

SHORT COMMUNICATION

Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice

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Abstract

Tea obtained from leaves of *Cymbopogon citratus* (DC) Stapf is used for its anxiolytic, hypnotic and anticonvulsant properties in Brazilian folk medicine. Essential oil (EO) from fresh leaves was obtained by hydrodistillation and orally administered to Swiss male mice 30 min before experimental procedures. EO at 0.5 or 1.0 g/kg was evaluated for sedative/hypnotic activity through pentobarbital sleeping time, anxiolytic activity by elevated plus maze and light/dark box procedures and anticonvulsant activity through seizures induced by pentylenetetrazole and maximal electroshock. EO was effective in increasing the sleeping time, the percentage of entries and time spent in the open arms of the elevated plus maze as well as the time spent in the light compartment of light/dark box. In addition, EO delayed clonic seizures induced by pentylenetetrazole and blocked tonic extensions induced by maximal electroshock, indicating the elevation of the seizure threshold and/or blockage of seizures spread. These effects were observed in the absence of motor impairment evaluated on the rotarod and open field test. Our results are in accord with the ethnopharmacological use of *Cymbopogon citratus*, and after complementary toxicological studies it can support investigations assessing their use as anxiolytic, sedative or anticonvulsive agent.

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Introduction

Numerous herbal medicines are recognized as active in the central nervous system (CNS), and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to conventional treatments (Phillipson, 2001; Carlini, 2003;). *Cymbopogon citratus* (DC) Stapf – Poaceae, an herb known worldwide as lemongrass (local name: capim-cidrao) is widely used in

tropical countries as a source of ethnomedicines (Di Stasi et al., 1989; Duke, 1989; Tortoriello and Romero, 1992). In spite of the strong popular indication and a short communication about its action published many years ago (Seth et al., 1976), there are few controlled experimental studies on their CNS activity, with some discrepant results. As pointed out by Viana et al. (2000a), negative results obtained in rodents (Carlini et al., 1986; Souza-Formigoni et al., 1986) and in human beings (Leite et al., 1986) could be due to different chemotypes of lemongrass evaluated, since there are at least two varieties: East Indian (roughly equal amounts of myrcene and citral) and the West Indian type (little myrcene but high amount of citral). The aim of the present study was to investigate the presence of CNS activity of the essential oil with high citral content, obtained from fresh leaves of *C. citratus*,

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using different experimental models to access anxiolytic, sedative and anticonvulsant activities in mice.

Materials and methods

Plant material and essential oil (EO) extraction: Leaves were collected from the garden of medicinal plants (Lageado Farm, UNESP, Botucatu, SP, Brazil) and a voucher specimen (#23031) has been deposited in the *Irina D. Gemtchujnirov* – BOTU herbarium. EO was obtained by hydrodistillation of fresh leaves (yield: 0.45% w/v) and stored, protected against light and heat, until behavioral assays. EO was analyzed by gas chromatography coupled with mass spectrometry according to these experimental conditions: injection of 1 μ l of a solution made with 5 μ l of EO suspended in 1 ml of ethyl acetate; silica capillary column: DB-5 (30 m \times 0.25 mm \times 0.25 μ m), electron impact: 70 eV, carrier gas: helium at 1.7 ml/min, injector temperature: 240 $^{\circ}$ C, detector temperature: 230 $^{\circ}$ C, temperature program: 50 $^{\circ}$ C (5 min)–250 $^{\circ}$ C, 5 $^{\circ}$ C/min. The identification of the components was made through comparison of substance mass spectrum with the database of the GC/MS (NIST 62.lib), literature and retention index (Adams, 1995; McLafferty and Stauffer, 1989).

Animals: Adult male Swiss mice (35–45 g) from the colony at the UNESP were maintained under controlled environmental conditions of temperature (21 \pm 2 $^{\circ}$ C) and light (12/12 light/dark cycle) with food and water ad libitum until 2 h before experimental procedures. Experimental protocols were designed according to the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Bioscience Institute – Ethics Committee for Animal Research (CEEAA).

