



Evaluation of anti-ulcer activity of *caesalpinia crista linn.* seeds on pylorus ligation and indomethacine induced gastric lesions in albino rats

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ABSTRACT

To evaluate the anti-ulcer activity of ethanolic and aqueous extracts of seeds of *Caesalpinia crista linn.* on pylorus ligation and Indomethacine induced gastric lesions in albino rats. 72 adult albino rats of either sex were used in the study. They were classified into 12 equal but random groups (n=6) and used six groups in each model. Extracts of *Caesalpinia crista linn.* were evaluated for anti-ulcer activity by using pylorus ligation and Indomethacine induced ulcer models in experimental animals. Ranitidine (20mg/kg ip) was used as a standard drug in both the models. Percentage yield of ethanolic and aqueous extracts was found to be 8.7 and 13.3 which reflects reduced ulcer incidence in both Ethanolic and aqueous extracts at both doses. There is decrease in ulcer score and ulcer index in both the groups but the maximum effect was shown by the ethanolic extract at high dose. The extracts of *Caesalpinia crista linn.* seeds exhibited significant anti-ulcer activity by Pylorus ligation and Indomethacine induced ulcer models, but the maximum effect was shown by the ethanolic extract at 200 mg/kg b.w. dose.

Key words: *Caesalpinia crista*, Antiulcer, Karanjwa, Fever nut

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors¹. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs². 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 motility³. The success of commercially available antiulcer drugs in the treatment of gastric ulcer is usually overshadowed by various side effects. eg. H₂- receptor antagonists (e.g. cimetidine) may cause gynecomastia in men and galactorrhoea in women⁴ while proton-pump inhibitors (e.g. omeprazole and lansoprazol) can cause nausea, abdominal pain, constipation and diarrhoea⁵. Due to these side effects, there is a need to find new antiulcerogenic compound with potentially less or no side effects. *Caesalpinia crista*

belonging to the plant *Caesalpinaceae* is a medicinal plant growing widely throughout India and tropical countries of the world⁶. It is a large straggling and very thorny shrub. Traditionally, in Ayurveda, this plant was used for the treatment of gynecological disorders, skin diseases, constipation, piles and ulcers⁷. Most widely used part is seed kernel which is reported as a rich source of cassane- and norcassane-type diterpenoids. Some new diterpenoids are also isolated from stems and root of this plant^{8,9}. The stem part and root part constituents two novel peltogynoids, pulcherrimin and 6- methoxypulcherrimin, one novel homoisoflavonoid, 8-methoxybonducellin,^{10,11} and the known compounds bonducellin, 2, 6-dimethoxybenzoquinone, 2', 4', 4-trihydroxychalcone and 2', 4-dihydroxy-4'- methoxychalcone^{12,13}. Its seeds are reported as anthelmintic, antipyretic, anti-inflammatory and antimalarial agent antidiuretic, antibacterial, antianaphylactic, antidiarrhoeal, antiameobic & antiviral properties. It has been reported that the methanol extract of *C. crista* seed and seed kernel possess antifeedant and anthelmintic property^{14,15}.

MATERIALS AND METHODS

Plant material:

The seeds of *C. crista* were procured from the local market of Ropar, Punjab in the month of December 2011. The seeds were authenticated by Mr. Madan Pal, Botanist, Chandigarh dated 31st January, 2012.

Preparation of extract:

Shade dried part (seeds) of plant were powdered (200g) coarsely and firstly extracted with petroleum ether for defatting and then ethanol (99.99%) by Soxhlet apparatus for 72 hr. and aqueous extracted was prepared by maceration process. The extracts were concentrated until dryness under reduced pressure and controlled temperature (40-50°C). Then Preliminary Phytochemical screening was performed. % yield of all extracts were calculated. The % yield of ethanolic and aqueous extracts was found to be 8.7% and 13.3%¹⁶. The LD₅₀ determination of *Caesalpinia crista* seed extract was reported by Sunil N Kshirsagar¹⁷.

