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Abstract

Clove (*Syzygium aromaticum*) is an aromatic tree that is used to treat gastric ulcers because of its analgesic, strong anti-infective, anti-inflammatory, and antioxidant properties. So, the primary objective of this research was to investigate the anti-ulcer potential and underlying mechanisms of clove extract in an ethanol-induced gastric ulcer rat model. Five groups of 30 adult male albino rats were prepared, each consisting of six rats. The third, fourth, and fifth sets received clove extract (CE) pretreatment in the form of 50, 100, and 200 mL/kg/b.wt, respectively, whereas the first and second groups received a standard diet. Rats in the second, third, fourth, and fifth groups were given a single oral dosage by gavage of 95% ethyl alcohol at a rate of 10 mL/Kg b.wt to cause gastric ulcer. The negative control group received a single oral dose of 0.9% w/v of saline. Based on the results, pretreatment with clove extract of all tested groups significantly reduced the ulcer length, number, score, and index, while markedly enhancing the curative ratio in ethanol-induced gastric ulcerated rats. For the curative ratio, the present data showed a significant increase in rats pretreated with 50, 100, and 200 mL/kg/b.wt CE by 28.6, 54.5, and 78.6%, respectively. Pretreatment of clove extract with different concentrations resulted in a significant ($P \leq 0.05$) increase in GPX, GSH, SOD, CAT, and TAC activity compared to the positive control group. The highest increase in hemoglobin was observed in gastric ulcer rats pretreated with 200 mL/kg/b.wt CE. Using 200 mL/kg/b.wt CE had a better ulcer-pretreated effect. The notable fat content, primarily composed of essential oils such as eugenol, eugenol acetate, and β-caryophyllene, is responsible for the characteristic aroma and exhibits potent antimicrobial, anti-inflammatory, and antioxidant activities. Also, the high levels of total phenols (167.5 mg/g) and flavonoids (163.75 mg/g) in clove, which reflect its antioxidant and anti-inflammatory activities. The product exhibited promising sensory attributes, particularly in terms of taste and color, and may offer a natural dietary approach to gastric protection. These findings provide evidence that clove extract possesses gastroprotective properties by stimulating antioxidant enzymes, suggesting potential therapeutic relevance in humans.

Key words: Inflammation; pH value; Ulcer index; Curative ratio; Haemoglobin; functional beverage.

التأثير الوقائى والمضاد للأكسدة لمستخلص القرنفل ضد قرحة المعدة الناتجة عن الإيثانول فى الفئران واستخدامه في مشروب وظيفي

الملخص العربى

يُعد القرنفل(*Syzygium aromaticum*) شجرةً عطرية تُستخدم في علاج قرحة المعدة نظرًا لخصائصها المسكنة، والمضادة للعدوى، والمضادة للالتهابات، والمضادة للأكسدة. لذلك، كان الهدف الرئيسي من هذا البحث هو دراسة القدرة المحتملة لمستخلص القرنفل في الوقاية من قرحة المعدة، والآليات الكامنة وراء ذلك، فى الفئران المصابة بقرحة معدية مستحثة بالإيثانول. تم إعداد خمس مجموعات من ٣٠ فأرًا بالغًا من الذكور، بحيث تضم كل مجموعة ستة فئران. تلقت المجموعات الثالثة والرابعة والخامسة معالجة وقائية بمستخلص القرنفل (CE) بجرعات قدرها ٥٠ و١٠٠ و٢٠٠ مل/كجم من وزن الجسم على التوالي، بينما تلقت المجموعتان الأولى والثانية الغذاء القياسى . وقد تم إعطاء الفئران في المجموعات الثانية والثالثة والرابعة والخامسة جرعة فموية واحدة من الكحول الإيثيلي بتركيز ٩٥% وبمعدل ١٠ مل/كجم من وزن الجسم لإحداث الإصابة بقرحة معدية. أما المجموعة الضابطة السالبة فقد تلقت جرعة فموية واحدة من محلول ملحي بتركيز ٠.٩% وزن/حجم. أظهرت النتائج أن المعالجة الوقائية بمستخلص القرنفل في جميع المجموعات المختبرة أدت إلى انخفاض ملحوظ في طول القرحة وعددها ودرجتها ومؤشرها، مع زيادة واضحة في نسبة الشفاء في الفئران المصابة بقرحة المعدة الناتجة عن الإيثانول. وفيما يخص نسبة الشفاء، أظهرت النتائج زيادة معنوية في الفئران التي عولجت مسبقًا بمستخلص القرنفل بتركيزات ٥٠ و١٠٠ و٢٠٠ مل/كجم من وزن الجسم، بنسبة بلغت ٢٨.٦%، ٥٤.٥%، و٧٨.٦% على التوالي. كما تسببت المعالجة الوقائية بمستخلص القرنفل بتركيزات مختلفة في زيادة معنوية ($P \leq 0.05$) في نشاط كل من إنزيمات GPX و GSHو SOD و CATو TACمقارنةً بالمجموعة الضابطة الموجبة. وسُجل أعلى ارتفاع في نسبة الهيموجلوبين لدى الفئران المصابة بقرحة المعدة التي عولجت بمستخلص القرنفل بتركيز ٢٠٠ مل/كجم من وزن الجسم. وقد أظهرت هذه الجرعة (٢٠٠ مل/كجم من وزن الجسم) أفضل تأثير وقائي ضد القرحة. إن المحتوى الدهني الملحوظ في القرنفل، والمكوَّن أساسًا من الزيوت الطيّارة مثل الأوجينول، وأوجينول أسيتات، و-βكاريوفيلين، هو المسؤول عن رائحته المميزة، ويُظهر نشاطًا قويًا مضادًا للميكروبات، ومضادًا للالتهاب، ومضادًا للأكسدة. كما أن المستويات المرتفعة من الفينولات الكلية (١٦٧.٥ ملجم/جم) والفلافونويدات (١٦٣.٧٥ ملجم/جم) في القرنفل تعكس نشاطه المضاد للأكسدة، والمضاد للالتهابات. وقد أظهر المنتج خصائص حسية واعدة، خاصة من حيث الطعم واللون، وقد يوفّر نهجًا غذائيًا طبيعيًا لحماية المعدة. وتُقدم هذه النتائج دليلاً على أن لمستخلص القرنفل خصائص واقية للمعدة من خلال تحفيز إنزيمات مضادة للأكسدة، مما يشير إلى أهميته العلاجية المحتملة لدى الإنسان.

