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Phytochemical screening and anticonvulsant property of Ocimum basilicum leaf essential oil

[Tamizaje fitoquímico y propiedad anticonvulsivante del aceite esencial de hojas de Ocimum basilicum]

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Abstract

Ocimum basilicum (Lamiaceae) and other species of the same genus are used as medicines for the treatment of central nervous system (CNS) diseases. In this report, we have investigated the possible CNS depressant and anticonvulsant effects of Ocimum basilicum (access “Maria Bonita”) leaf essential oil (EO) in different experimental models. GC-MS and GC-FID analysis of the essential oil resulted in the identification of 7 compounds constituting 98.8% of the total oil. 1.8-cineole, linalool, and geraniol were the principal components, comprising 92.9% of the oil. EO, at all doses, showed depressant CNS activity as revealed in the general pharmacological screening: decrease of spontaneous activity, ptosis, ataxia, and sedation. Additionally, all doses of EO induced a significant increase of sleeping time (p < 0.05) and decrease in the latency to sleep (p < 0.01). EO also increased the latency for development of convulsions in pentyleneetetrazol (PTZ) and picrotoxin tests (p < 0.05). For PTZ, the effects of EO were reversed by flumazenil. EO did not interfere with the convulsions induced by strychnine (p > 0.05). Our data suggests that EO possesses CNS depressant and anticonvulsant properties which could be mediated by an interaction with central GABAergic receptors.

Keywords: Ocimum basilicum; CNS depressant; Anticonvulsant; Pentyleneetetrazole; Picrotoxin.

Resumen

Ocimum basilicum (Lamiaceae) y otras especies del mismo género son usadas como medicamentos en el tratamiento de enfermedades del sistema nervioso central (SNC). En este reporte, hemos investigado los posibles efectos anticonvulsivantes y depresivos del SNC del aceite esencial (EO) de las hojas de Ocimum basilicum (conocida como “Maria Bonita”) en diferentes modelos experimentales. Los análisis por GC-MS y GC-FID del aceite esencial permitieron la identificación de 7 compuestos constituyendo el 98.8% del aceite total. Los principales componentes fueron 1.8-cineno, linalol y geranilo que comprendieron el 92.9% del aceite. EO, a todas las dosis, mostró actividad depresora del SNC revelado en el tamizaje farmacológico general: decrecimiento de la actividad espontánea, ptosis, ataxia y sedación. Además, todas las dosis de EO indujeron un incremento significativo del tiempo de sueño (p < 0.05) y disminuyeron la latencia de sueño (p < 0.01). EO también incrementó la latencia para el desarrollo de convulsiones en las pruebas de pentilenetetrazol (PTZ) y picrotoxina (p < 0.05). Para PTZ, los efectos de EO fueron revertidos por flumazenil. EO no interfirió con las convulsiones inducidas por estricnina (p > 0.05). Nuestros datos sugieren que EO posee propiedades anticonvulsivantes y depresoras del SNC lo que podría estar mediado por una interacción con los receptores GABAérgicos centrales.

Palabras Clave: Ocimum basilicum; Depresores del SNC; Anticonvulsivante; Pentilenetetrazol; Picrotoxina.

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INTRODUCTION

Epilepsy continues to be a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness, as currently available drugs fail to provide adequate control of epileptic seizures in about one-third of patients and do not prevent progressive epileptogenic changes (Jeub et al., 2002). Traditional systems of medicine are popular in developing countries, and up to 80% of the population relies on traditional medicines or folk remedies for primary health care needs (Akerele, 1988). A great number of scientists and organizations turn their attention to traditional therapies in order to find and conserve important resources (Akerele, 1990). However, medicinal plants have been an important source of new drugs with biological activity (Almeida et al., 2005; Quintans-Júnior et al., 2008).

Several Ocimum species (Lamiaceae) are used to treat central nervous system (CNS) disorders in various parts of the world and its antidepressive activity is frequently reported (Corrêa, 1984). Leaves from Ocimum species release a pleasing odor when squashed between the fingers and could be used as a culinary condiment (Mäkinen and Paakkonen, 1999) and for insect control (Holm, 1999). Brazilian Tropical Atlantic Forest inhabitants use a decoction of Ocimum gratissimum L. roots as a sedative for children (Di Stasi et al., 2002). Sedative and anticonvulsant activities were experimentally detected in Ocimum tenuiflorum (Ocimum sanctum) (Pérez de Alejo et al., 1996).

