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Pharmacodynamic interaction of the sedative effects of *Ternstroemia pringlei* (Rose) Standl. with six central nervous system depressant drugs in mice☆

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ABSTRACT

Ethnopharmacological relevance: The decoction of dried fruits of *Ternstroemia pringlei* (Rose) Standl. (Theaceae), commonly known as "Flor de Tila", is used in the Mexican traditional medicine to diminish insomnia and fear.
Aim of the study: To examine the sedative effects of the dried fruits of *Ternstroemia pringlei* and investigate a possible synergistic pharmacodynamic interaction between the sedative effect of aqueous extract of this plant and six central nervous system (CNS) depressant drugs.
Materials and methods: The sedative effect was performed using the exploratory cylinder test in ICR mice. The extracts and drugs were intraperitoneally administered 30 min before testing in different doses, with exception of ethanol and buspirone which were administered 5 and 20 min before testing, respectively. An isobolographic analysis was used to characterize the interaction between *Ternstroemia pringlei* extract and six CNS depressant drugs.
Results: The intraperitoneal administration of the hexane, dichloromethane, methanol and aqueous extracts of *Ternstroemia pringlei* showed a dose-dependent sedative effect. *Ternstroemia pringlei* aqueous extract combined with buspirone, diazepam, diphenhydramine, haloperidol or pentobarbital exerted a super-additive (synergistic) sedative interaction. Whereas the combination *Ternstroemia pringlei* extract plus ethanol resulted in a sub-additive (attenuate) sedative interaction.
Conclusions: These findings are in agreement with the traditional use of *Ternstroemia pringlei* in the treatment of insomnia, however it is a plant that interacts in a complex form with CNS depressant drugs. It may represent an advertence on the use of this plant concomitantly with other neuroactive drugs.

1. Introduction

Ternstroemia is the largest genus in the Theaceae family with an estimated 130 species worldwide. The species are distributed almost equally between the eastern and western hemispheres and are tropical and subtropical in distribution (Boom, 1989). The species of *Ternstroemia* are very frequent in the cloud forest, oak forest and pine-oak forest of the "Sierra Madre Oriental", "Serranías Meridionales", and "Serranías Transístmicas" of México with elevations above 1000 m over the sea (Alcántara et al., 2002). It has been reported that nine species occur in México (Boom, 1989; Alcántara et al., 2002). Several species of *Ternstroemia* that grow in México, commonly known as "Flor de Tila", are reputed to possess sedative, anxiolytic and anticonvulsant effects (Tortoriello and Romero, 1992; Aguilar-Santamaría and Tortoriello, 1996). Aqueous extracts of dried fruits of *Ternstroemia pringlei* (Rose) Standl. synonym *Ternstroemia lineata* DC (Kobuski, 1942; Bartholomew and McVaugh, 1997) and *Ternstroemia sylvatica* Schltdl. and Cham., which are the two major *Ternstroemia* species, are used as decoctions in the Mexican traditional medicine to diminish insomnia and fear (Molina et al., 1999). Pharmacological studies have showed that *Ternstroemia pringlei* (Aguilar-Santamaría and Tortoriello, 1996) and *Ternstroemia sylvatica* (Molina et al., 1999) produce sedative effects in rats. No chemical studies have been performed on these two species; however, from Asian species of *Ternstroemia* (principally *Ternstroemia japonica* and *Ternstroemia gymnanthera*) oleanane- and ursane-type triterpenoids, triterpenoid saponins,

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carotenoids, monoterpenoids, tannins and other aromatic compounds have been identified (Kikuchi and Yamaguchi, 1974; Ikuta et al., 2003; Shin et al., 2003; Jo et al., 2005; Tori et al., 2005).

On the other hand, drug–drug interactions and drug–dietary supplements, including herb–drug interactions are emerging as important issues to consider in the evaluation of new drug candidates. However, herb–drug interaction studies are very limited (Fugh-Berman and Cott, 1999; Fugh-Berman and Ernst, 2001; Huang and Lesko, 2004). There is a dearth of well-documented data in this area and there are few studies that have specifically evaluated herb–drugs interactions (Rotblatt and Ziment, 2002).

Both preclinical and clinical studies of the drug interactions have been performed using the isobolographic analysis. This analysis offers a rigorous evaluation of the interactions between two drugs that act together to produce overtly similar effects (Tallarida, 2000). The effect of the combination may be a simple addition of the individual effects (additivity). In contrast, the effect of the combination can be exaggerated or even attenuated. The exaggerated effect is termed super-additive or synergistic, whereas the attenuated effect is termed sub-additive (Tallarida et al., 1997).

