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Original Research Article

Evaluation of anti ulcer activity of ethanolic root extract of *Beta vulgaris* in rats

K. Samyuktha*, Krishna Mohan Chinnala, G. Prathiba, D. Rajendhar, P. Smana Reddy

Department of Pharmacology, School of Pharmacy, Nalla Narasimha Reddy Group of Institutions, Hyderabad, Telangana 500088, India

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***Correspondence to:**
Dr. Samyuktha Kadiyala,
Email: samyu35@gmail.com

ABSTRACT

Background: *Beta vulgaris* (chenopodiacea) is a plant reported for its variety of ethnic medicinal uses. Hence we have planned to screen anti ulcer activity of root of the plant with the alcoholic extract. Root powders successively extracted with alcohol and were subjected for phytochemical screening to identify different phytoconstituents.
Methods: Anti ulcer activity was evaluated in various animal models like pylorus ligation and ethanol induced ulcer models in rats.
Results: Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, polyphenols. No mortality was observed with root extract up to maximum dose level of 4g/kg. Further alcoholic extract of 200 and 400mg/ kg / p.o significantly ($p<0.01$) reduced the ulcer score, ulcer number, ulcer index, free acidity and total acidity in pylorus ligation and ethanol induced ulcer models in rats.
Conclusions: The present study revealed that the root extract of *Beta vulgaris* has antiulcer activity.

Keywords: Antiulcer, *Beta vulgaris*, Ethanol, Pylorus ligation

INTRODUCTION

Beta vulgaris also known as red beet (Family: Chenopodiaceae) is a small sized plant, cultivated in many parts of India. It is popularly known as 'chukandar' or 'beet root', is an erect annual herb with tuberous root stocks. It is native to Mediterranean region and widely cultivated in America, Europe and throughout India.[1] The leaves of *Beta vulgaris* possess diuretic, purgative and anti-inflammatory activity, seeds known to possess expectorant and carminative properties, roots possess sedative, anti-ulcer and emenogogue effects.[2] The root is rich source of phytochemical compound, glycine, betaine,it has the property of lowering Homocystenine one of the toxic metabolite.[3]

Ulcers are defined histological as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa in to the sub mucosa.[4] Peptic ulcer disease is an excoriated segment of the gastrointestinal mucosa, typically in the stomach (gastric ulcer) or first few centimetres of the duodenum (duodenal ulcer), which penetrates through the muscularis mucosa. It generally occurs due to the imbalance between mucosal defence factors and injurious factors.[5] Drug treatment of peptic ulcer aims at controlling gastric acidity, hypermotility and spasm and thus relieving the associated pain, promoting ulcer healing, prevention of complication and recurrence.[6] Currently available treatments for peptic ulcers include antacids (systemic and nonsystemic) and drugs which reduce acid secretion such as H2 anti-histaminics, proton pump inhibitors, anticholinergics, prostaglandin analogues, ulcer protectives, ulcer healing drugs and anti H. pylori drugs.[7] These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often

expensive for the poor.[8,9] In view of the above, the present study was planned to evaluate the antiulcer activity of ethanolic root extract of *Beta vulgaris*.

METHODS

Collection of plant material

The root part of plant *Beta vulgaris* were collected from surroundings of Uppal area, Hyderabad, Telangana.

Preparation of plant extract

The root pieces were shade-dried and made into a coarse powder to get a uniform particle size and then used for extraction. A weighed quantity of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with ethanol and the residual marc was collected. The extract was filtered through a cotton plug, followed by Whatman filter paper (no.1). The extract was evaporated under reduced pressure using an evaporator at a low temperature (40-60°C) until all the solvent had been removed to give an extract sample.

Experimental animals

Healthy S.D. albino rats weighing between 160-210g were used for the study. The animals were procured from Sainath agencies, Hyderabad (282/99/CPCSEA). The animals were housed at CPCSEA approved animal house of Nalla Narasimha Reddy School of Pharmacy, Hyderabad. The animals were kept in polypropylene cages (6 in each cage). Animal house was maintained under standard hygienic conditions, at 25 ± 2^0C, humidity (60 ±10%) with 12 hrs light and 12 hrs dark cycles, with food and water ad libitum. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Nalla Narasimha Reddy School of Pharmacy (006/IAEC/NNRG/2016).