The genus Ocimum is included in the Lamiaceae family and is distributed worldwide from the tropical and subtropical regions of Asia, Africa, and Central and South America (Suppakul, et al., 2003). This genus is characterized by a great variability in its morphology and chemotypes (Lawrence, 1988). The ease of its cross-pollination contributes to a myriad of subspecies, varieties, and forms (Guenther, 1975). The reason for this complexity stems from the fact that botanists have assigned several designations to the same varieties and, in some instances, have confused some varieties with forms of other species. Different chemotypes have been reported for Ocimum basilicum (Grayer et al., 1996), Ocimum canum, and Ocimum gratissimum (Martins et al., 1999). Two morphological varieties of Ocimum gratissimum (gratissimum var. and macrophyllum var.) were found to have their volatile oil constituents based into six groups, which were aggregated into three chemotypes (eugenol, thymol, and geraniol) according to genetic markers and volatile oil constituents (Vieira et al., 2001). According to Mazutti et al. (2006), the volatile oil constituents of O. basilicum were: δ-cadinol (10.2%), estragole (22.6%), and linalool (47.3%).

The aim of this work was to perform phytochemical screening of the essential oil of Ocimum basilicum (access “Maria Bonita”) and to investigate its possible anticonvulsant effects in mice.

MATERIALS AND METHODS

Animals

Male Swiss mice (30-35 g), with 2-3 months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 25±2 °C on a 12 h light/dark cycle (lights on 06:00-18:00) with free access to food (Purina) and water. They were used in groups of 10 animals each. All experiments were carried out between 9.00 h and 16.00 h in a quiet room. Experimental protocols and procedures were approved by the Universidade Federal de Sergipe Animal Care and Use Committee (CEPA/UFS Nº010/07).

Plant material and essential oil (EO) extraction

Leaves were collected from the cultivation of the O. basilicum access “Maria Bonita” obtained at the Research Station "Campus Rural da UFS" of the Universidade Federal de Sergipe, Brazil. O. basilicum access “Maria Bonita” was derived from the accession PI 197442 of the Germplasm Bank “North Central Regional PI Station”, USA. It’s a basil cultivar with a rounded canopy, rose petals and purple sepalas and is indicated for brazilian northeast region (Blank et al., 2008). The leaves of O. basilicum were dried in an oven with air renewal and circulation (model MA-037/18) at 40 °C until complete dehydration has been achieved. The essential oil was obtained by hydrodistillation in a Clevenger-type apparatus using 100 g of dried leaves. The oil obtained was dried over anhydrous sodium sulphate, producing yields of 2.34% (v/w).

Gas Chromatography – Mass Spectrometry

Oil sample analysis was performed on a Shimadzu QP5050A (Shimadzu Corporation, Kyoto, Japan) system comprising a AOC -20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column J&W Scientific DB-5MS fused silica capillary
column (30 cm x 0.25 mm i.d, 0.25 μm coating thickness, composed of 5% phenylmethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.99%) was used as carrier gas at a constant flow of 1.2 mL/min and an injection volume of 0.5 μL was employed (split ratio of 1:83) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 50 °C (isothermal for 2 min), with an increase of 4 °C/min., to 200 °C, then 10 °C/min to 300 °C, ending with a 10 min iso-thermal at 300 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

**Gas – Chromatography (GC-FID)**

Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID), using a Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) equipment, under the following operational conditions: capillary ZB-5MS column (5% dimethylpolysiloxane) fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm coating thickness) from Phenomenex (Torrance, CA, USA), under the same conditions GC-MS. Quantification of each constituent was estimated by area normalization (%). Compounds concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

**Identification of essential oil constituents**

Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in NIST21 and NIST107 mass spectral library of the GC-MS data system. Retention indices (RI) for all compounds were determined according to the Van den Dool and Kratz (1963) for each constituent as previously described (Adams, 2007).

**Drugs**

Diazepam (DZP), Pentylentetrazole (PTZ), picROTOXIN (PIC), phenytoin (PHE), Strychnine (STR), polyoxyethylene-sorbitan monolated (Tweens 80) and cremophor was purchased from Sigma (USA), Diazepam (DZP), Flumazenil (FLU) from Roche (Brazil) and sodium thiopental from Cristália (Brasil). Agents were injected intraperitoneally (ip) at a dose volume of 0.1 mL/10 g.

**Behavioural effects**

The behavioural screening of the mice was performed following the parameters described in Oliveira et al. (1999) and animals were observed at 0.5, 1, and 2 h after administration of EO (100, 200 and 400 mg/kg, ip).

