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Gastroprotective Effects of Cabbage Extracts on Ethanol-Induced Gastric Ulcer in Wistar Rats

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Abstract
Introduction: This study investigated the gastroprotective effects of *Brassica oleracea* (cabbage) extracts on ethanol-induced gastric ulcers in Wistar rats, exploring a potential natural alternative to conventional medications. Various extracts of *Brassica oleracea* were evaluated for their ability to prevent gastric ulcers, comparing their efficacy to that of standard treatments such as cimetidine and rabeprazole.
Methods: Fifty Wistar rats were divided into ten groups, including controls and those pretreated with different doses of cabbage (Hexane, Ethyl acetate and Methanol) extracts (HEBO 500 and 1000 mg/kg, EtBO 500 and 1000 mg/kg, and MEBO 500 and 1000 mg/kg) and standard drugs (Cimetidine 50 mg/kg and Rabeprazole 20 mg/kg). Ulcer induction was performed using ethanol, and the gastroprotective effects were assessed.
Results: The HEBO 500 mg/kg and EtBO 500 mg/kg body weight extracts demonstrated significantly higher ulcer inhibition when compared to other pretreatment groups. Notably, the EtBO 500 mg/kg group exhibited the highest gastroprotective effect.
Conclusion: This research highlighted the potential of Brassica oleracea as a natural source for developing novel gastroprotective agents. Further studies are currently ongoing in our laboratory to isolate and characterise the bioactive compounds responsible for these effects.

Keywords: Anti-ulcer, Gastroprotective, Phytochemicals, Brassica oleracea, Antioxidants, Cimetidine, Rabeprazole

Introduction
Extreme stomach discomfort and possible gastrointestinal bleeding are signs of Peptic Ulcer Disease (PUD), a serious gastrointestinal condition marked by erosions in the mucosal lining (1, 2). Ethanol can damage blood vessels by reducing mucus production and increasing reactive oxygen species (ROS), according to several studies. Ethanol also results in oxidative stress, which is associated with a decrease in the activity of antioxidant enzymes and an increase in the production of free radicals obtained from oxygen (3). When used as a medication to treat serious illnesses, herbal therapy is both clinically beneficial and less harmful than currently available medications.
Despite their overall effectiveness, PUD treatments are known to have several drawbacks and restrictions, including incomplete healing, recurrence, and antibiotic resistance in cases involving *Helicobacter pylori* (4). Similarly, gastrointestinal disorders like gastritis, gastric ulcer (GU), and gastric cancer are tightly linked to *Helicobacter pylori* (5). *H. pylori* infection was identified as a Class I carcinogen in the 2017 preliminary list of carcinogens released by the International Agency for Cancer Research of the World Health Organisation (6). To effectively manage and prevent

peptic ulcers, these deficiencies must be addressed by continued research and development of novel treatments, improved diagnostic instruments, and all-encompassing, less costly strategies.

Herbal medicine is widely used in primary healthcare in developing countries due to its greater acceptance, better compliance, and fewer adverse effects (7). Because they are a part of the metabolic processes of living plants, the chemical components in plant extracts are more like those in the human body (8, 9). Endoscopy is considered the gold standard for diagnosing PUD and is the most accurate test available (10). A member of the Brassica family, cabbage is an herbaceous, biennial, dicotyledonous flowering plant (11, 12). Due to its high flavonoid and anthocyanin content, cabbage has long been used in herbal therapy to treat gastrointestinal disorders, such as peptic, gastritis, duodenal ulcers and irritable bowel syndrome, sores, and mastitis (13). Because of their chemical components' ability to prevent and treat a wide range of ailments, medicinal plants are being used (14).

Several studies have shown that compounds with anti-inflammatory and antioxidant properties can help prevent stomach ulcers caused by alcohol (15). Studies have demonstrated the anti-ulcer properties of phytochemicals such as glycosides, alkaloids, flavonoids, and tannins. Phytochemicals such as tannins, alkaloids, flavonoids, glycosides, and saponins have been found in cabbage (16).

