Programa de Pós-graduação em Ciências Farmacêuticas[1], Laboratório de Pesquisa em Neuroquímica Experimental da Universidade Federal do Piauí; Programa de Pós-graduação em Farmacologia da Universidade Federal do Piauí[2]; Departmento de Química da Universidade Federal do Piauí[3], Teresina, Piauí, Brazil

Sedative, anxiolytic and antidepressant activities of *Citrus limon* (Burn) essential oil in mice

L. M. Lopes Campêlo[1], C. Gonçalves e Sá[1], A. A. C. de Almeida[2], J. Pereira da Costa[1], T. H. Costa Marques[2], C. Mendes Feitosa[1,3], G. Barros Saldanha[1], R. Mendes de Freitas[1,2]

Received January 14, 2011, accepted March 11, 2011

Dr. Rivelilson Mendes de Freitas, Campus Universitário Ministro Petrônio Portella, Curso de Farmácia, Bairro Ininga, Teresina, Piauí, Bratil
rivelilson@pq.cnpq.br

Pharmazie 66: 623–627 (2011) *doi: 10.1691/ph.2011.1508*

We examined the sedative, anxiolytic and antidepressant effects of essential oil (EO) of leaves from *Citrus limon,* which has been used as one of the most popular compounds in Brazilian traditional herbal medicine. The effects of EO were demonstrated by open-field, elevated-plus-maze, rota rod, pentobarbital-induced sleeping time, and forced swimming tests in mice. In the open-field test, EO at the doses of 50, 100 and 150 mg/kg, after oral administration, significantly decreased the number of crossings, grooming, and rearing. In the elevated-plus-maze (EPM) test, EO increased the time of permanence and the number of entrances in the open arms. On the contrary, the time of permanence and the number of entrances in the closed arms were decreased. In the rota rod test, EO did not alter motor coordination and, thus, was devoid of effects, as related to controls. In the pentobarbital-induced sleeping time test, EO at the same doses significantly increased the animals sleeping time duration. Since EO, at the doses of 50, 100 and 150 mg/kg, did not show a sedative effect in the open field test, these three doses were used in the forced swimming test, producing a decrease in the immobility time, similarly to that of imipramine (positive control). However, the antidepressant effects of EO were not altered by the previous administration of paroxetine. In addition, effects of EO in the forced swimming test were totally blocked by reserpine pretreatment. In conclusion, the present work evidenced sedative and anxiolytic effects of EO that might involve an action on benzodiazepine-type receptors, and also an antidepressant effect where noradrenergic and serotoninergic mechanisms will probably play a role.

1. Introduction

People from different regions of the world have used herbal medicines to alleviate emotional disorders for many years. In addition, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade (Zhang 2004; Santos et al. 2008). An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines, and there is also a large number of herbal medicines whose therapeutic potential has been assessed in a variety of animal models (Zhang 2004; Xavier et al. 2007).

In fact, these models have contributed to the screening of new psychopharmacological tools and to the understanding of their biological activity (Buller and Legrand 2001). A lot of those tools are commonly used to evaluate the effects of herbal medications for the prevention or treatment of neurodegenerative diseases. They have demonstrated efficacy and safety not only in animal models but also in clinical trials (Wong et al. 1998). In Brazil, several medicinal plants are used to alleviate insomnia, anxiety and depressed mood (Gomes et al. 2008).

High-resolution gas chromatography/mass spectrometry analysis of the essential oil from *Citrus limon* has led to the identification of twenty-eight volatile constituents (Campêlo et al. 2011a). Among them, mono- and sesqui-terpenoids such as limonene, linalool, *cis*-limonene-oxide, *trans*-limonene-oxide, citronellal, neral, geranial, nerol e geranyl acetate were predominant components.

