



Antinociceptive and gastroprotective effects of inhaled and orally administered *Lavandula hybrida* Reverchon “Grosso” essential oil

E. Barocelli^{a,*}, F. Calcina^a, M. Chiavarini^a, M. Impicciatore^a, R. Bruni^b,
A. Bianchi^b, V. Ballabeni^a

^aDipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università di Parma,
Parco Area delle Scienze 27/a, 43100 Parma, Italy

^bDipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parco Area delle Scienze 11/a, 43100 Parma, Italy

Received 13 February 2004; received in revised form 7 July 2004; accepted 27 August 2004

Abstract

In this study the antinociceptive and the gastroprotective effects of orally administered or inhaled *Lavandula hybrida* Reverchon “Grosso” essential oil, and its principal constituents linalool and linalyl acetate were evaluated in rodents. Either when orally administered (100 mg/kg) or inhaled for 60 min lavender essential oil significantly reduced the acetic acid-writhing response in a naloxone-sensitive manner. In the hot plate test, analgesic activity observed after oil inhalation was inhibited by naloxone, atropine, mecamylamine pretreatment suggesting the involvement of opioidergic as well as cholinergic pathways. Regardless of the administration route and the experimental model used both linalool and linalyl acetate did not produce significant analgesic response. Oral or inhalatory treatment with analgesic doses of essential oil did not affect mice spontaneous locomotor activity. Concerning the gastric effects, lavender oil, linalool and linalyl acetate oral administration protected against acute ethanol-induced gastric ulcers but did not prevent indomethacin-induced lesions indicating no interference with arachidonic acid metabolic cascade. In conclusion, besides this gastroprotection, lavender oil reveals an interesting analgesic activity mainly relevant after inhalation, at doses devoid of sedative side effect, suggesting the interest for potential application of this oil in aromatherapy.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Antinociceptive activity; Gastroprotection; Essential oils; *Lavandula hybrida*; Writhing test; Hot plate test

* Corresponding author. Tel.: +39 521905090; fax: +39 521905091.

E-mail address: barocell@unipr.it (E. Barocelli).

Introduction

Essential oils obtained from different species of *Lavandula* (*L. latifolia*, *L. angustifolia*, *L. stoechas*) are frequently used in aromatherapy and massage to obtain many clinical benefits traditionally ascribed to their antibacterial, antifungal, carminative, sedative and antidepressant actions. Several studies have been conducted in order to validate these applications. In clinical trials lavender essential oil demonstrated to improve sleep (Graham et al., 2003) and to reduce anxiety (Dunn et al., 1995). Furthermore, in animal models, it has been proved to possess anticonvulsant (Yamada et al., 1994) and local anesthetic activity (Ghelardini et al., 1999). Essential oil showed spasmolytic effect on smooth muscle in vitro (Lis-Balchin and Hart, 1997) supporting its use as carminative, antifatulence and anticolic agent.

In the recent years, the study of natural products, as lavender essential oils, continues to attract researchers' attention in order to detect possible clinical uses and in particular, the list of lavender biological activities is increasing. Indeed, we have recently highlighted for lavender essential oil an interesting antiplatelet activity associated with a promising protective effect in an animal model of acute pulmonary thromboembolism (Ballabeni et al., 2004). Although a conspicuous number of investigations have been conducted in these last years, no conclusive data are reported about lavender oil mechanisms of action. In fact, lavender oil ability to block Na^+ and/or Ca^{++} channels could account for oil local anesthetic activity (Ghelardini et al., 1999), while lavender spasmolytic effect has been attributed to a rise in intracellular cAMP (Lis-Balchin and Hart, 1999). These whole oil activities are shared by its major components, linalool and linalyl acetate. Experimental studies conducted with the terpenic alcohol linalool and its ester linalyl acetate revealed a significant anti-inflammatory activity in carrageenin-induced rat paw oedema (Peano et al., 2002). More recent investigations demonstrated that linalool prevents nociception in different experimental models of thermal, chemical and inflammatory pain (Peano et al., 2003; Peano et al., 2004) suggesting analgesic and anti-inflammatory potentials for linalool producing plant species.

