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Artigo

Pharmacological effects of the monoterpene α,β-epoxy-carvone in mice

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RESUMO: "Efeitos farmacológicos do monoterpeno α,β-epoxi-carvona em camundongos". O monoterpeno α,β-epóxi-carvona (EC) nas doses de 200, 300 e 400 mg/kg administrado por via i.p. em camundongos diminuiu significativamente a atividade motora dos animais, quando comparado aos controles, até 120 minutos após a administração. As doses de 300 e 400 mg/kg induziram um aumento significativo do tempo de sono dos animais não alterando, no entanto, a sua latência. O EC na dose de 400 mg/kg induziu uma redução no tempo de permanência dos animais na barra giratória (teste do rotarod). Os resultados sugerem um possível efeito central.

Unitermos: Efeito farmacológico, óleo essencial, monoterpeno, α,β-epóxi-carvona, camundongos.

ABSTRACT: The monoterpene α,β-epoxy-carvone (EC) in doses of 200, 300 or 400 mg/kg injected by i.p. route in mice caused a significant decrease in the motor activity of animals when compared with the control group, up to 120 minutes after the administration. The doses of 300 or 400 mg/kg had induced a significant increase of in the sleeping time of animals not having modified, however, the latency. The EC in the dose of 400 mg/kg reduced the remaining time of the animals on the rotating rod (Rotarod test). These results suggest a possible central effect.

Keywords: Pharmacological effects, essential oil, monoterpene, α,β-epoxy-carvone, mice.

INTRODUCTION

Several were the historical examples of the use of medicinal plants in the attainment and study of new substances with action in Central Nervous System (CNS), such as the morphine, codeine and caffeine (Huang; Kutchan, 2000; Henman, 1986).

Inside of this context we can cite essential oils with different applications, especially in the area of the medicine and cosmetic. They contribute to the pleasures of natural flavors and fragrances. In addition, many of them are found to exhibit varied biologic properties (Craveiro et al., 1981), such as spasmolytic (Lis-Balchin; Hart, 1999), antinociceptive and anti-inflammatory (Sousa et al., 2004), antimicrobial (Lima et al., 2006; Oliveira et al., 2006), larvicidal (Costa et al., 2005), and anticonvulsant (Almeida et al., 2003) activities. These effects are probably due to great structural diversity of the essential oils constituents. This notion is supported by previous studies which showed that some monoterpenes present in many essential oils possess anticonvulsant activity in animal experiments, such as linalool (Elisabetsky et al., 1995), limonene (Viana et al., 2000) and citronellol (De Sousa et al., 2006a). The derivative compounds of monoterpenes also exhibit several types of pharmacologic properties, such as antinociceptive (De Sousa et al., 2004), sedative (De Sousa et al., 2006b) and antidepressant (De Sousa et al., 2006c).

α,β-Epoxy-carvone (EC), Figure 1, is a monoterpene that can be found in the essential oil of *Carum carvi* (Iacobellis et al., 2005), *Kaempferia galanga* (Jirovetz et al., 2001) and other plants (Kaiser, 1997). In earlier studies, the antimicrobial effect of EC was investigated against *Staphylococcus aureus* and *Candida albicans* (Arruda et al., 2006). EC has functional groups and structural similarities with several monoterpenes with pharmacologic activity as limonene, menthol, menthone, pulegone, citronellol and hydroxydihydrocarvone (Umezu et al., 2001; De Sousa et al., 2006a; De Sousa et al., 2006b). This led us to verify the effects of EC in CNS using animal models.

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MATERIAL AND METHODS

Chemical

Compound EC was prepared in Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba as already described (Klein; Ohloff, 1963), and dissolved in 5% Tween 80 as an emulsion. Pentobarbital and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA).

Animals

Swiss male mice (3 months of age), weighing 28-35 g were obtained from the vivarium Prof. Thomas George of Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba. The animals were maintained at constant room temperature (21 ± 2 °C) and on a 12/12 h light-dark cycle (light from 06:00 to 18:00 h), with free access to food pellets and water. They were transferred to the laboratory at least 30 min before the start of experiments. All experiments were performed between 08:00-12:00 h to avoid circadian influences and carried out in accordance with ethical committee acts CEPA Nº. 1105/06.

