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Gastroprotective Effect of Abelmoschus esculentus (Ex-Maradi Okra Fruit Variety) Against Ethanol-Induced Ulcers in Rats

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Abstract

Peptic ulcer disease, a notable gastrointestinal disorder, results from an imbalance between gastric acid secretion and the factors maintaining gastric mucosal integrity. Abelmoschus esculentus, commonly known for its mucilaginous and nutraceutical properties, also exhibits an antacid effect. This research aimed to examine the antacid properties of fresh okra fruit mucilage (FOM) and dried okra fruit powder (DOP) of the Ex-Maradi okra fruit variety against ethanol-induced gastric mucosal damage in Wister rats. Rats were randomly assigned to seven groups consisting of six rats each. Rats in the FOM and that of the DOP group were pretreated orally with 250 and 500 mg/kg body weight of the FOM and DOP, respectively; the drug control (DC) group was pretreated orally with 20 mg/kg body weight of Cimetidine while the normal control (NC) group and the ulcer control (UC) group were pretreated orally with normal saline (2 mL/kg body weight). All the treatments were done for seven days before the induction of the ulcer. Ulcer index (UI), percentage inhibition (PI), gastric volume, gastric pH, total acidity, and total antioxidant power (TAP) were evaluated to assess the gastro-protective effect of the FOM and DOP in the rats. Both FOM and DOP groups demonstrated significant ($P < 0.05$) protection with a low ulcer index (2.41 ± 0.12) and high ulcer inhibition (75.6 %) against the damaging effect of ethanol on the gastric mucosa of the animals. Additionally, DOP also exhibited a strong antioxidant effect with a good percentage inhibition value (56.53 ± 2.1%) compared to the ulcer control group. These results were further supported by the histopathological findings from the rats' stomachs. In conclusion, the Ex-Maradi okra fruit, especially the DOP_{500}, demonstrated significant ($P < 0.05$) gastro-protective effects and maintained a relatively intact and continuous epithelial surface of the rats' stomachs. Overall, its gastroprotective effects may be possibly mediated by its potential to modulate the antioxidant system and gastric acid levels. Hence, the dried okra fruit could be suitable for the development of green anti-ulcer formulations.

Keywords: Abelmoschus esculentus, Antioxidants, Ethanol-induced-ulcer rats, Gastroprotection

INTRODUCTION

Peptic ulcer disease is a significant condition affecting the entire gastrointestinal tract (Ardalani *et al.*, 2020). It predominantly manifests in the stomach as gastric ulcers and in the proximal duodenum as duodenal ulcers due to a persistent imbalance between the secretion of gastric acid (aggressive factors) and the integrity of the gastric mucosa (defensive factors) (de Lira Mota *et al.*, 2009; Ahmed, 2019; Yaghoobi & Armstrong, 2022). It is described as a break in the normal gastric mucosal integrity (mucosal erosion in an area of the alimentary canal lining) exposed to the secretion of gastric acid and pepsin (Liju *et al.*, 2015; Abumunaser, 2021). Such ulcers are marked by the presence of neutrophil infiltration, decreased blood flow, heightened oxidative stress, and inflammatory response, as well as suppurating lesion, which could result in necrosis (da Silva *et al.*, 2015;

Liju *et al.*, 2015; Djanaev *et al.*, 2023). The gastric aggressive factors may include hydrochloric acid, gastric acid, pepsin secretions, free radicals, infectious agents such as *Helicobacter pylori*, and, to a lesser extent, bile salts and pancreatic enzymes, while the mucosal defensive (protective) factors include the adherent mucin, prostaglandins, mucus and bicarbonate barrier and adequate mucosa blood flow (Djanaev *et al.*, 2023). Besides all these factors, stress, smoking, inadequate nutrition, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), and infection with *Helicobacter pylori* are all significant causes contributing to the development of gastric ulcers (Djanaev *et al.*, 2023). A peptic ulcer is considered the most common gastrointestinal disorder ever known (Shristi *et al.*, 2012; Ibraheem, 2021). It is estimated to cause 15 deaths per 15,000 complications annually worldwide (Ardalani *et al.*, 2020). The incidence of peptic ulcers varies based on age, gender, and geographic location (Milivojevic & Milosavljevic, 2020; Ibraheem, 2021). In developed countries, peptic ulcer has a prevalence exceeding 40%, whereas in developing countries, it reaches up to 80% (Adinortey *et al.*, 2013; Milivojevic & Milosavljevic, 2020; Sperber *et al.*, 2021).

It has been established that ethanol can cause ulcers in humans, and it has long been used for the induction of ulcers in experimental animals and clinical studies, as some of its effects lead to the erosion of the gastric mucosa with severe gastric hemorrhagic lesions (Ortac *et al.*, 2018). The widely used ethanol-induced ulcer model is suitable for studying the gastroprotective and antioxidant properties of plant extracts as well as their other related therapeutic effects (Ortac *et al.*, 2018). Gastric ulcers induced by ethanol result from various mechanisms, including the depletion of gastric mucus. Gastric acid secretion reduces mucosal blood flow and impaired mucosal permeability, which causes increased leakage of hydrogen ions from the lumen and decreased transluminal membrane potential difference (Bongu & Vijayakumar, 2012).

