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Original Research Article

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# Antioxidant and Antiulcer Potential on leaves of *Brassica nigra L.* against Gastric Ulcer

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## Abstract

In the present study, anti-ulcerative effects of extract of *Brassica nigra* L. leaves were investigated in pylorus ligation and aspirin-induced gastric ulcer models in rats. In present study antiulcer effect of ethanolic extract of *Brassica nigra* leaves is well understood by total acidity, free acidity, and ulcer index of different groups of animals in both the model viz. Pylorus ligation model & Aspirin induced gastric ulcer in experimental rats when compared the test group with control and standard group. The percentage of ulcer inhibition was observed in pylorus ligated model was as follows: standard, BNLE 100mg/kg, BNLE 200mg/kg and BNLE 300mg/kg 55.76%, 30.96%, 49.54% and 51.35% respectively. The mechanism of gastric ulcer healing is not well understood and further study is needed to evaluate the specific phytoconstituents responsible for the gastric ulcer healing but on the basis of present study it can be concluded that this gastric healing potential may be due to the pharmacological effect played by the different phytoconstituents present in the ethanolic extract of *Brassica nigra* leaves for prevention of gastric mucosa or by the antioxidant potential of the leaves which is evaluated during the DPPH free radical scavenging activity.

**Keywords:** *Brassica nigra L.* (Brassicaceae), Gastric ulcer,Pylorus ligation, Antioxidant, *Brassica nigra* leaf extract (BNLE)

## Introduction

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors [1]. Consequently, reduction of gastric acid production as well as re-inforcement of gastricmucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to $H_2$ antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia and haemopoeitic changes [2]. There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune gastrointestinal inflammation and gastric ulcer [3]. Drugs with multiple mechanism of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases.It has been demonstrated that many drugs and formulations possess potent antioxidant action and are effective in healing experimentally induced gastric ulcers [4-6]

## Materials and methods

### Plant material

The leaves of *Brassica nigra* L. was collected in the month of January from the local market, Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia UlHasan (Professor, Department of Botany), Safia College Bhopal and the specimen voucher no. assigned was 454/Bot/Safia/14.

#### Preparation of the extract

Fresh leaves were collected, shade-dried and powdered mechanically. For defatting about 500 g of the coarsely powdered leaves soxhlatation with Pet. Ether for 72 hours at 50-60$^0$C. Powder was dried in hot air oven at 40-50$^\circ$C soxhlatation with Pure Ethanol for 36 hours at 60-70$^\circ$C, Extract was dried at room temperature for 5 days and extract was collected.

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### Phytochemical screening

Preliminary phytochemical screening of the powdered leaves was performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides [7].

### Animals

Albino rats of *Wistar* strain of either sex weighing between 150 to 200 g were used. They were housed in standard cages at room temperature (25±2°C) and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation, but had free access to drinking water. The animals used for experimentation were obtained from the authorized animal house of Sapience Bioanalytical Research Lab, Bhopal (M.P.).

### Drugs and chemicals

Omeprazole and Aspirin were obtained from Sapience Bioanalytical Research Lab, Bhopal, India and Diphenyl Picryl Hydrazyl (DPPH) from Sigma Aldrich. All other chemicals used in this study were obtained commercially and were of analytical grade.

### Acute toxicity studies

Rats were kept overnight fasting prior to drug administration. A total of three animals were used which received a single oraldose (2000 mg/kg, b.w.) of *Brassica nigra* leaf extract (BNLE). After the administration of BNLE, food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation)and central nervous system (drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks. [8]

### Selection of dose of the extract

$LD_{50}$ was done as per OECD guidelines for fixing the dose for biological evaluation. The $LD_{50}$ of the extract as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The biological evaluation was carried out at doses of 100, 200 and 300 mg/kg body weight.