Thus, in the present study we investigated the antinociceptive profile of the lavender essential oil, linalool and linalyl acetate, orally administered or inhaled in mice, in experimental models of chemical and thermal pain. Furthermore, based on the antiulcer activity previously reported for 1,8-cineole (Santos and Rao, 2001), another lavender oil's component, we assayed also the so far unexplored gastroprotective action of lavender oil in ethanol- and indomethacin-induced gastric ulcers in rats. Preliminary results of this research were reported to 3rd International Symposium on Natural Drugs (Calcina et al., 2003).

Methods

Plant material and essential oil extraction

Lavandula hybrida Reverchon cv. Grosso plants were cultivated by La Gachore, Puimoisson, Plateau de Valensole, Alpes de Haute Provence, France. A voucher specimen of the plant (Voucher n° OPR1) was deposited at Giardino delle Erbe Officinali, Casola Valsenio, Italy. The essential oil was obtained from steam distillation of fresh flowers few minutes after their machine harvesting, using the same

harvesting container as an industrial large-scale steam distiller (yield 1.55%). Essential oil characteristics were in accordance with literature data.

Essential oil analysis

Essential oil samples were analyzed using a Fisons (Rodano, Milano, Italy) 9130-9000 gas-chromatograph equipped with a FID detector and a MEGA SE52 (Mega, Legnano, Italy) column (i.d.=0.32 mm, length 30 m, film thickness=0.15 μm). Operating conditions: injector temperature 280 °C; FID temperature 280 °C, Carrier (Helium) 2 ml/min, split ratio 1:40. Oven temperature was initially 45 °C, then raised to 100 °C (1 °C/min), then raised to 250 °C (5 °C/min) and finally held at 250 °C for 10 min. 1 μl of oil dissolved in CH_2Cl_2 was injected. GS-MS analysis was performed on a Hewlett Packard HP5890 series II plus GC equipped with a HPMS 5989b mass spectrometer using EI. The MS conditions were as follows: ionization voltage, 70 eV; emission current, 40 μA ; scan rate, 1 scan/s; mass range, 35–300 Da; ion source temperature, 200 °C. Identification of compounds was based on relative retention times (Adams, 2001), matching with NBS MS library and comparison of fragmentation patterns with literature data (Adams, 2001). The oil composition is reported in Table 1.

Table 1

Chemical composition and retention index of the constituents of the essential oil of *Lavandula hybrida* Reverchon “Grosso”^{sa}

Compounds	KI ^b	%
α -Pinene	941	0.1
Camphene	955	0.1
β -Pinene	974	0.2
β -Myrcene	990	1.3
Hexyl acetate	1010	0.1
1,8-Cineole	1034	5.8
<i>Cis</i> -Ocimene	1037	0.2
<i>Trans</i> -Ocimene	1049	0.5
Linalool	1099	33.4
Octen-3-ol Acetate	1122	0.3
Camphor	1048	7.6
Hexyl isobutanoate	1153	0.2
4-Terpineol	1176	2.1
α -Terpineol	1191	1
Hexyl butanoate	1194	0.4
Linalyl acetate	1260	36.2
Lavandulyl acetate	1291	3
Neryl acetate	1363	0.7
Geranyl acetate	1384	1.4
β -Caryophyllene	1421	0.6
Farnesene	1455	0.6
Caryophyllene Oxide	1585	0.6
α -Bisabolol	1690	0.2
Total identified		96.6

^a Compounds listed in order of elution from a SE52 column.

^b Kovats indices were calculated against n-alkanes on a SE52 column.

Animals

Experiments were performed on adult male Swiss mice (20–30 g) and female Wistar rats (150–200 g), purchased from Charles River, Italy. All animals were fasted but had free access to water 18 hours before the experiments. Experiments were carried out in accordance to the Italian law (DL 116/92) and approved by Ministero della Salute.

Experiments lasted as briefly as possible and the number of animals was kept to the minimum to demonstrate the effects of the drug treatment.

Drugs

The following substances were used: linalool, linalyl acetate, atropine sulfate, naloxone hydrochloride, mecamlamine hydrochloride, methylcellulose, acetic acid and ethanol (purchased from SIGMA, Italy), indomethacinate meglumine (kindly given by Chiesi farmaceutici, Italy).

