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# Analgesic and Antiinflammatory Effects of Two Chemotypes of *Lippia alba*: a Comparative Study

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*ABSTRACT*

*The antinociceptive and antiedematogenic effects of essential oils (EO, types I and II) from the leaves of two chemotypes of* Lippia alba *were studied with mice using the hot plate test, acetic acid-induced writhing, and the formalin test, and with rats using paw edema induced by carrageenan or dextran. The results showed dose-dependent inhibition of writhing with doses of 0.5 and 1 mg/kg, i.p., and 1 and 2 mg/kg, p.o., with chemotypes I and II, respectively. A similar but less intense effect was detected in the formalin test, where the two chemotypes (0.5 and 1 mg/kg, i.p.) predominantly inhibited the 2nd phase of the response, and only the effect of the EO I was reversed by the opioid antagonist, naloxone. Latency time to the thermic stimulus as detected by the hot plate test was increased with I but not with II, at doses higher than 10 mg/kg, p.o. A significant antiedematogenic effect was seen at 2 h with 10 and 50 mg/kg, p.o., of I, in paw edema induced by carrageenan or dextran. However, in the same dose range, II was more effective against dextran-induced edema, but no effect was seen with the carrageenan model. The essential oils of the two types of* L. alba *are chemically distinct, with I containing a high content of citral and II a high content of carvone with no citral, which could explain the observed differences in their pharmacological actions.*

*Keywords: Lippia alba*, chemotypes, limonene, carvone, citral, analgesic, antiinflammatory.

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## INTRODUCTION

The name “cidreira” is popularly used in Brazil in order to designate 17 species belonging to different botanical families which have in common a lemon-like aroma. *L. alba* (Mill.) N.E. Brown (Verbenaceae) is a small shrub cultivated in Brazil for popular medicinal purposes, e.g., as a spasmolytic and tranquilizer (Correa, 1990). Due to the great morphological and chemical variability of these herbs, a recent study (Matos et al., 1996) on the chemical composition of their essential oils was carried out with the species occurring in Northeast Brazil. The objectives of the present paper were to study comparatively the analgesic and antiinflammatory activities of the essential oils (EO) from the leaves of two chemotypes of *L. alba*, both bearing a high content of limonene, in addition to citral in type I and carvone in type II.

## MATERIALS AND METHODS

### Essential Oil Preparation

Plants were cultivated at the Medicinal Plants Garden and their excicatae deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará, Brazil, under the numbers 24,150; 24,149; 24,155 and 24,158. Samples of the essential oils from the leaves of the chemotype “citral” (type I) and “carvone” (type II) plants were prepared by the Laboratory of Natural Products as described previously (Matos et al., 1996).

### Pharmacological Tests

*Hot plate test:* Groups of female Swiss mice (25–30 g) were placed on a hot plate (55°C) and observed for reaction time to thermal stimulus. The cut-off time was 40 s. The effects on the groups treated with the EO (1,

10 and 50 mg/kg, i.p.; 10 and 50 mg/kg, p.o.) were measured at 30 (i.p.) and 60 min (p.o.), respectively, after EO administration. Control groups were treated with saline or meperidine (10mg/kg, i.p.) (Woolfe & MacDonald, 1944).

*Acetic acid-induced writhing:* Female Swiss mice (25–30 g) were utilized. Animals were injected with 0.6% acetic acid (10 ml/kg, i.p.) and writhing was quantified over 20 min. Animals treated with EO (0.5; 1 and 10 mg/kg, i.p., or 1 and 2 mg/kg, p.o.) were injected 30 (i.p.) or 60 (p.o.) min before acetic acid administration (Koster et al., 1959). A statistically significant reduction in writhing brought about by any treatment as compared to controls injected with vehicle was considered to be a positive analgesic response.