The elucidation of the active components found in herbs and their mechanisms of central action, has been a major challenge for pharmaceutical chemistry, biochemistry, pharmacology and pharmaceutical industry. Herbs contain many constituents and their essential oils, when tested, may show synergistic effects among the different active compounds due to the presence of different classes or structures contributing to the same activity. In this study of the central biological activity of essential oil of *C. limon,* we selected, in our laboratory, bioassays to detect the effects on Central Nervous System (CNS). Bioassays were performed in animal models in order to identify possible pharmacological effects of essential oil from *C. limon*, and from these initial results further experiments have been conducted on neurochemical and design for the pharmaceutical development of new herbal products with application in the treatment of neurodegenerative diseases, since they affect a large population. The present study was undertaken to investigate whether the essential oil of leaves from *Citrus limon* has potential activities on the CNS and if it is able to induce behavioral alterations in mice. Spontaneous motor responses were monitored

Table 1: Chemical composition and retention indices of the constituents of the *C. limon* essential oil NI = Not identified. [a]Retention time; [b]Compounds listed in order of elution from an DB-5MS column; [c]Kovats indices were calculated against *n*-alkanes (C9-C18) on a DB-5MS column.

Compounds[b]	(%)	RT (min)[a]	IK[c]
Limonene	52.77	4.785	1025.5
Linalool	1.73	6.365	1100
cis-Limonene-oxide	2.68	7. 137	1129.3
trans-limonene-oxide	7.13	7.253	1133.7
Citronellal	2.77	7.686	1150
Neral	6.85	10.141	1238.5
Geranial	5.49	11.030	1268.9
NI	6.62	13.062	1337.8
Nerol	4.04	13.857	1363.3
Geranyl Acetate	9.92	14.441	1384.2
Total identified	93.38		

and the sedative-, anxiolytic- and antidepressant-like effects were assessed in the open field, elevated-plus-maze, rota rod, pentobarbital-induced sleeping time, and forced swimming tests.

2. Investigations and results

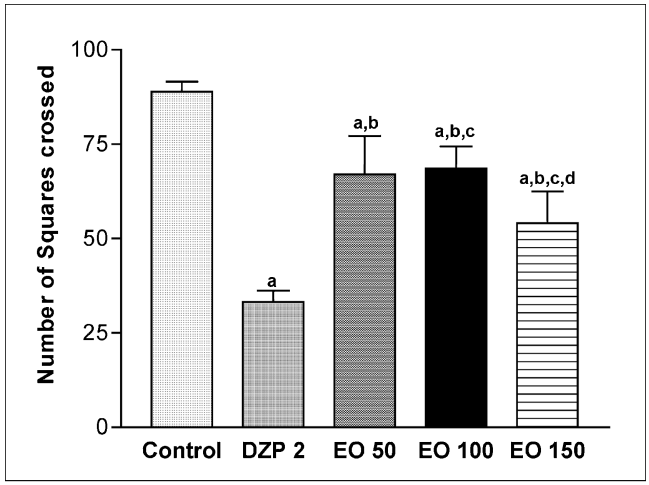
GCMS analysis showed a mixture of monoterpenes among which limonene (52.77%), geranyl acetate (9.92%) and trans-limonene-oxide (7.13%) were the main compounds in *C. limon* essential oil (Table 1).

Essential oil from *C. limon* at doses of 50, 100 and 150 mg/kg p.o. caused behavioral changes in animals during 30 days of treatment: decrease of spontaneous activity, palpebral ptosis, ataxia, analgesia, and sedation. Behavioral changes were more evident in the second day of treatments.