الكلمات المفتاحية: الإلتهاب – قيمة الرقم الهيدروجينى – معامل القرحة – نسبة الشفاء – الهيموجلوبين – مشروب وظيفى.

Introduction

The characteristic signs of peptic ulcer disease include mucosal lesions that extend into the muscularis mucosae, creating a cavity and causing acute or chronic inflammatory responses **Beiranvand, (2022)**. In 2019, almost 8 million individuals worldwide suffered from gastric ulcers, a condition of the digestive system that has become a public health concern and lowers the quality of life for those who have it **Xie *et al*., (2022)**. Millions of people worldwide are impacted by PUD, which is thought to affect one case among every 1000 people annually **Kumadoh *et al*., (2021)**. Additionally, it is 5–10% prevalent in the general population and has a major impact on health care costs and quality of life **Kuna *et al*., (2019)**. Gastric ulcers arise from an imbalance between aggressive (secretion of acid and pepsin) and protective (production of mucus, bicarbonate, and prostaglandins) agents, which causes necrosis in the stomach tissue glands and is characterized by redox imbalance and exudative inflammation **Tarnawski and Ahluwalia, (2021).** Lifestyle factors such as smoking and alcohol consumption, and bacterial infections, as well as gastric injury induced by NSAIDs and the Zollinger-Ellison syndrome, are linked to the development of PUD **Narayanan *et al*., (2018)**. Furthermore, greater rates of mortality and morbidity are linked to gastric ulcer complications such as penetration, hemorrhage, obstruction, and perforation (often as a result of multi-organ failure and cardiopulmonary complications) **Sreekumar *et al*., (2021)**. An increased risk of gastrointestinal malignancies, including pancreatic cancer, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma, is also connected to PUD associated with *H. Pylori* infection **Lanas and Chan, (2017)**. An untreated stomach ulcer may progress to gastritis, gastrointestinal hemorrhage, or even gastric cancer, all of which could be fatal to the patient **Scherubl, (2020)**. Using antacids, proton pump inhibitors, antibiotics, and histamine H2 receptor antagonists to protect and/or heal the stomach mucosa is part of treating and managing gastric ulcers **Clarke *et al*., (2022).** Adverse side effects from these medications are common and include headaches, constipation, diarrhea, abdominal discomfort, impotency, arrhythmia, hematological changes, hypersensitivity, and gynecomastia **Kuna *et al*., (2019)**. However, the long-term use of these drugs is linked to complications such as acute kidney injury, modifications to the structure and function of the stomach mucosa, and *H. pylori* resistance **Kim and Chung, (2020)**. Herbal remedies and other complementary therapies are thought to help treat stomach disorders **Azimi and Zahedi, (2021)**. Their accessibility, reduced adverse effects, increased environmental compatibility, positive treatment outcomes, and enhanced quality of life are the reasons for this **Singh and Easwari, (2022)**. Though their use as pharmaceutically active agents has been continuously adjusted to achieve optimal therapeutic action, natural products can have an inhibitory effect on the many inflammatory processes linked to the initiation of stomach ulcers **Jabbar *et al*., (2022)**.

Clove (*Syzygium aromaticum*) is a member of the *Myrtaceae* family. There are numerous antibacterial, antioxidant, antiviral, anticancer, anti-inflammatory, and anti-nociceptive activities associated with clove **El-shouny *et al*., (2020)**. Clove has enormous potential for use in food, medicine, cosmetics, and agriculture, and is one of the best sources of phenolic chemicals such as gallic acid, eugenol, and eugenol acetate **UHUO *et al*., (2022)**. Eugenol's gastroprotective mechanism has been characterized, showing that its anti-ulcer

activity was mediated by scavenging free radicals, opening K(ATP) channels, reducing acid-pepsin secretion, and enhancing mucin synthesis **Jung *et al*., (2011)**. Eugenol also promotes prostaglandin production and mucus secretion, which fortify the gastric mucosal barrier and aid in healing. These protective mechanisms are essential for preventing acid penetration and epithelial damage **Oliveira *et al.*, (2014).** Recently, the use of functional foods rich in antioxidants and phytochemicals for the prevention of ulcers has attracted great interest. In addition to their traditional nutritional benefits, functional foods have positive benefits on the body's health **Shah and Prajapati, (2013).** The primary objectives of this study were to evaluate the effects of clove extract as a pretreatment intervention against ethanol-induced gastric ulcer and to develop a beverage that utilizes these properties to support the daily dietary prevention of gastric ulcer.

Materials and Methods

Materials

Dry buds of cloves were acquired from Harraz for Food Industry and Natural Products, Bab Alkhalq, Cairo, Egypt, and then identified by the Department of Botanical Taxonomy, Faculty of Agriculture, Menoufia University. Banana, honey, ginger, and oat milk as a base were purchased from the local market in Cairo, Egypt.

Diet: Casein was provided by the Morgan Company for Chemicals, Cairo, Egypt. Vitamin as well as salt mixtures, cellulose, choline chloride, and L-methionine were purchased from EL-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Requirements, Cairo, Egypt.

Chemicals: Ethyl alcohol (95%), and all other chemicals were purchased from El-Gomhoreya Company in Cairo, Egypt. From Gama Trade for Company Pharmaceutical and Chemicals, Dokki, Giza, kits for biochemical analysis were obtained. Analytical grade or as pure as commercially available chemicals and reagents were used in this study.

Animals: Thirty adult male albino rats of the Sprague-Dawley strain, weighing 150 ±10 g and aged 10 weeks, were obtained from the Laboratory Animal Department of the College of Veterinary Medicine, Cairo College, Egypt. Individual rats were housed in stainless steel cages with adequate ventilation and controlled standard conditions, including a 12-h light-dark cycle, a temperature of 20–23 °C, and a humidity of 50–60%. Before the experiments began, the animals were given a week to adjust.