Pharmacological studies

Method I - Pylorus ligated model

The albino rats were randomly divided into five groups of six animals each.

Experimental design of Pylorus- ligation induced gastric ulcers

- Group I: Control animals
- Group II: Disease control
- Group III: EEBV (200 mg/kg p.o) suspended in 1%w/v CMC
- Group IV: EEBV (400mg/kg p.o) suspended in 1%w/v CMC
- Group V: Omeprazole (20mg/kg)

Experimental procedure

On the day of experiment, animals of Group III, IV and V were treated with low, high doses of beet root extract and omeprazole respectively with the help of an oral feeding tube. The control group was treated with normal saline only. After one hr of drug treatment, Group II, III, IV and V animals were anaesthetized with the help of anaesthetic ether the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by sutures. Rats were sacrificed by an over dose of anaesthetic ether after six hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube.

The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acid. Each stomach was examined for lesions in the fore stomach portion and indexed according to the severity.

Method II - Ethanol induced ulcers

The albino rats were randomly divided into five groups of six animals each.

Experimental design of Ethanol -induced gastric ulcer

- Group I: Control animals
- Group II: Disease control (1ml of 80% ethanol)
- Group III: EEBV (200 mg/kg p.o) suspended in 1%w/v CMC
- Group IV: EEBV (400mg/kg p.o) suspended in 1%w/v CMC
- Group V: Omeprazole (20mg/kg)

Experimental procedure

First group of animals were treated with saline and Group II animals were treated with 1ml of 80% ethanol orally. On the day of experiment with the help of an oral feeding tube III, IV and V groups of animals were treated with, low and high doses of beet root extract and omeprazole respectively. One hour after drug treatment III, IV and V groups of animals were treated with 1 ml of 80% ethanol by p.o, to induce ulcers. The animals were sacrificed after 1 hr of ethanol administration. The stomach was opened and the ulcer index and percentage inhibition of ulcer formation was calculated.

Biochemical estimations

Pylorus Ligated Model

Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred into the centrifuge tube, centrifuged and filtered. The supernatant liquid was then transferred to a measuring cylinder and the volume was measured.

Determination of pH of gastric content

One ml of the gastric juice was collected and the pH was directly measured by using Digital pH meter.

Determination of ulcer index

The stomachs were opened along the greater curvature; the number of ulcers was counted.

Ulcer scoring was done by the following scoring system:

- 0=no ulcer,
- 1=superficial ulcer,
- 2=deep ulcer,
- 3=perforation.

Ulcer index was calculated by using following formula

UI=UN+US+UP×10-1

Where,

UI=ulcer index,
UN=mean of ulcer number,
US=mean of ulcer score,
UP=ulcer probability for each group.

Determination of free acidity and total acidity

The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipetted out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of topfer's reagent as indicator, to the end point when the solution turned to yellowish orange colour was observed. This indicated the volume of NaOH required neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red colour appears. The difference between the two readings indicated the volume of NaOH required neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

Acidity was calculated by using formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Volume of gastric juice used}}$$

Ethanol induced ulcer

Ulceration index

The mucosa was flushed with saline and the stomach was pinned on frog board and their lesions were examined and scored macroscopically using hand lens. Ulcer index was measured by the following formula.

$$\text{Ulcer Index} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}$$

Statistical analysis

The analytical data was expressed as mean ± S.E.M. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dennett's Multiple Comparison test using Graph Pad Prism version 5.0 Software. Differences between the data were considered significant at $P<0.05$.

RESULTS

Preliminary phytochemical screening

The results of preliminary phytochemical screening of ethanolic extract of *Beta vulgaris* roots are shown in Table 1. The extract showed the presence of alkaloids, steroid glycosides, polyphenolic compounds, saponins, flavonoids, terpenes, tannins, cardiac glycosides, coumarins and sterols.

Table 1: Phytochemical screening of *Beta vulgaris* roots.