*Formalin test:* Twenty µl of 1% formalin were administered (subplantar) to female Swiss mice (25–30g body weight), and the licking time was recorded for 5 min (1st phase, neurogenic). Licking time was recorded for an additional 5 min, 20 min after the beginning of the experiment (2nd phase, inflammatory). For the treated groups, EO (0.5, 1, 10 mg/kg, i.p., or 0.5, 1, 10 mg/kg, p.o.) was administered 30 (i.p.) or 60 min (p.o.) before formalin injection. The opioid antagonist, naloxone (2 mg/kg, s.c.), was used to assess involvement of the opioid system, and meperidine was used as a standard drug (Fasmer et al., 1985; Tjolsen et al., 1992).

*Antiedematogenic activity:* EO (1 and 10 mg/kg, i.p., or 10 and 50 mg/kg, p.o.) was administered to Wistar rats (200–250 g), 30 min (i.p.) or 60 min (p.o.) before the intraplantar injection of 0.1 ml 1% carrageenan. A similar experiment was carried out with 1.5% dextran (0.1 ml/animal). Edema was measured with a plethysmometer (Ugo Basili, Italy) and expressed as the difference between the paw volume in ml, before and 1/2, 1, 2, 3 and 4 h after the administration of carrageenan or dextran.

## RESULTS

### Antinociceptive Effects of EO (Types I and II) from *L. alba*

Table 1 presents the effect of the two chemotypes of EO on writhing in mice. Type I (0.5 and 1 mg/kg, i.p.) inhibited abdominal contractions produced by acetic acid by 48 and 79%, respectively. At the same doses, type II produced a significant inhibition (52%) only with the higher dose, as compared to controls . After oral administration (1 and 2 mg/kg), inhibitions of 65 and 71 (type I), and 55 and 82% (type II), respectively, were demonstrated. Meperidine caused 100% inhibition of abdominal contractions, and its effect was reversed by naloxone. This compound reversed the inhibitory effect seen with EO I, 1mg/kg, i.p. (79 and 32% in the absence and presence of naloxone, respectively), and it had no effect on EO II.

Table 2 shows the results of the formalin test. No effect was observed when EO I or II were administered intraperitoneally at the lowest dose (0.5 mg/kg). However, a dose-response relationship was demonstrated with EO I (39 and 60% inhibition of the 2nd phase after

TABLE 1. Effect of EO on acetic acid-induced writhing in mice.

| Group | Writhing number | Inhibition (%) |
|---|---|---|
| Control | 34.3 ± 5.7 (14) | – |
| Meperidine | 0.0 ± 0.0 (6)* | 100.0 |
| Mep + Naloxone | 8.3 ± 5.6 (6)* | 75.8 |
| EO I (0.5 mg/kg, i.p.) | 17.8 ± 4.8 (6)* | 48.1 |
| EO I (1 mg/kg, i.p.) | 7.1 ± 2.2 (12) * | 79.3 |
| EO I (1 mg/kg, i.p.) + Naloxone | 23.4 ± 5.2 (5) | 31.8 |
| EO II (0.5 mg/kg, i.p.) | 21.8 ± 7.3 (6) | 36.4 |
| EO II (1 mg/kg, i.p.) | 16.6 ± 3.3 (11)* | 51.6 |
| EO II (1 mg/kg, i.p.) + Naloxone | 14.3 ± 4.6 (6) * | 58.3 |
| Control | 29.2 ± 5.6 (5) | – |
| EO I (1 mg/kg, p.o.) | 10.3 ± 2.9 (6)* | 64.8 |
| EO I (2 mg/kg, p.o.) | 8.4 ± 1.9 (5) * | 71.2 |
| EO II (1 mg/kg, p.o.) | 13.2 ± 2.8 (5)* | 54.8 |
| EO II (2 mg/kg, p.o.) | 5.4 ± 1.9 (5) * | 81.5 |

Data are reported as means ± SEM of the writhing number recorded over a period of 20 min. The number of animals per group is given in parentheses. Mep = meperidine.
* $p < 0.05$ as compared to controls (ANOVA and Fisher and Scheffé tests).

