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Sedative and anxiolytic efficacy of *Tilia americana* var. *mexicana* inflorescences used traditionally by communities of State of Michoacan, Mexico

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Abstract

Aim of the study: Increasing demand of herbal products acquired in stores and markets, as well as medicinal plants collected for personal consume are a known modern tendency. In this study, the ethnomedicinal use of *Tilia americana* var. *mexicana* inflorescences as sedative and anxiolytic is reinforced by examinating inflorescences used by communities of the State of Michoacan, Mexico.
Materials and methods: Experimental mouse models were used to evaluate the sodium pentobarbital (SP)-induced hypnosis potentiation, ambulatory activity, as well as sedative and anti-anxiety responses via oral administration of the aqueous extracts (10, 30 and/or 100 and 300 mg/kg).
Results: All samples tested produced a lengthening in the time of SP. Moreover, a significant attenuation in the anxiety-response in the plus-maze test and a diminution in both the head dipping response and ambulatory activity were observed resembling the response to diazepam (0.3 mg/kg, i.p.). TLC profiles of the samples showed similar pattern of flavonoids; HPLC-DAD exhibited peaks identified as derived of quercetin and kaempferol that may be responsible for the plant activity.
Conclusions: Our results demonstrate that inflorescences of stored specimens obtained from popular local markets show the same effectiveness with regard to sedative and anxiolytic-like actions than freshly collected samples. Since no toxicity was observed through this route of administration (up to 5000 mg/kg); therefore, it suggests that this plant is secure when used as tranquilizer in folk medicine.

Keywords: Anxiolytic; Central nervous system; Sedative-hypnotic; *Tilia americana* var. *mexicana*

1. Introduction

Tilia americana var. *mexicana* is distributed in 14 states of Mexico, from the northern states of Chihuahua and Coahuila to the southern states of Guerrero and Oaxaca. Even though this plant has a relatively large geographical distribution, the populations of this species are confined to the lower mountainous forest, which covers less than 1% of the Mexican territory (Flores et al., 1971). Pátzcuaro in the State of Michoacán is one of the most popular regions with lower mountainous forests in which

Abbreviations: ANOVA, analysis of variance; CNS, central nervous system; LD_{50}, lethal dose 50; SP, sodium pentobarbital; S.E.M., standard error of the mean; i.p., via intraperitoneal; p.o., via oral.

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plant biodiversity includes *Tilia* trees. Furthermore, Michoacán is the region of Mexico where most significant collections for taxonomy of this species have been assembled (Jones, 1968).

Tilia is a tree used in traditional medicine primarily as a non-narcotic sedative for sleep disorders or anxiety (Martínez, 1969; Adame and Adame, 2000). Flower infusions (teas) of this species have generally been regarded as non-toxic; diluted teas are commonly given to overanxious children as a mild sedative (Arteche and Vanaclocha, 1998). The inflorescences of *Tilia* are sold year-around in the popular markets of towns located in several regions of Mexico, but mainly in Morelia, in the State of Michoacán (Luna, 1964; Hardin, 1990; Soto and Sousa, 1995). *Tilia* is locally known as "cyrimbo", "flor de tila", "sirima", "tirimo", "jonote" or "tilio" (Hardin, 1990; Martínez, 1994; Pavón and Rico-Gray, 2000). It has been observed that during the flowering months (April–June) there is an increase in the marketing of inflorescences of this species, since it is believed that the medicinal effect is greater when the infusion is made during this period (Pavón and Rico-Gray, 2000). Moreover, *Tilia* inflorescences are sold in markets where it is stored for almost a year; these samples may even be adulterated by mixture with other species such as *Ternstroemia pringlee* (Theaceae), which is known as *Tilia* too.