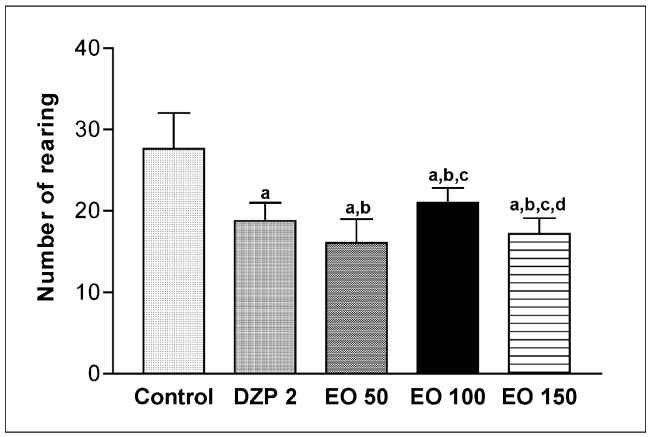
In the open field test (Fig. 1), EO (50 mg/kg, p.o.) decreased the number of rearings (16.09 $\pm$ 2.91) as compared to control (27.64 $\pm$ 4.42). Similarly, EO 100 (100 mg/kg, p.o.) and EO 150 (150 mg/kg, p.o.) decreased the number of rearings (21.00 $\pm$ 1.86 and 17.17 $\pm$ 1.95, respectively) and also grooming (3.80 $\pm$ 0.76 and 4.00 $\pm$ 1.41, respectively), as compared to control (rearing, 38.00 $\pm$ 7.59; grooming, 5.33 $\pm$ 0.72). In the number of crossings was observed in EO (50 mg/kg, p.o.) a decrease (67.00 $\pm$ 10.20) as compared to control (88.88 $\pm$ 2.68). Similarly, EO 100 (100 mg/kg, p.o.) and EO 150 (150 mg/kg, p.o.) decreased the number of crossings (68.60 $\pm$ 5.80 and 54.08 $\pm$ 8.47, respectively) as compared to control (88.88 $\pm$ 2.68). Diazepam, as expected, showed sedative effect at the dose used (2 mg/kg, i.p.).

Table 2 shows the effects of EO from *C. limon* in the elevated plus maze test. The parameters used for evaluation of the anxiolytic activity were number of entries in the open arms (NEOA) percentage of entries in the open arms (PEOA); time of permanence in the open arms (TPOA); and percentage of time of permanence (PTOA). EO 50 mg/kg (p.o.) not only decreased PEOA but also increased TPOA and PTOA. Similarly, EO, at a higher dose (150 mg/kg, i.p.) decreased PEOA ($p < 0.01$), and increased TPOA ($p < 0.01$) and PTOA ($p < 0.01$). NEOA, PEOA, TPOA and PTOA were decreased by EO, 100 mg/kg, i.p. Diazepam (0.75 mg/kg, i.p.) significantly increased all these parameters.

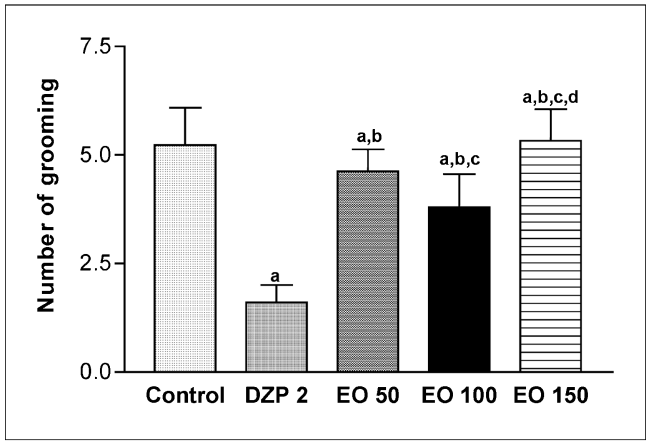
In the barbiturate-induced sleeping time test, the oral ($p < 0.01$) administration of EO (50, 100 and 150 mg/kg respectively) increased the sleep latency time in 67, 22 and 94%, respectively, suggesting an enhanced pentobarbital effect (Table 3).



(A)



(B)



(C)

Fig. 1: Effects of EO from *C. limon* on number of squares crossed, rearing and grooming in the open field test in mice. Values are the mean $\pm$ S.E.M. for number of squares crossed, rearing and grooming of 7 mice (per group) used in the experiments. [a]$p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b]$p < 0.001$ significantly different from DZP 2. [c]$p < 0.001$ significantly different from EO 50 group. [d]$p < 0.001$ significantly different from EO 100 group.

