

ANTI ULCER ACTIVITY OF ETHANOLIC, AQUEOUS AND TOTAL AQUEOUS EXTRACTS OF *COCCINIA GRANDIS* LINN. VOIGT IN PYLORIC LIGATURE INDUCED ULCERS IN ALBINO RATS

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ABSTRACT

Objectives: *Coccinia grandis* Linn, otherwise called *Coccinia indica* has been used in ayurvedic medicine in India and Sri Lanka to treat diabetes. As there is no indication of publications regarding the anti ulcer property of the plant, the present study was designed to investigate the antiulcer potential of ethanolic, aqueous and total aqueous extracts of *Coccinia grandis* Linn.

Methods: Ulcer was induced by pylorus ligation in Wistar albino rats. Drugs were administered in two different dose levels (200mg/Kg bwt, 400mg/ Kg bwt).

Results: Though all three extracts of *Coccinia grandis*, dose dependently reduced, the total acidity, ulcer index, and increased pH of gastric juice, ethanol extracts exhibited markedly significant results. However, ethanol extract has shown (78.57%) a highly significant ulcer curative potential and decreased ulcer formation also. A preliminary phytochemical analysis revealed the presence of different phytoconstituents such as alkaloids, carbohydrate, glycosides, phyto sterol, saponins, volatile oil, tannins etc which may impart their anti ulcer activity by acting as anti secretory and cytoprotective agents.

Conclusion: The present result suggests that both anti-secretory and cytoprotective mechanisms of different extracts of *Coccinia grandis* exerted protective effect. However further studies are required to propose the mechanism of action of the extracts for their human use.

Keywords: Antiulcer activity, *Coccinia grandis*, Pylorus ligation, Antisecretory, Cytoprotective.

INTRODUCTION

Gastric ulcer is one of the most prevalent gastrointestinal disorders, affecting approximately 5-10% of people during their life [1]. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach [2]. Herbal medicine is fast emerging treatment as an alternative to available synthetic drugs for treatment of ulcer, possibly due to lower costs and reduced side effects. Various chemical compounds have been isolated from medicinal plants with anti ulcer activity [3-4]. Fruits of *Coccinia grandis* Linn. (Cucurbitaceae) is commonly used as a vegetable throughout India especially in southern parts. Apart from their use as vegetable, leaves of the plant are reported to possess medicinal values such as anti convulsant anti pyretic, anti-inflammatory, anti helminthic, digestive, liver tonic, depurative and expectorant[5-7]. The present study assesses the effect of ethanolic, aqueous and crude aqueous extracts of leaves of *Coccinia grandis* Linn. on pyloric ligation induced gastric ulcers in rats to determine its effect on gastric secretion, and development of gastric ulcers in rats.

MATERIALS AND METHODS

Plant material

The leaves of *Coccinia grandis* Linn. were collected from Sivakasi, Virudhunganar district, Tamil Nadu and was authenticated by Mrs. M. Shanthi, Botanist, Department of Botany, S.F.R. College of Arts and Science, Sivakasi. The collected leaves were washed with tap water followed by distilled water to remove adhering dust, shade dried, and size reduced into small pieces. Dried materials were coarsely powdered and macerated with alcohol and aqueous solvents for 72 hours. The total aqueous extract was also prepared separately by cold maceration method [8]. The final product was filtered dried and the percentage yield was calculated.

Animals

Albino Wistar rats of either sex weighing between 150-250g were used. Animals were housed under standard conditions of temperature (24± 2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were given standard diet supplied by Kamadhenu agencies, Bangalore and water *ad libitum*. All

procedures involving animals were carried out under the institutional animal ethical committee approval (SBCP/ 2009-2010/ IAEC/ CPSCA/ 13).

Toxicity studies

Toxicity studies of all the three extracts were carried out in Swiss albino mice weighing between 20-25 g. All three extracts were found to be safe till 2000 mg/kg Bwt.

