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Pharmacological Evaluation of Anti-ulcer Activity of *Caesalpinia crista* in Rats

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ABSTRACT

In present study, we evaluated the antiulcer activity of the herbal preparation of *Caesalpinia crista* in rat models. Experimental animals were divided into four groups. Rats of group I (disease control) treated with normal saline only, group II (standard group) treated with Omeprazole (2 mg/ kg; p.o.), group III and IV served as test groups and were treated with *Caesalpinia crista* extract (CE) in the dose of 250 mg/ kg and 500 mg/ kg orally respectively. Peptic ulcer was induced by ligating the pyloric portion of rat stomach and was done 45 min after the respective treatment. After 4 hour of pylorus ligation, rats were sacrificed. Parameters like ulcer index, percent ulcer protection, total and free acidity were estimated for evaluation of anti-ulcer activity. Histopathological evaluation was also performed. The aqueous extract of *Caesalpinia crista* seeds reduced the volume of gastric juice, free acidity, total acidity and ulcer index. It increased the pH of the gastric acid. Histopathology of the rat stomach revealed the presence of lesions and infiltration of inflammatory cells in control group. Moreover, animals treated with test drug and standard drug did not reveal any microscopic lesions. These findings suggest that *Caesalpinia crista* seeds may have anti-secretory and anti-ulcer activity and may be helpful for ulcer therapy.

KEYWORDS: Peptic ulcer; Pylorus ligation; Omeprazole; C Crista; Rat.

Introduction

Peptic ulcer disease (PUD) is the most prevalent gastrointestinal disorder (Dharmani and Patil, 2006). Peptic ulcer disease is broadly refers to a group of disorders characterized by the presence of ulcer in any portion of the gastro intestinal tract exposed to acid in sufficient duration and concentration (Herfindal et al., 2006).Ulcers may occur anywhere in the alimentary canal, none are as prevalent as the peptic ulcers that occur in the duodenum and stomach. The causes of PUD involves various factors like *H.pylori* infection, NSAIDs, hypersecretory condition, stress related erosive syndrome and other factors (Herfindal et al., 2006). Pathophysiology of peptic ulcer disease involves an imbalance between the gastro duodenal mucosal defense and countervailing aggressive forces that overcome such defenses (Longo et al., 2005).

Currently available treatments for peptic ulcers include antacids (systemic and nonsystemic) and drugs which reduce acid secretion such as H2 anti-histaminic, proton pump inhibitors, anticholinergics, prostaglandin analogues, ulcer protective, ulcer healing drugs and drugs for H. pylori infection. These drugs are reported to decrease the morbidity rates, but produce many adverse effects. The major adverse effect is relapse of the disease, and such treatment is often expensive for the poor people (Panda and Sonkamble, 2012). H_2 receptor antagonists, Cimetidine produce side effects like, galactorrhea, reduced sperm count and gynecomastia. While proton pump inhibitors increase the risk of carcinoid tumours and increase the concentration of viable bacteria in stomach (Howland and Mycek, 2006).

In light of the above, it is pertinent to study natural products from food/plants as effective anti-ulcer compounds. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care because of the less side effect of herbal drugs as compared to the synthetic drugs (Tripathi et al., 2010).Various herbal plants like *Alstoriascholaris, Annonasquqmoza, Asparagus racemosus willed, Ficusarnottiana, Butea frondosa and Carica papaya L.* possess ulcer healing as well as anti-ulcerogenic properties (Gadekar et al., 2010).Various marketed herbal formulations are available for treating peptic ulcer, including Tricid (Trio HealthcarePvt. Ltd.) and Lucer (Tonix Healthcare Pvt. Ltd.) which are claimed to cool or inhibit the acidity (Shah and Patel, 2012).

