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### Phytochemical and antiulcer activity of *Cajanus cajan* leaves against pylorus ligation-induced gastric ulcer in rats

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ORIGINAL RESEARCH ARTICLE	ABSTRACT
<p>ARTICLE INFORMATION</p> <hr/> <p><i>Article history</i>            Received: 15 July 2015            Revised: 8 August 2015            Accepted: 10 August 2015            Early view: 13 August 2015</p> <hr/> <p>*Author for correspondence            E-mail: <a href="mailto:venku07177@gmail.com">venku07177@gmail.com</a></p> <hr/>  <p>Q R C o d e</p>	<p><b>Background:</b> The present study was designed to investigate the antiulcer potential of ethanolic leaves extract of <i>Cajanus cajan</i>.</p> <p><b>Material and methods:</b> Antiulcer activity was evaluated by pylorus ligation induced ulcer models in rats. The ethanolic extract of the leaves of <i>Cajanus cajan</i> was given by oral route at a dose of 250 and 500mg/kg b.w.</p> <p><b>Results:</b> Ethanolic extract of <i>Cajanus cajan</i>, dose dependently reduce, the total acidity, ulcer index, and an increase in pH of gastric juice in pylorus ligated ulcer model.</p> <p><b>Conclusion:</b> Extract of <i>Cajanus cajan</i> may be useful as a natural antioxidant in the treatment of ulcer.</p> <p><b>Keywords:</b> Antiulcer Activity, <i>Cajanus cajan</i>, Pylorus ligation ethanolic extract.</p>
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#### INTRODUCTION

Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity. There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune, gastrointestinal inflammation and gastric ulcer. "Peptic ulcer disease" refers to breaks in the mucosa at the stomach and small intestine, principally the proximal duodenum, which are produced by the action of gastric secretion. Peptic ulcer is one of the major gastro intestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. Consequently, reduction of gastric acid production as well as re-improvement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result drugs, of both herbal and synthetic origin are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitors to H<sub>2</sub> antagonist or a cytoprotective agent. Medicinal plants provide an

important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases (Wahida et al., 2007).

*Cajanus cajan* (L) Millsp. (Sanskrit: Adhaki, Hindi: Arhar, English: Pigeon pea, Bengali: Tur) (family: Fabaceae) is the most important grain legume crop of rain-fed agriculture in semi-arid tropics. It has been used widely for many years for treating diabetes, sores, skin irritations, hepatitis, measles, jaundice, dysentery and many other illnesses; for expelling bladder stones and stabilizing menstrual period (Yuan et al., 2010). *C. cajan* being a forage crop has been utilized as an important remedy for various ailments. The garo tribal community of Bangladesh utilizes it for the treatment diabetes and as an energy stimulant. In Trinidad and Tobago the leaves of *C. cajan* are used in food poisoning, as colic and in constipation (Lans, 2007). In Chinese folk medicine pigeon pea leaves are used to staunch blood, as an analgesic and to kill parasites. In some parts of Tamil Nadu, India, the leaf, seeds and young stems are used to cure gingivitis, stomatitis and as a toothbrush (Ganeshan, 2008). It is also an important folk medicine in eastern