Furthermore, it is becoming increasingly evident that dietary modifications are necessary for both managing and preventing peptic ulcers (17). The stated prevalence of peptic ulcers, which are generally non-fatal, primarily depends on the diagnostic skills of the doctor (18). Up to 50% of people in the West take herbal medicines, and 10% of them are used for curing or preventing digestive problems (19). Therefore, this research aimed to determine the effect of cabbage extracts on ethanol-induced gastric ulcers in Wistar rats.

Methods

Plant authentication

The *Brassica oleracea var capitata* was assigned the UIH-23516 authentication number by the Department of Botany, University of Ibadan, Oyo State, Nigeria.

Chemicals

All chemicals used were of analytical grade.

Preparation of Samples

Cabbage was purchased in Ilishan-Remo, Ogun State. It was washed separately under a running tap to remove adhering dirt, debris, extraneous materials and foreign matter. After washing, the cabbage was sliced separately into tiny pieces with a sharp kitchen knife, then they were transferred to a clean container, which was properly sealed and labelled, before storing in the freezer. It was oven dried at 40 °C. When completely dried, they were ground into fine powder using an electric grinder and stored in a tightly sealed glass jar.

Five hundred grams of the pulverised cabbage were macerated in 2000 mL n-hexane for 72 hours. Similarly, the n-hexane extract was decanted, and the residue was soaked in 2,000 mL of ethyl acetate for an additional 72 hours. The residue of the ethyl acetate was macerated in 2000 mL of methanol and decanted. The filtrate was concentrated using a rotary evaporator. The choice of solvents was based on these facts: n-hexane is a non-polar solvent, suitable for extracting lipophilic (fat-soluble) compounds, such as oils, waxes, and some pigments, ethyl acetate is moderately polar solvent, suitable for extracting a wide range of compounds, including flavonoids, alkaloids, and some glycosides and methanol is a polar solvent, suitable for extracting polar compounds, such as phenolic acids, glycosides, and some alkaloids.

Research procedure

Forty-nine Wistar rats with an average weight of 140 g were obtained from Babcock University, Ilishan-Remo, Ogun State. The rats were housed in polyethene cages with a constant temperature of 25 ± 2°C and a 12-hour light-dark cycle. They had free access to food and water. Ten groups, each consisting of five rats, were formed following a two-week acclimatisation period. Pretreatment (Table 1) was done one hour before challenging them with ethanol. The animals were sacrificed one hour after being challenged with ethanol.

Table 1: Pretreatment groups

Group	Treatment
1	Distilled water
2	Ethanol ulcer induction (without pretreatment)
3	Cimetidine (50 mg/kg) + ulcer induction
4	Rabeprazole (20 mg/kg) + ulcer induction
5	500 mg/kg *b.wt* n-hexane extract of *Brassica oleracea* (HEBO) + ulcer induction
6	1000 mg/kg *b.wt* n-hexane extract of *Brassica oleracea* (HEBO) + ulcer induction
7	500 mg/kg *b.wt* ethyl acetate extract of *Brassica oleracea* (EtBO) + ulcer induction
8	1000 mg/kg *b.wt* ethyl acetate extract of *Brassica oleracea var. capitata* (EtBO) + ulcer induction

9	500 mg/kg *b.wt* methanol extract of *Brassica oleracea* (MEBO) + ulcer induction
10	1000 mg/kg *b.wt* methanol extract of *Brassica oleracea* (MEBO) + ulcer induction

The male Wistar rats used had their stomachs removed and sliced open along the larger curvature to examine and measure lesions manually (mm2). The stomach juice was collected and stored in a standard container at 4°C until needed (20).

The scoring method, as described by reference (16), assesses the severity of damage or bleeding based on lesion size. A score of 0 indicates no observable damage or bleeding. A score of 1 is assigned when there is minimal damage or bleeding, with lesions measuring between 0.5 and 1.0 mm. Lesions ranging from 1.0 to 1.5 mm receive a score of 2. More severe lesions, measuring between 1.5 and 2.5 mm, are scored as 3. Finally, lesions indicative of perforation, with sizes between 2.5 and 3.5 mm, are given the highest score of 4.

$$\text{Ulcer index (UI)} = \frac{\text{total ulcer score}}{\text{No. of rats ulcerated}}$$

Percentage inhibition of ulceration

$$\frac{\text{Ulcer index}_{\text{control group}} - \text{Ulcer index}_{\text{test group}} \times 100}{\text{Ulcer index}_{\text{control group}}}$$

The gastric content was analysed for pH, total acidity, and ulcer index/or percentage inhibition (21).

