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Bioassay-guided isolation of an anti-ulcer chromene from *Eupatorium aschenbornianum*: Role of nitric oxide, prostaglandins and sulfydryls

María Elena Sánchez-Mendoza [a], Benito Reyes-Trejo [b], Paula Sánchez-Gómez [a], Juan Rodríguez-Silverio [a], Carlos Castillo-Henkel [a], Humberto Cervantes-Cuevas [c], Jesús Arrieta [a,*]

[a] *Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, Colonia Santo Tomás, Delegación Miguel Hidalgo 11340, México D. F., México*
[b] *Laboratorio de Productos Naturales, Área de Química, Universidad Autónoma Chapingo, Apartado 74 Oficina de Correos Chapingo, Texcoco, México, 56230, México*
[c] *Área de química, Universidad Autónoma Metropolitana, Unidad Azcapotzalco, Av. San Pablo, Colonia Reynosa Tamaulipas 02200, México D. F., México*

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ABSTRACT

Eupatorium aschenbornianum is considered useful in the treatment of gastric ulcer. In the current study the validity of this practice was tested by using the experimental model of an ethanol induced gastric ulcer in rats. The results show that *E. aschenbornianum* had gastroprotective activity, that the hexane extract had the highest protective activity (85.65 ± 4.76%), and that encecanescin isolated from this extract was the main active gastroprotective agent. The effect elicited by encecanescin was attenuated by N^G-nitro-L-arginine methyl ester, N-ethylmaleimide and indomethacin, which suggests that NO, prostaglandins and sulfydryl groups are involved in the mechanisms of gastroprotective action.

1. Introduction

A peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by the action of gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal disorders. It is generally recognized that peptic ulcers are caused by a lack of equilibrium between gastric aggressive factors and mucosal defensive factors [1]. A gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive factors, such as acid, pepsin, bile acid, food ingredients, bacterial products, and drugs. These agents increase gastric acid and pepsin secretion, decrease the gastric blood flow, suppress the endogenous generation of prostaglandins, inhibit mucosal growth and cell proliferation, and modify the gastric motility [2,1]. On the other hand, defensive mechanisms of the gastric mucosal consist mainly of functional, humoral and neural factors. Mucus-alkaline secretions, microcirculation and motility act as functional factors, prostaglandins (PGs) and nitric oxide (NO) as humoral factors, and capsaicin sensitive sensory neurons (CPSN) as neural factors [3,4]. Although many drugs have been effectively employed in the treatment of gastroduodenal ulcer and peptic diseases, all of these compounds have shown major shortcomings, such as the therapeutic failures observed in certain cases, or the adverse effects and high cost [2]. In the search for new therapeutic options, traditional medicinal plants are a source of natural products, such as triterpenes, diterpenes and flavonoids, among others with gastroprotective activity [5]. The genus *Eupatorium* belongs to the Eupatorieae family, one of the 13 families of Asteraceae, the latter of which includes nearly 1200 species that are distributed mainly in the tropical regions of the Americas, Europe, Africa and Asia. In addition to their antiulcerogenic uses, plant species in this genus have been used for many decades in folk medicine as antimalarial, antibacterial, antifungal and anti-inflammatory agents [6]. These species are well known for such components as sesquiterpene lactones, diterpenes, triterpenes, flavonoids, pyrrolizidine alkaloids, and

* Corresponding author. Tel.: +55 57 29 63 00x62 827; fax: +55 56 22 53 29.
E-mail address: maestrojesus@hotmail.com (J. Arrieta).

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many different types of monoterpene derivatives, among others. Some of these substances have cytotoxic, antimicrobial and immunomodulating properties [6].

In traditional medicine of the state of Morelos, Mexico, the leaves of *Eupatorium aschenbornianum*, locally known as axihuitl, are commonly prepared by healers and shamans as infusions and used to treat tumors, skin problems, wounds, aphthae and gastric ulcers [7,8]. However, there is no scientific report either validating or invalidating the empirical use of this plant to cure gastric ulcers. Therefore, we tested the gastroprotective activity of *Eupatorium aschenbornianum* and, upon validating such protective action, proceeded to identify the active compounds. A bioassay-guided fractionation was performed and the compounds obtained were tested with the absolute ethanol-induced gastric ulcer experimental model in Wistar rats. In addition, the role of endogenous NO, sulfydryl groups and prostaglandins in the gastroprotective effect was evaluated in order to provide information about the mechanism of action of these compounds. The results were compared to the effect of carbenoxolone.

