Anti-ulcer activity of *cassia auriculata* leaf extract

Mohammed Fazil Ahmed*, Hameed Thayyil, Rasheed Ahemad.S and Mohammed Ibrahim

[1]Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near Ramoji Film City, Nalgonda,(AP), INDIA-508284. & Center for Liver Research and Diagnostics, Deccan College of Medical Sciences and Allied Hospitals Kanchanbagh, Hyderabad, Andhra Pradesh, 500 058, India

ABSTRACT

The present study was carried out to evaluate the anti-ulcer activity of *cassia auriculata* leaf extract against pylorous ligation induced gastric ulcer. The methanolic leaf extract of cassia *auriculata* at dose of 300 mg/kg p.o. markedly decrease the incidence of ulcers in pyloric ligatied rats. In pyloric ligated rats, there was an increase in the gastric volume, free and total acidity and ulcerative index as compared to the control group. The methanolic leaf extract of *cassia auriculata* at dose of, 300 mg/kg showed significant reduction in the above parameters which was comparable to the standard drug famotidine(10 mg/kg). *Cassia auriculata* extract showed protection index 79.4 %, whereas standard drug famotidine showed protection index 90.7%.

Key words: *Cassia auriculata*, Methanolic extract, Anti-ulceractivity, Pylorous ligation induced gastric ulcer and Famotidine

INTRODUCTION

Peptic ulcer disease (PUD) is a spectrum of diseases consisting of gastritis, gastric ulcers, and duodenal ulcers.[1] It is known to occur when the endogenous defense mechanisms of the protective mucosal barrier have failed to sufficiently counteract the aggressive factors (hydrochloric acid, pepsin, and *Helicobacter pyroli*) and is characterized by gnawing or burning sensation in the abdomen.[2] These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.[3] Duodenal ulcers occurs more frequently (about 80% of PUDs) than gastric ulcers.

Medicinal herbs are significant source of pharmaceutical drug. Latest trends have shown increasing demand of phytodrugs and some medicinal herbs have been proven antiulcer activity .This paper describes the study of *Cassia auriculata* L. (Cesalpinaceae, common name: Tanner's Cassia) a common plant in Asia, has been widely used in traditional medicine as a cure for rheumatism, conjunctivitis and diabetes.[4] In addition, *Cassia auriculata* has been widely used in Ayurvedic medicine as 'Avarai Panchaga Choornam' and the main constituent of Kalpa herbal tea, has come under extensive study in the light of its antidiabetic effects. Recently had reported the antiperoxidative effect of *Cassia auriculata* flowers in streptozotocin diabetic rats.[5] The antidiabetic activity of aqueous extract of *C. auriculata* flowers has been documented previously.[6] The present study is an attempt to test the antiulcer activity of the *cassia auriculata* leaf extract.

Address for correspondence:
E-mail: mohdfazil_pharma@yahoo.co.in

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MATERIALS AND METHOD

Plant collection and identification

The basic plant material of *cassia auriculata* leaves used for the investigation was obtain from Mount Opera Garden, Near Ramoji Film City, Nalgonda dist,Andhra Pradesh, India. The plant can be identified and authenticated by department of Botany research office P. SUJATHA (Botanist), HOD, Bhavans New Science Degree College, Narayanaguda, Hyderabad.

Preparation of methanolic extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of *cassia auriculata* were used for extraction.

The shade dry coarsely leaves of *Cassia auriculata* were used for extraction with methanol. *Cassia auriculata* leaf powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with methanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration,

extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

EXPERIMENTAL PROTOCOL

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of NIZAM INSTITUTE OF PHARMACY, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registeration number. (1330/AC/10/CPCSEA)

Acute Oral Toxicity Studies

Cassia auriculata at the dose range of 100 mg-2000 mg/kg were administered orally to different group of rats comprising of ten rats in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of Litchfield and Wilcoxon.

ANTIULCER ACTIVITY

Experimental design for pyloric ligation induced gastric ulcer

Animals are divided into 4 groups, each comprising 6 rats.

