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Protective potential of the combination of cucumber (*Cucumis sativus*) and cabbage (*Brassica oleracea*) on ethanol-induced ulcer in male Wistar rats

Deborah Imisioluwa Makanju[1], Omowumi Titilola Kayode[2], Abolanle Abideen Azeez Kayode[1]*

[1]Phytomedicine Research, Drug Discovery & Development Laboratory, Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria; [2]Department of Biochemistry, College of Basic and Applied Sciences, Mountain Top University, Ogun State, Nigeria.

***Corresponding Author:** Abolanle A. A. Kayode, PhD, Associate Professor and HOD, Department of Biochemistry, Babcock University, PMB 4003, Ilishan Remo, Ogun State, Nigeria.

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ABSTRACT

Background: Peptic ulcer is an ulcerative disorder characterized by abdominal pains which often lead to gastrointestinal bleeding and/or perforation with a high mortality risk.

Objective: This study aimed to provide scientific validation for the folkloric use of vegetables (cucumber and cabbage) in Nigerian ethnomedicine to treat peptic ulcers.

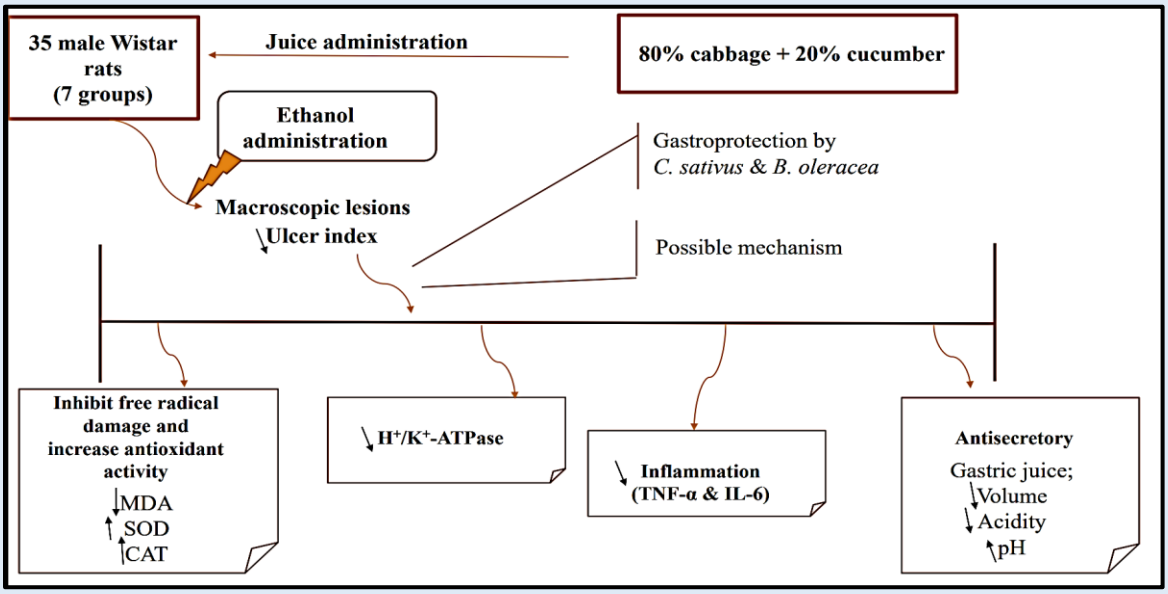
Methods: Cucumber and cabbage were used to formulate juice extract (JE) consisting of 80% cabbage and 20% cucumber. Thirty-five rats were randomly divided into 7 groups: Group 1 (naïve control) and group 2 (negative control) received distilled water, groups 3 – 7 were pre-treated with cimetidine (50 mg/kg b.wt), rabeprazole (20 mg/kg b.wt), 1.0 mL, 1.5 mL and 2.0 mL of JE, respectively, for 14 days after which the animals were fasted overnight and induced with gastric ulcer using 1 mL of absolute ethanol on day 15. Percentage inhibition of ulceration, gastric volume, total acidity, malondialdehyde, TNF– α and interleukin – 6 levels, H^+/K^+-ATPase, SOD and CAT activities were determined. Data were analyzed using one-way ANOVA at $p < 0.05$.

Results: The 2.0 mL of JE had the highest percentage ulcer inhibition (94.7%) when compared to cimetidine (52.6%) and rabeprazole (73.7%). Groups 3-7 significantly ($p < 0.05$) reduced gastric volume, total acidity, malondialdehyde, TNF– α, interleukin – 6 and H^+/K^+-ATPase activity/level, as well as significantly ($p < 0.05$) increased SOD and CAT activities when compared to the negative control.

Novelty: This study uniquely validates the traditional use of a specific combination of cucumber and cabbage (80% *Brassica oleracea*, 20% *Cucumis sativus*) in Nigerian ethnomedicine for treating peptic ulcers. Demonstrating a significantly higher gastro-protective effect with the juice extract compared to standard pharmaceutical treatments (cimetidine and rabeprazole), this research provides a novel scientific basis for this folkloric remedy. Furthermore, the comprehensive evaluation of the extract's impact on various biochemical markers, including inflammatory cytokines, oxidative stress enzymes, and gastric acidity, offers a detailed insight into its mechanism of action.

Conclusion: The 2.0 mL of JE exhibited the highest gastro-protective effect. Further studies to identify the main gastro-protective compound(s) are recommended.

Keywords: Anti-inflammatory, Antioxidants, *B. oleracea*, *C. sativus*, Gastro-protective, Peptic ulcer



Graphical Abstract: Proposed mode of action of the combination of cabbage and cucumber juice extract on ethanol-induced gastric ulcer in Wistar Rats

INTRODUCTION

Peptic ulcer disease (PUD) is a significant gastrointestinal disorder characterized by erosions in the mucosal lining, leading to symptoms such as severe stomach pain and potential gastrointestinal bleeding [1-2]. PUD comprises duodenal and gastric ulcers [3]. Duodenal ulcer occurs when the duodenal muscularis surface has any form of disruption [4], while gastric ulcer forms when the

mucosal lining is exposed to acid, alcohol, and pepsin [5]. As of 2017, the estimated prevalence of peptic ulcer disease (PUD) in the general population was between 5% and 10%. However, epidemiological studies have shown a decline in the incidence, hospital admission rates, and mortality associated with peptic ulcer [6]. The most accurate PUD diagnostic test, tagged as a golden standard for PUD diagnosis, is endoscopy [2, 7].

Standard treatments, including proton pump inhibitors (PPIs) and histamine type-2 (H2) receptor antagonists, are commonly employed but often have adverse effects and high recurrence rates. For instance, recurrence is common, with rates exceeding 60% in most series. This has prompted a growing interest in alternative therapies, particularly the use of functional foods with medicinal properties. Fibers and probiotics also play an important role in treating peptic ulcer, as they reduce the side effects of antibiotics and help reduce treatment time [8].

