Gastroprotective activity and pharmacological safety evaluation of *Eupatorium aschenbornianum*

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Abstract. *Eupatorium aschenbornianum* has been widely used in traditional Mexican and folk medicine for the treatment of wounds, skin lesions, hemorrhages and gastric ulcers in humans. Phytochemical studies have indicated that hexane extracts of *E. aschenbornianum* have anti-microbial and anti-fungal activities. In the present study, an accurate and reliable approach using a murine model was pursued to evaluate the anti-ulcer activity, lipid peroxidation properties and acute toxicity of powdered dried stem of *E. aschenbornianum*. The results indicated that administration of *E. aschenbornianum* exerted an anti-ulcerative effect and decreased lipid peroxidation in gastric ulcers induced by acetylsalicylic acid. An acute toxicity assay indicated normal behavior and no significant variations in the weight and food consumption of animals. In addition, quantitative analysis of biochemical parameters did not indicate any liver or kidney damage. The results indicated that *E. aschenbornianum* may be a safe therapeutic agent for the prevention of gastric ulcers.

Introduction

Mexico is considered one of the most biodiverse countries in the world. In this country, a wide variety of flowers grow and an estimated 23,000-30,000 species of vascular plants have been reported (1), 3,000-5,000 of which have medicinal properties (2). Since pre-Hispanic times, it has been noted that plants are an excellent source of therapeutic compounds and have been used in Mexican Traditional Medicine (3-5). The Eupatorium genus of the Asteraceae family comprises ~1,200 species that mainly grow in tropical regions (4). *Eupatorium aschenbornianum* is an endemic herb from Morelos, Mexico; it grows in altitudes of >2,000 meters above sea level, specifically around the Tepozteco National Park, and is known locally as Axihuitl. In traditional medicine, the leaves of Axihuitl are commonly prepared as a tea taken orally to treat tumors, skin ailments, wounds, aphthae and gastric ulcers (6,7). Previous studies have indicated that hexane extracts of *E. aschenbornianum* have gastroprotective effects (7), as well as anti-microbial and anti-fungal activities on diverse strains (8,9). Those effects are attributed to the major active compounds of *E. aschenbornianum*, including terpenes, flavonoids and alkaloids (9-12).

Peptic ulcer disease represents an important public health problem worldwide. This disease arises from acid peptic injury of the digestive tract, which results in mucosal breaks that reach the submucosal epithelium (13). *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs (NSAIDs) are the major risk factors for the development of gastric ulcers; however, it has been reported that 70% of gastric ulcers are linked to *H. pylori* and only a small proportion of individuals taking NSAIDs develop ulcers (13,14). This suggests that other risk factors, including the consumption of pepsin, certain foods, drugs, excessive alcohol intake, bacterial or viral infections, and physiological and psycho-sociological stresses may contribute to the development of ulcers (15-19). Treatments for gastric ulcers include proton pump inhibitors, which reduce the production of stomach acid. Although these inhibitors help to decrease mortality and morbidity rates, these pharmaceutical products are costly and may cause adverse effects. Therefore, products obtained from natural sources may represent therapeutic alternatives for the treatment of this condition (20). To date, certain studies have determined the potential toxicity of plants used in traditional medicine. Recent surveys have

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indicated that numerous medicinal plants applied in traditional medicine have adverse effects (21); thus, it is important to determine the toxicology of these medicinal plants. Therefore, the possible anti-ulcerative effects, as well as the acute toxicity of the powdered dried stem of *E. aschenbornianum*, were assessed in a rat model of ASA-induced gastric ulcers.

Materials and methods

Plant material. The stem of *E. aschenbornianum* plants were harvested from Morelos, Mexico, which was made in 2015 during the spring season and were identified by Professor Carlos Francisco Cortés García, a Specialist in the Department of Taxonomy at the University of Guadalajara, Mexico. Leaves, branches and flowers of the plant were excluded, the remaining stem was dried at room temperature until the moisture content was ~7%, the stems were then immediately chopped and pulverized with a blender, the resultant powder obtained was filtered through a 30-mesh (0.595 mm). Finally, the *E. aschenbornianum* powder was vacuum-packed at room temperature for one week to avoid alteration of the sample (22).

