

Butea frondosa as a gastro protective against induced gastric lesions

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Abstract: The study was undertaken to evaluate the potential of alcoholic extract of *Butea frondosa* Koen. ex Roxb against gastric lesions induced in rats using acetic acid plus pyloric ligation. The impact of alcoholic extract of *Butea frondosa* in doses of 100, 200 and 400 mg/kg as single dose schedules and 200 mg/kg for 7, 14, 21 and 28 days was determined. Parameters assessed were ulcer index, total acidity, acid volume, total protein and pH, non protein sulfhydryls and gastric wall mucus. Extract in dose of 400 mg/kg as a single schedule significantly reduced ulcer severity, total protein and pH as against the control ($p < 0.05$). Concurrent findings were also observed with 200 mg/kg administered for 21 and 28 days. Treatment with 400 mg/kg of the extract as a single dose and 200 mg/kg for 28 days produced an elevation in the content on non protein sulfhydryls. Gastric wall mucus was enhanced with 200 mg/kg of the extract administered for 28 days with a value of $186 \pm 2.74 \mu\text{g}$ Alcian blue/g wet weight compared with the control ($p < 0.05$). A palpable decline in incidence of ulcers was observed with the extract which might be largely due to the presence of flavonoids.

Introduction

The biological design of the gastroduodenal lining maintains a harmonious balance between the offensive and defensive factors. One of the most common gastroduodenal disorders is peptic ulcer responsible for a high rate of morbidity particularly in population of non-industrialized countries. Gastric mucosa is constantly exposed to exogenous and endogenous irritants which impinge the integrity of the gastroduodenal lining. In contrast to the conventional opinion that only gastric acid is a culprit for ulceration, contemporary views revolve around the fact that acid/pepsin mixture disrupts the mucosal barrier. Several factors are implicated in the pathogenesis of gastric ulcer. Increased acid-pepsin secretion, impaired bicarbonate neutralization and impaired mucus secretion are the most prominent of them. A staunch balance exists between the aggressive (acid, pepsin, active oxidants, *Helicobacter pylori*) and the mucosal protective (mucus, bicarbonate, prostaglandins) factors in stomach (Kent-Lloyd & Debas, 1994). Drug therapy for peptic ulcers commonly targets counteracting the aggressive factors or stimulating defensive ones. Considerable progress is being made in the field of

pharmacology with the emergence of newer therapeutic agents; however the adverse effects associated with their use are many. In view of this, herbs might provide new anti-ulcer compounds or can serve as simple dietary adjuncts to existing therapies.

Butea frondosa K.D. Koenig ex Roxb., Fabaceae, commonly referred to as the 'flame of the forest' is extensively used in the Indian traditional system of medicine against ulcers, skin diseases, herpes, acne, boils, gas colic, worms and piles (Nadkarni et al., 2002). Phytochemically, it is rich in flavanoids, terpenoids and lipid constituents (Mishra et al., 2000). The anti-inflammatory (Mengi & Deshpande, 1999), antistress and nootropic activity (Soman et al., 2004) of *Butea frondosa* has already been established so far. Although *B. frondosa* has been widely advocated in folk medicine for the treatment of ulcers as well as for gas colic, no studies have been undertaken so far to ascertain its role in preventing gastric ulceration. Secondly, the presence of abundant flavanoids in *B. frondosa* might play a role in preventing the formation of ulcers. Therefore the present study examined the anti-ulcer potential of stem of *Butea frondosa*.

Article

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Materials and Methods

Drugs

Bovine serum albumin was purchased from Sigma Chemical Company, Saint Louis, MO, USA; DTNB (5-5 dithiobis-2 nitrobenzoic acid) was procured from M/S Sisco Research Laboratories Pvt. Ltd, Mumbai, India. All solvents and chemicals utilized were of analytical grade.

Plant material and preparation of extract

The fresh stem of *Butea frondosa* K.D. Koenig ex Roxb., Fabaceae, was collected locally in the month of November 2004 and identified by Prof. Dr Sidappa. A voucher specimen is maintained in our herbarium bearing number BF/2. The biomass was dried in the shade, pulverized to a coarse powder and 50 g of stem powder was weighed. Extraction was then carried out with 100 mL of 50% v/v ethanol using soxhlet continuous extractor by maintaining the temperature at 60 °C for 6 h. The extract was evaporated under vacuum (4.3% w/w) and stored in a refrigerator. The required quantity of alcoholic extract of stem of *Butea frondosa* (AESBF) was suspended in 1.0% aqueous solution of tragacanth and used.