Rajasthan as fresh juice/boiled leaves are given orally to nullify the effect of intoxication and as a laxative. Leaf paste is applied in oral ulcers and inflammations. Leaves and seeds are applied as poultice over the breast to induce lactation (Upadhyay et al., 2010). A new natural coumarin cajanus lactone has been isolated from the leaves of *C. cajan* which is a potential antibacterial agent against Gram-positive micro-organisms (Luo et al., 2010). The three stilbenes, cajanin, longistylin C and longistylin A from leaves have been found to possess hypocholesterolemic effects (Chen et al., 1985). Anti-plasmodial activities have also been confirmed in betulinic acid isolated from roots and longistylin A and C obtained from leaves (Ezike et al., 2010). Pinostrobin, a substituted flavanone isolated from leaves possesses anti-inflammatory activity and inhibits sodium channel-activated depolarization of mouse brain synaptoneuroosomes (Nicholson et al., 2010). Two isoflavanoids genistein and genistin isolated from the roots were found to possess antioxidant activity (Zhang et al., 2010). Cajanol an isoflavanone found in the roots is found to possess anticancer activity (Luo et al., 2010). Four important compounds, pinostrobin, cajaninstilbene acid, vitexin and orientin isolated from ethanolic extracts of leaves were found to possess significant antioxidant properties. Isoflavanoids isolated from ethanolic extract of leaves also showed significant antimicrobial activities. Some protein fraction isolated from leaves also showed hepato-protective effects (Ahsan et al., 2009) and the presence of phenolics (flavanoids and tannins) impart anthelmintic activity (Singh et al., 2010).

## MATERIAL AND METHODS

### Collection and authentication of plant material

The leaves of *Cajanus cajan* were collected in the month of May from the surrounding fields of Acharya N.G. Ranga Agricultural University, Rajendra Nagar, Hyderabad, India and Authenticated by Dr. A. Manohar Rao.

### Extract preparation

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with ethanol solvent and the residual marc was collected. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1). The extract was evaporated under reduced pressure using a rotovac evaporator at a low temperature (40-60 °C). Preliminary phytochemical analysis was carried out to identify presence of phytoconstituents in the crude extract.

### Phytochemical screening of ethanolic leaves extract of *Cajanus cajan* leaves

It is planned to carry out the preliminary phytochemical investigation for leaves extracts of *Cajanus cajan* for detection of various phytoconstituents and the tests is

done to find out the presence of common phytochemicals by following standard methods.

### Experimental animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house. 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 experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

### Pylorus ligation method induced gastric ulcer

Animals were divided into 5 groups, each comprised 6 rats.

Group I: Control

Group II: Pyloric ligation

Group III: Pyloric ligation + ethanolic leaves Extract of *Cajanus cajan* 250 mg/kg body weight

Group IV: Pyloric ligation + ethanolic leaves Extract of *Cajanus cajan* 500 mg/kg body weight

Group V: Pyloric ligation + Standard drug (Famotidine 10 mg/kg)

Group I was fed with normal saline for 7 days. Ethanolic leaves Extract of *Cajanus cajan* (250 mg/kg and 500 mg/kg) was administered for a period of 7 days (Group III and IV). Group V were fed with standard famotidine for 7 days. Group II, III, IV & V were induced ulcers by Pyloric Ligation. On the 7th day normal saline, Famotidine and Ethanolic leaves extracts 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 vessels. The stomach was replaced carefully and the abdomen wall was closed with interrupted sutures.

After 4hrs 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 (Khayyum et al., 2009). Gastric juice is collected and drains into test tubes and then centrifuged at 1000 rpm for 10 minutes and the volume noted. The pH of the gastric juice is recorded by pH meter. Then the contents are subject for the analysis of free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The number of ulcers per stomach is noted and severity of the ulcer scores microscopically with the help of hand lens (10x).

In this model the following parameters are plan to study.

- pH of gastric juice.
- Volume of gastric secretion
- Free acidity
- Total acidity
- Ulcer index

### Estimation of gastric volume and free and total acidity in pylorus ligation model

Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml 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 the endpoint when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued using 1% solution of phenolphthalein till the solution gained pink color. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100 \text{ mEq/l}}{0.1}$$

### Estimation of gastric ulcerative index changes in pylorus ligation model

Ulcerative index is measured by method of Takagi et al. (1969), briefly the stomach was opened along the greatest 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:

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

Where X = total mucosal area/total ulcerated area

Percentage ulcer protection was calculated using the formula:

$$\text{Ulcer protection (\%)} = 100 - \frac{U_t}{100 - U_c} \times 100$$

Where:  $U_t$  = Ulcer index of treated group

$U_c$  = Ulcer index of control group

**Table 1. Effect of ethanolic leaves extract of *Cajanus cajan* on gastric secretion, free acidity, total acidity in pyloric ligated rats**