Group I: Control group (without pyloric ligation).
Group II: Pyloric ligation for the induction of ulcers.
Group III: Pyloric ligated group + Extract (300 mg/kg) 1 hr before ligation
Group IV: Pyloric ligated group + Standard drug (Famotidine 10 mg/kg)

Methanolic leaf extract (300 mg/kg) was administered for a period of 7 days. On the 7th day normal saline, famotidine and methanolic extact of leaf were administered 1hr prior to pyloric ligation. Animals were anaesthetized using diethyl ether and the abdomen was opened and pylorus was ligated without causing any damage to its blood vassels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures.[7,8] After 4hr of ligation, the animals were sacrificed by cervical dislocation. The abdomen was opened and a ligature was placed around the cardiac sphincter. The stomach was removed.[9] Gastric volume, free and total acid content of gastric juices were determined. Mean ulcer score for each animal was expressed as ulcerative index and the percentage ulcer protection was also calculated.

Estimation of gastric volume and free and total activity changes in pl model

Gastric volume

Four hours of ligation, stomachs were centrifuged and subjected to titration for estimation of free and total acid. One millimeter of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using topfer's reagent as indicator, to endpoint when the solution turned to orange colouyr. The volume of NaOH needed was taken as corresponding to free acidity. Titration was further continued by adding 1% solution of phenolphthalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to total acidity. The sum of two titrations was total acidity.[10] Acidity was expressed as:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{normality} \times 100\ \text{mEq/L}/100\ \text{g}}{0.1}$$

Estimation of gastric ulcerative index changes in PL and model:

Ulcerative index was measured by method of takagi et al,[11] Briefly, the stomach was opened along the greater curvature. The stomach was washed with running tap water. Then it was placed on a flat wooden plate to count the ulcerative area.

The ulcer index was determined using the formula:

$$\text{Ulcer index} = \frac{10}{X}$$

Where, X = total mucosal area/total ulcerated area
Percentage ulcer protection was calculated using the formula:

$$\text{Ulcer protection (\%)} = \frac{100 - Ut}{Uc \times 100}$$

Where:

Ut = Ulcer index of treated group

Uc = Ulcer index of control group

STATISTICAL ANALYSIS

All the biochemical results were expressed as mean ± standard error of means(SEM). Data were analysed by tukey's multiple range tests using sigma stat version-3.5 software. A probability value of $p < 0.05$ was considersd to be statistically significant.

RESULTS

Experimental Results

Phytochemical screening of *Cassia auriculata* .of leaf extract shows the presence of Alkaloids, Flavonoids, Tannins, Carbohydrate, Glycosides and Saponin .The acute oral toxicity study of *Cassia auriculata* showed no mortality upto 2000 mg/kg. (**Table 1).**The antiulcer activity of *Cassia auriculata* is shown in **Table 2 and Table 3.** In pyloric ligated rats, there was an increase in the gastric volume, free and total acidity and ulcerative index as compared to the control group, extract showed reduction in gastric secretion free and total acidity and ulcerative index, at dose of, 300 mg/kg.(**Table 2 and Table 3**) showed significant reduction in the above parameters which was comparable to the standard drug famotidine.

Table 1. Acute toxicity studies of *Cassia auriculata*

S.no	Group	Dose	Wt of animal (gm)		Sing of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before treatment (1st day)	after treatment (4th day)				
1	*Cassia auriculata*	2gm/kg	155	157	No Sign of toxicity	Nil	Nil	3 days
2	*Cassia auriculata*	2gm/kg	159	162	No Sign of toxicity	Nil	Nil	3 days
3	*Cassia auriculata*	2gm/kg	165	166	No Sign of toxicity	Nil	Nil	3 days
4	*Cassia auriculata*	2gm/kg	153	155	No Sign of toxicity	Nil	Nil	3 days
5	*Cassia auriculata*	2gm/kg	170	171	No Sign of toxicity	Nil	Nil	3 days
6	*Cassia auriculata*	2gm/kg	173	174	No Sign of toxicity	Nil	Nil	3 days

Table 2: Effect of extract on gastric secretion, free acidity, total acidity in pyloric ligated rats

