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ARTICLE *in* JOURNAL OF ETHNOPHARMACOLOGY · DECEMBER 2003

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Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill.

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Received 26 June 2002; received in revised form 18 June 2003; accepted 4 July 2003

Abstract

Extracts obtained from the leaves of *Lavandula angustifolia* Mill. (Lamiaceae) are used in Iranian folk medicine as remedies for the treatment of various inflammatory diseases. For evaluation of its probable analgesic and anti-inflammatory effects, hydroalcoholic extract, polyphenolic fraction and essential oil of the leaves of the herb were prepared and their analgesic effects were studied in mice using formalin and acetic acid-induced writhing tests. Carrageenan test in rats was used for assessment of anti-inflammatory activity of above-mentioned fractions. Results showed that while the hydroalcoholic extract (400–1600 mg/kg, p.o.) inhibited only the second phase of formalin test, the polyphenolic fraction (800 and 1600 mg/kg, p.o.) and essential oil (100 and 200 mg/kg, p.o.) suppressed both phases. In acetic acid-induced writhing test, polyphenolic fraction (400 and 800 mg/kg, p.o.) and essential oil (100 and 200 mg/kg, p.o.) reduced the number of abdominal constrictions. Essential oil at a dose of 200 mg/kg also inhibited carrageenan-induced paw edema. Results of the present study confirm the traditional use of *Lavandula angustifolia* for the treatment of painful and inflammatory conditions and calls for further investigations to determine the active chemical constituent(s).

Keywords: *Lavandula angustifolia*; Lamiaceae; Analgesic; Anti-inflammatory

1. Introduction

Lavandula angustifolia Mill. (Lamiaceae), commonly known in Iran as “Ostokhoddous”, is a widely distributed aromatic herb (Omidbaigi, 2000). The plant flowers and essential oils are principally used in the toiletry and perfumery industries (Evans, 1989). *Lavandula angustifolia* is well known among people as a powerful aromatic and medicinal herb. The plant is used in traditional and folk medicines of different parts of the world for the treatment of several gastrointestinal, nervous and rheumatic disorders (Duke, 1989; Evans, 1989; Leung and Foster, 1996). The infusions of the plant have also been used in Iranian folk and traditional medicine as carminative, diuretic, anti-epileptic, anti-rheumatic and pain reliever especially in nervous headache and migraine. Some Iranian practitioners such as Rhazes and Avicenna were also familiar with this plant and mentioned its medicinal uses in their valuable books, “Continens” and “The Canon”, respectively (Ebn-e Sina, 1988; Nafisy, 1989; Razi, 1990; Zargari, 1990). In some regions of Iran, the leaves of this plant are claimed to be especially effective against pain and inflammatory diseases including rheumatism and lumbago.

The chemical composition and pharmacological evaluation of *Lavandula angustifolia* have been the subject of several studies over the years. Most of these studies were focused on the extracts, fractions and essential oils of the aerial parts and flowers of the plant. In pharmacological and biological tests, extracts, fractions, and essential oil of *Lavandula angustifolia* are reported to have CNS-depressant, anti-convulsive, sedative, spasmolytic, local anaesthetic, antioxidant, anti-bacterial and mast cell degranulation inhibitory effects (Leung and Foster, 1996; Kim and Cho, 1999; Hohmann et al., 1999; Lis-Balchin and Hart, 1999; Ghelardini et al., 1999). Phytochemical studies revealed that linalool, linalyl acetate and some other mono- and sesquiterpenes, flavonoids like luteolin, triterpenoids like ursolic acid and coumarins like umbelliferone and coumarin were the main components of the aerial parts and

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doi:10.1016/S0378-8741(03)00234-4

flowers of the plant (Duke, 1989; Leung and Foster, 1996; Omidbaigi, 2000; Renaud et al., 2001).

The purpose of the present study was to evaluate the analgesic and anti-inflammatory activities of the hydroalcoholic extract, polyphenolic fraction and essential oil of the plant leaves in mice and rats using the formalin, acetic acid-induced writhing and carrageenan tests. In addition, we describe the identification of the oil constituents by GC and GC/MS analyses, since some of these compounds have been reported to possess anti-inflammatory and anti-nociceptive activities.