Acute toxicity (LD_{50})

This test was performed according to a method described by Lorke (1983), with modifications, where the acute toxicity of EC was assessed by intraperitoneal (i.p.) route. Groups of 10 animals each were separated and received doses of 500, 750, 1000 or 2000 mg/kg of EC. One group received 5% tween 80 solution (vehicle). The animals were observed daily for 7 days and a number of deaths of animals were registered day by day and lethal dose 50% (LD_{50}) calculated (Litchfield; Wilcoxon, 1949).

Behavioural effects

The behavioural screening of the mice was performed following parameters described by Almeida (2006) and Almeida et al. (1999) and animals were observed at 0.5, 1, and 2 h after administration of EC (200 or 300 mg/kg i.p.).

Locomotor activity

Mice were divided into four groups of 8 animals each. Vehicle (control) and EC (200, 300, or 400 mg/kg i.p.) were injected. The spontaneous motor activity of the animals was assessed in a Cage Activity (controller model 7441 an Grid-Floor Detecting Arrangement Cage model 7432, Ugo Basile, Italy) in 30, 60, 90 and 120 minutes after administration (Mattei; Carlini, 1995; Almeida, 2006).

Pentobarbital-induced hypnosis

Sodium pentobarbital at a hypnotic dose of 40 mg/kg i.p. was injected into four groups ($n = 8$) of mice 30 min after pretreatment with water/tween-80 5% (control) and EC (200, 300 or 400 mg/kg) i.p., respectively. The latency (the interval between the injection of sodium pentobarbital and loss of the righting reflex) and duration of sleeping time (the interval between loss and recovery of the righting reflex) was recorded (Elisabetsky et al., 1995; Mattei et al., 1998; Almeida, 2006).

Motor coordination (Rotarod Test)

The effect of motor coordination was assed using a Rotarod apparatus (Rotarod model 7750, Ugo Basile, Italy) and consists of evaluating the motor coordination of the animal, through the time of permanence of the mouse on a revolving bar (Capasso, 1996). Groups of 8 mice each were previously selected for their ability to successfully remain on the revolving bar (2.5 cm diameter, 7 r.p.m., 25 cm from floor) of a Rotarod apparatus over a 3-min period (Mendes et al., 2002). These animals were treated i.p. with 5% tween 80 or doses (200, 300 or 400 mg/kg) of EC and tested at 10-min intervals up to 3 h after treatment. Mice failing more than once to remain on the rotating rod for 3 min constituted a positive result (Dunham; Miya, 1957).

Statistical analysis

The results were expressed as mean ± S.E.M. and tested with one-way analysis of variance (ANOVA) followed by Dunnet's test and Student "t" test. A probability level of 0.05 was accepted as significant. LD_{50} was obtained by non-linear regression. All data were analyzed with the software package GraphPad Prism version 3.02 (GraphPad Sotware Incorporated, San Diego, CA 92121 USA).

RESULTS

Acute toxicity (LD_{50})

Was observed that the dose of 500 mg/kg after 7 days did not promote death in treated animals and doses of 750, 1000 or 2000 mg/kg promoted death in 1, 7 and 10 animals, respectively. The LD_{50} calculated was 923 mg/kg with confidence interval of 820 to 1037 mg/kg.

Behavioural screening

EC at doses of 200 or 300 mg/kg, i.p. showed depressant activity on CNS based on the following behavioral alterations in animals after 30 and 60 minutes after treatment: decrease of the spontaneous activity, palpebral ptosis, ataxia and sedation.

Locomotor activity

In doses of 200, 300 or 400 mg/kg EC caused a significant decrease of ambulation at 30, 60, 90 and 120 min after administration (Figure 2).

Pentobarbital-induced hypnosis

The figure 3A shows that EC at doses of 200, 300 or 400 mg/kg did not affect the latency of pentobarbital-induced hypnosis. Figure 3B shows that EC at 200mg/kg did not modify the sleeping time, but at 300 and 400 mg/kg it increased the sleeping time compared to control animals.

