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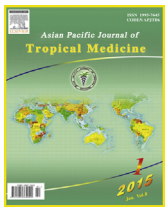

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Anti-inflammatory evaluation and acute toxicity of three food supplements that contain *Moussonia deppeana*

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ABSTRACT

Objective: To identify the anti-inflammatory activity through two murine models and in the median Lethal Dose (LD_{50}) of three dietary supplements that contain *Moussonia deppeana*.

Methods: The anti-inflammatory activity of three dietary supplements (Cicatrisan/Gastricus®, Gastinol®, and Gastrovita®) EtOH extracts was evaluated by TPA and by carrageenan murine models; also, median Lethal Dose (LD_{50}) was determined. Verbascoside was quantified by High-Performance Liquid Chromatography. β-sitosterol, stigmasterol and the mixture of ursolic and oleanolic acids were identified in all supplements by TLC; however, none of these dietary supplements contain verbascoside.

Results: For the TPA model, Cicatrisan/Gastricus® generated a notable effect with 38.24% inhibition. While in the carrageenan model, it also exhibited noteworthy anti-inflammatory activity of ear edema with 66.39% of paw edema inhibition at 150 mg/kg, followed by Gastinol® and Gastrovita® with ≈50% at 300 mg/kg. Finally, LD_{50} was >2 g/kg for all supplements, when was administered intragastrically and Body Weight (BW) gain in mice was not altered after 14 days.

Conclusions: Of the three food supplements containing *M. deppeana*, only the EtOH extract from Cicatrisan/Gastricus® formulation (tablets) showed significant anti-inflammatory activity in both experimental models and the LD_{50} was >2 g/kg.

1. Introduction

Currently, worldwide commercialization and use of medicinal plants and their derivatives for treatment of several human illnesses is in expansion. The majority of these products, obtained from vegetal species, are labeled and distributed as food or dietetic supplements [1,2], thus avoiding the need for scientific evidence of their alleged pharmacological benefits or possible toxic effects. Therefore, companies decrease production costs but maintain high sales destined to a growing sector of the population who are in the search of more economical and accessible alternatives to conventional allopathic medicine [3,4].

Due to this high demand, many countries (mostly developed ones) have propagated countless strategies to regulate and support the use of medicinal plants and their derivatives, first through regional institutions funded by federal governments, and second, by the World Health Organization (WHO). This is due to majority of herbal remedies are registered for sale under a very lax legal framework, in which production quality is required but not the effectiveness and scientific verification of their pharmacological effects [2,3].

In this context, legislation and regulation of herbal remedies in Mexico is behind compared with those of developed countries such as the U.S. and Germany. The Federal Commission of Protection against Health Risks in Mexico defines herbal remedies as follows: 'an herbal remedy only has to prove its safety (sometimes this parameter is not even evaluated), and should not, under any circumstances, guarantee its therapeutic efficacy against a specific disease'. Due to this definition, there are gaps

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in the registration process of many products containing medicinal plants in their formulation [5].

Mexico City and some Mexican states are the main consumers of medicinal plants and their derivatives; it is estimated that daily, nearly 250 species are sold (fresh or dehydrated) in markets, these species are delivered from the center and south of the country, while the products elaborated with them, such as food supplements, are distributed and sold in naturist stores and pharmacies [4].

Due to that wide sales without regulation of these polyherbal remedies (mixture of 5–6 medicinal plant extract powders), in addition to supporting their ethnomedicinal use, it is also of great importance to study their possible toxic effects in order to ensure the safety of their consumption by the population. There are many medicinal plants that give rise to severe adverse effects, mainly hepatic and renal damage, such as chaparral (*Larrea divaricata*) or the purple passionflower (*Passiflora incarnata*); these cause tachycardia and ataxia when both are consumed for long periods of time [6,7]. Another example of how food supplements may cause adverse effects is described by Höllerhage *et al*. [8], who demonstrated that the intake of dietary supplements containing the plant materials of *Annonaceae* species (*Annona muricata* L., *Annona squamosa* L., *Annona mucosa* Jacq., *Annona squamosa* x *cherimola* Mabb.) could provoke neurotoxicity. For example, the Ethyl Acetate (EtOAc) extract from these supplements generated 67% of cellular death of human mesencephalic neurons *in vitro*.