As a result, medicinal plants are being used, as their chemical compounds are beneficial in preventing and treating numerous diseases [6]. Additionally, it is increasingly clear that dietary changes are essential for preventing and managing peptic ulcer [9]. Generally, peptic ulcer is a non-fatal condition whose reported prevalence largely depends on the physician's diagnostic abilities [10]. In the West, up to 50% of people use herbal medications, with 10% of these used for treating or preventing digestive issues [11].

Functional foods are those that provide health benefits beyond basic nutrition, often due to the presence of bioactive compounds. These compounds can play a pivotal role in disease prevention and management. For instance, certain medicinal plants have demonstrated gastroprotective effects through mechanisms such as enhancing antioxidant activity, increasing gastric pH, and reducing gastric lesions. A comprehensive review highlighted various plants with such properties, underscoring the potential of natural remedies in PUD management [12].

Cucumber (*Cucumis sativus*) and cabbage (*Brassica oleracea* var. capitata) are widely consumed vegetables that are known for various potential health benefits. Cucumber is a warm-season vining plant of the genus Cucumis, belonging to the family cucurbitales [13], while cabbage – an herbaceous, biennial, dicotyledonous flowering plant [14], belongs to the Brassica family [15]. Cucumber is rich in polyphenolics and cucurbitacins, which have been shown to have antioxidant, anti-inflammatory, anti-hyaluronidase, anti-hyperglycemic, diuretic, amylolytic, anti-microbial, and analgesic properties [16], whereas, Cabbage has historically been used in herbal therapy to address gastrointestinal ailments, including peptic, gastritis, duodenal ulcers and irritable bowel syndrome, sores, and mastitis due to its high flavonoid and anthocyanin content [17]. These statements show that combining cucumber and cabbage can positively affect peptic ulcers. While there is limited research on the combined effects of cucumber and cabbage on peptic ulcers, both vegetables possess properties that may benefit individuals with ulcers.

No scientific reports were found in the literature supporting the combined impact of *Cucumis sativus* and *Brassica oleracea*. Studies on other plant-based remedies further support the integration of functional foods in managing PUD. For example, rat models' methanolic extract of *Ipomoea batatas* (sweet potato) tubers exhibited significant gastroprotective activity. The extract inhibited ulcer formation and enhanced antioxidant enzyme levels, suggesting its potential as a natural anti-ulcer agent [18].

Moreover, the role of bioactive compounds in disease management extends beyond PUD. Compounds with anticancer activity have been studied for their mechanisms of action, providing insights into how natural products can modulate disease pathways [19].

Thus, this study aimed to evaluate the effectiveness of these combined vegetables for their gastro-protective effects in rats.

MATERIALS AND METHODS

The cucumber and cabbage were purchased from a local market, Ogere, Ogun State. Cimetidine and Rabeprazole were obtained from Agram and Kayfaris pharmacies respectively, (accredited pharmacies) in Ilishan-Remo, Ogun State. The experiment utilized analytical grade chemicals including phosphate buffer, formaldehyde, Sodium hydroxide (NaOH), absolute ethanol, diethyl ether, and phenolphthalein. Commercial kits were used for the determination of MDA, SOD and CAT while enzyme linked immunosorbent assay (ELISA) kits were used for TNF-α, IL-6 and H^+/K^+-ATPase activity assay.

Processing of Cucumber and Cabbage: The cucumber and cabbage were washed separately under a running tap to remove adhering dirt, debris, extraneous materials, and any foreign matter. After washing, they were sliced into tiny pieces with a sharp kitchen knife and blended separately with a high-speed kitchen blender. Afterwards, they were transferred to a clean container, which was properly sealed, labelled, and stored in the freezer.

Preparation of Juice Extract: After weighing the sliced cabbage and cucumber, 80 % of Cabbage and 20% of cucumber were mixed together to form a juice extract, i.e., a ratio of 1:4. The juice extract was stored at 4°C.

Experimental Animals: Thirty-five male Wistar rats of average weight of 100 g were obtained from Adewuyi Research Animal Farm, Ibadan, Oyo State. The rats were kept in polyethylene cages with wood shavings under standard laboratory conditions, in a 12-hour light-dark cycle in a clean and well-ventilated room (25 ± 2°C and 45 – 55% relative humidity). They were allowed to acclimatize for 13 days. Thereafter, they were randomly selected into seven groups (5 rats per group). They were fed with standard laboratory rodent diet and drinking water *ad libitum.* Pre-treatment was done once daily for fourteen days (figure 1).

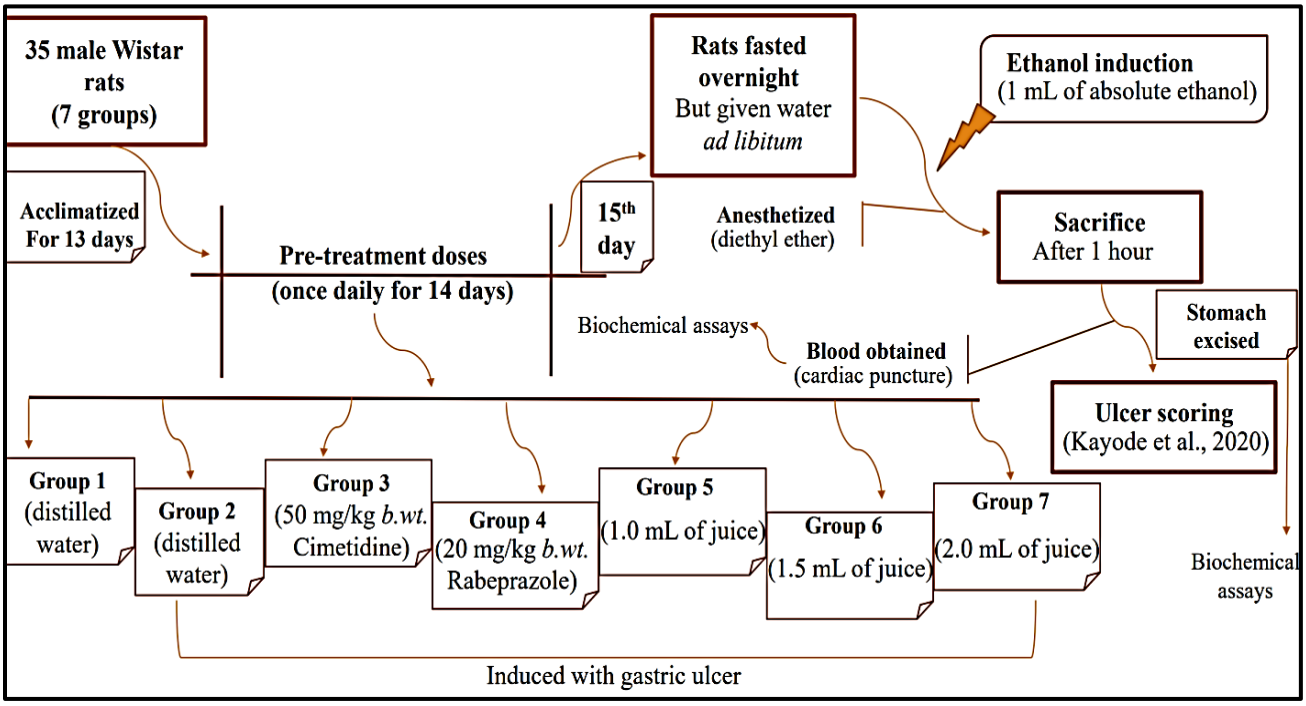


Figure 1: Experimental Design

Ethical Aspects: The experimental procedures were conducted in accordance with 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 955/23.

