



Anti-ulcer activity of *Caesalpinia bonducella* (Linn.) flem. leaves

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Abstract

The purpose of the present study is to evaluate the anti-ulcer activity of *Caesalpinia bonducella* (Linn.) Flem. The methanolic extract of *Caesalpinia bonducella* (Linn.) Flem. leaves were evaluated for its anti-ulcer activity against aspirin plus pylorus ligation induced gastric ulcer (antisecretory) in rats, water immersion stress induced ulcer models in rats and HCl-Ethanol induced ulcer (cytoprotective) in mice at two different doses viz. 100 and 200 mg/kg. Biochemical parameters like volume, pH, free acidity, total acidity of the gastric juice, ulcer index and percentage protection were studied. The methanolic extract of *Caesalpinia bonducella* leaves in aspirin plus pylorus ligation showed significant ($P < 0.05$, $P < 0.01$) reduction in gastric volume, free acidity, total acidity and ulcer index at dose of 100 and 200 mg/kg as compared to control, there was marked increase in pH at dose of 100 and 200 mg/kg. The plant extract at both dose of 100 and 200 mg/kg showed significant gastro protective activity in water immersion stress induced ulcer and HCl-Ethanol induced ulcer. Thus, the present results indicate that the methanolic extract of *Caesalpinia bonducella* (Linn.) Flem. leaves have significant anti-ulcer activity.

Keywords: *Caesalpinia bonducella*; anti-ulcer; aspirin pylorus ligation ulceration; HCl-ethanol ulceration; water stress ulcer.

1. Introduction

Numerous plants and herbs are used to treat gastrointestinal disorders in traditional medicine. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects of modern medicine [1] indigenous drugs with fewer side

effects should be looked for as a better alternative for the treatment of peptic ulcer. *Caesalpinia bonducella* (Linn.) Flem. (Family: Caesalpiniaceae) commonly known as Nata Karanja (Hindi), Fever nut (English) is a prickly shrub found throughout the hotter regions of

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India, Myanmar and Sri Lanka [2]. The plant *Caesalpinia bonducella* (Linn.) Flem. contains bonducin, proteins [3], saponin, starch, sucrose, an enzyme, two phytosterols namely sitosterol and heptacosane [4], fatty acids such as palmitic, stearic, lignoceric, oleic, linolenic acids [5]. It contains α -, β -, γ -, δ - and ψ -caesalpins, caesalpin-F and amino acids [6]. The leaves of *Caesalpinia bonducella* are traditionally used for the treatment of inflammation and toothache [7]. It has also been found to possess multiple therapeutic properties like anti-inflammatory [8], antipyretic, antidiuretic, anthelmintic and antibacterial [9], anticonvulsant [10], anti-anaphylactic and antidiarrheal [11], antiviral [12], antiasthmatic [13], antiamebic and antiestrogenic [14]. It has been also reported for hepatoprotective and antioxidant properties of this plant [15]. Many anti-inflammatory drugs are often ulcerogenic, thus restricting their use in long term therapy. Drugs that reduce acid secretion effectively promote healing of ulcers. All these factors promoted us to study in detail the anti-ulcer effect of *Caesalpinia bonducella*.

2. Materials and Methods

2.1. Plant material

The leaves of *Caesalpinia bonducella* (Linn) Flem. were collected from Jath, Sangli District of Maharashtra during June 2008 and was authenticated by Dr. Harsha Hegade, Research Officer, Regional Medical Research Centre (ICMR) Belgaum. A voucher specimen of plant material [RMRC-472] is kept at Pharmacognosy Museum of K.L.E.S's College of Pharmacy, Belgaum for future reference.

2.2. Preparation of extracts

The leaves of *Caesalpinia bonducella* were air-dried and the powdered plant material was extracted in soxhlet extractor with 70% methanol at 60°C for 72 hours. The extract was

concentrated using rotary vacuum to get the solid mass. The yield obtained was 13.73%.

2.3. Preparation of test sample

Two doses (100 mg & 200 mg) of the extract were prepared by using 1% sodium carboxy methyl cellulose (SCMC). Omeprazole was suspended in 1% SCMC and aspirin in 1% SCMC.

2.4. Acute oral toxicity studies

Acute toxicity studies were carried out following OECD guidelines [16] and were found to be safe up to 2000 mg/kg body weight in albino Wistar rats.

2.5. Animals

Wistar albino rats of either sex weighing 150-200 gm and Swiss albino mice weighing 25-30 g were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet (Gold mohur food and feeds Ltd. Vikroli East, Mumbai) and water *ad libitum*. The experiments were performed followed by approval from Animal Ethical Committee of the establishment [CPCSEA No. 25/1/99-AWD].

