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Toxicological and cytotoxic evaluation of standardized extracts of *Galphimia glauca*

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Abstract

Galphimia glauca Cav (Malpighiaceae) has been widely used in Mexican traditional medicine as a remedy for the treatment of mental disorders, principally as a sedative and tranquilizer. The sedative activity of extracts obtained from this plant has been demonstrated with different neuropharmacological models. Different triterpenes, known as galphimines, have been identified from the active extract. Galphimine-B (G-B) possesses anxiolytic activity and is able to selectively inhibit discharges of dopaminergic neurons in the ventral tegmental area in rats. Nevertheless, there have been no toxicological investigations carried out with products obtained from this plant. In this work three different extracts (aqueous, ethanolic, and methanolic) of *Galphimia glauca*, standardized in the content of three galphimines, were evaluated for their behavioral and pharmaco-toxicological effects. After administering the extracts to mice for 28 days (2.5 g/kg, p.o.), no deaths were found and the histopathological analysis of different organs did not show alterations; only the behavioral parameters analyzed showed a diminution of spontaneous activity. The administration of these extracts for 56 days (same doses and route) in mice did not cause any changes in the biochemical parameters that evaluate liver function. On the other hand, no cytotoxic effects were found on KB, UISO, and OVCAR-5 transformed cell lines, but all extracts specifically inhibited colon cancer cell line growth with an ED_{50} lower than 2 μg/ml. The extracts were also evaluated in genotoxicity tests *in vitro* (250, 100 and 50 μg/ml), which demonstrate that none of the three extracts from *Galphimia glauca* showed a genotoxic effect.

Keywords: *Galphimia glauca*; Malpighiaceae; Anxiolytic; Toxicology; Galphimine-B; Complementary and alternative medicine; Citotoxicity; Genotoxicity

1. Introduction

An infusion prepared with the aerial parts from *Galphimia glauca* Cav., commonly known as "calderona amarilla", has been used in Mexican traditional medicine for the treatment of mental disorders and for diminishing nervous excitement (Estrada, 1985). Previous reports demonstrated the sedative and anticonvulsant effects produced by the methanolic extract of the aerial parts from this plant (Tortoriello and Lozoya, 1992). A bio-guided chemical separation allowed the identification of one of the active compounds, the nor,seco-triterpene known as galphimine-B (G-B) (Toscano et al., 1993). This compound was able to reproduce the sedative effects displayed by the methanolic extract, but in this case, it was necessary to use lower doses. However, this compound was unable to produce any anticonvulsant effect (Tortoriello and Ortega, 1993). In electrophysiological experiments, local and systemic administration of G-B on rats produced modifications of the extracellular spiking activity records in the ventral tegmental area (VTA) neurons (Tortoriello et al., 1998). Later, it was proved that this effect is not a result of the interaction with the GABAergic system (Prieto-Gómez et al., 2003).

Abbreviations: CNS, central nervous system; G-B, galphimine-B; G-A, galphimine-A; G-E, galphimine-E; VTA, ventral tegmental area; HPLC, high precision liquid chromatography; NMR, nuclear magnetic resonance; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ANOVA, analysis of variance; MN, micronuclei; SCE, sister chromatid exchange

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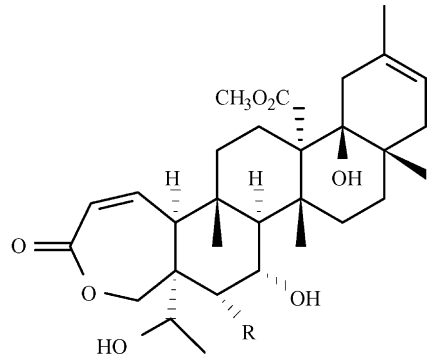

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Galphimine-B = H
Galphimine-A = OH
Galphimine-E = OAc

Fig. 1. Chemical structure of galphimine-B, galphimine-A and galphimine-E.