Several commercial products contain mixtures of the *Ternstroemia* species that grow in México, however there are no studies related to the potential interaction between these products and central nervous system (CNS) depressant drugs. Therefore in continuation with our studies on the potential pharmacological interaction between Mexican medicinal plants with reputed CNS depressant effect and commonly prescribed CNS depressant drugs (Ugalde et al., 2005), this study was designed to investigate a possible synergistic pharmacodynamic interaction between the sedative effect of aqueous extract of *Ternstroemia pringlei* and six CNS depressant drugs (diazepam, ethanol, pentobarbital, buspirone, haloperidol and diphenhydramine) by use of an isobolographic analysis in the exploratory cylinder test, a model to test sedative effect in mice (Ugalde et al., 2005; González-Trujano et al., 2006).

2. Materials and methods

2.1. Plant material

The dried fruits of *Ternstroemia pringlei* were acquired from a local market (Mercado de Sonora, México City) in January 2004. The homogeneity and authenticity of the plant material were certified by one of the authors (A. Aguilar), botanist from the Herbarium of the Instituto Mexicano del Seguro Social. A sample was deposited in this herbarium with the voucher number IMSSN117.

2.2. Extraction and isolation

After grinding, using a manual miller, 443 g of fruits were extracted at room temperature with hexane (3 × 2 L, 24 h each), then with CH_2Cl_2 (3 × 2 L, 24 h each) and finally with MeOH (3 × 2 L, 24 h each); evaporation of the solvents in vacuum gave 0.94 g (0.21% yield), 2.1 g (0.47% yield) and 152.7 g (34.46% yield) of syrupy residues, respectively. Aqueous extract was prepared with 10 g of dried and powdered fruits by boiling in 90 ml of distilled water for 10 min. Afterwards, the extract was filtered by gravity and concentrated through air current at room temperature (22 ± 2 °C) obtaining 1.52 g (15.2% yield) of a breakable reddish solid.

2.3. Drugs

Haloperidol, diphenhydramine, buspirone and Tween 80 were purchased from Sigma Co. (Sigma St. Louis, MO). Absolute ethanol was of HPLC grade (Fisher Scientific). Sodium pentobarbital (Anestesal™) was purchased from Pfizer S.A. de C.V., México, as a pharmaceutical solution for veterinary use.

2.4. Animals

All experiments were performed on adult male ICR mice (25–34 g; Centro UNAM-Harlan, Harlan México, S.A. de C.V.). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999) adopted in our laboratory, and in compliance with international rules on care and use of laboratory animals.

The experimental groups consisted of six (interaction study) or ten animals (calculus of ED_{50} of individual drug). They were maintained at constant room temperature (22 ± 2 °C) and submitted to a 12 h light/dark cycle with free access to food and water. All behavioral evaluations were carried out between 10:00 and 14:00 h.

Diazepam (Roche S.A.) and the organic extracts were suspended in 0.5% Tween 80 in saline solutions, all other compounds, including the aqueous extract, were dissolved in saline solution (0.9%). The drugs were freshly prepared each time and intraperitoneally injected in a volume of 0.1 ml/10 g body weight. Control animals received the same volume of vehicle (0.5% Tween 80 in saline or saline solution only).

2.5. Procedure

The apparatus consisted of a glass cylinder (30 cm in height, 11 cm in diameter, with wall of 3 mm). The cylinder is placed on filter paper in a room with constant lighting and isolated from external noise (Hiller and Zetler, 1996; Oliva et al., 2004; Ugalde et al., 2005).

An individual naïve mouse was put on the filter paper-covered floor of the glass cylinder; the number of rears performed over a 5-min period was recorded. The inner side of the apparatus and floor were cleaned with alcoholic solution and filter paper was changed between each animal test session (Oliva et al., 2004). The crude organic and aqueous extracts were tested for activity at different doses (10–1000 mg/kg i.p.) in the exploratory cylinder assay. The aqueous extract showed the major sedative activity. Therefore, the aqueous extract was used in the interaction study and it was freshly prepared each time in the same day of the experimentation. The aqueous extract and drugs were administered 30 min before testing in different doses, with exception of ethanol and buspirone which were administered 5 and 20 min before testing, respectively (Ugalde et al., 2005). When drugs were given in combination, aqueous extracts were injected first in the right side of the peritoneum, followed by the injection of the test drug in the left side. When only one of the drugs was given, the missing drug injection was substituted with the injection vehicle. During observation, the experimenter stood next to the apparatus always at the same place. The observations were made without prior knowledge of the experimental conditions applied to the animal. Reduced exploratory rearing showed by naïve mice after placement in an unfamiliar environment reveals a sedative effect (Rolland et al., 1991; Hiller and Zetler, 1996; Oliva et al., 2004).

Dose–response curves were constructed to assess the sedative effect of *Ternstroemia pringlei* extracts and the other CNS depressant drugs using ten animals at each of at least five doses. The ranges of doses used of the CNS depressant drugs were: diazepam (0.3–7.5 mg/kg), ethanol (1000–4000 mg/kg), pentobarbital (2.5–40 mg/kg), buspirone (0.15–10 mg/kg), haloperidol (0.1–3 mg/kg) and diphenhydramine (10–25 mg/kg). The dose that produced 50% of sedation (ED_{50}, 50% of reduction in the rears