2.6. Anti-ulcer studies

2.6.1. Aspirin plus pylorus ligation induced gastric ulcer in rats

The Wistar albino rats weighing 150-200 gm of either sex were divided into 4 groups, each group consists of 6 animals. All the animals received 100 mg/kg of aspirin once daily for three days. Group-I served as control received 1.0 ml/kg p.o 1% SCMC, group-II treated with Omeprazole (20 mg/kg p.o.) served as standard, group-III and group-IV treated with methanolic extract (100 & 200 mg/kg p.o.) of *Caesalpinia bonducella* leaves respectively. On the fourth day pylorus part was ligated following 36 hours fasting. Four hours after the pyloric ligation the animals were sacrificed by

Table 1. Effects of methanolic extract of *Caesalpinia bonducella* leaves on gastric secretion, acidity, pH and ulcer index in Aspirin plus pylorus ligated rats

Groups	Dose mg/kg	Volume ml/100gm	pH	Free acidity mEq/L	Total acidity mEq/L	Ulcer index	Percentage protection
Control	1	4.18±0.09	1.95±0.04	35.67±1.33	58.00±0.73	4.50±0.42	-
Standard	50	2.28±0.08**	3.48±0.13**	17.00±0.57**	33.83±1.27**	1.10±0.42**	75.55
Methanolic extract	100	3.76±0.08*	2.51±0.06**	31.17±1.75*	53.67±1.52*	2.10±0.42*	53.33
	200	2.75±0.09**	3.00±0.05**	22.50±0.95**	47.33±1.11**	1.26±0.55**	72.00

Values are expressed as mean±S.E.M, (n=6), ANOVA followed by Dunnett's 't' test. *P<0.05, **P<0.01, as compared to control group.

Table 2. Effects of methanolic extract of *Caesalpinia bonducella* leaves against HCl-ethanol induced gastric lesion in mice.

Treatment	Dose (mg/kg)	Mean lesion index	% Ulcer inhibition
Control	1% SMC	5.67 ± 0.49	-
Sucralfate	1	0.5 ± 0.22	91.18
Methanolic extract	100	2.5 ± 0.56**	55.90
	200	1.0 ± 0.25 **	82.36

Each value is the mean±S.E.M of 6 determinations. **P < 0.01. Dunnett's 't' as compared to control value.

Table 3. Effects of methanolic extract of *Caesalpinia bonducella* leaves on water immersion stress induced ulcer in rats

Treatment	Dose (mg/kg)	Mean ulcer score	% Ulcer inhibition
Vehicle control	1% SMC	121.0 ± 0.68	-
Omeprazole	20	0.5 ± 0.22	99.58
Methanolic extract	100	19.0 ± 0.89**	84.29
	200	9.5 ± 0.61 **	92.14

Each value is the mean±S.E.M. of six determinations. **P < 0.01 Dunnett's 't' test as compared to control value.

decapitation [17]. The stomach was opened and the ulcer index, percentage inhibition of ulcer was determined [18, 19]. A score for the ulcer was made as follows:

- ◆ 0: normal coloured stomach.
- ◆ 0.5: red colouration.
- ◆ 1: spot ulcers.
- ◆ 1.5: haemorrhagic streak.
- ◆ 2: ulcers.
- ◆ 3: perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

Inhibition of ulcer (%) = (control mean ulcer index – test mean ulcer index) x 100/ control mean ulcer index.

The gastric juice was titrated against 0.01N sodium hydroxide using Topfer's reagent as indicator to find out the free acidity and total acidity [20].

2.6.2. Ulcer lesion Index method: HCl - Ethanol induced ulcer [21, 22]

Swiss albino mice weighing 24-30 gm of either sex were divided into 4 groups, each group consists of 6 animals. Group-I served as a control received 1.0 ml/kg p.o. 1% SCMC; group-II received Sucralfate (100 mg/kg p.o.) served as standard; group-III & IV received methanolic extract (100 and 200 mg/kg p.o.) of *Caesalpinia bonducella* leaves respectively. After 1 hour all the animals were treated with 0.2 ml of HCl - Ethanol mixture p.o. (0.3 M Hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were sacrificed by cervical dislocation one hour after administration of HCl - Ethanol mixture. The stomach was excised and lesion index was determined.