The possible antidepressant effect of EO after oral administration was studied in the forced swimming test (Table 4). Under this condition, EO was used at higher doses (50, 100 and 150 mg/kg, p.o.), since at these three doses the antidepressant effect is masked by the sedative and anxiolytic effects of EO. The results have shown that EO presents a significant antidepressant effect, at the doses of 50, 100 and 150 mg/kg, suggested by the decrease in 44, 49 and 70%, respectively, of the time of immobility. The smallest dose (25 mg/kg) was devoid

Table 2: Effects of EO from *C. limon* on the plus maze test in mice each values represents mean ± S.E.M. of NEOA, number of entries in the open arms; PEOA, percentage of entries in the open arms; TPOA, time of permanence in the open arms; PTOA, percentage of time in the open arms. Values are the mean ± S.E.M. for 7 mice (per group) used in the experiments. [a] $p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b] $p < 0.001$ significantly different from DZP. [c] $p < 0.001$ significantly different from EO 50 group. [d] $p < 0.001$, significantly different from EO 100 group.

Groups	NEOA	PEOA	TPOA	PTOA
Control	6.75 ± 0.90	39.14 ± 0.87	89.08 ± 9.97	29.68 ± 3.32
DZP 0.75	15.71 ± 0.28^{a}	51.00 ± 0.31^{a}	206.90 ± 2.70^{a}	68.95 ± 0.90^{a}
EO 50	$4.60 \pm 0.82^{a,b}$	$38.39 \pm 2.86^{a,b}$	$96.00 \pm 18.01^{a,b}$	$31.99 \pm 6.00^{a,b}$
EO 100	$9.90 \pm 1.36^{a,b,c}$	$48.84 \pm 3.51^{a,b,c}$	$132.3 \pm 15.66^{a,b,c}$	$44.10 \pm 5.21^{a,b,c}$
EO 150	$4.50 \pm 0.47^{a,b,c,d}$	$38.28 \pm 1.79^{a,b,c,d}$	$70.83 \pm 8.15^{a,b,c,d}$	$23.60 \pm 2.71^{a,b,c,d}$

Table 3: Effects of EO from *C. limon*, on the barbiturate-induced sleeping time test values are the mean ± S.E.M. of number of falls and time of permanence for 7 mice (per group) used in the experiments. [a] $p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b] $p < 0.001$, significantly different from EO 50 group. [c] $p < 0.001$, significantly different from EO 100 group.

Groups	Sleeping time (min)	Increase (%)
Control	40.55 ± 0.60	–
EO 50	67.65 ± 0.82^{a}	67
EO 100	$49.62 \pm 1.36^{a,b}$	22
EO 150	$78.82 \pm 0.47^{a,b,c}$	94

Table 4: Effects of EO from *C. limon* in mice in model of forced swimming values are the mean ± S.E.M. of time of immobility for 7 mice (per group) used in the experiments. [a] $p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b] $p < 0.001$ significantly different from EO 50 group. [c] $p < 0.001$ significantly different from EO 100 group. [d] $p < 0.001$ significantly different from EO 150 group. [e] $p < 0.001$ significantly different from IMI 50 group. [f] $p < 0.001$ significantly different from PAROX 20 group. [g] $p < 0.001$ significantly different from RESERP 0.25 group. EO = essential oil; IMI = imipramine; PAROX = paroxetine; RESERP = reserpine. Drugs were administered 10 min before EO and the test performed 30 min later.

Groups	Time of immobility(s)
Control	184.60 ± 26.15
EO 50	103.70 ± 27.76^{a}
EO 100	$93.75 \pm 20.65^{a,b}$
EO 150	$54.83 \pm 19.72^{a,b,c}$
IMI 25	115.00 ± 0.58^{a}
IMI 50	75.00 ± 0.68^{a}
PAROX 10	82.67 ± 1.08^{a}
PAROX 20	133.00 ± 3.28^{a}
EO 150 + IMI 50	$52.00 \pm 21.41^{a,e}$
EO 150 + PAROX 20	$70.60 \pm 15.24^{a,f}$
RESERP 0.25	263.70 ± 2.16^{a}
RESERP 0.25 + EO 150	$16.00 \pm 4.44^{a,d,g}$

Table 5: Effects of EO from *C. limon* in the rota rod test in mice. Values are the mean ± S.E.M. of number of falls and time of permanence for 7 mice (per group) used in the experiments. [a] $p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b] $p < 0.001$ significantly different from DZP. [c] $p < 0.001$ significantly different from EO 50 group. [d] $p < 0.001$ significantly different from EO 100 group.

