Natural Product Radiance, Vol. 7(1), 2008, pp.15-18

Research Paper

Antiulcerogenic and antioxidant effects of *Coccinia grandis* (Linn.) Voigt leaves on aspirin-induced gastric ulcer in rats

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Received 31 January 2007; Accepted 23 April 2007

Abstract

The effect of ***Coccinia grandis* (Linn.)Voigt** leaves powder, its methanol and aqueous extracts were investigated on aspirin-induced gastric ulcer model in rats. The leaf powder showed a significant dose related decrease in ulcer index, with significant increase in mucus secretion and decrease in level of Lipid peroxidation (LPO) and Superoxide dismutase (SOD) activity. Methanol extract at an equivalent dose to that of the powder also showed a significant decrease in ulcer index with significant changes in mucus secretion, LPO and SOD. However, aqueous extract was found to be non-significant in reducing ulcer index. The group, receiving standard drug Famotidine, showed no effect on the mucus secretion induced in this experimental model. These observations confirm the antiulcerogenic potential of this plant, probably due to increased mucus secretion and antioxidant property.

Keywords: Ivy Gourd, *Coccinia grandis*, Gastric ulcer, Antiulcerogenic, Antioxidant.

IPC code; Int. cl.[8]— A61K 36/00, A61K 36/42, A61K 127/00, A61P 1/04

Introduction

The indigenous system of medicine makes a substantial contribution to the public health in India. Millions of households in rural and urban areas in our country consume traditional diets, use home remedies and follow health customs based on the principles of the traditional medicine. The Indian systems are known to be effective for muscular and nervous disorders, skin ailments, gastrointestinal tract diseases, conditions like diabetes and arthritis to name a few. Several plants are used in G.I. disorders and many are used effectively for their antiulcer properties in folklore medicine. ***Bacopa monnieri* (Linn.)Penn.**, ***Centella asiatica* (Linn.) Urban**, ***Convolvulus pluricaulis* Choisy**, ***Pongamia pinnata* Pierre** and ***Musa sapientum* Linn.** are few such plants which have immense potential as antiulcerogenic agents or have ulcer protective effects[1-8].

Ivy Gourd, ***Coccinia grandis* (Linn.) Voigt** syn. *C. indica* Wight & Arn. (Family—Cucurbitaceae) is cultivated as well as found wild throughout India, Sri Lanka, Malaysia and Tropical Africa. It is a rapidly growing, perennial climber or trailing vine. Traditionally different parts of this plant namely the roots, leaves and fruits are used in folklore medicine for numerous purposes like healing wounds, ulcers, jaundice, diabetes, stomachach, as an antipyretic and an astringent. The leaf and its constituents have been reported to possess hypoglycaemic, hypolipidemic and antioxidant properties, and are also used

Coccinia grandis

Vertically cut fruits

to treat infective hepatitis[1-4].

Despite the popular use of this species as a medicinal plant, there is no data about its pharmacological effect on the gastrointestinal system. Thus, efforts have been made to evaluate the antiulcerogenic and antioxidant activity of the ***C. grandis*** leaf powder and its methanol and aqueous extracts on aspirin induced gastric ulcer model in rats.

Materials and Methods

Plant material and extraction

Fresh green aerial parts of ***C. grandis*** were collected from Ranchi and various areas of the Chotanagpur region and authenticated by the Division of Pharmacognosy, Department of

Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi. The leaves were shade dried for 4 days and then kept in a hot air oven for 5 h at 40°C. It was then powdered with an electric grinder and stored in well-closed airtight containers.

The methanol extract of leaves (CGM) was prepared by Soxhlet extraction for 36 h, after which, the solvent was evaporated by slow heating and stirring. To prepare the aqueous extract (CGA), the methanol-extracted powder was dried, soaked and stirred well in distilled water in a stoppered glass container and left for 24 hours. The contents were again stirred for about 15 min and the extract decanted and dried as earlier. For dosing, the semisolid extracts were suspended in aqueous 1% sodium carboxy methylcellulose(CMC).

Acute toxicity studies

Healthy adult Wistar rats of either sex, starved overnight were subjected to acute toxicity studies to determine the safe dose by up-and-down staircase method[5]. The rats were observed continuously for 2h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death [6].