Anti ulcer activity-Pyloric ligation induced ulcer

Animals were starved for 48hrs with free access to drinking water in individual cages with raised bottoms of wide wire mesh to avoid cannibalism and coprophagy [9]. Animals were divided into 8 groups of six animals each. Group I served as solvent control and group II was administered with Omeprazole 2 mg/kg *p.o*. The remaining six groups were treated with ethanolic, aqueous and total aqueous extracts of *Coccinia grandis* at 200 and 400mg/kg *p.o*. respectively. Sixty minutes after the administration of drug or vehicle, a midline abdominal incision was made just below the xiphoid process, under ether anaesthesia. The stomach was lifted and the pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The abdominal wall was closed by interrupted sutures.

The animals were deprived of food and water post operatively and were sacrificed after 19 hours of pyloric ligation. Blood samples were withdrawn from the marginal tail vein and subjected to estimation of serum alkaline phosphatase and serum calcium. The stomach were dissected out along the greater curvature and examined for lesions and the gastric contents were collected. The mucosa was then washed and extent of ulceration was scored as per the method suggested by Kunchandy *et al* [10].

According to the following scale 0 = normal grey coloured stomach, 0.5 = pink to red coloration of the stomach, 1 = spot ulcer, 1.5 = haemorrhagic streak, 2 = number of ulcer < 5, 3 = number of ulcer > 5, 4 = ulcer with bleeding.

Ulcer index was calculated using formula, ulcer index = 10/X

$$\text{Where X} = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Estimation of gastric volume and pH

The gastric juice collected from each stomach was centrifuged at 100 rpm for 10 min and the gastric volume was measured. The supernatant was then collected and pH was determined in gastric juice [11].

Estimation of total acidity

Free and total acidity were estimated titrimetrically with 0.1 N NaOH using Topfer's reagent and Phenolphthalein as indicator. Acidity of gastric juice was expressed as mEq/l [12].

Statistic analysis

Mean values \pm S.E.M. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analysed separately and one-way analysis of variance (ANOVA) was carried out followed by Dunnet's t test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The colour for the ethanolic, aqueous and crude aqueous extracts of *Coccinia grandis* Linn. were dark green, brown and light brown respectively and the consistency of all three extracts were sticky. The percentage yield of the extracts found to be 13.64, 18.38 and 19.12% w/w respectively.

Pylorus ligation induces gastric ulcers due to accumulation of gastric secretion in the stomach [13]. The ethanolic extract of *Coccinia grandis* significantly decreased the total acidity and free acidity and increased the mucus content demonstrating that its gastric cytoprotective effects.

The higher doses of all three extracts of *Coccinia grandis* L. significantly reduced the free acidity and total acidity as the drug control group (Table 1). Even though the aqueous extracts could reduce the gastric acidity, they were less potent when compared to ethanol extract. However all three extracts at both dose levels and the standard drug could produce a significant increase in the pH when compared to the control group.

It is well known that gastric cytoprotective property alone in rats does not produce significant ulcer healing in humans [14]. The present study also evaluated the effect of *Coccinia grandis* L. leaves on gastric ulcer healing and gastric secretion, apart from the gastric cytoprotective effect. All three extracts reduced the gastric volume significantly when compared to the control group in a dose dependant manner (Table 1). However higher concentration of ethanol extract was nearly equipotent to Omeprazole in the reduction of gastric volume. The results of this study demonstrate the anti secretory effect of *Coccinia grandis* L. leaves.