Oral treatment

Groups of 8–10 animals received by gavage lavender oil (100 mg/kg), linalool (33 mg/kg) and linalyl acetate (36 mg/kg) in a final volume of 1 ml/100 g body weight 1 hour before the experiments. Linalool and linalyl acetate were administered at doses chosen in accordance to the percentages of these constituents in the natural oil as detected by gas chromatography. Control animals received vehicle alone (0.1 % methylcellulose). A 1% aqueous emulsion of lavender oil in methylcellulose was prepared immediately before use.

Inhalation

When inhaled, 200 µl of lavender oil, linalool or linalyl acetate, contained in a 10 ml glass baker, were positioned at the bottom of plastic cages (height 20 cm, width 30 cm, depth 20 cm) suddenly covered with plastic film in order to saturate the ambient. At saturation, the concentration of the oils in the cage was 2.4 µl/l. Mice introduced into the cage were allowed to inhale oil vapors for controlled time periods (15, 30 and 60 min) prior to performing the final experiments. Control animals were caged in the same conditions but in the absence of the tested oils. All experiments were conducted between 9.00 and 15.00.

Acetic acid writhing test

The writhing test was performed according to [Koster's method \(1959\)](#); briefly, concluded the inhalation time or passed 1 hour from the oral administration of the oils under study or the vehicle, mice were intraperitoneally injected with 0.2 ml of 0.6% acetic acid. After treatment with the algogen agent, mice were placed in observational chambers and the number of writhes of each mouse was counted over a period of 30 min. Different sets of mice were pretreated with the opioid antagonist naloxone (5 mg/kg i.p.), the muscarinic antagonist atropine (5 mg/kg i.p.) and the nicotinic antagonist mecamlamine (1 mg/kg i.p.) 10 min before the tested oils or vehicle challenge.

Hot plate test

The hot plate test was performed according to the method described by Eddy and Leimbach (1953). Mice were individually placed on the 55 °C hot plate apparatus [Model 475, Technical Lab Instruments Inc, Pequannock (NJ), USA] and the time between the placement and the occurrence of anterior paw licking, shacking or jumping was recorded as Latency Time (s). In order to exclude hypo- or hyper-sensitive mice, two hours before the final experiment all the animals were tested and those with latency time shorter than 10 sec or longer than 18 sec were eliminated from the study. Basal Latency Time (T_0) was measured before the administration of drugs or vehicle. Forward Latency Times (T_1) were measured starting 1 hour after oral treatment or after 15, 30 and 60 min of exposure to oils vapor with intervals of 15, 30 and 60 min. Different groups of mice were pretreated with naloxone (5 mg/kg i.p.), atropine (5 mg/kg i.p.) and mecamlamine (1 mg/kg i.p.) 10 min before the tested oils or vehicle administration.

Time of 30 sec was arbitrarily chosen as cut-off time (T_2). Results were expressed as percentage of analgesic effect as follows: % MPE (percent maximal possible effect) = $(T_1 - T_0) / (T_2 - T_0) \times 100$ (latency time after treatment - basal latency time) / (time of cut off - basal latency time).

Locomotor activity

Locomotor activity was measured by means of an activity cage [height 35 cm, width 23 cm, depth 19 cm, Model 7401, Ugo Basile, Comerio (VA), Italy]. Passed one hour from oral administration of lavender oil or vehicle or at the end of inhalation period times, mice were placed singularly into the activity cage and locomotor activity was recorded every 5 minutes for 90 min. All experiments were conducted from 9.00 to 13.00.

Acute gastrointestinal ulcerogenicity

Acute gastrointestinal ulcerogenicity was assessed following Rainsford's method (1982). Briefly, rats were treated orally with lavender oil 100 mg/kg. After 5 hours, animals were sacrificed by CO₂ inhalation, the stomachs were removed, fixed in 4% formaldehyde solution and processed for microscopic analysis using an image analyzer system (Leitz, ASM 68K). The total damaged area (mm²) and the number of gastric ulcers were counted for each stomach by an observer unaware of the treatment given to the animals.

Protection against acute indometacin- and ethanol-induced gastric lesions

Lavender oil, linalool and linalyl acetate were tested as potential gastroprotective drugs in two different models of acute gastric ulcers. For this purpose, rats were randomly assigned to 4 groups of 8–10 animals each one.