Experimental animals:

Wistar albino rats weighing 180-200g of either sex maintained under standard husbandry conditions at temp. 23±2°C, relative humidity 55±10% and 12 hours light dark cycle. Animals were fed with standard laboratory food and *ad libitum*. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2012 under the registration no. NU/PH/M/COL/12/71.

Preliminary Phytochemical Constituents:

Ethanolic and Aqueous extract of *C. crista* were subjected to preliminary phytochemical screening for the detection of various plants constituents¹⁶.

Experimental design:

A) Pylorus ligation in rats:

Animals were divided into six groups of six animals each. The animals in group I served as pylorus ligated control. Group II were served as standard control (ranitidine 20mg/kg). The animals in group III, IV, V and VI served as experimental and were treated orally with ethanol & aqueous extract of *C. crista* of 100 mg and 200 mg/kg body weight. After six hours of test drug and ranitidine treatment, pyloric ligations were performed by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After surgery, rats were sacrificed and ulcer scoring was done. The total volume of gastric content was measured and acidity was determined^{18,19}.

Scoring of ulcer was made as follows:

0 = no ulcer; 1 = superficial ulcers; 2 = deep ulcers; 3 = perforation

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as: $UI = UN + US + UP \times 10^{-1}$

Where, *UI* = ulcer index; *UN* = average of number of ulcers per animal; *US* = average of severity score; *UP* = percentage of animals with ulcers.

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{\text{Ulcer index (Control)} - \text{Ulcer index (Test)} \times 100}{\text{Ulcer index (Control)}}$$

Estimation of pH:

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

Estimation of free acidity:

One ml of the supernatant liquid was pipetted 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 colour. The volume of NaOH needed was taken as corresponding to the free acidity.

Estimation of total acidity:

Instead of Topfer's reagent, phenolphthalein indicator was used. An aliquot of gastric juice was titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed. The total acidity was expressed as mEq/L by the following formula:

$$\text{Acidity} = \text{Volume of NaOH} * \text{Normality} * 100 / 0.1 \text{ m Eq.l}$$

B) Indomethacinee induced ulcer:

The following groups of animals were used. Group I was served as Indomethacinee control (20 mg/kg b.w, p.o), Group II was served as standard control (ranitidine 20 mg/kg). The animals in group III-VI served as experimental and were treated orally with ethanol & aqueous extract of *C. crista* of 100 mg and 200 mg/kg body weight, for 5 days + Indomethacinee (20 mg/kg b.w., p.o).

Experimental procedure:

After 24 h of fasting at the end of the experimental period with water provided, Indomethacinee at an oral dosage of 20 mg/kg body weight was given. After the treatment period all groups underwent surgery after 5 days of pre-treatment and ulcer induction and calculate the ulcer index and ulcer score²⁰.

Statistical analysis:

The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison tests. The experimental results are represented as ± SEM (standard error mean).

RESULTS AND DISCUSSION

Phytochemical Screening:

The results of phytochemical screening showed the presence of Phytosterols, alkaloids, triterpenoids, saponins, flavonoids, tannins, carbohydrates, reducing sugars, proteins. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed²¹.

Pylorus ligation induced ulcer:

Effect of ethanolic extract of *C. crista* on pyloric ligation induced ulceration is shown in Table 1 and 2. The pyloric ligation has caused the accumulation of gastric secretions of 8.21 ± 0.075 ml with pH 2.15 ± 0.017 in a ligated control group. The total acidity and free acidity of the gastric secretions were found to be 44.2 ± 0.148 and 19.4 ± 0.122 mEq/l respectively. Pretreatment with the *C. crista* extract, significantly ($P < 0.001$) reduced the volume of gastric secretions 4.26 ± 0.021 at the dose of 200 mg/kg b.w respectively. pH of the gastric fluid was significantly ($P < 0.001$) elevated up to 3.60 ± 0.023 only at higher dose of the extract. In addition, total acidity and free acidity were also reduced significantly ($P < 0.001$) in a dose dependent manner. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with *C. crista* extract has reduced them significantly ($P < 0.001$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 68.6% at dose of 200mg/kg b.w. The protection offered by the test extract was comparable to that of the standard drug, ranitidine (20 mg/kg) (Table 1 & 2) (Fig.1).