Statistical Analysis

Statistical analysis was done using GraphPad Prism® 8.0. Statistical comparison of the data was carried out using one-way Analysis of Variance (ANOVA). Post hoc comparison was done using the Tukey test. Values of $p<0.05$ were considered significant.

Results

The macroscopic effect of extracts on the gastric mucosa

Figure 1 shows that the stomach mucosa of the negative control group suffered significant harm from absolute ethanol. The distilled water group (A) shows no lesions with intact gastric mucosa. Ulcer control group (negative control) (B) shows severe damage to the stomach. Cimetidine (C) shows gastric lesions. Rabeprazole (D) shows milder lesions. (E): HEBO 500 mg/kg shows milder lesions; (F): HEBO 1000 mg/kg shows milder lesions; (G): EtBO: 500 mg/kg shows no lesions; (H): EtBO: 1000 mg/kg shows milder lesions; (I): MEBO 500 mg/kg shows severe lesions; (J): MEBO 1000 mg/kg shows severe lesions.

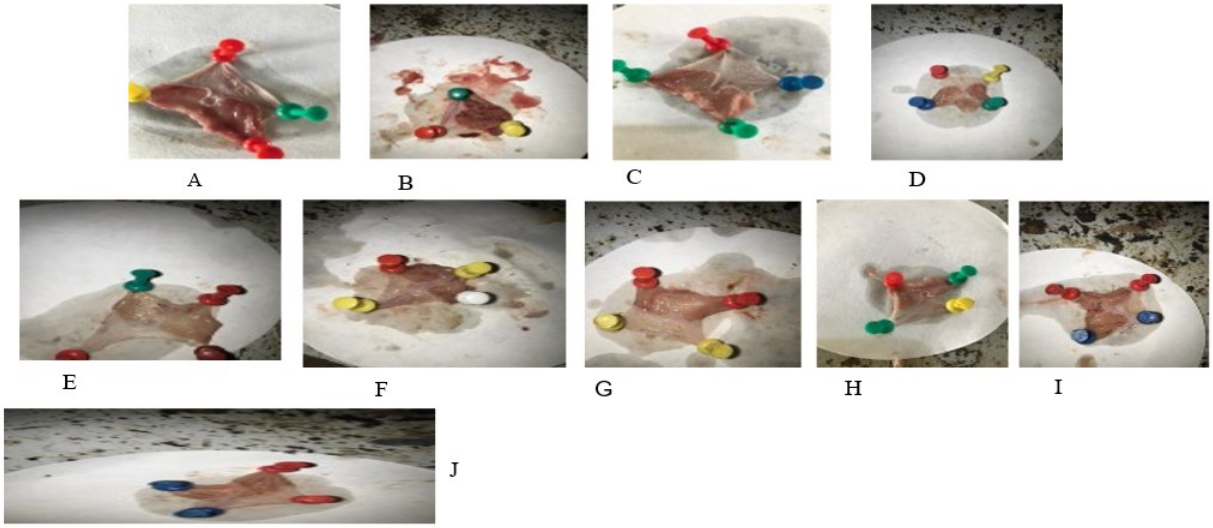

Figure 1: Examination of the gastric mucosa of treated rats after ulcer induction

A: Distilled water; B: Ulcer control group (negative control); C: Cimetidine; D: Rabeprazole; E: HEBO 500mg/kg; F: HEBO 1000 mg/kg; G: EtBO: 500 mg/kg; H: EtBO: 1000 mg/kg; I: MEBO 500 mg/kg; J: MEBO 1000 mg/kg

Effect of Cabbage extracts on ulcer index and percentage inhibition of ulceration

Figure 2 Shows that the stomach mucosa of the negative control group suffered significant injury from absolute ethanol. The ulcer index (UI) of the negative control was much higher at 2.25 ± 0.10. Pre-treated rats with 50 mg/kg body weight of cimetidine, 20 mg/kg body weight of rabeprazole, 500 mg/kg and 1000 mg/kg body weight of HEBO, 500 and 1000 mg/kg of EtBO, had significantly ($p < 0.05$) reduced ulcer index, 1.60 ± 0.05, 1.40 ± 0.11, 0.80 ± 7.85e-017, 1.0 ± 0.11, 0.4 ± 0.11, 0.6 ± 0.11, respectively, unlike that of 500 mg/kg and 1000 mg/kg of MEBO which did not show any significant difference (1.8 ± 0.11 and 2.0 ± 0.11) when compared to the Ulcer control group (negative control).