S. no.	Constituents	Test	Ethanolic extract
1	Alkaloids	Dragendroff's reagent	Present
2	Steroid glycosides	Acetic anhydride+$CHCL_3$+ H_2SO_4	Present
3	Polyphenolic compounds	Ferric chloride	Present
4	Saponins	Water	Present
5	Flavonoids	Methanol+ Magnesium +HCL	Present
6	Terpenes	Tin+Thionyl chloride	Present
7	Tannins	10% lead acetate	Present
8	Cardiac glycosides	Glacial acetic acid+$FECL_3$	Present
9	Coumarins	10%Ammonium hydroxide	Present
10	Sterols	5%potassium hydroxide	Present

Acute oral toxicity

The acute oral toxicity study was done according to the OECD 420 guidelines. A starting dose of 2000 mg/kg bodyweight/p.o of ethanolic extract of *Beta vulgaris* roots was administered to three rats and observed for 3 days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose 2000 mg/kg p.o of ethanolic extract of *Beta vulgaris* roots for more three days and observed for 14 days, no changes were observed from the first set of experiments.

Table 2: Acute oral toxicity studies.

S. No	Treatment group	Dose	Wt. of animals (in gm)		Signs of Toxicity	Onset of Toxicity	Reversible /Irreversible	Duration
			Before test (1st day)	After test (14th day)				
1	EEBV	2g/kg	160	165	No signs of toxicity	Nil	Nil	14 days
2	EEBV	2g/kg	170	174	No signs of toxicity	Nil	Nil	14days
3	EEBV	2g/kg	180	185	No signs of toxicity	Nil	Nil	14 days

Table 3: Effect of gastric volume in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus Ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Gastric Volume (ml/100g)	2.45 ± 0.32	7.85± 0.85 a**	6.50 ± 0.18 b**	4.92 ± 0.16 b**	3.67 ± 0.64 c**

Table 4: Effect of gastric pH in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus Ligated	III 200mg/kg	IV 400mg/kg EEBV	V OME
pH	2.96±0.16	2.10 ±0.20 a**	2.49 ± 0.24 b*	3.12 ± 0.26 b**	3.62 ± 0.62 c**

Comparisons were made between: a-(Group I vs II), b-(Group II vs III, IV), C-(Group II vs V) values were expressed as mean ± SEM of 6 animals. Statistical Significance test for comparison was done by ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P <0.01, * P <0.05 (Table 3).

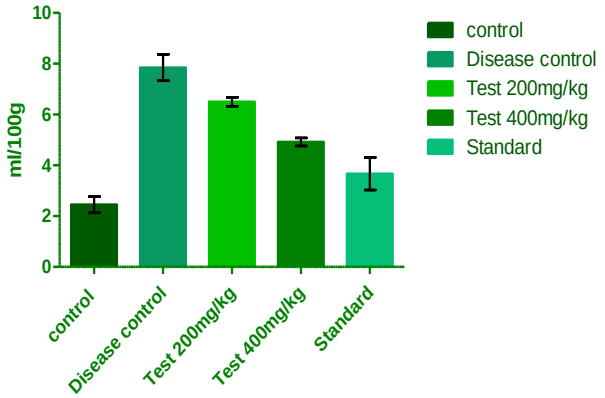

Figure 1: Effect of gastric volume in pylorus ligated gastric ulcer.

Comparisons were made between: a- (Group I vs II), b-(Group II vs III, IV), c-(Group II vs V) values are expressed as mean±SEM of 6 animals. Statistical Significance test for comparison was done by ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P <0.01, * P <0.05 (Table 4).

Comparisons were made between: a-(Group I vs II), b-(Group II vs III, IV), c- (Group II vs V). Values are expressed as mean±SEM of 6 animals. Statistical Significance test for comparison was done by ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P < 0.01, * P < 0.05 (Table 5).

Comparisons were made between: a- (Group I vs II), b-(Group II vs III, IV), C-(Group II vs V). Values are expressed as mean±SEM of 6 animals. Statistical Significance test for comparison was done by ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P < 0.01, * P < 0.05 (Table 6).

Comparisons were made between: a- (Group I vs II), b-(Group II vs III, IV), C-(GroupII vs V). Values are expressed as mean±SEM 6 animals. Statistical Significance test for comparison was done by one way

ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P < 0.01, * P <0.05 ns- non asignificant (Table 7).