Groups	Number of falls	Time of permanence (s)
Control	1.71 ± 0.28	52.71 ± 0.42
DZP 0.75	1.80 ± 0.37	52.40 ± 1.03
EO 50	1.80 ± 0.32	52.10 ± 0.92
EO 100	1.90 ± 0.33	52.90 ± 0.52
EO 150	1.890 ± 0.33	$44.80 \pm 0.88^{a,b,c,d}$

of any significant effect. The association of EO, at the doses of 150 mg/kg, with imipramine (IMI) showed a decrease of 5 and 31% in the immobility time, as related to the groups treated with EO alone (150 mg/kg, $p < 0.05$) or IMI ($p < 0.01$) alone, respectively. In addition, the association of EO with paroxetine ($p < 0.01$) alter the effect observed with EO or paroxetine alone ($p < 0.05$), respectively, suggesting that the serotonergic system is involved in the antidepressant effect of EO. On the contrary, the EO activity was totally blocked by the previous administration of reserpine. These data suggest that the noradrenergic and serotoninergic system participates in the EO antidepressant action.

In the rota rod test, used for evaluating motor coordination and presence of any muscle relaxation effect, there was no change in the number of falls after EO administration (50, 100 and 150 mg/kg, p.o.), when compared to controls (Table 5). In addition, in the rota rod, only EO at a dose of 150 mg/kg, p.o., decreased the time of permanence on the bar related to control. Diazepam (0.75 mg/kg, i.p.), similarly to EO 50 and 100, was devoid of effect.

Rectal temperatures of mice after administration of EO from *Citrus limon* are presented in Table 6. EO decreased the temperature in a dose-dependent manner. On the other hand, diazepam (0.75 mg/kg, i.p.) did not present any effect, when compared to control group.

3. Discussion

In the present work, the central effects of the essential oil (EO) of leaves from *C. limon*, were studied. EO was firstly evaluated on the open-field test which gives a good indication of the animal's emotional state. The findings have shown that EO was able to significantly decrease not only the number of crossings,

Table 6: Rectal temperature of mice after administration of EO from *Citrus limon* Values are the mean ± S.E.M. of rectal temperature for 7 mice (per group) used in the experiments. [a] $p < 0.01$ (ANOVA followed by Student-Neuman-Keuls *t*-test), significantly different from control. [b] $p < 0.001$ significantly different from DZP. [c] $p < 0.001$ significantly different from EO 50 group. [d] $p < 0.001$ significantly different from EO 100 group.

Groups	Rectal temperature (°C)
Control	37.30 ± 0.52
Diazepam 0.75	37.60 ± 0.37
EO 50	$35.32 \pm 0.70^{a,b}$
EO100	$34.29 \pm 0.36^{a,b,c}$
EO150	$34.14 \pm 0.35^{a,b,c,d}$

indicative of a possible sedative effect, but also grooming and rearing.

The phytochemical laboratories are rarely prepared to carry out elaborate biological tests made. Thus, in addition to the experiments already conducted in our pharmacology laboratory, we intend to investigate the general toxicity in animal models from rodents to determine the potential toxicity and its biological activity beneficial or detrimental in CNS. The development of new drugs from constituents of the essential oil from *C. limon* needs appropriate models to identify molecular targets that are critical in the brain. Among the large number of possible tests performed to obtain the active constituents of essential oil of *C. limon*, we selected some *in vivo* tests for evaluation of central activity, which constitute important therapeutic targets for neurodegenerative diseases.

