Chemico-Biological Interactions 222 (2014) 27–36

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats

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ARTICLE INFO

Article history:
Received 11 May 2014
Received in revised form 8 August 2014
Accepted 18 August 2014
Available online 26 August 2014

Keywords:
Gastroprotective
Patchouli alcohol
Prostaglandin E_2
Cyclooxygenase
Gastric blood flow
Gastric mucus

ABSTRACT

Pogostemonis Herba is an important Chinese medicine widely used in the treatment of gastrointestinal dysfunction. Patchouli alcohol (PA), a tricyclic sesquiterpene, is the major active constituent of Pogostemonis Herba. This study aimed to investigate the possible anti-ulcerogenic potential of PA and the underlying mechanism against ethanol, indomethacin and water immersion restraint-induced gastric ulcers in rats. Gross and histological gastric lesions, biochemical and immunological parameters were taken into consideration. The gastric mucus content and the antisecretory activity were analyzed through pylorus ligature model in rats. Results indicated that oral administration with PA significantly reduced the ulcer areas induced by ethanol, indomethacin and water immersion restraint. PA pretreatment significantly promoted gastric prostaglandin E_2 (PGE_2) and non-protein sulfhydryl group (NP-SH) levels, upregulated the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) mRNA expression, and considerably boosted the gastric blood flow (GBF) and gastric mucus production in comparison with vehicle. In addition, PA modulated the levels of interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α). The levels of glutathione (GSH), catalase (CAT) and malonaldehyde (MDA) were also restored by PA. However, the gastric secretion parameters (pH, volume of gastric juice and pepsin) did not show any significant alteration. These findings suggest that PA exhibited significant gastroprotective effects against gastric ulceration. The underlying mechanisms might involve the stimulation of COX-mediated PGE_2, improvement of antioxidant and anti-inflammatory status, preservation of GBF and NP-SH, as well as boost of gastric mucus production.

1. Introduction

Gastric ulcer remains a common gastrointestinal disorder in clinics, although its management has improved substantially following the introduction of proton pump inhibitors (PPIs) and therapy for *Helicobacter pylori* eradication [1]. Gastric mucosal injury may occur when noxious factors "overwhelm" an intact mucosal defense or the mucosal defensive mechanisms are impaired [2]. Noxious factors include acid and pepsin secretion, *H. pylori* infection, poor diet, alcohol ingestion, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs). The basic components and mechanisms that provide gastric mucosal defense include an intact mucus barrier, adequate mucus secretion and mucosal blood flow, prostaglandins, activity of antioxidant and anti-inflammatory compounds, etc. Drug treatment of gastric ulcer is targeted at either counteracting aggressive factors or stimulating the mucosal defenses.

Phytogenic agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of gastric ulcer. In recent decades, gastroprotection using medicinal plant products as possible therapeutic alternatives has become a subject of active scientific investigations [3]. Pogostemonis Herba, the dried aerial part of *Pogostemon cablin* (Blanco) Benth. (Labiatae), is a well-known traditional Chinese medicinal herb in southeast Asia. Pogostemonis Herba has been widely applied for gastrointestinal disease in China, Japan, and Korea as an aromatic stomachic to improve impairment of the digestive system [4]. To date, Pogostemonis

Abbreviation: PA, patchouli alcohol.

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http://dx.doi.org/10.1016/j.cbi.2014.08.008
0009-2797/

Herba has been used as the main compositions in more than 40 kinds of traditional Chinese medicine (TCM) preparations, such as Huoxiang Zhengqi Shui and Po Chai Pills, which are mainly used for acute gastrointestinal disorders [5]. Modern researches have demonstrated that the aqueous extract of Pogostemonis Herba could effectively protect the intestinal barrier function [6], relieve gastrointestinal spasms [7] and improve the digestion function [8].

Patchouli alcohol (PA, chemical structure shown in Fig. 1), a tricyclic sesquiterpene, is the major active ingredient of Pogostemonis Herba and has been officially listed as an indicator for the quality assessment of Pogostemonis Herba in the Chinese Pharmacopoeia [9]. In recent years, PA has proved to exhibit radical-scavenging [10], anti-colorectal cancer and Ca^{2+} antagonist activities [11], etc. Recent work from our laboratory has also demonstrated the effectiveness of PA as an anti-inflammatory agent *in vivo* and *in vitro* [12,13].

In spite of the reputed effectiveness of Pogostemonis Herba in gastrointestinal diseases, there has not been any reporting of gastroprotective activity and mechanism attributive to its components so far. In a pioneering effort to substantiate the active phytoconstituent responsible for its purported gastroprotective activity scientifically, the present study was therefore undertaken to evaluate PA for the possible gastroprotective effect against ethanol, indomethacin and water immersion and restraint-induced gastric ulcer in rats.