Tilia americana var. *mexicana* is an endemic Mexican species lacking scientific pharmacological description. In a preliminary study, we demonstrated that intraperitoneal administration of hexane and methanol extracts of its inflorescences produce sedative and anxiolytic-like effects in experimental in mice; however, the effective doses were near to the toxic effects (Aguirre-Hernández et al., 2007). Taking into consideration that *Tilia* has an ethnopharmacological use in Mexico as an infusion taken orally, we decided to validate its ethnomedicinal use by examinating the sedative and anxiolytic-like responses of an aqueous extract of this plant administered by oral route. Moreover, it is known that only few studies focus on herbal effectiveness of samples obtained from wild or cultivated medicinal plants in comparison to those stored in markets. In this study, we describe the effects of the *Tilia* inflorescences aqueous extracts prepared with samples obtained from popular local markets and compared them with those collected in two regions of Michoacán to assess their pharmacological effectiveness by using experimental models in mice. We also determined the acute toxicity by oral administration (LD_{50}, p.o.) and showed a preliminary flavonoid phytochemical analysis by using TLC and high performance liquid chromatography (HPLC)–diode array detection (DAD).

2. Materials and methods

2.1. Animals

Male Swiss albino mice (25–30 g, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñíz) were used in pharmacological tests and females of the same strain in the LD_{50} calculation. The animals were kept at constant room temperature (22 ± 1 °C) and maintained in a 12 h/12 h light/dark cycle. Experiments were carried out in accordance with the Ethical Committee Guidelines laid down by the local committee regarding the care and use of animals for experimental procedures and in compliance with international rules on care and use of laboratory animals. The animals were fed *ad libitum* with standard food and water except when fasting was required in the course of the study.

2.2. Plant material

Inflorescences of *Tilia americana* L. var. *mexicana* (Schltdl.) Hardin (Tiliaceae) were obtained from cultivation fields and from local markets of Morelia and Pátzcuaro, in the Mexican State of Michoacán, in June 2005. Samples were authenticated by M.Sc. Abigail Aguilar and Dr. Sergio Zamudio and a voucher specimen of each plant collected (sample 1: IMSSM-15103 and sample 2: IMSSM-15104 from Pátzcuaro) and samples bought in four independent markets (market 1: IMSSM-15105 and market 2: IMSSM-15106 from Morelia, while market 3: IMSSM-15107 and market 4: IMSSM-15106 from Pátzcuaro) were deposited in the herbarium of the Instituto Mexicano del Seguro Social in Mexico city for future reference.

2.3. Preparation of the plant extract

The air-dried powdered inflorescences (sample 1, 12.7 g; sample 2, 9.3 g; market 1, 26.8 g; market 2, 15.7 g; market 3, 22.4 g; market 4, 24.3 g) were extracted by infusion in boiled water (500 ml) for 1 h. The respective aqueous extracts were separated from its residues by gravity filtration, samples were freezed in liquid nitrogen and freeze-dried for 12 h in a lyophilizer model Heto (FD3 Lab). The final crude extracts were obtained as a yellow powder in percentage from dry weight (% d.w.) as follows: sample 1, 2 g (15.7% d.w.); sample 2, 2 g (21.5% d.w.); market 1, 1.3 g (4.9% d.w.); market 2, 2.4 g (15.3% d.w.); market 3, 2.0 g (8.9% d.w.) and market 4, 1.3 g (5.3% d.w.).

2.4. Drugs

Sodium pentobarbital (SP) and diazepam were purchased from Sigma–Aldrich Co. SP was dissolved in saline solution (s.s.) and diazepam was resuspended in 0.2% Tween 80 in s.s.; both compounds were administered intraperitoneally (i.p.). The extracts were resuspended in distilled water and administered via oral (p.o.). All drugs and extracts were freshly prepared on the day of the experiments and administered in a volume of 10 ml/kg body weight. Control animals received the same volume of the vehicle using the same route of administration.

2.5. Pharmacological evaluations

All mice were adapted to manipulation through a daily saline solution injection for 5 days before the treatments were initiated. For each experimental procedure, animal groups consisted of six mice. Diazepam was used as reference drug for both anxiolytic and sedative effects. The extracts and diazepam were evaluated 60 and 30 min after administration, respectively. One collected sample (sample 1) and one obtained from a local popular market