Group	Treatment	Gastric Volume (mL/100g)	Free acidity (mEq/100g)	Total acidity (mEq/100g)
Group I	Control	0.95±0.54 ml	16.2 ± 1.42	38.1 ± 2.74
Group II	Pylorus ligated	4.98±0.82 ml	41.2 ± 2.82	98.1 ± 2.62
Group III	Ehanolic extract of <i>Cajanus cajan</i> 250mg/kg + Pylorus ligated	3.85±0.6 ml	34.2 ± 1.44	74.2 ± 3.62
Group III	Ehanolic extract <i>Cajanus cajan</i> 500mg/kg + Pylorus ligated	2.05±0.7 ml	22.3 ± 1.28	48.2 ± 4.62
Group IV	Pylorus ligated + Standard famotidine 10mg/kg)	1.20±0.8 ml	19.2 ± 2.68	42.5± 4.52

Table 2: Shows the effect of ethanolic leaves extract on ulcer index and percentage protection. The Ulcer Index of ethanolic leaves extract 250 mg/kg and 500 mg/kg were significantly reduced when compared to pylorus ligated rats. Ulcer protective action at a dose of 500 mg/kg of ethanolic leaves extract of *Cajanus cajan* was found to be closer to the reference drug famotidine (10 mg/kg) respectively.

### Histopathology

Tissue samples from the stomach of rats in each group (I - V) of the experiment were fixed in 10% formalin saline for a minimum of 24 h and then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with a microtome, stained with hematoxyline and Eosin and mounted on Canada balsam. All sections were examined under light microscope (x10, x20 and x40) magnification.

### Statistical analysis

All the biochemical results were expressed as mean ± standard error of means (SEM). Data were analyzed by turkey's range tests using Sigma stat version-3.5 software. A probability value of  $P < 0.05$  was considered to be statistically significant.

### RESULTS

Phytochemical screening of saponins, flavanoids, phenols, tanins etc in *Cajanus cajan* leaves.

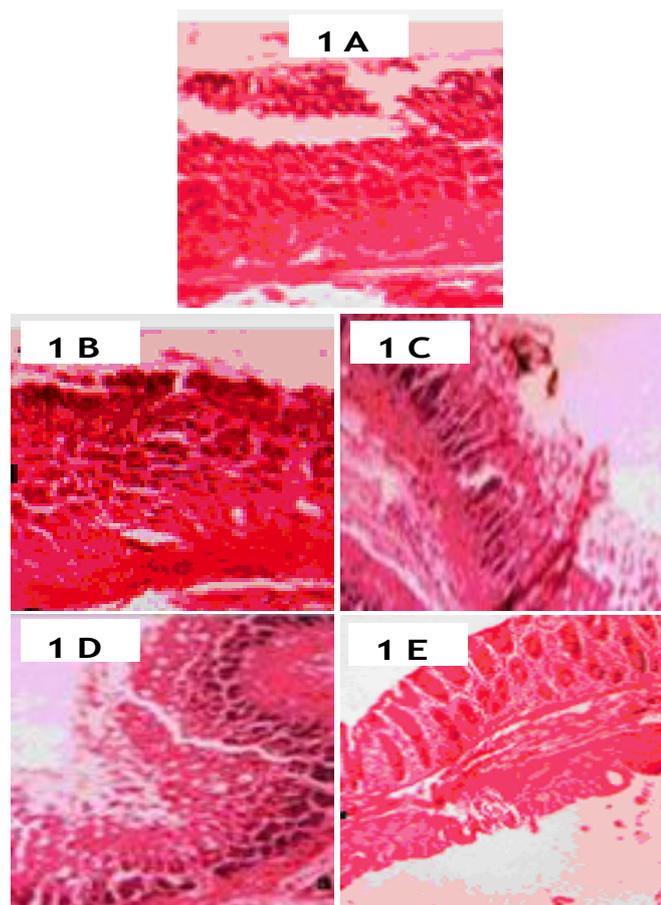
Table 1: Shows the effect of ethanolic leaves extract on gastric secretion, free acidity, total acidity in pyloric ligated rats. The volume of Gastric juice secretion was significantly reduced with a dose 250 mg/kg and 500 mg/kg of ethanolic leaves extract of *Cajanus cajan* when compared to pylorus ligated rats.