The treatment of ulcers primarily aims to enhance the gastrointestinal defense system. This involves preventing ulcer formation by inhibiting acid secretion, boosting gastro-protection, promoting epithelial cell proliferation, and halting apoptosis to ensure an effective ulcer healing process (Fu *et al.*, 2021). However, recent findings have underscored the multifactorial nature of peptic ulcer diseases, where it was recognized that secretion of gastric acid is a key factor in peptic ulcer diseases, making its control the primary therapeutic target. This is achieved using antacids, H_2 receptor blockers such as ranitidine and famotidine (Scarpignato, 2022), anticholinergics like pirenzepine, telenzepine (Tan *et al.*, 2023) and proton pump inhibitors such as omeprazole, lansoprazole, pantoprazole, etc. (Abed *et al.*, 2020; Das *et al.*, 2021). However, current gastric ulcer treatments nowadays face significant challenges due to the limited efficacy of many available drugs and their often severe side effects (Das *et al.*, 2021; Salari *et al.*, 2022).

Cimetidine is known as a histamine H_2-receptor antagonist. It works by binding to an H_2-receptor of Histamine, which is located on the basolateral membrane of the gastric parietal cells, thereby blocking the effect of histamine and its activity (El-Dakroury *et al.*, 2022). This competitive inhibition results in reduced basal and nocturnal gastric acid secretion as well as a reduction in gastric volume and amount of gastric acid released in response to stimuli including food, caffeine, insulin, betazole, or pentagastrin (Ohia *et al.*, 2022; Ithape *et al.*, 2023). Additionally, Cimetidine can also inhibit several isoenzymes of the hepatic CYP450 enzyme system. Other effects of Cimetidine include an increase in gastric bacterial flora, such as nitrate-reducing organisms. Moreover, Cimetidine has been indicated for the treatment of gastrointestinal disorders such as gastric or duodenal ulcers, gastroesophageal reflux disease, and pathological hypersecretory conditions (Omayone *et al.*, 2016; Das *et al.*, 2021; Focsa *et al.*, 2021; Maideen *et al.*, 2021).

The utilization of natural products for the prevention and treatment of various conditions, including ulcers, is steadily increasing worldwide (Nasri *et al.*, 2014; Chaachouay & Zidane, 2024). This trend is especially evident in the use of nutraceuticals (food plants that have both nutritional and medicinal values (Nasri *et al.*, 2014; Yaghoobi & Armstrong, 2022). Currently, one of the most significant challenges facing medical practice is the cure or prevention of peptic ulcers (Cemek *et al.*, 2010; Sidahmed *et al.*, 2015). Consequently, numerous studies have been conducted by researchers to extract new anti-ulcer agents from natural sources. (Cemek *et al.*, 2010; Roy *et al.*, 2014; Nasri *et al.*, 2014; Sidahmed *et al.*, 2015; Beiranvand, 2022). Okra fruit is commonly referred to as lady's finger in many English-speaking countries. It belongs to the flowering plant family known as the mallow family, and it is renowned for its mucilaginous properties (Okasha *et al.*, 2014). The fruit of this

plant gives nutritional benefits such as protein, niacin, riboflavin, phosphorus, zinc, copper, potassium, vitamins A, B, C, and K. Magnesium, folate, calcium, and manganese, etc. (Muhammad *et al.*, 2018; Yasin *et al.*, 2020). It has been widely recognized for various health benefits, including its potential as an anti-ulcer and antidiabetic agent (Muhammad *et al.*, 2018; Ortac *et al.*, 2018; Yasin *et al.*, 2020). Okra has been reported to possess several health benefits, including lowering blood cholesterol levels, relieving intestinal disorders, reducing inflammation of the colon, alleviating symptoms of diverticulitis, treating stomach ulcers, neutralizing acid, and lubricating the large intestine, treatment of irritable bowel, among others (Taiye *et al.*, 2013; Yasin *et al.*, 2020).

Ex-Maradi okra fruit (a native of Maradi from the Niger Republic) is a commercially and locally available variety of okra plant, distinguished by its high mucilaginous properties (Muhammad *et al.*, 2018). In this context, the global use of food plants like okra is steadily increasing for the prevention and management of various ailments including ulcers. To our knowledge, most anti-ulcer research involving okra fruits has primarily focused on the use of fresh okra fruit extract alone (Okasha *et al.*, 2014; Habtamu *et al.*, 2015; Yasin *et al.*, 2020). In view thereof, this study attempts to investigate the gastro-protective effect of both the fresh Ex-Maradi okra fruit mucilage (FOM) and the dry Ex-Maradi okra fruit powder (DOP) against ethanol-induced gastric mucosal damages in Wister rats as well as investigating the possible mechanisms underlying its gastroprotective effect.

MATERIALS AND METHODS

Chemicals and Reagents

Laboratory chemicals and reagents used throughout this study were of analytical grade.

Okra sample collection

Ex-Maradi, a commercially available okra fruit (Both dried and fresh) samples, were obtained from Maggi Market, Sokoto State, Nigeria, in June 2017. The sample was identified, authenticated, assigned a voucher number (UDUH/ANS/0066), and deposited at the herbarium by a taxonomist at the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The study was carried out at the General Laboratory of the Department of Biochemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, Katsina State, Nigeria, from July to August 2017.

Fresh okra fruit mucilage preparation

The okra fruit mucilage was prepared following the method described by Ortac *et al.* (2018), with slight modification. Briefly, 1 kg of the fresh cleaned Ex-Maradi okra fruit samples were blended with water using a domestic blender. The solid matter from the homogenized okra fruits was separated by passing the thick suspension through a muslin cloth to extract the mucilage. The obtained mucilage was then dried in a hot air oven at 40^{o}C for a sufficient period of time. The dried extract obtained was placed in a labelled airtight container and stored under normal laboratory conditions until needed for reconstitution and administration.

