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The effect of a minor constituent of essential oil from *Citrus aurantium*: The role of β-myrcene in preventing peptic ulcer disease

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ABSTRACT

The monoterpene β-myrcene has been widely used in cosmetics, food and beverages, and it is normally found in essential oil from citrus fruit. The aim of this study was to investigate the anti-ulcer effects of β-myrcene on experimental models of ulcers that are induced by ethanol, NSAIDs (non-steroidal anti-inflammatory drugs), stress, *Helicobacter pylori*, ischaemia–reperfusion injury (I/R) and cysteamine in order to compare with the essential oil of *Citrus aurantium* and its major compound limonene. The results indicate that the oral administration of β-myrcene at a dose of 7.50 mg/kg has important anti-ulcer activity with significantly decreased gastric and duodenal lesions as well as increased gastric mucus production. The results showed treatment with β-myrcene caused a significant increase in mucosal malondialdehyde level (MDA), an important index of oxidative tissue damage. The β-myrcene was also endowed with marked enhancement of antioxidant enzyme activity from GR system as evidenced by the decreased activity of superoxide dismutase (SOD) and increased levels of glutathione peroxidase (GPx), glutathione reductase (GR), and total glutathione in gastric tissue. Our results also shown that treatment with β-myrcene is not involved with thioredoxin reductase (TrxR) activity. Our results reveal, for the first time, the importance of β-myrcene as an inhibitor of gastric and duodenal ulcers and demonstrate that an increase in the levels of gastric mucosa defence factors is involved in the anti-ulcer activity of β-myrcene.

1. Introduction

Peptic ulcer disease (PUD) is an illness that affects many people worldwide. Despite advances in the understanding of PUD, its aetiology has not been completely elucidated. Factors that could increase the incidence of PUD in the global population include stress, high consumption of alcohol, smoking, *Helicobacter pylori* and ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) [1]. The use of NSAIDs, low-dose aspirin or dual anti-platelet therapy has become an important risk factor for recurrent ulcers and their associated complications [2]. The pathophysiology of PUD suggests that this disease is the result of a disruption in the normal balance between aggressive factors (gastric acid, pepsin, and bile salts) and defensive aspects [prostaglandin (PG), mucosal blood flow, mucus, and bicarbonate] [3]. However, oxidative stress is an important factor underlying PUD, and reactive oxygen species (ROS) can cause intracellular damage. Studies have shown that ulcers are commonly associated with lipid peroxidation and oxidative damage in the mucosa because ROS may cause the oxidative damage of biological macromolecules such as DNA, protein and lipids. There is evidence that an adequate balance among antioxidant enzymes is important to minimise the damaging effect of ROS [4]. Endogenous antioxidant systems can inhibit oxidation by scavenging free radicals, decreasing the action of endogenous free radicals, or preventing lipid peroxidation. The field of antioxidant enzymes

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; I/R, ischaemia–reperfusion injury; GPx, glutathione peroxidase; SOD, superoxide dismutase; GR, glutathione reductase; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; NEM, *N*-ethylmaleimide; L-NAME, *N*-nitro-L-arginine methyl ester; NO, nitric oxide; SH, sulfhydryl groups; MPO, myeloperoxidase; GSH, total glutathione content; TrxR, thioredoxin reductase.

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and free radicals is perceived to focus on the use of antioxidant supplements to prevent human diseases such as PUD [5].

β-Myrcene (7-methyl-3-methylene-1,6-octadiene) is a pleasant-smelling monoterpene that is found in many plants, including lemongrass, hops and verbena [6], as well as in the composition of the essential oil that is obtained from fresh fruit peels of *Citrus aurantium* [7]. It is used in the production of perfumes, and it is also a constituent of essential oils that have long been used as flavouring additives in the manufacture of alcoholic beverages and in the food industry [8].

Moraes et al. [7] have previously described the anti-ulcer activity of limonene, which is a major compound (97.83%) that is present in the essential oil of *C. aurantium*. They have also described the activity of the essential oil itself against gastric ulcers that were induced in young rats. The essential oil from *C. aurantium* at a dose of 250 mg/kg (p.o.) and limonene at 245 mg/kg had anti-ulcer activity against lesions that were induced by ethanol or NSAIDs in rats, and it did not interfere with gastric H^+ secretion, serum gastrin or the total glutathione level in the gastric mucosa. The gastric ulcer healing effects of the essential oil from *C. aurantium* was established in young and also middle-aged rats [9,10]. In addition to evidence of the anti-ulcer activity of the essential oil from *C. aurantium* and its major constituent limonene, the chromatographic analysis of samples from the essential oil indicated that β-myrcene was also present in the essential oil at a concentration of 1.43% [7].

According to Wagner [11], only compounds that were present in high concentrations in a drug could be identified, and research focused on these main constituents with regard to chemical standardisation and pharmacological testing, even if it was not clear whether the compounds included the main therapeutically relevant active ingredients of the extract. Chen et al. [12] described the important contribution of minor constituents from *Rhizoma coptis* extract to cytotoxicity and anti-hyperglycaemic effects.