### Antiulcer activity

### Aspirin+pylorus ligation-induced ulcer model

BNLE, aspirin and standard antiulcer drug: omeprazole [9,10] were prepared in 0.5%sodium carboxy methyl cellulose (CMC) suspension as vehicle and administered orally once daily at a volume of 10 ml/kg body weight. The animals were divided into five groups, consisting of six animals in each group. Group I received aspirin alone (200 mg/kg,p.o.). Group II received omeprazole orally at the dose of 20 mg/kg body [11], Group III, Group IV, and Group V received BNLE orally at the doses of 100,200 and 300 mg/kg body weight respectively. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The abdominal wall was closed by sutures. After 19 hours of ligation, animals were sacrificed by cervical dislocation. The abdomen was opened and a ligature was placed around the esophagus to close. The stomach was removed and the contents were drained in a centrifuge tube. Along the greater curvature the stomach was opened and pinned on a cork plate. The mucosa was examined with a3-fold magnifier.

**Table 1: Various groups**

| S.no | Groups | Treatment received | Dose |
|---|---|---|---|
| 1 | **Control** | Aspririn alone | 200mg/kg p.o. |
| 2 | **Standard** | Omeprazole**(Omeprazole)(20 mg/kg)** | 20 mg/kg body |
| 3 | **Test 1** | BNLE | 100mg/kg |
| 1.5 | **Test 2** | BNLE | 200mg/kg |
| 2.5 | **Test 3** | BNLE | 300mg/kg |

*Antiulcer Activity (Methods for Biochemical Estimations like Free Acidity, Total Acidity in Gastric Juice) Collection ofGastric Juice*[11]

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. The gastric juice was collected & centrifuged for 1000 rpm for10 minutes. pH and volume of gastric juice was observed and recorded.

### Determination of free acidity and total acidity[11]

Gastric juice (1ml) was taken into the 100 ml conical flask, then 2-3 drops of Tofer's added and titrated with 0.01 N NaOH until all traces of red color disappears and the color of the solution turn yellowish orange.

The volume of alkali added was noted, this volume corresponds to free acidity.

Then 2-3 drops of phenolphthalein solution was added and titration was continued until a definite pink tinge appears.

The volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by following formula.

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH}}{0.1} \text{ X } 100\text{mEq/L}$$

### In vitro antioxidant activity

### DPPH assay

The free radical scavenging activity of BNLE was measured in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Marsden S. Blois [12]. About 4.3mg of DPPH was dissolved in 3.3 ml methanol. Then different volume levels of test sample (50, 100, 150, 200, 250µl) were screened and made 250µl of each dose level by dilution with methanol. Diluted with methanol with up to 3 ml. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The $IC_{50}$ value of the crude extract was compared with that of ascorbic acid, which was used as the standard.

### Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for Tukey-Kramer Multiple Comparisons t-test ANOVA with post-hoc analysis by employing statistical software, GraphPad InStat 3.Differences between groups were considered significant at $P$ <0.05 levels.

### Phytochemical screening

Phytochemical screening of the powdered leaves showed the presence of tannins, flavanoids, alkaloidsetc.

### Acute toxicity studies

In $LD_{50}$ studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 200 and 300 mg/kg body weight.

### Aspirin+pylorus ligation-induced ulcer model

Animals in the Aspirin+pylorus ligation(APL) group showed a significant ($P$ < 0.01)increase in the ulcer index and acid secretory parameters like gastricvolume, pH, free and total acidity when compared with those of vehicle treated group. In the rats of this group, a number of perforated ulcers (score 50) were also observed. Administration of BNLE produced significant ($P$ < 0.01) decrease in ulcer index in a dose dependent manner. All the ulcers were of scores 10 and 20 and no perforated ulcers were observed. The extract also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid, proving its antisecretory activity. BNLE at a dose of 100,200 and 300 mg/kg body weight showed protection index of 30.96%, 59.54 and 51.35%, respectively, whereas omeprazole showed protection index of 55.76 % at a dose of 20 mg/kg body weight (Table 2).