Dry okra fruit powder preparation

The dry okra fruit powder was prepared following the previous method described by Muhammad *et al.* (2018). Briefly, the dried whole Ex-Maradi okra fruit sample obtained was thoroughly sorted to remove any unwanted organic residues and dirt to ensure purity. From the selected okra fruit, 1 kg was weighed, ground, and sieved into fine powder using a domestic grinder and sieves. The finely powdered sample was placed in a labeled airtight container and stored under normal laboratory conditions until needed for reconstitution and administration.

Procurement of experimental animals and ethics statement

The study was carried out in 46 young male and female Wistar rats of six weeks old, weighing approximately 250–300 g. The rats were procured from the School of Pharmacy, Usmanu Danfodiyo University Sokoto. The rats were housed in standard cages under standard conditions in the animal house. During the experimental period, all animals were provided with standard rodent pellets, chow, and water *ad libitum*. The protocol for handling and caring for the animals was meticulously followed according to the guidelines recommended by the Institute for Laboratory Animal Research and the Committee for the Update of the Guide for the Care and Use of Laboratory Animals (National Research Council [NRC], 2010).

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Grouping and treatment of the experimental rats

Wister rats of both sexes were distributed into seven (7) groups, each consisting of six (6) rats (3 males and 3 females). They were pretreated as follows before the induction of ulcers:

Group 1 (Normal Control [NC]): Rats in this group received normal saline at 2 mL/kg body weight orally for seven days in addition to their diet and drinking water.

Group 2 (Ulcer Control [UC]): Rats in this group received normal saline at 2 mL/kg body weight orally for seven days in addition to their diet and drinking water.

Group 3 (Drug Control [DC]): Rats in this group received Cimetidine at a dose of 20 mg/kg body weight orally for seven days in addition to their diet and drinking water.

Group 4 and 5 (Fresh Okra Mucilage [FOM_{250} and FOM_{500}]): Rats in this group received the diluted okra fruit mucilage orally at a dose of 250 and 500 mg/kg body weight, respectively for 7 days in addition to their normal diet and drinking water.

Group 6 and 7 (Dry Okra Powder [DOP_{250} and DOP_{500}]): Rats in this group received the diluted dry okra fruit powder orally at a dose of 250 and 500 mg/kg body weight, respectively, for 7 days in addition to their diet and drinking water.

Induction of ulcers and treatments

The animals in the drug control group were pretreated orally with the standard drug Cimetidine at a dose of 20 mg/kg body weight daily for seven days, while the animals in the test groups were pretreated orally with test samples (FOM and DOP), both at doses of 250 and 500 mg/kg body weight, daily for seven days respectively. After the final respective administration of the standard drug and the test samples, the rats were allowed free access to food and water for a period of 2 hours. Following this, they were fasted for 12 hours but allowed free access to water *ad libitum*. This step was taken to ensure an empty stomach, which helps in the clear observation of ulcer formation. Then, absolute ethanol (99.80% at the dose of 1 mL/200g body weight was administered orally to each animal except the normal control rats, which received normal saline at the dose of 2 mL/kg body weight. Sixty minutes after the ethanol administration, the animals were sacrificed with an excess of anesthetic ether, and the stomachs were isolated and cut open along the greater curvature. The stomach contents were respectively drained in labeled test tubes for gastric assay. The stomachs were gently rinsed with 0.9% saline solution to clean away any remnants of food substances and then pinned out on a flat surface. This was followed by a macroscopic examination of the stomach for the detection of any hemorrhagic lesions (ulcers) on the glandular mucosa. Ulcer scores, Ulcer index and Percentage inhibition, gastric volume, and gastric pH assays were carried out accordingly (Sahoo *et al.*, 2016).

Assessment of ulcer scores (US)

The ulcer score was determined based on the severity scores of mucosal lesions in millimeters (mm) following the criteria reported by Almasaudi *et al.* (2016) and Sahoo *et al.* (2016). Scores were assigned as follows: No ulcer = 0, Small ulcer (1-2 mm) = 1, Medium ulcer (3-4 mm) = 2, Large ulcer (5-6 mm) = 4, and Huge ulcer (> 6 mm) = 8.

Assessment of ulcer index (UI)

The ulcer index (UI) was measured following the method described by Almasaudi *et al.* (2016) and Sahoo *et al.* (2016). The average length of all lesions measured in millimeters (mm) was measured for each stomach to determine the mean UI by applying the formular below:

$$\text{Ulcer Index (UI)} = UN + US + UP \times 10^{-1}$$

Where:

UN = Average of number of ulcers per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Assessment of percentage inhibition (PI)

The percentage inhibition was calculated using the method of Mahmood *et al.* (2011) as follows:

$$\text{Percentage inhibition} = \frac{\text{Ulcer Index of Control} - \text{Ulcer Index of Test}}{\text{Ulcer index of Control}} \times 100$$

Determination of gastric volume (GV)

The isolated stomachs were opened mid-way along the greater curvature, and the gastric contents were drained directly into graduated centrifuge tubes. The tubes were centrifugated

at 3,000 rpm for 15 minutes at 25°C. After centrifugation, the volume of the supernatant was measured directly from the tubes and recorded (Ketuly *et al.*, 2011; AlRashdi *et al.*, 2012).

