medicine in western countries (1) as alternative sources of drugs for often intractable diseases. The genus *Stachys* (Lamiaceae) includes about 200–300 species in the world (2). In Iran, this genus is represented by 34 species including *Stachys lavandulifolia* Vahl. (3).

During past years pharmacological studies have confirmed that extracts or components of plants belonging to the genus *Stachys* exert significant anti-bacterial (4), antitoxic (5), antinephritic (6,7), antihepatitis (8) anti-anoxia (9), hypotensive (10) and antianxiety (11,12) effects. Also anti-inflammatory and analgesic activity has been reported for *S. inflata* (13) and *S. byzanthina* (14).

S. lavandulifolia Vahl is a plant widely distributed in different regions of Iran (15), being popularly named "Chai Koohi". Phytochemical analysis has shown that the plant contained flavonoids, saponins and bitter compounds (16). Boiled extracts obtained from the aerial parts of this plant is used in Iranian folk medicine in painful and inflammatory disorders (17) and to our knowledge, its analgesic and anti-inflammatory activity has not been

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pharmacologically elucidated. Considering the folk use of this plant to relieve some pains and also analgesic and anti-inflammatory activities reported for two other species of *Stachys*, we sought to investigate the analgesic and antiinflammatory effects of hydroalcoholic, polyphenolic and boiled extracts of *S. lavandulifolia* using standard animal models.

MATERIALS AND METHODS

Plant materials and preparation of extracts

The aerial parts of the plant were purchased from a local market. The scientific name of the plant was confirmed by Department of Botany (School of Sciences, Isfahan University, Isfahan, Iran). A voucher specimen was deposited in the herbarium of School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran (herbarium No. 1494).

For preparation of hydroalcoholic extract, air-dried and powdered aerial parts of the plant (50 g) were macerated with 500 ml of ethanol:water (7:3) for 48 h. The extract was shaken, filtered and then evaporated in a rotatory evaporator under reduced pressure until dryness (18). Polyphenolic fraction of the plant (100 g) was achieved in two steps, first, with ethanol:water (9:1) and then with ethanol:water (1:1). At each step sufficient solvent was added to make liquid slurry and the mixture was left for 12 h. The two extracts were combined and evaporated to about 1/3 of the original volume. The resultant aqueous solution was cleared by extraction in a separating funnel with chloroform and then evaporated to dryness under reduced pressure in a rotatory evaporator (18,19). Evaporation and solvent removal of hydroalcoholic extract and polyphenolic fraction gave semi-solid masses (yield 31.2% and 27.1%, respectively).

Boiled extract was prepared by adding 500 ml distilled water to 50 g powder of the plant. The mixture was boiled for 10 min., then filtered and evaporated in a rotatory evaporator under reduced pressure until dryness (yield 60%) (18).

Animal models and habituation

Male Wistar rats and male mice, weighing 200-300 and 25–30 g, respectively, were used in this study. Animals were housed in groups of six per standard makrolon cage, on 12 h light/12 h dark cycle; and air temperature was maintained at 22 ± 2 ^{o}C. They were provided with food and water *ad libitum*. All the experiments reported here were carried out in accordance with local guidelines for the care of laboratory animals of Isfahan University of Medical Sciences.

Antinociceptive tests

Acetic acid-induced writhing test

This test was performed in mice according to the method described by Ferreira et al. (20) with slight modifications. Thus, 1% acetic acid solution (10 ml/kg) was injected i.p. Animals were pretreated with *S. lavandulifolia* extracts or indomethacin (reference drug) orally 1 h prior to the peritoneal irritation. Control animals received the same volume of 1% aqueous solution of tween 20. The resulting writhes and stretching were observed and counted over a period of 10 min starting 10 min after acetic acid injection.

Formalin test

The method used in the present study was similar to that described previously by de Miranda et al. (2001) with slight modifications (21). It consists briefly of injecting s.c. 20 µl of 2.5% formalin into the right posterior paw of mice placed in a transparent enclosure. Throughout 5 min prior to this procedure, each mouse is allowed to adapt the testing box and left

freely moving and exploring (habituation). The formalin-induced licking of the paw was considered as indicative of the nociceptive behaviour. The total time spent in licking and biting the injected paw was recorded 0-5 and 20-25 min after formalin injection.