Treatment	Gastric volume (mL/100 g)	Free acidity (mEq/100 g)	Total acidity (mEq/100 g)
Group I	1.2 ± 0.6	24.1 ± 0.94	60.2 ± 2.06
Group II	8.65 ± 0.2	63.5 ± 1.99	102.2 ± 1.38
Group III	3.5 ± 0.5	32.3 ± 1.42	73.6 ± 1.65
Group IV	2.4 ± 0.3	27.8 ± 1.44	61.7 ± 1.81

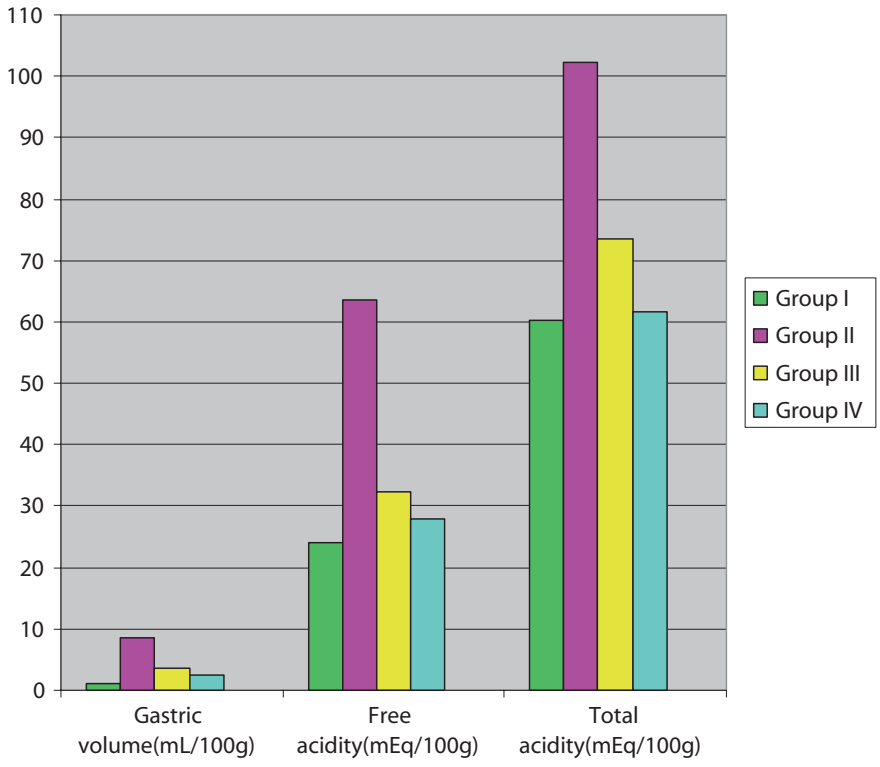
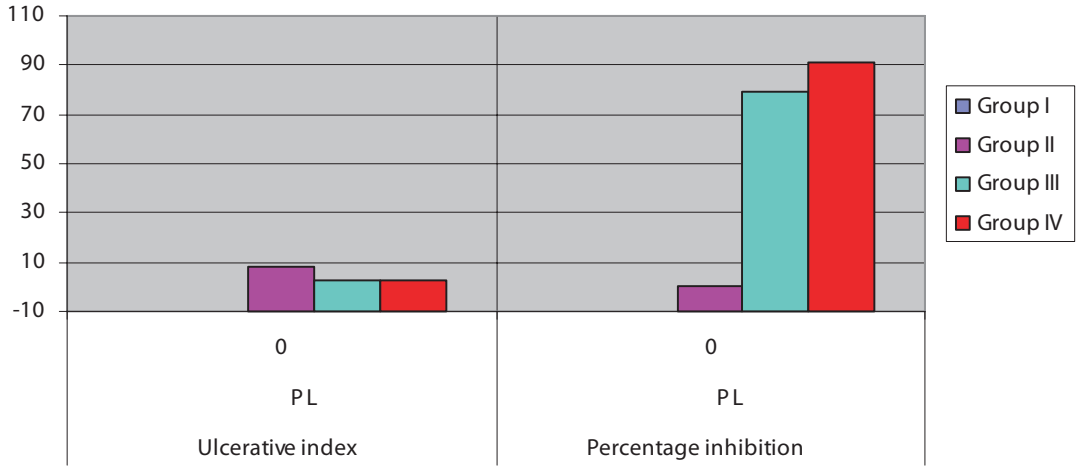