Methods

Clove extract preparation

Using 70% ethanol as the solvent, clove extract was prepared separately by exhaustive extraction during overnight maceration (24 h) over three consecutive days. The extract was filtered to separate the residue from the filtrate. The filtrate was then concentrated for 24 h using a vacuum oven and a rotary evaporator to create a thick, concentrated extract. The extract is kept at -20°C in a glass-wrapped container until it is needed for the experiment. This procedure was described by **Dzoyem *et al*., (2014).**

Preparation of a functional beverage containing clove extract for the prevention of gastric ulcer :

The banana was peeled, and the pulp was cut into small pieces. The base liquid (oat milk) was heated to approximately 45 °C. Clove extract and honey were dissolved into the warm liquid, after which banana pulp was added, and the mixture was blended until smooth. The beverage was allowed to cool, then bottled and stored at 4 °C **Alves *et al.*, (2015).**

Table (1): The formulation for preparing a functional beverage containing CE for gastric ulcer prevention is presented in :

Ingredients	Control	T_1 (50mL CE)	T_2 (100mL CE)	T_3 (200mL CE)
Banana pulp(g)	60	60	60	60
Oat milk (mL)	400	350	300	200
Honey (g)	15	15	15	15
Ginger Powder(g)	5	5	5	5
Clove extract (mL)	-----	50	100	200

Determination of clove chemical analysis

Cloves were analyzed for moisture, protein, fat, total ash, and fiber using the **AOAC, (2012)** method. Carbohydrates were calculated by difference. The vitamin and mineral contents (vitamin E, calcium, zinc, and iron) were determined according to **AOAC, (2015).** Using the Folin–Ciocalteu micro-method, the total phenolic content, represented as gallic acid equivalent (GAE), was determined **Saeedeh and Asna, (2007).** According to the method of **Ordon *et al.* (2006),** the total flavonoid content expressed as quercetin equivalent (QE) was determined.

Determination of the total antioxidant activity by DPPH

The method described by **Katalinic *et al.*, (2006)** was used to measure the DPPH (2,2-Diphenyl-1-picrylhydrazyl).

Basal diet

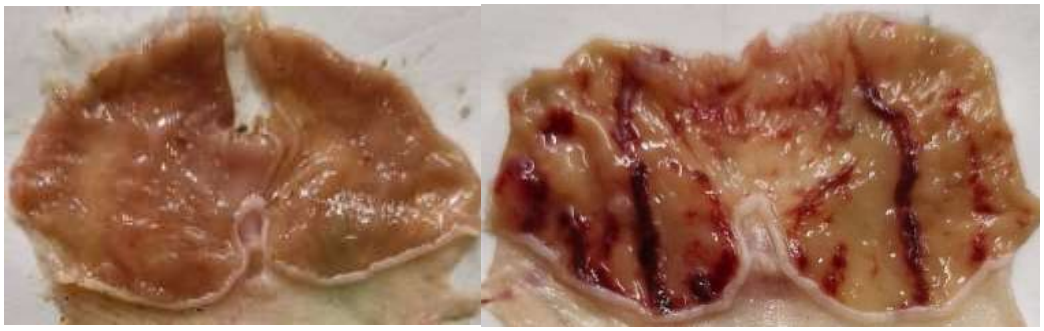
By the AIN-93 diet by **Reeves *et al.*, (1993)** the basal diet was prepared as follow: protein (10%), corn oil (10%), vitamin mix (1%), mineral mix (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remaining corn starch (69.5%). The preparation of the salt and vitamin mixture followed **Drury and Wallington, (1980)**.

Experimental design

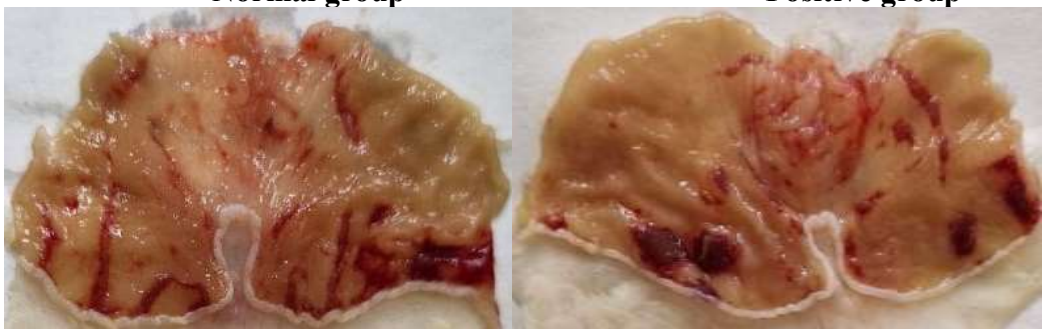
Five groups of 30 male albino rats were created (6 rats of each). The first negative group and the second positive group were given a basal diet, while groups 3, 4, and 5 were given CE at doses of 50, 100, and 200 mL/Kg/b.wt, respectively, according to **Magaji *et al.*, (2007).** The rats were given unrestricted access to water and fasted for 24 hours on day 30 of the experiment.

Induction of gastric ulcer

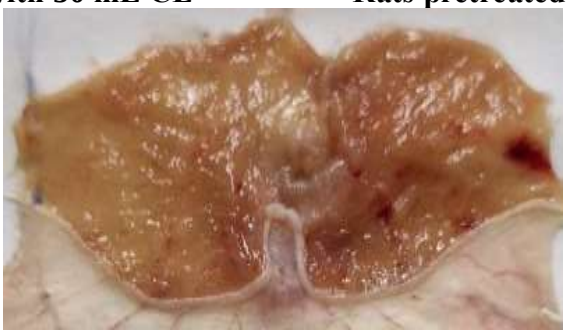
To induce gastric ulcers, the rats in groups 2, 3, 4, and 5 were given a single oral dose of ethyl alcohol at a rate of 10 mL/kg/b.wt for 2 hours **Huang *et al.*, 2014)**. As in group 1 (negative control), rats were given one oral dose of saline (0.9%, w/v).



