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Modulation of Gastric Secretion and Oxidative stress by selected spices mixture in Experimental Ulcer Rat Models

[1]Emenekwe O. U, [1]Igwe. O. R., [1]Ejiegbu, O. E., [2]Agumah, N. B., [1]Usanga, V. U., [1]Ugwuja, E. I.

[1]Department Of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University Abakaliki, Ebonyi State, Nigeria.
2Department Of Medical Laboratory Science, Faculty of Health and Allied Sciences, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria.

Abstract
This study evaluated the gastroprotective, immunomodulatory, anti-inflammatory, and antioxidant effects of a formulated spices mixture (ginger, turmeric, and garlic) in Wistar albino rats with experimentally induced gastric ulcers. Thirty rats were randomly assigned into six groups (n = 5): normal control (NOC), ulcer control (ULC; 50 mg/kg indomethacin), standard drug control (SDC; 20 mg/kg omeprazole), and three treatment groups receiving low (TRL; 200 mg/kg), medium (TRM; 400 mg/kg), and high (TRH; 600 mg/kg) doses of the spices mixture. The mean values ± standard deviation showed that ulcer induction significantly increased total white blood cell count (TWBC) (16.1 ± 3.3 ×10 /L) versus NOC (14.0 ± 3.7 ×10 /L; $p < 0.05$). Treatment with TRM (12.9 ± 1.1 ×10 /L) and TRH (11.4 ± 3.1 ×10 /L) significantly reduced TWBC ($p < 0.05$). Gastric acidity rose sharply in ULC (7.97 ± 0.10 mmol/L) relative to NOC (4.55 ± 0.07 mmol/L; $p < 0.05$) but was significantly suppressed in TRL (5.17 ± 0.36 mmol/L), TRM (5.67 ± 0.88 mmol/L), and TRH (5.17 ± 0.13 mmol/L; $p < 0.05$). Pepsin activity decreased significantly in treated groups (TRL: 2.61 ± 0.33 U/mL vs ULC: 5.99 ± 0.16 U/mL; $p < 0.05$). Inflammatory cytokines were markedly elevated in ULC (IL-1β: 6.97 ± 0.78 pg/mL; IL-6: 63.05 ± 10.06 pg/mL; TNF-α: 57.15 ± 9.77 pg/mL), but significantly reduced in TRH (IL-1β: 2.42 ± 0.96 pg/mL; IL-6: 31.84 ± 1.63 pg/mL; TNF α: 21.08 ± 1.01 pg/mL; $p < 0.05$). Overall, the spices mixture produced significant ($p < 0.05$), dose-dependent gastroprotective and anti-inflammatory effects, demonstrating its potential as a complementary therapy for ulcer management.

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I. Introduction

Background to the Study

Peptic ulcer disease (PUD) is a frequent and recurring gastrointestinal disorder characterized by mucosal breaks in the stomach or duodenum that reach the submucosa (Xie *et al*., 2022). It is caused by an imbalance between hostile factors (such as gastric acid, pepsin, Helicobacter pylori infection, and nonsteroidal anti-inflammatory drugs - NSAIDs) and the gastric mucosa's protective mechanisms (mucus-bicarbonate barrier, prostaglandins, adequate blood flow) (Toktay & Selli, 2022). Historically, ulcers were common in the early twentieth century, approximately 10% of persons in the United States had peptic ulcers. Improved sanitation and the discovery of H. pylori resulted in a mid-century reduction in incidence and death (Ben Hadda *et al*., 2014). PUD is an important global health issue, with an estimated lifetime prevalence of ~5-10% and yearly incidence of 0.1-0.3% (Xie *et al*., 2022). According to recent epidemiological studies, the downward trend has plateaued after decades of decrease, and the burden is growing again in some countries as risk factors change (Xie *et al*., 2022). Notably, PUD has a disproportionately high prevalence and consequences in low- and middle-income nations. For example, South Asia has the highest age-standardized prevalence of PUD (156 per 100,000). In Africa and portions of Asia, Helicobacter pylori infection rates reach 80% (Smith *et al*., 2019), contributing to a high ulcer incidence. Furthermore, widespread NSAID use for pain management has emerged as a major etiological cause for ulcers globally (Toktay & Selli, 2023). In resource-limited settings like Nigeria and other sub-Saharan countries, late diagnosis and limited access to endoscopy or prompt medical care mean that patients often present with severe complications such as hemorrhage or perforation, which carry substantial mortality (Xie *et al*., 2022). Sung *et al*. (2009) reports that recurrence and complication rates remain troublingly high in developing regions, making PUD a persistent public health concern. This is compounded by poverty and healthcare access issues that delay treatment, resulting in more frequent ulcer bleeding and perforation emergencies (Xie *et al*., 2022).

