

EVALUATION OF ANTIULCEROGENIC ACTIVITY OF METHANOL EXTRACTS OF *BRASSICA OLERACEA* VAR. *CAPITATA RUBRA* ON ALBINO RAT GASTRIC ULCERATION

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Received: 18 November 2016, Revised and Accepted: 05 December 2016

ABSTRACT

Objective: To investigate the antiulcerogenic activity of methanolic extract of *Brassica var. capitata rubra* in albino rats.

Methods: To evaluate the antiulcer activity by pyloric ligation models experimentally induced gastric ulcer by ranitidine (10 mg/kg) subcutaneously. The parameters taken to assess antiulcer activity were free acidity, total acidity, volume of gastric juice, PH, ulcer score, and ulcer index.

Results: The methanolic extract of *Brassica oleracea var. capitata rubra* in the dose of 0.50 mg/kg produced significant antiulcer activity. The control animals had ulcers and hemorrhagic streaks, whereas in animals administered with extracts of *B. oleracea* there was a significant reduction in ulcer index ($p < 0.05$).

Conclusion: This study concluded that methanol extract of *B. oleracea var. capitata* has healing property of gastric ulcers in albino rats.

Keywords: *Brassica*, Methanol extract, Antiulcer activity, Pyloric ligation model.

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INTRODUCTION

A peptic ulcer is the most common gastrointestinal disorder in clinical practice. Prolonged use of synthetic antiulcer drugs leads to adverse drug reactions. Hence, search for new antiulcer agents that retain therapeutic efficacy and are devoid of drug reactions lead to the usage of natural medicine [1]. *Brassica oleracea* belongs to *Brassicaceae* or *Cruciferae* family comprised approximately 3500 described species apportioned among 350 genera including cauliflower, broccoli, kohlrabi, kale, cabbage, and Brussels sprouts.



Red cabbage is a rich source of phenolic compounds, with anthocyanins being predominant over other flavonoids [2,3]. The highest stability and strongest antioxidant properties in red cabbage extract were assigned to anthocyanins [4]. Numerous studies have highlighted the potential importance of brassica vegetables as a source of antibacterial [5-7] and antioxidant substance [8-10]. Due to its antioxidant, anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal diseases (gastritis, peptic and duodenal ulcers, and irritable bowel syndrome) as well as in the treatment of minor cuts and wounds and mastitis [11].

In our study, methanol extract of *B. oleracea* showed significant results regarding photochemical [12], antimicrobial [13], and antioxidant

studies [14] and pharmacologic studies namely anti-inflammatory and antipyretic activity [15]. Hence, in this study, pharmacologic activity of antiulcer has been investigated in the methanol extract of *Brassica* plant.

METHODS

This project was conducted in the Department of PG Biochemistry at V.V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India. The preliminary work (extraction) was conducted in V.V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India. The pharmacologic activity of drug on rat study was conducted in the Sankaralingam Bhuvanewari College of Pharmacy, Anaikuttam, Tamil Nadu, India.

Vegetable collection

Red cabbage (*B. oleracea var. capitata rubra*) was collected from local markets of Virudhunagar, Tamil Nadu, India, and was authenticated by Dr. B. Karunai Selvi, Assistant Professor, Department of Botany, V. V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India. The vegetable was washed thoroughly under running tap water to remove dirt and then shade dried at room temperature for a week. They were ground into fine particles after drying and kept in closed container.

Extraction and sample preparation

About 10 g of ground sample of *B. oleracea var. capitata rubra* was weighed and homogenized with 100 ml of methanol. The crude preparation was left for 72 hrs in shaker at room temperature. The extract obtained by cold extraction was then concentrated by evaporating the solvent at room temperature.

Animal model

Wistar rats of either sex weighing between 130 and 170 g were procured from animal house of Sankaralingam Bhuvanewari College of Pharmacy (Regd. No. 622/02/C/CPCSEA) used for this study. They were maintained under standard conditions ($28 \pm 2^\circ\text{C}$; 55-60% relative humidity) and fed a standard diet for rats and given water *ad libitum*.