Treatments: Immediately before use, EO was suspended in a vehicle (polyoxyethylenesorbitan monooleate – Tween 80[®] 12% v/v in saline, Synth, Brazil) to achieve the proper dosage. The control group received vehicle (TW) at the same volume as the treated groups. Chlordiazepoxide (CDZ – 10 mg/kg, Psicosedin[®], Farmasa, Brazil), valproic acid (ACV – 400 mg/kg: Depakene[®], Abbott, Brazil) or diazepam (DZP – 1.0 mg/kg: Valium[®], Roche, Brazil) was used as the standard drug. All treatments were made at 10 ml/kg orally by gavage, except the animals that received DZP, which was administered intraperitoneally.

Neurobehavioral evaluation: Neurobehavioral effects were evaluated according to classical procedures, previously described in detail (Carvalho-Freitas and Costa, 2002; Pultrini et al., 2006). Shortly, acute toxicity and effects on gross behavior were evaluated in groups of five animals treated with EO at 0.25, 0.5, 1.0, 3.0 or 5.0 g/kg, and observed 30 min, 1 h, 3 h and 5 h after treatment and intermittently for 15 days during which their bodyweights were taken. Complementary evalua-

tion of the exploratory activity and motor system integrity was made after 30 min, 2 h and 24 h in mice treatment with EO or TW, through 3 min of observation in the open field procedure followed by evaluation on rota-rod apparatus, where the total time performed was registered on a stopwatch. Mice were previously selected in order to avoid a bias due to bad performance on rota-rod not related to treatments. Specific procedures were made in independent groups of mice treated with 0.5 or 1.0 g/kg of EO 30 min before induction of event. Hypnosis was induced by sodium pentobarbital (40 mg/kg, ip) and the latency and the duration of the sleep was individually recorded. Anxiolytic activity was evaluated for 5 min in the elevated plus maze and in the light/dark box. Convulsive episodes were induced by pentylenetetrazole (PTZ, 85 mg/kg, sc) or by maximal electroshock (MES – 50 mA, 60 Hz, 0.15 s, corneal).

Statistical analysis: Quantitative data were submitted to Kruskal–Wallis non-parametric variance analysis, followed by the Mann–Whitney test, when appropriate. Proportions were compared by Fisher's exact test. Contrasts were made between treated and TW (vehicle) group and differences were considered significant when $p \leq 0.05$.

Results

The chromatogram obtained by gas chromatography coupled with mass spectrometry is presented in Fig. 1. The analyses indicated the monoterpene citral, a mixture of the stereoisomers geranial (40.8% – peak 14) and neral (36.3% – peak 12), and beta-myrcene (13.2% – peak 2) as the main compounds in the EO.

Acute toxicity: Mice treated with different doses of EO presented some depressant effects such as reduction in general activity, righting and auricular reflexes, placing reactions, equilibrium, touch responses and strength grasping. These inhibitory effects, mainly related to disturbances of the motor system and coordination, were classified as discrete until 1.0 g/kg dosage and moderate to serious at higher doses. Treatment with EO did not present signs of toxicant

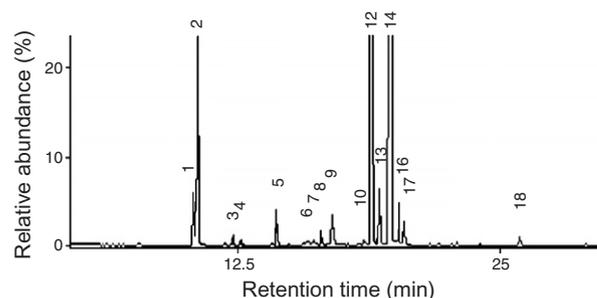


Fig. 1. Chromatogram obtained by gas chromatography coupled with mass spectrometry. Peaks: 2 – beta-myrcene; 12 – neral; 14 – geranial.

effects which could be detected by alteration in body weight measured for 15 days (data not shown). One death was registered in the group treated with 5.0 g/kg and 3 deaths in the group treated with 3.0 g/kg, which would indicate the classification of slightly toxicant according to Hodgson (1997).

