A Comparative Evaluation of anti-ulcer activity of plant *Leucas aspera spreng.* & *Caesalpinia crista linn.* seeds in albino rats

Nakul Gupta[1]*, Mohammed M. Safhi[1], Yousra Nomier[1], Meetu Agarwal[1]

[1]*,[1] Department of Pharmacology College of Pharmacy, Jazan University, Jazan, Kingdom of Saudi Arabia
E-Mail: drnakulmgupta76@gmail.com

Abstract-To compare the anti-ulcer activity of extracts of plant Leucas *aspera spreng.* and seeds of Caesalpinia *crista linn.* by pylorus ligation induced gastric lesions in albino rats. 36 adult albino rats of either sex were distributed in six groups (n=6) in each model. Extracts of plant Leucas *aspera spreng.* and seeds of Caesalpinia *crista linn.* were evaluated for anti-ulcer activity by using pylorus ligation induced ulcer models in experimental animals. Ranitidine (20mg/kg ip) was used as a standard drug. The percentage yield of ethanolic extracts was found to be 8.7% for C. *crista* and 6.2% for L. *aspera*. Pretreatment with ethanolic extracts of plant L. *aspera* & seeds of C. *crista* significantly reduced the volume of gastric secretions, total acidity, free acidity, and elevated pH of the gastric fluid in a dose dependentmanner, but the maximum benefit was observed by the ethanolic extracts of C. *crista* at a dose level of 200mg/kg body weight. Similarly protection against ulcers was offered by the both test extracts but maximum percentage inhibition of ulceration was given by C. *crista*. The standard drug used was ranitidine at a dose level of 20 mg/kg body weight. It can be concluded that the ethanolic extracts of plant L. *aspera* and seeds of C. *crista*. have anti-ulcer effect, but extract with more ulcer protection belongs to seeds of C. *crista*.

Key words-Leucas *aspera,* Caesalpinia *crista*, Antiulcer

INTRODUCTION

Gastric ulcer is one of the most widespread and common problem throughout the world, it is believed to be due to an imbalance between aggressive and protective factors [1]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs [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]. 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 women [4] while proton-pump inhibitors (e.g. omeprazole and lansoprazol) can cause nausea, abdominal pain, constipation and diarrhoea [5]. Due to these side effects, there is a need to find new antiulcerogenic compound with potentially less or no side effects.

Leucas *aspera* belonging to the family Labiate is used traditionally as anti-inflammatory, stimulant, in jaundice, cough, asthma, peptic ulcers, conjunctivitis, diabetes, malaria, headache, otalgia, skin diseases, snake bite, toothache, and wound healing etc.[6] Leucas *aspera* is studied for anti-inflammatory activity[7-9],analgesic activity[10], cobra venom induced mortality in mice[11], anti-parasitic activity[12], antibacterial activity against Micrococcus pyrogenes, V.*aureus* and E. *Coli*[13], toxic to the filarial vector mosquito[14], antinociceptive, antioxidant and cytotoxic activity[15]. Preliminary chemical examination of entire plants of L. *aspera* revealed presence of triterpenoids[16], contains oleanolic acid, ursolic acid and 3-sitosterol[17], and aerial parts are reported to contain nicotine, sterols, two new alkaloids (compound A m.p. 61.2°c, α-sitosterol and β-sitosterol m.p. 183.4^{o}c), reducing sugars (galactose), glucoside (230.1^{o} c)[18]. Caesalpinia *crista* belonging to Caesalpiniaceae is a medicinal plant growing widely throughout India and tropical countries of the world[8]. 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 ulcers [19]. 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[20,21].

The stem part and root part constituents two novel peltogynoids, pulcherrimin and 6-methoxypulcherrimin, one novel homoisoflavonoid, 8-methoxybonducellin, and the known compounds bonducellin, 2, 6-dimethoxybenzoquinone, 2', 4', 4-trihydroxychalcone and 2', 4-dihydroxy-4'-methoxychalcone.[22,23]

Its seeds are reported as anthelmintic, antipyretic, anti-inflammatory and antimalarial agent antidiuretic, antibacterial, antianaphylactic, antidiarrhoeal, antiameobic & antiviral properties [24,25]. It has been reported that the methanol extract of C. *crista* seed and seed kernel possess antifeedant and anthelmintic property[26].

