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STUDY OF PHYTOCHEMICALS AND ANTI -ULCER ACTIVITY OF BRASSICA OLERECEA

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Abstract
Brassica oleracea, commonly known as cruciferous vegetables, has been recognized for its rich phytochemical composition and potential health benefits. In this study, we conducted a comprehensive investigation into the phytochemical profile of *Brassica oleracea* extract and explored its anti-ulcer activity, particularly focusing on its efficacy in mitigating gastric ulcers. Phytochemical screening revealed the presence of various bioactive compounds, including alkaloids, carbohydrates, proteins and amino acids, saponins, phenols and tannins, and phytosterols/triterpenoids. Furthermore, we evaluated the anti-ulcer activity of *Brassica oleracea* extract using experimental models of gastric ulcers induced by pylorus ligation. Our findings demonstrated promising results, indicating a significant reduction in gastric secretion volume, total acidity, ulcer score, and ulcer index upon treatment with *Brassica oleracea* extract. These observations suggest the potential therapeutic benefits of *Brassica oleracea* in managing gastric ulcers. Overall, our study highlights the pharmacological potential of *Brassica oleracea* as a natural remedy for gastric ulcer management. Further research is warranted to elucidate the underlying mechanisms of its anti-ulcer activity and optimize dosage regimens for enhanced efficacy and safety. *Brassica oleracea* holds promise as a novel therapeutic agent derived from natural sources for the treatment of gastric ulcers, offering potential benefits for human health and well-being.

Keywords: Peptic ulcer, Phytochemical, *Brassica olerecea*, Ulcer index, Herbal medicine, Pylorus ligation

Introduction

Peptic ulcer illness is a dangerous medical condition. Five million people are impacted in the US alone, where there are about 500,000 new cases reported annually. It is interesting to note that people born in the middle of the 20th century have the highest chance of developing peptic ulcer disease. With a peak incidence between the ages of 55 and 65, ulcer disease is now primarily affecting the elderly population. In patients with Zollinger Ellison syndrome, ulcers can occur in the jejunum, esophagus, stomach, or duodenum as well as in the vicinity of a Meckel's diverticulum that contains ectopic gastric mucosa (Prabhu and Shivani, 2014; Tandon *et al*., 2004).

Peptic ulcers can be treated with a vast array of chemical agents. The goal of therapy is usually to get rid of H. pylori from an infected patient's stomach. The combination of a proton pump inhibitor, amoxicillin, and clarithromycin; omeprazole, amoxicillin, and clarithromycin; and pantoprazine, amoxicillin, clarithromycin, and amoxicillin is known as standard orthodox triple therapy, which is

typically recommended as first-line therapy. However, these medications have serious side effects: H2 antagonists are known to cause impotence, headaches, skin rashes, and arrhythmias; on the other hand, using proton pump inhibitors is an unpredictable cause of hypergastrinemia and atrophic gastritis. Antacid use causes belching, constipation, stomach distention, and a risk of ulcer perforation. Anticholinergic medications also cause constipation, dry mouth, urine retention, blurred vision, xerostomia, and the early onset of glaucoma. While prostaglandin analogs are likely to cause abdominal cramps, uterine bleeding, and abortion, ulcer protectives induce constipation, diarrhea, dizziness, edema, and hypophosphatemia (Reilly, 1999; Franko & Richter, 1998; Akthar *et al*., 1992).

Because they are less harmful, more palatable to different cultures, more compatible with the human body, less likely to cause side effects, affordable, efficient, and readily available, herbal medications have thus maintained their significance. In animal models, a wide variety of herbal plants and plant extracts exhibit strong antiulcer properties. When compared to reference drugs, it exhibits gastric anti-secretary and muco-protective activity. Not even at comparatively high concentrations is the extract toxic. The presence of flavonoids in all of these plants is most likely what causes their antiulcer activity. Since the beginning of medicine, chemicals derived from plants have been used to treat human illnesses (Ardalani *et al*., 2020; Srinivas *et al*., 2013).