Motor coordination (Rotarod Test)

In this test, 30 min after administration of EC only at the dose of 400 mg/kg the remaining time of animals on the rotating rod was decreased (Figure 4).

DISCUSSION

In the present study the pharmacological effects of the monoterpene EC were investigated in animal models and it characterized a psychopharmacological effect of this substance on the CNS. The results obtained and the LD_{50} value represent a low toxicity of EC and they were similar to the ones observed for other monoterpenes (Umezu et al., 2001; Farhat et al., 2001). In addition, EC did not produce writhing, tremor, convulsion, stereotyped behaviors and catalepsy, but did ataxia, which is recoverable, suggesting that the effect is not a result of toxic effect.

The mice treated with EC (200 or 300 mg/kg) presented behavioural alterations as reduction of the ambulation, palpebral ptosis, ataxia and sedation. These signals show possible evidence that the effects on CNS are similar to drugs that reduce the CNS activity (Fernández-Guasti et al., 2001; Morais et al., 2004; Argal; Pathak, 2006; Martinez et al., 2006).

EC caused a significant reduction of ambulation of animals in the test of spontaneous movement after 30, 60 and 90 minutes of its administration in the doses of 200, 300 or 400 mg/kg, that corroborates with the hypothesis of the EC reduces the CNS activity, it was reported that reduction of the ambulation of the animals is characteristic of psychopharmacological drugs (Fernández-Guasti et al., 2001; Argal; Pathak, 2006).

The reduction of the locomotive activity was observed by many essential oils (Umezu et al., 2001) and it can be due to either through an inhibitory effect of the EC in CNS or by muscular relaxant activity in the periphery. We suggest that EC which could possess a neuro-sedative activity or a profile for hypnotic drug (Santos et al., 1996).

The EC 300 or 400 mg/kg had an increase in the total time of sleep of the animals, but did not have an increase in the latency for the induction of sleep comparing to the control group. It is established that the potencialization of the time of sleep induced by pentobarbital must be a sedative or hypnotic action that is attributed to the involvement of central mechanisms in the regulation of sleep (N'Gouemo et al., 1994) and involves the inhibition of the GABAergic system (Steinbach; Akk, 2001; Sivam et al., 2004).

The lack of motor coordination in the test of the Rotarod is characteristic of a drug that reduces the CNS activity such as neuroleptics, anxiolytics, sedatives and hypnotics (Sen; Chaudhuri, 1992).

The animals treated with EC in the doses of 200 or 300 mg/kg did not present significant alterations in the time of performance in the bar, therefore not interfering with the motor coordination of the animals, therefore discarding muscular relaxant effect or even common neurotoxicity of some drugs with a depressant profile on the CNS. Only at the dose of 400 mg/kg the animals presented a significant reduction in the time of permanence on the revolving bar, what could be attributed to the elevated dose that would reflect a possible muscular relaxant action of the EC at this dose.

Taken into account all the results we can conclude that the monoterpene EC presents pharmacological effects on CNS characterizing it as a psychopharmacological drug and those new studies using specific methodologies are necessary to better characterize its pharmacological effect.

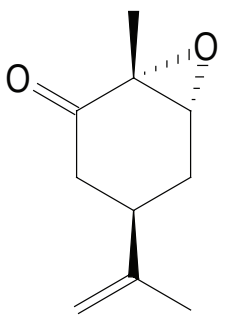

Figure 1. Structure of α,β-epoxy-carvone.

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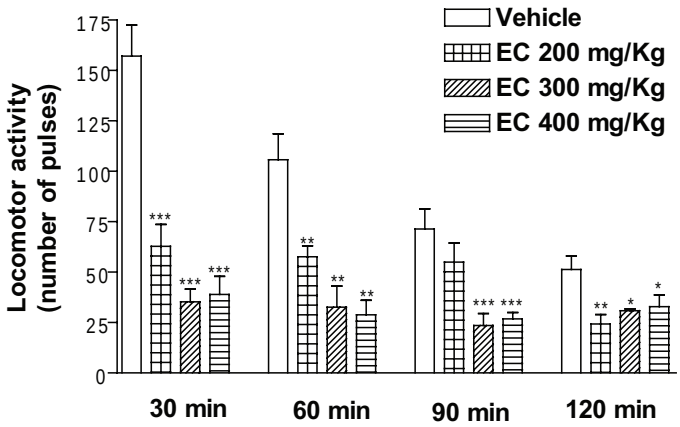

Figure 2. Effect of EC on locomotor activity of of mice. The parameters evaluated were the total number of pulses measured in Activity Cage. Values are the mean ± SEM for 8 mice; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to vehicle (control), one-way ANOVA followed by Dunnet's test.