Phytochemical screening

AESBF was subjected to preliminary phytochemical screening to detect the presence of alkaloids, tannins, saponins, terpenes, coumarins and anthraquinones according to established methods (Harborne, 1973).

Animals

Albino rats weighing 180-200 g of either sex were used in this study. They were maintained in a temperature controlled animal house (28±2 °C) on a 12 h light-dark cycle. Food and water was provided *ad libitum*. They were divided into five groups for the single dose and six groups for the time dependent studies. Each group contained six animals. Clearance to carry out the work was obtained from the Institutional animal ethical committee bearing no. IAEC/Clear/ 27/2004-05 dated 3 Feb 2005.

Dose fixation

Wistar rats were treated with AESBF up to a dose of 2 g/kg *p.o.* as per OECD guidelines No. 425. Observations were made for gross behavioural changes such as locomotion, rearing, respiration, tremors, gait, passivity, righting reflex, lacrimation and mortality in the first 3 h and monitored continuously for fourteen days (OECD, 2001).

Grouping of animals

In the single dose regimen, Group-1 was the control and received 1% aqueous tragacanth (1 mL/kg), group-2 represented the reference standard and received famotidine (3 mg/kg) orally, groups-3, 4 and 5 received orally 100, 200 and 400 mg/kg body weight (b.w.) dose of AESBF respectively. In the time dependent schedule, group-1 received aqueous tragacanth for fourteen days and served as the control, group-2 received famotidine (3 mg/kg) orally for fourteen days, groups-3, 4, 5 and 6 were administered with 200 mg/kg b.w. of AESBF orally for 7, 14, 21 and 28 days respectively.

Acetic acid and pyloric ligation induced ulcer model

Thirty minutes after the administration of the extract, 15% acetic acid was administered orally at a dose of 0.05 mL/rat and served as an ulcerogen (Okabe & Pfeiffer, 1992). A midline incision was then made under light enflurane anaesthesia and the pylorus was ligated. The abdominal wall was closed by sutures and 3 h after pyloric ligation the animals were sacrificed. Stomach was dissected out, small nick was made along the greater curvature and contents were drained into a graduated centrifuge tube from which acid volume was determined. The contents were centrifuged at 806.4 g for 10 min. Total acidity and pH was analysed from the decanted supernatant. pH was determined using digital pH meter (Type DPH - 100- Data instruments). Total acidity was ascertained by titrating with 0.1 N NaOH using phenolphthalein as indicator and expressed as mEq/L (Parmar & Desai, 1993).

Ulcer index

The stomach was opened along the greater curvature and fixed on a cork plate. The number and severity of ulcers was registered using scores (Kulkarni, 2002). Ulcer index was calculated as: $UI = UN + US + UP \times 10^{-1}$, where UI: ulcer index; UN: average number of ulcers per animal; US: average of severity score; and UP: percentage of animals with ulcer.

Estimation of total protein

Total protein was quantified using bovine serum albumin as a standard (Lowry et al., 1951).

Determination of gastric wall mucus (GWM)

The weighed glandular segment of the stomach was transferred immediately to 10 mL of 0.1% w/v Alcian blue solution. Staining of tissue with Alcian blue was carried out for 2 h and excess dye was removed after 15 and 45 min by two successive rinses with 10 mL of 0.25

mmol/L sucrose. Dye complexed with gastric wall mucus was subjected to extraction with 10 ml of 0.5 mmol/L magnesium chloride which was intermittently shaken for 1 min at 30 min intervals for 2 h. Blue extract (4 mL) was vigorously shaken with an equal volume of diethyl ether. After centrifugation of the resulting mixture at 1433.6 g for 10 min, the absorbance of the aqueous layer was read at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated (Corne et al., 1974).

Estimation of non-protein sulfhydryls (NP-SH)

Gastric mucosal non-protein sulfhydryls were determined by homogenization of the glandular part of the stomach in ice-cold 0.02 mmol/L ethylenediaminetetraacetic acid (EDTA). Homogenates (5 mL) were mixed with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 806.4 g. Two milliliters of supernatant were mixed with 4 mL of 0.4 mol/L Tris buffer at pH 8.9. 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added in a volume of 0.1 mL and the sample was shaken. The absorbance was measured within 5 min of DTNB addition at 412 nm against a reagent blank (Sedlak & Lindsay, 1968).