To evaluate the ability of oils to protect against NSAIDs-induced gastric ulcers, animals were treated simultaneously with indomethacin (40 mg/kg i.p.) and oils per os. The animals were killed 5 hours later. The protection against ethanol-induced gastric lesions was tested administering orally 1 ml of 90% ethanol to animals which 1 hour previously had been treated orally with essential oils. The animals were killed 1 hour later. Stomachs were then removed and processed as described before.

Statistical analysis

All data are expressed as mean \pm S.E.M (n=8–10 observations per group). Results were analyzed statistically using Student's t-test for unpaired data (two tailed). P values less than 0.05 or 0.01 were considered as indicative of significance or high significance respectively.

Results

Chemical composition of the essential oil

Table 1 shows the composition and the relative abundance of the constituents identified in the lavender essential oil. The major components are linalool (33.44%) and linalyl acetate (36.15%).

Acetic acid writhing test

Administration of lavender oil 100 mg/kg os significantly reduced the writhing response to acetic acid treatment to 51% over the control group (P=0.0002) (Fig. 1). This antinociceptive effect was significantly prevented by opioid antagonist naloxone pretreatment but it was completely unaffected by either nicotinic antagonist mecamlamine or muscarinic antagonist atropine administered at doses by themselves unable to modify nociceptive response. When the effects of the two major components,

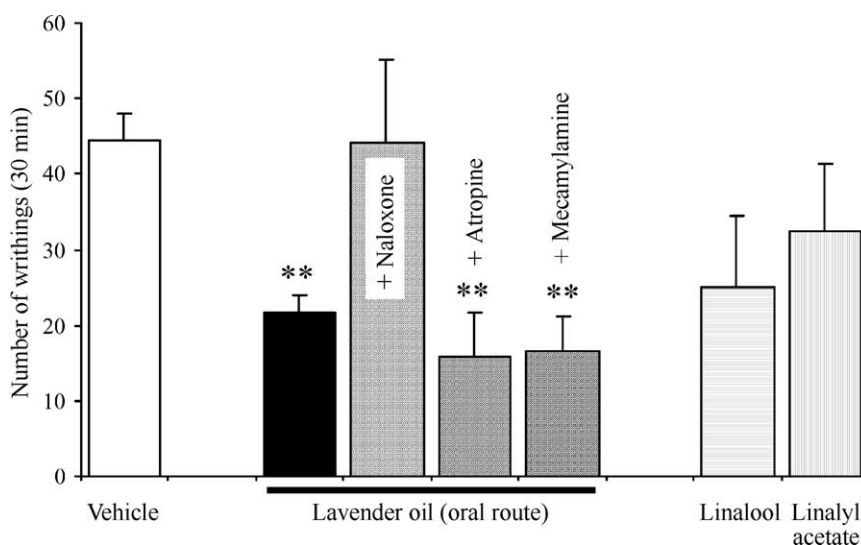


Fig. 1. Effect of oral administration of lavender essential oil (100 mg/kg), linalool (33 mg/kg) and linalyl acetate (36 mg/kg), on the acetic acid-induced writhings and effect of pretreatment of animals with naloxone (5 mg/kg/ip), atropine (5 mg/kg/ip) and mecamlamine (1 mg/kg/ip) on lavender antinociceptive effect. The number of writhings was counted for 30 min following acetic acid injection. The vertical bars indicate the standard error of the mean. The number of mice used for each group was 8–10. **P < 0.01 compared to vehicle-treated mice.

linalool and linalyl acetate, were separately considered, a modest antinociception was observed only with linalool oral administration.

Inhalation of lavender essential oil attenuated the writhing numbers in a time dependent manner producing a significant antinociception (61% reduction over control, $P < 0.0001$) only after 60 minutes of exposure. In this case, the lavender oil antinociceptive effect was completely prevented by the administration of all the three different antagonists (Fig. 2).

Linalool and linalyl acetate inhalation for 60 minutes caused only a partial reduction of writhing response (Fig. 2).