Determination of gastric pH value (GpH)

The respective stomach contents were centrifuged at 2,000 rpm for 10 minutes at 25°C. Aliquots of 1 mL from the corresponding supernatant (gastric juice) were taken and diluted with 1 mL of distilled water, and the pH was measured directly using an automated pH meter (Mahmood *et al.*, 2011; Sidahmed *et al.*, 2015; Yasin *et al.*, 2020).

Determination of total acidity (TA)

An aliquot of 1 mL from the corresponding supernatant liquid (gastric juice) was taken and diluted with 1 mL of distilled water. This mixture was transferred into a 50 mL conical flask, followed by the addition of two drops of phenolphthalein indicator. It was then titrated against 0.01N NaOH until a permanent pink color appeared. The volume of 0.01N NaOH used was noted and recorded, following the method described by Ketuly *et al.* (2011). The total acidity (expressed as mEq/L) was calculated using the following formula:

$$\text{Total Acidity} = \frac{\text{The volume of NaOH} \times \text{Normality}}{0.1} \times 100$$

Determination of total antioxidant power (TAP) in the serum

The free radical scavenging activity (FRSA) of the serum samples was assessed using the 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) reduction assay, based on the reduction of the purple DPPH$^{\bullet}$ to 1,1-diphenyl-2-picryl hydrazine, as described by Cecchini and Fazio (2000). Briefly, 25 µL of the serum samples were mixed with 475 µL of 10 mM phosphate buffered saline (PBS), pH 7.4, and mixed. Then, 500 µL of a 0.1 mM DPPH solution prepared in absolute methanol was added to the mixture. The mixture was incubated for 30 min in darkness at ambient temperature to allow the reaction between the serum components and DPPH to take place. The absorbance reading was taken at 520 nm against the blank, using a spectrophotometer. The absorbance of the sample was compared with that of a reference sample containing only PBS and DPPH solution without the serum (baseline absorbance [Ao]). The percentage of decrease in DPPH discoloration was calculated by applying the following equation:

$$\% \text{ Scavenging Activity} = \frac{1 - (\text{As})}{\text{Ao}} \times 100$$

Where: As is the absorbance of the sample, and Ao is the absorbance of the DPPH solution. The percentage scavenging activity of the serum samples was compared to assess their total antioxidant power (TAP) (Cecchini & Fazio, 2020).

Histopathological examination of the stomach tissues

For histopathological examination, the stomach tissues were initially fixed in a 10% formalin solution to preserve them until when required for the histopathological studies. Tissue processing was conducted using an automatic tissue processing machine, where the formalin-fixed stomach specimens were embedded in paraffin wax and serially sectioned (3-5 μm) micron thickness. These sections were mounted on a slide and stained with hematoxylin & eosin (H&E) and then observed under a light trinocular microscope (Almasaudi *et al.*, 2016).

Statistical analysis

Obtained data were presented as means ± standard error of the mean (SEM). Statistical analysis was performed using the Statistical Package for Social Sciences (version 20). Differences between means were evaluated using one-way analysis of variance (ANOVA), with a P value ≤ 0.05 considered statistically significant.

RESULTS

Effect of Ex-Maradi Okra Fruit Administration on the Ulcer Index and % Inhibition in Ethanol-induced Ulcer Rats

The results of the effect of administration of the FOM or the DOP on UI and percentage inhibition in the rats are presented in Table 1. The results of the pre-treatments of the rats with 250 and 500 mg/kg body weight of FOM or 250 and 500mg/kg body weight of DOP and that of the Cimetidine (20 mg/kg body weight) for seven days resulted in significant ($P < 0.05$) changes in the ulcer index (UI) and percentage of Ulcer Inhibition (PI). Based on the results, it was found that there were significant ($P < 0.05$) differences in the percentage number of rats with ulcers in the ulcer untreated group (88.00 ± 1.15)% compared to the normal control group, which

showed no ulcers (0.00 ± 0.00)% (Table 1). Treatments of rats with FOM_{500} and DOP_{500} significantly ($P < 0.05$) resulted in a lower percentage of rats with ulcers (39.33 ± 7.05 and 22.00 ± 1.15), respectively (Table 1). The results also showed that all the treatments with FOM and DOP produced significant ($P < 0.05$) reduction in the mean UI compared to that of the ulcer untreated group (UC), which has a higher mean UI (9.67 ± 0.15), where FOM treated groups at dose of 500mg/kg had 4.36±0.70 UI, while DOP treated group at dose of 500 mg/kg had 2.41 ± 0.12 UI. This indicates a significant ($P < 0.05$) difference in the ulcer preventive effects between FOM and DOP. DOP_{500} demonstrated superior gastroprotective and ulcer inhibitory effects with a 75.65% inhibition rate, compared to 55.90% inhibition observed with FOM_{500}. In addition to that, DOP_{500} also demonstrated a similar gastroprotective effect with an ulcer preventive index of 75.65% inhibition, comparable to the effect of Cimetidine treated group, which exhibited an ulcer preventive index of 72.82% inhibition (Table 1).