In order to study the possible anxyolitic effect of EO, the elevated-plus-maze test was used, and the results have demonstrated that EO was also able to significantly increase the time of permanence as well as the number of entrances in the open arms, indicating a positive response. Our results point out that the sedative as well as the anxyolitic effects of EO possibly involve the $GABA_A$ receptor complex. A sedative action was already shown by the essential oil of leaves from *C. limon* (Campêlo et al. 2011b). *Lavandula angustifolia* P. Miller (Lis-Balchin and Hart 1999) was assessed by the elevated-plus-maze test in rodents. Active constituents of *C. limon* are primarily monoterpene compounds and, thus, chemically similar to EO. These monoterpenes exhibit antianxiety activity, which is thought to be due to GABAergic mechanisms. Furthermore, an essential oil from *C. limon* was shown to exert a dose-dependent increase in antioxidant parameters, in mice hippocampus (Campêlo et al. 2011a). In our study, the forced swimming test was performed to determine the possible antidepressant effect of EO after oral administration. Our result suggests that the noradrenergic and serotoninergic system participates in the EO antidepressant action.

In the rota rod test, used for evaluating motor coordination and presence of any muscle relaxation effect, there was no change in the number of falls after EO administration. In addition, in the rota rod, only EO at a dose of 150 mg/kg, decreased the time of permanence on the bar.

It was observed that the oral treatment of mice with *Citrus limon* essential oil had significant effect on normal mice body temperature. These findings appear to be in contrast to reports with other medicinal plants (Woode et al. 2009). Possible explanations for this contradiction may be the different plant extraction methods used in both studies for extraction of bioactive compounds, and the time/season in which the leaves were harvested. These variations have been reported to exert differences in activities of extracts and essential oil of medicinal plants (Houghton and Raman 1998).

Our results support the idea that EO interacts with the $GABA_A$ receptor, probably at the receptor subtypes that mediate BDZ effects, to produce sedative and hypnotic activities, and also acts to increase the noradrenergic and serotoninergic activities that is the main factor responsible for its antidepressant activity. Additional studies, however, should be carried out to fully clarify the mechanism of anxiolytic and antidepressant effects of ethanolic extract of leaves from *C. limon*. Furthermore, EO could manifest these effects at some doses without showing either sedative or hypnotic activities, being thus potentially useful in clinical practice.

4. Experimental

4.1. Plant material

Citrus limon was identified and collected by Chistiane Mendes Feitosa in February 2010, at the city of Picos, state of Piaui, Brazil; and their excicatae deposited at the Graziella Barroso Herbarium of the Federal University of Piaui under the voucher number 26.453. Sample of *Citrus limon* essential oil leaves were prepared by Laboratory of Chemistry of Natural Products of this University (Matos et al. 1996).

4.2. Plant extraction of Citrus limon essential oil

Leaves (0.3 kg) from "limoeiro" were collected on the same day and hour, from flowering plants. The same leaf samples collected were steam distilled for 1 h, and the essential oil obtained from these leaves were analyzed by GC/MS using a GC-17 A/MS-QP505A (Shimadzu) instrument under the following conditions: column: dimethylpolysiloxane DB-1 fused-silica capillary column (30 m x 0.25 mm); carrier gas, helium (1 ml/min); injector temperature, 35–180 °C at 4 °C/min, then 180–250 °C at 10 °C/min; mass spectra electron impact, 70 eV (Matos et al. 1996). Individual components were identified by spectrometric analyses using two computer library MS searches, and Kovacs indices as a preselection aid. Visual mass spectra comparison data from the literature were used for confirmation.

4.3. Animals and behavioral tests

Male Swiss mice (25–30 g) were used. All animals were maintained at a controlled temperature (23 ± 1 °C) and a 12-h dark/light cycle. Animals had free access to water and food. All behavioral tests were conducted in quiet rooms at the same controlled conditions referred above and isolated from external noise. Different groups of mice were used for each behavioral task. For each experiment, mice were randomized into five groups (7 mice per group): one control group treated with the vehicle, three groups treated with EO at 50, 100 and 150 mg/kg, respectively, and a fifth drug reference group treated with imipramine 25 or 50 mg/kg, paroxetine 10 or 20 mg/kg and reserpine 0.25 mg/kg (forced swimming) or DZP 0.75 or 2 mg/kg (open field, rota rod, forced swimming). Treatment with EO or vehicle was given orally (p.o.) via gastric gavage (1 ml/kg) for 30 days.