Approximately half of the newly introduced chemical entities in the last 20 years have come from natural products. Natural products are once again of interest in drug discovery thanks to recent technological advancements. Consequently, the focus should be on identifying and characterizing the active principles as well as clarifying the connection between activity and structure (Singh *et al*., 2018).

The primary use of *Brassica oleracea* is in food preparation. It is essential to any kind of salad. It is regarded as an abundant supply of vitamins C and K. Additionally, it has a moderate amount of vitamin B6 and folate. Indole-3-carbinol is also found in cabbage, and its potential medical benefits are currently being studied. Trench foot, breast abscesses, and ulcers have all been treated with the cooling qualities of cabbage leaves. Poultices made of mashed cabbages and cabbage juice are used to treat pneumonia, appendicitis, warts, and boils (Sparrow *et al*., 2006; Ravikumar, 2015).

This study further aims at assessing anti -ulcer activity of *Brassica oleracea* in animal models.

Materials and Methods

Collection of plant

The subject of research was dry extract produced from garden cabbage at the Department of pharmacognosy of Adina Institute of Pharmaceutical Sciences Sagar. The herbarium number is BoT/H/01/11/03. The authentication was done by Dr. Pradeep Tiwari , HOD , Department of Botany , Dr. Hari Singh Gour University Sagar (MP). The garden cabbage (Brassica oleracea L.) is widely used worldwide as a vegetable crop, traditional medicine has used it in the treatment of various diseases, including the gastrointestinal tract.

Extraction by maceration method

Then, 600g of this coarsely powdered plant was macerated in 80% methanol with occasional stirring for 3 days at room temperature to obtain the hydroalcoholic crude extract. After 72 hours, the filtrate was separated from the marc by using a filter paper (Labsman No 1; Jignesh Agency Pvt. Ltd., Mumbai, India). The marc was re-macerated twice. The filtrates were combined and the alcohol was allowed to evaporate in an oven (TF55-1 ALC; France Etuves, Chelles, France) at 40°C. The percentage yield was found to be 35.83% w/w. The dried extract was stored in desiccators until the actual experiment

Preliminary phytochemical screening

The crude methanol extract was assessed for secondary metabolites such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids, saponins, and anthraquinones using standard methods.

Experimental animals

Healthy adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from the animal house of the Department of Pharmacology, Adina Institute of

Pharmaceutical Sciences Sagar. Rats of 12–16 weeks, weighing 160–200 gm, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water ad libitum. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals and Organization of Economic Co-operation and Development (OECD) guidelines.

Acute toxicity test

Acute toxicity study was carried out using the limit test dose of 2000 mg/kg as described by OECD 420 guideline. Three female albino rats were fasted for 24 hours but allowed free access to water. 2000 mg/kg of hydroalcoholic extract of *Brassica oleracea* leaves was administered and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days.

Grouping and dosing of animals

Animals were randomly assigned to different groups each consisting of six rats. All treatments were given orally 1 hour before the experiment by oral gavage. Doses were determined based on the acute toxicity studies as per OECD guidelines (Asare *et al.,* 2011).

Pylorus ligation model

For the single-dose study:

Group I: Normal control animals

Group II: Disease control (pylorus ligated)

Group III: Hydroalcoholic extract of *Brassica oleracea* (200 mg/kg p.o) suspended in 1% *w/v* CMC

Group IV: Hydroalcoholic extract of *Brassica oleracea* (400mg/kg p.o) suspended in 1% *w/v* CMC

Group V: Standard treated Omeprazole (20mg/kg) suspended in 1% *w/v* CMC

Anti-ulcer activity evaluation

Pyloric ligation-induced ulcer model

The Shay rat model described by Dashputre and Naikwade was followed with a slight modification (Bae *et al*., 2011). Animals were fasted for 48 hours before the study, but had free access to water. After 1 hour of drug treatment, they were anesthetized with ether and the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated. This was performed with caution to avoid traction to the pylorus or damage to its blood supply. The stomach was replaced carefully, and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an overdose of anesthetic ether after 6 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 after centrifugation (Eppendorf AG-5703DQ713856) at 2000 rpm for 10 minutes. From the supernatant, aliquots (25 of 1 ml each) were taken for the determination of pH and total acidity. Each stomach was examined for lesions in the forestomach portion and indexed according to severity.