Normal group Positive group



Rats pretreated with 50 mL CE Rats pretreated with 100 mL CE



Rats pretreated with 200 mL CE

Photo 1: The changes in rats with ethanol-induced gastric ulcer on the last day of the trial, after CE pretreatment

Collection of gastric secretions and ulcer index determination

Rats were anesthetized with diethyl ether and given ethyl alcohol for two hours. They were then sacrificed, and their stomachs were ligated around the pyloric and cardiac openings. After injecting 4 mL of distilled water into the stomach, the gastric juice was collected in a sterile tube. The juice was centrifuged at 500 rpm for 5 minutes to estimate gastric secretion parameters, including volume (mL) and total acidity. The stomachs were examined for ulcers. The severity of ulceration was assessed using the ulcer score, which is calculated by dividing the total number of ulcers in each group by the number of rats in that group **Robert *et al.*, (1968)**. According to **Khanavi *et al.*, (2012)**, the ulcer score was multiplied by 100 to determine the ulcer index. The curative ratio was determined for each treated group, following the procedure outlined by **Akhtar and Ahmad, (1995),** using the following equation:

Curative ratio (CR) = (LC-LT/LC) x100

LC: The length of the ulcer in the control positive group

LT: The length of the ulcer in the treated group

Determination of the volume, pH value, and total acidity of the gastric juice

The gastric juice volume was measured using a graduated cylinder following the procedure detailed by **Anandan *et al*., (2004)**. **Debnath *et al*., (1974)** method was used to determine the pH value. Using the method outlined by **Anson, (1938)**, the total acidity of gastric juice was measured by titrating 1 mL of gastric juice in 10 mL of distilled water with 0.01 N NaOH and using 2 drops of phenolphthalein as an indicator. The following equation was used to determine the percentage of decrease in total acidity of the gastric juice of the treated group compared to the positive control group:

Percentage of the decrease = (TAC-TAT/TAC) x 100

TAC =Total acidity of gastric juice of the positive control group

TAT =Total acidity of the gastric juice of the treated group

Following the procedure outlined by **Agrawal *et al*., (2000)**, the following equation was used to determine the percentage of decrease in volume of the gastric juice of the treated groups compared to the positive control group:

Percentage of the decrease = (VJC-VJT/VJC) x 100

VJC = volume of gastric juice of the positive control group

VJT = volume of gastric juice of the treated group

Biochemical Analysis

Glutathione peroxidase (GPx), Glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) were assayed by **Jentezch *et al*., (1996), Jollow *et al.*, (1974), Habig *et al*., (1974), Hu, (1994), and Erel, (2004)**, respectively. Serum total antioxidant capacity (TAC) was determined colorimetrically according to **Koracevic *et al*., (2001).** Using the Rat TNF-α Immunoassay, R&D system (USA), serum TNF-α (tumor necrosis factor-alpha) was analyzed **Juhász *et al*., (2013).** Blood hemoglobin was calculated using **Drabkin's (1949) method**.

Histological examination of the stomach

Samples of stomach tissue were taken from rats in different groups and preserved for 24 hours in a 10% phosphate-buffered formaldehyde solution (pH 7.4). After these samples were embedded in paraffin, 5 µm-thick sections were sliced and stained with hematoxylin and eosin. High-resolution images were obtained using light microscopy according to **Carleton, (1980)**.

Sensory evaluation of a functional beverage containing clove extract for gastric ulcer pretreatment

Sensory evaluation was performed using a score sheet to detect color, odor, taste, texture, and overall acceptability. Organoleptic characteristics were evaluated from 1 to 5 degrees (1 represents very poor and 5 represents very good general acceptability) according to **Penfield and Campbell, (1990).** The evaluation was carried out on 10 well-trained panelists from the Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt, using a score sheet.

Ethical approval

The Institutional Animal Care and Use Committee (IACUC) at Menoufia University in Sheibin El-Kom, Egypt, approved all experimental and animal care procedures. The IACUC's recommendations for the use and care of laboratory animals were followed in every biological experiment **(Approval no. MUFHE /F/NFS / 19/25).**

Statistical analysis

The findings were displayed using the mean ± standard deviation (SD). An analysis of variance (ANOVA) was performed on the experimental data using a statistical analysis system for a completely randomized design. Duncan's multiple range tests were used to evaluate differences between means at the 5% level according to **Artimage and Berry, (1987).**

Results and Discussion

Proximate chemical composition of clove buds (% dry weight basis)

Table 2 displays the proximate chemical composition of clove buds. The proximate analysis of clove buds revealed high carbohydrate content (64.2%), protein (6.94%), fat (16.16%), dietary fiber (6.9%), and ash (4.6%) content. These values reflect the rich nutritional and functional profile of clove, which contributes to its extensive use in food and traditional medicine **Banerjee *et al.*, (2020).** The high carbohydrate content suggests that clove buds serve as a potential energy source and may contain significant amounts of polysaccharides with bioactive properties, including antioxidant and prebiotic effects **Zhang *et al.*, (2021).** Dietary fiber enhances gastrointestinal health and may contribute to cholesterol-lowering and glycemic-regulation effects, which supports its use in managing metabolic disorders **Slavin, (2013).** The notable fat content, primarily composed of essential oils such as eugenol**,** eugenol acetate**,** and β-caryophyllene, is responsible for the characteristic aroma and exhibits potent antimicrobial, anti-inflammatory, and antioxidant activities **Cortés-Rojas *et al.*, (2014).** These bioactive compounds have been linked to therapeutic effects in gastrointestinal and cardiovascular health **Gülçin, (2011).**

Analysis of clove shows that it is a valuable source of vitamin E (24.68 mg/100 g)**,** which is a potent lipid-soluble antioxidant that helps protect cells from oxidative damage and supports immune function **Traber and Atkinson, (2007).** Clove also contains essential minerals, such as calcium (Ca: 4.37 mg/100g)**,** which contributes to bone health and muscle function**.** Although the levels of zinc (0.008 mg/100g) and iron (0.05 mg/100g) are relatively low, their presence adds to clove's micronutrient diversity. Iron is vital for Hb formation**,** whereas zinc supports immune response and enzymatic activity**.** Together, these micronutrients enhance the nutritional value of clove**,** making it a functional ingredient in food systems, especially when used for its antioxidant and therapeutic benefits **Ravindran *et al.*, (2018).**

Table (2): Proximate chemical composition of clove buds (% dry weight basis)

Component	Clove
Carbohydrates (g/100g)	64.2±6.42
Protein (g/100g)	6.94±0.69
Fat (g/100g)	16.16±1.5
Dietary fiber (g/100g)	6.9±0.6
Ash (g/100g)	4.6±0.42
Moisture (g/100g)	1.2±0.1
Vitamin E (mg/100g)	24.68±1.5
Ca (mg/100g)	4.37±0.29
Zinc (mg/100g)	0.008±0.001
Iron (mg/100g)	0.05±0.005

The mean ± (SD) of three replicates is used to present the findings.

