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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT *BETA VULGARIS* L. LEAVES

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Abstract:

Gastroesophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer, and other peptic illnesses are frequent in today's lifestyle. This could be the result of a hectic lifestyle or an unbalanced diet. The pathophysiology underlying these illnesses could be a mismatch between offensive and defensive mechanisms, either due to excessive acid and pepsin secretion or a decreased ability of the gastro-duodenal mucosal barrier to guard against acid-pepsin secretion from the stomach. Non-steroidal anti-inflammatory drugs (NSAIDs) are a type of therapy that has been shown to be beneficial in treating a variety of ailments. Some people take NSAIDs on a daily basis to keep themselves healthy. NSAIDs, on the other hand, can cause a wide range of serious adverse effects. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. As a result, the purpose of this study was to assess the antiulcer activity of an ethanolic extract of Beta vulgaris leaves in rats. The well-known test technique in the literature was used to determine the qualitative analysis of several phytochemical elements. The anti-ulcer efficacy of ethanolic extract was tested in vivo. The pH of stomach fluid, ulcer index, and percent inhibition of ulcer index were used as outcome measures, depending on the model. Because of one or more of the secondary metabolites included in Beta vulgaris extract, the results of this investigation indicated that it possesses anti-ulcer pharmacologic efficacy. As a result, this research backs up its anti-ulcer use in Indian folk medicine. More research is needed into isolating particular phytochemicals and understanding mechanisms of action.

Keywords:*Beta vulgaris, Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs*

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INTRODUCTION:

Ulcers are open sores on the skin or mucous membrane that are characterized by the sloughing of inflammatory dead tissue [1]. Ulcers are sores that cause a superficial loss of tissue on the skin's surface or a mucous membrane. Ulcers most commonly occur on the skin of the lower limbs and in the gastrointestinal tract, but they can occur everywhere. Mouth ulcers, esophageal ulcers, peptic ulcers, and genital ulcers are among the many varieties of ulcers. Many people suffer from peptic ulcers as a result of them. Peptic ulcers are caused by erosion of the stomach or duodenal lining [2]. Gastric ulcer and duodenal ulcer are the two most prevalent kinds of peptic ulcer. Both stomach and duodenal ulcers can occur at the same time. Gastric ulcers are painful ulcers that occur in the stomach. They are more common in those over the age of 50. Eating may aggravate rather than alleviate pain. Nausea, vomiting, and weight loss are possible side effects. Despite the fact that patients with stomach ulcers have normal or reduced acid production, ulcers can develop even in the absence of acid [3]. Duodenal ulcers are present at the start of the small intestine and cause intense discomfort and a burning sensation in the upper abdomen, waking patients up. Pain is most common when the stomach is empty and subsides after eating. Duodenal ulcers are more common in younger people and primarily affect men. Ulcers can occur on both the anterior and posterior walls of the duodenum [4]. Peptic ulcers can be life-threatening in some situations, with symptoms such as bloody stool, severe stomach discomfort and cramps, and blood vomiting [5]. A balance between offensive (acid, pepsin, and Helicobacter pylori) and defensive (mucin, prostaglandin, bicarbonate, nitric oxide, and growth hormones) components is involved in the pathogenesis of peptic ulcer disease [6].Spicy food and stress were once thought to be the causes of peptic ulcers; however, research has discovered that the true causes of peptic ulcers are bacterial infection (Helicobacter pylori) or pharmacological reactions, notably to NSAIDS (non-steroidal anti-inflammatory drugs) [7]. The main etiological variables related with peptic ulcer are Helicobacter pylori, NSAIDS medications, emotional stress, alcohol usage, and smoking [8, 9]. Helicobacter pylorus, a Gram-negative bacteria, lives between the mucous layer and the gastric epithelium and is specifically engineered to thrive in the stomach's hostile environment. Helicobacter pylorus is found in the antrum at first, but it migrates to the stomach's more proximal parts over time [10]. Peptic ulcer disease is one of the most common gastrointestinal problems, affecting 10% of the global population [11]. Duodenal ulcers account for 19 out of every 20 peptic ulcers. Each year, an estimated 15000 people die as a result of a peptic ulcer. Peptic ulcer bleeding and perforation had annual incidence estimates of 19.4-57 and 3.8-14 per 100,000 people, respectively. The average 7-day recurrence of hemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2% [12]. In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects [13]. Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity [14]. Beetroot (*Beta vulgaris* L.), locally known as Shamandar, is a vegetable plant belonging to the family Amaranthaceae. Beetroot have long been used in traditional Arab medicine to treat a wide variety of diseases and it has been used for its carminative, emmenagogue and hemostatic and renal protective properties and for the treatment of cardiovascular diseases [15]. Hence, the objective of the present investigation is to evaluate the anti-ulcer activity of *Beta vulgaris*in Wister albino rat model.

MATERIALS AND METHODS:

Plant material:

Leaves of *Beta vulgaris*were collected from local area of sagar in the month of January, 2021.

Chemicals and reagents:

All the drugs, solvents and chemicals used in the study were of analytical grade. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India.

Extraction by maceration process:

Leavesof *Beta vulgaris* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether. The extraction was continued till the defatting of the material has been taken place. 50 gm of dried powdered leaves of *Beta vulgaris* has been extracted with ethanol using maceration process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the

percentage yields were calculated of the dried extracts.

Phytochemical screening:
Ethanolic extract of *Beta vulgaris* leaveswas subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures [16].