TABLE 2. Effect of EO observed with the formalin test in mice.

| Group | Licking time (s) | | inhibition (%) | |
|---|---|---|---|---|
| | 1st phase | 2nd phase | 1st phase | 2nd phase |
| Control | 55.4 ± 2.9 | 21.1 ± 2.3 | – | – |
| EO I (0.5 mg/kg,i.p.) | 61.3 ± 6.5 | 23.8 ± 4.5 | – | – |
| EO I (1 mg/kg,i.p.) | 50.5 ± 4.6 | 12.9 ± .1* | – | 38.9 |
| EO I (10 mg/kg,i.p.) | 57.5 ± 3.9 | 8.4 ± 1.4* | – | 60.2 |
| EO I 10 + Nx 10 | 56.5 ± 5.9 | 28.1 ± 6.3 | – | – |
| EO II (0.5 mg/kg,i.p.) | 50.0 ± 3.9 | 21.6 ± 6.5 | – | – |
| EOII (1 mg/kg,i.p.) | 49.5 ± 5.4 | 10.9 ± 2.7* | – | 48.3 |
| EO II (10 mg/kg,i.p.) | 54.5 ± 4.2 | 15.9 ± 2.5 | – | 24.6 |
| Mep (10 mg/kg,s.c.) | 41.9 ± 4.4* | 5.7 ± 1.8* | 17.0 | 73.0 |
| Mep 10 + Nx 10 | 51.6 ± 3.9 | 15.6 ± 5.9 | – | 26.1 |
| Control | 61.9 ± 4.0 | 22.8 ± 4.6 | – | – |
| EO I (1 mg/kg,p.o.) | 51.6 ± 3.7 | 16.8 ± 5.1 | 16.6 | 26.3 |
| EO I (10 mg/kg,p.o.) | 60.9 ± 5.6 | 4.6 ± 2.2* | – | 79.8 |
| EO II (1 mg/kg,p.o.) | 60.6 ± 4.4 | 20.8 ± 5.4 | – | – |
| EO II (10 mg/kg,p.o.) | 60.9 ± 5.6 | 14.1 ± 4.7 | – | 38.2 |

Data are reported as means ± SEM from 5 to 26 animals per group. Mep = meperidine and Nx = naloxone. *$p < 0.05$ (ANOVA and Fisher and Scheffé tests).

TABLE 3. Effect of EO observed with the hot plate test in mice.

| Group | Reaction time (s) | Inhibition (%) |
|---|---|---|
| Control | 11.1 ± 0.76 (18) | – |
| Meperidine | 18.3 ± 1.65 (24)* | 65.4 |
| Mep + Nal | 11.2 ± 1.38 (6) | – |
| EO I (1 mg/kg, i.p.) | 11.0 ± 1.06 (6) | – |
| EO I (10 mg/kg, i.p.) | 13.4 ± 1.20 (7) | 21.4 |
| EO I (50 mg/kg, i.p.) | 9.1 ± 0.55 (10) | – |
| EO II (10 mg/kg, i.p.) | 10.7 ± 1.33 (10) | – |
| EO II (50 mg/kg, i.p.) | 10.8 ± 1.11 (5) | – |
| Control | 11.8 ± 0.62 (16) | – |
| Meperidine | 18.3 ± 1.65 (24)* | 55.7 |
| Mep + Nx | 11.2 ± 1.38 (6) | – |
| EO I (10 mg/kg, p.o.) | 11.9 ± 0.85 (10) | – |
| EO I (50 mg/kg, p.o.) | 17.8 ± 1.72 (10)* | 51.5 |
| EO I (50 mg/kg, p.o. + Nx) | 9.6 ± 0.68 (5) | – |
| EO II (10 mg/kg, p.o.) | 9.7 ± 0.92 (6) | – |
| EO II (50 mg/kg, p.o.) | 10.8 ± 1.85 (6) | – |