Antiulcer activity

The animals were starved overnight and the first group of animals was given saline orally - 5 ml/kg (control), second was injected ranitidine - 10 mg/kg subcutaneously and the next two groups were given different concentrations of extract (T₁ - 0.25 mg/kg and T₂ - 0.50 mg/kg) orally. The rat was anesthetized with anesthetic ether. After 15 minutes of injection, pyloric ligation was performed. The rat was secured on the operating table. An incision of 1 cm length is given in the abdomen just below the sternum. The stomach was exposed. Passed a thread around the pyloric sphincter and applied a tight knot. While putting the knot care should be taken so that no blood vessel is tied along the knot. The abdomen wall was closed by putting the sutures. Cleaned the skin from any blood spots and bleeding. The collodion was applied over the wound. The rat was kept in the separate cage and allowed to recover. After 4 hrs of pyloric ligation, the animals are sacrificed by decapitation. Opened the abdomen and tied the esophageal end (cardiac end) of the stomach. Cut and removed the entire stomach from the body of the animal. Given a small cut to the pyloric region just above, the knot and collected the contents of the stomach in a graduated centrifuge tube. Opened the stomach along the curvature and washed it slowly under the running tap water. Put it on the slide glass and observed under 10× magnification for ulcers. The ulcers are scored as below.

- 0 = Normal colored stomach
 0.5 = Red coloration
 1 = Spot ulcers
 1.5 = Hemorrhagic streaks
 2 = Ulcers ≥3 but ≤5
 3 = Ulcers >5.

Mean ulcer score for each animal was expressed as ulcer index. Gastric content was centrifuged at 1000 rpm for 10 minutes. pH of this solution was noted with the help of pH meter. The solution was titrated against 0.01 N sodium hydroxide using Topfer's reagent as indicator. (It is dimethyl amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids). The end point is the appearance of orange color. The volume of sodium hydroxide was noted which corresponds to the free acidity. The solution was titrated further, till it regained pink color. The total volume of sodium hydroxide which corresponds to the total acidity was noted [16].

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

$$\text{Acidity} = \text{Volume of sodium hydroxide} \times \text{normality} \times 100 / 0.1.$$

Statistical analysis was performed by one-way ANOVA followed by student's test using SPSS software.

RESULTS AND DISCUSSION

The plant *B. oleracea* var. capitata rubra was collected from local markets of Virudhunagar, Tamil Nadu. The material was dried under shade and then powdered. The dried powders of *B. oleracea* were extracted with methanol solvents using cold extraction. The extracts were allowed to evaporate to dryness.

We chose methanol extract for further pharmacological study in antiulcer activity. The methanolic extract of *B. oleracea* var. capitata rubra in the dose of 0.50 mg/kg produced significant antiulcer activity. The control animals had ulcers and hemorrhagic streaks, whereas in animals administered with extracts of *B. oleracea* there was a significant reduction in ulcer index ($p < 0.05$). The results of antiulcer activity are tabulated in Table 1 and diagrammatically represented in Plate 1.

The methanol extract of the *B. oleracea* has significantly shown reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration [17]. The antiulcer activity of methanol extract of *B. oleracea* in pylorus ligation model was evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index, and increase in pH of gastric juice. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanisms [18]. The excess gastric formation of prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid, and pepsin [19]. Acute gastric ulcers were induced in rats by the oral administration of acetylsalicylic acid. The gastroprotective potential of aqueous extract (0.250, 0.500, and 1.0 mg/kg body weight) was compared with omeprazole (20 mg/kg body weight). The stomach analysis indicated the gastric damage. The gastroprotective activities were evidenced by its significant inhibition in the formation of ulcers induced by chemical agent with a maximum of 99.44% curative in acetylsalicylic acid-induced ulcer [20]. The effect of methanolic, chloroform and diethyl ether extracts of *Mimosa pudica* were investigated in rats to evaluate the antiulcer activity using three models, i.e., aspirin, alcohol, and pyloric ligation models experimentally induced gastric ulcer. The parameter assessed was gastric volume, pH, free acidity, total acidity, and ulcer index. The results indicated that there is a significant decrease in all the above-mentioned parameter in alcohol extract with respect to control [21]. The patients who utilized the fresh cabbage juice obtained a healing action for gastric disorders, particularly for the peptic ulcer, presenting healing effects of the lesion [22]. The preliminary phytochemical studies revealed the presence of flavonoids in methanolic extract of *B. oleracea*. A possible mechanism of antiulcer action of *B. oleracea* may be due to its flavonoids content. Flavonoids may be recognized as active compounds against gastric lesions [23]. Our results are coincides with this.