Table 3: Effect of MECS on ulcerative index and percentage inhibition in pl and wis induced gastric ulcer in rats

Groups (mg/kg)	Ulcerative index PL	Percentage inhibition PL
Group I	0.00 ± 0.00	0.0
Group II	8.1 ± 0.01	0.0
Group III	2.8 ± 0.01	79.4
Group IV	2.3 ± 0.01	90.7



DISCUSSION

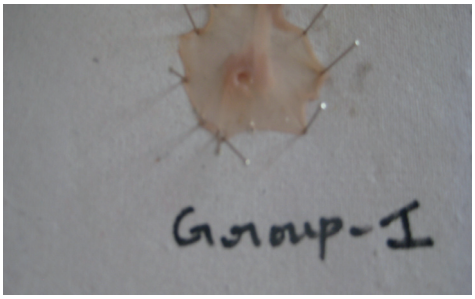
Various mechanisms are thought to be involved in the ulcer production in different experimental models.[12, 13] Hence it is not possible to produce a single mechanism for antiulcer effect of a particular drug. Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in pyloric ligated rats. In addition to gastric acid secretion, reflex neurogenic effect has also been suggested to important role in the formation of gastric ulcers in this model.[13, 14] Ulcer index parameter was used for the evaluation of anti ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity.[15]

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms.[16] The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin.[17] Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells.[18] To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilising the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.[19] The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach.

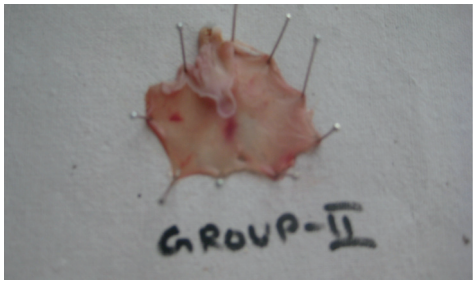
It is well known that free radicals are involved in the progression of ulcers. Pyloric ligated models increase in the oxidative and decrease in the antioxidative biomarkers have been reported.[20,21] Hence, as our results indicated maximum *in vitro* free radical scavenging activity along with ameliorative effect on various ulcerative parameters of *Cassia auriculata* extract so, this antioxidant potential may be responsible for its anti-ulcerogenic activity. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

CONCLUSION

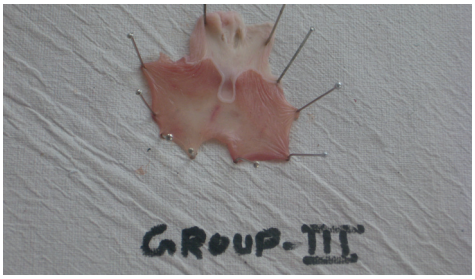
From the results obtained, it was observed that, the extract shows decrease in percent of incidence of ulcer and ulcer index in a dose dependent manner when compared with control group (Figure 1). After calculating the healing index (percent improvement), there was an increase in healing index in dose dependent manner when compared with



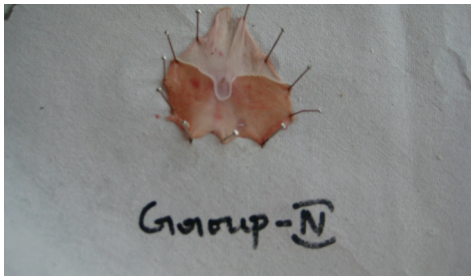
GROUP 1: Stomach of normal rat



GROUP II: Stomach of an ulcer control rat



GROUPIII: Stomach of a rat treated with 300 mg kg-1 **C. auriculataa** leaf methanolic extract



GROUP IV: Stomach of a rat treated with standerd drug famotidine (10 mg kg-1)

Figure 1

control group. So it was considered that the plant *cassia auriculata* has significantly decreased the no of ulcers in pyloric lygation induced gastric ulcers in rats. Thus the result from our study suggested that the plant *cassia auriculata* has the potent anti-ulcer property.

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