Intraperitoneal (i.p.) administration with imipramine and oral treatment with DZP were done 30 and 60 min before the tests, respectively, following the schemes of other authors (Tadano et al. 2000; Griebel et al. 2001; Kulkarni and Dhir 2007).

Animal care followed the official governmental guidelines in compliance with the Society Policy and was submitted by the Ethics Committee of the Federal University of Piaui, Brazil. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All doses are expressed in milligrams per kilogram and were administered in a volume of 10 ml/kg injected intraperitoneally (i.p.).

4.4. Drugs and reagents

The essential oil (EO) was emulsified with polyoxyethylenesorbitan monooleate (Tween 80 - 0.5% v/v in saline, Synth, Brazil) in distilled water. Diazepam (DZP) was purchased from the (União Química, Brazil) and used as standard. Reserpine sulphate (RESERP) was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Imipramine (IMI) and paroxetine (PAROX) were purchased from the Novartis Biociências S.A. (São Paulo, Brazil) and Glaxo Smith Kline Brasil Ltda (Rio de Janeiro, Brazil), respectively. All other drugs were of analytical grade.

4.5. Experimental protocol and behavioral screening

After the treatment, each animal was submitted to a series of tests in the manner described below. Firstly, the animal was observed in a closed room,

at constant temperature (23 ± 1 °C). The animal was then placed inside a plus maze and observed for 5 min. Immediately after the plus maze test, it was placed in the open field area for 5 min. After that, the animal was removed to the rota rod where it was evaluated for 1 min. Finally, its temperature was taken with a digital thermometer. All the tests were performed between 08:00 a.m. and 18:00 p.m.

Behavioral screening of the mice was performed following parameters described by Almeida et al. (1999) and animals were observed during 24 h after 30 days of treatment with EO of *C. limon* (50, 100 and 150 mg/kg, p.o.). During these 30 days were observed the occurrence of the following general signs of toxicity: piloerection, prostration, writhing, increased evacuation, grooming, discrete groups, dyspnea, sedation, analgesia and palpebral ptosis.

4.6. Elevated plus maze test (EPM)

The plus maze for mice consisted of two perpendicular open arms (30 × 5 cm) and two closed arms (30 × 5 × 25 cm) also in perpendicular position (Lister 1987). The open and closed arms were connected by a central platform (5 × 5 cm). The platform and the lateral walls of the closed arms were made of transparent acrylic. The floor was made of black acrylic. The maze was 45 cm above the floor. After treatment, the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence measures the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the natural animal's aversion to the open arms and promotes the exploration thereof. On the other hand, the forced or voluntary passages of the animal into the open arms of the EPM are associated with hormonal and behavioral changes indicative of increased anxiety (Hogg 1996). These tests were done in different places, justifying the use of this apparatus.

4.7. Open field test

The open field area was made of acrylic transparent walls and black floor (30 × 30 × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the animal (Archer 1973). The observed parameters were: number of squares crossed (with the four paws) and numbers of grooming and rearing.

4.8. Rota rod test

For the rota rod test, the animal was placed with the four paws on a 2.5-cm diameter bar, 25 cm above the floor, which was turning at 12 rpm. For each animal, the number of falls (up to three falls) and the time of permanence on the bar for 1 min were registered (Dunham and Miya 1957).

4.9. Barbiturate-induced sleeping time

In this test, performed according to the method of Ferrini et al. (1974) the mouse sleep was induced by an i.p. administration of 40 mg/kg body wt of pentobarbital, and the duration of sleep (min) of each animal was observed. The sleeping time was recorded as the period for recovering the righting reflex.

4.10. Forced swimming test

This test is the most widely used and recognized pharmacological model, for assessing antidepressant activities (Porsolt et al. 1977a). In the present work, we employed that described by Porsolt et al. (1977b; 1978) relying on the development of immobility when mice were placed inside an inescapable cylinder filled with water.