**Thiopental-induced hypnosis**

Sodium thiopental at a hypnotic dose of 50 mg/kg i.p. was injected into four groups (n= 8) of mice 30 min after pretreatment with saline/tween-80 (vehicle), EO (10, 50, 100 and 200 mg/kg, ip) and DZP (3 mg/kg, ip). The latency (the interval between the injection of sodium thiopental and the loss of the righting reflex) and the duration of sleeping (the interval between loss and recovery of the righting reflex) was recorded (Elisabetsky et al., 1995).

**PTZ- and PIC-induced convulsion**

A modified method from Vellucci and Webster (1984) was used to assess the anticonvulsant effect of the EO. Mice were kept individually in transparent mice cages (25 cm × 15cm × 15cm) for 30 min to acclimatize to their new environment before the beginning of the experiments. Seizures were induced with PTZ (60 mg/kg, ip) or PIC (8 mg/kg, ip) and the animals were observed for a period of 15 or 20 min., respectively (Oliveira et al., 2001; Ngo Bum et al., 2001). The onset of tonic-clonic convulsion and the number of animals convulsing or not convulsing within the observation period were recorded. Experiments were repeated following the pretreatment of animals with either (EO 100, 200 or 400 mg/kg), DZP (3 mg/kg, ip) or control vehicle thirty minutes prior to the administration of one of the convulsant agents. The ability of the EO to prevent or delay the onset of tonic and tonic-clonic convulsions was taken as an indication of anticonvulsant activity.

**STR-induced convulsion**

This method has been described previously (Lehmann et al., 1988). In brief, strychnine (STR) seizures followed by death were induced in male mice by the ip injection of 3 mg/kg of the STR nitrate. The treatment of the EO was similar to those used in the PTZ- and PIC-tests. A protective effect of the EO (100, 200 or 400 mg/kg) given (ip) 0.5 h prior to STR (3 mg/kg, ip) was recorded and compared to the one of 30 mg/kg PHE and with the control group (vehicle). The number of animals, which survived more than 10 min served as criterion of protection. The time to onset of death was recorded in non-protected mice.
Statistical analysis

The data on the thiopental-induced hypnosis and on the convulsion experiments (PTZ, PIC and STR) were expressed as the mean ± S.E.M. The differences of the means among the groups were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test (thiopental-induced hypnosis) or the Dunnett’s t test (for the convulsion experiments). The incidence (%) of clonic or tonic-clonic seizures as well as the mortality were evaluated by the Fisher’s Exact Test. Differences were considered to be statistically significant when p<0.05.

RESULTS

Phytochemical screening

GC-MS and GC-FID analysis of the essential oil resulted in the identification of 7 compounds, constituting 98.8% of the total oil. 1,8-cineole, linalool, and geraniol were the principal components, comprising 92.9% of the oil (Table 1). This result is similar to Lee et al. (2005).

Behavioral effects

Animals treated with EO, at all doses, showed signs of CNS depressant activity. They decreased in spontaneous activity and to the response to touch; they also showed palpebral ptosis, ataxia, and sedation. These effects were observed from thirty minutes to two hours after the treatment.

Table 1. Chemical composition of the essential oil of Ocimum basilicum

<table>
<thead>
<tr>
<th>TR (min)</th>
<th>Compound</th>
<th>(%)</th>
<th>RRI(^a)</th>
<th>RRI(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.458</td>
<td>sabinene</td>
<td>0.89</td>
<td>974</td>
<td>975</td>
</tr>
<tr>
<td>10.358</td>
<td>1,8-Cineole</td>
<td>7.90</td>
<td>1030</td>
<td>1031</td>
</tr>
<tr>
<td>12.908</td>
<td>linalool</td>
<td>72.14</td>
<td>1099</td>
<td>1096</td>
</tr>
<tr>
<td>16.383</td>
<td>unknown</td>
<td>0.68</td>
<td>1192</td>
<td>-</td>
</tr>
<tr>
<td>18.382</td>
<td>geraniol</td>
<td>12.95</td>
<td>1248</td>
<td>1252</td>
</tr>
<tr>
<td>22.900</td>
<td>linalool isobutanoate</td>
<td>2.30</td>
<td>1375</td>
<td>1375</td>
</tr>
<tr>
<td>24.750</td>
<td>α- trans- bergamotene</td>
<td>1.21</td>
<td>1430</td>
<td>1434</td>
</tr>
<tr>
<td>31.358</td>
<td>epi-α-cadinol</td>
<td>0.80</td>
<td>1638</td>
<td>1640</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>98.87</td>
</tr>
</tbody>
</table>

RT Retention time; RRI relative retention indices
\(^a\)Experimental (Van Den Dool and Kratz 1963)
\(^b\)As compared with those reported by Adams (2007)

Figure 1. Effect of EO on thiopental-induced hypnosis in mice. The parameters evaluated were the onset of sleeping (A) and duration of sleeping (B).