Drug treatment and induction of gastric ulcer

Wistar albino rats of both the sexes weighing 170-230g were used in the experiments. They were housed in standard cages at room temperature (25 ± 4°C), provided with commercial pellets (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Thirty-six hours before the experiment, the animals were deprived of food but not of water. Rats were divided into seven groups of six rats in each group. All groups received the drugs orally. Group 1 served as the Aspirin control group receiving aqueous suspension of aspirin (200mg/ kg body weight) in 1% sodium CMC (5ml/kg), Group 2 was treated with standard drug Famotidine at a dose of 20mg/kg body weight in 1% sodium CMC (5ml/kg)[7, 8]. Groups 3-5 received the leaf powder of ***C. grandis*** at doses of 0.5, 1 and 2g/kg body weight. Group 6 received the methanol extract at 2g/kg body weight and Group 7 received the aqueous extract at 2g/kg body weight in 1% CMC (5ml/kg). The test drugs were administered once daily 3h before aspirin treatment. This regime was continued for 3 days. Pylorus was ligated on the fourth day. All animal experiments were performed after approval from the Institutional Animal Ethics Committee.

Collection and analysis of gastric juice

The rats were anaesthetised with pentobarbitone (100 mg/kg body weight) and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures[9]. After the administration of aspirin, the abdominal wound was covered with cotton and the animals were kept under anesthesia. The gastric juice was allowed to collect for a period of 4 hours. The rats were then sacrificed by an over dose of chloroform vapours. The stomach was removed after clamping the esophagus. The gastric contents were collected and the gastric mucosa was washed with 4 ml distilled water. The gastric content and washings were centrifuged at 3500 rpm for 15 minutes. Aliquots from this were used for the estimation of hexosamines and sialic acids at the wavelengths of 492nm and 549nm, respectively against water used as blank. The stomach was cut open along the greater curvature and immersed in normal saline for 30 seconds. The lesions in the glandular part of the rats' stomach were examined under 10X magnification of the microscope and scored[10-12].

Analysis of the gastric mucus

The gastric mucosa was scraped with glass slides, weighed and properly homogenised in 8 ml of ice cold 0.9% saline for 30 seconds. The homogenate was then centrifuged at 800 rpm for 10 min followed by centrifugation of the supernatant at 12,000 rpm for 15 minutes[13]. Aliquots were taken from this preparation for analysis of catalase[14] (CAT) and superoxide dismutase[15] (SOD) activities. Lipid peroxidation(LPO) was determined by quantifying the thiobarbituric acid reacting substances[16]. Protein concentration was calculated following Lowry assay[17].

Statistical analysis

The results were expressed as means ± S.E. Statistical analysis was done using unpaired Student's t-test. A value of $P<0.05$ was considered significant.

Results

Acute toxicity studies revealed that the extract was safe up to a dose level of 3500 mg/kg body weight and no lethality or toxic reactions were found up to the end of the study period.

Antiulcer activity

Results for the determination of ulcer index, percentage inhibition of

ulcer, hexosamines and sialic acids levels in different treated groups are given in Table 1. An apparent dose related decrease in ulcer index was seen with the leaf powders of the plant (CGL) and this activity was also apparent using an equal dose of methanol extract of leaves. CGL (2 g/kg) showed a significant decrease in ulcer index ($P<0.05$), significant increase in hexosamine ($P<0.05$) and sialic acid ($P<0.01$) contents in the gastric secretion. CGM also showed a significant decrease in ulcer index ($P<0.05$), significant increase in hexosamine ($P<0.01$) and sialic acid ($P<0.05$) levels. Aqueous extract of the leaves showed no significant change in any of the above parameters when compared to control.