Animals. A total of 23 male Wistar rats (age, 8 weeks; weight, 180-220 g) were purchased from the animal facility of the University of Guadalajara. All animal procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health from 1985. Animals were housed, handled and cared for in accordance with the official Mexican standards for the care and use of laboratory animals (no. NOM-062-ZOO-1999). An internal bioethical committee at the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (Guadalajara, Mexico) reviewed and approved the animal study. Animals were housed with controlled conditions, light cycle (12-h light/dark cycle), relative humidity (46-50%) and temperature (22˚C). The animals had free access to rat chow and water. All were maintained individually in polyethylene cages. The rats were fasted for 24 h prior to the start of the experiment but had free access to water (23).

Animal experiment to assess anti-ulcer activity. The induction of gastric ulcers was performed according to the method described by Konturek *et al* (24), Lewis and Shaw (25) and Narayan *et al* (26). Rats were randomly divided into three groups containing six animals each. The first and second groups were induced by acetylsalicylic acid (ASA) by intragastric gavage at a dose of 150 mg/kg body weight to generate gastric ulcers, while the third group was administered saline solution. Immediately after induction, the second group was fed *E. aschenbornianum* powder at a dose of 400 mg/kg body weight (previous studies with few animals suggested anti-ulcerative effects at this dose) (22) mixed in standard rat chow, while the other groups were fed in the same manner without *E. aschenbornianum* powder. Animals were maintained individually in cages and were fed for a period of five days. The next day, all animals were euthanized by an intraperitoneal injection of sodium pentobarbital (120 mg/kg). Animals were dissected and the stomachs were carefully extracted. An incision was made on the stomach along the greater curvature, and the stomachs were then washed with PBS. Mucosal lesions were evaluated by macroscopic analysis.

Ulcerative index (UI) and protective effect. Taking into account the severity of damage in the gastric mucosa, the UI was determined as follows: UI=[1x (number lesions of ≤1 mm) + 2x (number lesions of 1-2 mm) + 3x (number lesions >2 mm)]/10.

The overall score was divided among the Ulcer Index designated as 10 (27,28), while the percentage of ulcer protection was calculated as follows: Ulcer protection (%)=[(UI ulcer-induced group)-(UI treated group)]/(UI ulcer-induced group) x100.

Lipid peroxidation. Gastric mucosal tissues (100 mg/ml) were placed into 20 mM Tris-HCl buffer (pH 7.4) at 4˚C and homogenized using a Tissue-Tearor (BioSpec Products Inc.). The homogenates were centrifuged at 2,000 x g, at 4˚C for 10 min; the supernatant was collected and stored at 4˚C until use. The degree of lipid peroxidation was determined as the amount of malondialdehyde (MDA) and 4-hydroxynonenal (HNE) using the Lipid Hydroperoxide Assay kit (Bioquochem SL). In brief, 200 μl of the supernatant and 650 μl 10.3 nM N-methyl-2-phenylindole were added to a 1:3 mixture of acetonitrile and methanol. Subsequently, 150 μl methanesulfonic acid was added and the reaction mixture was incubated at 40˚C for 40 min. Subsequently, the tubes were centrifuged at 5,000 x g at room temperature for 5 min. Finally, 200 μl of the supernatant was collected and the absorbance was measured at 586 nm. A standard calibration curve was generated simultaneously to establish the sample concentration.