In this test, polyphenolic, hydro-alcoholic and boiled extracts of *S. lavandulifolia* were administered at doses of 1, 2 and 4 g/kg and boiled extract at doses of 0.5, 1 and 2 g/kg one hour prior to formalin injection. Control group received 1% aqueous solution of tween 20 (10 ml/kg) and a group of animals received morphine (10 mg/kg, i.p.) as a standard analgesic drug.

Light tail flick test

Acute nociception was assessed using a tail flick apparatus (Pooya-armaghan, Iran) following the method of D'Amour and Smith (22). Briefly, each animal was placed in a restrainer, 2 min before treatment, and baseline reaction time was measured by focusing a beam of light on the distal one-third portion of the animal's tail. Extracts (2 g/kg) and vehicle were administered orally and 30 min later the post drug reaction time was measured at 15 min intervals until 2 h. A 12 s cut-off time was used in order to prevent tissue damage. The MPE% (percent of maximum possible analgesic effect) was calculated for each time interval.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the carrageenan-induced paw oedema test in the rat (23). Male Wistar rats (200-300 g) were briefly anaesthetized with ether and injected subplantarly into right hind paw with 0.1 ml of 1% suspension of carrageenan in isotonic saline. The left hind paw was injected with 0.1 ml saline and used as a control. Paw volume was measured prior and 4 h after carrageenan administration using a mercury plethysmorgraph (Ugo Basil, Italy).

The extracts were diluted in 1% tween 20 and administered 1 h prior to carrageenan injection. The control group received equivalent volume of the vehicle. Dexamethasone (1 mg/kg) was used as positive control.

Data analysis

Data were expressed as mean (± S.E.M.). Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan as the post hoc test. Significance was defined at the 5% level.

RESULTS

In acetic acid-induced writhing all of the examined extracts inhibited the abdominal twitches in a dose dependent manner. Indomethacin as a reference drug produced 67% inhibition of acetic acid-induced twitches. A dose of 1 g/kg of boiled extract had analgesic activity almost equal to 2 and 4 g/kg of hydroalcoholic and polyphenolic extracts, respectively (Table 1).

Table 1. Effect of *S. lavandulifolia* extracts on acetic acid-induced writhing in mice.

Treatment	Dose (g/kg, p.o.)	Number of writhes (Mean ± S.E.M.)	Percent inhibition
Control	-	56.3 ± 7.5	-
HE	1	38.8 ± 5.6*	31
	2	30.8 ± 2.9*	45
	4	29.2 ± 3.0*	48
PE	1	40.4 ± 2.9*	28
	2	35.2 ±3.0*	37
	4	30.0 ± 2.7*	47
BE	0.5	40.7 ± 5.9*	28
	1	31.3± 8.1 *	44
	2	27.0± 3.5*	52
Indomethacin	0.01	18.5 ± 6.3*	67

* $P<0.001$ compared with control group. HE, Hydroalcoholic Extract; PE, Polyphenolic Extract; BE, Boiled Extract. (n = 6)

The results of formalin test have been shown in Table 2. As it is shown, hydroalcoholic, polyphenoilc and boiled extracts of the plant significantly (P<0.001) reduced time spent for paw licking in both acute and chronic phases of formalin test. In this test, morphine, as a standard drug, also showed potent analgesia in both phases.

In light tail flick test while morphine showed a potent analgesic effect, none of the tested extracts were able to increase tail flick latency (data not shown).

Table 3 shows the results of carrageenan test. The hydroalcoholic extract (at doses of 0.5, 1 and 2 g/kg) when administered p.o. or i.p failed to produce any significant anti-inflammatory effect. Polyphenolic fraction only at a dose of 2 g/kg produced a significant (P<0.05) reduction of carrageenan-induced paw oedema (at a dose of 2 g/kg) and dexamethazone (1 mg/kg, i.p.), as a reference drug produced a greater (60%) inhibition of edema development.

DISCUSSION

The results of the present study indicated that hydroalcoholic, polyphenolic and boiled extracts of *S. lavandulifolia* had potent antinociceptive effect in formalin and acetic-acid writhing tests but not in light tail flick test. In the formalin test which is sensitive to various classes of analgesic drugs (24), our results showed that the time spent in licking the injured paw was significantly reduced by oral administration of all examined extracts in both phases. In this test, as it is observed in figures the centrally acting drugs such as morphine inhibits both phases equally, while peripherally acting drugs only inhibited the second phase (25). It is also well known that the formalin model may involve sensorial C-fibers (26) in early phase and a combined process generated by peripheral inflammatory tissue and functional changes in the dorsal horn in late phase (27,28). In fact, the considerable effect of the tested extracts on both phases showed that they probably contain active analgesic principles, acting both centrally and peripherally. According to these claims, it is expected that these extracts show some analgesic activity in light tail flick test, a central model which has selectivity for opioid-derived analgesics (29). Unlike this expectation, a relatively high dose of any of the extracts (2 g/kg) had no analgesic activity in this model and it seems that other mechanisms rather than

Table 2. Effect of *Stachys lavandulifolia* extracts in the formalin test.

