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Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil

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Abstract

Ocimum gratissimum L. (Lamiaceae) and other species of the same genus are used as medicines to treat central nervous system (CNS) diseases, commonly encountered in warm regions of the world. The chemical composition of *Ocimum gratissimum* essential oil varies according to their chemotypes: timol, eugenol or geraniol. In this study, the essential oil type eugenol was extracted by hydrodistillation in each of the four seasons of the year. Activity upon CNS was evaluated in the open-field and rota-rod tests; sleeping time induced by sodium pentobarbital (PBS, 40 mg/kg, intraperitoneally, i.p.) and anticonvulsant activity against seizures induced by both pentylenetetrazole (PTZ; 85 mg/kg, s.c.) and maximal electroshock (MES, 50 mA, 0.11 s) were determined. Essential oils obtained in each season were effective in increasing the sleeping duration and a preparation obtained in Spring was able to protect animals against tonic seizures induced by electroshock. In each season, eugenol and 1,8-cineole were the most abundant compounds, and in Spring the essential oil presented the greatest relative percentage of sesquiterpenes, suggesting that these compounds could explain the differences observed in the biological activity in essential oils obtained in different seasons of the year.

Keywords: *Ocimum gratissimum* L.; Phytochemistry; Seasonality; Anticonvulsant; Sedative

1. Introduction

Several *Ocimum* species (Lamiaceae) are used to treat central nervous system (CNS) disorders in various parts of the world and its depressive 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).

Published data from ethnopharmacological sources indicate the use of *Ocimum basilicum* as a sedative in Spain (Vázquez et al., 1997) and as a sedative and anticonvulsant in Mexico (Tortoriello and Romero, 1992). 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 from *Ocimum tenuiflorum* (*Ocimum sanctum*) (Sakina et al., 1990; Pérez de Alejo et al., 1996; Jaggi et al., 2003). Experimental procedures using preparations obtained from *Ocimum gratissimum* were able to detect relaxant effect on isolated intestinal smooth muscle (Madeira et al., 2002) and antinociceptive properties (Aziba et al., 1999; Rabelo et al., 2003), but there were no studies focusing on the central nervous system activity. Chemical composition of essential oil from *Ocimum* species is variable. Different chemotypes have been reported for *Ocimum basilicum* (Grayer et al., 1996) and for *Ocimum canum* and *Ocimum gratissimum* (Martins et al., 1999). Two morphological varieties of *Ocimum gratissimum* (var. *gratissimum* and var. *macrophyllum*) were found based on volatile oil constituents 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). In the present study with *Ocimum gratissimum* the major component is eugenol, followed by 1,8-cineole, which is present in very low amount in accessions studied by Vieira et al. (2001).

Experimental studies showed that eugenol has anesthetic, hypothermic, myorelaxant and anticonvulsant properties

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(Dallmeier and Carlini, 1981) and that 1,8-cineole presents stimulant activity (Umezu et al., 2001).

It is known that climatic conditions and water available in the soil can change the vegetal secondary metabolism and, consequently, alter the composition of essential oils, throughout the seasons of the year. Chemical variations in essential oils were associated with seasons for *Ocimum selloi* (Moraes et al., 2002) and with time of day for *Ocimum gratissimum* (Vasconcelos Silva et al., 1999).

Thus, the aim of the present study was to investigate whether seasonal variations in composition of essential oil of *Ocimum gratissimum* are accompanied by changes in pharmacological properties, using experimental procedures to investigate the central nervous system activity.

2. Materials and methods

2.1. Plant material and extraction

Leaves of *Ocimum gratissimum* (local name: alfavaca or manjericão) were collected from a cultivated field at the medicinal plants garden (Lageado Farm, UNESP, Botucatu, São Paulo, Brazil) and a voucher specimen (#23.279) was made for *Ocimum gratissimum* L. var. *gratissimum* and deposited at Irina D. Gemtchyniov—BOTU herbarium. The harvests were accomplished in the middle of each season of the year and the essential oil was immediately extracted by hydrodistillation. Fresh leaves were immersed in double their volume of distilled water and boiled for 4 h in the Clevenger apparatus. Essential oil was collected in amber flasks and stored at the temperature of +4 °C until use. Each extraction of essential oil is identified by the name of the season in which it was harvested.