## Results

**Table 2: -** Effect of ethanolic extract of *Brassica nigra L.* leaves on ulcer index and % protection on Pylorus Ligation induced ulcer in rats

| S. No | Treatment (mg/kg) | pH of gastric content | Volume of gastric content(ml/100g) | Free acidity (meq/L/100g) | Total acidity (meq/L/100g) | Ulcer index | %protect-tion |
|---|---|---|---|---|---|---|---|
| 1 | Control | 2.65±0.87 | 3.12±1.56 | 22.21±1.3 | 38.22±0.02 | 18.83± 0.9 | - |
| 2 | Standard(Omeprazole)(20 mg/kg) | 4.87±0.79 | 1.33±0.61 | 10.67±2.51 a*** | 15.32±0.12 a*** | 8.33±0.33aa*** | 55.76 |
| 3 | Test 1 group (100mg/kg) | 2.9±0.84 | 2.89±0.85 | 18.3±1.23 a**,b*** | 32.47±0.32a**,b*** | 13.0±0.36 a***,b*** | 30.96 |
| 4 | Test 2 group (200mg/kg) | 3.84±0.74 | 1.94±0.94 | 15.9±2.18 a***,b** | 21.5±0.14 a***,b** | 9.5±0.42 a*** | 49.54 |
| 5 | Test 3 group (300mg/kg) | 3.6±1.38 | 1.75±1.09 | 15.06±3.12 a***,b** | 19.89±1.09 a***,b* | 9.16±0.3 a*** | 51.35 |

All values are mean ± SEM, n = 6. $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001

a- Significance difference as compared to group-I (control).

b- Significance difference as compared to group-II (Standard).



**Table 2:-**Antioxidant activity of ethanolic extract of *Brassica nigra* leaves

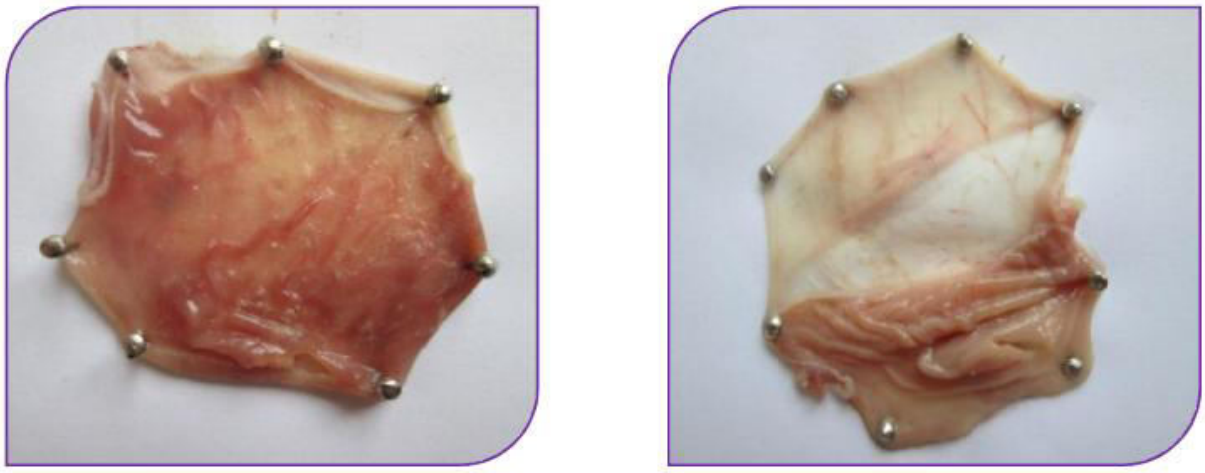
| S. No. | Absorbance at 517 nm | | | | % of activity | |
|---|---|---|---|---|---|---|
| | Standard | | Sample | | | |
| | Conc.(µg/ml) | Absorbance | Conc.(µg/ml) | Absorbance | Standard | Sample |
| 1 | 10 | 0.933 | 50 | 0.782 | 19.56 | 5.09 |
| 2 | 20 | 0.759 | 100 | 0.709 | 34.56 | 13.95 |
| 3 | 30 | 0.534 | 200 | 0.526 | 53.96 | 36.16 |
| 4 | 40 | 0.411 | 300 | 0.483 | 63.56 | 41.38 |
| 5 | 50 | 0.321 | 400 | 0.263 | 74.32 | 68.08 |