Table 1: Effect of different extracts of *Caesalpinia crista* on pylorus ligation induced ulcer

Treatment	Volume of gastric juice (ml/100g)	Free acidity (mEq/l)	Total acidity (mEq/l)	pH
Ligated control	8.21 ± 0.075	19.4 ± 0.122	44.2 ± 0.148	2.15 ± 0.017
Ranitidine 20mg/kg	4.07 ± 0.032 ***	9.80 ± 0.009 ***	16.90 ± 0.087 ***	3.70 ± 0.018 ***
CCAD 100 mg/kg	4.79 ± 0.057 ***	13.4 ± 0.015 ***	27.0 ± 0.097 ***	3.36 ± 0.018 ***
CCAD 200mg/kg	4.55 ± 0.033 ***	10.5 ± 0.015 ***	18.9 ± 0.150 ***	2.80 ± 0.060 ***
CCED 100 mg/kg	5.20 ± 0.051 ***	12.4 ± 0.011 ***	24.4 ± 0.092 ***	3.58 ± 0.043 ***
CCED 200 mg/kg	4.26 ± 0.021 ***	9.97 ± 0.083 ***	17.1 ± 0.118 ***	3.60 ± 0.023 ***

Values are expressed as mean \pm S.E.M. ($n = 6$); *** $p \leq 0.001$ when compared with ligated control group, CCAD (100mg/kg)- *Caesalpinia crista*, aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg)- *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg, CCED (100mg/kg) *Caesalpinia crista* Ethanolic extract at a dose of 100 mg/kg, CCED (200mg/kg)- *Caesalpinia crista* Ethanolic extract at a dose of 200 mg/kg.

Table 2: Effects of *Caesalpinia crista* extracts on ulcer incidence in pylorus ligation model

Treatment	Ulcer score	Ulcer Index	% Protection
Ligated Control	3.83 ± 0.023	10.9 ± 0.036	----
Ranitidine(20mg/kg)	0.17 ± 0.002 ***	1.70 ± 0.007 ***	84.5%
CCAD 100 mg	1.43 ± 0.121 ***	5.16 ± 0.002 ***	52.7%
CCAD 200mg	0.83 ± 0.001 ***	3.54 ± 0.031 ***	67.8%
CCED 100 mg	1.19 ± 0.002 ***	5.11 ± 0.004 ***	53.2%
CCED 200 mg	0.56 ± 0.033 ***	3.47 ± 0.037 ***	68.6%

Ulcers score values are mean \pm SD, CCAD (100mg/kg)- *Caesalpinia crista*, aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg)- *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg, CCED (100mg/kg) *Caesalpinia crista* Ethanolic extract at a dose of 100 mg/kg, CCED (200mg/kg)- *Caesalpinia crista* Ethanolic extract at a dose of 200 mg/kg. *** $p < 0.001$ - indicates comparison with control groups