The effect of control, pylorus ligated rats, ethanolic leaves extracts (250 mg/kg and 500 mg/kg) and standard drug famotidine (10 mg/kg) on volume of gastric juice secretions were 0.95, 4.98, 3.85, 2.05 and 1.20 ml respectively. Gastric volume, free and total acidity of ethanolic leaves extract of *Cajanus cajan* at a dose of 500 mg/kg was significantly reduced when compared to the pylorus ligated rats.

**Table 2. Effect of ethanolic leaves extract of *Cajanus cajan* on ulcer index and percentage protection.**

Treatment Groups	Ulcer index	% Protection
Group I	0.00±0.00	0.00
Group II	6.26±0.45	0.00
Group III	4.56±0.23	65.28
Group IV	2.52±0.51	90.04
Group V	1.8±0.06	97.40

## Histopathology of rat stomach



**Figure 1.** (1A) Control; (1B) Pylorus ligated; (1C) Pylorus ligated + extract 250mg/kg; (1D) Pylorus ligated + extract 500 mg/kg; (1E) Pylorus ligated + Standard (famotidine 12mg/kg).

Group I: shows normal microscopic structure of rat stomach, Group II: Pylorus ligated rats showing severe ulcer lesions, Group III: Shows acute desquamation of the surface epithelium, Group IV: Shows mild ulcer and Group V: Shows rat stomach fairly protected with famotidine.

## DISCUSSION

Plant extracts are some of the most attractive sources of new drugs, and have shown promising results for the treatment of gastric ulcers in several experimental models. The *Cajanus cajan* is used for various gastrointestinal diseases in the folk medicine. Since, this plant has been reported to contain flavonoids, tannins, carbohydrates, where flavonoids and tannins have shown potent anti ulcer activity (Mohanty et al., 2011), which are known to be altered in this intestinal condition. The preliminary phytochemical investigation of *Cajanus cajan* leaves showed the presence of tannins and flavonoids.

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanisms (Bhave et al., 2006). The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin (Gordan et al., 1990).

Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the gastric mucosal barrier. In case of pyloric ligation, ulcer

formation is mainly due to the stasis at the gastric juice and stress (George et al., 1999).

Peptic ulcer results due to overproduction of gastric acid (or) decrease in gastric mucosal production. Pylorus ligation induced ulcers occur because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion. In folk medicine, *Cajanus cajan* Linn is used for the various gastrointestinal diseases. The present study reveals that ethanolic leaves extract of *Cajanus cajan* extract treated groups showed a significant increase in gastric juice, reduces the gastric volume, free acidity and total acidity when compared to pyloric ligated group. *Cajanus cajan* extract decreased the ulcer index more effectively in a dose dependent manner.

From the results it has been revealed that pretreatment with 250 mg/kg and 500 mg/kg dose of ethanolic leaves extracts of *Cajanus cajan* had significantly produced Anti ulcer properties.

## CONCLUSION

In the present study ethanolic leaves extract of *Cajanus cajan* shows significant antiulcer activity. On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Cajanus cajan* leaves extract could be mainly due to its gastroprotective effect. The ethanolic extracts also possess free radical scavenging property on endogenous PGs. The ethanolic extract of *Cajanus cajan* leaves possess Anti ulcer activity could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

## CONFLICT OF INTEREST

None declared.

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