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Original article

Antiulcer activity of *Garcinia indica* fruit rind (kokum berry) in rats

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ABSTRACT

Garcinia indica Choisy (Family: Guttiferae/Clusiaceae) is a slender evergreen tree endemic to the west coast of India. The dried rind of the fruit of *Garcinia indica* known as "kokum" is an Indian spice and a food additive. Many therapeutic effects of the fruit have been reported in literature. The present study evaluates the antiulcer activity of the aqueous extract of *Garcinia indica* fruit rind (GIE) against absolute ethanol- (necrotizing agent), aspirin- (non-steroidal anti-inflammatory drug) and histamine- (gastric secretion stimulator via H_2 receptor) induced ulcers in rats. GIE (400 mg/kg and 800 mg/kg) was administered orally to the overnight fasted rats, 1 h prior to the absolute ethanol/aspirin/histamine challenge. The ulcer index, gastroprotective potential, status of the antioxidant enzymes {superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)} along with reduced glutathione (GSH), and lipid peroxidation were studied in all the three models. Pre-treatment with GIE showed a dose-dependent decrease in the ulcer index in all three models. GIE, at both doses, elicited significant antioxidant activity by attenuating the ulcer elevated levels of MDA and restored the ulcer-depleted levels of GSH, SOD, CAT, GPx and GR. In conclusion, GIE possesses potent antiulcer activity, which may be attributed to an underlying antioxidant activity.

1. Introduction

Peptic ulcer or peptic ulcer disease (PUD) is defined as a break in the mucosal lining of the gastrointestinal tract [1]. It occurs in that part of the gastrointestinal tract, which is exposed, to gastric acid and pepsin, i.e. the stomach and duodenum. The normal stomach mucosa maintains a balance between defensive and aggressive factors [2]. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, free radicals, use of alcohol and non-steroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (*Helicobacter pylori* and others). On the other hand, defensive factors, such as mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, endogenous nitric oxide and normal tissue microcirculation protect against ulcer formation. Although the etiology of ulcer is unknown yet, it is generally accepted that peptic ulcers develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms [3]. The incidence of PUD varies with age, gender, geographical location and is associated with severe complications, including haemorrhages, perforations, gastrointestinal obstruction, and malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss [4].

Modest approaches to control peptic ulceration include potentiation of the mucosal defense along with reduction of acid secretion and its neutralization, enhancement of antioxidant levels in the stomach, stimulation of gastric mucin synthesis and inhibition of *H. pylori* growth. The currently used drugs include antibiotics to kill *H. pylori*, acid blockers, which reduce acid secretion for a prolonged duration (ranitidine, famotidine), proton pump inhibitors (omeprazole), and tissue lining protecting agents (sucralfate, bismuth) [5]. These drugs have decreased the morbidity rates, but produce many adverse effects, including relapse of the disease, and are often expensive for the poor population.

In light of the above, it is pertinent to study natural products from food/plants as potential antiulcer compounds. Use of natural drugs in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa [6].

Garcinia indica (Family: Guttiferae; Clusiaceae), a slender evergreen tree, is endemic to the west coast of India [7]. It has many culinary, pharmaceutical and industrial uses. The dried outer rind of the fruit of *G. indica* is popularly known as kokum and is used for imparting flavor and taste to curries. The fresh fruits are steeped

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in sugar syrup to make a healthy soft drink to relieve sunstroke and to provide gastric relief during summer. Kokum juice is mixed with yogurt and salt to make a "natural antacid" and has a plausible function as an antiulcer agent [8]. Many therapeutic effects of the fruit have been described in Ayurveda, which include its usefulness in skin ailments, such as rashes caused by allergies; in treatment of burns, scalds and chaffed skin; as a remedy for dysentery and mucous diarrhoea; as an appetizer and a good liver tonic; as a cardiotonic and for bleeding, piles, dysentery, tumors and heart diseases [9].

The major phytoconstituents present in *G. indica* are anthocyanins, hydroxycitric acid (HCA), garcinol, isogarcinol and polyphenols. One phytoconstituent of kokum, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent [10]. Garcinol, a polyisoprenylated benzophenone purified from *G. indica* fruit rind, displays anti-cancer and antioxidant activities [11]. Apart from HCA and garcinol, kokum contains other compounds, like isogarcinol, ascorbic acid and polyphenols with potential antioxidant properties.