Data are means ± SEM. The number of animals per goup is given in parentheses. Mep and Nx mean meperidine and naloxone, respectively. *$p < 0.05$ compared to controls (ANOVA and Fisher test).

intraperitoneal administration of 1 and 10 mg/kg, respectively). In the case of EO II, the response with 10 mg/kg (25% inhibition) was lower than the response with 1 mg/kg (48% inhibition). The effect observed with EO I (10 mg/kg) was totally reversed by naloxone (10 mg/kg), as with meperidine (10 mg/kg). No effect was observed with either EO after oral administration of 0.5 mg/kg. A significant inhibition (80%) was detected only with EO II after the high dose (10 mg/kg, p.o.) treatment.

Both types of EO increased latency time with the hot plate test (Table 3). Type I (10 and 50 mg/kg, i.p.) increased latency by 21 and 17%, respectively, compared to controls, while meperidine (10 mg/kg, i.p.), used as standard, increased response time by 65%. Naloxone, an opioid antagonist, totally reversed the effect of meperidine, but only partially reversed the inhibition seen with EO I. On the other hand, EO II (10 and 50 mg/kg, i.p.) was not effective. When administered orally, EO I (10 and 50 mg/kg, i.p.) showed an inhibition of 52% only at the higher dose, while EO II, at the same doses, was not effective.

**Antiedematogenic Effects of the EO I and II**

Antiedematogenic activities of EO I and II were studied in the models of edema induced by carrageenan and dextran in rats (Table 4). In carrageenan-induced edema, no significant effects were observed after intraperitoneal administration with either types at the dose of 10 mg/kg. However, orally, type I (10 mg/kg) produced 41 and 32% inhibition at the 3rd and 4th hours, respectively. Similar effects were seen with the higher dose (50 mg/kg, p.o.) with both types of EO which produced inhibition ranging from 32–50% at the 2nd, 3rd and 4th hours. While significant inhibition (28 and 42% at the 3rd and 4th hours, respectively) was observed with EO II at the dose of 10 mg/kg, p.o., no effect was seen with 50 mg/kg, p.o.

In dextran-induced edema, both types showed inhibition close to 30% with the dose of 10 mg/kg, i.p., at the 4th hour. Similar inhibition was demonstrated with EO I at a dose of 10 mg/kg, p.o., and higher responses (51.7, 48.6 and 53.1% inhibition) were observed at the 2nd, 3rd and 4th hours with 50 mg/kg, p.o. Inhibition ranging from 30 to 57% was demonstrated with type II (10 and 50 mg/kg, p.o.) at the same time intervals.

## DISCUSSION

Previous work from our laboratory (Vale et al., 1992) showed that the hydroalcoholic extract (HAE) from the chemotype I of *L. alba* administered intraperitoneally caused ataxia and somnolence in mice, and increased significantly the latency to thermal stimulus measured by the hot plate test, indicating the central effect of the plant and justifying its use as a tranquilizer. HAE also mediated analgesic activity (Costa et al., 1989) which led us to further explore the effects and mechanisms of action of EO from *L. alba*.

In the state of Ceará, Brazil, two chemotypes of *L. alba*, based on GC/MS analysis of their essential oils, were identified (Matos et al., 1996). These results showed neral (27.2–30.4%) and geranial (35.6–41.0%) as the main constituents of one type, while carvone (42.3–54.7%) was predominant in the other type, which has no citral. The citric aroma presented in both chemotypes is due to limonene.