2. Materials and methods

2.1. Plant material and preparation of essential oil and extracts

The leaves of *Lavandula angustifolia*, cultivated near Isfahan, were collected in June 2000. The plant identity as *Lavandula angustifolia* was confirmed by the Herbarium Department of Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. A voucher specimen was deposited in the herbarium of Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

The essential oil was isolated by hydrodistillation of the air-dried powdered leaves of the plant for 3 h according to the method recommended in European Pharmacopoeia (2002).

For preparation of hydroalcoholic extract, air-dried and powdered leaves of the plant (100 g) were macerated with 500 ml of EtOH–H_2O (7:3) for 48 h. The extract was then shaked, filtered and evaporated in a rotating evaporator under reduced pressure until dryness (Sajjadi et al., 1998). Polyphenolic fraction of the plant (100 g) was extracted in two steps, firstly with EtOH:H_2O (9:1) and secondly with EtOH:H_2O (1:1). At each step sufficient solvent was added to make a liquid slurry and the mixture was left for 12 h. The two extracts were then combined and evaporated to about 1/3 of the original volume. The resultant aqueous solution then was cleared by extraction in a separating funnel with chloroform and then evaporated to dryness under reduced pressure in a rotating evaporator (Markham, 1982; Sajjadi et al., 1998). Evaporation and solvent removal of hydroalcoholic extract and polyphenolic fraction gave semi-solid masses (yield 42.0 and 12.2%, respectively).

2.2. Analysis of the essential oil

The oil was analyzed by GC and GC/MS. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The operating conditions were as follows: carrier gas, helium with a flow rate of 2 ml/min; column temperature, 60–275 °C at 4 °C/min; injector and detector temperature, 280 °C; volume injected, 0.1 μl of the oil; split ratio, 1:50.

GC/MS analysis was performed on a Hewlett Packard 6890 MS selective detector coupled with Hewlett Packard 6890 gas chromatograph equipped with a cross-linked 5% PHME siloxane HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and operating under the same conditions as described above. The MS operating parameters were as follows: ionization potential 70 eV, ionization current 2 A, ion source temperature 200 °C, resolution 1000.

Identification of components in the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the Wiley 275 L library as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Sandra and Bicchi, 1987; Mclafferty and Stauffer, 1991; Adams, 1995). The relative percentage of the oil constituents was calculated from the GC peak areas.

2.3. Analgesic activity

This was measured in 15 h fasted male swiss mice (25–35 g, six in each group) by formalin (Hunskaar and Hole, 1987) and acetic acid-induced writhing (Koster et al., 1959) methods.

2.3.1. Formalin test

The test carried out as described by Hunskaar and Hole (1987). Mice were injected 20 μl of 2.5% formalin (in 0.9% saline) into the subplantar space of the right hind paw and the duration of paw licking was determined 0–5 min (first phase) and 20–25 min (second phase) after formalin.

Hydroalcoholic extract, polyphenolic fraction and essential oil were given orally one hour prior to formalin injection. Control animals received vehicle (10 ml/kg of 1% solution of tween 20). Morphine (10 mg/kg, s.c.) pretreated animals were included in the study for comparison.

2.3.2. Acetic acid-induced writhing test

Groups of mice ($n = 6$) received orally different doses of hydroalcoholic extract, polyphenolic fraction and essential oil one hour prior to an intraperitoneal injection of 1% acetic acid in a volume of 10 ml/kg. Control group received vehicle (10 ml/kg of 1% solution of tween 20). Indomethacin (10 mg/kg, p.o.) pretreated animals were used as positive control.

2.4. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in the rat (Winter et al., 1962). 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

Table 1
Volatile constituents of the leaf oil of *Lavandula angustifolia*

Compound	Percentage	RI
Alpha-thujene	0.1	927
Alpha-pinene	3.6	935
Alpha-fenchene	0.5	948
Camphene	0.5	950
Beta-pinene	2.3	977
Delta-3-carene	2.1	1009
1,8-Cineole	65.4	1032
trans-Beta-ocimene	0.1	1049
Gamma-terpinene	0.2	1062
Terpinolene	0.3	1087
Linalool	0.4	1096
Camphor	9.5	1143
Borneol	11.5	1162
Lavandulol	0.2	1165
4-Terpineol	0.5	1178
Alpha-terpineol	0.6	1189
Linalyl acetate	0.6	1260
Bornyl acetate	0.2	1287
Beta-caryophyllene	0.1	1421
cis-Beta-farnesene	0.2	1446
Germacrene-D	0.2	1485

prior and 4 h after carrageenan administration using a mercury plethysmorgraph (Ugo Basil, Italy).