2. Materials and methods

2.1. General procedures

Infrared spectrum was determined on a Perkin-Elmer spectrometer (model 1310). High resolution mass spectroscopy was performed in a JEOL spectrometer (model 102 ASX). The ^{13}C and ^{1}H NMR spectra were recorded on a Bruker DPX-300 spectrometer operating at 75.4 MHz for ^{13}C and at 300 MHz for ^{1}H. Chemical shifts are quoted relative to internal TMS.

2.2. Plant material

The leaves of *Eupatorium aschenbornianum* were collected in San Juan, Morelos during August of 2007. A specimen of the original collection can be found in the biology area of the Hortorio Jorge Espinosa Herbarium at the Chapingo Autonomous University, with the voucher number 1835.

2.3. Extraction and preliminary fraction

The leaves of *E. aschenbornianum* were dried at room temperature (22 ± 2 °C) in the shade. After grinding 3.0 kg of leaves, compounds were successively extracted from them by maceration at room temperature (22 °C ± 2) during 3 days, first with hexane (15 L × 3), then dichloromethane (15 L × 3), and finally methanol (15 L × 3). Evaporation of the solvents in vacuum yielded 66.8 g, 75.3 g and 150.6 g of syrupy residues, respectively. The hexane extract obtained from the leaves of *E. aschenbornianum* showed the most active gastroprotective effect (Table 1). Thus 40 g of this extract was subjected to percolation over a silica gel column (0.063–0.200 mm, 250 g) by using a step gradient of hexane (1.7 L, F1), hexane/EtOAc (9:1, 1.7 L, F2), hexane/EtOAc (7:3, 1.7 L, F3), and hexane/EtOAc (1:1, 1.7 L, F4). Fraction 2 (F2), which was the most active, was chromatographed on a silica gel column (200 g). Elution was performed with the hexane and hexane/EtOAc mixtures, using 50 fractions of 20 mL each. Fractions 15–25 (Hexane/EtOAc, 95:5) yielded a white solid (2.30 g, mp 148–150 °C), which was identified as encecanescin (Fig. 1) by comparing its spectral data (IR, FAB^{+}-MS, ^{1}H and ^{13}C NMR) with that of the literature [9].

2.4. Animals

All the experiments were performed with male Wistar rats, weighing 180–220 g, obtained from the animal house of the Superior Medicine School (ESM) of the National Polytechnic Institute (IPN). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with international rules on care and use of laboratory animals. Unless otherwise specified, the rats were placed in single cages with wire-net floors and deprived of food 24 h before experimentation, but allowed free access to tap water throughout. All experiments were carried out using 7–10 animals per group.

2.5. Drugs and dosage

Carbenoxolone (Sigma-Aldrich Co.) was used as the gastroprotective reference drug. The drugs were prepared freshly each time, suspended in 0.5% Tween 80 and administered by the intragastric route. Control rats received the vehicle (0.5% Tween 80) in the same volume (0.5 mL/100 g) and by the same route. N^{G}-nitro-L-arginine methyl ester (L-NAME), N-ethylmaleimide (NEM) and indomethacin were purchased from Sigma Chemical Co., USA.

2.6. Acute gastric ulcer induced by absolute ethanol

A gastric ulcer was induced by orally administering absolute ethanol (1 mL), as described by Robert [10]. The extracts or drugs were administered to different groups 30 min before ethanol administration. Two hours after ethanol administration, the animals were killed in a CO_2

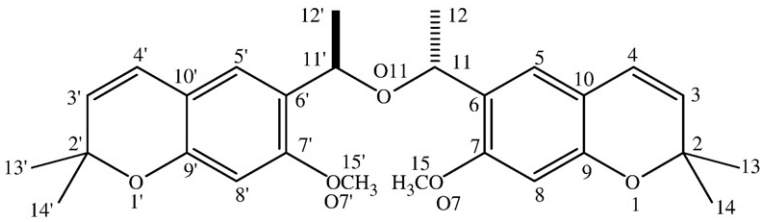

Fig. 1. The structure of encecanescin.