Hot plate test

Oral administration of lavender oil 100 mg/kg failed to prolong latency time compared with controls in mice hot plate test. On the other hand, inhalation of lavender oil produced an inhibition of the hot-plate response proportional to the time of exposure to oil vapours, yielding a significant delay ($P < 0.01$) in reaction time after 60 minutes inhalation. This analgesic activity peaked at the suspension of inhalation and progressively diminished disappearing at 60 min. This lavender oil antinociceptive effect was significantly prevented by pretreatment with naloxone, atropine and mecamlamine, administered at doses by themselves unable to modify nociceptive response (Table 2). No analgesia was accounted after 60 minutes inhalation of linalool and linalyl acetate.

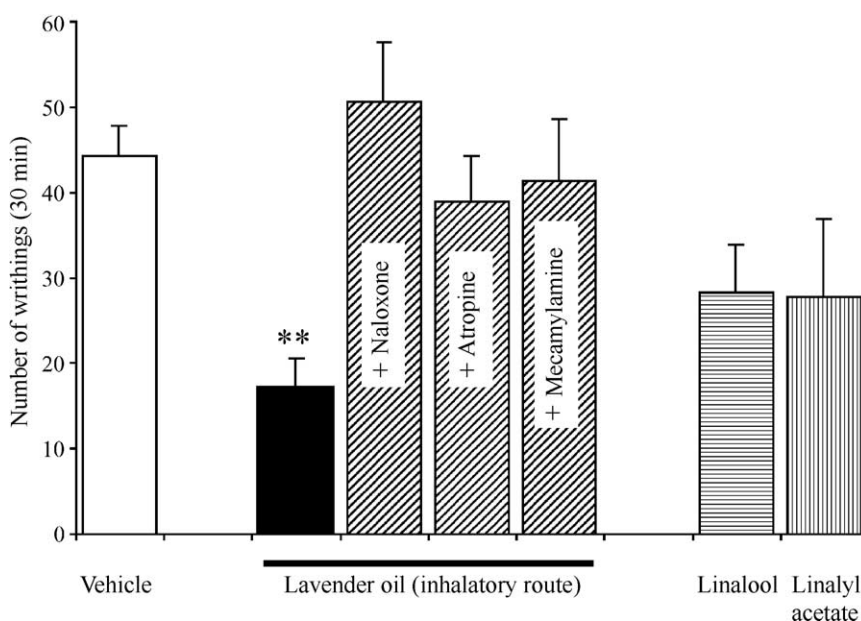


Fig. 2. Effect of lavender essential oil, linalool and linalyl acetate inhaled for 60 min on the acetic acid-induced writhings and effect of pretreatment with naloxone (5 mg/kg/ip), atropine (5 mg/kg/ip) and mecamlamine (1 mg/kg/ip) on antinociceptive effect produced by lavender oil. The number of writhings was counted for 30 min following acetic acid injection. The vertical bars indicate the standard error of the mean. The number of mice used for each group was 8–10. ** $P < 0.01$ compared to vehicle-treated mice.

Table 2

Time dependence of analgesic effect of inhaled lavender oil, linalool and linalyl acetate in hot plate test in mice in the absence and in the presence of pretreatment with naloxone (5 mg/kg/ip), atropine (5 mg/kg/ip) and mecamlamine (1 mg/kg/ip)

Treatment	time after the suspension of inhalation			
	0 min	15 min	30 min	60 min
Vehicle	-2.7 ± 6.8	-3.6 ± 9.7	-2.0 ± 8.9	-1.8 ± 7.2
Lavender oil	38.7 ± 13.6**	32.4 ± 10.2*	16.9 ± 7.8	-6.5 ± 7.2
Lavender oil + naloxone	1.6 ± 8.2	-26.8 ± 7.7	-14.9 ± 5.5	-11.3 ± 8.3
Lavender oil + atropine	-16.4 ± 6.3	-11.2 ± 8.8	-29.2 ± 11.2	18.9 ± 8.6
Lavender oil + mecamlamine	-10.3 ± 4.3	-20.3 ± 9.9	-24.5 ± 7.4	24.8 ± 8.4
Linalool	-4.9 ± 7.9	-18.8 ± 8.6	-6.4 ± 5.4	-12.5 ± 5.8
Linalyl acetate	4.2 ± 5.9	2.3 ± 7.1	-3.5 ± 7.2	17.3 ± 7.2

Results are expressed as mean ± S.E.M of percent Maximal Possible Effect.