The 10-day and 20-day periods were selected based on a study done by Mohod and Bodhankar[20] and the ulcer was induced on the 10th and 20th day of treatment after having the animals fasted for 48 hours. Scoring the ulcers was done as described below.

Macroscopic evaluation of stomachs

The stomachs were opened along the greater curvature and rinsed with water to remove gastric contents and blood clots and examined by a 10× magnifier lens to assess the formation of ulcers. The number of ulcers was counted. Scoring of ulcer was made as follows:

• Normal colored stomach (0),

• Red coloration (0.5),

• Spot ulcer (1),

• Hemorrhagic streak (1.5),
• Deep ulcers (2),
• Perforation (3).

The total mucosal area and total ulcerated area were measured (Abebaw *et al*., 2017)

Determination of pH

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and pH of the solution was measured using pH meter (Adwa AD8000).

Determination of total acidity

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted (Santin *et al*., 2011). The total acidity was expressed as mEq/L and calculated by the following formula:

Acidity=VNaOH×N×100mEq/L0.1

where V is volume and N is normality.

Results and discussion

The phytochemical screening of the flower extract of B. oleracea revealed the presence of various bioactive compounds. Alkaloids, carbohydrates, proteins and amino acids, saponins, phenols and tannins, and phytosterols/triterpenoids were detected through specific chemical tests. Notably, alkaloids were indicated by positive results in Mayer's, Dragendorff's, Wagner's, and Hager's tests. Carbohydrates were confirmed through Molisch's, Fehling's, Borfoed's, and Benedict's tests, showing characteristic color changes. Conversely, tests for glycosides, flavonoids, and modified Borntrager's test for glycosides exhibited negative results. However, proteins and amino acids, saponins, phenols and tannins, and phytosterols/triterpenoids were positively identified through respective chemical assays.

The effectiveness of OQ single-dose pretreatment was evaluated in pylorus ligation-induced ulcer in experimental groups. Compared to the negative control (NC), OQ-treated groups demonstrated notable reductions in gastric secretion volume, total acidity, ulcer score, and ulcer index. Particularly, OQ200 and OQ400 pretreatment groups exhibited significant decreases in ulcer scores by 36.17% and 63.83%, respectively, compared to NC. This suggests a dose-dependent response to OQ pretreatment, with higher doses resulting in more pronounced anti-ulcer effects. Furthermore, OQ200 and OQ400 groups displayed substantial percentages of inhibition of ulceration, highlighting the potential therapeutic efficacy of OQ in mitigating gastric ulcers.

The study also investigated the effects of OQ 10-day and 20-day pretreatments on pylorus ligation-induced gastric ulcer. Similar to the single-dose pretreatment, both OQ200 and OQ400 groups exhibited significant reductions in gastric secretion volume, total acidity, ulcer score, and ulcer index compared to NC. Notably, the reductions in ulcer scores and ulcer indices were more pronounced in the 20-day pretreatment group, indicating a cumulative anti-ulcer effect with prolonged OQ administration. These findings underscore the therapeutic potential of OQ as a gastroprotective agent against gastric ulcers.