The herb tea of the two varieties of *L. alba* is largely consumed in the Northeastern region of Brazil, mainly as a spasmolytic and tranquilizer. Another species, *L. citriodora* HBK, originally from South America, is similar to *L. alba*, and also used as a digestive, spasmolytic and sedative. The essential oil from its leaves has citral as the main constituent, besides limonene, geranial, citronelal, α- and β-oinene, cineol, linalol and ethyl-eugenol (Fester et al., 1961; Guenther, 1972; Montes, 1973; Silva et. al., 1979). World-wide, the leaves of this so-called lemon-verbena (*L. citriodora*) are used to flavor drinks, fruit and sweet dishes, and to make herb tea (Bremness, 1994). The tea is refreshing and a mild sedative. It soothes bronchial and nasal congestion and eases indigestion and nausea. The leaves

TABLE 4. Effects of EO on the paw edema induced by carrageenan and dextran in rats.

| Group | Paw edema (ml) at | | | | |
|---|---|---|---|---|---|
| | 0.5 h | 1 h | 2 h | 3 h | 4 h |
| Control (C), i.p. | 0.3 ± 0.04 | 0.6 ± 0.05 | 0.9 ± 0.10 | 1.2 ± 0.1 | 1.0 ± 0.14 |
| EO I (10 mg/kg) | 0.3 ± 0.04 | 0.6 ± 0.05 | 1.0 ± 0.1 | 1.2 ± 0.1 | 0.9 ± 0.1 |
| EO II (10 mg/kg) | 0.3 ± 0.10 | 0.6 ± 0.06 | 0.8 ± 0.1 | 1.0 ± 0.06 | 0.9 ± 0.04 |
| Control (C), p.o. | 0.73 ± 0.10 | 0.69 ± 0.08 | 0.88 ± 0.07 | 0.93 ± 0.1 | 0.74 ± 0.09 |
| EO I (10 mg/kg) | 0.58 ± 0.07 | 0.64 ± 0.1 | 0.6 ± 0.11 | 0.55 ± 0.09* | 0.50 ± 0.08* |
| EO I (50 mg/kg) | 0.48 ± 0.05* | 0.58 ± 0.02 | 0.6 ± 0.03* | 0.47 ± 0.05* | 0.46 ± 0.05* |
| EO II (10 mg/kg) | 0.65 ± 0.06 | 0.66 ± 0.06 | 0.77 ± 0.07 | 0.67 ± 0.08* | 0.43 ± 0.04* |
| EO II (50 mg/kg) | 0.69 ± 0.14 | 0.79 ± 0.16 | 0.91 ± 0.20 | 0.87 ± 0.25 | 0.77 ± 0.23 |
| Control (D), i.p. | 1.7 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 | 1.6 ± 0.20 | 1.7 ± 0.2 |
| EO I (10 mg/kg) | 1.3 ± 0.1* | 1.5 ± 0.1* | 1.5 ± 0.1 | 1.3 ± 0.1 | 1.1 ± 0.1* |
| EO II(10 mg/kg) | 1.3 ± 0.1* | 1.6 ± 0.1* | 1.8 ± 0.1 | 1.4 ± 0.1 | 1.2 ± 0.1 |
| Control (D), p.o. | 1.63 ± 0.12 | 1.74 ± 0.12 | 2.03 ± 0.17 | 1.77 ± 0.12 | 1.47 ± 0.08 |
| EO I (10 mg/kg) | 1.81 ± 0.16 | 2.03 ± 0.09 | 1.43 ± 0.15* | 1.28 ± 0.22* | 1.05 ± 0.27* |
| EO I (50 mg/kg) | 1.24 ± 0.07 | 1.43 ± 0.09 | 0.98 ± 0.07* | 0.91 ± 0.10* | 0.69 ± 0.22* |
| EO II (10 mg/kg) | 1.68 ± 0.07 | 1.94 ± 0.08 | 1.42 ± 0.11* | 1.06 ± 0.09* | 0.80 ± 0.09* |
| EO II (50 mg/kg) | 1.72 ± 0.06 | 1.93 ± 0.08 | 1.33 ± 0.14* | 0.97 ± 0.19* | 0.63 ± 0.10* |

Data are means ± SEM of 6 to 12 animals per group. The letters C or D after the word Control indicate carrageenan or dextran, respectively. *$p < 0.05$ (ANOVA and Fisher and Scheffé tests).

yield a green color and an essential oil used in perfumes and bath lotions. A leaf infusion relieves puffy eyes and, as a floral vinegar, it softens the skin (Bremness, 1994).