* P < 0.05 compared to vehicle-treated mice.

** P < 0.01 compared to vehicle-treated mice.

Effect on locomotor activity

No significant alterations of locomotor activity were observed in mice after treatment with lavender oil either orally administered at the dose of 100 mg/kg (2440 ± 544 counts in 90 min) or inhaled for 60 minutes (2815 ± 872 counts in 90 min) with respect to vehicle-treated animals (1974 ± 598 counts in 90 min).

Gastrolesivity and gastroprotection

Acute oral treatment with lavender oil (100 mg/kg os) did not produce any damage on rat gastric mucosa. Regarding the gastroprotection, neither lavender oil oral administration nor oil inhalation

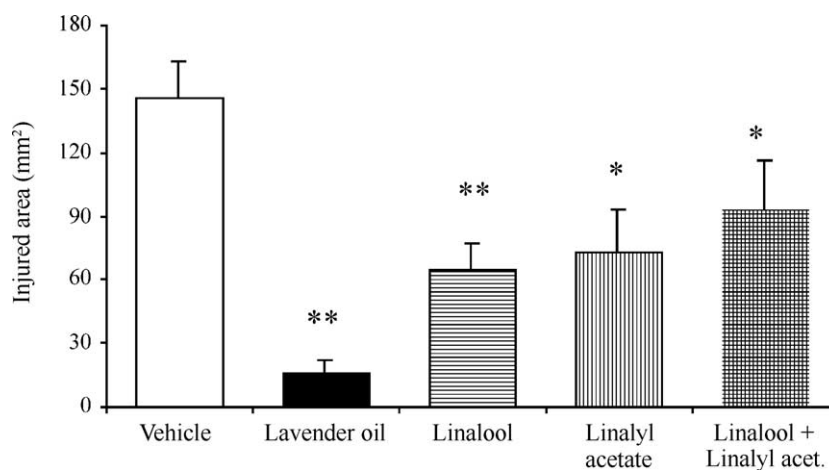


Fig. 3. Effect of oral administration of lavender essential oil (100 mg/kg), linalool (33 mg/kg), linalyl acetate (36 mg/kg) and linalool (33 mg/kg) plus linalyl acetate (36 mg/kg) on ethanol-induced gastric ulcers. Results are expressed as total injured area. Vertical bars indicate the standard error of the mean. The number of rats used for each group was 8–10. *P < 0.05 and **P < 0.01 compared to vehicle-treated rats.

protected against indomethacin-induced gastric ulcers being the number and the area of gastric lesions observed in lavender treated rats comparable to vehicle-treated animals (ulcer number 14.7 ± 4.8 and 18.7 ± 4.3 respectively and injured area $8.2 \pm 4.4 \text{ mm}^2$ and $6.9 \pm 2.3 \text{ mm}^2$ respectively).

A significant prevention of acute ethanol-induced gastric lesions was elicited by lavender oil oral administration (100 mg/kg) as it reduced the total injured area by an 89% ($P = 0.003$) compared with control. A lower but still significant gastroprotection was obtained with the administration of linalool (33 mg/kg os) and linalyl acetate (36 mg/kg os) which diminished the hemorrhagic erosion areas of about 56% ($P = 0.003$) and 49% ($P = 0.013$) respectively (Fig. 3). Co-administration of the two components did not enhance the gastroprotection produced by the oils singularly administered. Essential oil inhalation for 60 min failed to protect gastric mucosa from necrotizing action of ethanol (data not shown).