## Discussion

After the extraction, pharmacognostic evaluation was done including determination of Ash value in which percentage of acid insoluble ash, water soluble ash, total ash and moisture content, foaming index were determined. Extract was subjected to various chemical tests for preliminary identification of various phytochemicals. The extract contains carbohydrates, glycosides, alkaloids, flavonoids, etc.In the present study, anti-ulcerative effects of extract of *Brassica nigra* L. leaves were investigated in pylorus ligation and aspirin-induced gastric ulcer models in rats. The pylorus ligation induced ulcer was used to study the effect on gastric secretion. The ligation of the pyloric end of stomach causes accumulation of gastric acid in the stomach that produces ulcers. Agents that reduce secretion of gastric aggressive factors such as acid and pepsin (anti-secretary) and/or increase secretion of mucin (cytoprotective) are effective in reducing development of gastric ulcers–in this model. In this model, ethanolic extract of *Brassica nigra* L. leaves and omeprazole showed a significant reduction in Free acidity, Total acidity and Ulcer index ($p<0.001$, $p<0.01$, $p<0.05$) when compared to control. The percentage of ulcer inhibition was observed in pylorus ligated model was as follows: standard, BNLE 100mg/kg, BNLE 200mg/kg and BNLE 300mg/kg 55.76%, 30.96%, 49.54% and 51.35% respectively.

Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice [13], or decrease the volume of gastric juice and its acid output [14]. Prostaglandins have protective effects against various gastric injury models [15,16]. Aspirin has been shown to reduce the mucosal PGE2 content [17,18].In this model, ethanolic extract of *Brassica nigra* leaves and omeprazole showed a significant reduction in Ulcer index ($p<0.001$, $p<0.01$) when compared to control. The percentage of ulcer inhibition was observed in this model as follows: standard, BNLE 100mg/kg, BNLE 200mg/kg and BNLE 300mg/kg 48.92%, 22.48% ,41.29% and 47.81% respectively.

