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Gastroprotective effect of leaf extracts of *Basella alba* var. *alba* against experimental gastric ulcers in rats

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Abstract: The aqueous and ethanol extracts of the leaves of *Basella alba* L. var. *alba* Wight, Basellaceae, were investigated for antiulcer activity on rats employing the pylorus ligation and ethanol induced ulcer models. The various gastric secretion parameters such as total acidity, free acidity, gastric acid volume, pH and histopathological parameters such as ulcer index and percent protection were comparatively examined between control, test and standard groups. The antiulcer activity of aqueous extract of *B. alba* (AEBA) and ethanol extract of *B. alba* (EEBA) were studied in rats treated with the doses of 1 mL/kg of absolute ethanol, 200 and 400 mg of test extracts and 20 mg/kg of famotidine for control, test and standard groups respectively in both the models. The animals pretreated with AEBA and EEBA showed a dose-dependent protection against gross damaging action of ethanol and pylorus ligation on gastric mucosa of animals. Histopathological evaluation also revealed that Group I treated with absolute ethanol showed severe gastric mucosal damage. The AEBA and EEBA showed 68.25 and 58.11% protection in gastric mucosal damage as compared to control group. Both the extracts of *B. alba* var. *alba* were able to decrease the gastric acidity and increase the mucosal defense in the gastric mucosal area. This study indicate that *B. alba* var. *alba* possesses significant gastroprotective effect and the same is substantiated by the histopathological examination of the ulcerated stomachs of the animals.

Introduction

Basella alba L. var. *alba* Wight, Basellaceae, is known as “Poi” (Hindi), “Potaki” (Sanskrit) and “Indian spinach” in English (Nandkarni, 19760). The stems and leaves of *B. alba* are thick, sweet, mucilaginous and used in constipation, as diuretic, in urticaria, as demulcent, antiulcer, and as cooling application for burn (Pareek et al., 2010). Peptic ulcer occurs in two main forms *i.e.* acute peptic ulcer and chronic peptic ulcer. Acute peptic ulcer penetrates the lamina muscularis mucosa but does not extend more deeply than the submucosa. It is mainly related to stress and present in the form of severe burns (Curlings ulcer) and brain damage (Cushing’s ulcer). The chronic peptic ulcer which penetrates the full thickness of the muscularis propria and has its base in the serosal layer of the organ involved or out with the gut altogether. The common forms of peptic ulcer are duodenal ulcer (DU), gastric ulcer (GU), stress ulcer, non steroidal anti-inflammatory drug (NSAID) induced ulcers and recurrent oral ulceration also known as aphthous ulceration (Vyawahare et al., 2009). The etiology of gastro duodenal

ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori* may be responsible for the development of peptic ulcer (Khandare et al., 2009). A number of synthetic antiulcer drugs such as H₂-receptor antagonists, proton pump inhibitors and cytoprotectants are available for the treatment of ulceration. All these drugs have a number of side effects and limitations. We have studied the antiulcer potential of *B. alba* leaf extracts and compared it with famotidine as standard drug. *B. alba* is used as vegetable in Tripura and in traditional medicinal preparations especially for the treatment of constipation, as diuretic, in urticaria, as demulcent, antiulcer, as cooling application for burns and in toothache. This study revealed a significant anti-ulcer effect of the leaf extract of *B. alba* justifying its use in traditional system of medicine for the treatment of ulcer.

Materials and Method

Plant material

The fresh leaves of *Basella alba* L. var. *alba* Wight, Basellaceae, were purchased from vegetable market of Tripura in the month of Nov 2008. The plant material was identified (Ref. - PARC/2009/258) by Prof. P Jayaraman; Director, PARC, National Institute of Herbal Science, W. Tambaram (Chennai).

Preparation of extracts

The fresh leaves of *B. alba* var. *alba* were shade dried and powdered. The powdered leaf material was cold extracted separately with ethanol and distilled water. After filtration, the filtrate obtained was concentrated using rotary vacuum evaporator and then dried in lyophilizer (Lobcono, USA) under reduced pressure to get solid mass. The yield of aqueous and ethanol extracts obtained were 8.06 and 6.18 % respectively.