One of the well-known medicinal plants in Mexican traditional medicine, and one that is widely used in the formulation of many food supplements and polyherbal remedies, but with few scientific studies, is *Moussonia deppeana* (Schldl. & Cham) Hanst; syn. *Kohleria deppeana*, *Gesneria deppeana*, or *Moussonia elongata*, commonly known as *tlachichinole*. This plant is frequently employed for the treatment of arthritis, colon and intestinal inflammation, stomach ache, kidney failure, vaginal infection, ulcers, diarrheas, burns and flu [9–11]. Some ethnopharmacological uses have been demonstrated through scientific research with some extracts obtained from its aerial parts or from the whole plant, such as antioxidant and anti-inflammatory [12,13], antimycobacterial [14], anti-*Helicobacter pylori* [15,16], antiprotozoal [17], and antitrypanosomal properties [18]. Also, in mouse, acute and sub-acute toxicity of the Ethanolic (EtOH) extract from aerial parts has been described; in this case, this extract demonstrated no adverse effects or lethality. In addition, verbascoside, a main metabolite, was identified in the EtOH extract and can be utilized as a marker in polyherbal formulations and dietary supplements that contain *M. deppeana* [13].

Although *M. deppeana* is a medicinal plant used widely as a food supplement, to date, the pharmacological from this plant alone has been reported, and not for food supplements that contains it. In this paper, we described the anti-inflammatory activity determined in two murine models and the median Lethal Dose (LD_{50}) of three dietary supplements in which this medicinal plant comprises part of their formulation.

2. Materials and methods

2.1. Description of food supplement formulation

The commercial name, the composition of each supplement, and the main use are described as follows:

Cicatrisan/Gastricus®: *Medicago sativa* (Fabaceae), *Malva sylvestris* (Malvaceae), *Kohleria deppeana* (Gesneriaceae), *Conyza filaginoides* (Asteraceae), and *Matricaria recutita* (Asteraceae), for stomach and intestinal inflammation.

Gastinol®: *Amphipterygium adstringens* (Julianaceae), *Salvia hispanica* L. (Lamiaceae), *Trigonella foenum-graecum* (Fabaceae), *Calendula officinalis* (Asteraceae), *Kohleria deppeana* (Gesneriaceae), and *Aloe barbadensis* (Xanthorrhoeaceae), for gastritis and stomach inflammation.

Gastrovita®: *Gentiana lutea* (Gentianaceae), *Croton niveus* (Euphorbiaceae), *Amphipterygium adstringens* (Julianaceae), *Mentha piperita* (Lamiaceae), and *Kohleria deppeana* (Gesneriaceae), for intestinal inflammation and gastric ulcers.

Formulation of three food supplements that contain *Moussonia deppeana* are as follows: Net content/pharmaceutical presentation of Cicatrisan/Gastricus®, Gastinol® and Gastrovita® are 353 mg/50 tablets, 220 mg/40 capsules and 500 mg/40 capsules, respectively; *M. deppeana* formulation dose of Cicatrisan/Gastricus® and Gastinol® are 37.5 mg/tablet, and 20.0 mg/capsule, respectively. Food supplements are used 3 times/day each meal.

The polyherbal preparations were purchased in naturist stores in Mexico city.

2.2. Ethanolic extract preparation

The capsules were emptied until reaching 20 g of each Gastinol® and Gastrovita® supplements, while Cicatrisan/Gastricus® tablets were ground into powder (20 g). Each sample was macerated with EtOH (200 mL) for 1 week under constant shaking at room temperature. Final extracts were filtered and concentrated at 40 °C using a rotary evaporator (Buchii RE-11) coupled to a vacuum system (BuchiiVac V-153) and a cooling system (ECO 20). The extracts were maintained under conditions of darkness until their use. Yield was calculated for each compared with its respective dried-powder initial weight.

2.3. Qualitative phytochemical analysis and verbascoside quantification through HPLC

In the EtOH extracts of each sample, β-sitosterol, stigmasterol, ursolic acid, and oleanolic acid were detected by Thin Layer chromatography (TLC) utilizing as mobile phases and chromogenic agents described previously [13]. For the identification of verbascoside, EtOAc:EtOH:H_2O (100:13.5:10) or EtOAc:Formic acid:Acetic acid:H_2O (10:1.1:1.1:0.3) as mobile phase were employed and were detected with 2-aminoethylester diphenyl boric acid 1% in MeOH with polyethylene glycol 5% in EtOH as chromogenic agent. β-sitosterol, stigmasterol, ursolic acid, and oleanolic acid were compared with Sigma pure standards and standard verbascoside was obtained previously from the *M. deppeana* EtOH extract [13].

For verbascoside identification and quantification, High-Performance Liquid Chromatography (HPLC) analysis was carried out in Waters equipment (Waters, USA) comprising a 600E multi-solvent delivery system with a 486 UV detector, as described by Gutiérrez-Rebolledo *et al*. [13]. Equipment control, data acquisition, and the processing and management of HPLC information were performed by Empower 2 software (Waters). Analytical conditions were employed: column ZORBAX Eclipse XDB-C18 (5 μm, 4.6 × 250 mm i.d.) with pre-column

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