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Chemical characterization, toxicology and mechanism of gastric antiulcer action of essential oil from *Gallesia integrifolia* (Spreng.) Harms in the *in vitro* and *in vivo* experimental models

Karuppusamy Arunachalam[a], Sikiru Olaitan Balogun[a,b], Eduarda Pavan[a], Guilherme Vieira Botelho de Almeida[a], Ruberlei Godinho de Oliveira[a], Theodoro Wagner[c], Valdir Cechinel Filho[c], Domingos Tabajara de Oliveira Martins[a,*]

[a] *Área de Farmacologia, Departamento de Ciências Básicas em Saúde, Faculdade de Medicina, Universidade Federal de Mato Grosso (UFMT), Cuiabá, Brazil*
[b] *Curso de Farmácia, Faculdade Noroeste do Mato Grosso, Associação Juinense de Ensino Superior (AJES), Juína, Mato Grosso, Brazil*
[c] *Programa de Pós-Graduação em Ciências Farmacêuticas e Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí, Itajaí, Santa Catarina, Brazil*

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ABSTRACT

Gallesia integrifolia is a Brazilian Amazon tree whose bark decoction is popularly used to treat peptic ulcer. The essential oil from the inner stem bark of *G. integrifolia* (EOGi) was chemically characterized by GC/MS. The *in vitro* cytotoxicity and genotoxicity were evaluated in CHO-K1 cells, while the *in vivo* oral acute toxicity was performed in mice. The gastroprotective effect of EOGi was assessed in acidified ethanol and piroxicam and ulcer healing on acetic acid -induced ulcer models in rodents. Anti-secretory, mucus, K^+-ATP channels, prostaglandins (PGs), nitric oxide (NO), TNF-α, IL-1β, IL-10, catalase (CAT) and myeloperoxidase (MPO) activities and *in vitro Helicobacter pylori* action by EOGi were evaluated. EOGi exhibited cytotoxic effects only at 72 h and no acute toxicity. EOGi showed gastroprotective and ulcer healing effects. EOGi gastroprotection was attenuated by indomethacin pre-treatment. Gastric volume and total acidity were reduced, while gastric pH was elevated. EOGi increased mucus and NO productions and CAT activity, and inhibited MPO activity, TNF-α and IL-1β concentrations and augmented IL-10. EOGi was not active against *H. pylori*. These results indicated that EOGi is safe and exerts preventive and curative gastric ulcer effects by multitarget actions. Twenty compounds were identified and (−)-alpha-santalene was the main compound.

1. Introduction

Peptic ulcers are lesions in the gastrointestinal tract that usually occur in the stomach and duodenum affecting millions of people around the world and has been considered a major cause of morbidity and mortality [1]. In Brazil, there are no accurate estimates, the incidence of the disease in the population ranges from 1 to 20%, whereas in the USA, it is estimated that about 25 million people will develop some kind of ulcer during their lifetime. When untreated, complications can occur in up to 30% of patients with ulcer and include upper gastrointestinal bleeding, perforation and pylorus-duodenal obstruction, which are important causes of mortality [2].

Gastric ulcer is a lesion characterized by necrosis, neutrophil infiltration, reduction in blood flow, increased oxidative stress, and inflammation [3]. It occurs due to an imbalance between aggressive injurious factors (hydrochloric acid, pepsin, refluxed bile, leukotrienes, and reactive oxygen species (ROS)) and defensive mucosa-protective factors (prostaglandins – PGs, mucus and bicarbonate barrier and adequate blood flow, surface active phospholipids, nitric oxide (NO) and antioxidant enzymes). Besides, stress [4], smoking, nutritional deficiencies, prolonged ingestion of nonsteroidal-anti-inflammatory drugs (NSAIDs), and *Helicobacter pylori* (*H. pylori*) infection are all relevant etiological factors for the development of gastric ulcer [5].

* Corresponding author at: Universidade Federal de Mato Grosso, Av. Fernando Correa da Costa, No. 2367, Boa Esperança, Cuiabá, Mato Grosso, 78060-900, Brazil.
E-mail address: taba@terra.com.br (D.T. de Oliveira Martins).

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Nowadays, the existing treatment options available for patients suffering from gastric ulceration include antacids, sucralfate, PGE_1 analogue; muscarinic antagonists, histamine-2 receptor antagonists (H_2-RAs), and proton pump inhibitors (PPIs). However, it has been observed that long-term use of H_2-RAs and PPIs induces hyperplasia in enterochromaffin-like cells, which may lead to a recurrence of the ulcer disease and induction of gastric cancer [6]. In addition, some of these treatments may cause side effects like hypersensitivity, arrhythmia, impotence, gynecomastia, and hematopoietic disturbances [7]. Therefore, there is a pressing need for development of effective and safer anti-ulcerogenic agents with fewer side effects, through scientific studies like the present.

The advancement of phytochemical and phytopharmacological investigations has enabled elucidation of the composition and biological activities of several medicinal plant products including plant extract and essential oils. Plant essential oils, as well as similar compounds, have been considered as promising natural drug and food additives or preservatives [8].

Some natural compounds such as essential oils from herbs are additionally used for food protection [9]. These products have been widely used around the world since ancient time for the treatment of various disorders such as peptic ulcer disease, microbial infection, diabetes, hypertension and among others [10]. Therefore, the investigation of compounds bearing antiulcer effects in medicinal plants may lead to the discovery of new antiulcer drugs [11]. The pharmaceutical properties of some of these plants have been partially attributed to essential oils [12].