Bioactive compounds from essential clove oil

Table 3 presents the chemical composition of clove essential oil, with eugenol as the major constituent, accounting for 46.70% of the total oil content. Other prominent compounds include β-caryophyllene (33.61%), α-humulene (6.44%), copaene (4.62%), eugenol acetate (2.67%), and β-ocimene (0.62%). Minor constituents such as δ-cadinene and 2-heptyl acetate were also detected at smaller concentrations. The high concentrations of eugenol and β-caryophyllene confirm their dominant role in the chemical profile of clove oil. These results are in agreement with **Kaur *et al.*, (2019)**, who identified eugenol as the primary bioactive compound in clove. However, the concentration of eugenol may vary among studies, possibly due to differences in extraction methods. **Ćavar Zeljković *et al.*, (2022)** emphasized that factors such as genetic variation, environmental conditions, and processing techniques can significantly affect the essential oil composition.

Table 3. Bioactive compounds from essential clove oil

Component	(%)
Eugenol	46.70
β-Caryophyllene	33.61
α-Humulene	6.44
Copaene	4.62
Eugenol acetate	2.67
β-Ocimene	0.62
δ-Cadinene	0.39
2-Heptyl acetate	0.33

Total phenolic content, total flavonoid content, and clove antioxidant activity

Table 4 shows that the high levels of total phenols (167.5 mg/g) and flavonoids (163.75 mg/g) in clove, which reflect its antioxidant and anti-inflammatory activities. The DPPH value of 4.73 µg/mL indicates very strong antioxidant activity, as lower IC_{50} values denote higher radical-scavenging efficiency. This supports clove's traditional and modern use in functional foods and natural preservative systems. In terms of the foods with the highest polyphenol content, the results showed that clove was the spice with the highest concentration of polyphenols and antioxidant compounds **Pérez-Jiménez *et al.*, (2010)**.

Table (4): Total phenolic content, total flavonoid content, and clove antioxidant activity

Component	Clove
Total phenol (Gallic acid, mg/g)	167.5±12
Total flavonoid (Quercetin, mg/g)	163.75±5
Antioxidant activity (DPPH) (%)	4.73±0.24

Means±SD is used to express values. **DPPH**: 2,2-Diphenyl-1-picrylhydrazyl.

Impact of clove extract on the gastric juice analysis in GU rats

Table 5 shows the impact of clove extract on the gastric juice analysis of GU rats. Rats injected with ethyl alcohol had significantly ($P\leq 0.05$) higher gastric juice volume and total acidity and a lower ($P\leq 0.05$) pH value compared with the negative group. The pH provides information about the volume and acidity level of stomach secretions. A reduced hydrogen ion concentration in the gastric juice is indicated by a low pH value. It has been linked to the pathogenesis of stomach injury and ulcers in animals. Additionally, the increase in acidity and decrease in mucin secretion showed altered hydrophobicity and a diminished mucosal membrane's capacity to prevent hemorrhagic erosion, which led to tissue damage **UHUO *et al*., (2022).** On the other hand, pretreatment of rats with CE led to a significant ($P\leq 0.05$) increase in pH value and decrease in gastric juice volume and total acidity compared with the positive group. These findings were consistent with **El-Metwally *et al*., (2014),** who indicated that the treatment with clove produced a significant reduction in gastric juice volume and total acidity. Pretreating rats with 200 mL/kg/b.wt CE led to gastric juice volume and pH values similar to those of negative rats. Rats pretreated with 100 and 200 mL/kg/b.wt CE did not differ in their effect on the pH value. Moreover, pretreatment of rats with 100 and 200 mL/kg/b.wt CE was more effective ($P\leq 0.05$) in reducing gastric juice volume, total acidity, and increasing pH value than pretreatment with 50 mL/kg/b.wt CE. The present data showed a significant decrease in gastric juice volume and total acidity in rats pretreated with 50, 100, and 200 mL/kg/b.wt CE by (45.12, 59.07, and 73.02%) and by (24, 51.4, and 73.7%), respectively.

Table (5): Impact of clove extract on the gastric juice analysis in GU rats

Variables	Negative Control	GU groups				LSD
		Positive control	50 mL CE	100 mL CE	200 mL CE	
pH Value	2.86^{b}±0.4	1.63^{c}±0.32	2.8^{b}±0.36	3.8^{a}±0.36	4.4^{a}±0.46	0.67
Gastric volume (mL)	0.9^{d}±0.36	4.3^{a}±0.72	2.36^{b}±0.3	1.76^{bc}±0.25	1.16^{cd}±0.25	0.68
Decrease in volume(%)	——	——	45.12	59.07	73.02	
Total acidity (meq/L)	0.46^{e}±0.02	3.5^{a}±0.1	2.66^{b}±0.15	1.7^{c}±0.1	0.92^{d}±0.01	0.16
Decrease in total acidity(%)	——	——	24	51.4	73.7	

The values are presented as means ± SD. Means with different letters in the same row indicate significant differences ($P\leq 0.05$).
CE: clove extract, and **GU**: gastric ulcer.