Marketed herbal formulations include number of the herbal drugs that affect the costing of the formulation due to unnecessary addition of the plants. *Caesalpinia crista* belonging to the family Caesalpiniaceae can be used in peptic ulcer as it was reported traditionally (Mndal et al., 2009). The anti -ulcer activity was not proven yet. The present study was carried out to evaluate the antiulcer activity of *Caesalpinia crista* Linn. in rats. Phytochemical analysis of *Caesalpinia crista* seeds showed the presence of tannins flavonoids,

glycosides, alkaloids, saponins and triterpenoids (Gaur et al., 2008). Traditionally, in Ayurveda, this plant was used for the treatment of gynaecological disorders, skin disease, constipation and piles. *Caesalpinia crista* showed anti-malarial (Kalauni et al., 2006), anticancer (Bodakhe et al., 2011), cytotoxic activity (Billah at al., 2013) and analgesic and anti-inflammatory activity (Mahfoozurrahman et al., 2012). *Caesalpinia crista* seeds exhibited wound healing (Patil, 2005) and in vitro anti-oxidant activity (Jaykrishnan et al., 2014) and anti-diabetic activity (Gupta et al., 2013).

In the present, we evaluated the potential antiulcer activity of the herbal preparation of *Caesalpinia crista* in experimal rat models.

Materials and Methods

Plant Material

The seeds of Caesalpinia crista were collected from Gandhinagar. Plant was authenticated by D.B. Patel, Botanist at Anand Agriculture University, Anand, Gujarat, India.

Extraction Procedure

Seeds of *Caesalpinia crista* were cleaned and dried. They were milled into powder by a mechanical grinder and were extracted with distilled water by using cold maceration process. It was shaked frequently at specific interval. At the end of three days, extract was filtered and concentrated. The final filtrate is *Caesalpinia crista* seed extract (CE) (Sabrin et al., 2011).

Phytochemical Screening

The phytochemical tests were done for preliminary screening of the *Caesalpinia crista* seed extract (Kokate et al., 1990) (Table 1).

TABLE 1

Phytochemical test for preliminary screening of C.crista extract.

Constituent	Tests
Tannin	I. Ferric chloride test II. Gelatin test
Triterpinoids	I. Libermann- Burchard test II. Salkowski test
Flavanoids	I. Shinoda test II. Alkaline reagent test
Alkaloids	I. Dragendroff's test II. Hager's test III. Mayer's test IV. Wagner's test
Carbohydrates	I. Molish test II. Barfoed test III. Benedict test
Proteins	I. Millon's test II. Biuret test
Glycosides	I. Baljet test II. Keller-killiani test

Animals

Male wistar rats of body weight range between 150-250g were used for induction of Peptic ulcer. All animals were housed in cages (6 in each cage) and maintained under controlled room temperature (20-25 ± 2º C) and relative humidity (50± 15%) with 12 h light / 12 h darkness (day / night) cycle. All the rats were fed with commercially available normal pellet diet and water ad libitum. All rats used in this study were allowed to adapt to the housing conditions for one week prior to the commencement of the study. Experiment was conducted according to the CPCSEA guidelines and the study was approved by the Institutional Animal Ethics Committee (SPCP/IAEC/RP-01/2014)

Chemicals

The standard drug Omeprazole was procured from Zydus cadilla, Ahmadabad, Gujarat, India. All the reagents and chemicals used in the study were of analytical grade and were procured from the Chemdyes Corporation, Rajkot.

Induction of Peptic Ulcer by Pylorus Ligated Rat Model

After the period of acclimatization, rats were divided into five groups containing 6 animals in each group. Group I and group II animals were received vehicle and served as a normal and disease control group in which pylorus ligation was carried out respectively. Rats in group III administered with standard drug Omeprazole (2 mg/kg; p.o.) and served as a standard group. Animals of group IV and V were treated with the CE in the dose of 250 and 500 mg/kg respectively and served as a Test group- I and Test group- II respectively (Table 2).

TABLE 2

Experimental design of pylorus ligated rat model.

Groups	Treatment
Normal control (NC)	Distilled water
Disease control (DC)	Distilled water + Pylorus Ligation
Standard group (STD)	Omeprazole (2 mg/kg; p.o.) + Pylorus Ligation
Test group I	CE (250 mg/kg; p.o.) + Pylorus Ligation
Test group II	CE (500 mg/kg; p.o.) + Pylorus Ligation

Induction Procedure

All animals were fasted for the 48 hour before the pylorus ligation. During fasting condition, there was free access to water. After 48 hour, Gastric ulcer was induced by ligating the pyloric portion of the stomach of the rat. All animals were anaesthetised with ether anaesthesia and pylorus ligation was done 1 hour after the respective treatment except normal control. For surgery, biodegradable thread and for suturing needle no. 14 was used. Four hour after the pylorus ligation the animals were sacrificed and stomach removed. The gastric content was collected by dissecting the greater curvature of stomach, centrifuged and supernatant was measured (Agrawal et al., 2010).