Treatment	Dose (g/kg)	Paw licking time(s)			
		First phase (0-5 min) (Mean ± S.E.M.)	Inhibition (%)	Second phase (20-25 min) (Mean ± S.E.M.)	Inhibition (%)
Control	-	75.5 ± 11.6	-	68.0 ± 12.9	-
HE	1	5.8 ± 4.3[b]	92	18.2 ± 7.9[b]	73
	2	3.8 ± 3.1[b]	95	12.5 ± 8.4[b]	82
	4	6.0 ± 1.2[b]	92	0.5 ± 1.2[b]	99
PE	1	11.7 ± 8.6[b]	85	31.8 ± 10.8[b]	53
	2	11.2 ± 7.5[b]	85	25.7 ± 11.9[b]	62
	4	7.7 ± 7.5[b]	90	27.8 ± 15.1[b]	59
BE	0.5	45.0 ± 10.5[a]	40	46.2 ± 13.0[a]	32
	1	33.8 ± 14.4[b]	55	35.3 ± 7.8[b]	48
	2	19.2 ± 10.0[b]	75	14.5 ± 9.4[b]	79
Morphine	0.01 (i.p.)	2.6 ± 1.1[b]	97	1.2 ± 0.5[b]	98

[a]P<0.01 [b]P<0.001 Compared with control group. HE, Hydroalcoholic Extract; PE, Polyphenolic Extract; BE, Boiled Extract. (n = 6)

opioid receptors are involved in analgesic activity of these extracts.

The plant polyphenolic extract had also anti-inflammatory activity against carrageenan. Consistent with our results, anti-inflammatory and analgesic activities have also been reported for two other species of *Stachys* (13,14). Considering that the production of arachidonic metabolites via the COX-2 enzyme is the main factor responsible for carrageenan-induced inflammation, these findings demonstrate that the anti-inflammatory activity of polyphenolic extract of *S. lavandulifolia* is probably related to the inhibition of the synthesis or release of COX-2 products. There is also evidence that inflammation processes are accompanied by increase in free radical activity (30). Previous investigations also showed that extracts of the some species of *Stachys* have appreciable levels of antioxidant activity (30). On the basis of this function, the extracts may exert their anti-inflammatory effects at least partially through the relative antioxidant activity. However, further experiments are needed to confirm these mechanisms. Other mechanisms including corticosteroid-like effects, interaction with tachykinin or other inflammatory mediators should also be considered (31).

Although a preliminary study has shown that the plant contains flavonoids, saponin and bitter compounds (16), further phytochemical and biological tests are suggested to determine the active chemical constituent(s) responsible for these activities.

In conclusion, our results clearly demonstrated that hydroalcoholic, polyphenoilc and boiled extracts of *S. lavandulifoliai* displayed a potent analgesic effect in acetic acid and formalin tests and these results provided a validation of the plant popular use as a remedy in painful conditions.

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Table 3. Effect of oral and i.p. administration of *S. lavandulifolia* extracts on carrageenan-induced rat paw edema

Treatment	Dose (g/kg)	Increase in paw volume (ml)	Percent inhibition of paw edema
Control	-	0.295 ± 0.03	-
HE (p.o.)	0.5	0.271 ± 0.05	8
	1	0.251 ± 0.03	15
	2	0.209 ± 0.03	29
HE (i.p.)	0.5	0.282 ± 0.05	4
	1	0.262 ± 0.07	11
	2	0.257 ± 0.03	13
PE (p.o.)	0.5	0.275 ± 0.04	7
	1	0.257 ± 0.03	13
	2	0.147 ± 0.01	50*
PE (i.p.)	0.5	0.262 ± 0.03	11
	1	0.207 ± 0.04	30
	2	0.166 ± 0.02	44*
Dexamethazone (i.p.)	0.001	0.117 ± 0.03	60**

*$P<0.05$; **$P<0.01$ compared with control group. HE, Hydroalcoholic Extract; PE, Polyphenolic Extract. (n = 6)

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