2.2. Animals

All experimental procedures were carried out using adult male Swiss mice (30–45 g) from the colony at the Central Animal House of UNESP and maintained under standard environmental conditions: temperature 21 ± 2 °C, 12-h light:12-h dark cycle at the Animal House of the Department of Pharmacology for at least 1 week before the experiments. The experimental protocols were designed according to Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Bioscience Institute/UNESP—Ethics Committee for Animal Research (CEEA).

2.3. Treatments

Independent experimental procedures were accomplished to evaluate the activity of essential oil obtained in each season of the year. Immediately before use, the essential oil was suspended in a vehicle (polyoxyethylenesorbitan monooleate—Tween 80®—12% (v/v) in saline, Synth, Brazil) to achieve the proper doses. Experimental sessions were completed with a negative control group treated with the vehicle (TW) and a positive control group treated with standard drugs: chlordiazepoxide (CDZ, 10 mg/kg: Psicosedin®, Farmasa, Brazil) or valproic acid (ACV, 400 mg/kg: Depakene®, Abbott, Brazil), in order to validate the experimental conditions. Essential oils were assayed at 0.5 and 10 g/kg and treatments with essential oil, vehicle or standard drug were made by oral route and the volume administered was always adjusted to 10 ml/kg. Food and water were available until 2 h before treatments and experimental procedures were begun after 30 min of treatments.

2.4. Essential oil composition

Samples of the essential oil obtained in each season were suspended in 1 ml of ethyl acetate (P.A., Merck, Germany) and 1 μl of this solution was analyzed by gas chromatography coupled with mass spectrometry (GC/MS, Shimadzu, model QP-5000) equipped with a fused silica capillary column DB-5 (30 m × 0.25 mm × 0.25 μm). The electron impact technique (70 eV) was used with the injector temperature at 240 °C and that of the detector at 230 °C. The carrier gas was helium at the working rate of 1.7 ml/min. The column temperature was initially 60 °C and then was gradually increased at the rate of 3 °C/min up to 240 °C. For detection of the oil components, we used a flame ionization detector set up at 230 °C. The identification of the components of the essential oils was effected through comparison of substance mass spectrum with the database of the GC/MS (NIST 62.lib), literature and retention index (McLafferty and Stauffer, 1989; Adams, 1995).

2.5. Pentobarbital sleeping time

After treatment with TW, CDZ or essential oil, sleep was induced by sodium pentobarbital (SPB; 40 mg/kg, i.p., Hypnol® 3%, Fontoveter, Brazil) administration. Immediately after SPB administration, each animal was placed in an individual cage and observed to record the time elapsed from the injection to the loss of the righting reflex (induction time) and the time between the loss and recovery of righting reflex (sleeping time) (Dandiya and Cullumbine, 1959).

2.6. Anticonvulsive evaluation

Convulsive episodes were induced using chemical or electrical stimuli recognized as models to identify drugs that contribute to the elevation of the seizure threshold or blocking spread of seizures (Swinyard and Kupferberg, 1985).

Chemical stimulus was provided in animals treated with TW, CDZ or essential oil by administration of pentylenetetrazole (PTZ; 85 mg/kg, Sigma, Brazil) dissolved in saline and injected by subcutaneous route (6 ml/kg) into the loose fold on the dorsal neck. Immediately, each animal was placed in an individual cage and observed for 40 min in order to register the percentage of occurrence and latency for clonic and tonic convulsive episodes and for death.

Corneal electrodes were used for bilateral delivery of electrical stimulus (maximal electroshock seizures, MES—50 mA; 0.11 s). The percentage of occurrence of hind-limb tonic exten-