Experimental Design

The rats were randomized into 7 groups:

Group 1 (naïve control): The normal control group rats received 1 mL of distilled water orally for 14 days.

Group 2 (negative control): Received 1 mL of distilled water by oral gavage for 14 days and were induced with gastric ulcer on the 15th day.

Group 3 (positive control 1): Cimetidine, freshly dissolved in distilled water, at a dose of 50 mg/kg body weight was orally given for 14 days and was induced with a gastric ulcer on the day 15.

Group 4 (positive control 2): Rabeprazole, freshly dissolved in distilled water, at a dose of 20 mg/kg body weight was orally administered for 14 days and was induced with gastric ulcer on the 15th day.

Group 5: Received 1.0 mL of juice extract for 14 days and were induced with gastric ulcer on the 15th day.

Group 6: Received 1.5 mL of juice extract for 14 days and were induced with gastric ulcer on the 15th day.

Group 7: Received 2.0 mL of juice extract for 14 days and were induced with gastric ulcer on the 15th day.

Experimental Protocol for the Induction of Gastric Ulcer in Experimental Animals: On the 15th day, the rats were fasted overnight but given water *ad libitum*. The animals were administered an oral dose of absolute ethanol (1 mL) by intra-gastric gavage, using ball-tipped 18-gauge stainless steel feeding needle (76 mm length). The animals were sacrificed one hour later to obtain their stomach, after being anesthetized by diethyl ether to obtain blood samples. The blood was obtained by drawing it into plain sample bottles using cardiac puncture and then centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to obtain the serum. The serum was collected into plain sample bottles and was stored at -20°C or lower before biochemical assay.

Gross Evaluations of the Stomachs: The stomachs of each experimental animal were excised and opened along the greater curvature, rinsed with 0.01 M phosphate-buffered saline (PBS) at pH 7.4, and examined for lesions. The lesions were measured manually (mm²) using a calibrated ruler, and the stomachs were pinned to cardboard for photographing with a digital camera to evaluate the gastro-protective effects of the different treatments. The stomach juice was collected for measuring gastric secretion parameters and stored in a standard container at 4°C until needed [1]. Meanwhile, the mucosal content was used to determine macroscopic ulcer scoring, index, and inhibition percentage [20].

Ulcer Scoring, Index and Percentage Inhibition

The scoring method was adopted from Kayode et al. [1]:

i) No damage or bleeding = 0
ii) Little damage or bleeding (0.5 – 1.0 mm) = 1
iii) Lesions between 1.0 and 15 mm in size = 2
iv) Lesions of severe severity (1.5 – 2.5 mm) = 3
v) Lesions of perforation (2.5 – 3.5 mm) = 4

The ulceration index and percentage inhibition of ulceration was calculated according to Kayode et al. [1]:

$$\text{Ulcer index (UI)} = \frac{Sum of ulcer scores}{Rats ulceration count}$$

$$\text{Percentage inhibition of ulceration} = \frac{UI untreated category - UI treated category}{UI untreated category} \times 100$$

After collecting the gastric tissues, each stomach was divided into two parts: one part was fixed in 10%

formalin-buffered solution for histopathological analysis, and the other part was homogenized in normal saline (0.9% sodium chloride, NaCl) to prepare 10% gastric tissue homogenates (20 mg tissue in 180 µL normal saline) for further biochemical measurements [21].

Formulation of Stomach Tissue Homogenate: The second part of the rats' stomachs was homogenized in cold PBS solution according to the methods of Al-Nadaf et al. [21] and Ibrahim et al. [22]. A minor segment of the stomach wall was pulverized (10% w/v) in cold PBS supplemented with a mammalian protease inhibitor combination. The stomach homogenate underwent centrifugation for 10 minutes at 4°C. The transparent supernatant was used to measure the concentrations of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA), according to the manufacturer's guidelines.

Histopathology Examination: Following macroscopic inspection, the preserved stomachs were submerged in a 10% formalin saline solution for 24 hours for histopathological study. A histopathological investigation with hematoxylin and eosin was conducted as described by Dabburu et al. [23] and Al-Nadaf et al. [21]: A segment of the gastric wall was excised from the fore stomach to the pylorus, traversing the whole glandular mucosa and inevitably included areas of erythema or ulceration. The tissue samples were dried using graded alcohol (70°, 95°, and 100°). The tissue was immersed in paraffin and then sectioned into 5 µm-thick slices. The slices were stained with hematoxylin and eosin, thereafter analyzed, and photographed using a light microscope at magnifications of x100 and x400.

Measurement of Gastric Juice Volume, pH and Total Acidity: Gastric Juice Volume: Gastric contents were obtained using the technique described by Bhattamisra et al. [24], which included ligating the pyloric and cardiac ends of the stomach with a single thread. A little incision was performed along the greater curvature, and the stomach contents were collected into a centrifuge tube. To determine the total volume of gastric juice, the Ansari and Doshi [20] method was used, measuring the gastric volume with a measuring cylinder. The collected content was then centrifuged at 1000 rpm for 10 minutes.

Gastric Juice pH: The pH of the supernatant from the centrifuged gastric juice was measured using an automated pH meter.

Gastric Juice Total Acidity: Total acidity was assessed using the method of Belmamoun et al. [25], titration with 0.01 N sodium hydroxide (NaOH) utilizing phenolphthalein as an indicator. The data were presented as mEq/L/100 g. Total acidity = $n \times 0.01 \times 40 \times 1000$

Where:

N = volume of NaOH consumed

0.01 = normality of NaOH

40 = molecular weight of NaOH

1000= factor represents in liters

Protein Content: The protein concentration was determined using the Biuret method, as described by Gornall et al. [26], and slightly modified as described by Kayode, Owolabi, et al. [27]. The modification was the addition of potassium iodide to prevent the precipitation of Cu^{2+} ions as cuprous oxide.

H^+/K^+-ATPase Activity: The H+/K+-ATPase activity assay was determined using a spectrophotometer kit (H^+K^+-ATPase activity assay kit; Elabscience, USA) according to the manufacturer's protocol.

Assessment of SOD, CAT and MDA Measurements: After centrifugation, the supernatant was collected and the MDA concentration, SOD and CAT activities in tissue samples were measured with commercial assay kits following manufacturers' protocols [28, 29, 30].

TNF– α and IL – 6 Evaluation: According to the manufacturer's protocol, TNF-α and IL-6 levels were determined using ELISA kits (Rat TNF-α and IL-6 ELISA Ready-SET-Co; Elabscience, USA).

Statistical Data: The statistical analysis was conducted with Graphpad Prism® 8.0. A one-way ANOVA was used to compare the results systematically. The Bonferroni multiple comparison test was used for post-hoc analysis. Differences with $p < 0.05$ were deemed significant.