Impact of clove extract on gastric ulceration in GU rats

Table 6 shows the impact of clove extract on gastric ulceration in GU rats. The GU rats had the highest ulcer length, number, score, and index compared with the negative group. Pretreatment of rats with 50, 100, and 200 mL/kg/b.wt CE led to a decrease in ulcer length, number, score, and index and an increase in the curative ratio. The highest reduction in ulcer length, number, score, and index, and the highest increase in curative ratio were observed in the GU group pretreated with 200 mL/kg/b.wt CE. These enhancements might be due to the high total flavonoid and total phenolic compound concentrations in clove extract. For the curative ratio, the present data showed a significant increase in rats pretreated with 50, 100, and 200 mL/kg/b.wt CE by 28.6, 54.5, and 78.6%, respectively. Using 200 mL/kg/b.wt CE increased the ulcer healing effect. The antigastric ulcer effect may be due to the high amount of total phenols and flavonoids in CE. These results are completely in line with those of **UHUO *et al.*, (2022),** who showed that the extract of clove demonstrated its ability to heal mucosal epithelial cell damage, indicating a better ulcer healing capacity. Eugenol from clove has a gastroprotective effect by increasing mucus secretion on the stomach mucosal barrier **Oliveira *et al.*, (2014)**.

Table (6): Impact of clove extract on gastric ulceration in GU rats

Variables	Negative Control	GU groups				LSD
		Positive control	50 mL CE	100 mL CE	200 mL CE	
Ulcer Length(mm)	———	$5.94^{a}\pm0.75$	$4.24^{b}\pm0.7$	$2.7^{c}\pm0.85$	$1.27^{d}\pm0.25$	1.27
Ulcer number	———	$9^{a}\pm2.64$	$6.33^{ab}\pm1.53$	$4.66^{bc}\pm1.15$	$2.33^{cd}\pm0.58$	2.99
Ulcer score (US)	———	9	6.33	4.6	2.3	
Ulcer index (UI)	———	900	633	466	233	
Curative ratio (CR)%	———	———	28.6	54.5	78.6	

The values are presented as means ± SD. Means with different letters in the same row indicate significant differences ($P\leq0.05$). **CE**: clove extract, and **GU**: gastric ulcer.

Impact of clove extract on antioxidant enzymes in GU rats

Table 7 illustrates how various clove extract concentrations affect antioxidant enzyme levels in normal and GU rats. The levels of GPX, GSH, SOD, CAT, and TAC were significantly ($p \leq 0.05$) reduced in the positive group after oral exposure to ethyl alcohol, while MDA had the reverse trend compared to the negative group. This might be the effect of oxidative stress on the stomach caused by ethyl alcohol exposure. These results were consistent with **Hobani *et al.*, (2022),** who showed that rats with ethanol-induced ulcers had significantly lower levels of GSH than normal rats, and their MDA levels were greater. In rats with ethanol-induced ulcers, elongated hemorrhagic lesions, submucosal edema, leukocyte infiltration, inflammatory cell accumulation, and high ROS production are all associated with the development of severe oxidative damage **Raish *et al.*, (2021)**. Inflammation and oxidative stress are interrelated phenomena. Increased production of reactive free radicals, which can cause oxidative stress in cells, results in the suppression of TAC levels **El-Metwally, (2014)**. It is interesting to note that

pretreatment of clove extract with different concentrations resulted in a significant (*P≤0.05*) increase in GPX, GSH, SOD, CAT, and TAC activity compared to the positive control group. The antioxidant activity of clove may be due to the presence of phenolic compounds as thymol, eugenol, and eugenol acetate **Abukhalil *et al*., (2021)**. Also, **Issac *et al*., (2015)** reported that clove extract's potential for acting as antioxidants by upregulating the activities of the enzymes catalase, glutathione, and superoxide dismutase is linked to its antiulcerogenic impact. Additionally, flavonoids can scavenge OH•, O_2, and peroxyl radicals and inhibit LPO activity **Pourlak *et al*., (2020)**. The highest increases in GPX, GSH, SOD, CAT, and TAC values were observed in rats with gastric ulcers pretreated with 200 ml of CE. On the other hand, MDA was significantly (*P≤0.05*) decreased in GU rats pretreated with 50, 100, and 200 mL/kg/b.wt CE compared to the positive group. Moreover, pretreatment of rats with 100 and 200 mL/kg/b.wt CE was more effective (*P≤ 0.05*) in decreasing MDA levels than pretreatment with 50 mL CE. From the previous results, clove extract can be considered to protect against oxidative stress in gastric ulcers. These results showed that pretreatment with CE protected the gastric mucosa against ethanol-induced gastric injury by elevating the levels of GSH, GPX, SOD, CAT, and TAC. Finally, clove ethanolic extract can increase the antioxidant system enzymes and reduce oxidative stress **Abtahi-Eivari *et al*., (2021)**. It has already been shown that eugenol is a highly effective antioxidant **Nagababu *et al*., (2010).**

Table (7): Impact of clove extract on antioxidant enzymes in GU rats

Variables	Negative Control	GU groups				LSD
		Positive control	50 mL CE	100 mL CE	200 mL CE	
GPX (mU/mL)	92^{a}±3	47.33^{d}±6.5	67^{c}±4	80^{b}±4	86.33^{ab}±4.5	9.57
GSH (mmol/L)	1.9^{a}±0.04	0.64^{d}±0.07	1.09^{c}±0.17	1.54^{b}±0.21	1.72^{ab}±0.09	0.27
SOD (U/mL)	190.66^{a}±3.51	77.5^{e}±9.5	113.5^{d}±5.5	140.66^{c}±2.51	175^{b}±6	12.39
MDA(nmol/mL)	4.5^{c}±0.6	20.2^{a}±2.2	11.4^{b}±1.1	7.53^{c}±1.35	5.83^{c}±1.55	3.07
CAT (U/L)	2.4^{a}±0.1	0.87^{e}±0.06	1.34^{d}±0.07	1.68^{c}±0.18	2.02^{b}±0.24	0.27
TAC (mmol/L)	1.56^{a}±0.04	0.44^{e}±0.12	0.81^{d}±0.06	1.15^{c}±0.05	1.33^{b}±0.05	0.15

The values are presented as means ± SD. Means with different letters in the same row indicate significant differences (*P≤0.05*). **CE:** clove extract, **GU**: gastric ulcer, **GPX:** Glutathione peroxidase, **GSH**: glutathione, **SOD**: superoxide dismutase, **MDA**: malondialdehyde, **CAT**: catalase, **TAC**: total antioxidant capacity.