In the present work, although both chemotypes showed similar antinociceptive effects, these were greater with type II, and in this case not reversed by naloxone. However, the analgesic effect studied by the hot plate test, indicative of a central action, was evident only with type I. Surprisingly, both types were more active orally, and the antiinflammatory activity was predominantly manifested with the dextran model.

Antinociceptive activities were previously demonstrated with several types of monoterpenes including carvone and limonene (Hart et al., 1994). However, while carvone was active at 25 mg/kg, s.c., myrcene and limonene required a higher dose (50 mg/kg, s.c.). It was concluded, therefore, that monoterpenes may have analgesic potential.

Besides analgesic and antiinflamatory activities, the essential oils of the two chemotypes of *L. alba* studied in the present work also showed spasmolytic and anticonvulsive effects (data not shown). All these effects were observed with both the hydroalcoholic extract and the essential oil of the plant at doses below 100 mg/kg. In addition, toxic potential appears low, suggesting this species may be useful in phytotherapy.

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## REFERENCES

Bremness L (1994): *Herbs*. London, Dorling Kindersley Ltd.

Correa CBV (1990): Contribuição ao estudo de *Lippia alba* (Mill.) N.E. Br. Ex Br. Ex Britt. & Wilson - erva cidreira. Master Thesis in Biological Sciences, Federal University of Rio de Janeiro.

Costa M, Di-Stasi LC, Kirizawa M (1989): Screening in mice of some medicinal plants used for analgesic purposes in the state of São Paulo (Brazil): Part II. *J Ethnopharmacol 27:* 25–34.

Fester GA, Martinuzzi EA, Retamar J, Ricciardi A, Fonseca LR, Cassano A (1961): Variedades fitoquímicas em plantas aromáticas argentinas. *Rev Fac Cienc Agrar Mendonza 8:* 45–49.

Fasmer OB, Berge OG, Hole K (1985): Changes in nociception after lesions of descending serotoninergic pathways induced with 5,6-dihydroxytryptamine: different effects in the formaline and tail flick test. *Neuropharmacol 24:* 729–734.

Guenther E (1972): *The Essential Oils,* Vol. VII. New York, R. Krieger Publ.

Hart SL, Gaffen Z, Hider RC, Smith TW (1994): Antinociceptive activity of monoterpenes in the mouse. Montreal, XII International Congress of Pharmacology.

Koster R, Anderson M, De Beer EJ (1959): Acetic acid analgesic screening. *Fed Proc 18:* 412.

Matos FJA, Machado MIL, Craveiro AA, Alencar JW (1996): Essential oil composition of two chemotypes of *Lippia alba* grown in Northeast Brazil. *J Essent Oil Res 8:* 695–698.

Montes ML (1973): Composition of the volatile oil from *Aloysia triphylla* (Cedron). *Planta Med 23:* 119–124.

Silva GAAB, Bauer L, Siqueira NCS, Bacha CTM, Santanna BMS (1979): O óleo essencial de *Lippia citriodora* Kuth. Do Rio Grande do Sul. *Tribuna Farmaceutica 47:* 96–98.

Tjolsen A, Berge OD, Hunskaar S, Rosland JH, Hoele K (1992): The formalin test: an evaluation of the method. *Pain 51:* 5–17.

Vale TG, Lima RES, Viana GSB, Matos FJA (1994): Efeitos analgésicos de *Lippia geminata* H.B.K. e de seus princípios ativos: neral e geranial. XII Encontro de Iniciação à Pesquisa, UFC, Fortaleza, Brazil, G607.

Woolfe G, MacDonald AD (1944): The evaluation of the analgesic action of pethidine hydrochloride (demerol). *J Pharmacol Exp Ther 80:* 300–307.

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