RESULTS

Photographic Images of the Gastro-protective Effect of the Different Treatment Groups: Figure 2 illustrates the macroscopic observations of the gastro-protective effects across several treatment groups. The treatment of ethanol resulted in significant damage to the stomach mucosa in the negative control group relative to the naïve control group. Gastric mucosal edema and widespread erosion are shown as elongated, severe hemorrhagic lesions in the negative control. The pre-treatment groups exhibited significantly less stomach damage than the negative control, with the 2.0 mL juice group demonstrating a comparable trend to the normal control. No macroscopic alterations were seen in the naïve control.

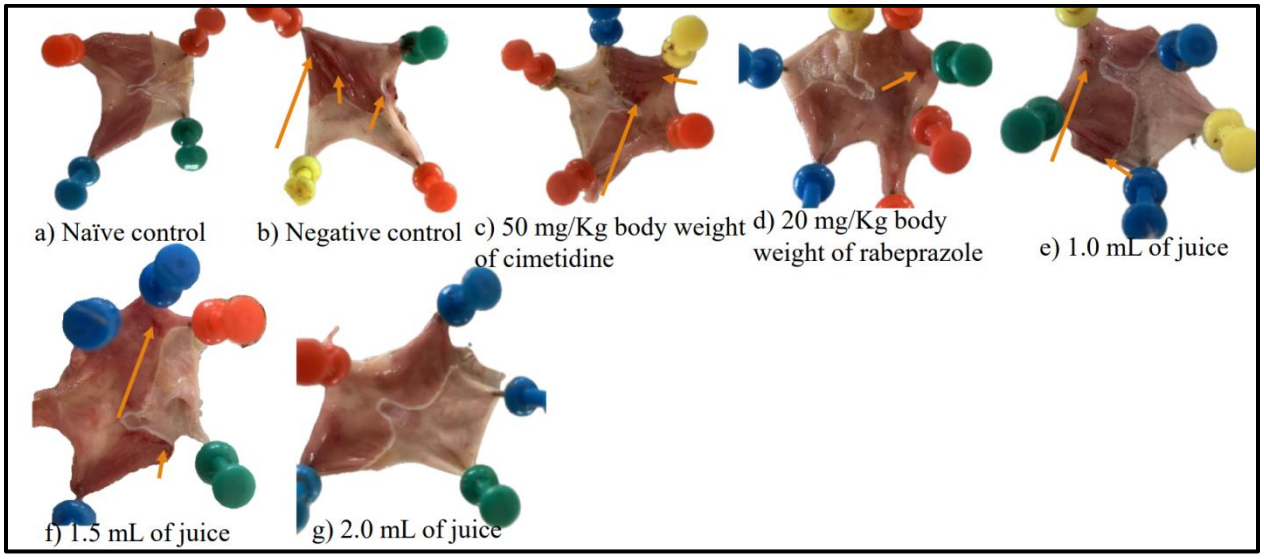


Figure 2: Macroscopic Effect of Juice extract on Ethanol-Induced Gastric Ulcer. (a) Naïve control (b) Negative control (c) Positive control 1: Cimetidine + ulcer (d) Positive control 2: Rabeprazole + ulcer (e) 1.0 mL of Juice (f) 1.5 mL of Juice (g) 2.0 mL of Juice. The direction of arrows indicates ulcer lesions

Effect of the Juice Extract on Ulcer Index and Percentage Inhibition of Ulceration: Figure 3a showed that the stomach mucosa of the negative control group suffered significant harm from absolute ethanol. The ulcer index (UI) of the negative control group was much higher at 1.90 ± 0.12. Pre-treated rats with 50 mg/kg body weight of cimetidine, 20 mg/kg body weight of rabeprazole, 1.0 mL of juice, 1.5 mL of juice, 2.0 mL of juice had significantly ($p < 0.05$) reduced ulcer index, 1.0 ± 0.2, 0.5 ± 0.14, 0.80 ± 0.20, 0.5 ± 0.14 and 0.10 ± 0.14

respectively, compared to the negative control group (1.90 ± 0.12).

Figure 3b demonstrated that the stomach mucosa of the negative control group suffered significant harm from absolute ethanol administered 1 hour before the sacrifice. With an inhibition rate of 52.63 ± 2.47, 73.68 ± 2.48, 57.90 ± 7.21, 73.68 ± 2.48 and 94.74 ± 2.48, the rats pre-treated with 50 mg/kg body weight of cimetidine, 20 mg/kg body weight of rabeprazole, 1.0 mL of juice, 1.5 mL of juice and 2.0 mL of juice respectively had less mucosal hemorrhagic foci. The results indicated that 50 mg/kg body weight of cimetidine, 20 mg/kg body weight of rabeprazole, 1.0 mL of juice, 1.5 mL of juice, and 2.0 mL of juice conferred significant ($p < 0.05$) resistance against ethanol-induced stomach ulcers.

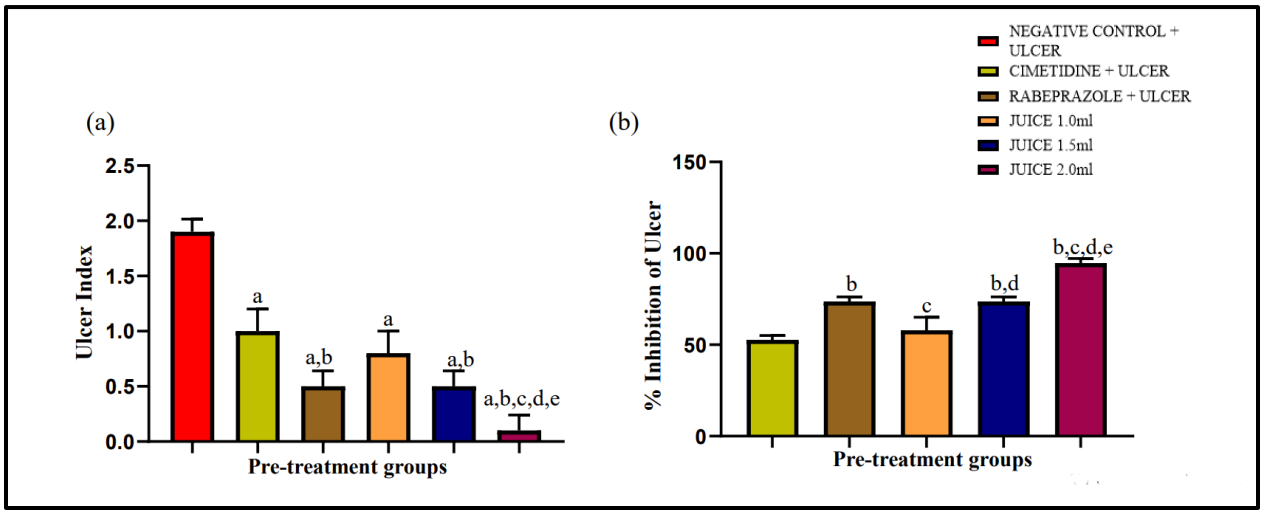


Figure 3: Effect of Juice Extract on Gastric Ulcer Index. The values in the bars represent the mean ± standard error of the mean (n=5). When comparing groups; a = significant difference ($p < 0.05$) from negative control; b = significant difference ($p < 0.05$) from 50 mg/kg body weight of Cimetidine; c = significant difference ($p < 0.05$) from 20 mg/kg body weight of Rabeprazole; d = significant difference ($p < 0.05$) from 1.0 mL of Juice; e = significant difference ($p < 0.05$) from 1.5 mL of Juice