Assessed Parameters

Volume of gastric juice (mL)

The stomach was removed from the sacrificed animal and the gastric juice was collected by opening the greater

curvature of the stomach. The volume of the collected juice was measured (Reddy et al., 2012).

pH of gastric acid

An aliquot of 1mL gastric juice was diluted with 1 mL of distilled water and pH of the solution was measured using pH meter (Toshcon industries Pvt. Ltd. Hardwar; 220 V, 50 Hz) (Reddy et al., 2012).

Determination of total acidity

An aliquot of 1mL gastric juice was taken into a 50 mL conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as meq/L by the following formula:

$$n \times 0.01 \times 36.45 \times 1000$$

Where,

n is volume of NaOH consumed, 36.45 is molecular weight of NaOH, 0.01 is normality of NaOH, 1000 is the factor (to be represented in litre) (Reddy et al., 2012).

Determination of free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity (Reddy et al., 2012).

Ulcer score and ulcer index *(Reddy et al; 2012)*

The scoring system was used for indicating the severity of disease condition. (Table 3)

TABLE 3

Scoring of ulcer in pylorus ligated rat model.

Parameter	Ulcer score
Normal stomach	0
Spot ulcer	0.5
Superficial ulcer	1
Deep ulcer	2
Perforation	3

Ulcer index

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where,

U_N = average no. of ulcers per animal

U_S = average of severity score

U_P = % of animals with ulcer.

Histopathology of stomach

The stomach was fixed in 10% aqueous buffered formaldehyde for the storage purpose and for histopathology paraffin - embedded sections were stained with haematoxylin and eosin (Jainu et al., 2006)

Statistical Analysis

The observations in various groups were expressed as mean (+/-) SEM. The ulcer score an index of various groups was compared with disease control group. The group comparison was analyzed by using one-way ANOVA test followed by Dunnett's post hoc test. The difference of mean was considered significant at $p<0.05$.

Results

Phytochemical Screening

The aqueous extract of *Caesalpinia crista* was subjected to phytochemical investigation, the results revealed the presence of Glycosides, saponins, Carbohydrates, Alkaloids, Flavanoids, Tanins and Terpenes. The same was summarised in Table 4.

TABLE 4

Preliminary phytochemical screening of aqueous extract of *C.crista* seeds.

S. No.	Test	Inference
1	Glycoside	+
2	Saponins	+
3	Carbohydrates	+
4	Alkaloids	+
5	Flavanoids	+
6	Tannins	+
7	Terpenes	+

Volume of Gastric Juice and pH of Gastric Acid

Animals from the standard group showed significant reduction in the volume of gastric juice and significant increase in the pH of gastric acid as compared to the disease control group. Test group I and Test group II animals showed significant reduction in the volume of gastric juice and significant increase the pH of acid as compared to the disease control group (Table 5).

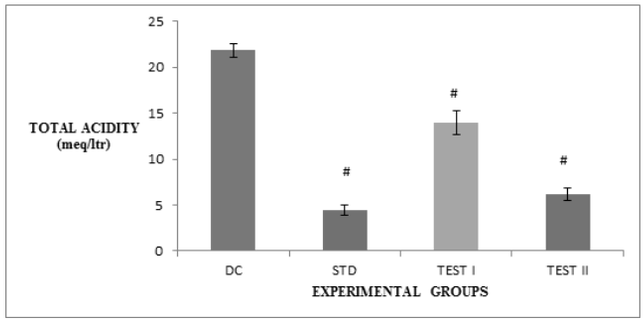
TABLE 5

Effect of CE on volume and pH of gastric juice.

Groups	Volume of gastric juice (ml)	pH of gastric acid
DC	6.317 ± 0.157	2.43 ± 0.13
STD	2.217 ± 0.124#	6.26 ± 0.11#
TEST I	4.267 ± 0.158#	3.2 ± 0.085#
TEST II	2.683 ± 0.094#	5.56 ± 0.088#

Total and Free Acidity

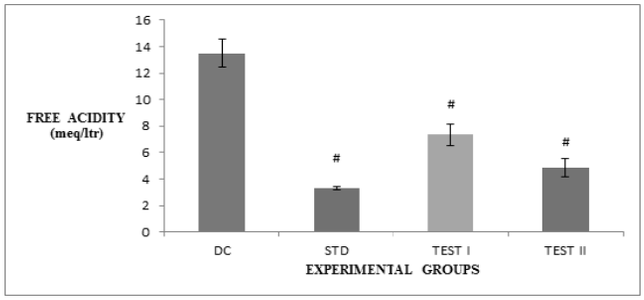
The standard group, Test group I and Test group II animals showed significant reduction in the Total and Free acidity as compared to the Disease control animals (Figure 1 and 2).