In Brazil, medicinal plants are a source of medicine, and sometimes the only affordable to low-income people, especially rural communities, constituting the basis for the research and discovery of new drugs from ethnobotanical information [13]. Some studies have shown the anti-ulcerogenic effect of essential oils extracted from various medicinal plants [14–16], which have been generally attributed to the presence of the terpenes, the main constituents of many essential oils [17].

Gallesia integrifolia (Spreng.) Harms (*G. integrifolia*), (Phytolaccaceae) is a large tree, native and endemic to Brazil and is found in several states in the regions of North (Acre and Amazon), Northeast (Bahia, Ceará, Paraíba and Pernambuco), Midwest (Mato Grosso), Southeast (Minas Gerais, Rio de Janeiro and São Paulo) and South (Paraná) of Brazil [18]. It is popularly known as *pau-d'alho* meaning "garlic plant", due to its semblance with garlic smell [19].

This species was first described by Sprengel in 1821 and named *Thouinia integrifolia* Spreng. The *G. integrifolia* species is cited by some authors as a synonym of *G. acorododendron*, *G. gorarema* and *Crataeva gorarema* [19,20], It presents as large arboreal habit, with a height of 15–30 m high, wide and dense canopy with trunk diameter of 70–140 cm. The wood and other plant parts exude a sharp odor of garlic, hence its local name [21].

In traditional medicine, teas made from its leaves and stem bark are used to treat ulcers [22]. Its boiled leaves, wood shavings and stem bark are used to bath tumors, treatment of the respiratory and lymphatic systems diseases and intestinal worms. The crushed fresh leaves are used topically to treat abscesses, orchitis and gonorrhea [23]. The infusion made from its roots is used in the treatment of rheumatism and ulcers in some Brazilian local communities. In addition, essential oil obtained from it is used in the treatment of gonorrhea [24–26].

Several classes of phytochemicals have been reported from different parts of the plant, which include terpenes, porphyrins, branched alcohols, phenols, aromatic ketones and sulfur-containing natural substances. Several biological activities, such as antiulcer, antioxidant, antimicrobial and cytotoxic properties against certain cancer cells have been reported [27,28]. De Jesus Silva Junior [29] isolated 28-hydroxyoctacosyl ferulate, a novel natural product, which displayed strong antiviral activity against HSV-1. Recently, our laboratory reported that the hydroethanolic extract obtained from the inner stem bark of *G. integrifolia* (HEGi) contains pharmacologically significant compounds such as gallic acid and rutin [30].

The majority of pharmacological and toxicological studies with this plant refers to antinociceptive and anti-inflammatory [29] activities of leaf extracts. Also antimicrobial activities of extracts derived from leaves, bark and root are cited [30–34].

Although some medicinal properties of this plant have been reported, the antiulcer activity of *G. integrifolia* essential oil has not been investigated until now. Therefore, the present study was aimed to investigate the mechanism of gastric antiulcer action of the essential oil of the inner stem bark of *G. integrifolia*, its potential toxicity and to undertake chemical characterization of the essential oil.

2. Material and methods

2.1. Animals

Swiss albino mice (25–40 g, 6–8 weeks) of both sexes and adult female Wistar rats (150–200 g) obtained from the Animal House of Universidade Federal de Mato Grosso, Cuiabá (UFMT) were used for the studies. The animals were maintained in propylene cages at $25 \pm 1\,^{\circ}C$ in a 12 h dark/12 h light cycle experimental room, with free access to standard laboratory feeds (NUVILAB®, Quimtia, PR, Brasil) and water *ad libitum* for at least three days prior to each experiment. For the *in vivo* acute toxicity experiment, male and female mice were used and in other studies (antiulcer activity and mechanistic studies) female mice or female rats were used.

The animals were used according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS/ICLAS). All of the procedures described had prior approval from the Institutional Committee for Ethics in the Use of Animals (CEUA) – UFMT (Process no. 23108.138731/2016-08) and conducted in agreement with the "Principles of Laboratory Animal Care" (NIH Publication 85-23, revised 1985).

2.2. Drugs and reagents

Alcian Blue® 8GX, Bovine serum albumin (BSA), carbenoxolone, cimetidine, clarithromycin, penicillin, streptomycin, Dulbecco's Modified Eagle's Medium (DMEM), Mueller Hinton (M-H) Broth, hexadecyl trimethyl ammonium bromide (HTAB), Fetal Bovine Serum (FBS) were obtained from Sigma-Aldrich Co., St. Louis, Missouri, USA. and ethanol from Tedia Company, Inc Fairfield, Ohio, USA, alamar blue® (Invitrogen, Life Technologies, New York, USA), tetramethylbenzidine (TMB) (eBioscience Inc., California, USA) sodium acetate, anhydrous sodium sulphate (Sigma, São Paulo, Brazil), sodium hydroxide (Vetec®, Quimica Fina Ltda, Rio de Janeiro, Brazil, Brain-Heart Infusion (BHI) Broth (Newprov Produtos para Laboratórios, Pinhais, Paraná, Brazil), Fetal Calf Serum (FCS) (Cultilab, São Paulo, Brazil), cytokines ELISA Ready-SET-Go kit (eBioscience®, Inc.Science Center, A 92121, CA, USA) sucrose (Dinâmica®, Química Contemporânea Ltda, São Paulo, Brazil); panoptic dye (Panótico RÁPIDO, Paraná, Brazil), ketamine and xylazine (Syntec/R.I. Farmacêutica Ltda, Brasília, DF, Brazil). All other chemicals, drugs and reagents used were of analytical grade and obtained from standard commercial suppliers.

2.3. Collection of plant material and extraction of essential oil

The inner stem bark of *G. integrifolia* (10 kg) was collected freshly from Bom Jardim, Nobres, Mato Grosso (MT), Brazil,