2. Materials and methods

2.1. Drugs and chemicals

Poloxamer 407 (Lutrol®F127) was provided by BASF Chemical Ltd., (Ludwigshafen, Germany). Indomethacin and Alcian Blue were obtained from Sigma–Aldrich. Lansoprazole tablets were purchased from Tuobin Pharmaceutical Factory (Shantou, China). All the chemicals and reagents used were of analytical grade.

2.2. Preparation of patchouli alcohol

Patchouli alcohol was isolated from patchouli oil at purity of 99.0% and further confirmed by melting point, infrared spectroscopy (IR), as well as by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) in our previous work [14]. In this investigation, patchouli alcohol solid dispersions (SDs) were prepared with poloxamer 407 by melting method as described in our previous studies [15].

2.3. Animals

Male Sprague–Dawley rats (6–7 weeks old, 200–250 g) were obtained from Laboratory Animal Center of Guangzhou University of Chinese Medicine (Guangzhou, China). Rats were housed in environmentally controlled conditions (22 ± 2 °C, relative humidity of 50 ± 5%) with a 12-h light/dark cycle and had free access to food and water. Prior to experimentation, all rats were fasted and housed in cages with raised floors of a wide mesh to prevent coprophagy. Experimental protocols were approved by the Animal Experimental Ethics Committee of Guangzhou University of Chinese Medicine (Guangzhou, China).

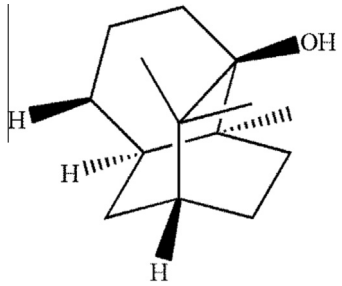

Fig. 1. The structure of (−)-patchouli alcohol.

2.4. Drug administration and dose selection

Male Sprague–Dawley rats were randomly divided into 6 groups ($n = 8$) and pretreated orally with the vehicle (200 mg/kg poloxamer 407), lansoprazole (30 mg/kg) and SDs of PA (equivalent to PA 10, 20 and 40 mg/kg) for 7 consecutive days. The sixth group (Intact) received just distilled water. The dose of PA was selected based on the previous investigation and preliminary experiment. In our previous investigation, 10, 20 and 40 mg/kg of PA was adopted for its possible anti-inflammatory effect in rats and 20, 40 and 80 mg/kg for anti-virus activity in mice [13,16]. The preliminary experiment in ethanol-induced ulceration showed that PA at a higher dose (80 mg/kg) did not display superior anti-ulcerogenic potential than 40 mg/kg concerning the ulcer area. At the same time, considering the dosage of Pogostemonis Herba used in Chinese medicine, 40 mg/kg was selected as the highest test dose. In our pilot trial, a lower dose (5 mg/kg) had been actually tested in ethanol and indomethacin-induced ulcer. However, PA pretreatment failed to show significant reduction in the ulcer areas at the dose of 5 mg/kg (data not shown). Therefore, the subsequent biochemical and immunological parameters were not taken into consideration at 5 mg/kg.

2.5. Ethanol-induced gastric ulcer

All rats were fasted for 24 h with free access to drinking water before receiving absolute ethanol. Then, the rats were treated with distilled water (Intact), vehicle (200 mg/kg poloxamer 407), lansoprazole (30 mg/kg) and SDs of PA (equivalent to PA 10, 20 and 40 mg/kg). One hour later, the rats except the intact group received an oral dose of 1 ml of absolute ethanol. All rats were euthanized after 1 h and their stomachs were removed. Serum was collected for follow-up analyses before euthanasia. The stomachs were then opened along the greater curvature and rinsed with cold saline to remove the gastric contents and blood clots. The flattened stomach samples were photographed and the ulcer area (mm^2) was measured using Image J software (developed by the National Institutes of Health). The inhibition percentage was calculated according to the formula: $[(\text{Ulcer Area}_{(\text{Vehicle})} - \text{Ulcer Area}_{(\text{Treated})})/\text{Ulcer Area}_{(\text{Vehicle})}] \times 100\%$.

2.6. Indomethacin-induced gastric ulcer

The rats were fasted for 24 h with free access to drinking water prior to indomethacin administration. The rats were treated with distilled water (Intact) vehicle (200 mg/kg poloxamer 407), lansoprazole (30 mg/kg) and SDs of PA (equivalent to PA 10, 20 and 40 mg/kg). Thirty minutes later, the rats except the intact group were orally administered indomethacin (100 mg/kg) and all rats were euthanized after 5 h [17]. The stomachs were removed, opened along the greater curvature, and then photographed as described in Section 2.5. The ulcer area (mm^2) was determined using Image J software. The inhibition percentage was calculated in the same manner.

2.7. Histological analysis

Samples of the stomach of each rat were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections were then