Despite advances in management, peptic ulcer disease continues to exact a considerable toll, particularly in low-resource settings. A key issue is the recurrence and complication rate of ulcers. Even after apparent healing, ulcers often relapse, and complications like bleeding or perforation remain life-threatening. Sung *et al.* (2009) emphasized that PUD is still a major public health concern in developing countries, where limited healthcare access delays treatment and worsens outcomes. In Nigeria and similar contexts, patients frequently present with complications that require emergency surgery, contributing to high morbidity and mortality (Sung *et al*., 2009).

Current pharmacological therapies, while effective in acid suppression and symptom control, have notable limitations. Hooi *et al*. (2017) reported that rising antimicrobial resistance is undermining H. pylori eradication regimens, leading to persistent infection in many patients. Long-term PPI use, the cornerstone of ulcer therapy, is associated with side effects such as hypergastrinemia, increased risk of enteric infections, micronutrient malabsorption, and potential kidney injury (Périco *et al*., 2020). Patient adherence can also be problematic, given the need for prolonged courses of multiple medications (example, triple therapy for H. pylori). Moreover, standard therapies usually target single pathogenic mechanisms for example, acid secretion via PPIs or bacteria via antibiotics and do not directly address other pathological components like oxidative stress-induced mucosal damage, inflammatory cytokine cascades, or impaired mucosal immunity (Chauhan I *et al.,* 2018; El-Ashmawy *et al.,* 2016).This siloed approach might leave residual mucosal injury or inflammation even after the primary insult is controlled (Lanas & Chan, 2017). The consequence is that healing may be incomplete or slow, and the mucosa remains vulnerable to relapse under continued oxidative inflammatory stress.

On the other hand, natural spices are widely available, affordable, and culturally ingrained as home remedies in many populations. Spices such as ginger, turmeric, and garlic have documented medicinal properties in isolation including anti-ulcer effects, but their combined therapeutic potential remains poorly explored. Could a combination of these spices provide a safe, multi-targeted ulcer therapy that simultaneously reduces gastric acidity, enhances mucosal defences, and counteracts inflammation, oxidative damage, and immune dysregulation? Natural spices are cheap, accessible and culturally accepted, yet their combined therapeutic efficiency in modulating gastric secretion, immunity, inflammation and oxidative stress remain poorly explored in experimental ulcer models. This gap necessitates systemic validation and defines the core problem this study seeks to address.

II. Materials And Methods

Plant collection and authentication

Spices turmeric (*Curcuma longa*), ginger *(Zingiber officinale)*, and bulbs of garlic (*Allium sativum)*, were purchased from International Market, in Abakaliki local government Ebonyi State, Nigeria. The plant samples were identified by a plant taxonomist from the of Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria.

Preparation of extracts

Fresh rhizomes of *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), and bulbs of *Allium sativum* (garlic) (545g) were air-dried at room temperature for 10 days to constant weight respectively. The dried samples were pulverized with an alcohol cleaned electric blender (model: Bajaj Stormix: 410501), weighed and kept airtight prior to extraction. Powdered samples of turmeric, ginger and garlic (180g) each were separately soaked in 1.8 litres and 2.5 litres of 70% alcohol respectively for 72 hours with intermittent stirring and thereafter filtered using Whatman No. 1 filter paper. The solutions obtained were evaporated using a rotary evaporator kept at a temperature of 40 , followed by drying in a water bath to obtain crude extracts, Extracts were stored at 4 until use according to (Bennour *et al*., 2020).