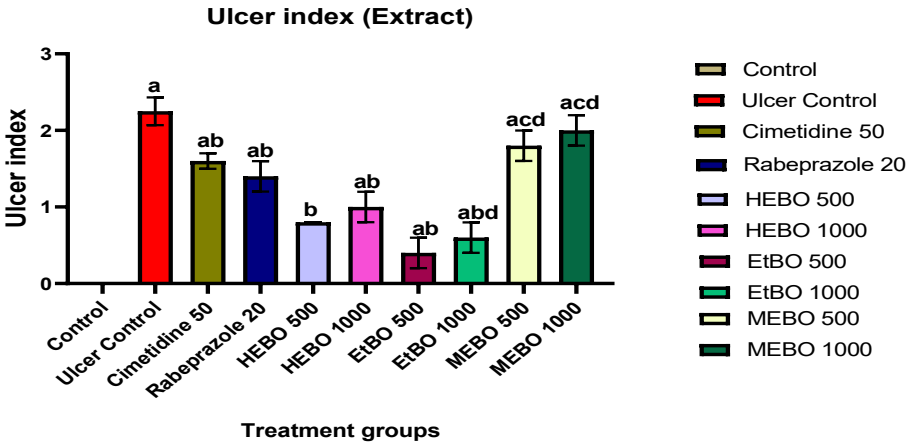

Figure 2: Effect of the different doses of the extracts on the ulcer index in the gastric mucosa of rats
Each bar represents Mean ± SEM (n=5). Similar letters represent no significant difference between groups. Different letters represent a significant difference at $p < 0.05$

Figure 3 shows that there was 0% inhibition of ulceration in the ulcer control rats. Inhibition rate of 50.0 ± 1.15, 58.0 ± 1.15, 72.0 ± 1.15, 60.66 ± 0.66, 82.0 ± 1.15, 62.0 ± 1.15 were observed in the rats pretreated with 50 mg/kg of cimetidine, 20 mg/kg of rabeprazole, 500 mg/kg of HEBO, 1000 mg/kg of HEBO, 500 mg/kg EtBO and 1000 mg/kg EtBO, respectively. They had fewer mucosal haemorrhagic foci. These findings showed that 50 mg/kg of cimetidine, 20 mg/kg of rabeprazole, 500 mg/kg of HEBO, 1000 mg/kg of HEBO, 500 mg/kg EtBO and 1000 mg/kg EtBO provided significant ($p < 0.05$) protection against ethanol-induced gastric ulcer. Similarly, 500 mg/kg and 1000 mg/kg of MEBO had the lowest protective function against the ethanol-induced ulceration on the gastric mucosa of the rats, with 22.00 ± 1.15, 10.33 ± 0.11 when compared to the other treatment groups.

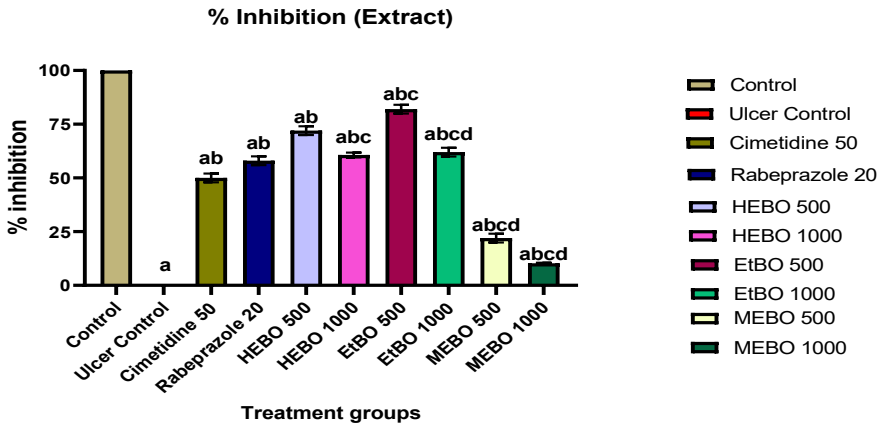

Figure 3: Effect of the different doses of the extracts on the inhibition of ulceration in the gastric mucosa of rats
Each bar represents Mean ± SEM (n=5). Similar letters represent no significant difference between groups. Different letters represent a significant difference at $p < 0.05$