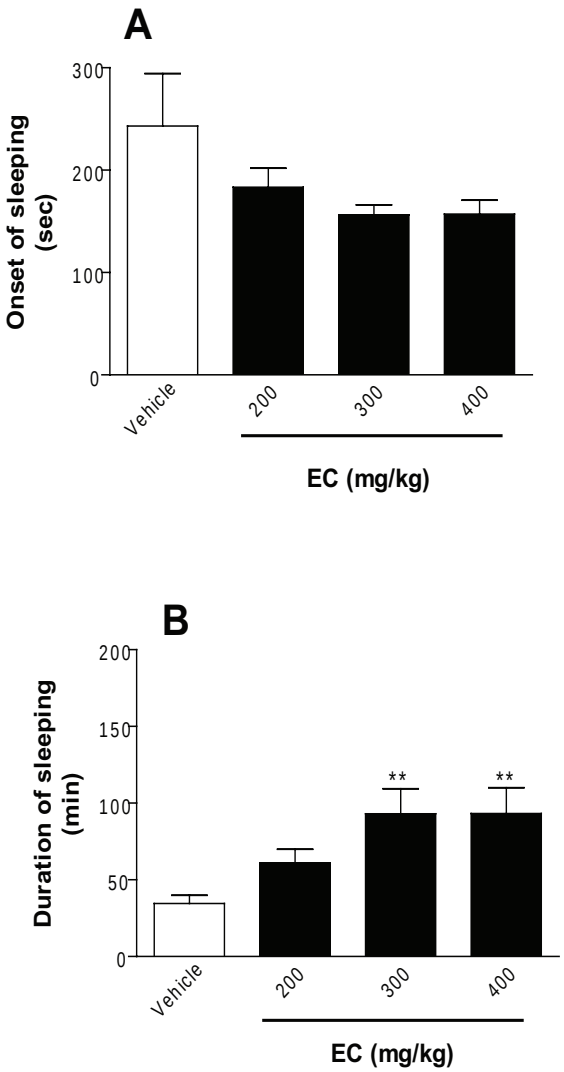

Figure 3. Effect of EC on pentobarbital-induced hypnosis in mice. The parameters evaluated were the onset of sleeping (A) and duration of sleeping (B). Values are mean ± SEM for 8 mice, **$p < 0.01$, as compared to vehicle (control), one-way ANOVA followed by Dunnet's test.

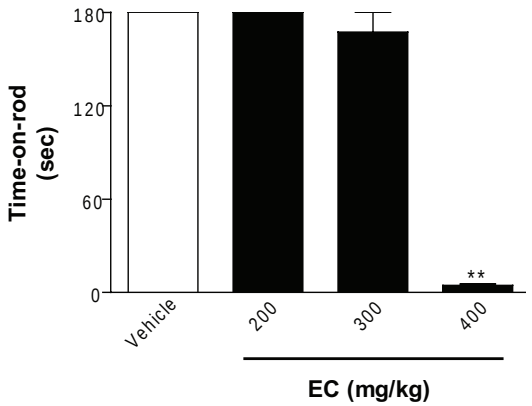

Figure 4. Effect of EC on motor coordination of mice in the Rotarod Test. Values are the length of time that each animal remained on the rotating rod (time-on-rod) recorded after 30 minutes of the administration of agents. Values are the mean ± SEM for 8 mice; $^{**}p < 0.01$, as compared to vehicle (control), one-way ANOVA followed by Dunnet's test.