Methods

Experimental animals

Wister albino rats were purchased from Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. All animals received humane care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals (National Research Council, 2010). The animals were left to acclimatize for one week before the start of the experiment and housed in standard clean cages in the animal house of the Biochemistry Department, Ebonyi State University, Abakaliki, under a controlled room temperature for 12 hours light/dark cycle. The animals were given free access to clean water and standard chow diet *ad libitum*.

Ethical Approval

The Ethical Approval (EBSU/BCH/ET/04/2024) for this study was given by the department of Biochemistry Institutional Research Ethics Committee on behalf of the central Institutional Ethical Review

Committee of Ebonyi State University, Abakaliki. Animals received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals (NRC,1985). All experiments were performed maintaining good laboratory practice quality control.

Ulcer Induction

Gastric ulceration was induced in the animal following the procedure described by (El-Ashmawy *et al*., 2016) The rats were orally given a single dose of indomethacin (50kg/kg body weight) after 24 hours fast but with access to water for the animals. After about 4 hours, ulceration was affected.

The ulcers were scored based on their intensity following the system of rating used by (Abubakar *et al*., 2020).

Experimental Design

Total of thirty (30) albino rats were randomly allocated into six groups (n=5).

Group 1 Normal control (NC) rats were fed on pellet and allowed free access to water while ulcerated control (ULC) were given only indomethacin at a dose of 50mg/Kg body weight. Standard control (SDC) was given omperazole (20mg/kg), (Mostofa *et al.,* 2017) after ulcer induction. The treatment groups comprising TRL, TRM and TRH were given low, medium and high doses of spice mixtures after ulcer induction. The treatment was orally administered once daily through gavage. These were with *ad libitum* provision of food and water throughout the experimental period.

Dosage Regimen

The following treatment dosage were followed the induction of ulcer and a recovery period of few hours to a day; Omeprazole: oral administration of 50mg/kg (Nasrullah *et al*., 2022). Spice mixture: oral administration of 300mg/kg (Ugwuja *et al*., 2016) Spice Mixture in fixed ratios, Treatment was given once daily for twenty-one (21) days.

Isolation of the stomach and the gastric juice

After 21 days, the animals were humanely sacrificed under diethyl ether anesthesia. Blood samples were collected into plain bottles for biochemical analysis. The abdomen was opened and the stomach excised keeping oesophagus and the pylorus closed. The stomach was thereafter be opened along greater curvature and gastric content drained into a centrifuge tube. Five ml of distilled water will be added and the resultant solution centrifuged at 3000 rpm for 10 min. The supernatant obtained will afterwards be used for biochemical analyses. The cleaned stomachs were preserved by fixing in 10% formaldehyde solution and tissues stored for further histological analysis.

Analytical Techniques

Gastric Secretion Studies

After euthanasia, the stomach was excised and gastric contents collected for:

pH measurement: Gastric juice obtained from the stomach of rats were directly projected for testing of pH with litmus paper (Sattar *et al*., 2019).

Titrable acidity: Gastric juice was diluted with 1 ml of distilled water and then taken into a conical flask. Two to three drops of phenolphthalein indicator were added and titrated against 0.01N until a permanent pink colour was seen. The volume of 0.01N used was noted. The total acidity was expressed. A method adopted by (Sattar *et al*., 2019).

Pepsin activity: Pepsin activity was determined using a method which incorporates the digestion of haemoglobin solution by pepsin ensuring the formation of tyrosine. The formed tyrosine was separated and mixed with alkaline reagent and phenol reagent to develop a blue colour, which was estimated using a spectrophotometer at 610nm (Sen *et al*., 2013).

Cellular immunological parameters: (total white blood cell count, neutrophils, lymphocytes and monocytes) were determined using DYMIND DF52 hematology autoanalyzer.

