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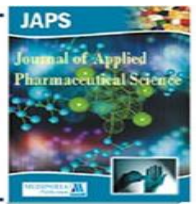

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Antiulcer activity of *Garcinia indica* linn fruit rinds

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ABSTRACT

Garcinia indica Linn (Clusiaceae), a medicinal plant mentioned in Ayurveda has been used for treatment of liver disorders, dysentery, sunstroke, cancer and heart diseases. The present study was undertaken to investigate ulcer protective effect of aqueous and ethanolic extract of *Garcinia indica* Linn fruit rind. The aqueous and ethanol extract of *Garcinia indica* Linn were investigated for ulcer protective activity against indomethacin induced ulcerogenesis and HCl/ethanol induced gastric lesion. Oral administration of the aqueous and ethanol extracts of *Garcinia indica* fruit rind at the dose 500 mg/kg provided significant ($p<0.001$) reduction of ulcer index in the HCl/ethanol and indomethacin induced gastric lesion rat models.

Key words: Gastric lesion; Ethanol; Indomethacin; Ulcerogenesis; Garcinia indica; ulcer index.

INTRODUCTION

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world and they are induced by several factors like stress, smoking, alcohol consumption, nutritional deficiencies and ingestion of non steroidal-antiinflammatory drugs (NSAIDs) e.g. Indomethacin, has side effects such as gastrointestinal irritation, erosion, bleeding, ulceration and perforation (Basil and Howard,1995; Loguercio et al.,1993; Naito et al.,1998; Nash et al.,1994; Sagar and Ahamed,1999; Suleyman et al.,2002). Although a number of antiulcer drugs such as H_2 receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have various undesirable effects such as arrhythmias, impotence and hematopoietic changes and limitations (Ariyoshi et al.,1986; Del et al.,1985; Satoh et al.,1988).

Garcinia indica Linn belonging to family Clusiaceae commonly recognized as 'Kokum' is found in Maharashtra and particular in Konkan, Goa and the western region of India. The fruits of Garcinia indica Linn have been suggested in the Indian system of medicine for a number of diseases. These include its usefulness as an infusion, in skin rashes caused by allergies, treatment of burns, to relieve sunstroke, remedy for dysentery and mucous diarrhea, an appetizer, antiulcer, liver tonic, to allay thirst and as a cardiotonic. The outer rind of the fruits of Garcinia indica Linn has been shown to be antioxidant activity (Devasagayam et al.,2006; Khare,2007; Kirtikar and Basu,1991; Sheth et al.,2006). Garcinol a polyisoprenylated benzophenones, has antioxidative, chelating, free radical scavenging, antiglycation, anticancer, antiinflammatory, and antiulcer activities (Lin and Liao,2005; Ho et al.,2002; Yamaguchi et al.,2000a; Yamaguchi et al.,2000b). One of the ingredients of kokum, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent (Heymsfield et al.,1998; Mattes and Bormann,2000; Sakariah et al.,2002). Kokum contains other compounds with potential antioxidant properties include citric

acid, malic acid, polyphenols, carbohydrates, anthocyanin flavonoids and ascorbic acid (Cadenas and Packer,1996; Peter,2001; Einbond et al.,2004; Rastogi and Nayak,2010; Yoshikawa,2000). Based on reported traditional and chemical constituents we selected Garcinia indica fruits for the present study.

MATERIAL AND METHOD

Plant material

The Garcinia indica Linn fruits were collected from the Devrukh region in Ratnagiri district and authenticated from Dr.Yadav, Department of Botany, Willington College, Sangli.(voucher specimen no.BN86)

Animals

Male/female wistar albino rats weighing 180-200 g, procured from the animal house of Pharmacology Department., Appasaheb Birnale College of Pharmacy, Sangli, were used with the approval of The Institutional Animal Ethics Committee of CPCSEA, Govt. of India. During the complete course of the experiment, rats were maintained at room temperature in the animal house. The animals had free access to food pellets (Amrut Laboratories animal feed, Sangli) and water ad libitum.

Preparation of aqueous extract of Garcinia indica Linn fruit rind

Fruits were cut open and the seeds were separated from the pulp. Then the fruit rinds were allowed to dry in the shade. The fruits rinds were cut into pieces and shade dried at room temperature. The dried fruits were subjected to size reduction to a coarse powder by using mixer grinder. The coarsely powdered form of shade dried fruit rinds was placed in a conical flask containing distilled water and closed with cotton plug for 7 days at room temperature. Then it was filtered using a piece of clean, sterile, white cotton cloth and evaporated to dryness to yield aqueous extract. The semisolid extract obtained was stored in an airtight container in refrigerator for further use. The solution of aqueous extract was prepared by using normal saline as solvent for experiment.