Statistical analysis

The results are expressed as mean±SEM and analysed by ANOVA followed by Dunnet's post hoc test using Graph InStat, version 3.01, GraphpadSoftware Inc. $p < 0.05$ was considered statistically significant compared with the control.

Results

Phytochemical screening

Preliminary screening of AESBF revealed the presence of flavonoids and terpenes with absence of alkaloids, saponins and anthraquinones.

Safety evaluation

AESBF was safe up to a dose of 2000 mg/kg as animals failed to exhibit any behavioral changes. Therefore 100, 200 and 400 mg/kg of the safe dose were utilized in order to obtain geometric increase in the dose.

Effect of AESBF on acid volume, total acidity, ulcer index, total protein and glutathione content in acetic acid plus pyloric ligation induced ulcer model

Macroscopic evaluation of the gastric mucosa following treatment with acetic acid plus pyloric ligation (control) revealed elevation in total acid output, acid volume, ulcer index with values of 114.1±0.70 mEq/L, 8.25±0.53 ml and 11.6±1.07 respectively. A change in the pH to 2.6±0.19 and total protein to 0.80±0.03 g/dL was also observed in the control group. 100 mg/kg of the extract elicited a nominal change in the levels of total acid, acid volume, pH, ulcer index and total protein. Treatment with 200 and 400 mg/kg body weight of AESBF produced a pronounced change in these profiles compared with the control ($p < 0.05$) (Table 1, Figure 1).

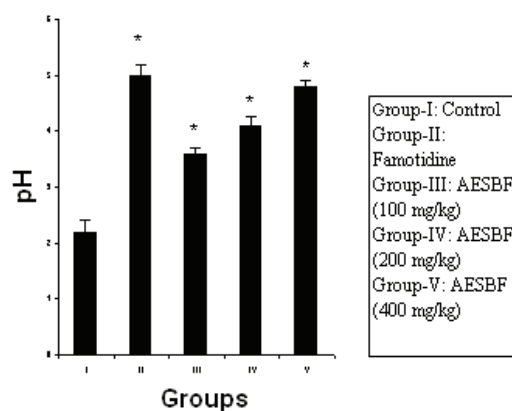


Figure 1. Effect of alcoholic extract of stem of *Butea frondosa* on pH in dose dependent studies. Values seen are mean±SEM, n=6, * $p < 0.05$ compared with control. AESBF: alcoholic extract of stem of *Butea frondosa*.

Table 1. Effect of various doses of alcoholic extract of stem of *Butea frondosa* on biochemical parameters in rats in acetic acid plus pyloric ligation induced ulcer model.

S.No	Treatment	Dose (mg/kg)	Ulcer Index	Total Acidity (mEq/L)	Acid Volume (mL)	Non-protein sulfhydryls (mol/g wet weight)	Total protein (g/dL)
1	Control	-----	11.6±1.07	114.1±0.70	6.25±0.53	1.89±0.01	0.90±0.03
2	Famotidine	3	4.1±0.70*	56.4±1.41*	3.75±0.35*	3.65±0.01*	0.61±0.06*
3	AESBF	100	8.6±0.42*	80.8±1.12*	6.2±0.45	1.95±0.09	0.85±0.05*
4	AESBF	200	5.6±0.28*	75.0±0.90*	5.58±0.60	2.3±0.02*	0.67±0.02*
5	AESBF	400	2.4±0.18*	59.1±1.23*	3.31±0.33*	3.2±0.01*	0.63±0.02*

Values seen are mean±SEM, n=6, * $p < 0.05$ compared with control. AESBF: alcoholic extract of stem of *Butea frondosa*.

Table 2 represents the impact of 200 mg/kg of AESBF following treatment for 7, 14, 21 and 28 days. Continued treatment for 28 days produced a significant decrease in total acidity, acid volume and total protein with values of 55.0 ± 1.05 mEq/L, 3.0 ± 0.37 mL and 0.52 ± 0.02 g/dL respectively compared with the control ($p < 0.05$). Concurrently, a significant change was also seen in the pH compared with the control ($p < 0.05$, Figure 2).