Figure 4 shows that the ulcer control rats (3.10 ± 0.05 mL) had significantly ($p < 0.05$) elevated gastric juice volume when compared with the normal rats (0.79 ± 0.02 mL). However, rats treated with 50 mg/kg b.wt. Rabeprazole, 20 mg/kg b.wt, cimetidine, 500 mg/kg and 1000 mg/kg HEBO, 500 mg/kg and 1000 mg/kg EtBO. 500 mg/kg and 1000 mg/kg MEBO significantly ($p < 0.05$) reduced the gastric juice volume of 1.89 ± 0.01, 1.81 ± 0.01, 1.66 ± 0.02, 1.74 ± 0.20, 1.13 ± 0.04, 1.45 ± 0.05, 2.4 ± 0.02, and 2.65 ± 0.07 mL, respectively, when compared with the ulcer control rats. Further analysis showed that rats treated with HEBO and EtBO had significantly (0.05) reduced gastric juice volume than the 500 mg/kg and 1000 mg/kg MEBO.

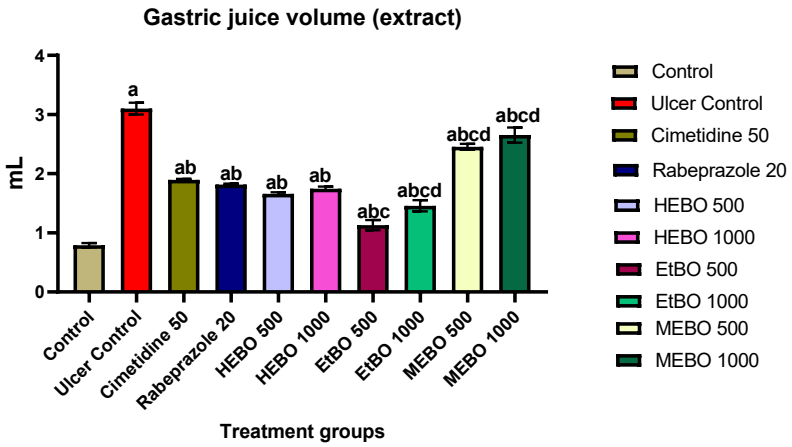

Figure 4: Effect of the different doses of the extracts on gastric juice volume

Each bar represents Mean ± SEM (N=5). Similar letters represent no significant difference between groups. Different letters represent a significant difference at $p < 0.05$

Figure 5 shows that there was a significant ($p < 0.05$) elevation in the gastric juice pH of the ulcer control rats (7.09 ± 0.04) when compared with the normal rats (1.63 ± 0.02). Rats treated with 50 mg/kg b.wt. rabeprazole, 20 mg/kg b.wt. 500 mg/kg and 1000 mg/kg HEBO, 500 mg/kg and 1000 mg/kg EtBO and 500 mg/kg and 1000 mg/kg MEBO b.wt. had significantly ($p < 0.05$) elevated gastric juice pH of 5.76 ± 0.07, 5.25 ± 0.06, 4.29 ± 0.02, 4.87 ± 0.03, 3.12 ± 0.08, 4.59 ± 0.06, 6.21 ± 0.12, 6.61 ± 0.03, respectively, when compared with the ulcer control rats. The 500 mg/kg and 1000 mg/kg MEBO had higher gastric juice volume than the other treatment groups.

