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Effect of *Caesalpinia bonducella* extract in pylorus ligation induced ulcers in wistar rats

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ABSTRACT

Caesalpinia bonducella (L.) Fleming (Syn. Caesalpinia bonduc (L.) Roxb, Syn. Caesalpinia cristaLinn.), belonging to the family Fabaceae /Caesalpiniaceae, traditionally used in treating ailments of gastrointestinal tract. The phytoconstituents suggest the possible anti-ulcer property of the plant. Based on which, the present study has been conducted to evaluate the effect of Caesalpinia bonducella dry nut extract in Wistar rats. The animals were divided into six groups of 6 animals each. The treatments were administered for 10 days. On day 10, the animals were fasted overnight, the pylorus was ligated and after 19 hours, the animals were sacrificed, gastric contents were removed. The pH, volume, total and free acid of the gastric contents were determined. The stomach was excised and observed for the ulcer index. Treatment with Caesalpinia bonducella dry nut extract significantly decreased the gastric volume, decreased the total and free acids while there was no change in pH. However, there was a significant decrease in the ulcer index. The Caesalpinia bonducella dry nut extract was found to decrease pylorus ligation induced ulcers. The present study had validated the traditional use of Caesalpinia bonducella as an anti-ulcer agent.

Key words: *Caesalpinia bonducella*, ulcer, Pylorus ligation

INTRODUCTION

Peptic ulcer disease is a group of disorders characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid in sufficient concentration and duration. An ulcer is a crater like lesion in a membrane; ulcers that develop in areas of the GIT exposed to acidic gastric juice are called peptic ulcers. Peptic ulcer is due to exposure of stomach and duodenum to pepsin and gastric acid. Imbalance occurs between aggressive factors like acid, pepsin, *H. pylori* and defensive factors such as gastric mucus, bicarbonate ions, and prostaglandins along with innate resistance of mucosal cells. Gastroduodenal mucosa utilizes these defense mechanisms against the aggressive factors such as hydrochloric acid and pepsin [1]. Peptic ulceration is the most predominant gastro-intestinal disease with a worldwide prevalence of about 80% in developing countries and 40% in developed countries. Epidemiological studies show that males have about three times as many ulcers as females between the ages of fifty-five and seventy years. It is estimated that 14.5 million of the worldwide population are affected by gastric ulcers with a mortality rate of 4.08 million. World Health Organization has reported that about 500,000 new cases of Peptic Ulcer diseases are reported each year. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of hemorrhage was 13.9%

and the average long-term recurrence of perforation was 12.2%. In developing nations, the majority of children are infected with *H. pylori* before the age of 10 and adult prevalence peaks at more than 80 percent before age 50. In contrast, in developed countries such as the United States, serologic evidence of *H. pylori* is uncommon before age 10, increasing to 10 percent in those between 18 and 30 years of age, and to 50 percent in those older than age 60 .However, there has been a significant decrease in the prevalence of duodenal ulcers over the past decades but little change in the prevalence of gastric ulcers [2].

Although recent advances in understanding the multifactorial pathogenesis of peptic ulcers has been recognized, secretion of gastric acid is still considered as a central component of this disease and therefore the main therapeutic target is the control of this secretion using antacids, H2 receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telezipine or proton pump blockers like omeprazole, lansoprazole, cytoprotective agents like sucralfate *etc*. However, ulcer therapy faces nowadays a major drawback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects like hypomagnesia, osteoporosis related fractures, erythema multiforme, etc. Drugs of plant origin are gaining popularity and are being investigated for a number of gastric disorders including peptic ulcer. Indian medicinal plants and their derivatives have been used as an invaluable source of therapeutic agents to treat various gastric disorders [3].