Preparation of ethanolic extract of Garcinia indica Linn fruit rind

The fruits rinds were cut into pieces and shade dried at room temperature. The dried fruits were subjected to size reduction to a coarse powder by using mixer grinder. This powder was defatted with petroleum ether then filtered. The residue was allowed to dry at room temperature. This residue was extracted with ethanol (95%) into soxhlet apparatus. The extract was dried at room temperature till semisolid mass was obtained. The sweet scented, chocolate colored semisolid residue formed after the complete dryness was kept in an airtight and waterproof container, which is stored in the refrigerator. The suspension of ethanolic extract of Garcinia indica Linn fruit rind was prepared in 0.5% w/v carboxymethylcellulose (cmc) in normal saline.

Toxicity study

Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300, 2000 and 5000 mg/kg as per OECD guidelines. 1/10th dose of 5000 mg/kg i.e. 500 mg/kg has been selected for the present study.

HCl/ethanol induced gastric lesion

Wistar rats of either sex weighing 120-150 g were used for present study. The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on standard pellet diet and water ad libitum. The food was withdrawn 24 h before the experiment, but the animals were allowed free access of water. The vehicle control group animals received suspension 0.5% carboxymethylcellulose (cmc) by means of a gavage. Both fruit rind extracts (500 mg/kg, p.o.) and standard drug ranitidine (50 mg/kg p.o.) were administered orally 15 min before oral administration of 1.0 ml HCl/ethanol mixture containing 0.15 N HCl in 70% v/v ethanol (Anadan et al.,1998) for ulcer induction in to their corresponding groups constituting each of six rats. Later (4 h), the animals were sacrificed with an over-dose of ether. The stomachs were then removed and inflated with 10ml of 1% formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was opened along the great curvature, rinsed with tap water to remove gastric contents and blood clots and examined inner surface with a magnifying lens. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the inhibition percentage was calculated by the following formula;

$$\mathbf{UI = (n\ lesion\ I) + (n\ lesion\ II)\ 2 + (n\ lesion\ III)\ 3}$$

Where:

I = presence of edema, hyperemia and single, submucosal, punctiform hemorrhages;

II = presence of submucosal, hemorrhagic lesions with small erosions;

III = presence of deep ulcer with erosions and invasive lesions (Szelenyl and Thiemer,1978).

$$\textbf{Percentage inhibition} = [(UI_{Control} - UI_{Treated}) / UI_{Control}] \times 100.$$

Indomethacin induced ulcerogenesis

Male Wistar rats (200-250 g), fasted for 24 h, with free access to water, and were divided in four groups. The vehicle control group animals received suspension 0.5% carboxymethylcellulose (cmc) by means of a gavage. Both fruit rind extracts (500 mg/kg, p.o.) and standard drug ranitidine (50 mg/kg p.o.) were administered. After 30 min of oral treatment, indomethacin (30 mg/kg, p.o.) was administrated subcutaneously to all groups of animals, according to reported methodology (Morimoto et al.,1991). After 4 h, the animals were sacrificed; their stomachs were removed, and opened along the greater curvature. The ulcerative lesion index of each animal was calculated by

adding the following values, according to reported methodology (Gamberini et al.,1991).

Loss of normal morphology	1 point
Discoloration of mucosa	1 point
Mucosal edema	1 point
Hemorrhages	1 point
Petechial points (until 9)	2 points
Petechial points (>10)	3 points
Ulcers up to 1 mm	*n×2 points
Ulcers>1 mm	*n×3 points
Perforated ulcers	*n×4 points

* Number of ulcers found

Statistical analysis of data

Results were expressed as mean±/S.E.M. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by using one-way ANOVA and Tukey-/Kramer multiple comparison tests.

RESULT

HCl/ethanol-induced gastric lesion

Aqueous extract of Garcinia indica significantly showed reduction in gastric lesion (52.94%) where as standard drug ranitidine showed inhibition of gastric lesion by 58.26% in experimental rats. Ethanol extract showed inhibition up to 34.45%.