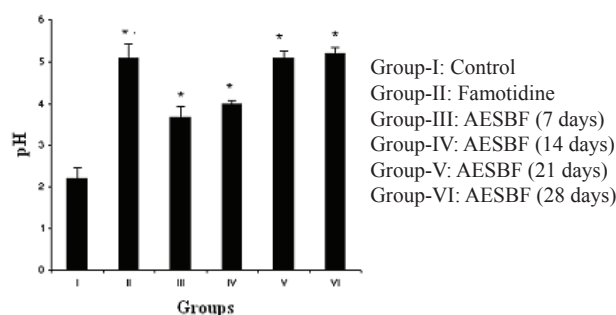


Figure 2. Effect of alcoholic extract of stem of *Butea frondosa* on pH in time dependent studies. Values seen are mean±SEM, n=6, * $p < 0.05$ compared with control. AESBF: alcoholic extract of stem of *Butea frondosa*.

Effect of AESBF on non-protein sulfhydryls (NP SH) in acetic acid plus pyloric ligation induced ulcer model

The levels of non protein sulfhydryls were not significantly altered following single dose treatments (Table 1). Treatment for 21 and 28 days with AESBF produced an elevation in the value to 3.96 ± 0.08 and 5.13 ± 0.05 mol/g wet weight. Both these values were significant compared with the control ($p < 0.05$) (Table 2).

Effect of AESBF on gastric wall mucus (GWM) in acetic acid plus pyloric ligation induced ulcer model

Control rats subjected to pylorus ligation plus acetic acid treatment exhibited a significant decrease in the Alcian blue binding capacity of gastric wall mucus depicting a value of 125.8 ± 2.26 µg Alcian blue/g wet

weight of tissue. Treatment with AESBF at dose of 100 mg/kg produced only a scarce increase in Alcian binding; however treatment with 400 mg/kg of AESBF depicted a significant enhancement in Alcian blue binding capacity of gastric mucus to 145.2 ± 2.60 µg Alcian blue/g wet weight which was significant compared with the control ($p < 0.05$, Figure 3). Treatment with 200 mg/kg AESBF for 28 days exhibited a significant increase in gastric wall mucus binding to 186 ± 2.7 µg Alcian blue/g wet weight which was significant with the control ($p < 0.05$, Figure 4).

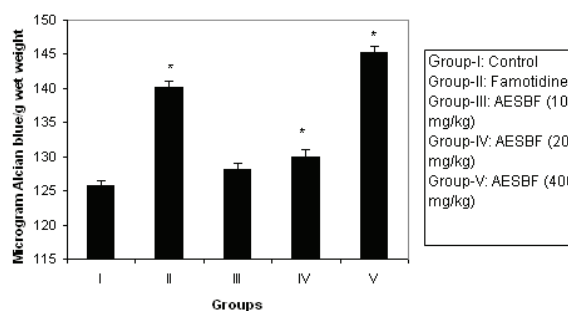


Figure 3. Effect of alcoholic extract of stem of *Butea frondosa* on gastric mucus in dose dependent studies. Values seen are mean±SEM, n=6, * $p < 0.05$ compared with control. AESBF: alcoholic extract of stem of *Butea frondosa*.

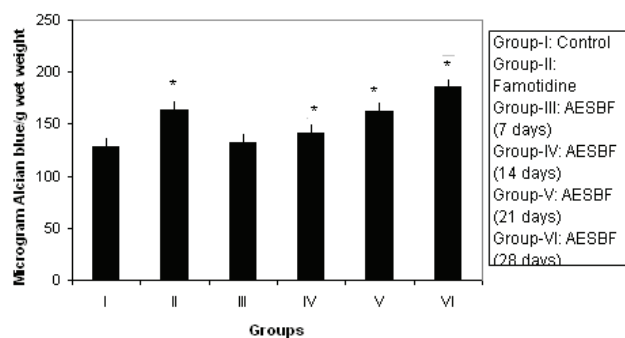


Figure 4. Effect of alcoholic extract of stem of *Butea frondosa* on gastric mucus in time dependent studies.

Table 2. Effect of the alcoholic extract of stem of *Butea frondosa* on biochemical parameters in rats in acetic acid plus pyloric ligation induced ulcer model following treatment for different durations.