Caesalpinia bonducella (L.) Fleming (Syn. *Caesalpinia bonduc* (L.) Roxb, Syn. *Caesalpinia crista*Linn.), belonging to the family Fabaceae /Caesalpiniaceae, is a prickly shrub widely distributed all over the world specially, in India, Sri Lanka and Andaman and Nicobar Islands, in India specially found in tropical regions [4]. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in traditional system of medicine. *Caesalpinia bonducella* (L.) is claimed to be styptic, purgative and anthelmintic and antiinflammatory, useful in colic, malaria, hydrocele, skin diseases and leprosy. The seeds are considered tonic, febrifuge, anthelmintic, antiblennorrhagic and specific in the treatment of hydrocele. The oil from the seeds is used in convulsions and paralysis [5,6]. The different extracts of *Caesalpinia bonducella* has been found to possess multiple therapeutic properties like anthelmintic [7, 8], antiamyloidogenic [9], immunomodulatory [10], antipyretic and anti inflammatory [11], antitumor [12], antioxidant [13], antidiabetic [14], hypoglycemic [15], antidiarrhoeal [16], antifilarial activities [17], antiestrogenic [18], etc. *Caesalpinia bonducella* (Linn.) contains bonducin, proteins, saponins, starch, sucrose, an enzyme, two phytosterols namely sitosterol and heptocosane, fatty acids such as palmitic, stearic, lignoceric,oleic, lonolenic acids. It contains α-, β-,γ-,δ- caesalpins, caesalpin- F and amino acids. [19, 20].

Caesalpinia bonducella extract contains saponins and tannins as one of its major phytoconstituents. The saponins are reported to activate the factors which produce protective mucous membrane like improvement of blood flow. Tannins protect the outermost layer of the mucosa by rendering it less permeable to chemical and mechanical injury or irritation by reacting with the proteins of the layer of the tissue and thus exerts its astringent property [21]. Drugs that reduce acid secretion and those which provide cytoprotection to the gastric mucosa is considered effective in promoting healing of ulcers. Considering all these factors, seeds of *Caesalpinia bonducella* prompted further investigation for its effect in pylorus ligation induced ulcers.

MATERIALS AND METHODS

Drugs and chemicals

Sucralfate, Naproxen hydrochloride, Rabeprazole were obtained as gift samples from Dr, Reddy's Laboratories, Hyderabad. All other reagents and chemicals used in the study were of analytical grade.

Plant Extract

Caesalpinia bonducella Linn Standardized dry nut extract (CAE) was procured from Amsar Private Limited, Indore as a gift sample. The procedure of extraction followed by Amsar Private Limited, Indore is as follows: Seeds were collected, powdered and extracted with petroleum ether (60-80°C) in a soxhlet apparatus. Then the obtained seed was extracted using ethanol by cold maceration process with intermittent shaking. Each day the ethanol was filtered from the macerate and fresh ethanol was added till filtered ethanol does not show any colour. The crude extract was evaporated under reduced pressure and subjected for preliminary testing and screening.

Animals

Albino Wistar rats of either sex weighing between (150-280 g) were obtained from National Toxicology Centre, Pune. All the animals were housed in animal house in groups of six animals in polypropylene cages. The animals were maintained at 25 ± 2°C, relative humidity of 45 to 55% and under standard environmental conditions (12 h light 12 h dark cycle). All the animals were acclimatized for 10 days to the animal house conditions prior to the start of experimental protocol. The Wistar rats had free access to feed pellets (Amrut Laboratory Animal Feed manufactured by Nav Maharashtra Chakan oil mills Ltd., Purchased from Pranav Agro Industries Ltd.,Sangli, Maharashtra) and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Registration No. 1703/PO/c/13/CPCSEA. Protocol Approval No. (CPCSEA/PCL 21/2014-2015).

Acute Oral Toxicity

Adult female Albino Wistar rats (200-250g) were subjected to acute oral toxicity studies as per guideline (AOT No. 423) suggested by Organization for Economic Co-operation and Development (OECD) [22]. Dose of the extract were administered orally. Rats were observed individually after administration of extract during the first 30 minutes, and periodically for 24 hours with special attention given during the first 4 hours and daily thereafter for a total of 14 days for toxic symptoms and mortality. All observations were systematically recorded with individual records being maintained for each animal. The study was performed in a step wise manner was found that the extract was not toxic up to 2000 mg/kg dose level. One-tenth dose of the maximum dose used in the acute toxicity study was considered as therapeutic dose for further pharmacological study.

