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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.

Keywords: Anxiolytic; Sedative; Anticonvulsant; *Cymbopogon citratus*; Essential oil; Lemongrass

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-cidrão) 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. Gemtchujniov* – 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 µl of a solution made with 5 µl of EO suspended in 1 ml of ethyl acetate; silica capillary column: DB-5 (30 m × 0.25 mm × 0.25 µm), electron impact: 70 eV, carrier gas: helium at 1.7 ml/min, injector temperature: 240 °C, detector temperature: 230 °C, temperature program: 50 °C (5 min)–250 °C, 5 °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 ± 2 °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 (CEEA).

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 evaluation 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 $p \leqslant 0.05$.

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

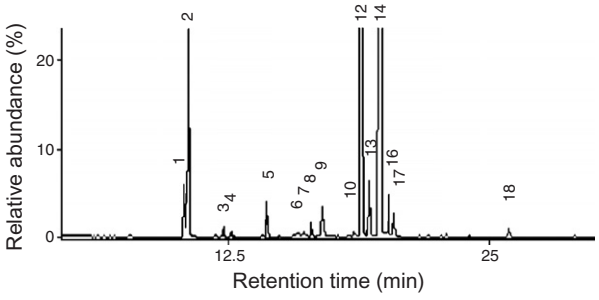

Fig. 1. Chromatogram obtained by gas chromatography coupled with mass spectrometry. Peaks: 2 – beta-myrcene; 12 – neral; 14 – geranial.