Effect of Ex-Maradi okra fruit administration on the gastric juice, gastric pH, total acidity, and serum TAP in ethanol-induced ulcer rats

The result of the effect of administration of the FOM or DOP on gastric juice volume, gastric pH, and total acidity in the ethanol-induced ulcer rats are presented in Table 2. The administration of the ethanol to the rats significantly ($P < 0.05$) resulted in over-secretion and accumulation of gastric juice, with a volume of 7.48 ± 0.16 mL and a pH of 2.17 ± 0.12 in the ulcer untreated group of rats, compared to the normal control group of rats, which had a gastric juice volume of 4.25 ± 0.08 mL and a pH of 4.32 ± 0.06 (Table 2). Furthermore, the total acidity of the gastric secretions was found to be 122.57 ± 1.09 mEq/L in the ulcer control group, which was significantly ($P < 0.05$) higher than that of the normal control group, which was found to be 31.30 ± 0.72 mEq/L (Table 2). Pre-treatments with different doses of the FOM or DOP for seven days resulted in a significant ($P < 0.05$) reduction in the volume of gastric secretions, ranging from 4.02 ± 0.01 to 4.45 ± 0.06 mL in the okra-treated groups, compared to the ulcer untreated group which had a volume of 7.48 ± 0.16 mL. Additionally, treatment with different doses of FOM or DOP significantly ($P < 0.05$) elevated the pH of the gastric juice to 4.74 ± 0.04 in the DOP_{500} treated group, compared to the UC, which had an acidic pH of 2.17 ± 0.12. Furthermore, total acidity was also observed to be significantly ($P < 0.05$) reduced in all the okra-treated groups, which ranges from (37.49 ± 36 to 48.31 ± 0.06 mEq/L) compared to the ulcer untreated group with a total acidity of 122.57 ± 1.09 mEq/L (Table 2).

Table 1: Effect of Ex-Maradi okra fruit administration on the ulcer index and % inhibition in ethanol-induced ulcer rats

Group	N	UN	US	UP (%)	UI	PI (%)
NC	5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	100^{d}
UC	5	4.40 ± 0.05^{e}	4.36 ± 0.08^{d}	88.00 ± 1.15^{e}	9.67 ± 0.15^{d}	0.00^{a}
DC	5	1.23 ± 0.08^{b}	1.00 ± 0.01^{b}	24.66 ± 1.76^{b}	2.69 ± 0.18^{b}	72.82^{c}
FOM_{250}	5	2.06 ± 0.08^{d}	2.00 ± 0.02^{c}	41.33 ± 1.76^{d}	4.54 ± 0.18^{c}	54.13^{b}
FOM_{500}	5	2.30 ± 0.11^{d}	2.00 ± 0.01^{c}	39.33 ± 7.05^{d}	4.36 ± 0.70^{c}	55.90^{b}
DOP_{250}	5	1.53 ± 0.14^{c}	1.00 ± 0.01^{b}	30.66 ± 2.90^{c}	3.32 ± 0.30^{b}	66.45^{c}
DOP_{500}	5	1.10 ± 0.05^{b}	1.00 ± 0.01^{b}	22.00 ± 1.15^{b}	2.41 ± 0.12^{b}	75.65^{c}

Values were expressed as Mean ± S.E.M., Mean values having different superscript letters in the same column are significantly ($P < 0.05$) different.
Key: N: Number of animals in the group; UN: Average number of ulcers per animal; US: Average severity score; UP: Percentage of animals with ulcer; UI: Ulcer index; PI: protective inhibition; N: Number of animals in the group; NC: normal control, UC: ulcer control, DC: drug control, FOM and DOP: fresh okra fruit mucilage and dry okra fruit powder, while the subscripts 250 and 500 denote the doses in mg/kg rats' body weight respectively.

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Table 2: Effect of Ex-Maradi okra fruit administration on the gastric juice, gastric pH, total acidity, and serum TAP in ethanol-induced ulcer rats

Group	N	GV(mL)	GpH	TA (mEq/L)	TAP (%)
NC	5	4.25 ± 0.08^{a}	4.32 ± 0.06^{c}	31.30 ± 0.72^{a}	52.61 ± 2.3^{d}
UC	5	7.48 ± 0.16^{c}	2.17 ± 0.12^{a}	122.57 ± 1.09^{f}	23.84 ± 17^{a}
DC	5	4.05 ± 0.03^{a}	5.35 ± 0.09^{e}	34.09 ± 0.62^{b}	48.91 ± 23^{c}
FOM_{250}	5	4.45 ± 0.06^{b}	3.42 ± 0.15^{b}	48.31 ± 0.06^{e}	44.21 ± 31^{b}
FOM_{500}	5	4.02 ± 0.01^{a}	3.63 ± 0.11^{b}	40.73 ± 0.29^{d}	47.62 ± 10^{c}
DOP_{250}	5	4.06 ± 0.03^{a}	4.17 ± 0.11^{c}	39.26 ± 0.59^{c}	52.82 ± 16^{d}
DOP_{500}	5	4.03 ± 0.02^{a}	4.74 ± 0.04^{d}	37.49 ± 0.36^{c}	56.53 ± 21^{d}

Values are expressed as Mean ± S.E.M., Mean values having different superscript letters in the same column are significantly ($p < 0.05$) different.
Key: N: Number of animals in the group; GV: gastric volume; GpH: gastric pH; TA: total acidity; TAP: total antioxidant power; NC: normal control, UC: ulcer control, DC: drug control, FOM and DOP: fresh okra fruit mucilage and dry okra fruit powder, while the subscripts 250 and 500 denote the doses in mg/kg rats' body weight respectively.