Values are mean ± SEM of 8 mice, ** p < 0.01, *** p < 0.001, as compared to vehicle (control), one-way ANOVA followed by Tukey’s test.
Effect of EO on PTZ- and PIC-induced convulsion

One way ANOVA revealed differences between the experimental groups for the onset of convulsions induced by pentilenotetrazole (p<0.0001) (Table 2) and picrotoxin (p<0.0001) (Table 3). Diazepam (p<0.01) and EO, at the doses of 200 and 400 mg/kg (p<0.01), increased the latencies for the onset of the tonic-clonic convulsions induced by PTZ. The percentage of animals that convulsed was decreased by diazepam (p<0.01) and by EO at the doses of 200 (p<0.05) and 400 mg/kg (p<0.01). The number of animal deaths was also decreased by diazepam (p<0.01) and by EO at the three doses tested (100 mg/kg and 200 mg/kg, p<0.05; 400 mg/kg, p<0.01). The effects of diazepam and EO (200 and 400 mg/kg), for the three parameters, were reversed by flumazenil 10 mg/kg (Table 2).

Effect of EO on STR-induced convulsion

One way ANOVA revealed differences between the experimental groups for the onset of convulsions induced by strychnine (p<0.0001). Phenytoin increased the latency for the onset of convulsions (p<0.01), and decreased the percentage of animals that convulsed (p<0.01) and the number of animal deaths (p<0.05). These parameters were not changed by EO in any of the doses tested (p>0.05) (Table 4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency (s)a</th>
<th>% Convulsion</th>
<th>% Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>212.0 ± 41.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DZP</td>
<td>3</td>
<td>835.1 ± 12.9c</td>
<td>10e</td>
<td>0e</td>
</tr>
<tr>
<td>EO</td>
<td>100</td>
<td>369.0 ± 209.3</td>
<td>90</td>
<td>70d</td>
</tr>
<tr>
<td>EO</td>
<td>200</td>
<td>421.2 ± 132.8c</td>
<td>70d</td>
<td>50d</td>
</tr>
<tr>
<td>EO</td>
<td>400</td>
<td>459.1 ± 149.7b</td>
<td>60e</td>
<td>40e</td>
</tr>
<tr>
<td>DZP + FLU</td>
<td>3 + 10</td>
<td>341.3 ± 112.8</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>EO + FLU</td>
<td>200 + 10</td>
<td>310.5 ± 87.9</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>EO + FLU</td>
<td>400 + 10</td>
<td>285.4 ± 94.1</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Effect of EO on convulsions induced by PTZ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency (s)a</th>
<th>% Convulsion</th>
<th>% Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>534.0 ± 121.3</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>DZP</td>
<td>3</td>
<td>1200.0 ± 0.0c</td>
<td>0e</td>
<td>0e</td>
</tr>
<tr>
<td>EO</td>
<td>100</td>
<td>454.0 ± 94.2</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>EO</td>
<td>200</td>
<td>781.2 ± 93.4b</td>
<td>70d</td>
<td>30e</td>
</tr>
<tr>
<td>EO</td>
<td>400</td>
<td>1045.3 ± 109.6c</td>
<td>30e</td>
<td>20e</td>
</tr>
</tbody>
</table>

Table 3. Effect of EO on the convulsions induced by PIC.

n = 10

a Values represent mean ± S.E.M.
b P < 0.05 (one-way ANOVA and Dunnett’s test), significantly different from control
c P < 0.01 (one-way ANOVA and Dunnett’s test), significantly different from control
d P < 0.05 (Fisher’s test), significantly different from control
e P < 0.01 (Fisher’s test), significantly different from control.
Oliveira et al. Phytochemical screening and anticonvulsant property of Ocimum basilicum

Table 4. Effect of EO on the convulsions induced by STR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency (s)</th>
<th>% Convulsion</th>
<th>% Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>121.3 ± 12.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PHE</td>
<td>30</td>
<td>532.1 ± 23.7</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>EO</td>
<td>100</td>
<td>164.2 ± 41.4</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>EO</td>
<td>200</td>
<td>203.4 ± 82.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>EO</td>
<td>400</td>
<td>189.3 ± 78.2</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

n = 10

<sup>a</sup> Values represent mean ± S.E.M.
<sup>b</sup> P < 0.01 (one-way ANOVA and Dunnett’s test), significantly different from control
<sup>c</sup> P < 0.05 (Fisher’s test), significantly different from control.
<sup>d</sup> P < 0.01 (Fisher’s test), significantly different from control.