METHODS

Plant material

The plants of Leucas *aspera* were collected from local areas around the Mangalore, Karnataka, India and seeds of C. *crista* were procured from local market of Ropar, Punjab, India.

Preparation of extract

Shade dried seeds of C. *crista* and whole plant of L. *aspera* including root, stem, leaves and flowers were chopped into small pieces separately. The powder of both was extracted with petroleum ether for defatting and then by ethanol (99.99%) by using Soxhlet apparatus for 72 hr.

The extracts were concentrated until dryness under reduced pressure and controlled temperature (40-50ºC). Then preliminary phytochemical screening was performed. Percentage yield of both the extracts were calculated. The LD50 of C. *crista* seed and L. *aspera* extracts was reported by Sunil N Kshirsagar and Nakul Gupta respectively [27,28].

Experimental animals

Wistar albino rats weighing 180-200g of either sex maintained under standard husbandry conditions at temp. 23±2^{o}C, relative humidity 55±10% and 12 hours light dark cycle. Animals were fed with standard laboratory food and ad libitum.

Preliminary Phytochemical Constituents

Ethanolic extracts of L. *aspera* and C. *crista* were subjected to preliminary phytochemical screening for the detection of various plants constituents [29,30].

Experimental design (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 ethanolic extracts of L. *aspera* and C. *crista* at two different dose levels i.e. 100 mg and 200 mg/kg body weight by oral route for a period of 5 days before pyrolus ligation.

On sixth day, after six hours of test drug and ranitidine administration, pyloric ligations were performed by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia. 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 [31,32].

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 follows:

UI = UN + US+ UP × 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:

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

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 color. The volume of NaOH needed was taken as corresponding to the free acidity.

Estimation of total acidity

An aliquot of gastric juice was titrated with 0.01N NaOH until a permanent pink color was observed. Acidity was expressed as mEq/L and calculated by:

Acidity= Volume of NaOH * Normality*100/0.1 m Eq.l

Statistical analysis

The significance of difference among the various treated groups and control group were

analyzed by means of one-way ANNOVA followed by Dunnett's multiple comparison tests. The experimental results are represented as mean ± SEM.

RESULTS Phytochemical Screening

The % yield of ethanolic extracts was found to be 8.7% for C. *crista* and 6.2% for L. *aspera*. The results of phytochemical screening of plant L. *aspera spreng*. & Caesalpinia *crista linn.* seeds showed the presence of phytosterols, like steroids, alkaloids, glycosides, saponins, flavonoids, tannins, carbohydrates. As tabulated in table 1.Flavonoids and tannins are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed.

Table 1: Phytochemical screening of ethanolic extracts of plant L. *aspera & seeds of* C. *crista*

Ethanolic Extracts	Steroids	Alkaloids	Glycosides	Saponins	Flavonoids	Tannins	Carbohydrates
Leucas *aspera*	+	+	+	+	+	+	+
Caesalpinia *crista*	+	+	+	+	+	+	+

(+) = present; (-) = absent

Pylorus ligation induced ulcer

Effect of ethanolic extract of L. *aspera* & C. *crista* on pyloric ligation induced ulceration is shown in Table 2 and 3. The pyloric ligation has caused the accumulation of gastric secretions of 8.21±0.075ml 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 ethanolic extracts of plant L. *aspera &* seeds of C. *crista* significantly (P<0.001) reduced the volume of gastric secretions, significantly (P<0.001) elevated pH of the gastric fluid and in addition to these, significantly reduced (P<0.001) total acidity and free acidity in a dose dependent manner for both the drug extracts, but the maximum benefit was observed by the ethanolic extracts of C. *crista* at a dose level of 200mg/kg body weight.

Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with ethanolic extracts of L. *aspera &* C. *crista* has reduced them significantly (P<0.001) in a dose dependent manner. Maximum percentage inhibition of ulceration was found to be 68.6% by ethanolic extract of C. *crista* at a dose of 200mg/kg b.w. The protection offered by the both test extracts was comparable to that of the standard drug, ranitidine (20 mg/kg) (Table 2 & 3).