Impact of clove extract on tumor necrosis factor-alpha and hemoglobin in GU rats

Data in **Table 8** indicated that the exposure to ethyl alcohol led to a significant decrease in hemoglobin, while there was a significant increase in serum TNF-α level compared to the negative group. Also, such data concur with **Hobani *et al*., (2022),** who found that GU rats given ethanol significantly increased plasma TNF-α compared with normal rats. TNF-α overproduction raises the risk of cancer and stomach ulcers **Mitsushige *et al*., (2007)**. Pretreatment with CE showed a significant increase in hemoglobin, while serum TNF-α levels were significantly lower than those of the positive control group. These findings are completely in line with those of **El-Metwally, (2014),** who showed that the treatment groups with clove oil showed a significant decrease in TNF-α, while hemoglobin levels were significantly higher than in the ulcerated positive group.The highest increase in hemoglobin was observed in gastric ulcer rats pretreated with 200 mL/kg/b.wt CE. Maintaining hemoglobin levels is crucial in patients

experiencing ulcer bleeding, as significant blood loss can lead to anemia and further complications **Babiuc *et al*., (2013)**. Moreover, it was observed that pretreatment of GU rats with 100 and 200 mL/kg/b.wt CE was more effective ($P \leq 0.05$) in decreasing TNF-α level than that pretreated with 50 mL/kg/b.wt CE. These findings were consistent with **Magalhães *et al*., (2019),** who showed that TNF-α and other inflammatory cytokines were inhibited by eugenol in rats. Also, **Rusmana *et al*., (2015)** indicated that ethanol extract of clove and eugenol demonstrated inhibitory effects on TNF-α production.

Table (8): Impact of clove extract on tumor necrosis factor-alpha and hemoglobin in GU rats

Variables	Negative Control	GU groups				LSD
		Positive control	50 mL CE	100 mL CE	200 mL CE	
TNF-α (pg/mL)	$39^{d}\pm7$	$107.66^{a}\pm9.5$	$82.33^{b}\pm1.53$	$64.33^{c}\pm8.5$	$53^{c}\pm4$	13.82
HB (g/dl)	$18.05^{a}\pm0.65$	$13.83^{e}\pm0.35$	$14.9^{d}\pm0.1$	$15.93^{c}\pm0.35$	$16.8^{b}\pm0.2$	0.73

The values are presented as means ± SD. Means with different letters in the same row indicate significant differences ($P \leq 0.05$). **CE:** clove extract, **GU**: gastric ulcer, **TNF-α:** tumor necrosis factor-alpha, and **HB**: hemoglobin.

Sensory evaluation of a functional beverage

The sensory quality attributes of the functional beverage containing clove extract for gastric ulcer prevention (color, odor, taste, viscosity, and overall acceptability) are presented in **Figure 1.** The data demonstrated that there were no significant differences ($p \leq 0.05$) in viscosity between T_1, T_2, T_3, and the control. Viscosity remained consistent across all formulations, indicating that the use of banana pulp and oat milk as a base was effective in maintaining a desirable mouthfeel.

However, both T_1 and T_2 (clove-treated samples) showed significantly higher ($p \leq 0.05$) scores for color, odor, taste, and overall acceptability compared to the control. Notably, sample T_1 was the most preferred in terms of color and taste, as well as overall consumer acceptability**.** This may be attributed to the balanced concentration of clove extract, which effectively enhanced flavor without overpowering it, and contributed to a more appealing visual appearance.

In addition, sample T_3, which contained the highest concentration of clove extract (200 mL), also received high scores in all attributes, particularly odor and color. Although its taste score was slightly lower than T_1, the overall acceptability of T_3 remained favorable. This could be attributed to the strong aroma and noticeable flavor profile of clove at higher concentrations. While these characteristics enhance the functional benefits of the beverage, they may slightly reduce palatability for some individuals.

These findings are consistent with **Gülçin, (2011),** who reported that clove extract, particularly due to its rich content of eugenol and phenolic compounds**,** significantly improves flavor and provides antioxidant and antimicrobial benefits. These compounds may also play a role in maintaining sensory quality during storage at 4 °C for 15 days.

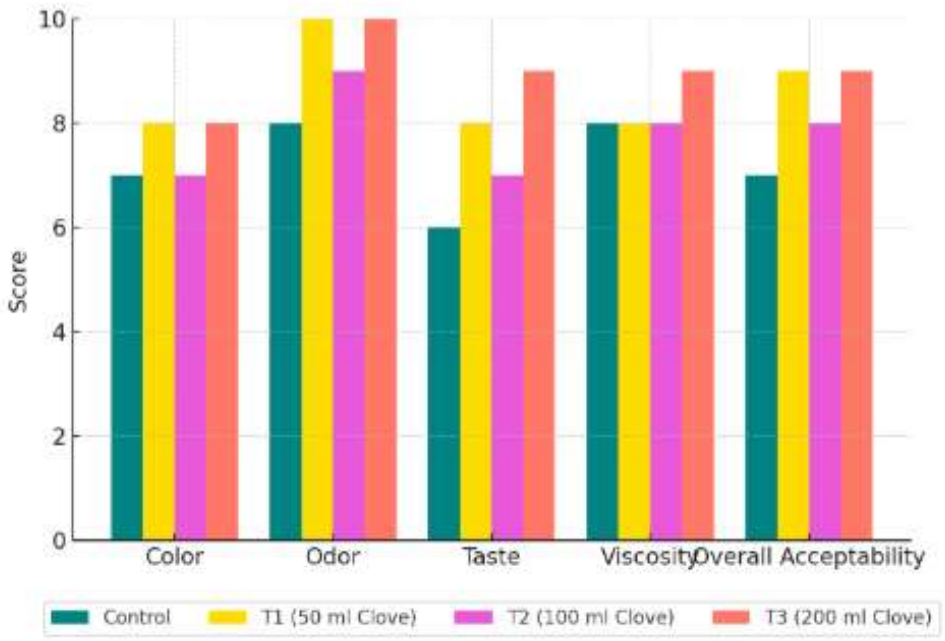


Fig.1 Sensory evaluation of a functional beverage containing clove extract for gastric ulcer prevention at refrigerator temp (2-6℃).