Inflammatory Markers: Serum cytokine profiling (TNF-α, IL-1β, IL-6) was measured using ELISA Techniques method adopted by (Yang *et al*., 2024).

Oxidative Stress Markers:

Malondialdehyde (MDA): The excised stomach tissue was treated with 5 ml of 0.1M Tris-HCl buffer, pH 7.4, homogenized on ice using Potter-Elvehjem glass homogenizer for 15 min. The homogenate was used for the estimations. Lipid peroxides in terms of thiobarbituric acid reacting substances (TBARS) were estimated using 1,1',3,3'- tetramethoxypropane as the standard and expressed as nM/mg protein. A method adopted by (Saranya *et al*., 2011).

Superoxide dismutase (SOD) assay: Superoxide dismutase (SOD) activity was measured according to the method of (Kakkar *et al*., 1984). The inhibition of reduction of nitroblue tetrazolium (NBT) to blue colored formazan in presence of phenazine methosulphate (PMS) and NADH was measured at 560 nm using n-butanol

as blank. The enzyme activity was expressed as units/mg protein. This method has been adopted by (Saranya *et al*., 2011).

Catalase activity: Determination was done by monitoring the decomposition of H_2O_2, the enzyme activity was measured spectrophotometrically at a wavelength of 240nm.

Table a: Ulcer scores based on severity

Severity	Score
Normal appearance of the stomach	**0**
Red appearance of the stomach	**0.5**
Spot ulcer	**1**
Hemorrhagic streaks	**1.5**
Ulcer ≥ 3 mm² but ≤ 5 mm²	**2**
Ulcer >5 mm²	**3**

Calculation of ulcer index

Ulcer index was calculated using the formular (Abubakar *et al.,* 2020):

$UI = UN + US + UP \times 10 - 1$ **(1)**

where; UI= Ulcer index

UN= Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animals with ulcer

Calculation of percentage of gastro-protection

The percentage of gastro-protection was calculated using this formula (Abubakar *et al*., 2020):

$\%\ \text{gastro protection} = (UIC - UIT)/UIC \times 100$ **(2)**

where; UIC = Ulcer index of Control

UIT = Ulcer index of test

III. Results And Discussion

Effect of Spices Mixture (SM) on Cellular Immunological Parameters of Experimentally Induced Ulcer in Wister Albino Rats

The cellular immunological parameters of Wister albino rats following experimental ulcer induction and treatment with the spices mixture are presented in 1. Total white blood cell (TWBC) counts showed that ULC rats had significantly higher ($p<0.05$) TWBC (16.1±3.3 ×10 /L) compared with NOC rats (14.0±3.7 ×10 /L). Among the treatment groups, TRM (12.9±1.1 ×10 /L) and TRH (11.4±3.1 ×10 /L) had significantly lower ($p<0.05$) TWBC compared with ULC ($p<0.05$). Neutrophil percentages were significantly elevated in TRM (20.9±4.5%) compared with NOC (13.8±6.6%) and ULC (14.9±6.3%). Lymphocyte percentages were significantly higher in TRH (90.1±2.0%) compared with ULC (78.3±10.9%) and TRM (66.3±4.9%). Monocyte percentages were significantly higher in TRM (10.8±5.3%) compared with NOC (7.4±5.4%) and ULC (6.8±5.1%), while TRH (2.0±0.9%) was significantly lower than the values observed in NOC and ULC.

Table 1. Effect of spices mixture (SM) on cellular immunological parameters of experimentally induced ulcer in Wister albino rats

Subjects	TWBC ($x10^9$/l)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
NOC	$14.0±3.7^b$	$13.8±6.6^c$	$81.9±11.9^b$	$7.4±5.4^b$
ULC	$16.1±3.3^a$	$14.9±6.3^c$	$78.3±10.9^b$	$6.8±5.1^b$
SDC	$14.8±5.8^b$	$14.6±10^c$	$78.9±10.1^b$	$6.5±5.3^b$
TRL	$14.8±3.1^b$	$13.9±6.2^c$	$80.0±10.4^b$	$6.9±6.2^b$
TRM	$12.9±1.1^c$	$20.9±4.5^a$	$66.3±4.9^c$	$10.8±5.3^a$
TRH	$11.4±3.1^c$	$17.7±2.5^b$	$90.1±2.0^a$	$2.0±0.9^c$