Since the VTA is a region with high density of dopaminergic neurons responsible for the innervation of the prefrontal cortex, nucleus accumbens and entorhinal region, and because these areas are targets for the action of antipsychotic drugs, the pharmacological effects produced by G-B have greater importance. With this background, the extract of *Galphimia glauca* (standardized for G-B content) was evaluated in behavioral animal models, and showed interesting anxiolytic effects in ICR mice (Herrera-Ruiz et al., 2006). More recently, other galphimines (Fig. 1) with similar structures and with spasmolytic effect, were identified in this species (González-Cortazar et al., 2005). In these galphimines, G-B and G-A also showed significant anxiolytic properties, and thus are considered the active compounds in the methanolic extract (Herrera-Ruiz et al., 2006b).

Despite the interesting results obtained from the pharmacological and chemical studies and their potential therapeutic usefulness, no toxicological investigation of this species has been reported. In this work, based on the demonstrated pharmacological effects, three different extracts were prepared and submitted to different *in vivo* and *in vitro* toxicological tests. Previously, these extracts were monitored by the HPLC analytical method in order to quantify the G-B, G-A and G-E concentrations.

2. Materials and methods

2.1. Plant material

Leaves of *Galphimia glauca* Cav. (Malpighiaceae) were collected in an experimental field in Xochitepec, Morelos, Mexico on August 18th, of 2000 and identified by M.Sc. Abigail Aguilar, Director of the IMSSM Herbarium, where a botanical voucher was deposited for reference.

2.2. Preparation of the extracts

Plant material was dried under dark conditions during 10 days and milled to obtain 2–5 mm particles. This material was divided into three portions (1.7 kg, each), which were extracted by water (17 l, at 90 °C for 50 min), or exhaustive maceration with methanol, or ethanol. The aqueous (420 g, 24.7%), methanol (451 g, 26.5%) and, ethanol (179 g, 10.5%) extracts were filtered and the solvent was evaporated under vacuum to dryness. The residue of each extract was re-dissolved in water for its successively lyophilized process. The lyophilized extracts were subjected to toxicological tests and quantitative analysis.

2.3. HPLC analysis

Galphimia glauca extracts were analyzed on a Merck-Hitachi modular HPLC system, consisting of an L-6200A intelligent pump, connected with a L-4500 photodiode array detector, equipped with an AS-2000 auto sampler and Chromatography data station software (Merck-Hitachi). Acquisition was set at 232 nm (spectral acquisition in the range 200–400 nm). The analyses were carried out on a Chromolith Performance RP-18e column (4.6 mm × 100 mm, Merck). The eluent was an isocratic acetonitrile/water 35:65 system. The mobile phase flow-rate was 1.7 ml/min for 12 min.

2.4. Quantitative determination of galphimines

The galphimines G-A, G-B, and G-E, used as standards, were previously isolated from *Galphimia glauca* leaves. Their identity and purity were confirmed by comparison with published spectral data (IR, 1D and 2D NMR). Calibration curves were constructed using four points dilutions of each compound: 50, 100, 200 and 400 μg/ml in methanol (R^2 = 0.99, 0.98 and 0.99 for G-A, G-B and G-E, respectively). All compounds were detected at 232 nm (Fig. 2). A volume of 50 μl was injected. The calibrations curves were based on the peak areas of the HPLC chromatograms. The experiments were performed in triplicate. The values were expressed in terms of percent on the basis of the dry weight in grams.

Methanol and ethanol samples extracts were each prepared by elution of 10 mg of the extract on LC-18 Supelclean cartridge with 5 ml of methanol. The aqueous extract (10 g) was partitioned by $CHCl_3/H_2O$ for obtaining the water sample. The organic fraction (10 mg) was evaporated to dryness and dissolved in 5 ml of methanol. Samples of 70 μl of each solution (2 mg/ml) were injected in triplicate.

2.5. In vivo toxicological evaluation

2.5.1. Animals

Animal experimentation was performed observing "official regulations of experimental animal care" (NOM-062-ZOO-1999), and in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23 revised in 1985). The experimental protocol was approved by the Institutional Research and Ethics Committee. Male and female Balb-C mice (18 ± 2 g) were used. Animals were maintained under regular husbandry conditions; 23 ± 2 °C, 12 h light-dark cycle with *ad libitum* access to water and standard rodent chow (2018S, Harlan Teklad).