Acute toxicity. According to the OECD 425 guidelines, it is possible to perform a limit test (a maximum fixed dose) with five animals using a dose of 2,000 mg/kg *E. aschenbornianum* as a suggestion (29); it was then decided to use a single administration of 400 mg/kg *E. aschenbornianum* in order to demonstrate that intake at this dose is safe. *E. aschenbornianum* powder was administered to five rats by intragastric gavage at a dose of 400 mg/kg body weight. Animals were observed for one week to detect any toxicity signs and an additional week for any delayed toxicity. Mortality, body weight and clinical signs were recorded. Blood samples were collected from the tail vein 14 days after *E. aschenbornianum* administration and separated to obtain serum, which was stored at -80˚C until use for the analysis of biochemical parameters. Bilirubin was measured using the method of Jendsrassik and Grof (30), while the activities of alanine transaminase (ALT) and aspartate transaminase (AST) were measured according to the Reitman and Frankel method (31). Albumin was estimated by the method of Doumas *et al* (32), while alkaline phosphatase (ALP) activity was estimated by the method of Bessey *et al* (33).

Statistical analysis. Values are expressed as the mean ± standard error of the mean. Statistically significant differences between groups were determined by one-way analysis of variance, followed by a Tukey's post-hoc test. $P<0.05$ was considered to indicate statistical significance. Statistical analyses were performed using SigmaStat 8.0 software (Systat Software, Inc.).

Results

Analysis of gastric lesions. In the ulcer-induced group, gastric mucosal lesions with a diameter of >2 mm were observed in

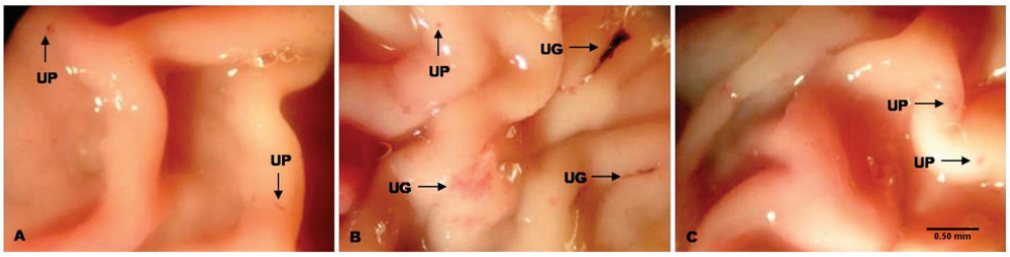

Figure 1. Macroscopic analysis of the gastroprotective effect of *E. aschenbornianum* on gastric mucosal lesions induced in rats by ASA. (A) Non-induced group; (B) ulcer-induced group (ASA at 150 mg/kg); (C) treated group administered with *E. aschenbornianum* powder (400 mg/kg) after gastric ulcer induction (ASA at 150 mg/kg). UG, lesions >2 mm; UP, lesions <1 mm; ASA, acetylsalicylic acid.

the glandular regions of the stomach. These mucosal lesions appeared to be black and dark red with elongated bands. Of note, a gastroprotective effect was observed in rats administered *E. aschenbornianum* powder after the development of ASA-induced gastric ulcers. The severity of these mucosal gastric lesions was similar to that of the non-induced group, and the lesions were <1 mm in diameter (Fig. 1).

Anti-ulcerative effects of E. aschenbornianum. Administration of ASA caused severe gastric lesions to develop with a UI value of 9.65±0.89 in the ulcer-induced group. Rats of the ulcer-induced group administered *E. aschenbornianum* powder exhibited a statistically significant reduction in the severity and number of gastric lesions with a UI of 4.52±0.14. Unexpectedly, this group also exhibited a significant reduction in the UI value compared with the non-induced group (UI=5.04±0.30; Fig. 2).

Effect of E. aschenbornianum on lipid peroxidation. Treatment with *E. aschenbornianum* powder in animals with gastric ulcers induced by ASA resulted in significantly reduced levels of MDA-HNE aldehydes (33.79±1.84 μM) in the gastric mucosa compared with those in the ASA-induced ulcer group (46.00±1.65 μM); of note, the MDA-HNE aldehydes in the treatment group were not significantly different compared with those in the non-induced group (39.16±2.15 μM; Fig. 3).

Acute toxicity. No mortality, clinical signs of toxicity or effects on behavior or appearance, including lethargy and immobility, were observed throughout the study. All animals survived during the whole study period. Similar food intake and consistent changes in body weight were observed in the non-induced and ulcer-induced groups (data not shown). The ulcer-induced group administered 400 mg/kg of *E. aschenbornianum* powder did not exhibit any alterations in the biochemical parameters analyzed compared with the non-induced group and the reference range (Table I) (34).