Discussion

In the current study we demonstrate that oral treatment with whole lavender oil produces significant antinociception and gastroprotective activity in animal models. This pharmacological activity could derive from the contribution of various active principles composing the whole oil such as linalool, myrcene and 1–8 cineole, previously proved to possess antinociceptive activity (Peana et al., 2003; Aydin et al., 1999; Rao et al., 1990). Higher analgesic efficacy was exhibited by lavender oil when administered through inhalatory route being the nociceptive responses to chemical (writhing test) and thermal (hot plate test) stimuli significantly reduced. At variance with lavender oil, both linalool and its metabolic precursor linalyl acetate produce only scarce or no analgesic effect in the two pain models here adopted. The different dosage applied in this study with respect to previous investigations (Peana et al., 2003, 2004) can account for the lack of antinociceptive activity of linalool both in writhing and hot plate tests. The absence of any modification of spontaneous locomotor activity after oral/inhaled administration of antinociceptive doses of whole oil, led us to rule out the occurrence of sedative effect confounding analgesia studies. It must be pointed out that in literature the sedative effect of lavender oil upon inhalation in mice have been clearly described (Buchbauer et al., 1991, 1993). However present findings are not conflicting with previous data describing the sedative effect of lavender since in this work we studied locomotor activity by exposing mice to lavender oil lower air concentration than that proved to produce sedative serum oils levels after inhalation (Buchbauer et al., 1993; Letizia et al., 2003a,b). As for the mechanism underlying lavender oil analgesic action it is noteworthy that opioidergic neurotransmission seems to be primary involved in oral induced analgesia since only naloxone pretreatment prevents lavender effect in writhing test. Also cholinergic system appears to play a significant role in lavender oil analgesia displayed after inhalation since also the blockade of muscarinic and nicotinic receptors prevented antinociception. The involvement of cholinergic transmission could be ascribed to some component terpenes of the oil in addition to linalool, since potent in vitro anticholinesterase activity was reported for terpenoids such as 1,8-cineole, a constituent of various essential oils (Savelev et al., 2003).

As concerns antiulcer activity, interestingly, linalool as well as linalyl acetate demonstrate to contribute to the gastroprotective effect of lavender oil which, orally administered, caused a dramatic reduction of ethanol-induced gastric injury in rats. The involvement also of additional active principles, such as the gastroprotective agent 1,8-cineole (Santos and Rao, 2001), cannot be ruled

out since the antiulcer effect of the co-administration of linalool and linalyl acetate is lower than that of whole oil.

The lack of protective effect against gastric mucosal damage caused by indomethacin led us to hypothesize that gastroprotection afforded by lavender oil cannot be attributed to interference with arachidonic acid metabolic cascade. Actually, we have already described an interesting ability of lavender oil to prevent experimental thrombus formation with an ASA-unlike mechanism of action (Ballabeni et al., 2004). The amelioration of gastric microcirculation could be the mechanism underlying the lavender gastroprotection against ethanol injury which is known to be dependent on microvasculature engulfment in the gastric mucosa (Oates and Hakkinen, 1988).

In conclusion the results of this study reveal a remarkable analgesic and gastroprotective activities of oral lavender oil at doses 100–400 fold lower than those proved to be acutely toxic for the main components of the phytocomplex (Letizia et al., 2003a,b). Furthermore, the effectiveness of oil inhalation in controlling chemical and thermal pain without evidence of central adverse effects supports the interest for potential application of lavender essential oil in aromatherapy.

Acknowledgements

The authors wish to thank Dr. Giuseppe Domenichini for the valuable technical assistance and J. P. Féraud, M. Zunino and M. Vernet (S. Jours, Plateau de Valensole, France) for the donation of *Lavandula hybrida* Reverchon cv. Grosso essential oil. This work was supported by a grant from the University of Parma (FIL 2002).