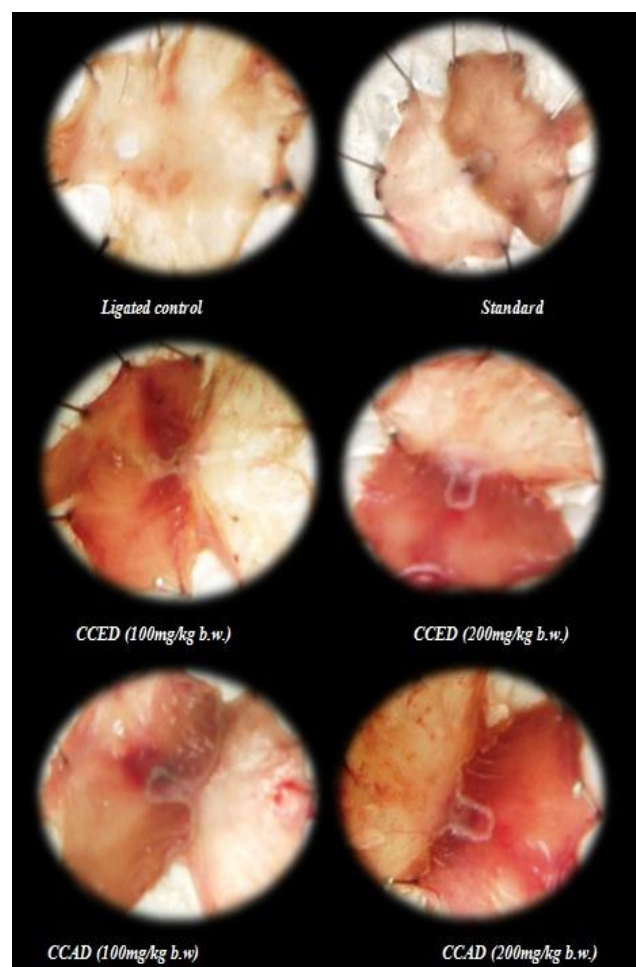


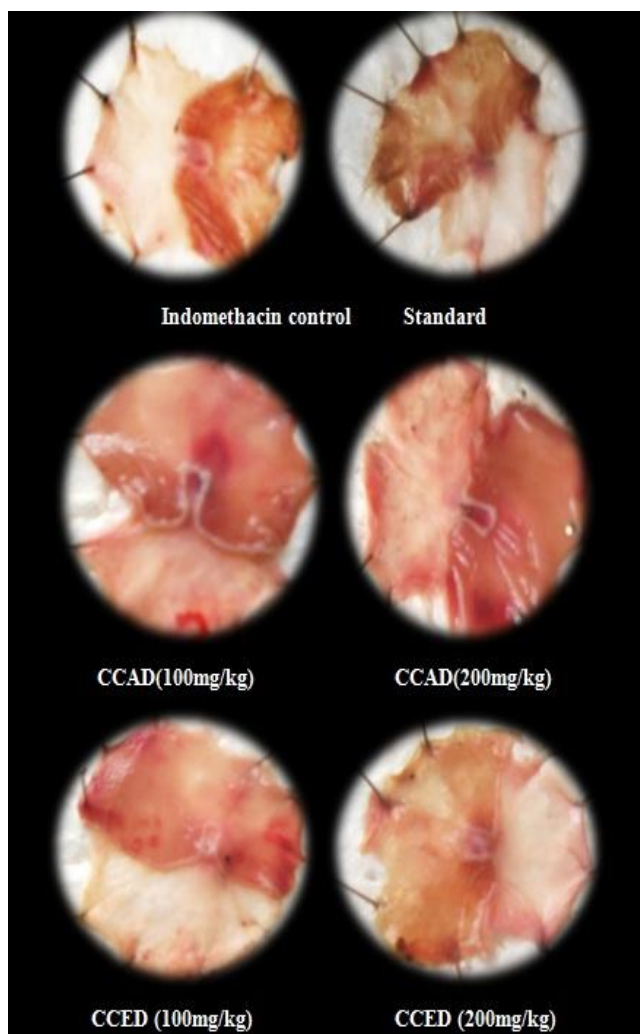
Figure 1: Effect of different extracts of *Caesalpinia crista* on pylorus ligation induced ulcer Indomethacinee induced ulcer

Oral administration of ethanolic extract of *Caesalpinia crista* at 200 mg/kg b.w showed the significant ($p < 0.001$) reduction in ulcer index 3.44 ± 0.005 as compared to control group 11.1 ± 0.055 . It showed the protection index of 69.07% in comparison to control whereas Ranitidine as reference standard drug showed ulcer reduction as 84.76% (Table 3). Effect of different extracts of *Caesalpinia crista* on Indomethacinee induced ulcer is also observed in fig. 2.