Screening of *Caesalpinia bonducella* Linn Extract in pylorus- ligation induced ulcer:

Male Wistar rats weighing between 200 – 250 g were divided into following groups each consisting of six animals each.

Group I: Normal group received distilled water, p.o
Group II: Pylorus ligation ulcer control group received distilled water, p.o
Group III: Pylorus ligation + Standard treated group (Rabeprazole 20mg/kg,p.o)
Group IV: Pylorus ligation + *Caesalpinia bonducella* extract (100 mg/kg,p.o)
Group V: Pylorus ligation + *Caesalpinia bonducella* extract (200 mg/kg,p.o)
Group VI: Pylorus ligation + *Caesalpinia bonducella* extract (400 mg/kg,p.o)

The method of "Shay rat ulcer" [23] described by Bafna and Balaraman [24] was adopted with some modification. The rats were pretreated with respective treatments for a period of ten days. Rats were deprived of food, but not water, for 24 h prior to the experiment. On 10^{th} day, 1 h after the respective treatments, animals were anaesthetized with ketamine (80 mg/kg, i.p). The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. The animals were housed in individual cages with mesh bottom to prevent coprophagy. The size of the mesh (4 x 4 mm) allowed to fall to the floor of the cage below the mesh. Nineteen hours after pylorus ligation the rats were sacrificed. The gastric content was collected and centrifuged. The volume, pH, total and free acidity of gastric fluid were determined. The stomach was then incised along the greater curvature. Stomach was washed with normal saline and each stomach was photographed using a CCD scanner at a magnification of 2400 (dots per inch) DPI . The ulcerated area was digitally determined by the method mentioned by Shuai *et al.*, [25]. The pyloric antrum area of stomach was selected and used for determination of ulcer area using Image J (National Institute of Health, U. S. A.)

Determination of free and total acidity:

The gastric content was centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipeted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N sodium hydroxide using 2-3 drops of Topfer's reagent until all traces of red colour disappears and the colour of the solution turns yellowish orange. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and continued until a definite red tinge appears. The total volume of alkali added was noted. Acidity was calculated by using the formula given below: Acidity = (Volume of NaOH × Normality of NaOH × 100) / 0.1

Total acidity was expressed as mEq/l/ 100g.

Statistical Analysis
Data analysis was performed using GraphPad Prism 5.0 software (Graph Pad, San Diego, CA). The data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test.

RESULTS

Acute Toxicity Studies:
In adherence of OECD guidelines 2001 [25], the test animals were observed individually, after dosing at least once during the first 30 minutes, periodically during the first 24 h with special attention during first 4 h. The test animals did not exhibit any visible change and survived beyond recommended duration of observation i.e. 14 days. Hence *Caesalpinia bonducella* dry nut extract was safe up to 2 g/kg. Based upon the limit dose of 2 g/kg, three doses – high dose (400 mg/kg), middle dose (200 mg/kg) and low dose (100 mg/kg) were selected for the study.

Effect of *Caesalpinia bonducella* dry nut extract on ulcer index in pylorus ligation induced ulcer in Wistar rats:
There is a significant increase in ulcer index of ulcer controlgroup when compared to normal group. Rabeprazole (20 mg/kg, p.o.) produced significant ($p<0.001$) decrease in ulcer index to 58.65% when compared with ulcer control group. Treatment with CAE (100,200 and 400 mg/kg) significantly ($p< 0.05$,$p< 0.01$ and $p<0.001$) decreased ulcer index by 21.99%, 49.79% and 65.50% respectively when compared with ulcer control group (Table 1, Figure 1).