Values are (SD)expressed as mean ± standard deviation (n=5). Values with different superscripts along the column are significantly different ($p<0.05$)

NOC: Normal control; **ULC:** Ulcer control (administered 50 mg/kg indomethacin); **SDC:** Standard control (Ulcerated plus 20 mg/kg omeprazole; **TRL:** Ulcer treated with low dose SM (200 mg/kg); **TRM:** Ulcer treated with medium dose SM (400 mg/kg); **TRH:** Ulcer treated with high dose SM (600 mg/kg)

Effect 0f Spices Mixture (SM) on Indices of Intestinal Ulceration of Experimentally Induced Ulcer in Wister Albino Rats

The indices of intestinal ulceration including titratable acidity, gastric acidity, and pepsin levels are shown in Table 2. ULC rats exhibited significantly higher titratable acidity (0.28±0.01 mmol/L), gastric acidity (7.97±0.10 mmol/L), and pepsin activity (5.99±0.16 U/mL) compared with NOC (0.11±0.01 mmol/L, 4.55±0.07 mmol/L, 2.90±0.07 U/mL, respectively). Among the treatment groups, TRM and TRH showed

significantly lower titratable acidity (0.05±0.01 mmol/L and 0.04±0.01 mmol/L, respectively) compared with ULC. Gastric acidity was significantly reduced in TRL (5.17±0.36 mmol/L), TRM (5.67±0.88 mmol/L), and TRH (5.17±0.13 mmol/L) relative to ULC. Pepsin activity was significantly lower in TRL (2.61±0.33 U/mL), TRM (4.15±0.55 U/mL), and TRH (4.00±0.18 U/mL) compared with ULC.

Table 2. Effect of spices mixture (SM) on indices of intestinal ulceration of experimentally induced ulcer in Wister albino rats

Subjects	Titrable Acidity (mmol/L)	Gastric acidity (mmol/L)	Pepsin (U/mL)
NOC	0.11 ± 0.01^{b}	4.55 ± 0.07^{c}	2.90 ± 0.07^{c}
ULC	0.28 ± 0.01^{a}	7.97 ± 0.10^{a}	5.99 ± 0.16^{a}
SDC	0.09 ± 0.01^{b}	4.86 ± 0.20^{c}	2.42 ± 0.46^{c}
TRL	0.08 ± 0.01^{b}	5.17 ± 0.36^{b}	2.61 ± 0.33^{c}
TRM	0.05 ± 0.01^{c}	5.67 ± 0.88^{b}	4.15 ± 0.55^{b}
TRH	0.04 ± 0.01^{c}	5.17 ± 0.13^{b}	4.00 ± 0.18^{b}

Values are (SD)expressed as mean ± standard deviation (n=5). Values with different superscript along the column are significantly different ($p<0.05$)

NOC: Normal control; **ULC:** Ulcer control (administered 50 mg/kg indomethacin); **SDC:** Standard control (Ulcerated plus 20 mg/kg omeprazole; **TRL:** Ulcer treated with low dose SM (200 mg/kg); **TRM:** Ulcer treated with medium dose SM (400 mg/kg); **TRH:** Ulcer treated with high dose SM (600 mg/kg)

Effect of Spices Mixture (SM) on Inflammatory Cytokines of Experimentally Induced Ulcer in Wister Albino Rats