**Figure 1.A:- Healing of Pylorus Ligated Induced Gastric Ulcer**



CONTROL OMEPRAZOLE 20mg/kg

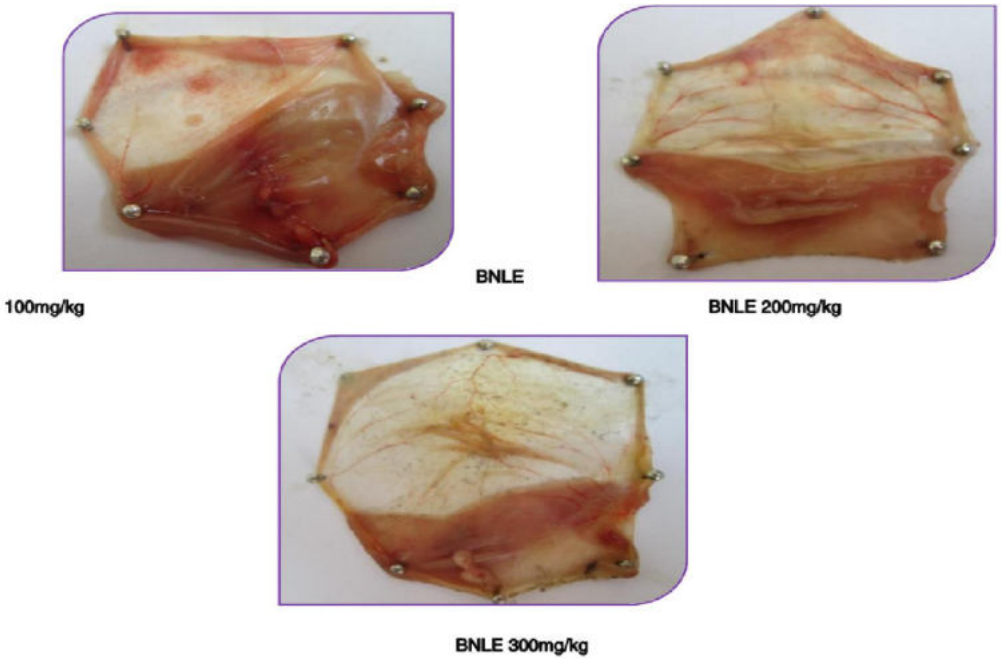
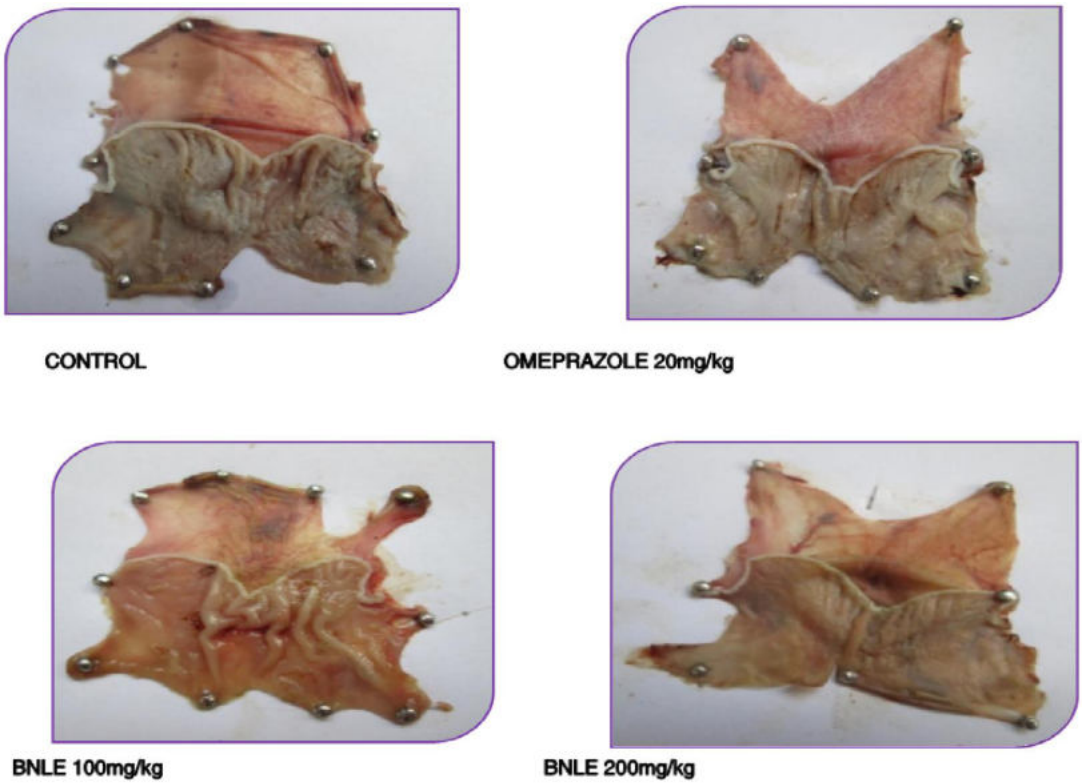
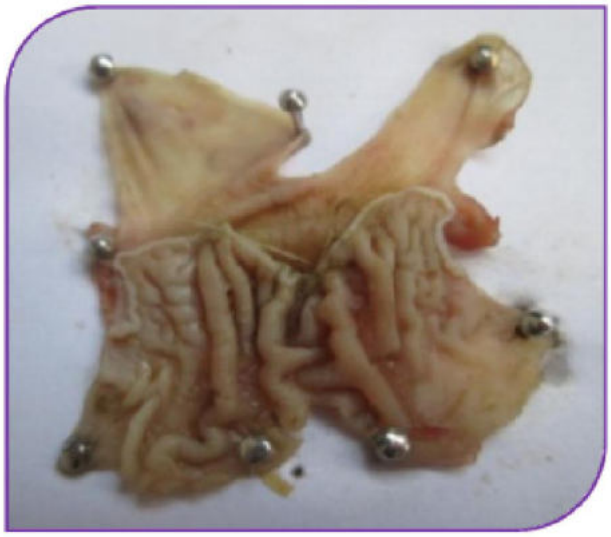



Figure 1.B:- Healing of Aspirin Induced Gastric Ulcer





BNLE 300mg/kg

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