REFERENCES

Almeida RN, Falcão ACGM, Diniz RST, Quintans-Júnior LJ, Polari RM, Barbosa-Filho JM, Agra MF, Duarte JC, Ferreira CD, Antoniolli AR, Araújo CC 1999. Metodologia para avaliação de plantas com atividade no sistema nervoso central e alguns dados experimentais. *Rev Bras Farm 80*: 72-76.

Almeida RN, Motta SC, Leite JR 2003. Óleos essenciais com propriedades anticonvulsivantes. *Bol Latinoam Caribe Plantas Med Aromat 2*: 3-6.

Almeida RN 2006. *Psicofarmacologia. Fundamentos práticos.* Rio de janeiro: Guanabara-Koogan.

Argal A, Pathak AK 2006. CNS activity of *Calotropis gigantea* roots. *J Ethnopharmacol 106*: 142-145.

Arruda TA, Antunes RMP, Catão RMR, Lima EO, De Sousa DP, Nunes XP, Pereira MSV, Barbosa-Filho JM, Da Cunha EVL 2006. Preliminary study of the antimicrobial activity of *Mentha x villosa* Hudson essential oil, rotundifolone and its analogues. *Rev Bras Farmacogn 16*: 307-311.

Capasso A, De Feo V, De Simone F, Sorrentino L 1996. Pharmacological effect of the aqueous extract from *Valeriana adscendeus*. *Phytother Res 10*: 309-312.

Costa JGM, Rodrigues FFG, Angélico EC, Silva MR, Mota ML, Santos NKA, Cardoso ALH, Lemos TLG 2005. Estudo químico-biológico dos óleos essenciais de *Hyptis martiusii*, *Lippia sidoides* e *Syzigium aromaticum* frente às larvas do *Aedes aegypti*. *Rev Bras Farmacogn 15*: 304-309.

Craveiro AA, Fernandes AG, Andrade CHS, Matos FJA, Alencar JW, Machado MIL 1981. *Óleos Essenciais de Plantas do Nordeste.* Fortaleza: UFC.

De Sousa DP, Raphael E, Brocksom U, Brocksom TJ 2004. Antinociceptive profile of 2-phenylselenenyl-1,8-cineole in mice. *Biol Pharm Bull 27*: 910-911.

De Sousa DP, Gonçalves JCR, Quintans-Júnior L, Cruz JS, Araújo DAM, De Almeida RN 2006a. Study of anticonvulsant effect of citronellol, a monoterpene alcohol, in rodents. *Neurosci Lett 401*: 231-235.

De Sousa DP, Oliveira FS, Almeida RN 2006b. Evaluation of the central activity of hydroxydihydrocarvone. *Biol Pharm Bull 29*: 811-812.

De Sousa DP, Schefer RR, Brocksom U, Brocksom TJ 2006c. Synthesis and antidepressant evaluation of three para-benzoquinone mono-oximes and their oxy derivatives. *Molecules 11*: 148-155.

Dunham NW, Miya TS 1957. A note on simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm 46*: 208-209.

Elisabetsky E, Coelho-De-Souza GP, Santos MAC, Siqueira IR, Amador TA 1995. Sedative properties of linalool. *Fitoterapia 66*: 407-414.

Farhat GN, Affara NI, Gali-Muhtasib HU 2001. Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. *Toxicon 39*: 1601-1605.

Fernández-Guasti A, Ferreira A, Picazo O 2001. Diazepam, but not buspirone, induces similar anxiolytic-like actions in lactating and ovariectomized Wistar rats. *Pharmacol Biochem Behav 70*: 85-93.

Henman AR 1986. *Vida Natural. O Guaraná: sua cultura, propriedades, formas de preparação e o uso*. São Paulo: Global/Ground.

Huang FC, Kutchan TM 2000. Distribution of morphinan and benzo[c]phenanthridine alkaloid gene transcript accumulation in *Papaver somniferum*. *Phytochemistry 53*: 555-564.

Iacobellis NS, Cantore PL, Capasso F, Senatore F 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J Agric Food Chem 53*: 57-61.

Jirovetz L, Buchbauer G, Shafi PM, Abraham GT 2001. Analysis of the essential oil of the roots of the medicinal plant *Kaempferia galanga* L. (Zingiberaceae) from South-India. *Acta Pharmaceutica Turcica 43*: 107-110.