4.11. Rectal temperature

The rectal temperature of the mice was determined with a digital thermometer Modelo HI 98501 (SP Labor Equipments, São Paulo, Brazil) at the distance of 2.5 mm from the anus. The measurement was performed at room temperature of 24.0 ± 2.0 °C. Mice with normal rectal temperature 37.0–38.0 °C were selected and used in the experiment.

4.12. Statistical analysis

Results were expressed as means ± S.E.M. and were compared by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls *t*-test ($p < 0.05$) (Graphpad program Intuitive, Software for Science, San Diego, CA).

Acknowledgments: We would like to thank the National Council of Technological and Scientific Development (CNPq/Brazil) [grant number 503354/2009–0] and the Research Supporting Foundation of State of Piaui (FAPEPI/Brazil) for the financial support.

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 Farmacogn 80: 72–76.

Archer J (1973) Tests for emotionality in rats and mice: a review. Anim Behav 21: 205–235.

Buller R, Legrand V (2001) Novel treatments for anxiety and depression: Hurdles in bringing them to the market. Drug Discov. Today 6: 1220–1230.

Campêlo LML, Feitosa CM, Freitas RM (2011a) Evaluation of central nervous system effects of essential oil from *Citrus limon* (Burms) in mice. Rev Bras Farmacogn - *in press*.

Campêlo LML, Gonçalves FCM, Feitosa CM, Freitas RM (2011b) Antioxidant activity of Citrus limon essential oil in mice hippocampus. Pharm Biol - *in press*.

Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 46: 208–209.

Ferrini R, Miragoli G, Taccardi B (1974) Neuropharmacological studies on SB 5833, a new psychotherapeutic agent of the benzodiazepine class. Arzneimittel-Forschung 24: 2029–2032.

Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M (2001) SL651498: an anxioselective compound with functional selectivity for 2- and 3-containing -aminobutyric acidA ($GABA_A$) receptors. J Pharmacol Exp Ther 298: 753–768.

Gomes PB, Noronha EC, Melo CTV, Bezerra JNS, Neto MA, Lino CS, Vasconcelos SMM, Viana GSB, Sousa FCF (2008) Central effects of isolated fractions from the root of *Petiveria alliacea* L. (tipi) in mice. J Ethnopharmacol 120: 209–214.

Hogg S (1996) A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 54: 21–30.

Houghton PJ, Raman A (1998) Laboratory Handbook for the Fractionation of Natural Extracts. Chapman and Hall, London, UK, pp. 39–45.

Kulkarni SK, Dhir A (2007) Effect of various classes of antidepressants in behavioral paradigms of despair. Prog Neuropsychopharmacol Biol Psychiatry 31: 1248–1253.

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.

Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92: 180–185.

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.

Porsolt RD, Bertin A, Jalfre M (1977a) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229: 327–336.

Porsolt RD, Le Pichon M, Jalfre M (1977b) Depression: a new animal model sensitive to antidepressant treatments. Nature 266: 730–722.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: A new model sensitive to antidepressant treatments. Eur J Pharmacol 47: 379–391.

Santos LFL, Freitas RLM, Xavier SML, Saldanha GB, Freitas RM (2008) Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures. Pharmacol Biochem Behav 89: 1–5.

Tadano T, Nakagawasai O, Niijima F, Tan-No K, Kisara K (2000) The effects of traditional tonics on fatigue in mice differ from those of the antidepressant imipramine: a pharmacological and behavioral study. Am J Chin Med 28: 97–104.

Xavier SM, Barbosa CO, Barros DO, Silva RF, Oliveira AA, Freitas RM (2007) Vitamin C antioxidant effects in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine. Neurosci Lett 420: 76–79.

Wong AHC, Smith M, Boon HS (1998) Herbal Remedies in Psychiatric Practice. Arch Gen Psychiatry 55: 1033–1044.

Woode E, Poku RA, Ainooson GK, Boakye-Gaisi E, Abotsi WKM, Mensah TL, Amoh-Barimah AK (2009) An evaluation of the anti-incammatory, antipyretic and antinociceptive effects of *Ficus exasperata* (Vahl) leaf extracts. J Pharm Toxicol 4: 138–151.

Zhang, ZJ (2004) Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci 75: 1659–1699.