DISCUSSION

Major volatiles detected in this study (Table 1) were different with those of previously published studies in which volatile components were isolated by various organic solvent extractions (Lee et al., 2005; Zhang et al., 2009). However, in our study of essential oil produced from Ocimum basilicum, access “Maria Bonita’, linalool (72.14% of total quantified volatile compounds), geraniol (12.95%), and 1,8-cineole (7.9%) were also determined as major constituents (Hasegawa et al., 1997). This result showed that essential oil produced from Ocimum basilicum, access “Maria Bonita’, is very rich in linalool.

In the present study, the CNS depressant and anticonvulsant activities of Ocimum basilicum leaf essential oil (EO) were investigated in different animal models. Additionally, the chemical composition of the oil was evaluated. Our results demonstrate that the acute administration of EO (200 and 400 mg/kg, i.p.) increases the hypnosis induced by sodium thiopental and prevents the convulsions induced by -PTZ and -PIC, but not by STR. 1,8-cineole, linalool, and geraniol were the main components of the essential oil.

Mice treated with EO (100, 200, and 400 mg/kg, i.p.) presented behavioral alterations, such as: reduction of spontaneous activity, decrease of response to touch, palpebral ptosis, ataxia, and sedation. These behaviors suggest that the effects of EO are similar to those of known CNS depressant natural products (De Sousa et al., 2006; 2007). The general depressant activity of EO was confirmed by the decrease in the latency to sleep and its tendency to increase thiopental-induced sleeping (De Sousa et al., 2006).

The PTZ test represents a valid model for human generalized myoclonic convulsions (Malawska, 2003). PTZ induces convulsions in rodents by blocking the Cl⁻ channel of GABA<sub>A</sub> receptors. It has been reported that GABAergic neurotransmission plays an important role in stress, anxiety (Zwanzger and Rupprecht, 2005), pain (Rode et al., 2005), and epilepsy (Perucca, 2005). In fact, drugs that inhibit convulsions or increase the latency of PTZ-induced convulsions are suggested as having anticonvulsant activity (Haruna, 2000). Benzodiazepines and many barbiturates potentiate the inhibitory action of GABA<sub>A</sub> receptors, reducing neuronal excitability and increasing the threshold for convulsions (Löschler and Schmidt, 2006).

According to Nicoll (2001), picrotoxin, a GABA<sub>A</sub>-receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA<sub>A</sub>-receptors, thus preventing the entry of chloride ions into the brain and, consequently, inhibitory transmission in the brain (Löschler and Schmidt, 1988). The findings of the present study, therefore, suggest that EO might have inhibited and/or attenuated the PIC-induced convulsions of mice by interfering with GABAergic neurotransmission. In addition, the antagonism of PTZ and its reversion by flumazenil (Table 2), an agonist of GABA<sub>A</sub> receptors, further supports the hypothesis of an interaction of EO with these receptors.

Strychnine causes convulsions by antagonizing the activity of glycine receptors and increasing postsynaptic excitability and ongoing activity in the brain stem and spinal cord (Webb and Lynch, 2007). Since EO did not prevent the convulsions induced by strychnine, an interaction of the extract with glycine receptors is unlikely.

According to Quintans-Júnior et al. (2005), linalool, the major monoterpene found in Ocimum basilicum leaf essential oil (Table 1), possesses anticonvulsant properties and Elisabetsky et al. (1995) showed sedative effects. However, Re et al. (2000) showed that linalool had inhibitory properties in acetylcholine (ACh) release and induces a reduction of the ACh-evoked release. Nevertheless, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), characterized by partial seizures that occur predominantly during sleep, has been
associated in some families with mutations involving either the α or β subunit in αβ2 nicotinic acetylcholine receptors (Meldrum and Rogawski, 2007). Carbamazepine is a noncompetitive inhibitor of nicotinic receptors that blocks acetylcholine-evoked currents at concentrations in the therapeutic range (Picard et al., 1999). On the other hand, linalool is a competitive antagonist of NMDA receptors (Elisabetsky et al., 1999; Silva Brum et al., 2001) and blockade of the NMDA subtype of glutamate receptor has been reported to contribute to the antiepileptic effects of felbamate (Deckers et al., 2003).

CONCLUSION

All together, the data presented in this study suggests that EO extracted from the leaves of Ocimum basilicum, access “Maria Bonita”, possesses CNS depressant properties and anticonvulsant effects in mice. However, the precise mechanisms involved in these properties are not clear, although our evidence points to an involvement of EO with GABAergic neurotransmission. Nevertheless, further studies will be required for elucidation of the mechanisms involved. Patch-clamping experiments would be highly recommendable.

REFERENCES