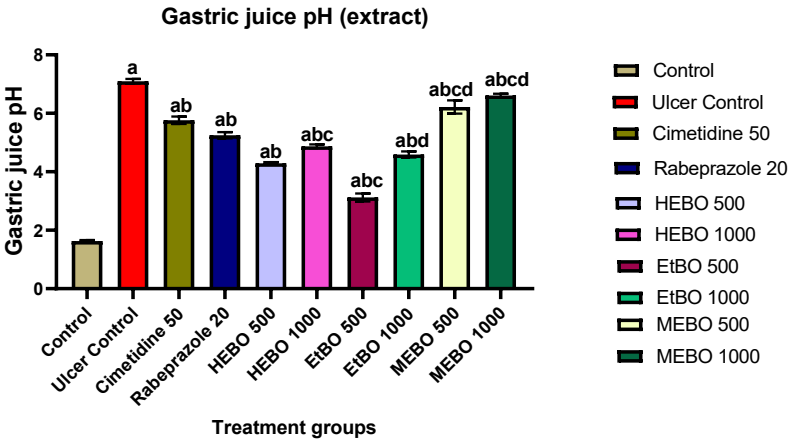

Figure 5: Effect of the different doses of the extracts on the gastric juice pH of rats

Each bar represents Mean ± SEM (N=5). Similar letters represent no significant difference between groups. Different letters represent a significant difference at $p < 0.05$

Figure 6 shows that there was a significant ($p < 0.05$) elevation in the total acid output of ulcer control rats (610.00 ± 5.77 mEq/1/100 g) when compared with the normal rats (125.0 ± 2.88). Similarly, rats treated with 50 mg/kg cimetidine, 20 mg/kg rabeprazole, 500 mg/kg and 1000 mg/kg HEBO, 500 mg/kg and 1000 mg/kg EtBO and 500 mg/kg and 1000 mg/kg MEBO b.wt 383.33 ± 8.81, 360.0 ± 5.77, 320.0 ± 5.77, 361.0 ± 4.40, 262.66 ± 3.71.

The rats treated with HEBO 500 mg/kg and EtBO 500 mg/kg b.t.w ($p < 0.05$) reduced total acid output when compared with rats in the other treatment groups. The 500 mg/kg and 1000 mg/kg MEBO had increased total acid output than the other treatment groups, 290.0 ± 5.77 vs 500.0 ± 5.77.

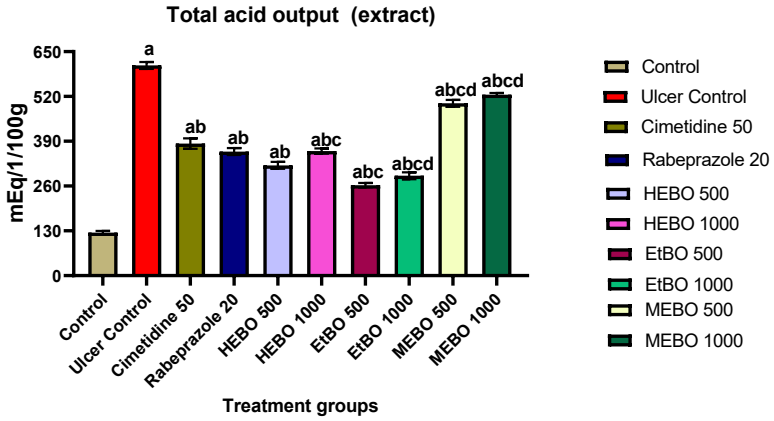

Figure 6: Effect of the different doses of the extracts on total acid output

Each bar represents Mean ± SEM (N=5). Similar letters represent no significant difference between groups. Different letters represent a significant difference at $p < 0.05$

Discussion

The ethanol-induced Gastric Ulcer (GU) model is a frequently used technique in laboratory studies (22). Ulcers are more common in people who consume ethanol (23). The ethanol-induced GU model has an advantage over other GU models since it is like acute peptic ulcer illness in humans in many ways (24). The aetiology of alcohol-induced stomach ulcers is complex and multifaceted. Like histamine, ethanol damages mucosal cells by reducing mucus production, which limits blood flow to the mucosa and acid-producing cells. Because mucus is soluble in ethanol and the mucosa is susceptible to the proteolytic and hydrolytic effects of pepsin and hydrochloric acid, ethanol readily penetrates the gastric mucosa and breaks down the protective layer (25).