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

2.5. Statistical analysis

The results are presented as mean ± S.E.M. and statistically analyzed by one-way ANOVA followed by Duncan test.

3. Results

3.1. Analysis of the essential oil

The plant leaves yielded 1.1% of a pale-yellowish essential oil with a fresh pleasant odor. Twenty-one components were characterized, representing 99.1% of the total oil components detected, which are listed in Table 1 with their percentage composition and retention indices.

3.2. Pharmacological study

The results of formalin test have been summarized in Table 2. The hydroalcoholic extract failed to produce any significant analgesia in the first phase. However, its effect on late phase was significant ($P < 0.001$) so that at doses of 400, 800 and 1600 mg/kg paw licking time was reduced by 69, 72 and 93%, respectively. The polyphenolic fraction and essential oil in a dose-dependent manner inhibited paw licking of both phases of formalin test.

In acetic acid-induced writhing test, hydroalcoholic extract could not exert a significant decrease of abdominal twitches, while the polyphenolic fraction at doses of 400 and 800 mg/kg and the essential oil at doses of 100 and 200 mg/kg significantly ($P < 0.05$) reduced writhes (Table 3). However, these effects were not dose-dependent since a two-fold increase in dose did not alter the response. Table 4 shows the results of carrageenan test. The hydroalcoholic extract and polyphenolic fraction, even in high doses (e.g. 4000 mg/kg) had not a considerable anti-inflammatory effect. The essential oil at a dose of 200 mg/kg produced a 48% inhibition of carrageenan-induced paw edema. Dexamethazone (1 mg/kg), a reference drug produced a greater (60%) inhibition of edema development.

Table 2
Effect of *Lavandula angustifolia* extracts and essential oil in the formalin test ($n = 6$)

Treatment	Dose (mg/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	–	46.7 ± 5.6	–	21.8 ± 5.6	–
HE	400	40.0 ± 3.8	14	6.7 ± 2.7***	69
	800	39.5 ± 4.1	15	6.0 ± 2.5***	72
	1600	41.0 ± 4.9	12	1.5 ± 1.0***	93
PF	400	34.2 ± 4.0	27	11.7 ± 2.9**	47
	800	18.8 ± 4.0**	60	11.7 ± 1.9**	47
	1600	15.8 ± 2.6**	66	6.2 ± 2.1***	70
EO	50	39.2 ± 3.8	15	3.5 ± 1.5***	83
	100	14.7 ± 1.3**	69	3.0 ± 1.5***	86
	200	5.7 ± 1.1***	88	2.0 ± 1.1***	90
Morphine	10 (s.c.)	5.0 ± 3.5***	90	1.0 ± 0.1***	95

HE, hydroalcoholic extract; PF, polyphenolic fraction; EO, essential oil.
** $P < 0.01$.
*** $P < 0.001$ compared with control group.

Table 3
Effect of *Lavandula angustifolia* extracts and essential oil on acetic acid-induced writhing in mice ($n = 6$)

Treatment	Dose (mg/kg, p.o.)	Number of writhes (mean ± S.E.M.)	Percent inhibition
Control	–	31.3 ± 1.9	–
HE	400	29.0 ± 1.1	7
	800	28.0 ± 1.0	14
	1600	26.2 ± 3.3	17
PF	200	29.2 ± 1.6	7
	400	26.7 ± 0.5*	15
	800	26.8 ± 0.7*	15
EO	100	23.2 ± 0.7*	27
	200	23.0 ± 2.9*	27
Indomethacin	10	7.0 ± 1.2**	78

HE, hydroalcoholic extract; PF, polyphenolic fraction; EO, essential oil.
* $P < 0.05$.
** $P < 0.01$ compared with control group.