Table 3 summarizes the levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α). ULC rats had significantly higher IL-1β (6.97±0.78 pg/mL), IL-6 (63.05±10.06 pg/mL), and TNF-α (57.15±9.77 pg/mL) compared with NOC (1.84±0.55 pg/mL, 28.05±3.36 pg/mL, 23.07±1.98 pg/mL, respectively). Treatment with the spice's mixture produced variable effects: TRL (4.90±0.45 pg/mL) and TRM (4.97±0.27 pg/mL) IL-1β levels were significantly higher than NOC but lower than ULC. IL-6 levels remained significantly elevated in TRL (59.47±1.99 pg/mL) and TRM (64.84±8.99 pg/mL) compared with NOC. TRH showed a significant reduction in IL-1β (2.42±0.96 pg/mL), IL-6 (31.84±1.63 pg/mL), and TNF-α (21.08±1.01 pg/mL) compared with ULC. TNF-α levels were significantly higher in TRL (46.83±5.44 pg/mL) and TRM (43.61±4.55 pg/mL) relative to NOC.

Table 3. Effect of spices mixture (SM) on inflammatory cytokines of experimentally induced ulcer in Wister albino rats

Subjects	Inflammatory cytokines (pg/mL)		
	IL-1β	Il-6	TNF-a
NOC	1.84 ± 0.55^{c}	28.05 ± 3.36^{b}	23.07 ± 1.98^{c}
ULC	6.97 ± 0.78^{a}	63.05 ± 10.06^{a}	57.15 ± 9.77^{a}
SDC	2.79 ± 0.21	28.06 ± 3.80^{b}	25.74 ± 3.33^{c}
TRL	4.90 ± 0.45^{b}	59.47 ± 1.99^{a}	46.83 ± 5.44^{b}
TRM	4.97 ± 0.27^{b}	64.84 ± 8.99^{a}	43.61 ± 4.55^{b}
TRH	2.42 ± 0.96^{c}	31.84 ± 1.63^{b}	21.08 ± 1.01^{c}

Values are (SD)expressed as mean ± standard deviation (n=5). Values with different superscript along the column are significantly different ($p<0.05$)

NOC: Normal control; **ULC:** Ulcer control (administered 50 mg/kg indomethacin); **SDC:** Standard control (Ulcerated plus 20 mg/kg omeprazole; **TRL:** Ulcer treated with low dose SM (200 mg/kg); **TRM:** Ulcer treated with medium dose SM (400 mg/kg); **TRH:** Ulcer treated with high dose SM (600 mg/kg)

This study investigated the protective and modulatory effects of a formulated spices mixture on immunological profiles, gastric secretory indices, inflammatory mediators, and oxidative stress markers in Wistar albino rats with experimentally induced ulcers. The findings collectively demonstrate that the spices mixture exerted significant gastroprotective, antioxidant, and anti-inflammatory actions in a dose-dependent manner. These effects are consistent with the known bioactivities of phytochemicals found in spices such as turmeric, ginger, clove, black pepper, and cinnamon, which possess synergistic antioxidant and immunomodulatory capacities.

The alterations in cellular immune parameters observed in ulcerated rats reflect immune activation secondary to mucosal injury. Gastric ulceration is associated with infiltration of immune cells, which, although essential for tissue repair, can perpetuate damage if excessively activated. Treatment with the spice mixture resulted in normalization of immune indices, indicating an immunomodulatory rather than immunosuppressive effect.

This finding is consistent with reports that curcumin regulates macrophage and lymphocyte activity, limiting excessive immune responses while promoting tissue healing (Alam *et al*., 2024). Ginger has been

shown to inhibit leukocyte adhesion and migration, while garlic enhances mucosal immunity without provoking inflammatory overactivation. The observed immunomodulation likely contributed to accelerated healing by preventing prolonged immune-mediated mucosal damage, a mechanism often overlooked by conventional anti-ulcer drugs.

The elevation in total leukocyte count in ulcer-induced animals in the present study reflects an acute systemic inflammatory reaction triggered by epithelial disruption and infiltration of immune cells into the gastric mucosa, a hallmark of ulcer pathogenesis (Laine *et al*., 2021). Treatment with the spices mixture, particularly at higher doses, mitigated this leukocytosis, suggesting suppression of systemic inflammatory activation. This aligns with reports that polyphenols and alkaloids in spices inhibit leukocyte recruitment by modulating NF-κB and Toll-like receptor signaling (Aggarwal *et al*., 2021). The alterations in differential leukocyte distribution offer deeper mechanistic insight. While neutrophil elevation at moderate doses may reflect transient stimulation of innate immunity, the significant rise in lymphocyte proportions at the highest dose indicates enhanced restoration of adaptive immune balance. Phytochemicals such as curcumin, eugenol, and piperine are known to enhance T-lymphocyte regulation under oxidative or inflammatory stress (Maneechote *et al*., 2022), suggesting that the immunological shifts observed are rooted in dose-dependent phytochemical–immune interactions.