For the therapeutic strategies of gastroduodenal ulcer disease, it is an important to find antioxidant compounds that are able to inhibit the gastric acid secretion, boost the mucosal defense mechanisms by increasing mucosal production, and stabilizing the surface epithelial cells [24]. Natural products were considered as a rich source of compounds for drug discovery [25]. Therefore, by scavenging free radicals, antioxidants from plant sources may play an important role in gastric ulcer therapy [26]. In accordance with this report, methanolic extract showed the highest scavenging capacity for 2,2-diphenyl-1-picrylhydrazyl radical, superoxide radical scavenging activity recorded in our study [14]. Gastric acid oversecretion is one of the

Table 1: Antiulcer activity by pyloric ligation method

Drug and dose	Acidity (mEq/l)		Volume of gastric juice (ml)	pH of gastric juice	Ulcer score	Ulcer index (%)
	Free acidity	Total acidity				
Control Normal saline 5 ml/kg	25.5±1.0	70.75±0.5	6.73±0.51	1.85±0.03	-	-
Standard Ranitidine 10 mg/kg	11.25±0.96	23±0.82	4.85±0.31	4.37±0.24	80.5±1.0	80.5*
BOME 1 0.25 mg/kg	19.25±0.5	52.5±1.0	5.88±0.40	4.16±0.04	61±1.0	61*
BOME 2 0.50 mg/kg	11.75±0.5	34±0.82	4.55±0.21	4.39±0.36	74.25±0.55	73.25*

Values are expressed as mean±SD (n=4); At 95% confidence interval * $p < 0.05$ were considered significant. BOME - *B. oleracea* methanolic extract

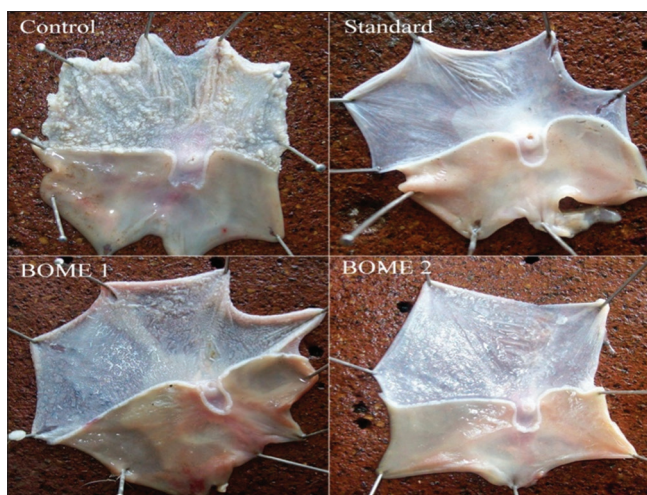


Plate 1: Antilucer activity by pyloric ligation method

key pathogenic factors for gastric ulcer induction [27]. In this study, pyloric ligation model caused a significant increase in gastric juice discharge, total acidity, and marked peptic ulcer lesions. In consonance with the report of [28], gastric ulcer lesion recorded in this study may be due to inhibition of PG synthesis that induced gastric acidity, and consequently, stomach susceptibility to mucosal injury. Therapeutic agents of peptic ulcers generally depend on the inhibition of gastric acid secretion by histamine H₂ antagonists [29,30]. Similar to our results, the most active subfraction of *Buchanania lanzan* ethyl acetate (BLE) exerted a significant dose-dependent decrease in the ulcerative lesion index produced by APL ulcer model in rats as compared to the standard drugs omeprazole (30 mg/kg, b.w orally) and ranitidine (32 mg/kg, b.w orally), respectively. The reduction in gastric fluid volume, total acidity, and an increase in the pH of the gastric fluid in APL treated rats proved the antisecretory activity of most active subfraction of BLE. The oral administration of most active subfraction (F4) of ethyl acetate fraction of methanolic leaf extract of *B. lanzan* Spreng produced significant antilucer activity [31]. The antilucer activity was investigated in *M. pudica*. Among the methanolic, chloroform and diethyl ether extracts, methanolic extract showed significant activity [21] which coincides with our results. 200 mg/kg methanolic extract was significant in the range $p < 0.001$.

SUMMARY AND CONCLUSION

The methanolic extract of *B. oleracea* var. *capitata rubra* in the dose 0.50 mg/kg produced significant ($p < 0.05$) antilucer activity. On the basis of the present results, it can be finally concluded that *B. oleracea* var. *capitata rubra* can be used as an effective herbal medicine for ulcer conditions. Hence, further research is required to isolate individual components, characterize the active phytochemical constituents responsible for the activity and formulation of a potent antilucer drug from *B. oleracea* var. *capitata rubra*.

ACKNOWLEDGMENT

The authors are thankful to the Principal and the Management of V.V. Vanniaperumal College for Women, Virudhunagar, Tamil Nadu, India, and Sankaralingam Bhuvanewari College of Pharmacy, Anaikuttam, Sivakasi, Tamil Nadu, India for providing necessary facilities to carry out this research work.

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