Table 3: Effect of different extracts of *Caesalpinia crista* on Indomethacine induced ulcer

Treatment	Ulcer score	Ulcer Index	Percentage protection
Indomethacin (control)	4.16±0.003***	11.1±0.055***	-----
Ranitidine(20 mg/kg)	0.16±0.004***	1.68±0.002***	84.76%
CCAD 100 mg	0.81±0.156***	3.46±0.005***	68.9%
CCAD 200mg	1.26±0.089***	5.16±0.002***	53.5%
CCED 100 mg	0.83±0.001***	5.14±0.003***	53.67%
CCED 200 mg	0.52±0.007***	3.44±0.005***	69.07%

Values are expressed as mean ± S.E.M. (n = 6); *** p ≤ 0.001 when compared with Indomethacine control group, CCAD (100mg/kg)- *Caesalpinia crista*, aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg)- *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg, CCED (100mg/kg) *Caesalpinia crista* Ethanolic extract at a dose of 100 mg/kg, CCED (200mg/kg)- *Caesalpinia crista* Ethanolic extract at a dose of 200 mg/kg.

**Figure 2: Effect of different extracts of *Caesalpinia crista* on Indomethacine induced ulcer**

Peptic ulcer and gastritis have been associated with multi pathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal

integrity through the endogenous defense mechanism (e.g. of defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier)²². *Caesalpinia crista* extract is one such herbal drug used in present study primarily to evaluate the anti-ulcerogenic in pylorus ligation and Indomethacine induced ulcers in rats. 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 acid²³. In the present study, antiulcer activity of ethanolic extract of *Caesalpinia crista linn.* was studied by using pyloric ligation model in rats. Results showed that CCED (200mg/kg) shows maximum ulcer protection as compared to ligated control as shown in figure no.-1 and there is a significant (p<0.001) reduction in free acidity, total acidity, number of ulcers and ulcer index. *C. crista* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both concentrations and increased the pH. Therefore, it is suggested that *C. crista* can suppress gastric damage induced by aggressive factors. Anti-inflammatory drugs like Indomethacine administered in toxic doses (20 mg/kg), produce visible gastric ulcers in animals. Indomethacine is a potent inhibitor of prostaglandin biosynthesis. Prostaglandins are known to play an important role in maintaining mucosal integrity. An increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents. The mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output, Gastro duodenal bicarbonate secretion eliminate, gastric mucosal blood flow decreasing gastric motility, increasing the release of endogenous mediators of gastric injury-vasoactive amines and LT, stimulation of cellular growth and repair [24]. The ulcers and ulcer index were significantly (p<0.001) increased in the Indomethacine control group. After the pretreatment with the ethanolic extract of *C. crista* there were a significant (p<0.001) decrease in the both parameters which were comparable to the standard group as shown in table 3.

CONCLUSION

The study was taken up to evaluate ethanolic and aqueous extracts of seeds *Caesalpinia crista* for antiulcer activities. *Caesalpinia crista* seeds extracts exhibited significant anti-ulcer activity by Pylorus ligation and Indomethacine induced ulcer models. Ethanolic and aqueous extracts at both the doses, reduced ulcer incidence, when compared to the control as evident by decrease in ulcer score and ulcer index in both the models but the maximum effect was shown by the ethanolic extract high dose. There was decrease in gastric volume and reduction in free and total acidity, pH in the animals treated with extracts. Hence, it can be concluded that extracts of *Caesalpinia crista* have anti-ulcer effect. So, further research work is required to isolate the compound responsible for this activity.

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