Aside from the widespread use of β-myrcene in the food industry, there are no reports about its effects on peptic ulcers. Therefore, the aim of the present study was to evaluate the anti-ulcer effects of β-myrcene in experimental models of peptic ulcers that were induced by different agents in rats and to evaluate the mechanisms of action underlying the effects of β-myrcene.

2. Material and methods

2.1. Animals and evaluation of the lesions

Adult male Wistar rats (150–250 g) were supplied by the Central Animal House of the UNESP. The animals were fed a certified Nuvilab® (Nuvital) diet with free access to tap water under standard conditions of dark/light (12 h/12 h) and temperature (22 ± 1 °C). The animals were fasted before all experimental procedures because standard drugs and β-myrcene were administered orally (gavage). The animals were housed in cages with raised wide mesh floors to prevent coprophagy. All experiments were performed in the morning and followed the recommendations of the Canadian Council on Animal Care [13]. The employed protocol was approved by the Institutional Animal Care and Use Committee from UNESP (CEUA no. 18/05). After each experiment, the animals were sacrificed with CO_2 gas, their stomachs or duodenums were excised and cut along the greater curvature, gently rinsed under tap water, pressed onto a glass plate, and scanned so that the lesions could be counted with the aid of the AVSoft® program. The results were expressed as the total ulcerated area (mm^2). In addition to the animal groups that were treated with vehicle (negative control), anti-ulcer drugs (positive control), or β-myrcene. We also included a group called Sham, in which the animals were subjected to the same procedure as the other groups but without the use of agents that induced injury.

2.2. Drugs and treatment

Ketamine hydrochloride, xylazine hydrochloride, ethanol, lansoprazole, *N*-nitro-L-arginine methyl ester (L-NAME), indomethacin, carbenoxolone, *N*-ethylmaleimide (NEM), Alcian Blue, cysteamine hydrochloride, and cimetidine were purchased from Sigma Co (USA). β-Myrcene was purchased from Acros Organics (USA) and dissolved in an 8% Tween-80 aqueous solution that was administered orally (p.o.). The vehicle control group was administered an 8% Tween-80 aqueous solution (10 mL/kg).

2.3. Anti-ulcer activity

2.3.1. Ethanol-induced gastric ulcer in rats

The method described by Robert et al. [14] was followed with slight modifications. Fasted rats (12 h) were randomly divided into five groups ($n = 7$) and given β-myrcene at doses of 3.75, 7.50 and 11.25 mg/kg body weight, 100 mg/kg carbenoxolone (positive control) or 8% Tween-80 (vehicle). One hour later, all groups were treated orally with 1 mL absolute ethanol to induce gastric ulcers. After 1 h of induction, the animals were sacrificed and their stomachs were removed to evaluate the extent of the lesions, as previously described. Strips of the stomach (effective dose) were removed, weighed and stored for subsequent biochemical procedures.

2.3.2. Indomethacin-induced gastric ulcer in rats

The procedure was performed according to the method described by Rainsford [15]. Rats were randomly divided into three groups ($n = 7$), and each group was given vehicle (8% Tween-80), cimetidine (100 mg/kg) or β-myrcene (7.50 mg/kg). Thirty minutes later, all groups were orally given indomethacin (50 mg/kg) solubilised in sodium carbonate 0.5% [16]. Six hours following the administration of the harmful agent (indomethacin), all animals were sacrificed, their stomachs were removed, and the gastric lesions were evaluated.

2.3.3. Stress-induced gastric ulcer in rats

The method of Alder [17] was followed with slight modifications. The rats were randomly divided into 3 groups, and each group ($n = 7$) was treated orally with vehicle (8% Tween-80), lansoprazole (30 mg/kg) or β-myrcene (7.50 mg/kg) for 14 consecutive days. On day 15, all groups were subjected to stress by forced swimming for 3 h in a glass cylinder (height 45 cm, diameter 25 cm) containing water at a height of 35 cm that was maintained at 23 ± 1 °C. Subsequently, the animals were sacrificed, and their stomachs were removed for evaluation of the gastric lesions, as previously described.

2.3.4. Gastric ulcer induced by ischaemia–reperfusion in rats

The rats were divided into three groups ($n = 7$) and treated with vehicle, lansoprazole (30 mg/kg) or β-myrcene (7.50 mg/kg) 30 min before surgery (artery clamp). Then, the animals were anaesthetised with ketamine hydrochloride and xylazine hydrochloride, and they remained sedated throughout the procedure [18]. An incision was made approximately 3 cm on the left side of the abdomen where the aorta and vena cava were first identified to facilitate the location of the coeliac artery. After identification, the coeliac artery was isolated and subjected to a process of cleaning and the removal of adhesions and any adipose tissue. A microvascular clamp was placed in the artery, and the artery remained clamped for 30 min. After this period of ischaemia, the clamp was removed (to allow reperfusion), and reperfusion was allowed to proceed for 60 min. At the end of this period, the animals were killed and their stomachs were removed for evaluation of the gastric lesions, as previously described.