Table 1: Effect of Famotidine and different doses of *Coccinia grandis* leaf powder(CGL), methanol and aqueous extracts(CGM and CGA) on ulcer index, hexosamines and sialic acids levels in aspirin treated rats

Treatment	Ulcer index	% Inhibition of ulcer	Hexosamines (μg/ml)	Sialic acids (μg/ml)
Aspirin control 200mg/kg	18.57 ± 1.35	-	122.7 ± 10.46	26.08 ± 3.4
Famotidine 20mg/kg	14.1 ± 1.26	35.30	188.2 ± 8.53[b]	49.89 ± 5.6[b]
CGL 0.5g/kg	17.7 ± 1.41	4.68	137 ± 12.28	31.44 ± 7.14
CGL 1g/kg	15.44 ± 1.4	16.85	155 ± 14.9	37.48 ± 5.79
CGL 2g/kg	13.6 ± 1.96[a]	26.76	172.6 ± 17.22[a]	46.86 ± 7.46[b]
CGM 2g/kg	12.1 ± 1.15[b]	34.84	180.4 ± 16.18[b]	47.60 ± 6.1[b]
CGA 2g/kg	18.3 ± 1.44	1.45	112.8 ± 11.35	21.96 ± 3.21

n = 6 in each group

Values (mean ± SE), [a] $P<0.05$; [b] $P<0.01$ as compared to aspirin control

Table 2 : Effect of Famotidine and different doses of *Coccinia grandis* leaf (CGL), methanol and aqueous extract (CGM and CGA) on LPO, SOD and CAT activities in rat gastric mucosal homogenates

Treatment	LPO (nmol MDA/ mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Aspirin control 200mg/kg	0.533 ± 0.012	292.9 ± 7.61	16.58 ± 0.86
Famotidine 20mg/kg	0.476 ± 0.027	277.94 ± 9.5	15.72 ± 1.59
CGL 0.5g/kg	0.512 ± 0.016	289.7 ± 11.74	16.62 ± 1.92
CGL 1g/kg	0.5 ± 0.02	271.82 ± 12.61	17.88 ± 2.14
CGL 2g/kg	0.466 ± 0.02[b]	225.4 ± 19[b]	18.22 ± 2.54
CGM 2g/kg	0.468 ± 0.018[b]	238 ± 16.45[b]	18.04 ± 1.67
CGA 2g/kg	0.554 ± 0.013	283 ± 11.85	15.82 ± 1.51

n = 6 in each group

Values (mean ± SE), [b] $P<0.01$ as compared to aspirin control.

Antioxidant activity

Results for the determination of the level of LPO, SOD and CAT activities in different treated groups are given in Table 2. CGL (2g/kg) and CGM treated groups showed a significant decrease in LPO ($P<0.01$) and SOD ($P<0.01$) activities when compared to the aspirin treated control group. No significant change in LPO and SOD was observed in other treatment groups. None of the treatment groups showed significant change in CAT activity when compared to control group.

Discussion

CGL and CGM were found to be effective in decreasing the ulcer index in acute ulcers induced by aspirin, but CGA failed to do so, therefore, it reveals that antiulcer principles may be methanol soluble. Aspirin induced ulcer model is suitable for detecting those drugs which support the mucosal defence[7, 12]. Mucosal prostaglandins are known to afford cytoprotection. Aspirin inhibits prostaglandin synthesis which is also the basis for its anti-inflammatory action. However, inhibition of prostaglandin synthesis, therefore, results in gastroduodenal mucosal damage and

ulceration of the gastric mucosa. The significant increase in hexosamines and sialic acids levels by CGL and CGM with decrease in ulcer index reveals the protective action of the drug may be due to increased mucus secretion.

The role of reactive oxygen species in the pathophysiology of gastrointestinal injury induced by stress, ethanol and NSAIDs is well known. The increase in damage due to O_2^- is contained by dismutation with SOD[18,19]. SOD converts the reactive O_2 to H_2O_2, which if not scavenged by the CAT can generate increased amount of hydroxyl radicals and cause lipid peroxidation[13]. Treatment with CGL and CGM significantly decreased the LPO and SOD activity when compared to the aspirin control group, which shows the antioxidant property of the drug. However, no significant change was observed in CAT activity.

Conclusion

On the basis of the results, it appears that ***C. grandis*** leaves powder and methanol extract possess antiulcerogenic principles, which stimulates gastric mucus secretion and has antioxidant activity. However, further investigations are required to isolate the active principles of this plant drug to elucidate the exact mechanism (s) of antiulcerogenic activity.

Acknowledgement

The authors are thankful to the Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, for providing the necessary facilities during the course of this research work.

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