References

- Adams, R.P., 2001. Identification of essential oil compounds by GC-quadrupole mass-spectrometry. Allured Publishing Corporation, Carol Stream Illinois, USA.
- Aydin, S., Demir, T., Ozturk, Y., Baser, K.H., 1999. Analgesic activity of *Nepeta italica* L. *Phytotherapy Research* 13 (1), 20–23.
- Ballabeni, V., Tognolini, M., Chiavarini, M., Impicciatore, M., Bruni, R., Bianchi, A., Barocelli, E., 2004. Novel antiplatelet and antithrombotic activities of essential oil from *Lavandula hybrida* Reverchon “Grosso”. *Phytomedicine* (In press).
- Buchbauer, G., Jirovetz, L., Jager, W., Dietrich, H., Plank, C., 1991. Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. *Zeitschrift fur Naturforschung* 46 (11–12), 1067–1072.
- Buchbauer, G., Jirovetz, L., Jager, W., Plank, C., Dietrich, H., 1993. Fragrance compounds and essential oils with sedative effects upon inhalation. *Journal of Pharmaceutical Sciences* 82 (6), 660–664.
- Calcina, F., Chiavarini, M., Bianchi, A., Albasini, A., Bruni, R., Barocelli, E., 2003. Antinociceptive effects of inhaled and orally administered *Lavandula hybrida* Reverchon “Grosso” essential oil. The Third International Symposium on Natural Drugs, Naples, Italy, October 2–4, 2003, p. 229.
- Dunn, C., Sleep, J., Collett, D., 1995. Sensing an improvement: an experimental study to evaluate the use of aromatherapy, massage and periods of rest in an intensive care unit. *Journal of Advanced Nursing* 21 (1), 34–40.
- Eddy, N.B., Leimbach, D., 1953. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapy* 107 (2), 385–393.
- Ghelardini, C., Galeotti, N., Salvatore, G., Mazzanti, G., 1999. Local anaesthetic activity of the essential oil of *Lavandula angustifolia*. *Planta Medica* 65 (8), 700–703.
- Graham, P.H., Browne, L., Cox, H., Graham, J., 2003. Inhalation aromatherapy during radiotherapy: results of a placebo-controlled double-blind randomized trial. *Journal of Clinical Oncology* 21 (12), 2372–2376.

- Koster, R., Anderson, M., De Beer, E.J., 1959. Acetic acid for analgesic screening. *Federation Proceedings* 18, 412–421.
- Letizia, C.S., Cocchiara, J., Lalko, J., Api, A.M., 2003a. Fragrance material review on linalool. *Food and Chemical Toxicology* 41 (7), 943–964.
- Letizia, C.S., Cocchiara, J., Lalko, J., Api, A.M., 2003b. Fragrance material review on linalyl acetate. *Food and Chemical Toxicology* 41 (7), 965–976.
- Lis-Balchin, M., Hart, S., 1997. A preliminary study of the effect of essential oils on skeletal and smooth muscle in vitro. *Journal of Ethnopharmacology* 58 (3), 183–187.
- Lis-Balchin, M., Hart, S., 1999. Studies on the mode of action of the essential oil of lavender (*Lavandula angustifolia*). *Phytotherapy Research* 13 (6), 540–542.
- Oates, P.J., Hakkinen, J.P., 1988. Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology* 94 (1), 10–21.
- Peana, A.T., D'Aquila, P.S., Panin, F., Serra, G., Pippia, P., Moretti, M.D., 2002. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine* 9 (8), 721–726.
- Peana, A.T., D'Aquila, P.S., Chessa, M.L., Moretti, M.D., Serra, G., Pippia, P., 2003. (–)-Linalool produces antinociception in two experimental models of pain. *European Journal of Pharmacology* 460 (1), 37–41.
- Peana, A.T., De Montis, M.G., Nieddu, E., Spano, M.T., D'Aquila, P.S., Pippia, P., 2004. Profile of spinal and supraspinal antinociception of (–)-linalool. *European Journal of Pharmacology* 485 (1–3), 165–174.
- Rainsford, K.D., 1982. An analysis of the gastro-intestinal side-effects of non-steroidal anti-inflammatory drugs, with particular reference to comparative studies in man and laboratory species. *Rheumatology International* 2 (1), 1–10.
- Rao, V.S., Menezes, A.M., Viana, G.S., 1990. Effect of myrcene on nociception in mice. *Journal of Pharmacy and Pharmacology* 42 (12), 877–878.
- Santos, F.A., Rao, V.S., 2001. 1,8-cineol, a food flavoring agent, prevents ethanol-induced gastric injury in rats. *Digestive Disease Science* 46 (2), 331–337.
- Savelev, S., Okello, E., Perry, N.S., Wilkins, R.M., Perry, E.K., 2003. Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. *Pharmacology Biochemistry and Behavior* 75 (3), 661–668.
- Yamada, K., Mimaki, Y., Sashida, Y., 1994. Anticonvulsive effects of inhaling lavender oil vapour. *Biological Pharmaceutical Bulletin* 17 (2), 359–360.