S.No	Treatment	Dose (mg/kg)	Duration (days)	Ulcer index	Total acidity (mEq/L)	Acid volume (mL)	Non-protein sulfhydryls (mol/g wet weight)	Total protein (g/dL)
1	Control	-----	14	12.1±0.42	121.2±1.13	6.40±0.42	1.79±0.01	0.92±0.08
2	Famotidine	3	14	3.5±0.48*	58.3±0.95*	3.15±0.33*	3.90±0.19*	0.65±0.07*
3	AESBF	200	7	8.1±0.40	96.0±1.09*	5.20±0.50	2.72±0.03	0.68±0.01*
4	AESBF	200	14	3.6±0.50*	91.4±1.14*	5.0±0.43	2.81±0.02*	0.62±0.01*
5	AESBF	200	21	3.1±0.41*	61.3±1.01*	4.2±0.33*	3.96±0.08*	0.55±0.03*
6	AESBF	200	28	0.00*	55.0±1.05*	3.0±0.37*	5.13±0.05*	0.52±0.02*

Values seen are mean±SEM, n=6, * $p < 0.05$ compared with control. AESBF: alcoholic extract of stem of *Butea frondosa*.

Discussion

Amongst an array of disorders of the gastrointestinal tract, the most prevalent with a significant clinical impact are peptic ulcers. Disruption of mucosal integrity ensues due to an imbalance between aggressive factors (acid, pepsin, NSAID) and local mucosal defensive factors (mucus, bicarbonate, blood flow and prostaglandins). Mucosal barrier is relatively sturdy, resisting the back diffusion of acid under normal circumstances. However due to perturbation induced by ulcerogens, this barrier might be affected (Freston, 1960).

Acetic acid plus pylorus ligation model was utilized in our study. Acetic acid was employed as an ulcerogen and was capable of inducing severe haemorrhagic lesions due to penetration of the gastric mucosa when administered intragastrically. It also suppresses mucosal defense by reducing mucus secretion. Acetic acid induced lesions bear a close correspondence to clinical ulcers in terms of location, chronicity and severity (Okabe & Pfeiffer, 1992). A tangible impact on the gastric mucosa can be induced by acetic acid and pyloric ligation.

One of the major therapeutic targets in the management of peptic ulcer is to regulate the secretion of gastric acid. In light of this concept, famotidine which served as our reference standard exerted its antisecretory profile by inhibiting histamine induced secretion of hydrogen ions and significantly diminished peptic activity (Sener-Muratoğlu et al., 2001). AESBF administered in a dose of 400 mg/kg body weight in a single schedule reduced the volume of gastric acid, total acidity, ulcer index and altered pH of gastric contents possibly by controlling the secretion of gastric juice by the parietal cells and exerted an antisecretory effect. 200 mg/kg of the extract showed a fair response and therefore the impact of this dose for prolonged periods was determined in our study. A reduction in the gastric vascular permeability and suppression of the neutrophil cytokine cascade in the gastrointestinal tract might be an added advantage with AESBF (Alarcon et al., 1995), as *Butea frondosa* is already proved to possess anti-inflammatory action. However, it may be coherent to state that AESBF might not augment the formation of prostaglandins due to its anti-inflammatory action.

High protein content in gastric juice is reflective of leakage of plasma protein through the mucosal barrier during injury by ulcerogens (Mizushima & Kobayashi, 1967). AESBF minimises injury as evidenced from a reduction in protein content of gastric juice. Mucosal erosions are facilitated by proteolytic enzymes such as pepsin which is capable of hydrolyzing mucosal proteins. The gastric mucus coat is thought to be important in facilitating the repair of damaged gastric epithelium (Wallace & Whittle, 1986). AESBF was efficient in raising

the levels of gastric mucus in doses of 400 mg/kg and following 21 and 28 days administration.

As indicated in several studies, induction of severe gastric lesions causes the loss of non protein sulfhydryl compounds like glutathione (GSH) (Gunzler & Flohe, 1985). GSH exerts a pivotal role in maintenance of gastric integrity (Dutta et al., 2007). Oxidative damage to the mucosal lining could be minimized by superoxides scavenger like GSH. It has been documented that flavonoids stimulate mucus and bicarbonate secretion (Minaiyan et al., 2006). Bioflavonoids are particularly useful in soothing inflamed and irritated mucus membrane of the stomach. Bioflavonoids are also capable of scavenging free radicals (Pattipati et al., 2003). AESBF was found to elevate the levels of glutathione in doses of 200 mg/kg when treatment was continued for 28 days possibly due to the presence of bioflavonoids as a vital constituent. In conclusion, a palpable decline in the incidence of ulcers was observed following treatment with AESBF due to its anti secretory and mucosal defensive action.

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