Phytochemical screening

The AEBA and EEBA were screened for various phytoconstituents such as alkaloids, carbohydrates, glycosides, proteins, sterols and terpenoids as per described methods (Khandelwal & Kokate, 1995) and the same have been reported earlier as well (Kumar et al., 2011)

Experimental animals

Albino wistar rats of either sex weighing about 180-210 g were used for the study. The animals were housed in polyethylene cage at 26±2 °C and relative humidity of 55-60%, light and dark cycle of 12 h each respectively for one week before and during the experiments. Animals were provided with commercial rodent pellet diet (Hindustan Lever) and water *ad libitum*. All animal in experiments were carried out in accordance with guidelines of CPCSEA (Committee for the purpose of control and supervision on experiments on animals) and the study was approved by the Institutional Animal Ethical Committee as (Ref.-IAEC-48/2008).

Acute oral toxicity studies

Acute toxicity study was evaluated as per described guidelines (Ecobichon, 1997) in which the rats were fasted overnight and treated with the leaf extracts of *B. alba* var. *alba* at doses of 100-2000 mg/kg *p.o.* Increasing doses of extract were administered individually to groups of ten animals and the same were observed daily for seven days. Animals were observed

individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of seven days. All the animals remained alive and there were no significant behavioral and body weight changes during the observations period in which the animals were monitored on regular basis.

Antiulcer studies

Pylorus ligation model

Pylorus ligation was performed as described by method (Shay, 1945). Albino rats of either sex were divided into six groups, each group consists of six animals. Group-I represented the control group and was served distilled water (vehicle) orally. Group-II and Group-III served AEBA 200 and 400 mg/kg each, respectively. Group-IV and Group-V served EEBA 200 and 400 mg/kg each, respectively. Famotidine, 20 mg/kg, was administered orally for Group-VI as standard drug. All the drugs were administered once daily for five days. The doses were calculated with respect to the body weights of animals and administered orally. On day 6 after the last dose, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. After 4 h of pylorus ligation, stomachs were dissected out and cut open along the greater curvature and gastric juice was collected and centrifuged.

Gastric juice, total acidity and free acidity estimation

Four hours after pyloric ligation the rats were sacrificed and the stomach was removed. Gastric juice was collected, centrifuged for 5 min at 3000 x g, supernatant separated and then analyzed for volume, pH, free acidity and total acidity. Total acid was estimated by titrating against 0.01N sodium hydroxide using Topfer's reagent as indicator to find out the free acidity and total acidity expressed as mEq/L (Maity et al, 1986).

Ethanol induced ulcer

The experiment was performed as per described method (Deshpande et al., 2003). After 24 h of fasting, the rats were randomly divided into six groups of six animals each. Group-I represented the control group, which received distilled water + 1 mL/kg of absolute ethanol orally. Group-II and Group-III received AEBA 200 and 400 mg/kg, respectively. Group-IV and Group-V received EEBA 200 and 400 mg/kg, respectively. Famotidine, at a dose of 20 mg/kg, was administered orally to Group-VI as reference standard drug. After 45 min of AEBA, EEBA and famotidine treatment, all animals received 1 mL/kg of absolute ethanol orally. They were kept in specially

constructed cages to prevent coprophagia. The animals were anaesthetized 1 h later of ethanol administration with anesthetic ether and their stomachs removed immediately and fixed in 10% buffered formalin (Deshpal et al., 2003). Ulcer index was obtained by dividing the total area of the lesions in the stomach by the area of the glandular portion of stomach.

Histopathological evaluation of gastric lesions

All the groups of animals were fasted over-night prior to being sacrificed. The animals were anesthetized with ether and a midline incision was made in the abdomen to expose out the abdominal organs. The stomach tissue was taken out and preserved in 10% formalin before microscopic examination. The tissues were then fixed in normal saline for 24 h and processed through a series of ethyl alcohol of ascending strength (50, 60, 70, 80 and 95%) for period of 1 h, twice in absolute ethanol for 1 h each and twice in xylene for 1 h in order to render the tissue elements transparent. The transparent tissues after being cleared of all the elements were embedded in a solid mass of paraplast. The blocks were labelled, allowed to cool and the metal blocks were removed. The solid mass was then trimmed free of excess paraplast, leaving some free margins around the embedded tissues. Five microns thick longitudinal sections were cut with a rotary microtome. The sections were mounted on thoroughly cleaned gelatinized slides and were placed on hot plates at 37 °C for 24 h for proper fixation. The slides were then stained by hematoxylin and eosin stain according to the prescribed staining method (Bancroft & Stevens, 1990). The stain was prepared by dissolving hematoxylin in absolute ethanol. The mixture was boiled rapidly and mercuric oxide was then added. The stain was cooled rapidly in cold water bath; glacial acetic acid was then added and the stain was ready for immediate use. Several stained slides prepared, after drying and labeling were preserved and stored for histopathological studies before microscopic examination for comparative morphological and pathological changes in the control, test drug and standard drug treated gastric tissues of the

rat stomach, examined using arbitrary scale (Shah & Khan, 1973). Grossly (1.8x) with a square-grid eye piece (big square, length x width=10x10 mm²=ulcer area) to assess the formation of ulcer area (hemorrhagic lesions). The length and width of each lesions was determined as (Abdulla et al., 2009) and the sum of the area of all lesion for each stomach was expressed as the ulcer area (mm²). Protection ratio of each fraction was calculated using the formula:

$$\text{Protection ratio} = 100 - \frac{\text{Ulcer index (test)}}{\text{Ulcer index (control)}} \times 100$$

Statistical analysis

The data are expressed as mean±SEM. Statistical data comparisons were performed using one way ANOVA followed by Dunnet's 't' test. The results were considered statistically significant if ($p < 0.05$).

Results

Phytochemical screening

The AEBA showed the presence of amino acids, carbohydrates, flavonoids, proteins, mucilage and saponins whereas EEBA contained amino acids, carbohydrates, favonoids, fixed oil, mucilage, saponins and tannin.

Effect of AEBA and EEBA on pyloric ligation ulcer

The effect of AEBA and EEBA at dose levels of 200 and 400 mg/kg and famotidine (20 mg/kg) as standard drug on the gastric volume, total acidity, free acidity and pH are shown in Table 1. Rats pretreated with AEBA and EEBA showed dose dependent decrease in gastric volume (4.2±0.12 and 4.9±0.16 mL, respectively), reduced free acidity (41.6±0.97 and 50.4±0.44 mEq/L, respectively), decreased total acidity (48.07±0.88 and 55.66±0.33 mEq/L, respectively) and increased pH value of 6.7±0.08 and

Table 1. Effect of plant extract of *Basella alba* var. *alba* leaf extract on gastric secretion following pyloric ligation induced ulcer in rats.

Treatment	Dose (mg/kg)	Vol. of gastric juice (ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)
Control	--	9.3±0.12	2.6±0.12	96.2±1.24	102.3±1.94
Famotidine	20	3.3±0.08**	7.2±0.16**	31.2±0.16**	38.2±0.86**
AEBA	200	5.2±0.11*	5.9±0.12**	48.8±0.18*	57.1±0.27*
AEBA	400	4.2±0.12**	6.7±0.08**	41.6±0.97**	48.1±0.88**
EEBA	200	5.9±0.09*	5.0±0.12*	52.1±1.96*	59.5±0.11*
EEBA	400	4.7±0.16*	5.9±0.10**	50.4±0.44*	55.7±0.33*

Values are the mean±SEM of n=6 rats/treatment. Significance * $p < 0.05$, and ** $p < 0.01$.

5.9±0.10, respectively at 400 mg/kg *p.o.* as compared to control which showed much higher values with a gastric volume of 9.3±0.12 mL, free acidity value of 96.2±1.24 mEq/L, total acidity of 102.3±1.94 mEq/L and a pH value of 2.6±0.12. Famotidine showed the most significant activity as compared to control but the effect of EEBA and AEBA were almost similar to it.

Effect of AEBA and EEBA on ethanol induced ulcer

The antiulcer effect of AEBA, EEBA and famotidine were comparatively studied in ethanol induced gastric ulcer in albino rats. Group I treated with absolute ethanol alone showed gross gastric mucosal lesions in rat's stomach. Group II-V treated with aqueous and ethanol extract at doses 200 and 400 mg/kg showed dose dependent gastroprotection and simultaneously decreased the ulcer index. The highest gastroprotection and reduction in ulcer index (1.95±0.04) was obtained in rats treated with 20 mg/kg of famotidine. AEBA (400 mg) and EEBA(400 mg) showed an ulcer index value of 2.20±0.42 and 3.51±0.08, respectively. The control group showed an ulcer index value of 6.9±0.07 which was comparatively much higher than animals treated with AEBA, EEBA and famotidine. The percentage protection in ethanol induced ulceration were found to be 71.73, 68.11 and 51.28% for famotidine, AEBA and EEBA respectively (Table 2).

Table 2. Percentage (%) protection of plant extracts of *Basella alba* var. *alba* leaf extracts in ethanol induced ulcer model.

Groups	Dose (mg/kg/oral)	Ulcer index	% Protection
Control	1 mL	6.90±0.07	-
Famotidine	20	1.95±0.04**	71.73**
EEBA	200	4.64±0.43*	33.19
EEBA	400	3.51±0.08*	51.28*
AEBA	200	4.10±0.09*	40.98*
AEBA	400	2.20±0.42**	68.11**

All values are the mean±SEM (n=6 rats/treatment) **p*<0.05 and ***p*<0.01.

Histopathological examination of ulcerated stomach of experimental rats

The cytoprotective effect was confirmed by histological examination. In the rats treated with absolute ethanol, there was markedly extensive damage to the gastric mucosa due to severe disruption of the surface epithelium, deep penetration of necrotic lesions into mucosa and edema of the submucosa layer with leukocyte infiltration of ulcerative tissues (Figure 1). However, rats treated with famotidine, AEBA and EEBA showed gastric mucosal protection as compared to control. Rats

treated with EEBA 200 or 400 mg/kg showed a marked reduction in ulcer areas, milder disruption of the surface epithelium and inhibition of edema and leukocyte infiltration of the submucosal layer. The rats treated with AEBA 200 or 400 mg/kg showed a mild disruption of the surface epithelium and edema of the submucosa layer with leukocyte infiltration which is comparatively similar to the effects of famotidine (20 mg/kg). Grossly, the leaf extracts of *Basella alba* has shown a substantial and significant protection against gastric ulcers in rats.

Discussion

In order to probe the traditional claim of *Basella alba* leaf extracts in gastric ulcer, we studied the effectiveness of AEBA and EEBA in preventing gastric ulcer and also assess their antisecretory activity. They were tested against ethanol induced and pylorus ligation induced ulcer in rats. Pylorus ligation induced ulcers are results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCl in the stomach (Sairam et al., 2003). Gastric acid is an important factor for the genesis of ulceration in pylorus ligated rats (Shay et al, 1945). The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligation is believed to increase gastric acid secretion (Baggio et al., 2003). The current data clearly demonstrated that, AEBA is comparatively better than EEBA and showed a dose-dependent decrease in hydrogen ion concentration suggesting that the pharmacological mechanism has a relationship to antisecretory and cytoprotective activity (Table.1) in experimental models of gastric lesion induced by absolute ethanol. Ethanol causes severe gastric mucosal ulceration either by direct corrosive effect on the gastric mucosal epithelium or by the release of vasoactive products from mast cells (Szabo, 1987) or release of histamine (Oates & Hakkinen, 1988). The vascular changes in ethanol-induced gastric mucosal injury and severity of the damage in such injury are associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of blood flow in mucosa (Gaskil et al., 1982). Ethanol also produces massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. Flavonoids (like quercetin, catechin) seems to play a very important role in the prevention and treatment of peptic ulcer (Middleton et al., 2000). It acts by promoting mucus secretion, thereby serves as gastro protective agent. In addition to this, quercetin has been shown to inhibit the growth of *H. pylori* bacterium *in-vitro* studies. Catechin interferes with the formation of histamine in gastric mucosa and hence produces the protective effect