2.6.3. Water immersion stress induced ulcer in rats

Stress ulcers were induced by forced swimming in the glass cylinder [23] (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3 hours. Animals were fasted for 24 hours prior to the experiment and divided into 4 groups with 6 animals in each group. Group-I received 1.0 ml/kg p.o. 1% SCMC as vehicle control; group-II treated with Omeprazole (20 mg/kg p.o.) served as standard control; group-III & IV received methanolic extract (100 & 200 mg/kg p.o.) of *Caesalpinia bonducella* leaves respectively. After the drug treatment animals were allowed to swim in water for 3 hours. The stomach of each animal was removed and the extent of gastric damage was assessed [24, 25].

2.7 Statistical analysis

Results are expressed as the mean \pm SEM. The results were analysed for statistical significance by one - way analysis of variance (ANOVA) followed by Dunnett's test using computerized

Graph Pad InStat version 3.05, Graph Pad software Inc., San Diego, U.S.A

3. Results

In Aspirin plus pylorus ligation induced gastric ulcer the methanolic extract of *Caesalpinia bonducella* leaves at dose of 100 and 200 mg/kg showed significant ($p < 0.05$ and $P < 0.01$) reduction in gastric volume, free acidity, total acidity and ulcer index and there was marked increase in pH (table 1). In terms of percentage of ulcer inhibition, the methanolic extract of *Caesalpinia bonducella* at a dose of 100 and 200 mg/kg showed 53.33% and 72% activity respectively. In HCl- ethanol induced ulcer model, the plant extract at dose of 100 and 200 mg/kg showed significant gastro protective activity 55.9% and 82.36% respectively (table 2). Water immersion stress is one of the best models of stress in rats to induce ulcer. In water immersion stress, the plant extract at dose of 100 and 200 mg/kg showed significant gastro protective activity 84.29% and 92.14% respectively (table 3).

4. Discussion

In Aspirin-pylorus ligation induced gastric ulcer model the methanol extracts of *Caesalpinia bonducella* leaves attenuated the gastric volume, free acidity, total acidity and ulcer index thus showing the antiseecretory mechanism. NSAIDs can damage the gastrointestinal mucosa by local injury, when surface cells are damaged and allow acid diffusion into the submucosa, and by systemic injury, when systemic inhibition of prostaglandin synthesis occurs, thereby reducing gastric mucus production, bicarbonate secretion and mucosal blood flow. NSAIDs also delay the healing of peptic ulcers, interfere with the action of growth factors, decrease epithelial cell proliferation at the ulcer margin, decrease angiogenesis in the ulcer bed and slow the maturation of granulation tissue [26].

Omeprazole is standard control used here to test antisecretory mechanism. It is significant to note when the pH reached 3, the ulcer score appeared less. This is born out by the decrease in free acid, which might have contributed to the anti-ulcer property of the plant extract. In case of vehicle control, Aspirin plus pylorus ligation aggravated the acid secretion, which in turn caused increase in gastric volume, increased free and total acidity, low pH and increased ulcers are thought to be due to increase in offensive factors like gastric acid, pepsin and bile salts but it has been observed that gastric ulcer patients have either normal or below normal acid level in the stomach [27]. This indicates that other mechanisms are also involved in ulcer formation. Moreover the disturbance of defensive factors like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcers [28].

HCl-ethanol induced gastric damage in mice possibly through leukotrienes production and also involvement of 5-lipoxygenase in the formation of ulcer lesion. Prostaglandins also play a role in ethanol-induced ulcer. So the protective effect of the *Caesalpinia bonducella* extract against the gastric damage might be due to protection against 5-lipoxygenase or leukotriene pathway. The cytoprotective action possibly stimulates the prostaglandin synthesis, which in turn is involved in cytoprotection of the gastric mucosa. It is well known that HCl-

ethanol induced gastric lesions are not inhibited by antisecretory agents like ranitidine but are inhibited by agents who enhance mucosal defence factors such as sucralfate. The gastro protective effect in HCl-ethanol model indicates that the plant extract could enhance cytoprotective mechanism of the gastric mucosa.

In stress induced model stress increases histamine release with enhanced acid secretion which causes ulcer and reduces mucous production. Stress induced ulcers can be prevented partly or fully by vagotomy. Vagal over activity is suggested as the principle effector in stress induced ulcers [29].

Preliminary phytochemical investigations of the methanolic extract of *Caesalpinia bonducella* showed the presence of tannins, terpenoids and steroids. The results demonstrated that *Caesalpinia bonducella* extract produced antiulcerogenic effects possessing antisecretory, cytoprotective and proton pump inhibition mechanism. This study indicates that *Caesalpinia bonducella* leaves extract has potential antiulcer activity.

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