Table1: Effect of oral administration of Garcinia indica extracts on HCl/ethanol-induced gastric lesion in rats

Treatment groups	Dose	Ulcer index (mm)	% Inhibition
Vehicle control (0.5% cmc)	05 ml/kg(p.o.)	59.50±2.918	-
Ranitidine	50 mg/kg (p.o.)	24.83±1.327***	58.26
Aqueous extract	500 mg/kg(p.o.)	**28±2.774*** **	52.94
Ethanol extract	500 mg/kg(p.o.)	39±4.155***	34.45

Values are Mean ± SEM; *** p < 0.001 when compared all groups (by using one way ANOVA with Tukey's test); n=6.
UI = (n lesion I) + (n lesion II)2 + (n lesion III)3

Where: I = presence of edema, hyperemia and single, submucosal, punctiform hemorrhages; II = presence of submucosal, hemorrhagic lesions with small erosions; III = presence of deep ulcer with erosions and invasive lesions (Szelenyl and Thiemer, 1978). Percentage inhibition= $(UI_{Control} - UI_{Treated}) / UI_{Control} \times 100$.

Table 2: Effect of oral administration of Garcinia indica extracts on indomethacin induced ulcerogenesis in rats.

Treatment groups	Dose	Ulcer index mm (Mean ±S.E.M))	% Inhibition
Vehicle control (0.5% cmc)	05 ml/kg (p.o.)	73.83±3.11	-
Ranitidine	50 mg/kg (p.o.)	23.00±2.08***	68.84
Aqueous extract	500 mg/kg(p.o.)	46.66±2.37***	36.80
Ethanol extract	500 mg/kg(p.o.)	28.33±1.05***	61.62

Values are Mean ± SEM; *** p < 0.001 when compared all groups (by using one way ANOVA with Tukey's test); n=6.

Indomethacin induced ulcerogenesis

Aqueous extract of Garcinia indica significantly showed reduction in ulceroenesis (36.80%) where as standard drug ranitidine showed inhibition of ulceroenesis by 68.84% in experimental rats. Ethanol extract showed inhibition up to 61.62%.

DISCUSSION

It is evident from the Table 1 and 2 that the aqueous and ethanolic extract of Garcinia indica exhibited significant antiulcer activity ($p<0.001$) in HCl/ethanol induced gastric lesion and indomethacin induced ulcerogenesis in rats. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radical which are involved in mechanism of acute and chronic ulceration in the gastric mucosa (Pihan et al.,1987). Ethanol-induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhage and necrotic aspects of tissue injury (Guth et al.,1984). This action is direct on the gastric epithelium also causing perturbation of mast cells and release of a vasoactive mediator such as histamine (Oates and Hakkinen,1988). It has been shown that vascular changes in ethanol-induced gastric mucosal injury and severe damage in such injury is associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of blood flow in mucosa (Gaskil et al.,1982). The administration of HCl and ethanol produced ulcerative in the gastric mucosa. In present study, the aqueous and ethanolic extract of G. indica significantly prevented ($p<0.001$) acute, gastric mucosal injury by 52.94 % and 34.45% respectively in ethanol-acid induced ulcerative rats.

Indomethacin, one of the NSAIDs, is known to activate polymorphonuclear granulocytes and to induce gastrointestinal damage including bleeding, ulceration and perforation in both animal and human models (Sagar and Ahamed, 1999; Naito et al.,1998). These pathologies have been attributed to damage of the mucosa membranes. A well-known mechanism responsible for gastric damage induced by indomethacin is the inhibition of cycloxygenase (COX), a rate limiting enzyme in the synthesis of prostaglandins (Naito et al.,1998; Takeuchi et al.,1991). In the stomach, prostaglandins play a vital protective role, stimulating secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turn over and repair (Hayllar and Bjarnason,1995). Thus the suppression of prostaglandin synthesis by NSAIDS results in increased susceptibility to mucosal cell injury and gastroduodenal ulceration. In present experiments, administration of the aqueous and ethanolic extract of G. indica significantly prevented acute, gastric mucosal injury 52.94% and 61.62% respectively in indomethacin induced ulcerogenesis. These results suggest the possible involvement of prostaglandin and/ mucus in the ulcer healing action of extract.

CONCLUSION

The fruits of Garcinia indica Linn contain anthocyanin flavonoids, polyphenols, hydroxycitric acid, malic acid,

carbohydrates, ascorbic acid and phenolic compounds as major chemical constituents which can be considered responsible for the antiulcer activity. Further investigation regarding the isolation of these chemical constituents and pharmacological screening can prove the plant as useful antiulcer medicine.

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