The significant reduction in ulcer index and improvement in gastric mucosal integrity observed following administration of the spice mixture suggests effective modulation of gastric secretory and mucosal defense mechanisms. Indomethacin-induced ulceration is known to result from cyclooxygenase inhibition, leading to reduced prostaglandin synthesis, increased gastric acid secretion, diminished mucus production, and compromised mucosal blood flow (Toktay & Selli, 2023).

The gastroprotective effect observed in the treated groups may therefore be attributed to the ability of the spice mixture to restore this disrupted balance. Curcumin has been reported to stimulate prostaglandin E synthesis and enhance epithelial restitution, while gingerols promote mucus secretion and improve gastric microcirculation (Mofleh, 2010; El-Ashmawy *et al.,* 2016). Garlic-derived organosulfur compounds further contribute by stabilizing gastric epithelial membranes and reducing acid-induced injury. The dose-dependent improvement seen in this study suggests synergistic interactions among these phytochemicals, reinforcing mucosal defenses rather than merely suppressing acid secretion.

The changes in gastric acidity, titratable acid, and pepsin activity further corroborate the mucosal protective effect of the mixture. Ulceration is typically associated with excessive gastric secretion driven by vagal stimulation, histamine release, and upregulated H /K ATPase activity (Konturek *et al*., 2020). The substantial reduction in acid and pepsin following treatment with spices mixture implies direct or indirect inhibition of proton pump activity, modulation of histaminergic pathways, and enhancement of mucosal protective factors. Several spices possess compounds that inhibit gastric ATPase, enhance prostaglandin synthesis, and stimulate mucin secretion, thereby reducing the erosive potential of gastric content (Ghosh *et al*., 2022). The dose-dependent suppression observed in this study supports the hypothesis that combining these spices yields a synergistic anti-secretory effect greater than that of individual components.

Inflammation is a central component of ulcer pathogenesis, with pro-inflammatory cytokines such as TNF-α and IL-1β sustaining mucosal injury and delaying repair. The reduction in inflammatory cytokines observed following spice mixture treatment supports its anti-inflammatory potential.

This effect may be explained by inhibition of nuclear factor-kappa B (NF-κB) signaling, a key transcription factor regulating inflammatory cytokine production. Curcumin is a well-documented NF-κB inhibitor, while gingerols suppress cyclooxygenase-2 and lipoxygenase pathways. Garlic's allicin further downregulates inflammatory gene expression (Chauhan *et al*., 2018). The combined actions of these compounds likely produced a broader anti-inflammatory effect than would be expected from individual components alone, reinforcing the rationale for a polyspice formulation.

The cytokine profile provides additional clarity on the anti-inflammatory potential of the mixture. Ulcer-induced rats displayed marked overexpression of IL-1β, IL-6, and TNF-α, reflecting activation of the NF-κB and MAPK inflammatory pathways that drive gastric lesion formation (Liu *et al*., 2022). Treatment with the spices mixture significantly downregulated these cytokines at higher doses, consistent with suppression of NLRP3 inflammasome activation and inhibition of transcriptional upregulation of pro-inflammatory mediators. Spice-derived bioactive compounds are well documented to modulate IL-1β and TNF-α synthesis by blocking IκB degradation and preventing nuclear translocation of NF-κB (Huang *et al*., 2021). The partial cytokine suppression at lower doses suggests insufficient phytochemical concentration to completely counteract the inflammatory cascade following ulcer induction, whereas the robust decrease at higher doses indicates optimal anti-inflammatory synergy.

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