Table 4
Effect of *Lavandula angustifolia* extracts and essential oil on carrageenan-induced rat paw edema

Treatment	Dose (mg/kg)	Percent inhibition of paw edema
Control	–	–
HE	1000	2
	2000	5
	4000	5
PF	1000	5
	2000	8
	4000	10
EO	200	48
Dexamethazone	1	60***

HE, hydroalcoholic extract; PF, polyphenolic fraction; EO, essential oil.
*** $P < 0.001$ compared with control group.

4. Discussion

The results of the present study indicated that hydroalcoholic extract and polyphenolic fraction of *Lavandula angustifolia* at relatively high doses had moderate anti-nociceptive effect in formalin test. The polyphenolic fraction also showed some analgesic activity in acetic acid test. Among the fractions which were studied, the essential oil of the plant at doses of 100 and 200 mg/kg had considerable anti-nociceptive effect in both phases of formalin test as well as in acetic acid test and it seems that at least a large part of the analgesic effect of the plant is due to its essential oil content.

The plant essential oil had also potent anti-inflammatory activity against carrageenan. Consistent with our results, anti-inflammatory and analgesic activities have also been reported for different extracts, polyphenolic fractions and essential oils of some other Lamiaceae plants (Alcaraz and Jimenez, 1988; Shimizu et al., 1990; Amabeoku et al., 2001; Maleki et al., 2001; Bispo et al., 2001). There is also a report about anti-spasmodic and sedative effects of another *Lavandula* species (Gilani et al., 2000).

More than 65% of the essential oil components of *Lavandula angustifolia* were due to a terpenoid oxide,1,8-cineole. Other two major constituents of the leaf oil were borneol (11.5%) and camphor (9.5%). Many of the identified compounds in the oil, were present in the aerial parts and flower essential oils of *Lavandula angustifolia* reported before (Duke, 1989; Zargari, 1990; Leung and Foster, 1996; Omidbaigi, 2000; Renaud et al., 2001). It seems that 1,8-cineole has been partly associated with pharmacological findings of the present study (Santos and Rao, 2000), although it is not so clear whether this terpene oxide is the only contributing component of this plant or not. 1,8-Cineole displayed an inhibitory effect on some types of experimental inflammation in rats. It also inhibited in mice, the acetic acid-induced increase in peritoneal capillary permeability and the chemical nociception induced by intraplantar formalin and intraperitoneal acetic acid (Santos and Rao, 2000).

Significant anti-inflammatory and analgesic activities of the essential oil of the plant leaves have been found in these models, suggesting a rational basis for folk and traditional uses of this herb in Iran for some inflammatory ailments. Several mechanisms including corticosteroid-like effects, release of endogenous glucocorticoids, interaction with prostaglandin biosynthesis, interaction with tachykinin or other inflammatory mediators are involved in anti-inflammatory action of drugs (Barnes et al., 1990) and they should be studied for these fractions in future.

Also further phytochemical and biological tests are suggested to determine the active chemical constituent(s) responsible for these activities.

Acknowledgements

This work was supported by Research Council of the Isfahan University of Medical Sciences, Isfahan, Iran (Research project no. 79180). We are also grateful to Mr. I. Mehregan, Herbarium Department of Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran for his help in identifying the plant material.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Company, Carol Stream.

Alcaraz, M.J., Jimenez, M.J., 1988. Flavonoids as anti-inflammatory agents. Fitoterapia 59, 25–38.

Amabeoku, G.J., Eagles, P., Scott, G., Mayeng, I., Springfield, E., 2001. Analgesic and antipyretic effects of *Dodonaea angustifolia* and *Salvia africana-lutea*. Journal of Ethnopharmacology 75, 117–124.

Barnes, P.J., Belivisi, M.G., Rogers, D.F., 1990. Modulation of neurogenic inflammation: novel approaches to inflammatory disease. Trends in Pharmacological Sciences 11, 185–189.