Effect of Ex-Maradi okra Fruit Administration on Histopathology of the Stomach Tissues of ethanol-induced ulcer Rats

The photograph of rats' stomachs showing the ulcer effect of ethanol were respectively presented in Figure 2. The mucosa of the normal rat's stomach was observed to be intact (Figure 2a), while that of the ulcer control rat was observed to have severe ulcer lesions (Figure 2b). The photograph of Cimetidine pretreated rat stomach is presented in Figure 2c. Here, the mucosa was observed to be intact with minimal ulcer lesions (Figure 2c), while the photographs of FOM_{250}, FOM_{500}, DOP_{250}, and DOP_{500} pretreated rats' stomachs are presented in Figures 2d, 2e, 2f, and 2g respectively, showing a respective dose-dependent protection of rats' mucosae with minimal ulcer lesions.

To further investigate the level of the ethanol-induced ulceration in the rats' stomachs, histopathological evaluations were conducted. The results showed that the gastric mucosal tissue section of the normal rat stomach showed a condition of normal cytoarchitecture of gastric mucosa (continuous epithelial surface) with no pathological changes (Figure 3a). Administration of absolute (99.80%) ethanol at a dose of 1 mL/200 g body weight resulted in superficial, deep ulcerations and perforations in the ulcer-untreated animals (Figure 3b). The micrograph of ulcer untreated rat stomachs revealed numerous severe erosions with marked disorientation of the surface epithelium showing severe ulcer lesions and desquamation of the surface epithelium (Figure 3b). The drug control group, which was orally pretreated with 20 mg/kg Cimetidine before the ulcer induction, resulted in fairly protected mucosa, even though few areas of disorientation of the villi and crypts were visible (Figure 3c). However, there were traces of erosions with small ulcer lesions on the surface epithelium of the rats' stomachs that were pretreated with FOM at 250 and 500 mg/kg (Figure 3d & 3e). However, rats pretreated with DOP at 250 and 500 mg/kg did not show ulcer lesions or perforations and disorientation of the surface epithelium of the rats' stomachs as evidenced by the micrograph (Figure 3f & 3g) comparable to the micrograph of the normal control (NC) rats' stomachs which showed no injuries to the gastric mucosa (Figure 3a).

Figure 1a: fresh Ex-Maradi okra fruit sample. **Figure 1b:** dried Ex-Maradi okra fruit sample.

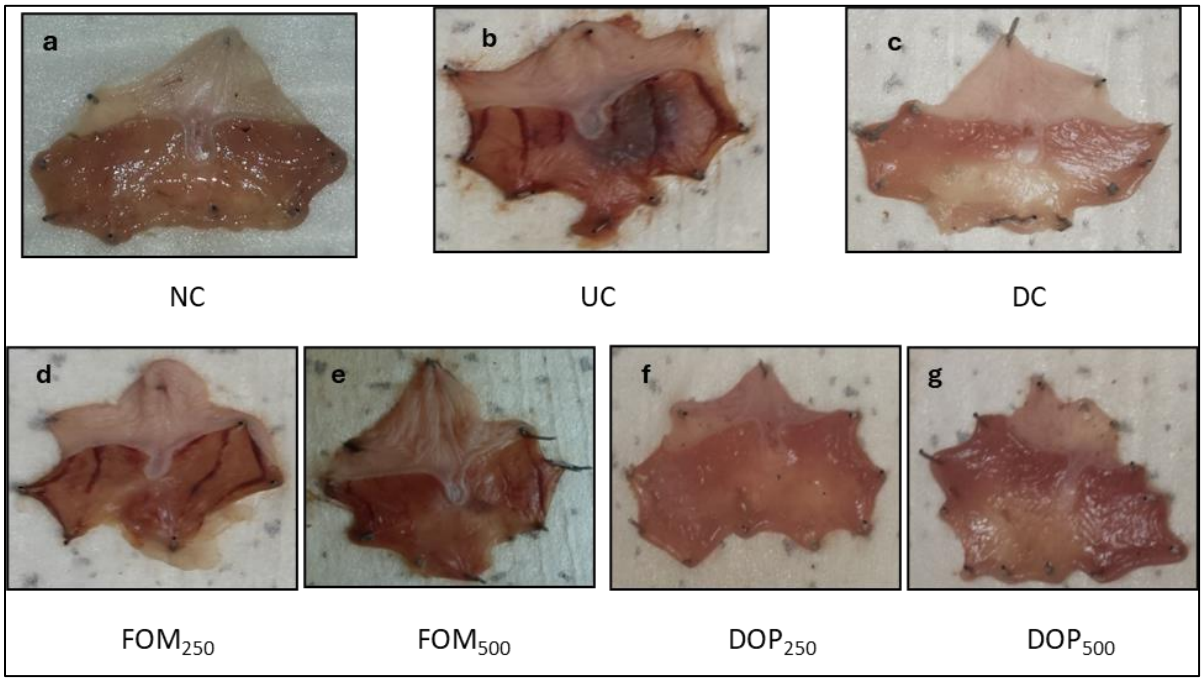

Figure 2a to 2g: Representative photographs of rats' stomachs illustrating the effect of Ex-Maradi okra fruit administration on ethanol-induced gastric ulcers in Wister rats.