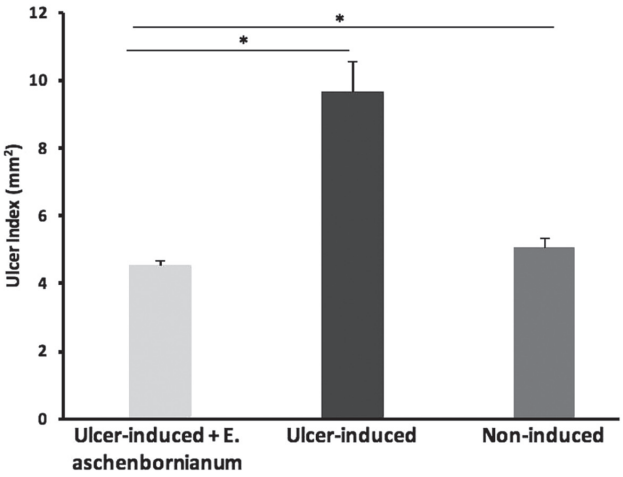

Figure 2. Anti-ulcerative effect of *E. aschenbornianum* powder at 400 mg/kg on gastric lesions induced by acetylsalicylic acid in rats (150 mg/kg). Values are expressed as the mean ± standard error of the mean (n=6). *P<0.05.

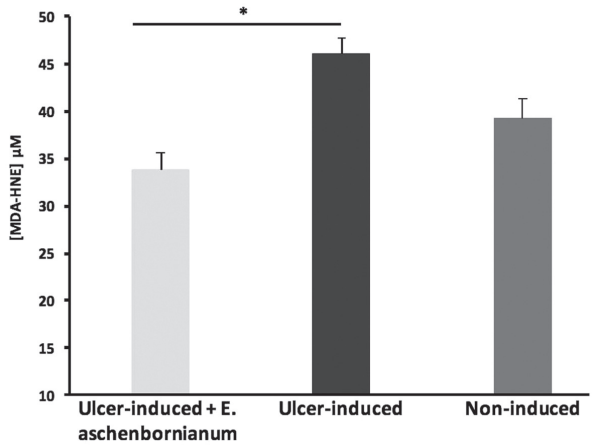

Figure 3. Effect of *E. aschenbornianum* powder on the levels of MDA + HNE in gastric mucosal tissue homogenate. Values are expressed as the mean ± standard error of the mean (n=6). *P<0.05. MDA, malondialdehyde; HNE, 4-hydroxynonenal.

Discussion

Medicinal plant studies are important for the identification of potential therapeutic agents in the treatment of gastrointestinal disorders. In the present study, the anti-ulcerative effects of *E. aschenbornianum* powder against ASA-induced gastric ulcers were investigated in rats. The rat model of ASA-induced gastric ulcers has been used to evaluate the gastroprotective effects of certain compounds, gastrointestinal irritation, stomach bleeding, lipid peroxidation and carbonylated protein content, as well as increases in gastric myeloperoxidase activity (35-37). Ingestion of *E. aschenbornianum* powder led to a significant reduction in the UI compared with that in the

Table I. Effect of *E. aschenbornianum* (400 mg/kg) on liver and kidney function 14 days after administration.

Parameter	*E. aschenbornianum* treatment	Control	P-value	Reference range
Total bilirubin (mg/dl)	0.19±0.02	0.23±0.03	0.127	0.04-0.23>
Direct bilirubin (mg/dl)	0.05±0.01	0.04±0.01	0.288	0.03-0.06
AST (U/l)	120.46±2.28	116.00±3.06	0.113	64-222
ALT (U/l)	71.10±0.40	70.20±0.50	0.072	14-64
ALP (U/l)	409.20±15.82	356±16.43	0.323	62-230
GGT (U/l)	1.89±0.18	2.20±0.21	0.124	<1
Creatinine (mg/dl)	0.83±0.03	0.79±0.04	0.238	0.3-0.60
Urea (mg/dl)	60.28±5.45	51.04±4.30	0.082	13.2-27.1
Albumin (g/dl)	3.69±0.35	3.32±0.24	0.206	3.6-4.7

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase. Reference range Giknis and Clifford (34).