DC = Disease control; STD = Standard; Test I = Test group – I; Test II = Test group- II

All values are expressed as a Mean ± S.E.M (n=6).Statistical analysis was done by one way ANOVA followed by *dunnett's post hoc test.* # ($P< 0.05$), as compared to DC Group.

Fig. 1. Effect of *Caesalpinia crista* seed extract on Total acidity in pylorus ligated rat model.



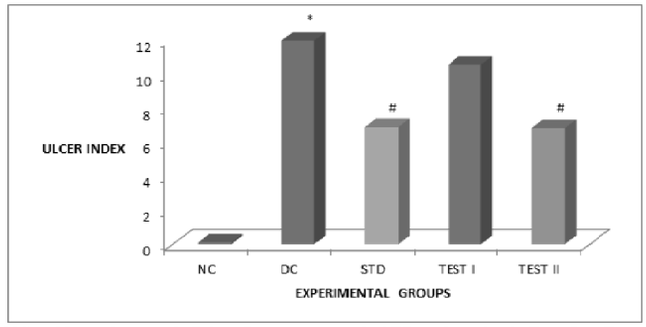
DC = Disease control; STD = Standard; Test I = Test group – I; Test II = Test group- II

All values are expressed as a Mean ± S.E.M (n=6). Statistical analysis was done by one way ANOVA followed by *dunnett's post hoc test.* # (P< 0.05), as compared to DC Group.

Fig. 2. Effect of *Caesalpinia crista* seed extract on free acidity in pylorus ligated rat model.

Ulcer index

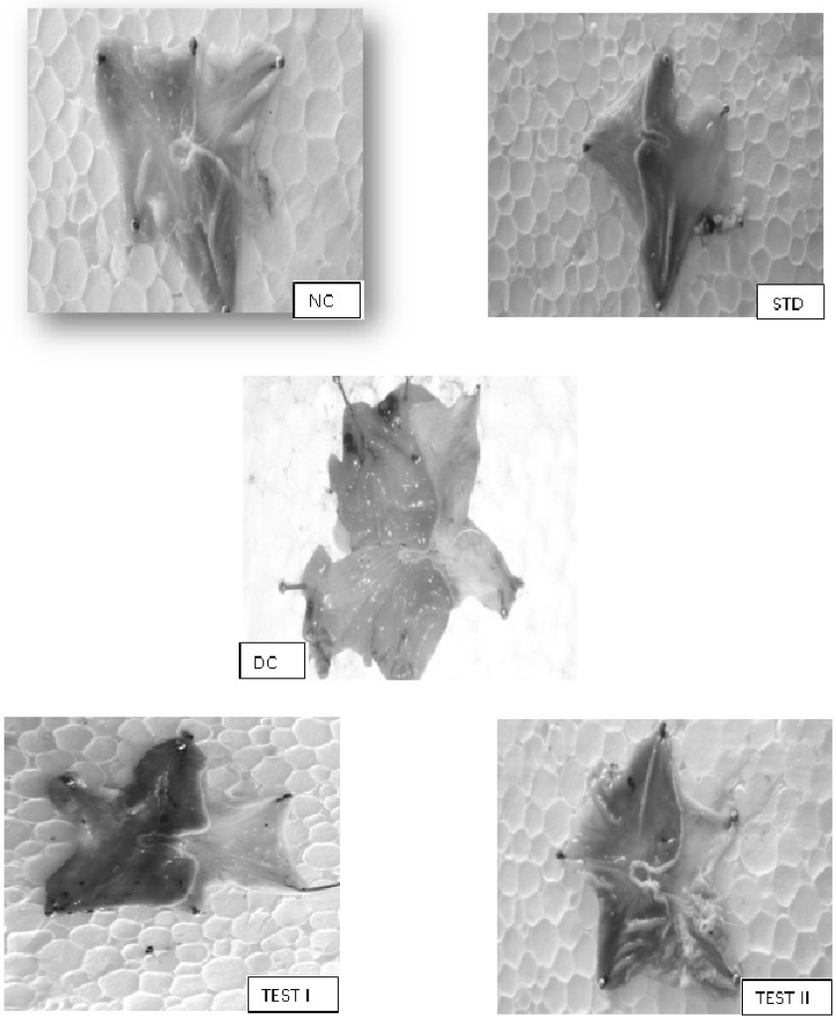
The disease control group animals showed significant reduction in the Ulcer index as compared to the Normal control group. Animals from the Standard group and Test group II showed significant reduction in the ulcer index as compared to the disease control group animals. While the animals from the Test group I did not show significant reduction in the ulcer index as compared to the disease control group animals (Figure 3 and 4).