Effect of *Caesalpinia bonducella* dry nut extract on volume of gastric juice in pylorus ligation induced ulcer in Wistar rats.
Rabeprazole (20 mg/kg, p.o.) significantly ($p<0.001$) decreased volume of gastric fluid upto 6l.46% as compared to ulcer control group. There was a significant decrease($p<0.01$) in volume of gastric fluid at the dose of 400mg/kg, p.o of CAE when compared against ulcer control group (40.67% decrease). However, non significant decrease in volume was observed in CAE (100mg/kg) (13.86%) and CAE(200mg/kg) (22.68%) when compared against pylorus ligated control group (Table 1).

Effect of *Caesalpinia bonducella* dry nut extract on pH of gastric fluid in pylorus ligation induced ulcer in Wistar rats.
Oral administration of rabeprazole at the dose of 20 mg/kg, p.o. significantly increased ($p<0.001$) the pH of gastric fluid by 64.41% as compared with ulcer control group. There was no change in the pH of gastric fluid in any of the CAE treated groups (Table 1).

Effect of *Caesalpinia bonducella* dry nut extract on total acidity of gastric fluid in pylorus ligation induced ulcer in Wistar rats.
Rabeprazole (20 mg/kg) significantly decreased($p<0.001$) total acidity of gastric fluid by 58.63% as compared with ulcer control group. The total acidity of gastric fluid was significantly reduced at the doses of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) of CAE by 23.34% and 50.06% as compared to pylorus-ligated control group. However, non-significant changes was observed in CAE (100mg/kg) (9.18%) when compared with ulcer control group (Table 1).

Effect of *Caesalpinia bonducella* dry nut extract on free acidity of gastric fluid in pylorus ligation induced ulcer in Wistar rats.
Rabeprazole (20 mg/kg) significantly decreased ($p<0.001$) acidity of gastric fluid by 65.52 % as compared with ulcer control group. The free acidity of gastric fluid was significantly reduced at the doses of 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) of CAE to 33.7% and 51.72% as compared to ulcer control group. However, non significant changes were observed in CAE (100mg/kg) (9.12%) when compared against pylorus ligated control group (Table 1).

Table No.-1 Effect of *Caesalpinia bonducella* dry nut extract (CAE) in pylorus ligation induced ulcer in Wistar rats

Treatment	Lesion index (mm)	pH	Vol (ml)	Free Acidity (mEq/l)	Total acidity (mEq/l)
Normal Control	0.00 ± 0.00	1.50 ± 0.08	7.12 ± 0.16	20.62 ± 2.14	38.32 ± 1.96
Ulcer Control	14.32 ± 0.81^{c}	1.00 ± 0.08	12.12 ± 1.25^{c}	82.16 ± 4.30^{c}	104.80 ± 5.48^{c}
Rabeprazole (20 mg/kg)	5.92 ± 0.74***	2.70 ± 0.28**	4.67 ± 0.57***	28.33 ± 4.20***	43.35 ± 3.24***
CAE (100 mg/kg)	11.17 ± 1.13*	1.54 ± 0.14	10.44 ± 0.83	74.66 ± 3.06	95.17 ± 5.76
CAE (200 mg/kg)	7.19 ± 0.33**	1.50 ± 0.08	9.37 ± 0.64	54.82 ± 1.72***	80.32 ± 9.12*
CAE (400 mg/kg)	4.94 ± 0.60***	1.56 ± 0.08	7.19 ± 0.98**	39.60 ± 2.52***	52.32 ± 6.32***

*All values are represented as mean ±SEM (n = 6). One way ANOVA followed by Dunnet's 't' test. *p<0.05, **p<0.01, ***p<0.001 compared to Ulcer control.*