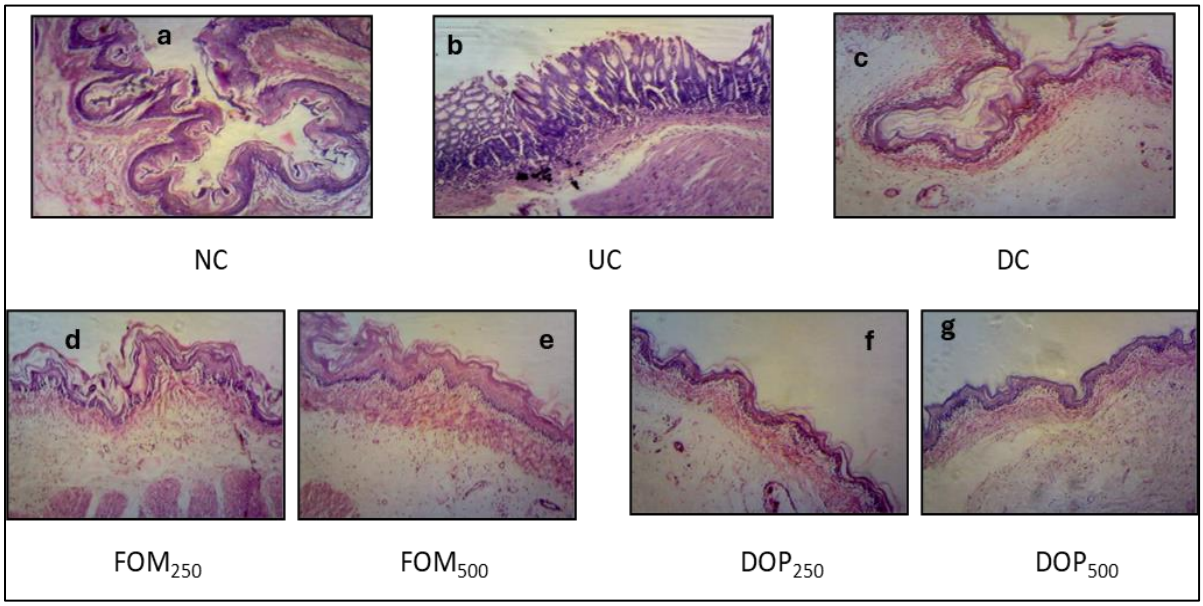

Figure 3a to 3g: Representative micrographs of gastric mucosal histology illustrating the effects of Ex-Maradi okra fruit administration on ethanol-induced gastric ulcers in Wistar rats.

Figure 2a **(Normal control):** Intact gastric mucosa with no signs of ulceration; Figure 2b **(Ulcer control):** Extensive ulceration and damage to the gastric mucosa; Figure 2c **(Drug control):** Fewer signs of ulceration compared to the ulcer control group, indicating partial protective effects of the drug; Figure 2d **and** 2e **(FOM_{250} and FOM_{500}):** Varying degrees of ulceration, suggesting a dose-dependent effect of FOM; Figure 2f **and** 2g **(DOP_{250} and DOP_{500}):** Fewer signs of ulceration compared to the ulcer control group, indicating better protective effect of DOP at both doses.

Figure 3a **(Normal control):** Normal and continuous epithelial surface; Figure 3b **(Ulcer control):** Severe ulcer lesions and discontinuous epithelial surface due to ethanol administration; Figure 3c **(Drug control):** Protected epithelium due to Cimetidine treatment, indicating protective effects of the drug; Figure 3d **and** 3e **(FOM_{250} and FOM_{500}):** Protected epithelium compared to the ulcer control group, suggesting

a dose-dependent effect of FOM; **Figure 3f and 3g (DOP$_{250}$ and DOP$_{500}$):** Better protected epithelium compared to the ulcer control group, indicating a better dose-dependent protective effect of DOP.

DISCUSSION

This study was carried out to evaluate the gastroprotective efficacy of fresh and dry Ex-Maradi okra fruits utilizing the ethanol-induced model of ulcers in rats. In this study, the observed significant ($P < 0.05$) increase in ulcer index, which was accompanied by severe congestion and hemorrhages in the epithelial surface of the rats' stomachs of the ulcer control (UC) rats compared to the normal control (NC) rats could be due to the damaging effect of ethanol on the stomach mucosal lining. Previous research has indicated that ethanol can induce lesions in the gastric mucosa (Fu *et al.*, 2021). These results align with earlier studies demonstrating ethanol's role in causing gastric mucosal injury by solubilization of stomachs' mucus constituents (Abebaw *et al.*, 2017; Fu *et al.*, 2021); and increase in xanthine oxidase activity causing extensive necrotic lesions and damages resulting in increased vascular permeability, oedema formation, reduction in gastric blood flow leading to cell death and exfoliation in the surface epithelium in the gastric mucosa of the animals (Almasaudi *et al.*, 2016). AlRashdi *et al.* (2012) have further reported that ethanol induces gastric mucosal damage by promoting vasoconstriction, releasing vasoactive substances like histamine, and generating free radicals that disrupt or damage the integrity of the mucosal cell membrane. In this study, administration of FOM or DOP, especially DOP at a higher dose (DOP$_{500}$) prior to administration of ethanol for ulcer induction significantly ($P < 0.05$) protected the rats' stomachs against ethanol-induced ulcers in the rats' stomachs compared to the ulcer control group (UC) suggesting its potent cytoprotective effect as showed by the photographs of the rats' stomachs and the results of the histopathological studies (Figures 2a to 2g and Figures 3a to 3g). This is in support of the previous findings of Ortac *et al.* (2018), where they documented that okra fruit mucilage might act as a mechanical barrier by creating a condition that makes it difficult to let ethanol penetrate into the gastric mucosae. The mucilage likely forms a protective layer that prevents the deep necrotic lesions and extensive exfoliation of surface epithelium induced by ethanol. This is similar to the action of sucralfate, an anti-ulcer drug, which forms a gel-like web over ulcerated or eroded tissues, serving as a protective bandage for the mucosa. Moreover, Ortac *et al.* (2018) reported that the gastroprotective effect of okra fruit is possibly due to the presence of polyphenolic and flavonoid components, such as quercetin, which is known for its antioxidant properties. The gastroprotective activity of quercetin has been reported in different animal studies, and most investigators have focused on its possible multiple mechanisms through which quercetin exerts its protective effects on the gastrointestinal tract, contributing to its anti-ulcer properties, which include antioxidant, anti-inflammatory effect and promotion of tissue repair (Sabitha *et al.*, 2011; Habtamu *et al.*, 2015; Omayone *et al.*, 2016).