ASA-induced ulcer group; treatment with *E. aschenbornianum* powder resulted in a 53.16±2.60% gastroprotective effect in animals with ASA-induced gastric ulcers. These results obtained with *E. aschenbornianum* treatment are consistent with those of a study by Sánchez-Mendoza *et al* (7), in which a hexane extract of the leaves of *E. aschenbornianum* provided a gastroprotective effect in rats with ethanol-induced gastric ulcers. Secondary metabolites of plants, including terpenes, flavonoids and alkaloids, have been documented to possess anti-ulcer activity (38), and are the major active components of plants of the *Eupatorium* genus (10-12).

ASA induces the production of reactive oxygen species, which leads to increased lipid peroxidation, damaging lipids of the cell membrane (36). MDA and HNE aldehydes are important toxic byproducts of lipid peroxidation and are used as indicators of tissue damage (38). The levels of MDA and HNE in gastric mucosal tissues of rats administered *E. aschenbornianum* powder were significantly lower compared with those in the ASA-induced ulcer group, these results about the possible antioxidant activity was similar to those reported by Tuluce *et al* 2011 (37) and Krishnan *et al* (39) using 50% aqueous-ethanolic small centaury in acute gastric ulcer model and methanolic fractions of *Eupatorium triplinerve* on acetic acid induced ulcerative colitis mice model, respectively. Although these observations suggest that *E. aschenbornianum* powder exerted gastroprotective effects at the dose applied, the ability of ASA to induce-ulcers could have been reduced in the present study by the diet intake due to quenching by the food provided to the rats immediately after ASA administration. Although numerous traditional medicines are widely used to treat and prevent diseases, their safety remains in question, as these medicinal plants also contain several bioactive ingredients that have the potential to cause harmful or detrimental effects. For this reason and due to their increasing use worldwide, the safety and pharmacological efficacy of traditional and alternative medicines require to be evaluated (10,40,41). The acute toxicity of *E. aschenbornianum* powder at 400 mg/kg was tested; all animals exhibited notable tolerance without any mortality or toxicity. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (42). No differences in body weight and food intake were noted in the present study. The levels of biochemical indicators used to evaluate liver function, including serum bilirubin, AST, ALT, ALP and gamma-glutamyl transferase (43) were not significantly altered compared with those in the non-induced group. Renal biomarkers, including creatinine, urea and albumin indicated that kidney function was comparable for the two groups (44,45). As no toxic effects were observed, the consumption of *E. aschenbornianum* powder applied at a unique dose may be considered safe. Further experiments comparing the effect of *E. aschenbornianum* with that of other anti-ulcer drugs are required.

In conclusion, the present study suggested that *E. aschenbornianum* powder had anti-ulcerative and gastroprotective effects in a rat model of ASA-induced gastric ulcers, suggesting the potential use of *E. aschenbornianum* in the prevention of gastric ulcers. In addition, the consumption of *E. aschenbornianum* powder was determined to be pharmacologically safe after acute oral administration, as no indications of liver or kidney damage were observed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JMF-F, CPB-A and PBR-R drafted the manuscript. PBR-R, EP-C and NED-M designed the study. CPB-A, OF-F, JMF-F

and NED-M analyzed and interpreted the data. CPB-A, OF-F and JMF-F performed the experiments, treated the animals and were responsible for statistical analysis. EP-C, PBR-R and JMF-F revised the manuscript. EP-C, JMF-F and PBR-R supervised and coordinated the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Animal Care and Use Committee of the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (Guadalajara, Mexico) and was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the official Mexican standard for the care and use of laboratory animals (approval no. NOM-062-ZOO-1999).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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