Table 5: Effect of number of ulcer in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Number of Ulcer	0.18±0.13	4.80±0.22 a**	3.6±0.24 b*	2.20±0.18 b**	1.52±0.26 c**

Table 6: Effect of ulcer index in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Ulcer index	2.80±0.21	26.26±0.26 a**	18.20±0.24 b*	16.20±0.20 b**	12.60±0.12 c**

Table 7: Effect of free acidity in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Free acidity (mEq/dl)	4.60± 0.24	7.86 ±0.22 a**	6.69±0.21 b**	5.01± 0.18 b**	3.62±0.20 c**

Table 8: Effect of total acidity in pylorus ligated gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Total acidity (mEq/dl)	5.60 ± 0.21	8.46 ± 0.16 a**	7.62 ±0.20 b*	5.21 ± 0.18 b**	4.02± 0.10 c**

Table 9: Effect of ulcer index in ethanol induced gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
Ulcer index (mm^2)	0.36±0.13	39.21 ±1.49 a**	26.21±0.86 b**	20.12±1.89 b**	10.11±1.20 c**

Table 10: Effect of gastric pH in ethanol induced gastric ulcer.

Groups	I Control (CMC)	II Pylorus ligated	III 200mg/kg EEBV	IV 400mg/kg EEBV	V OME
pH	2.98±0.16	2.02 ±0.26 a**	2.36 ± 0.18 b*	3.20 ± 0.26 b**	3.48 ± 0.20 c**

Comparisons were made between: a- (Group I vs II), b- (Group II vs III, IV), C-(GroupII vs V). Values are expressed as mean±SEM 6 animals.Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P < 0.01, * P < 0.05, ns- non a significant (Table 8).

Comparisons were between: a-(Group I vs II), b- (Group II vs III, IV),C-(GroupII vs V). Values are expressed as mean ± SEM of 6 animals. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. Symbols represent statistical significance: ** P < 0.01, * P < 0.05, ns- non a significant (Table 9).

Comparisons were between: a-(Group I vs II), b- (Group II vs III, IV), C-(GroupII vs V). Values are expressed as mean ± SEM of 6 animals. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. Symbols represent statistical

significance: ** P < 0.01, * P < 0.05, ns- non a significant (Table 10).

DISCUSSION

The anti ulcer activity of *Beta vulgaris* was evaluated by employing alcohol and pylorus ligation induced ulcer models. These models cause the gastric ulcer in humans [10]. Many factors and mechanisms are involved in the ulcerogenesis and gastric mucosal damage. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. The ethanol-induced ulcers are predominant in the glandular part of stomach and were reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa.[11] Alcohol rapidly penetrates the gastric mucosa causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. The beet root extract shows protection in dose dependent manner against characteristic lesions produced by ethanol and reduced values of ulcer index as compared control group suggesting its potent cytoprotective activity.

Pylorus ligation induced ulcer was used to study the effect of beet root extract on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The fasting of rats for 24 h followed by ligation of pyloric end of the stomach, the ulcer index is determined 4 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. The Ethanolic extract of *Beta vulgaris* and omeprazole significantly decreased the total acidity and free acidity; and significantly enhance the pH; this suggests that it having an anti secretory effect. Pylorus ligation induced ulcer control rats shown perforated ulcer, deep ulceration of granular epithelium and almost reducing the sub-mucosa. The Ethanolic extract of *Beta vulgaris* at 200 mg/kg dose has shown mucosal erosion, the partial healing of ulcer with few inflammatory cells and the dose 400 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells. Beet root extracts have been reported to possess antioxidant activity and to contain various types of compounds such as flavonoids, polyphenolic compounds, saponins and tannins. The gastro protective effect exhibited by Ethanolic extract *Beta vulgaris* is speculated to be attributed to its antioxidant property, which in turn could be linked to the presence of flavonoids, polyphenolic compounds, saponins and tannins . These compounds most likely inhibit gastric mucosal injury.

CONCLUSION

The preliminary phytochemical investigation of ethanolic extract of *Beta vulgaris* roots showed the presence of alkaloids, flavonoids, saponins, polyphenols. Ethanolic extract of *Beta vulgaris* roots was screened for acute oral toxicity and was found to be non toxic. Ethanolic extract of *Beta vulgaris* roots exhibited significant protection in both ethanol induced ulcer model and pylorus ligation ulcer model. Histopathological studies of stomach in pylorus ligation and ethanol induced ulcer models exhibit normal architecture of stomach respectively. Since flavonoids have shown to be represent in ethanolic extract of *Beta vulgaris* roots, it is possible that these constituents may be responsible for the anti ulcer activity of the ethanolic extract of *Beta vulgaris* roots. *Beta vulgaris* may be considered as a natural source in modern drug development areas for its versatile medicinal uses.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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