Impact of clove extract on the histological examination of stomach tissue in GU rats

Figure 2 demonstrates how CE affected the histological examination of the stomach tissue of both normal and GU rats. Microscopic pictures of H&E-stained rats' stomachs showing normal glandular stomach mucosa in the normal group. Rats' stomach from the positive group showing a large area of mucosal necrosis (thin black arrow) and ulceration (thick black arrow), disorganized glandular structures (curved arrow) associated with marked submucosal edema and leukocytic cells infiltration. These findings are completely in line with those of **Hobani *et al.*, (2022),** who showed that leukocyte infiltration, submucosal expansion and edema, severe disruption of the lamina epithelialis, and severe stomach mucosal injury were all observed in the ulcer control group.

On the other hand, the pretreated group with 50 mL/kg/b.wt CE in rats' stomach showed a smaller area of mucosal necrosis (thin black arrow) and focal ulceration (thick black arrow) linked to marked submucosal edema, congestion (red arrow), and leukocytic cell infiltration. Also, the rat's stomach from the group pretreated with 100 mL/kg/b.wt CE showed small focal areas of mucosal necrosis (thin black arrow) and focal ulceration (thick black arrow) associated with marked submucosal edema. Finally**,** the rat's stomach from the group pretreated with 200 mL/kg/b.wt CE showed a very small area of mucosal necrosis (thin black arrow) linked to some submucosal edema. Low magnification X: 100 bar 100 and high magnification X: 400 bar 50. These results were consistent with **Hobani *et al.*, (2022),** who showed that eugenol demonstrated better results for mucosal lesions and some submucosal blood vessel congestion in comparison with the ulcer control. Overall, pretreatment with clove extract reduced gastric acid production and protected the stomach mucosa.

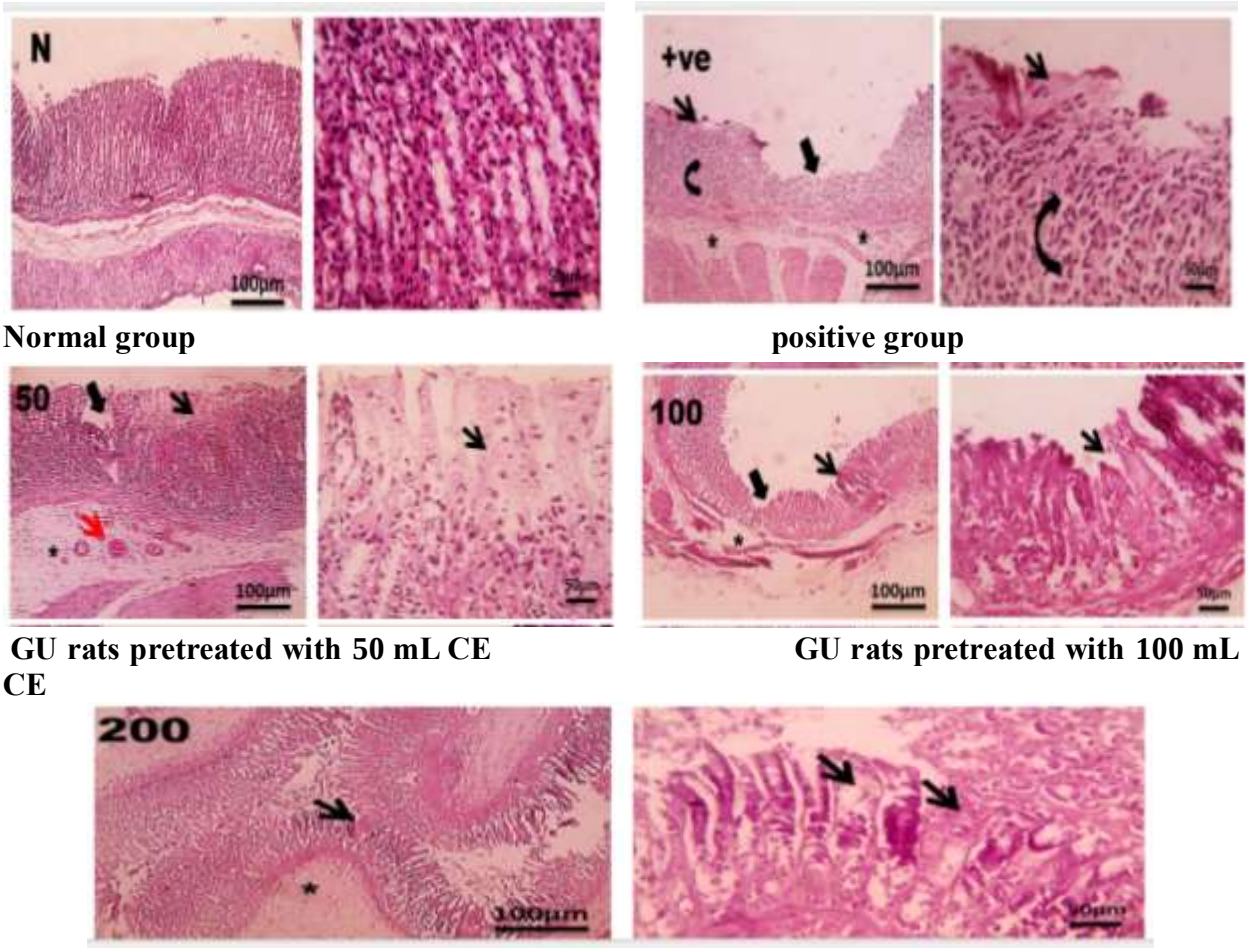


Figure (2): Effect of CE on histological examination of stomach tissue in GU rats

Conclusion

This study showed that clove is a source of natural antioxidants that could demonstrate significant gastroprotective and anti-inflammatory properties in a rat model of ethanol-induced gastric ulcer. Clove extract could protect biomolecules against oxidation and peroxidation and demonstrated high free radical scavenging property due to its content of phenolic compounds. Furthermore, the developed beverage represents a natural, functional option for gastric ulcer prevention, combining antioxidant and mucosal-protective properties with good sensory acceptability and simple preparation.

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