Effect of Juice Extract on Gastric Juice Volume, pH and Total Acidity: Figure 4a displayed the results on the measurement of gastric juice volume, showing that the difference between the control group and the negative group was statistically significant ($p < 0.05$). Except for 50 mg/kg body weight of cimetidine, the pre-treated groups are significantly ($p < 0.05$) different from the negative control. Naïve control = 0.26 ± 0.05; Negative control = 1.6 ± 0.2; 50 mg/kg body weight of cimetidine = 1.27 ± 0.31; 20 mg/kg body weight of rabeprazole = 0.43 ± 0.04; 1.0 mL of juice = 0.93 ± 0.12; 1.5 mL of juice = 0.70 ± 0.14; 2.0 mL of juice = 0.32 ± 0.04.

From Figure 4b, it is indicated that in comparison to the negative control group, the pre-treated groups displayed significant ($p < 0.05$) increase in pH. Still, in comparison to themselves, 2.0 mL of juice is significantly ($p < 0.05$) higher than 1.0 and 1.5 mL of juice. Naïve control = 5.64 ± 0.31; Negative control = 2.29 ± 0.29; 50 mg/kg body weight of cimetidine = 3.75 ± 0.26; 20 mg/kg body weight of rabeprazole = 4.50 ± 0.28; 1.0 mL of juice = 4.09 ± 0.10; 1.5 mL of juice = 4.35 ± 0.21; 2.0 mL of juice = 5.02 ± 0.08.

Figure 4c indicated that there was a significant ($p<0.05$) increase in the acidity of gastric juice of the negative control group (1293.97±25.77) when compared to the pre-treated groups. This may indicate that the negative control group's gastric mucosa may have been harmed by the overproduction of pepsinogen and gastric acid, ultimately resulting in ulcers. There was also a significant difference among the pre-treated group.

Although, when compared with 20 mg/kg body weight rabeprazole, 1.5 mL of juice is not statistically significant ($p < 0.05$). Naïve control = 236.93 ± 25.77; 50 mg/kg body weight cimetidine = 777.6 ± 42.09, 20 mg/kg body weight of rabeprazole = 425.3 ± 9.41, 1.0 mL of Juice = 583.2 ± 51.55, 1.5 mL of Juice = 470.7 ± 5.40, 2.0 mL of Juice = 340.2 ± 21.04.

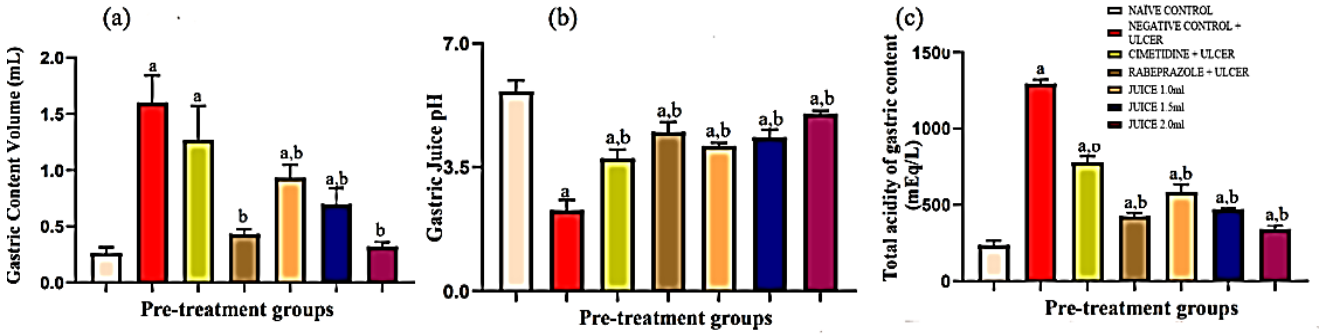


Figure 4: Effect of Juice Extract on Gastric Juice Volume, pH and Total Acidity. The values in the bars represent the mean ± standard error of the mean (n=5). When comparing groups; a = significant difference ($p < 0.05$) from naïve control; b = significant difference ($p < 0.05$) from negative control

Effect of Juice Extract on H^+/K^+-ATPase Activity: Figure 5 showed that the pre-treatment groups significantly ($p < 0.05$) decreased ethanol-induced H^+/K^+-ATPase activity in stomach tissue compared to the negative control. Compared to the naïve control, 20 mg/kg body weight of rabeprazole and 2.0 mL of juice displayed no significant ($p < 0.05$) difference, while the others are significantly different. Naïve control = 0.49 ± 0.06; negative control = 6.86 ± 0.31; 50 mg/kg body weight cimetidine = 3.40 ± 0.36; 20 mg/kg body weight of rabeprazole = 0.95 ± 0.04; 1.0 mL of Juice = 4.48 ± 0.36; 1.5 mL of Juice = 2.49 ± 0.06; 2.0 mL of Juice = 0.81 ± 0.00.

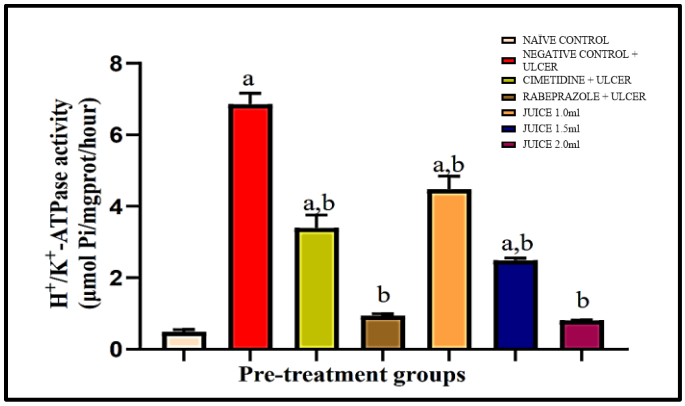


Figure 5: Effect of Juice Extract on H^+/K^+-ATPase Activity. The values in the bars represent the mean ± standard error of the mean (n=5). When comparing groups; a = significant difference ($p<0.05$) from naïve control; b = significant difference ($p<0.05$) from negative control

Effect of Juice Extract on SOD and CAT Activities, and MDA Concentration: Figure 6a indicates that all groups exhibited statistically significant differences ($p < 0.05$) when compared to one another. Relative to the negative control group (6.19 ± 0.54), the pre-treated groups and standard medications exhibited a significant ($p < 0.05$) elevation in SOD effectiveness. 50 mg/kg body weight of cimetidine = 10.28 ± 0.08, 20 mg/kg body weight of

rabeprazole = 16.96 ± 0.56, 1.0 mL of Juice = 12.99 ± 0.96, 1.5 mL of Juice = 15.04 ± 1.11, 2.0 mL of Juice = 19.00 ± 0.79, Naïve control = 21.31 ± 0.51.