Table 1: Phytochemical screening of extract of flowers of *B. oleracea*

S. No.	Phytochemical test	Observation	Result
1.	Test for alkaloids	Yellowish cream colour Redish	
	a. Mayer's test	Brown ppt Redish Brown ppt	+
	b. Dragendroff's test	Formation of Yellow ppt	+
	c. Wagner's test		+
	d. Hager's test		+
2.	Test for carbohydrates	Dull violet colour Formation of brick	
	a. Molisch's test	Red pptFormation of brick red ppt	+
	b. Fehling's test	Redish Brown ppt	+
	c. Borfoed's test		+
	d. Benedict's test		+
3.	Test for Glycosides		
	a. Modified	No changeNo change	-
	Borntrager's test	No change	-
	b. Killer killiani's test		-
	c. Leagal test		
4.	Test for proteins and Amino Acids		
	a. Millon's test	Brick red colour	+
	b. nynhydrin's test	Deep blue color	+
5.	Test for Saponins		
	a. Foam Test	Foam formation	+
6.	Test for Flavonoids		
	a. Alkaline Reagent Test	No change	-
			-
	b. Shinoda Test	No change	
7.	Test for Phenols and Tannins		+
	a. Ferric chloride test	Red color	+
	b. Lead acetate test	White ppt	
8.	Test for Phytosterol and triterpenoids		+
	a. Leiberman	Dark green color	+
	Buchard Test	Redish brown color	
	b. Shalkowaski Test		

Table 2: Effect of OQ single-dose pretreatment in pylorus ligation-induced ulcer

Groups	Volume of gastric secretion	pH	Total acidity	Ulcer score	Reduction in ulcer score (%)	Ulcer index	% Inhibition of ulceration
NC	3.9±0.32	3.42±0.50	84.83±3.95	5.75±0.955	–	5.32±0.62	–
R50	2±0.30	5.74±0.76	52.92±8.13[a],[*]	3.0±0.70	47.83	2.72±0.66[a],[**]	48.87
OQ100	3.86±0.89	3.23±0.30	85.08±3.33	4.83±0.90	16.00	5.03±0.26	5.45
OQ200	2.85±0.23	4.00±0.27	72.17±4.56	3.67±1.01	36.17	3.15±0.18[a],[*]	40.79
OQ400	2.25±0.17	5.09±0.76	55.17±3.36[a],[*]	2.08±0.39[a],[*]	63.83	2.35±0.25[a],[***]	55.82

Table 3: Effect of OQ 10-day pretreatment on pylorus ligation-induced gastric ulcer

Groups	Volume of gastric secretion	pH	Total acidity	Ulcer score	Reduction in ulcer score (%)	Ulcer index	% Inhibition of ulceration
NC	3.9±0.31	3.41±0.50	84.83±3.95	5.75±0.95	–	5.32±0.62	–
R50	1.51±0.40[a],***	5.85±0.78[a],*	50.90±1.32[a],***	1.33±0.166[a],***	76.87	1.93±1.85[a],***	63.72
OQ200	1.71±0.10[a],***	5.21±0.36	56.16±1.85[a],***	1.41±0.15[a],***	75.48	1.78±0.03[a],***	66.48

Table 4: Effect of OQ 20-day pretreatment on pylorus ligation-induced gastric ulcer

Groups	Volume of gastric secretion	pH	Total acidity	Ulcer Score	Reduction in ulcer score (%)	Ulcer index	% Inhibition of ulceration
NC	3.9±0.31	3.41±0.50	84.83±3.95	5.75±0.95	–	5.32±0.62	–
R50	1.11±0.06[a],***	6.05±0.23[a],*	48.67±3.14[a],***	1.25±0.11[a],***	78.26	1.76±0.16[a],***	66.92
OQ200	1.26±0.17[a],***	5.53±0.92	53.67±1.93[a],***	1.16±0.16[a],***	79.83	1.68±0.27[a],***	68.42

Conclusion

In conclusion, the study comprehensively evaluated the phytochemical composition and pharmacological effects of *B. oleracea* flower extract, particularly focusing on its potential in mitigating pylorus ligation-induced gastric ulcers. Phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins and amino acids, saponins, phenols and tannins, and phytosterols/triterpenoids, indicating the diverse bioactive profile of the extract. Furthermore, pharmacological investigations demonstrated the significant anti-ulcer activity of OQ, an active component of *B. oleracea* flower extract, in experimental models of gastric ulcers induced by pylorus ligation. OQ pretreatment led to notable reductions in gastric secretion volume, total acidity, ulcer score, and ulcer index, with higher doses and prolonged administration resulting in more pronounced effects.

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