Open field and rota rod tests: There were no differences between the EO and TW groups for parameters observed in the open field: ambulation; rearing; freezing and grooming, or in the rota-rod test when evaluated 30 min, 2 h and 24 h after treatments. Data obtained 30 min after treatments are presented in Table 1, and the same pattern of results was observed after 2 and 24 h (data not shown).

Assessment of anxiolytic activity: Results are presented in Fig. 2. In the elevated plus maze the treatment with EO or DZP was effective in increasing the frequency of open-arm entries, expressed as percentage of total arm entries. Treatment with 1.0 g/kg or DZP resulted in increase of time spent in the open arms in relation to total time spent inside arms (open + enclosed), as well as in decrease of time spent on the central platform. The total arm entries were not affected by any treatment compared to the TW group. In the light/dark box test, differences among groups were observed in time spent in the light compartment and in number of shuttle crossings. The time spent in the illuminated compartment was significantly enhanced due to treatment with 1.0 g/kg of EO or DZP. There was no observed effect after treatment with EO in transitions between white and black compartments.

Assessment of anticonvulsant activity: Occurrences of convulsive episodes after PTZ- and MES-induced seizures are presented in Table 2. In the MES test, 90% of vehicle-treated mice developed typical seizure patterns: the tonic flexion of the limbs occurred immediately after the shock, progressing to tonic extension of hind limbs followed by stupor and recovery. Mice treated with EO were protected against seizure episodes, reducing the occurrence to 40% in mice treated with 0.5 g/kg and to 20% in the group treated with 1.0 g/kg. In the PTZ test, treatment with EO was not able to reduce the occurrence of convulsive episodes. Due to its usual variability, the latency to the first

convulsive episode had a weak significance if the occurrence of convulsion was not prevented. However, a difference in latency to clonic episode was observed after treatment with 1.0 g/kg of EO in PTZ-test, in which the latency in seconds was significantly improved from [median (Q_1 – Q_3)] 221 (124–245) in the TW group to 422 (248–823).

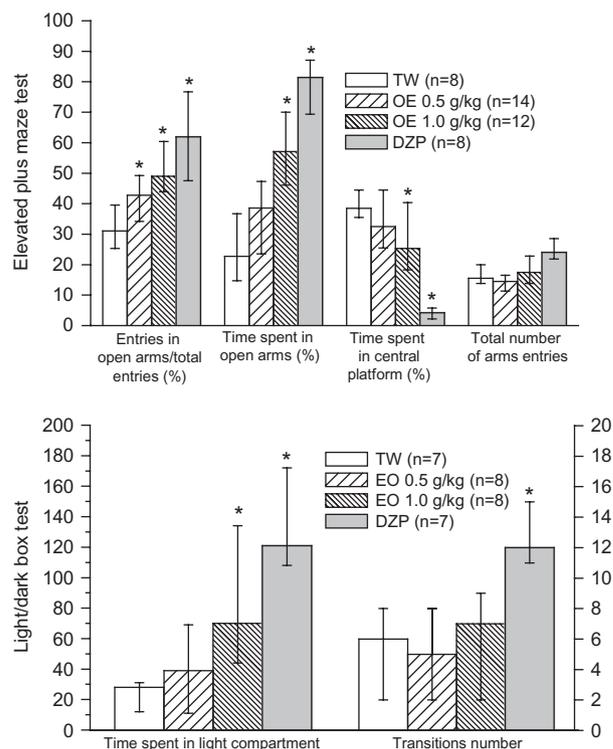


Fig. 2. Effect of essential oil on elevated plus maze and on the light/dark box tests. Upper panel: percentage of entries in open arms; percentage of time spent in open arms; percentage of time spent in central platform and total arm entries on elevated plus maze. Bottom panel: time (s) spent in the light compartment (left scale) and number of transitions between compartments (right scale) on the light/dark box. Results are expressed as median and interquartile range (Q_1 – Q_3). EO: essential oil of *C. citratus*; TW: control group (oral route); DZP: diazepam (1.0 mg/kg, ip). The number of animals in each group is in parentheses. * $p < 0.05$, compared with TW group (Kruskal–Wallis followed by Mann–Whitney test).