As shown in Figure 6b, the CAT activity was observed to have statistically significant ($p < 0.05$) differences when compared to each other. Compared to the negative and naïve control, all groups are significantly different at $p < 0.05$. Compared with the standard drugs, 1.5 mL of juice displayed not statistically significant ($p < 0.05$) difference with 50 mg/kg body weight of cimetidine. In comparison, 2.0 mL of juice is not significantly different from 20 mg/kg body weight of rabeprazole. The results of this investigation indicated that the CAT function in the pre-treated groups was considerably ($p < 0.05$) elevated compared to the negative control group. The results, however, demonstrated that the juice at different concentrations was able to suppress this ulceration, suggesting that the juice could keep the antioxidant defense in ethanol-induced stomach ulcers. Naïve control = 1.74 ± 0.08; negative control = 0.26 ± 0.05, 50 mg/kg body weight cimetidine = 1.07 ± 0.03, 20 mg/kg body weight of rabeprazole = 1.48 ± 0.03, 1.0 mL of Juice = 0.91 ± 0.01, 1.5 mL of Juice = 1.16 ± 0.06, 2.0 mL of Juice = 1.54 ± 0.06.

Figure 6c showed that the pre-treatment groups considerably ($p < 0.05$) decreased ethanol-induced MDA generation in stomach tissue compared to the negative control. This may imply that oxidative stress induced peroxidation in the negative control group following the induction of ulcers with ethanol. Compared to the naïve control, 20 mg/kg body weight of rabeprazole and 2.0 mL of juice displayed no significant ($p < 0.05$) difference, while the others are significantly different. Naïve control = 0.01 ± 0.02; negative control = 0.14 ± 0.02, 50 mg/kg body weight cimetidine = 0.05 ± 0.00, 20 mg/kg body weight of rabeprazole = 0.02 ± 0.00, 1.0 mL of Juice = 0.08 ± 0.01, 1.5 mL of Juice = 0.05 ± 0.00, 2.0 mL of Juice = 0.02 ± 0.00.

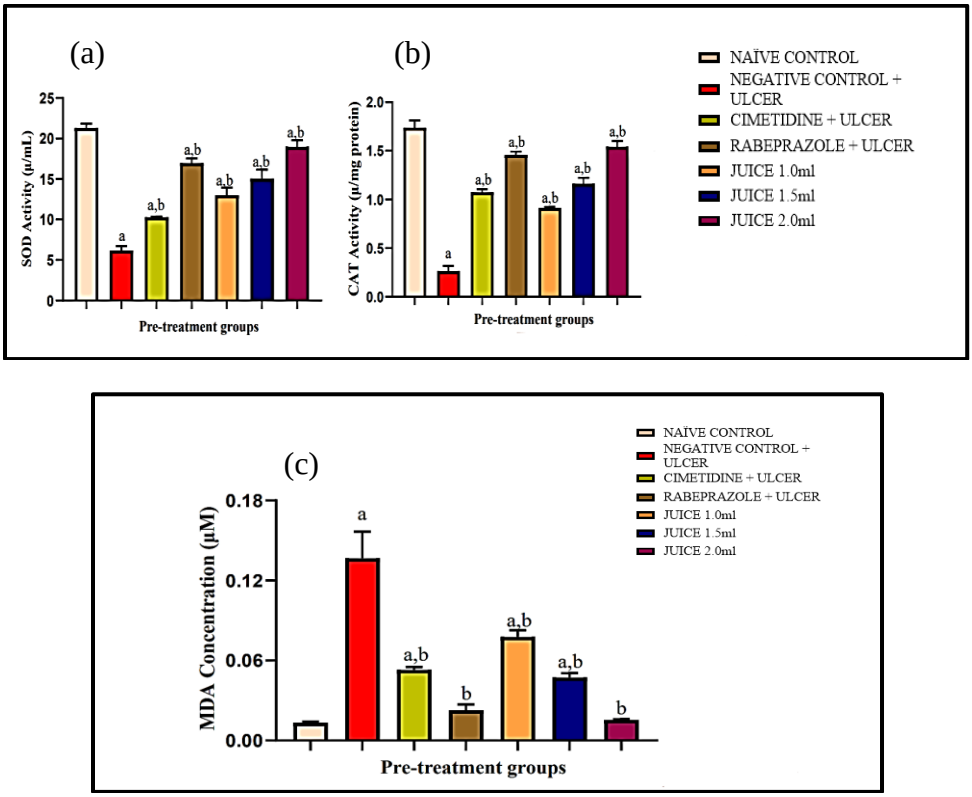


Figure 6: Effect of Juice Extract on SOD and CAT Activities, and MDA Level. The values in the bars represent the mean ± standard error of the mean (n=5). When comparing groups; a = significant difference ($p < 0.05$) from naïve control; b = significant difference ($p < 0.05$) from negative control

Effect of Juice Extract on TNF – α and IL – 6: Figure 7a demonstrated that the pre-treatment groups considerably ($p < 0.05$) reduced the serum levels of TNF-α in the experimental animals relative to the negative control. The negative control group substantially increased ($p < 0.05$) relative to the naïve control. Naïve control = 0.12 ± 0.01; negative control = 1.22 ± 0.07; 50 mg/kg body weight cimetidine = 0.66 ± 0.01; 20 mg/kg body weight of rabeprazole = 0.44 ± 0.00; 1.0 mL of Juice = 0.85 ± 0.02; 1.5 mL of Juice = 0.47 ± 0.01; 2.0 mL of Juice = 0.19 ± 0.01.

Figure 7b demonstrated that the pre-treatment groups considerably ($p < 0.05$) reduced the blood levels of IL-6 in experimental animals relative to negative control. All pre-treated groups, except for 2.0 mL of juice, exhibited a statistically significant change ($p < 0.05$) compared to the naïve control. The negative control group had a significant ($p < 0.05$) increase compared to the naïve control. Naïve control = 0.09 ± 0.01; negative control = 1.25 ± 0.07; 50 mg/kg body weight cimetidine = 0.53 ± 0.02; 20 mg/kg body weight of rabeprazole = 0.22 ± 0.01; 1.0 mL of Juice = 0.76 ± 0.03; 1.5 mL of Juice = 0.21 ± 0.02; 2.0 mL of Juice = 0.12 ± 0.01.