Table 2: Effect of ethanolic extracts of L. *aspera &* C. *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(20 mg/kg)	4.07±0.032***	9.80±0.009***	16.90±0.087***	3.70±0.018***
LAE 100 mg	7.02±0.017***	14.4±0.031***	28.0±0.071***	2.72±0.018***
LAE 200mg	5.65±0.023***	11.5±0.051***	21.9±0.125***	3.18±0.060***
CCE 100 mg	5.20±0.051***	12.4±0.011***	24.4±0.092***	3.58±0.043***
CCE 200 mg	4.26±0.021***	9.97±0.083***	17.1±0.118***	3.60±0.023***

Values are expressed as mean ± S.E.M. (n = 6); *** $p \leq 0.001$when compared with ligated control group, LAE (100mg/kg)- L *aspera* ethanolic extract dose 100 mg/kg, LAE (200 mg/kg)- L *aspera* ethanolic extract dose of 200 mg/kg. CCE (100mg/kg) C. *crista* ethanolic extract dose 100 mg/kg, CCE (200mg/kg)-C. *crista* ethanolic extract dose 200 mg/kg.

Table 3: Effect of ethanolic extracts of L. *aspera &* C. *crista* 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%
LAE 100 mg	1.41±0.161***	6.27±0.002***	42.5%
LAE 200mg	0.85±0.021***	4.65±0.031***	57.3%
CCE 100 mg	1.19±0.002***	5.11±0.004***	53.2%
CCE 200 mg	0.56±0.033***	3.47±0.037***	68.6%

Ulcers score values are expressed as mean ± S.E.M. (n = 6); *** $p \leq 0.001$when compared with ligated control group, LAE (100mg/kg)- L *aspera* ethanolic extract dose 100 mg/kg, LAE (200 mg/kg)- L *aspera* ethanolic extract dose of 200 mg/kg. CCE (100mg/kg) Caesalpinia *crista* ethanolic extract dose 100 mg/kg, CCE (200mg/kg)- Caesalpinia *crista* ethanolic extract dose 200 mg/kg.

DISCUSSION

According to Shetty et al., 2008 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) [33].

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 unprotected lumen of the stomach to the accumulating acid [34,35].

In the present study, antiulcer activity of ethanolic extracts of plant L. *aspera* and seeds of C *crista*. was studied by using pyloric ligation model in rats. Results showed that CCE (200mg/kg) has maximum ulcer protection as compared to ligated control as shown in tables. There is also a significant ($p<0.001$) reduction in free acidity, total acidity, number of ulcers and ulcer index. L. *aspera &* C. *crista* ethanolic extracts treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both concentrations and increased the pH dose dependently. But the maximum effect was observed by the C. *crista* ethanolic extract at a dose of 200mg/kg body weight. Therefore, it is suggested that C. *crista* can suppress gastric damage induced by aggressive factors better than L. *aspera*.

Conclusion

The study was taken up to comparatively evaluate antiulcer activity of ethanolic extracts of plant Leucas *aspera* and seeds of Caesalpinia *crista*. Ethanolic extracts of Plant Leucas *aspera* and seeds of Caesalpinia *crista* exhibited significant anti-ulcer activity in pylorus ligation induced ulcer model. Ethanolic extracts of both Plant Leucas *aspera* and seeds of Caesalpinia *crista*, reduced ulcer incidence at both the doses, when compared to the control as evident by decrease in ulcer score and ulcer index but the maximum effect was shown by the ethanolic extract of Caesalpinia *crista* at high dose (200mg/kg b.w.). A decrease in gastric volume and reduction in free and total acidity, pH in the animals treated with extracts was also noted.

Hence, it can be concluded that extracts of plant Leucas *aspera* and seeds of Caesalpinia *crista*. have anti-ulcer effect. But the extract with more ulcer protection belongs to seeds of Caesalpinia *crista*, a further research work is required to isolate the compound responsible for this activity and it may be a potential source of antiulcer drug.

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