Keeping in view the use of kokum as an effective home remedy for acidity and gastric discomfort, the present study was undertaken to evaluate the antiulcer activity of *G. indica* fruit rind.

2. Materials and methods

2.1. Plant material

The fruit rind of *G. indica* was collected from the Konkan region of Maharashtra, India and air dried under shade, powdered mechanically and stored in an airtight container. The powder was extracted using soxhlet apparatus and water as solvent, dried and stored in a refrigerator for further use. The plant was authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai after matching with the existing specimen (accession no. 03587).

2.2. Drugs and chemicals

Omeprazole, ranitidine and diclofenac sodium were gift samples from Cipla Laboratories, Unique Chemicals and Pharmaceuticals Ltd., and Themis Pharmaceuticals, India respectively. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione, oxidized glutathione and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Himedia Laboratories, Mumbai, India. Epinephrine, histamine and 5, 5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were obtained from local sources and were of analytical grade.

2.3. Experimental animals

Wistar Albino rats (180–200 g) of either sex were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5%), temperature (25 ± 2 °C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pune, India) and water *ad libitum*. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 25/1999/CPCSEA) and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

2.4. Acute toxicity study (ALD_{50})

Acute toxicity studies were carried out on Wistar rats by the oral route at dose levels up to 2000 mg/kg of the fruit rind extract of *G. indica* as per the OECD- guidelines No. 402.

2.5. Preparation of test and reference drug solutions

The aqueous extract of *G. indica* fruit rind (GIE) was dissolved in distilled water and the aqueous solution was used.

Reference drugs viz., diclofenac sodium, omeprazole and ranitidine were prepared as a suspension in 1% (w/v) aqueous carboxymethyl cellulose solution and used immediately.

Toxicant drugs viz., aspirin and histamine were also prepared as suspensions in 1% (w/v) aqueous carboxymethyl cellulose solution and administered immediately.

2.6. Antiulcer activity

The effects of GIE were evaluated in ethanol-, aspirin- and histamine-induced ulcer models in rats. Omeprazole was used as a standard drug for the ethanol and aspirin-induced ulcer models and ranitidine was used as a standard drug for the histamine-induced ulcer model for comparing the antiulcer potential of GIE.

2.7. Ethanol-induced gastric ulceration [12,13]

Albino Wistar rats weighing 180–200 g after acclimatization (6–7 days) in the animal quarters were randomly divided into five groups of 6 animals each and treated in the following way:

- group 1: served as normal control (untreated);
- group 2: served as toxicant control and received absolute ethanol (1 mL/200 g, p.o.);
- group 3: served as standard and received omeprazole (20 mg/kg, p.o.) 30 min prior to absolute ethanol treatment (1 mL/200 g, p.o.);
- group 4: received GIE (400 mg/kg, p.o.) 30 min prior to absolute ethanol treatment (1 mL/200 g, p.o.);
- group 5: received GIE (800 mg/kg, p.o.) 30 min prior to absolute ethanol treatment (1 mL/200 g, p.o.).

All rats were fasted for 24 h but allowed free access to water. The standard drug and the test drugs were administered orally to the respective groups. Thirty minutes after their pre-treatment, all animals were gavaged with absolute ethanol. They were humanely sacrificed 1 h later by cervical dislocation, the stomachs were excised and opened along the greater curvature. Ulcers formed in the glandular portion of the stomach were observed under a magnifying glass for measuring the ulcer score and the ulcer index. The stomachs were weighed, chilled and washed with ice-cold saline after evaluation of the above parameters. A stomach homogenate (10% w/v) was prepared in 1.15% (w/v) KCl. An aliquot of the homogenate was used for the estimation of lipid peroxidation (LPO). The homogenates were centrifuged at 7000 × *g* for 10 min at 4 °C and the supernatants were used for the assays of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx) and glutathione reductase (GR).

2.8. Aspirin-induced gastric ulceration [14]

Albino Wistar rats were randomly divided into five groups of 6 animals each and treated in the following way: