Antiulcer Activity of Methanolic Extract of Roots of Beta vulgaris, Chenopodiaceae

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ABSTRACT
Beta vulgaris (chenopodiacea) is a medicinal plant reported for its variety of ethnic medicinal uses. Beta vulgaris showed antioxidant, anticancer, hepatoprotective, nephroprotective, wound healing, and anti-inflammatory activities. Hence we have planned to screen antiulcer activity of root of the plant with the methanolic extract. Root powders successively extracted with methyl alcohol and were subjected for phytochemical screening to identify different phytoconstituents. The methanolic extracts of roots of Beta vulgaris were investigated for ulcer protective activity against pyloric-ligation, ethanol induced gastric lesion and cold restraint stress induced ulcers. Preliminary phytochemical screening revealed the presence of flavonoids, saponins, sterols, and alkaloids. The extract was tested for their lethal effect up to the dose level of 2000 mg/kg. None of them have produced abnormal behavior or mortality in rats. Further methanolic extract of 200 and 400 mg/kg/p.o significantly (p<0.01) reduced the ulcer score, ulcer number, ulcer index, free acidity and total acidity in pyloric-ligation, ethanol induced gastric lesion and cold restraint stress induced ulcer models in rats. The present study revealed that the root extract of Beta vulgaris has antiulcer activity.

Keywords: Pyloric-ligation, gastric lesion, ulceration, wound healing.

INTRODUCTION
Peptic ulcer (comprising gastric ulcer and duodenal ulcer) is a major health hazard both in terms of morbidity and mortality. A peptic ulcer results from an imbalance between some endogenous aggressive factors [hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS)] and cytoprotective factors, which include the function of the mucous-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood...

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flow, cell renewal and migration, nonenzymatic and enzymatic antioxidants and some growth factors. [1-4] Peptic ulcer disease affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs. [5] The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection. [6] Peptic ulcer disease and gastric dyspepsia-associated with chronic use of therapeutic agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and anticancer agents are the two major causes that adversely affect the life quality. Presently used antisecretory agents like proton pump inhibitors may represent a key option in peptic ulcer therapy [7] but their prolonged use seems to be associated with high incidence of hip fractures. NSAIDs induced gastropathy remains a major clinical problem [8] which has not been solved through the introduction of selective inhibitors of cyclooxygenase-2 (COX-2) due to cardiac side effects. [8] The World Health Organization (WHO) has estimated that there are about 2 billion people worldwide who consume alcoholic beverages and 76.3 million with diagnosable alcohol use disorders. Alcohol consumption is an important factor to induce the gastric ulcer.

Over recent years, abundant work has been accomplished to develop natural products to potentially provide rich sources of new agents with anti-ulcer activity. It is significant to clarify their prevention or management action against gastric ulcer. A few of plant extracts and plant-derived compounds have been found and proved to be safe, effective, relatively less expensive and globally competitive. [9-10] Beetroot (Beta vulgaris) is botanically classified as an herbaceous biennial from Chenopodiaceae family and has several varieties with bulb colors ranging from yellow to red. Deep red-colored beet roots are the most popular for human consumption, both cooked and raw as salad or juice. [11] It is cultivated as a vegetable almost throughout India. [12-14] B. vulgaris leaves stated diuretic, purgative, laxative, and aphrodisiac activity. [15-16] B. vulgaris leaves proved antioxidant, anticancer, antihypertensive, hepatoprotective, nephroprotective, wound healing, and anti-inflammatory activities. [16-22] Roots of Beta vulgaris are rich in valuable, active compounds such as carotenoids, glycine betaine, saponins, betacyanines, folates, betanin, polyphenols and flavonoids. [24-26] Consumption of red beet which are rich source of antioxidants can contribute to protection from age-related diseases. According to Vinson, Hao, Su, and Zubik [1998] Zříhánová et al. [2006] red beet is one of the most potent vegetables with respect to antioxidant activity. [29-30] Betacyanins are a group of compounds exhibiting antioxidant and radical-scavenging activities. [31] Several reports are available about the gastroprotective effects are associated with plant extracts that are rich in antioxidants. [32] Although many of the pharmacological and biochemical actions of flavonoids are attributed to their activities as antioxidants [33] Flavonoids were also reported to act in the gastrointestinal tract, having antiallergic potential. [34] Considering the important role of flavonoids in the prevention or reduction of gastric lesions induced by different ulcerogenic agents, the present study was aimed to determine the antiallergic activity of methanol extract of roots of Beta vulgaris using established methods.

MATERIALS AND METHODS
Drugs and chemicals
All the drugs, chemicals, and reagents were procured from Qualigens Fine chemicals, Mumbai, India. Omeprazole were obtained from Glaxo Pharmaceuticals, Mumbai. All the chemicals used were of analytical grade.

Preparation of extract
The roots of Beta vulgaris were collected from local market of Nashik and authenticated at the Agharkar Research Institute, Pune. The roots were cleaned with water. The chopped pieces were subjected to maceration with 1% methanolic HCl (methanol 495 ml and HCl 5 ml) for two days with frequent shaking the macerating flask. The macerated product was then air dried for removal of menthol. The extract

Phytochemical investigation
Phytochemical tests were carried out to find out the presence of phytoconstituents viz flavonoids, saponins, glycosides, alkaloids, carbohydrate, tannins, phenols etc. [35] Animals
Adult albino Wistar rats of either sex, weighting 150-200 g obtained from Serum Institute Pune, India, were used for the experiment. They were housed in polypropylene cages lined with husk, renewed every 48 hours, under 12:12 hours light dark cycle at around 25 ± 5°C. They were fed with commercial pellet rat chow and given water ad libitum. The animal care maintained according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India, and the protocol of this study was approved by the Animal Ethical Committee of College (Reg. No. MGVIPC/CPCSEA/XXIX/1/2014).

Acute Toxicity study in mice (LD₉₀ determination)
Individual animals were administered dosage at interval of 24 hours. The methanolic extracts of Beta vulgaris roots were given with minimum dose to animals and observed for next 24 hours. If no mortality was seen then the dose was increased. The extracts
were administered in doses 50, 100, 200, 500, 800, 1000, 1500, and 2000 mg/kg p.o. [38]

Experimental Design

Induction of acute ulcer by pyloric-ligation method
Adult albino rats of Wistar strain of either sex weighing 150-250 g were used for the study. All the animals were divided into the four groups with five animals in each group.

Group I: Control (pyloric ligation)
Group II: Standard receives drug Omeprazole (20 mg/kg, p.o.)
Group III: Methanolic extract of Beta vulgaris (200 mg/kg, p.o.)
Group IV: Methanolic extract of Beta vulgaris (400 mg/kg, p.o.)

They were fasted overnight and placed on wire mesh to avoid coprophagy. Next day they were anaesthetized with anaesthetic ketamine. Incision of 1 cm long was given in the abdomen just below the sternum and stomach was exposed. A thread was passed around the pyloric sphincter and a tight knot was applied. The abdomen wall was closed by putting the sutures. The methanolic extract of Beta vulgaris and standard drug Omeprazole were given orally. After 4 hours of pyloric ligation all the animals were sacrificed by large dose of chloroform. Abdomen was opened and oesophageal end was tied and stomach was removed and the contents were drained in a centrifuge tube. Stomach was cut opened along the greater curvature and pinned on a cork plate and observed under magnification (10X) for morphological evaluation of mucosa. [37]

The number of ulcers was noted and the severity recorded with the following scores:

Scores: 0.5- Red coloration, 1-Spot ulcer, 1.5- Hemorrhagic streak, 2-Ulcers, 3-Perforation

Mean ulcer score for each animal was expressed as ulcer index.

The volume of the gastric content was measured. After centrifugation, acidity was determined by titration with 0.1 N NaOH (sodium hydroxide)

Acidity (mEq/l/100g) can be expressed as:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality}}{0.1}
\]

Gastric volume, acidity of the gastric content, pH and ulcer index of treated animals were compared with controls.

Free Acidity: Pipette 1 ml of filtered gastric content into a small beaker. Add 2-3 ml of water & then a drop of Topfers indicator. It will turn pink in presence of free HCl. Titrate it with N/100 NaOH until pink colour disappear and colour becomes yellowish orange (pH 4). At this pH all free HCl is titrated. Take burette reading. The volume of alkali requires titration represent free HCl present in 1 ml gastric juice. Calculate as N/10 acid present in 100 ml of gastric juice.

Total acidity: Add drop of phenolphthalein to above content & continue titration with the alkali until a definite red reappear (pH 8.5). Take the difference between this reading & the initial reading represent the total acid present in 1 ml of gastric content.

Ethanol induced gastric lesion

Male Wistar rats weighing 250–300 g are deprived of food 24 h prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. All the animals were divided into the four groups with five animals in each group.

Group I: Control receives distilled water (50 ml/kg, p.o.)
Group II: Standard receives Omeprazole (20 mg/kg, p.o.)
Group III: Methanolic extract of Beta vulgaris (200 mg/kg, p.o.)
Group IV: Methanolic extract of Beta vulgaris (400 mg/kg, p.o.)

The rats are administered either the vehicle or the standard drug orally 30 min prior to administration of 1 ml absolute ethanol. Untreated animals are included as controls. One hour after administration of ethanol, the animals are euthanized with ketamine, the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues are recorded. [39]

The numbers of ulcers were noted and the severity recorded with the following scores:

- 0.5-Red coloration
- 1-Spot ulcer
- 1.5-Hemorrhagic streak
- 2-Ulcers
- 3-Perforation

Mean ulcer score for each animal was expressed as ulcer index.

The volume of the gastric content was measured. After centrifugation, acidity was determined by titration with 0.1 N NaOH.

Cold restraint stress induced ulcers

Male Wistar rats weighing 250–300 g are deprived of food 24 hours prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. All the animals were divided into the four groups with five animals in each group.

Group I: Control receives ethanol (50 ml/kg, p.o.)
Group II: Standard receives Omeprazole (20 mg/kg, p.o.)
Group III: Methanolic extract of Beta vulgaris (200 mg/kg, p.o.)
Group IV: Methanolic extract of Beta vulgaris (400 mg/kg, p.o.)

The rats after 1 hour of pre-treatment with standard drug and extract were subjected to cold stress in restrain cages that were placed at 2-4°C in refrigerator for 2 hours. The animals were sacrificed 2 hours later and the ulcer index was determined. Gastric juice was collected and its volume, pH, free acidity, total acidity and percentage inhibition was determined. [39]

The numbers of ulcers were noted and the severity recorded with the following scores:

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Table 1: Effect of Beta vulgaris on gastric content, pH, acidity and ulcer index in pylorus-ligated rats

| Treatment Groups (Dose in mg/kg) | Total gastric content (ml) | pH | Free acidity (mEq/L) | Total acidity (mEq/L) | Ulcer Index | % Inhibition |
|---------------------------------|---------------------------|----|----------------------|-----------------------|-------------|-------------|
| Control (pyloric-ligation)      | 3.20 ± 0.95               | 3.15 ± 0.19 | 45.0 ± 5.7           | 75 ± 3.10             | 40.3        | -           |
| Omeprazole (20)                 | 1.80 ± 0.20*              | 3.87 ± 0.28* | 27.5 ± 5.0*          | 35 ± 5.0*             | 1.4         | 96.52%      |
| MEBV (200)                      | 1.10 ± 0.77*              | 3.92 ± 0.45* | 22.5 ± 5.0*          | 40 ± 8.16*            | 12.5        | 68.96%      |
| MEBV (400)                      | 1.37 ± 0.47*              | 4.42 ± 0.44  | 12.5 ± 5.0*          | 25 ± 5.77*            | 0           | 100%        |

MEBV: Methanolic extract of Beta vulgaris; Values are expressed as mean ± S.E.M. One way analysis of variance (ANOVA- Dunnett test); * Statistically significant compared with control group (p <0.05).

Table 2: Effect of Beta vulgaris on gastric content, pH, acidity and ulcer index in ethanol induced rats

| Treatment Groups (Dose in mg/kg) | Total gastric content (ml) | pH | Free acidity (mEq/L) | Total acidity (mEq/L) | Ulcer Index | % Inhibition |
|---------------------------------|---------------------------|----|----------------------|-----------------------|-------------|-------------|
| Control (ethanol 5 ml/kg)       | 3.40 ± 0.46               | 2.30 ± 0.12 | 110.0 ± 12.2         | 185.0 ± 11.90         | 68.8        | -           |
| Omeprazole (20)                 | 1.90 ± 0.33*              | 3.87 ± 0.02* | 47.5 ± 12.5*         | 118.8 ± 9.65*         | 13.2        | 80.8%       |
| MEBV (200)                      | 2.10 ± 0.14*              | 3.65 ± 0.08* | 35.0 ± 6.45*         | 110.0 ± 21.60*        | 22.75       | 66.93%      |
| MEBV (400)                      | 1.65 ± 0.17*              | 4.42 ± 0.14  | 22.5 ± 6.29*         | 45.0 ± 5.70*          | 2.1         | 96.94%      |

MEBV: Methanolic extract of Beta vulgaris; Values are expressed as mean ± S.E.M. One way analysis of variance (ANOVA- Dunnett test); * Statistically significant compared with control group (p<0.05).

Table 3: Effect of Beta vulgaris on gastric content, pH, acidity and ulcer index in Cold stress induced rats

| Treatment Groups (Dose in mg/kg) | Total gastric content (ml) | pH | Free acidity (mEq/L) | Total acidity (mEq/L) | Ulcer Index | % Inhibition |
|---------------------------------|---------------------------|----|----------------------|-----------------------|-------------|-------------|
| Control                          | 4.25 ± 0.14               | 3.033 ± 0.03 | 70 ± 5.00           | 83.33 ± 8.81         | 24.5        | -           |
| Omeprazole (20)                  | 3.867 ± 0.03*             | 3.633 ± 0.13* | 16.67 ± 3.33*       | 43.33 ± 3.33*        | 0           | 100%        |
| MEBV (200)                       | 1.533 ± 0.31*             | 3.83 ± 0.20*  | 33.33 ± 3.33*       | 53.33 ± 3.33*        | 0           | 100%        |
| MEBV (400)                       | 1.33 ± 0.35*              | 4.60 ± 0.1*   | 26.67 ± 6.66*       | 46.67 ± 3.33*        | 0           | 100%        |

MEBV: Methanolic extract of Beta vulgaris; Values are expressed as mean ± S.E.M. One way analysis of variance (ANOVA- Dunnett test); * Statistically significant compared with control group (p<0.05).

0.5-Red coloration, 1-Spot ulcer, 1.5-Hemorrhagic streak, 2-Ulcers, 3-Perforation
Mean ulcer score for each animal was expressed as ulcer index.
The volume of the gastric content was measured. After centrifugation, acidity was determined by titration with 0.1 N NaOH.

Statistical analysis
The mean ± SEM values were calculated for each group. One-Way ANOVA followed by Dunnett’s multiple comparison tests were used for statistical analysis. The results were statistically analyzed by Graphpad Instat Software. Values p<0.05 was considered statistically significant.

RESULTS
The methanolic extract of roots of Beta vulgaris was subjected to phytochemical investigation and LD50 studies. It was found that methanolic extract contained flavonoids, saponins, sterols, and alkaloids. The extract was tested for their lethal effect up to the dose level of 2000 mg/kg. None of them have produced abnormal behavior or mortality in rats.
Animals in the control group (pyloric-ligation) showed ulceration of gastric mucosa and more than 5 ulcers were observed. Administration of methanolic extract of roots of Beta vulgaris produced significant (p<0.01) decrease in ulcer index and also significantly reduced the gastric volume, total acidity, and increased the pH of the gastric fluid, proving its anti-secretory activity when compared with control group. Omeprazole also significantly (p<0.01) reduced ulcer index, gastric volume, total acidity, and increased the pH of the gastric fluid, of pyloric-ligation induced gastric ulcers.
Pylorus-ligation produced gastric lesions in the gastric mucosa of the control group (Group I). Administration of methanolic extract of Beta vulgaris (Group III, Group IV) reduced these lesions as evidenced by a significant (p<0.01) reduction in the ulcer index when compared with the control group. Omeprazole (Group II) also significantly (p<0.01) reduced ulcer index of pylorus ligated-induced gastric ulcers.
Administration of ethanol produced haemorrhagic gastric lesions in the gastric mucosa of the control group (Group I). Administration of methanolic extract of Beta vulgaris (Group III, Group IV) reduced these lesions as evidenced by a significant (p<0.01) reduction in the ulcer index when compared with the control group. Omeprazole (Group II) also significantly (p<0.01) reduced ulcer index of pylorus ligated-induced gastric ulcers.
Cold stress induced produced gastric lesions in the gastric mucosa of the control group (Group I). Administration of methanolic extract of Beta vulgaris (Group III, Group IV) reduced these lesions as evidenced by a significant (p<0.01) reduction in the ulcer index when compared with the control group. Omeprazole (Group II) also significantly (p<0.01) reduced ulcer index of Cold stress -induced gastric ulcers.

DISCUSSION
Pyloric ligation induced ulcers caused due to imbalance between offensive and defensive mucosal factors are ideal model to infer the mechanism by which a drug works as an anti ulcerogenic agent. [40] 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. The causes of gastric ulcer in 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 the unprotected lumen of the stomach to the accumulating acid. Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced ulceration in rats. The present study demonstrated the potential of MEBV (200) and MEBV (400) to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in pyloric ligation model by 68.98% and 100% when compared to control.

Ethanol is responsible for disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. The generation of free radicals was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol. Ethanol induced gastric ulceration may be occurred due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic tissue injuries. Alcohol has ability to penetrate the gastric mucosa and causing the cellular damage which increases the permeability to sodium and water. In other hand, the accumulation of intracellular calcium causes the pathogenesis of gastric injury that leads to cell death and exfoliation of surface epithelium. The present study observed that the MEBV significantly reduced ethanol induced ulcer by cytoprotective action via antioxidant effect. The methanol extract showed cytoprotection against the ethanol induced ulceration by reducing the gastric acid secretion. The present study demonstrated the potential of MEBV (200) and MEBV (400) to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in ethanol induced ulceration by 66.93% and 96.94% when compared to control.

Cold restraint causes both psychological and physical stress to the rats. The induced stress releases histamine in the stomach, which leads to increased acid secretion and decreased mucus production, ultimately leading to ulcers. MEBV caused a dose-dependent significant reduction in the ulcer index in this model. The significant increase in the antiulcer activity of Beta vulgaris could be attributed to the presence of flavonoids, alkaloids, tannins, saponins glycosides and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. So the antiulcer activity of Beta vulgaris may be attributed to its flavonoids content. The present findings conclude that Root extract of Beta vulgaris has significant antiulcer activity as it exhibited protective effect on gastric ulcer in rats.

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