Kaiser R 1997. New or uncommon volatile components in the most diverse natural scents. *EPPOS Spec. Num. 15th Journees Internationales Huiles Essentielles*, 17-47.

Klein E, Ohloff G 1963. Der stereochemische verlauf der alkalischen epoxydation von α,β-ungesättigten carbonylverbindungen der cyclischen monoterpenreihe.

Tetrahedron 19: 1091-1099.

Lima IO, Oliveira RAG, Lima EO, Farias NMP, Souza EL 2006. Atividade antifúngica de óleos essenciais sobre espécies de *Candida. Rev Bras Farmacogn 16*: 197-201.

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

Litchfield LT, Wilcoxon F 1949. A simplified method of evaluation dose-effect experiments. *J Pharmacol Exp Ther 19*: 388-397.

Lorke DA 1983. New approach to acute toxicity testing. *Arch Toxicol 54:* 275–287.

Martínez AL, Domínguez F, Orozco S, Chávez M, Salgado H, González M, González-Trujano ME 2006. Neuropharmacological effects of an ethanol extract of the *Magnolia dealbata* Zucc. leaves in mice. *J Ethnopharmacol 106*: 250-255.

Mattei R, Dias RF, Espínola EB, Carlini EA, Barros SBM 1998. Guarana (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity in vitro. *J Ethnopharmacol 60*: 111-116.

Mattei R, Carlini EA 1995. Mazindol: anorectic and behavioral effects in female rats. *Arch Int Pharmacodyn Ther 330*: 279-287.

Mendes FR, Mattei R, Carlini EA 2002. Activity of *Hypericum brasiliense* and *Hypericum cordatum* on the central nervous system in rodents. *Fitoterapia 73*: 462-471.

Morais LCSL, Quintans-Júnior LJ, Franco CIF, Almeida JRGS, Almeida RN 2004. Antiparkinsonian-like effects of *Plumbago scandens* on tremorine-induced tremors methodology. *Pharmacol Biochem Behav 79*: 745-749.

N'Gouemo P, Nquemby-Bina C, Baldy-Moulinier M 1994. Some neuropharmacological effects of an ethanol extract of *Maprounea africana* in rodents. *J Ethnopharmacol 43*: 161-166.

Oliveira FP, Lima EO, Siqueira Júnior JP, Souza EL, Santos BHC, Barreto HM 2006. Effectiveness of *Lippia sidoides* Cham. (Verbenaceae) essential oil in inhibiting the growth of *Staphylococcus aureus* strains isolated from clinical material. *Rev Bras Farmacogn 16*: 510-516.

Santos FA, Rao VSN, Silveira ER 1996. Studies on the neuropharmacological effects of *Psidium guyanensis* and *Psidium pholianum* essential oils. *Phytother Res 10*: 655-658.

Sen T, Chaudhuri KN 1992. Studies on the neuropharmacological aspects of *Pluchea indica* root extract. *Phytother Res 6*: 175-179.

Sivam SP, Nabeshima T, Ho IK 2004. Acute and chronic effects of pentobarbital in relation to postsynaptic GABA receptors: A study with muscimol. *J Neurosci Res 7*: 37-47.

Sousa OV, Soares-Júnior DT, Del-Vechio G, Mattosinhos RG, Gattas CR, Kaplan MAC 2004. Atividades antinociceptiva e antiinflamatória do óleo essencial de cascas de *Duguetia lanceolata* St. Hil., Annonaceae. *Rev Bras Farmacogn 14(Supl)*: 11-14.

Steinbach JH, Akk G 2001. Modulation of $GABA_A$ receptor channel gating by pentobarbital. *J Physiol 537*: 715-733.

Umezu T, Sakata A, Ito H 2001. Ambulation-promoting effect of peppermint oil and identification of its active constituents. *Pharmacol Biochem Behav 69*: 383-390.

Viana GSD, Vale TG, Silva CMM, Matos FJD 2000. Anticonvulsant activity of essential oils and active principles from chemotypes of *Lippia alba* (MILL.) Ne Brown. *Biol Pharm Bull 23*: 1314-1317.