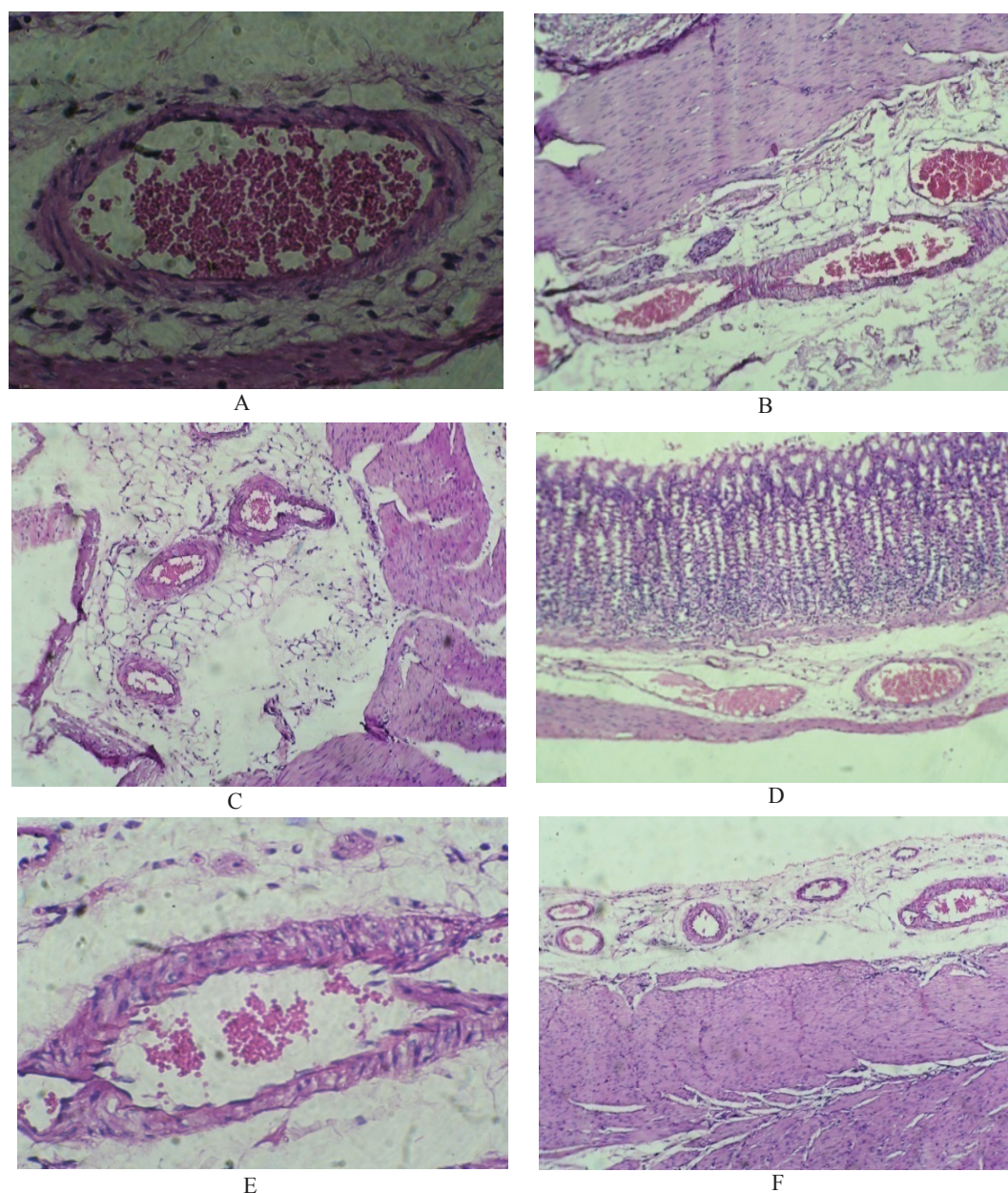


Figure 1. Histological evaluation of gastric lesions of ulcerated rats stomach. A. Control (distilled water), (H&E stain, 10 \times); B. AEBA (200 mg/kg), (H&E stain, 10 \times); C. AEBA (400 mg/kg), (H&E stain, 10 \times); D. EEBA (200 mg/kg), (H&E stain, 10 \times); E. EEBA (400 mg/kg), (H&E stain, 10 \times); Standard (20mg/kg), (H&E stain, 10 \times).

(Middleton et al., 2000). Preliminary phytochemical tests on the aqueous as well as alcoholic extracts of *Basella alba* have shown the presence flavonoids as well as tannins and this could be responsible for its anti-ulcer activity. The presence of mucilage in the plant could also play a role in cytoprotection. The exact mechanisms underlying the protective action of the leaf extract against ethanol induced gastric lesions are unclear. Further studies are in progress in this lab to explore the compounds responsible for the protective effect and the mechanism of this activity.

Conclusion

The present study reveal that the leaf extracts of *Basella alba* var. *alba* possesses statistically significant antiulcerogenic activity. The activity may be due to enhancement of defensive mechanism through an improvement in gastric cytoprotection or inhibition of acid secretion or both.

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