NC = Normal control; DC = Disease control; STD = Standard; Test I = Test group –I; Test II= Test group- II

All values are expressed as a Mean ± S.E.M (n=6). Statistical analysis was done by one way ANOVA followed by *dunnett's post hoc test.* # (P< 0.05), as compared to DC Group.

Fig. 3. Effect of *Caesalpinia crista* seed extract on Ulcer index in pylorus ligated rat model.



NC- Normal control; DC- Disease control; STD- Standard

Fig. 4. Figure of stomach for ulcer examination.

Histopathology of the stomach

The disease control group showed focal haemorrhage and infiltration of inflammatory cells in the sub mucosa. Rest all groups did not reveal any microscopic lesions (Table 6; Figure 5).

TABLE 6

Histopathological evaluation.

Groups	Organ	Observation
Normal control	Stomach	Did not reveal any microscopic lesions.
Disease control	Stomach	Sections revealed focal haemorrhage and infiltration of inflammatory cells in the sub mucosa.
Standard group	Stomach	Did not reveal any microscopic lesions.
Test group I	Stomach	Did not reveal any microscopic lesions.
Test group II	Stomach	Did not reveal any microscopic lesions.

Discussion

The etiology of peptic ulcer is unknown in most of the cases, though it is accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. For the restoration of the balance, different therapeutic agents including plant extracts and allopathic drugs may be used (Sharma et al., 2011). The extract of seeds of *Caesalpinia crista* is used in the present study primarily to evaluate the anti-ulcerogenic in pylorus ligated rat model.

Various herbal plants have been reported for its antiulcer activity, which includes *Aeglemarmelos* (Dutta et al., 2014), *Carissa corandas* (Merai and Jadhav, 2014), *Pisonia aculeate* (Madhulatha et al., 2013), *Garugapinnata* (Chitra et al., 2013), *Ocimum sanctum* (Kaur et al., 2012), *cyndondactylon* (Kaur et al., 2012). Bael (*Aeglemarmelos*) has a prominent gastroprotective effect due to the presence of Luvangetin which is a pyranocoumarin, tannin and the Cineole in the fruit. Oxidative stress usually leads to gastric ulcer. Luvangetin tends to lower the oxidative stress in the gastro duodenal mucosa preventing ulcer formation. The phenolic compounds are powerful antioxidants and have potent antiulcer activities (Dutta et al., 2014).