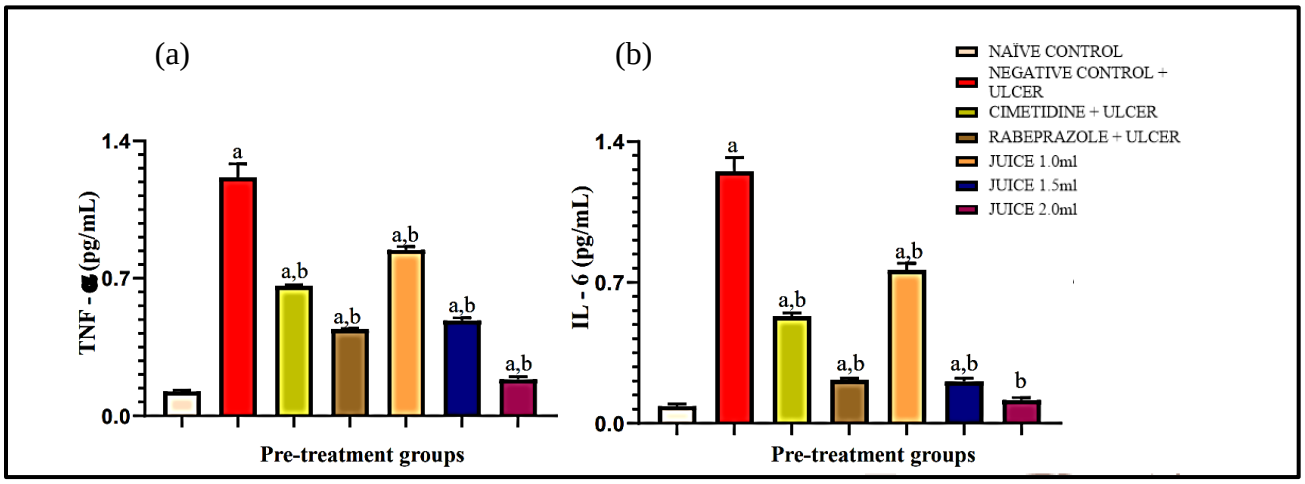


Figure 7: Effect of Juice Extract on TNF – α and IL – 6. Values are represented as mean ± SEM (n=5). a-represent significant differences ($p < 0.05$) from naïve control; b = significant difference ($p < 0.05$) from negative control

Histopathology: Figure 8a showed normal histo-architecture of the stomach having normal gastric pits, surface epithelium and parietal cells distributions. Figure 8 b expresses degenerated gastric glands with marked infiltration of inflammatory cells (red arrows) and pronounced parietal and chief cells (blue and green arrows respectively). Figure 8 c showed the necrosis of the chief cells and parietal cells and the luminal surface showed marked vacuolations with ulcerative changes. In Figure 8 d, there is degeneration of surface epithelium (black arrows), abnormal distribution of the gastric pits but well-defined gastric gland cells. Figures 8 e displays mild degeneration of surface epithelium but restoration of the glands and well distributed cells when compared to Figure 8 d. Figure 8 f showed a significant gastric cell restoration with its epithelium appearing close to normal, having also uniform gastric pits arrays compared to other experimental groups. Figure 8 g showed changes in parietal cells, karyolyed nuclei, marked ulcerative and erosive changes in the luminal surface.

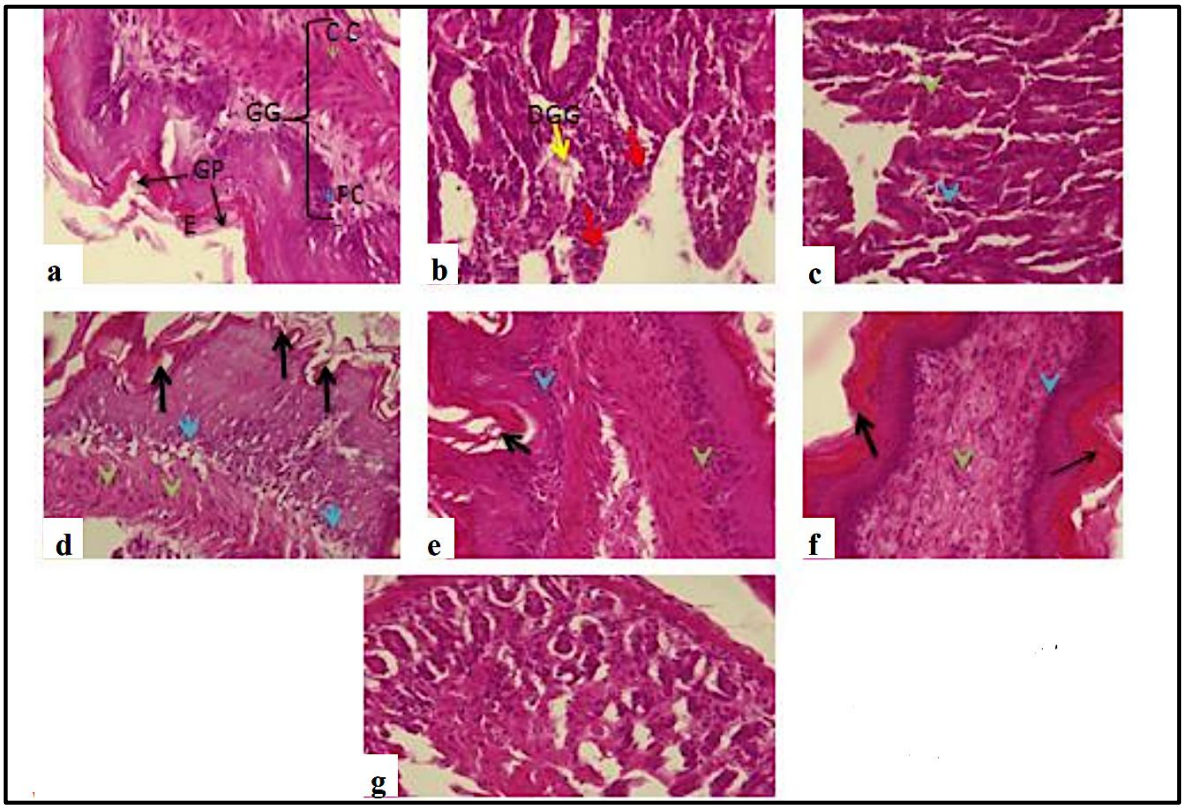


Figure 8: Photomicrograph Representation of Stomach Sections from Different Experimental Groups at Higher Magnification (x400). The entire group shows the general histo-morphology of the stomach at this magnification. Red, blue and green arrows signify inflammatory, parietal and chief cells respectively. a = Naïve control; b = Negative control; c = 50 mg/kg body weight of Cimetidine; d = 20 mg/kg body weight of Rabeprazole; e = 1.0 mL of Juice; f = 1.5 mL of Juice; G = 2.0 mL of Juice; GP = Gastric pit; GG = Gastric glands; DGG = Degenerated gastric glands; PC = Parietal cells; CC = Chief cells; E = Epithelium.

DISCUSSION

Peptic ulcer disease (PUD) is a prevalent global illness and a chronic disorder [31]. It is distinguished by a visible mucosal membrane defect that spreads into the submucosa or muscularis propria [32]. These issues arise from a disparity between protective factors for the mucosa and aggressive components. Endogenous aggressive forces include stomach acid, pepsin, and refluxing bile acids, while the mucus-phospholipid layer provides primary mucosal protection, which is further strengthened by prostaglandins and epidermal growth factor [33].