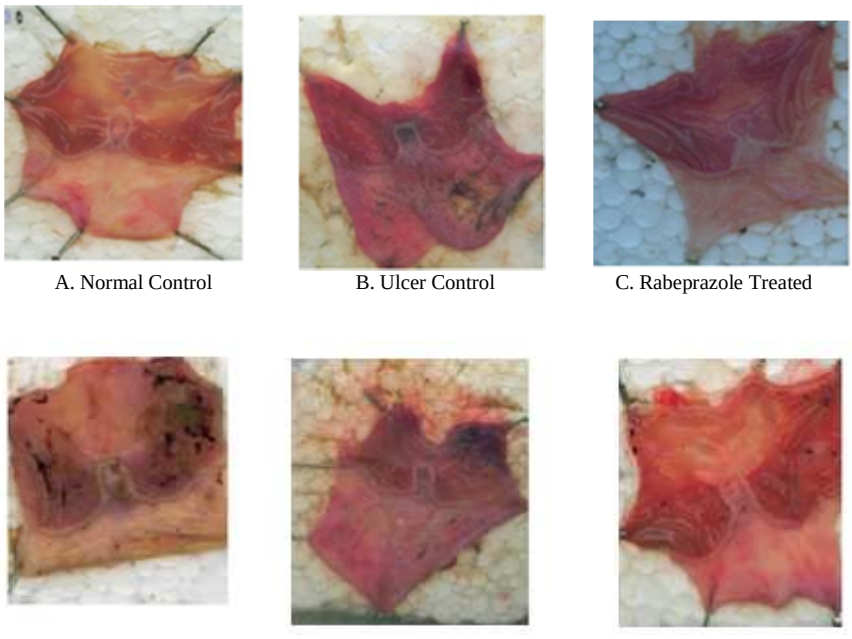
DISCUSSION

Peptic ulcers are a common disorder of the entire gastrointestinal tract. A peptic ulcer results from an imbalance between some endogenous aggressive factor(s) (hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS)) and cytoprotective factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, nonenzymatic and enzymatic antioxidants and some growth factors.The pathogenesis of peptic ulcer is attributed to disregulated oxido-nitrosative and necrotic changes in gastrointestinal mucosa [26].

Ligation of the pylorus induces ulcers that serve as a useful model for investigating the efficacy of drugs on gastric secretions. The balance between acid neutralizing mucous and erosive HCl is disturbed due to ligation of pyloric end of stomach. There is accumulation of gastric acid in the stomach leading to oxidative, nitrosative and ulcerative changes in the mucosa. This might cause disruption of mucosal defense barrier leading to lesions mirroring in human peptic ulcer disease [27]. Pyloric ligation-induced ulcers are mainly due to auto-digestion of the gastric mucosa, thus leading to the increased accumulation of acid–pepsin secretion in the stomach [28]. In the present study, gastric secretion is accrued over a period of 19 h. The secretion and accumulation of gastric acid are two important factors responsible for the production of gastric ulcer by the pylorus ligation. At pH 2 pepsinogen is converted to pepsin, whereas its inactivation occurs at pH 6. In presence of active pepsin, acid gets accumulated and causes the gastric ulcer in the pylorus ligated rat. Gastric acid accumulation due to pylorus ligation is accompanied by increased gastric volume, decreased gastric pH and elevation of total as well as free acidity. Acid neutralizing drug have been reported to reverse these changes leading to effective control of mucosal damage [29].

From the results of the present work it could be deduced that CAE has potent ulcer protective activity against pylorus ligation ulcers at a dose of 100, 200 and 400 mg/kg p.o. The ulcer index in CAE(100, 200 and 400 mg/kg p.o.) treated rats were comparable to those of Rabeprazole treated group.

Figure1: Morphological changes in the pyloric antrum tissue of rats in pylorus ligation induced ulcer



A. Normal Control B. Ulcer Control C. Rabeprazole Treated

D. CAE (100 mg/kg treated) E. CAE (200 mg/kg treated) F. CAE (400 mg/kg treated)

CONCLUSION

The present study concludes the antiulcer potential of *Caesalpinia bonducella* dry nut extract. The activity is mediated through the ability of *Caesalpinia bonducella* to decrease the gastric acid secretions.

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