Furthermore, the observed increase in the volume of gastric juice and total acidity, as well as the decrease in the pH of the gastric juice in the ulcer untreated rats (UC), could be attributed to the belief that gastric ulcers are primarily caused due to increase in gastric hydrochloric acid secretion and stasis of acid. Also, the volume of gastric secretion is of great significance for the formation of ulcers due to exposure of the unprotected lumen of the stomach to the accumulating acid, leading to tissue damage (Abebaw *et al.*, 2017). The effect of treatment with the FOM and DOP, especially DOP$_{500}$, resulted in a significant ($P < 0.05$) reduction in the gastric volume and total acidity with a concomitant increase in gastric pH. In addition, Treatment with DOP$_{500}$ resulted in a significant ($P < 0.05$) increase in total antioxidant power in the serum (Table 2). The protection offered by the FOM and DOP could be linked to some important bioactive compounds with antacid and antioxidant properties. These compounds may include phenols, flavonoids, and other polysaccharides present in the okra fruit, which could facilitate in the increase of bicarbonate secretion and promote the production of mucus membranes, which ultimately leads to a decrease in vascular permeability (Sabitha *et al.*, 2011; Gemede *et al.*, 2016; Uddin Zim *et al.*, 2021).

The histopathological examination of organs provides information to strengthen the findings of biochemical analysis. In the present study, the photographs of the rats' stomachs and the micrographs show the histopathological examinations of the rats' stomachs (Figures 2a to 2g and Figures 3a to 3g). The histopathological observations showed that the rats' stomachs of the UC group showed deep lesions and pathological changes (Figure 3b). This justifies the injurious effect of ethanol on

the stomach epithelia, while such lesions or pathological changes were not observed in the NC group (Kim *et al.*, 2021) (Figure 3a). However, such lesions, disorientation, and degenerations of the epithelial cell lining, as well as the observed pathological changes observed in the ulcer untreated group (Figure 3b), were not vividly observed in all the okra-treated (FOM and DOP) groups (Figures 2d to 2g and Figures 3d to 3g), especially the DOP_{500} treated group (Figure 2g and Figure 3g). This also justifies the results of the ulcer index and percentage protection obtained from the treatment with FOM and DOP and further supports the effect of treatments with FOM and DOP, especially the DOP_{500} in the ethanol-induced ulcer rats, which showed better ulcer protection (Figure 2g and Figure 3g). Similar observations of the effect of okra fruit against gastric ulcers have also been reported (Okasha *et al.*, 2014; Ortac *et al.*, 2018). A plausible explanation for this observation could be attributed to the higher dose of the dry okra fruit powder (DOP_{500}), possibly due to the advantage of its dry matter contents compared to that of the okra mucilage (Muhammad *et al.*, 2018). Also, the significant cytoprotection of gastric mucosa and inhibition of leucocyte infiltration of gastric walls in the rats pretreated with DOP_{500} could be attributed to the anti-inflammatory activities of okra fruit, as previously reported by (Sipahi *et al.*, 2022). This anti-inflammatory potential of the okra fruit could also be a key factor in the prevention of gastric ulcers (Xia *et al.*, 2015). In addition, the anti-ulcer effect shown by both FOM and DOP could be due to the reasons of their ability to modulate the antioxidant system, improving gastric cytoprotection and decreasing gastric acid secretion.

CONCLUSION

The anti-ulcer property of Ex- Maradi okra fruit (*Abelmoschus esculentus*) in ethanol-induced ulcer rat model is evident from its significant reduction in gastric volume, total acidity, number of ulcers and ulcer index as well as the increase in gastric pH and serum total antioxidant capacity in the okra treated rats. Although both DOP and FOM demonstrated good anti-ulcer effects, DOP had an overall greater impact than FOM. Even though okra fruits have been reported to have various medicinal values, including anti-ulcer effect, our findings suggest that the fruit (especially the DOP) can potentially suppress gastric damage, and it could be used to develop a useful therapeutic anti-ulcer agent. Moreover, these findings underscore the importance of exploring various forms of okra fruits (e.g., dry and fresh) to determine and compare their respective anti-ulcer effects.

RECOMMENDATIONS

The anti-ulcer effects of Ex-Maradi okra fruit may result from the synergistic actions of its bioactive compounds. However, the specific components responsible for these effects remain unknown. Thus, further phytochemical studies and *in vivo* anti-ulcer evaluations are recommended to characterize the pure compounds from the active fractions and to elucidate the underlying mechanisms behind these effects.

AUTHOR CONTRIBUTIONS

Muhammad I. Conceptualized and designed the study, conducted the research, analyzed obtained data, and drafted and revised the manuscript. Other colleagues assisted with animal procedures and biochemical assays.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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