As an aggressive component, ethanol significantly contributes to the formation of stomach ulcers. Due to its ability to create necrotic lesions and considerable erosion of the epithelial surface, so significantly disturbing the gastric mucosa, it has been extensively utilized in numerous investigations to assess gastroprotective effects. Ethanol generates reactive species, including hydroperoxy radicals and superoxide anions, during metabolic processes in the body. Alcohol dehydrogenase (ADH) enzymes are responsible for most of its breakdown, producing acetaldehyde, a very reactive and harmful metabolite that is involved in tissue damage to a considerable extent [34, 35]. The protracted activation of immune cells and the release of pro-inflammatory cytokines can cause tissue harm and malfunction, which is another consequence of chronic ethanol intake. Besides inflammation, ethanol-induced tissue damage and dysfunction can occur through other mechanisms, such as impairing nutrient absorption and metabolism, altering the structure and function of cell membranes by

affecting their fluidity and permeability, and generating toxic by-products like acetaldehyde. As a result of acetaldehyde's ability to weaken the antioxidant security system, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced. This results in a reduction in the overall antioxidant capacity of the cell [36]. When natural antioxidant safeguards such as superoxide dismutase, catalase, and glutathione peroxidase, are outmatched by the increased generation of ROS, an imbalance may favor oxidative stress and cellular damage. This imbalance may be caused by the fact that ROS production is increased.

The slogan "no acid, no ulcer" is a well-known idea that stresses how the inhibition or neutralization of acid released helps foster ulcer healing. In this study, the volume of gastric juice was measured to determine the extent to which ethanol stimulates H+/K+-ATPase, resulting in the generation of gastric acid in the stomach. The results demonstrated increased gastric juice volume in the negative control group, which agrees with earlier ethanol-induced investigations [1, 37-38]. Furthermore, the pre-treatment groups significantly ($p < 0.05$) reduced the gastric juice volume, showing their potential to block H+/K+-ATPase activity and decrease stomach acid secretion. It was noticed that the 2.0 mL juice therapy produced slightly less gastric juice than the 20 mg/kg body weight of rabeprazole, a proton pump inhibitor, indicating that the juice was extremely effective compared to rabeprazole and could serve as a preventative intervention against peptic ulcer.

Antioxidants that are preventative in nature, such as superoxide dismutase (SOD) and catalase (CAT), are the first line of protection toward (ROS). Superoxide dismutase (SOD) is responsible for catalyzing the transforming of SOD radicals into oxygen and hydrogen peroxide when oxidative stress is present. Catalase (CAT) is responsible for breaking down hydrogen peroxide into water and oxygen. For the purpose of this inquiry, the application of ethanol treatment led to a substantial decrease ($p < 0.05$) in the levels of superoxide dismutase (SOD) activity when compared to pre-treated groups. On the other hand, the levels of CAT performance were considerably greater ($p < 0.05$) in the pre-treated groups compared to the negative control. These data demonstrate that the juice treatment substantially minimized ulcer formation, suggesting its potential in sustaining antioxidant defense systems in ethanol-induced stomach ulcers.

Another significant element in ethanol-induced stomach tissue injury is inflammation, which increases ROS generation [39]. Pro-inflammatory cytokines, including TNF-α and IL-6, are crucial in immune responsiveness and inflammation. The findings of this study revealed substantially ($p < 0.05$) elevated levels of TNF-α and IL-6 in the negative control group relative to the naïve control, but the preliminary treatment groups had significantly ($p < 0.05$) reduced levels. Notably, the 2.0 mL juice treatment corrected TNF-α and IL-6 levels more successfully than other pre-treatment groups. These data reveal a good gastroprotective impact of the juice against ethanol-induced ulcers, hinting that it can alter TNF-α and IL-6 levels, serving as a preventive agent for gastric ulcers.

Malondialdehyde (MDA), a highly reactive chemical created as a result of lipid peroxidation, is produced when unsaturated fatty acids in cell membranes undergo oxidation, generally initiated by ROS. Measuring MDA levels can provide information into ethanol-induced stomach tissue damage [40]. Elevated MDA levels indicate increased oxidative stress and lipid peroxidation, which can affect the integrity of the stomach mucosal barrier, leading to erosion and ulceration. Increasing lipid peroxidation and MDA levels can also impair gastric ulcer healing by limiting epithelial cell proliferation and migration, lowering angiogenesis, and modifying extracellular matrix remodeling. It is commonly advised

that lowering oxidative stress is a helpful technique for treating stomach ulcers. This study indicated that pre-treatment with a combination of cucumber and cabbage juice significantly ($p < 0.05$) reduced ethanol-induced MDA generation in stomach tissue, suggesting its protective action against oxidative stress.

CONCLUSION

Peptic ulcer disease (PUD) remains a significant global health concern, prompting increased interest in dietary interventions that offer health benefits beyond basic nutrition [41]. Functional foods, rich in bioactive compounds such as flavonoids and antioxidants, have demonstrated potential in reducing oxidative stress, modulating inflammatory responses, and enhancing mucosal protection—key mechanisms in peptic ulcer prevention and healing [42]. *Brassica oleracea* (cabbage) and *Cucumis sativus* (cucumber) have been identified for their gastroprotective properties, attributed to their antioxidant protection, anti-inflammatory effects, and gastrointestinal mucosal protection.

The concept of functional foods encompasses products that provide additional health benefits beyond basic nutrition, often due to the presence of bioactive compounds [41]. These compounds in various plant-based foods play a crucial role in health promotion and disease risk reduction [42]. For instance, phytochemicals such as polyphenols have been associated with improved heart and metabolic health, reduced risk of neurodegenerative diseases, and anti-inflammatory effects. In the context of PUD, functional foods rich in mucilage, fiber, and phytochemicals can enhance gastric mucosal defense, reduce acid secretion, and promote tissue regeneration [41].

The combined usage of *Cucumis sativus* and *Brassica oleracea* has shown strong gastroprotective effects, benefiting both the prevention and repair of ulcers. This natural combination may help lessen the harmful effects of synthetic conventional medications, reinforcing the shift toward natural interventions supported by functional food science. The findings from this study highlight the potential of functional foods in gastroprotection, particularly the combined effects of these two plants. Their rich antioxidant, anti-inflammatory, and mucosal-protective compounds make them viable natural interventions for peptic ulcer prevention and treatment.

Finally, future studies should explore clinical applications and optimal dietary formulations to maximize these benefits. Integrating functional foods rich in bioactive compounds into the diet presents a promising strategy for managing and preventing peptic ulcer disease. As research advances, a clearer understanding of these foods' mechanisms and optimal applications will further solidify their role in promoting gastrointestinal health.

List of Abbreviations: BUHREC, Babcock University Health and Research Ethics Committee; CAT, catalase; H^+/K^+-ATPase, Hydrogen-potassium adenosine triphosphatase; IL-6, Interleukin-6; MDA, malondialdehyde; PUD, peptic ulcer disease; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; SOD, superoxide dismutase; TNF-α, Tumor necrosis factor – alpha.

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

Authors' contributions: KAA, developed the main idea and framework of the study (conceptualization), designed the methods and procedures for the research, supervised the research project and managed the overall progress and coordination of the research, MDI, conducted experiments and data collection, prepared the initial draft of the manuscript, secured financial

support for the project, KAA and MDI, analyzed and interpreted the data, MDI, KOT and KAA, provided materials, equipment, or other resources necessary for the research, KAA and KOT reviewed and edited the manuscript.

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