Table 1: Effect of various extracts of *Coccinia grandis* Linn. on the volume of gastric secretion, pH, free acidity, total acidity, ulcer index, and ulcer curative potential on pylorus ligated albino rats

Group no	Treatment	Dose mg/kg	Volume of Gastric Juice (ml)	pH	Free Acidity meq/litre	Total Acidity meq/litre	Ulcer Index	% Ulcer Curative Ratio
I	Solvent control-Saline	10ml/kg	8.5 \pm 0.1290	2.76 \pm 0.0697	46.25 \pm 0.1707	83.3 \pm 0.4301	3.5 \pm 0.288	-
II	Omeprazole	2mg/kg	5.5 \pm 0.1290	5.75 \pm 0.0188*	39.75 \pm 0.1707****	60.19 \pm 0.0774****	0.5 \pm 0.2041****	85.70%
III	Ethanolic Extract	400mg/kg	5.7 \pm 0.1081	5.61 \pm 0.0341****	40.85 \pm 0.1707****	62.80 \pm 0.0412****	0.75 \pm 0.1443****	78.57%
IV	Aqueous extract	400mg/kg	6.5 \pm 0.1290	5.34 \pm 0.0818****	42 \pm 0.4082****	67.57 \pm 0.1391****	1.25 \pm 0.322****	64.28%
V	Total aqueous extract	400mg/kg	6.0 \pm 0.1825	5.12 \pm 0.0360****	41.65 \pm 0.2629****	65.48 \pm 0.0678****	1.0 \pm 0.2041****	71.40%
VI	Ethanolic extract	200mg/kg	6.7 \pm 0.1290	5.14 \pm 0.0420****	43.4 \pm 0.1826****	70.54 \pm 0.2102****	1.5 \pm 0.288****	57.14%
VII	Aqueous extract	200mg/kg	7.3 \pm 0.1290	4.97 \pm 0.3109****	44.75 \pm 0.25****	76.70 \pm 0.3824*	2.2 \pm 0.324****	37.14%
VIII	Total aqueous extract	200mg/kg	6.9 \pm 0.1290	5.12 \pm 0.0360****	44.1 \pm 0.2236**	73.8 \pm 0.4339***	1.75 \pm 0.1443****	50%

a. Values are expressed as Mean \pm S.E.M.

b. One way ANOVA followed by Dunnet's t - test

c. **** $p < 0.001$, *** $p < 0.01$, ** $p < 0.02$, * $p < 0.05$ as compared to control group

All extracts significantly reduced the ulcer index when compared to the control in a dose dependant manner. Ethanol extract exhibited highest ulcer curative ratio next to Omeprazole, whereas aqueous extracts could not contribute much in curing of ulcer in

the present study. The results of the present study suggests that ulcer healing effect of *Coccinia grandis* L. leaves observed in rats may be due to both gastric anti secretory and gastric cytoprotective effects.

Table 2: Phytochemical constituents of various extracts of *Coccinia grandis* Linn. Leaves

S. No.	Phyto constituents	Ethanolic extract	Aqueous extract	Total aqueous extract
1	Alkaloids	+	+	+
2	Carbohydrate	+	+	+
3	Glycosides	+	+	+
4	Phyto sterol	+	+	+
5	Fixed oil & fats	-	-	-
6	Saponins	+	+	+
7	Phenolic compounds	-	-	-
8	Proteins and Amino acids	+	+	+
9	Gums & mucilage	-	-	-
10	Volatile oils	-	-	-
11	Tannins	+	+	+
12	Tri terpenoids	+	+	+
13	Flavonoids	+	-	-

However, ethanol extract has shown (78.57%) a highly significant ulcer curative potential, decrease in ulcer formation, and decrease in gastric secretion, total acidity and free acidity and increase in pH of the stomach. In a conclusion, this study provides evidence that the ethanolic extract of *Coccinia grandis* L. leaves possesses an anti gastric ulcer effect, which is related to a preservation of gastric mucus secretion, reduction in ulcer formation, gastric acid secretion, gastric pH, total acidity and free acidity and an increased ulcer curative potential. A preliminary phytochemical analysis performed in our laboratory revealed the presence of different phytoconstituents in the extracts of *Coccinia grandis* L. leaves (Table 2), which may contribute the anti-ulcerogenic activity of the plant in rats. However further investigation is mandatory to isolate the active principles and to elucidate the exact mechanism of anti-ulcer activity through the use of additional experimental models.

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