Bispo, M.D., Mourao, R.H., Franzotti, E.M., Bomfim, K.B., Arrigoni-Blank, M.F., Moreno, M.P., 2001. Antinociceptive and antidermatogenic effects of the aqueous extract of *Hyptis pectinata* leaves in experimental animals. Journal of Ethnopharmacology 76, 81–86.

Duke, J.A., 1989. CRC Handbook of Medicinal Herbs. CRC Press, Boca Raton, p. 273.

Ebn-e Sina, A., 1988. Ghanoon dar Teb, vol. 2. Soroosh Press, Tehran, p. 66.

European Pharmacopoeia, 2002. Council of Europe, Strasbourg, pp. 183–184.

Evans, W.C., 1989. Trease and Evans' Pharmacognosy, 13th ed. Bailliere Tindall, London, pp. 433–434.

Ghelardini, C., Galeotti, N., Salvatore, G., Mazzanti, G., 1999. Local anaesthetic activity of the essential oil of *Lavandula angustifolia*. Planta Medica 65, 700–703.

Gilani, A.H., Aziz, N., Khan, M.A., Shaheen, F., Jabeen, Q., Siddiqui, B.S., 2000. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas* L. Journal of Ethnopharmacology 71, 161–167.

Hohmann, J., Zupko, I., Redei, D., Csanyi, M., Falkay, G., Mathe, I., 1999. Protective effects of the aerial parts of *Salvia officinalis*, *Melissa officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. Planta Medica 65, 576–578.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114.

Kim, H.M., Cho, S.H., 1999. Lavender oil inhibits immediate-type allergic reaction in mice and rats. The Journal of Pharmacy and Pharmacology 51, 221–226.

Koster, R., Anderson, M., De Beer, E.J., 1959. Acetic acid for analgesic screening. Federal Proceedings 18, 412–417.

Leung, A.Y., Foster, S., 1996. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. Wiley, New York, pp. 339–342.

Lis-Balchin, M., Hart, S., 1999. Studies on the mode of action of the essential oil lavender (*Lavandula angustifolia* P. Miller). Phytotherapy Research 13, 540–542.

Maleki, N., Garjani, A., Nazemiyeh, H., Nilforoushan, N., Eftekhar Sadat, A.T., Allameh, Z., 2001. Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. Journal of Ethnopharmacology 75, 213–218.

Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, London, pp. 16–17.

Mclafferty, F.W., Stauffer, D.B., 1991. The Important Peak Index of the Registry of Mass Spectral Data. Wiley, New York.

Nafisy, A.T., 1989. A Review of Traditional Medicine in Iran. Isfahan University Publications, Isfahan, p. 122.

Omidbaigi, R., 2000. Production and Processing of Medicinal Plants, vol. 3. Astane Ghods Publications, Mashhad, pp. 106–122.

Razi, A., 1990. Al-Hawi, vol. 1. Al-Hawi Pharmaceutical Company Press, Tehran, pp. 358–359.

Renaud, E.N.C., Charles, D.J., Simon, J.E., 2001. Essential oil quantity and composition from 10 cultivars of organically grown lavender and lavandin. Journal of Essential Oil Research 13, 269–273.

Sajjadi, S.E., Movahedian Atar, A.M., Yektaian, A., 1998. Antihyperlipidemic effect of hydroalcoholic extract and polyphenolic fraction from *Dracocephalum Kotschyi* Boiss. Pharmaceutica Acta Helvetiae 73, 167–170.

Sandra, P., Bicchi, C., 1987. Capillary Gas Chromatography in Essential Oil Analysis. Huethig Publications, Heidelberg, pp. 259–274.

Santos, F.A., Rao, V.S.N., 2000. Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phytotherapy Research 14, 240–244.

Shimizu, M., Shogawa, H., Matsuzawa, T., Yonezawa, S., Hayashi, T., Arisawa, M., 1990. Anti-inflammatory constituents of topically applied crude drugs. IV. Constituents and anti-inflammatory effect of Paraguayan crude drug "alhucema" (*Lavandula latifolia* Vill.). Chemical and Pharmaceutical Bulletin (Tokyo) 38, 2283–2284.

Winter, Ch.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine 111, 544–547.

Zargari, A., 1990. Medicinal Plants, vol. 4. Tehran University Publications, Tehran, pp. 20–28.