Irritation of the stomach lumen stimulates increased production of gastric juice. One of the objectives of this study was to quantify this response by measuring the volume of gastric juice and evaluating the extent to which ethanol stimulates H^+/K^+-ATPase activity and histamine-mediated gastric acid secretion. In the untreated group, gastric acid levels were elevated. Previous studies have shown that mucosal injury enhances gastric acid secretion, leading to increased gastric juice output and a subsequent decrease in pH (26).

In this study, rats pretreated with EtBO 500 and 1000 mg/kg exhibited high potential in preserving gastric tissues and preventing ethanol-induced stomach injuries by significantly decreasing the amount of gastric juice in the stomach when compared to the other pretreatment groups. This suggests that EtBO may prevent H^+/K^+ ATPase/histamine from producing additional stomach acid. Since acid is crucial to the growth of peptic ulcers, EtBO's antiulcerogenic activity may be connected to its antisecretory function.

The stomach's acidity is gauged by its pH. Depending on how much stomach tissue can be harmed, the pH of the stomach may be low. Greater delivery of H+ and Cl-ions into the stomach lumen, which combine to produce gastric acid, is indicated by a low gastric pH.

Thus, the maximum secretion of acid and pepsin at low pH (high H^+ ions) results in mucus and cellular damage. In this study, the stomach pH was found to be considerably ($p < 0.05$) lower in rats treated with ethanol. Significantly ($p < 0.05$), higher results were obtained from the EtBO pretreated groups than from the groups pretreated with MEBO and HEBO.

It is essential to start digestion by triggering the release of stomach acid by pepsinogen because: (a) it eliminates dangerous bacteria and germs, and (b) it prevents their growth. However, some people may develop potentially fatal duodenal and stomach ulcers because of high levels of gastric acid and pepsinogen. Thus, hyperacidity can be avoided by activating parietal cell surface receptors that regulate acid production (27).

The results from this study may imply that there was excessive secretion of gastric acid and pepsinogen, which caused damage to the gastric mucosa of the negative control group (ulcer group), thereby producing ulcers. This investigation could also imply that all the pretreated groups had the potential to significantly reduce the damage caused by ethanol induction on the mucosa.

Previous phytochemical screening of *Brassica oleracea* revealed that they contain phenolic compounds, flavonols, carotenoids and alkaloids (28). The depletion in the gastric ulcer index of the ethyl acetate pretreated rats confirmed the effective ulcerogenic activity of ethyl acetate extract (EtBO). The gastroprotective importance of plant extracts in decreasing ulcer index, total acidity, gastric secretion and inhibition of ulceration in pre-treated animals has been reported (29).

Conclusion

This study demonstrated the gastroprotective effects of cabbage extracts in ethanol-induced gastric ulcers in Wistar

rats, highlighting their potential as a natural therapeutic option. The findings suggest that specific extracts, particularly EtBO at 500 mg/kg, exhibited significant ulcer inhibition and gastroprotection, comparable to or surpassing standard medications like cimetidine and rabeprazole. The results implied that *Brassica oleracea* (cabbage) contains bioactive compounds with potent anti-ulcer properties, warranting further research to isolate and characterise the compounds responsible, elucidate the underlying mechanisms of action, and explore potential applications in human gastroenterology.

This research has contributed to the growing interest in natural products for gastrointestinal health and may lead to novel therapeutic strategies for managing gastric ulcers.

Abbreviations

PUD: Peptic Ulcer Disease
MEBO: Methanol Extract of *Brassica oleracea*
EtBO: Ethyl Acetate Extract of *Brassica oleracea*
HEBO: n-Hexane Extract of *Brassica oleracea*
GU: Gastric Ulcer
BUHREC: Babcock University Health Research and Ethics Committee

Declarations

Ethics Approval: The experimental procedures were conducted following the ethical standards established by the Ethics Committee on Animal Research and Babcock University Health Research and Ethics Committee (BUHREC). BUHREC provided permission from an ethical standpoint with reference number BUHREC 345/24.

Data Availability: The data and materials associated with this research will be made available by the corresponding author upon request.

Competing Interests: The authors declare that they have no competing interests.

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Author contributions: AO: Methodology, Formal Analysis, Writing- Original Draft, Data Curation, Investigation, AK: conceptualisation, methodology, supervision, Writing - Review & Editing, Writing - Original Draft OA: Writing & Editing, OS: Investigation, Writing and Editing.

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