Table 1. Effects of essential oil on open field and rota-rod tests performed 30 min after oral treatment

Treatment (<i>n</i>)	Parameters in open field				Total time (s) on rota-rod
	Ambulation	Rearing	Grooming (s)	Freezing (s)	
TW (5)	94 (90–110)	24 (23–25)	12 (8–38)	4 (1–11)	160 (111–180)
EO 0.5 g/kg (5)	80 (66–87)	20 (16–20)	3 (1–33)	7 (3–7)	180 (124–180)
EO 1.0 g/kg (5)	85 (80–103)	15 (12–18)	7 (6–12)	8 (2–11)	160 (160–180)

Data are expressed as median and interquartile range (Q_1 – Q_3). EO: essential oil of *C. citratus*; TW: control group. Treatments were applied orally. Comparisons were made between treated and TW groups with Kruskal–Wallis Test ($p > 0.05$ in all cases).

Table 2. Effects of essential oil on anticonvulsive (pentylenetetrazole – PTZ and maximal electroshock – MES induced seizures) and sodium pentobarbital sleeping time

Treatment	PTZ test				MES test			Sleeping time		
	<i>n</i>	Clonic (%)	Tonic (%)	Death (%)	<i>n</i>	Tonic (%)	Death (%)	<i>n</i>	Latency (min)	Duration (min)
TW	19	100	80	80	10	90	10	8	3.6 (3.3–4.7)	28 (25–40)
EO 0.5 g/kg	10	90	80	90	10	40*	10	8	2.9 (2.4–3.2)#	121 (114–141)#
EO 1.0 g/kg	10	90	60	60	10	20*	0	7	2.0 (1.8–2.5)#	139 (127–188)#
Standard drug	20	0*	0*	0*	10	0*	0	11	3.0 (2.6–3.4)#	98 (57–131)#

Data are expressed as percentage [PTZ and MES tests] or median and interquartile range (Q_1 – Q_3) [sleeping time]. EO: essential oil of *C. citratus*; TW: control group. Standard drug for PTZ test and sleeping time: CDZ; for MES test: ACV. $p < 0.05$ when compared with TW group: *Fisher's exact test; #Kruskal–Wallis followed by Mann–Whitney test.

Pentobarbital sleeping time: Induction of sleep occurred in 100% of animals in the group treated with CDZ (total $n = 11$) and in 80% of animals in the TW group (total $n = 10$). EO-treated groups presented sleep occurrence of 73% (0.5 g/kg – total $n = 11$) and 78% (1.0 g/kg – total $n = 9$). There were no significant differences between groups related to sleeping occurrence. Treatment with EO was effective in decreasing the latency to sleep induction after treatment with 0.5 or 1.0 g/kg, as well as after CDZ treatment. Sleep duration was significantly increased after treatments with 0.5 or 1.0 g/kg of EO or CDZ (Table 2). It was observed the occurrence of a deep depression of the CNS in four mice treated with 1.0 g/kg, persistent until 4 h after barbiturate treatment. In this way, data from these animals were not considered on sleeping time.

Discussion and conclusions

C. citratus is popularly used in Brazil mainly due to its central nervous system action, with few controlled studies about this type of activity. The present study provided some data on the effects of the essential oil from fresh leaves on the mouse central nervous system, providing information about motor performance, sedative–hypnotic, anxiolytic and anticonvulsant activities.

The rota-rod and open field tests were used to assess the locomotor and exploratory activity and similar performance observed in mice treated with EO in relation to the control group suggests absence of impaired motor coordination, equilibrium or exploratory behavior due to treatment with EO. Absence of effect on motor activity was also observed in elevated plus maze, where treated mice performed similarly to the control mice in relation to total arm entries, a mixed measure that reflects changes in motor activity more than in anxiety (Lister, 1990; File, 2001). In this way, we can accept absence of deleterious effect on coordination and motor activity due to EO treatment at evaluated doses.