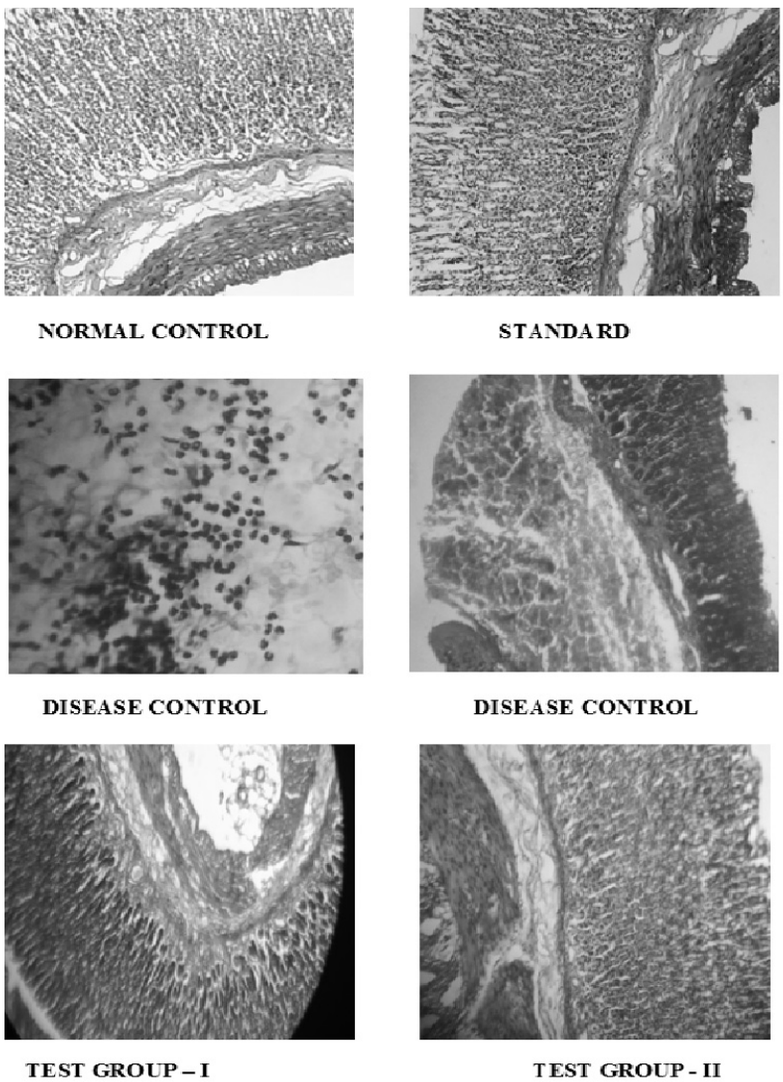


Fig. 5. Histopathology of stomach sections.

The alcoholic leaf extract of Garugapinnata possess anti-ulcer activity property in pylorus ligated model could be mainly due to modulation of defensive factors through an improvement of gastric cytoprotection and/or partly due to acid inhibition. The above effects of *Garugapinnata* may also due to the presence of Flavonoids and Tannins in the extract (Chitra et al., 2013). On preliminary phytochemical study the aqueous extract of *Caesalpinia crista* seed showed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. The plant was studied for the antiulcer activity.

Pylorus ligation induced gastric ulcer model is generally used to study the effect of various drugs on gastric secretions. Ulcers caused by pyloric ligation are due to amplified accumulation of pepsin and gastric acid, leading to the auto digestion of mucosa of the stomach and break down of the gastric mucosal barrier. Pylorus ligation increased the acid secretion that is why the gastric volume is increased. Thus low pH increases total acidity and free acidity and as a result ulcer index is increased.

The aqueous extract of seeds of *Caesalpinia crista* in the dose of 500 mg/kg showed significant increase in the pH of gastric acid and significant reduction in the volume of gastric juice, ulcer index and free and total acidity. However, in the dose of 250 mg/kg it did not show significant reduction in the ulcer index.The histo-pathological examination of gastric mucosa of animals pretreated with omeprazole (2 mg/kg; p.o.) and *Caesalpinia crista* seed extract (250 & 500 mg/kg p.o.) in standard and test groups respectively was done. In pylorus ligation model, after 4 hour both group animals showed decrease in necrosis, cellular infiltration, mucosal edema and haemorrhage as compared to disease control group in pylorus ligation induced ulcer model.

The antiulcer property of *Caesalpinia crista* in the dose of 500 mg/kg in pylorus ligation model is evident from its significant reduction in number of ulcers, total acidity, free acidity, and ulcer index. *Caesalpinia crista* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *Caesalpinia crista* (500 mg/kg) can suppress gastric damage induced by aggressive factors and may have anti-secretory effect.

The preliminary phytochemical studies revealed the presence of flavonoids in aqueous extract of *Caesalpinia crista;* various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. Therefore, the possible mechanism of antiulcer action of *Caesalpinia crista* may be due to its flavonoid content.

Conclusions

In summary, our results suggests that *Caesalpinia crista* seed extract could be useful component in prevention ulcer formation as well as it has antisecretory activity in the dose of 500 mg/kg. This study proved antiulcer and antisecretory effect of aqueous extract of Caesalpinia crista seeds in the dose of 500 mg/kg.

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