Animals:
Wistar rats (150-200 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum.* Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity test:
Preliminary experiments were carried out on rats (n=6). Ethanolicleaves extract of *Beta vulgaris* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD) [17]. Animals were kept fasting providing only water, Ethanolicleaves extract of *Beta vulgaris* were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 14 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

Experimental designs:
Aspirin-induced gastric ulcer:
Group –1: Control
Group –2: Ranitidine (Standard)
Group –3: Ethanolicleaves extract of *Beta vulgaris* (100mg/kg, p.o.)
Group –4: Ethanolicleaves extract of *Beta vulgaris* (200mg/kg, p.o.)
Group -5: Ethanolic leaves extract of *Beta vulgaris* (400mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anesthesia, ulcers were induced by applying aspirin (500 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Ranitidine (50 mg/kg, p.o.), low dose of Ethanolicleaves extract of *Beta vulgaris* (100 m/kg p.o.), Medium dose of Ethanolicleaves extract of *Beta vulgaris* (200 m/kg p.o.) and high dose of Ethanolic leaves extract of *Beta vulgaris* (400 m/kg p.o.) [once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5th day, the stomachs removed and cut open along the greater curvature [18].
The ulcer index was determined using the formula:
Ulcer index = 10/X
Where X = Total mucosal area/Total ulcerated area.
Based on their intensity, the ulcers were given scores as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

RESULTS AND DISCUSSIONS:

The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received Ethanolic extract (2000 mg/kg) of *Beta vulgaris* leaves. This indicates that 2000 mg/kg is maximum safe dose. 400, 200 and 100 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-ulcereffects. Aspirin induced ulcer was used to study the effect of Ethanolic extract of *Beta vulgaris* leaves on gastric acid secretion and mucus secretion. Ethanolic leaves extract of *Beta vulgaris* revealed that it has significant anti-ulcer activity. Usually, NSAIDs and corticosteroids are widely used in clinical practice as anti-inflammatory agents. With the exception of newer highly selective COX-2 inhibitors, NSAID's and corticosteroids produce significant gastric irritation resulting in gastritis and gastric ulceration, especially on long-term treatment. Present study revealed that Ethanolic leaves extract of *Beta vulgaris* has ulcer protective properties. The Ethanolic extract of *Beta vulgaris* and Ranitidine significantly decreased the ulcer index and significantly enhance the pH; this suggests that it having an anti secretory effect. Aspirin induced ulcer control rats shown perforated ulcer. There is a dose-dependent increase in anti-ulcer effect of Ethanolic leaves extract of *Beta vulgaris*.

Table 1: Result of phytochemical screening of extracts of *Beta vulgaris*

S. No.	Active constituents	Test	Result
1.	Fixed oils	Spot test	-
2.	Glycosides	Baljet's test Keller kilani test Brontrager's test	+ + +
3.	Saponins	Foam test	+
4.	Alkaloids	Mayer's test Dragendroff's test Wagner's test	- - -
5.	Phenolic compounds and tannins	$FeCl_3$ test Lead acetate test Potassium dichromate	+ + +
6.	Flavonoids	Dil. NaOH solution Conc. H_2SO_4	+ +

Table 2: comparison of antiulcer effect of ethanolic extract of *Beta vulgaris* leaves in pylorus ligation induced ulcer model in rats

S. No.	Treatment	Ulcer index Mean ± SEM	Ulcer protection Percentage
1	Control	10.30±0.148	-
2	Ranitidine	3.43±0.058**	66.6%
3	Low dose(100mg/kg)	6.71±0.110*	34.78%
4	Medium Dose(200mg/kg)	5.417±0.174**	47.40%
5	High dose (400mg/kg)	5.217±0.192**	50.11%

Value are mean ± SEM (n= 6) one way ANOVA followed by tukey's multiple comparison test wear, * significant at p <0.05,** represent highly significant at p <0.01 ,*** represent significant at p<0.001 and ns represent non significant , comparison to control.

Table 3: comparison of antacid activity of ethanolic extract of *Beta vulgaris* Leaves treated pylorus ligation induced ulcer model in rats

S. No.	Treatment	Vol. of gastric juice (ml)	pH	Free acidity Meq/1/100g	Total acidity Meq/1/100mg
1	Control	7.083±0.0872	2.167±0.08	32.0±1.390	82.5±2.4
2	Ranitidine	4.450±0.1688	3.800±0.19	18.0±0.5774	44.5±1.11
3	Low dose	5.917±0.0945	3.233±0.1054	25.5±1.118	59.1±0.945
4	Medium dose	4.883±0.1014	3.783±0.0703	23.17±0.948	47.67±1.856
5	High dose	4.833±0.730	3.750±0.076	20.0±0.88	46.67±0.7601

CONCLUSION:

The preliminary phytochemical investigation of Ethanolic extract of *Beta vulgaris* leaves showed the presence of Glycosides, Alkaloids, Phenolic compounds, tannins and Flavonoids . Ethanolic extract was screened for acute oral toxicity and was found to be non toxic. Ethanolic extract of *Beta vulgaris* leaves possesses significant anti-ulcer activity. In conclusion, our results showed that the anti-ulcer activity of the extract was a result of the probable gastric ulcer healing mechanism (anti-secretory, cytoprotective and the antioxidant properties) of its active phytoconstituents. These findings suggest the potential for use of *Beta vulgaris* as an adjuvant in the treatment of gastric ulcer. Further, studies are needed for the isolation of active constituents responsible for the anti-ulcer activity and

to elucidate the exact mechanism of action in gastric ulcer healing.

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