Decrease in sleeping latency and increase in sleeping time induced by pentobarbital, as observed after treatment with EO, are conventional measures related

to a sedative/hypnotic property. Nevertheless, it is a non-specific test, since compounds that decrease the rate of hepatic biotransformation of barbiturates can show the same behavioral effects as central nervous system depressant drugs (Fujimoto et al., 1960). The two main compounds in EO, citral (Nakamura et al., 2003) and myrcene (De-Oliveira et al., 1997) were recognized as enzyme inducers, suggesting central depressant activity instead of nonspecific action upon barbiturate metabolism. This activity was emphasized by the occurrence of deep depression, with respiratory failure, in animals treated with association of 1.0 g/kg of EO and pentobarbital. No similar effect was observed in mice evaluated daily for 15 days or during evaluation of anxiolytic and anticonvulsant activities, which were treated with the same dose of EO alone.

Results obtained on the elevated plus maze after treatment with EO reveal anxiolytic activity, since increases in open-arm parameters are the most representative indices of anxiolytic activity (Lister, 1990). Time spent on the central platform appears to be related to decision making and/or risk assessment, and the total arm entries is a contaminated measure reflecting changes in anxiety or in general activity (File, 2001). Anxiolytic-like activity was also observed in the light/dark box. In this test, the number of transitions between the light and dark compartments as well as the time spent in the light side are recognized as anxiety indices, despite the transition parameter being highly dependent on locomotor activity (Bourin and Hascoët, 2003). Mice treated with EO at 1.0 g/kg performed the test by increasing the time spent in the light compartment, without changes in the numbers of shuttle crossings, confirming activity upon the main anxiolytic parameter.

The anticonvulsant property was evaluated by experimental procedures widely used to investigate antiepileptic drugs, with high predictive value for detection of clinically effective drugs (White, 1997). EO was able to modify the progress of convulsive episodes induced by both PTZ and MES. The PTZ test identifies drugs with efficacy against non-convulsive absence or myoclonic

seizures, acting due to increase of the seizure threshold while the MES test identifies agents active against generalized tonic-clonic seizures due to blocking of seizure spread (Loscher and Schmidt, 1988). Our results suggest that EO from *C. citratus* has the potential to alter the course of convulsive episodes, interfering in seizure threshold and/or blocking seizure propagation. Since isolated citral or myrcene was not able to protect mice against convulsive episodes (Viana et al., 2000b), or to show anxiolytic activity (Vale et al., 2002), the results obtained with EO are probably due to synergistic action of more than one compound or on account of compounds present in small amounts. Considerations about synergistic action could also be supposed in relation to antinociceptive activity of essential oil from *C. citratus*, acting by central and peripheral mechanisms (Viana et al., 2000a).

The behavioral results obtained with EO from fresh leaves of *Cymbopogon citratus* are in accord with the ethnopharmacological use of this plant, suggesting a sedative/hypnotic, anxiolytic and anticonvulsant activity.

This results profile reinforces the biological and cultural value of traditional studies as sources for new drugs for treatment of central disturbances. Meanwhile, it should be pointed out that the effective dose of EO is inconsistent with the amount of fresh leaves used to make a cup of tea, usually 2–10 g. Considering only the extraction yield (0.45%), roughly 200 g of fresh leaves/kg was required for EO doses of 1.0 g/kg. This straightforward reasoning deserves some considerations. Firstly, due to several factors – including the interspecies differences in plasma clearance of drugs, which principally reflects hepatic and renal peculiarities – there is not a definitive rule for extrapolation of doses among species. Another point to consider is the difference in dosage regimen, typically in repeated or chronic intake when the population uses the tea, as opposed to acute treatment employed experimentally. Moreover, compounds extracted from leaves by warm water – as in tea preparation – could be different or present in unequal proportions in the essential oil; and differences in composition and/or concentration of compounds could interfere with absorption and distribution processes. In this way, the same compound could be present in a soluble form, easily absorbable from the tea, and in a less soluble form in the essential oil. These considerations could be assessable in the continuity of this study in order to improve the knowledge of its pharmacokinetics, pharmacodynamics, efficacy and safety in long-term use.

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