Published by
NIGERIAN SOCIETY OF PHYSICAL SCIENCES
Available online @ https://asr.nsps.org.ng/index.php/asr

African Scientific Reports 3 (2024) 189

African Scientific Reports

Attenuation of indomethacin-induced gastric ulceration by methanolic extract of *Cucumis melo (L. Indoro*us) seeds in male Wistar Rats

Grace Adebayo-Gege [a,*], James Adiwu[b], Abednego Ovey Angbashim[b], Toyin Dorcas Alabi[c], Murtala Ngabea Audu[a], Frank Abimbola Ogundolie[d]

[a]*Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Baze University, Abuja, FCT, Nigeria*
[b]*Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Bingham University, Nassarawa State, Nigeria*
[c]*Department of Biochemistry, Faculty of Computing and Applied Sciences, Baze University, FCT, Nigeria*
[d]*Department of Biotechnology, Faculty of Computing and Applied Sciences, Baze University, Abuja, FCT, Nigeria*

Abstract

Inflammatory markers has been implicated during NSAID-induced gastric ulcer as it enhances leukocyte adhesion which also contribute to lipid peroxidation and decrease in antioxidants. *Cucumis melo seeds (L. Indorous)* is well known to contain some active component with strong anti-inflammatory potentials. Therefore, this study aimed to investigate the effects of *Cucumis melo* seed extract (MECmS) on indomethacin-induced stomach ulcers in male Wistar rats. Twenty-five male Wistar rats (n = 5, 130–150 mg) were randomly assigned to five groups: group 1 (water); group 2 (indomethacin; 40 mg/Kg); group 3 (50 mg/Kg methanolic extract of *Cucumis melo* seeds; MECmS + indomethacin); group 4 (100 mg/kg MECmS + indomethacin); and group 5 (200 mg/kg MECmS + indomethacin). MECmS was administered 14 days prior to induction of ulcers. Organs' relative weights (RWO), total stomach acidity, ulcer score, and hematological parameters were assessed. MDA, catalase, protein level, nitrite, and TNF-alpha were also measured. Induction with indomethacin led to significant (p<0.05) increase in gastric acidity, ulcer score, relative stomach weight, MDA, TNF-alpha, and significant decrease in total protein, catalase, and nitrite level. Indomethacin also induced significant necrosis of the tunic mucosal. Treatment with Cucumis *melo* seed extract reversed tunic mucosal necrosis and significantly induced the proliferation of the gastric mucosal gland. *Cucumis melo* seed extract also significantly increased total protein, catalase, and nitrite level, while it significantly reduced ulcer score, gastric acidity, MDA and TNF-alpha levels. *Cucumis melo* possess antiulcer and anti-inflammatory properties; hence can be explored as a novel anti-ulcer drug.

DOI:10.46481/asr.2024.3.3.189

Keywords: Indomethacin, Ulceration, Catalase, TNF-A, Nitrite level

Article History :
Received: 27 February 2024
Received in revised form: 09 October 2024
Accepted for publication: 29 October 2024
Published: 17 December 2024

© 2024 The Author(s). Published by the Nigerian Society of Physical Sciences under the terms of the Creative Commons Attribution 4.0 International license. Further distribution of this work must maintain attribution to the author(s) and the published article's title, journal citation, and DOI.

1. Introduction

One of the things that contribute to the development of ulcers is inflammation. Gastric ulcer disease can be caused by three major factors; stress, non-steroidal anti-inflammatory drugs (NSAIDs), and *Helicobacter pylori* infection and they have an impact on the

*Corresponding author: Tel.: +234-806-621-9972.
Email address: funbimbola@gmail.com (Grace Adebayo-Gege)

etiology of ulcer which can be duodenal, gastric or both [1, 2]. It is believed that an imbalance between factors affecting mucosal integrity and aggressiveness causes stomach ulcers. Prostaglandin inhibition decreased bicarbonate concentration, and decreased stomach blood flow are examples of mucosal integrity variables that are correlated with a decrease in gastric ulcer development, while increased gastric acid and pepsin secretion are associated with an increase in aggressive factors [3].

Ibuprofen, naproxen, and indomethacin are frequently used as analgesics and anti-inflammatory and they act by preventing the creation of prostaglandins [3–5]. Numerous scientific and epidemiological research have demonstrated the role that NSAIDs play in reducing the rate of colorectal cancer (CRC) [6, 7]. In a study that employed meta-analysis, it was discovered that mortality rate and CRC incidence can be reduced by 30–40% through the use of aspirin for period of roughly five years [8, 9]. However, it is impossible to ignore its detrimental effects on the digestive system. NSAIDs generally are known for their ability to disruption prostaglandin production by blocking cyclooxygenases' ability to produce prostaglandin in the gastric mucosa [10]. Since Ibuprofen, indomethacin and aspirin have been reported as examples of NSAIDs to cause gastric ulcers, lipid peroxidation (which is triggered by free radicals and a reduced antioxidant system) is a crucial step in the toxicity mechanism of these drugs. This is linked to the radical destruction of cellular antioxidant defence systems, which in turn causes oxidative damage in the stomach [11].

Studies have shown that TNF-α is upregulated in response to gastric mucosal injury induced by NSAIDs. TNF-α contributes to the inflammatory response by promoting the recruitment of immune cells, enhancing the expression of adhesion molecules (like ICAM-1), and inducing the release of other inflammatory cytokines (such as IL-1 and IL-6). This cascade exacerbates mucosal damage and can lead to increased gastric permeability and epithelial cell apoptosis [12].

Notably, mucosal injury is also facilitated by nitric oxide (NO), a modulator of gastrointestinal (GIT) mucosal defense. NO synthesis is mediated by nitric oxide synthase (NOS). It can be either inducible (iNOS) or constitutive (cNOS). The iNOS is usually found in the neutrophils and macrophages [13], while the cNOS which has two isoforms, neuronal (nNOS) and endothelial (eNOS) can be found in the stomach mucosa [14]. The properties of the NOS synthesized vary in the digestive system, while iNOS has a cytotoxic property, cNOS is cytoprotective [15]. Low concentrations of nitric oxide produced by cNOS help to preserve the integrity of the epithelial tissues by enhancing the blood flow to the mucosa in the GIT system [16]. Nitric oxide's anti-inflammatory properties are due to its ability to prevent leucocyte activation, adhesion, and migration [17].

Mechanisms of interventions utilized in conventional medicine against ulcers include H2 histamine receptor inhibitors (such as Cimetidine), mucosal defense agents (such as Sucralfate), gene therapy, prostaglandin analogs and proton pump inhibitors (PPIS; such as Omeprazole) [18]. Each of these drugs comes with mild to severe side effects, which has led to a search for nontoxic, readily available, and affordable antiulcer drugs [19, 20]. Medicinal plants that have been researched for their antiulcer ability include; *Spondias mombin, Ficus exasperate*, and *Cucumis melo* (L. Inodorous) to name a few. It has been discovered that *Cucumis melo* also known as honeydew melon, is useful in the treatment of experimentally induced ulceration brought on by NSAIDS such as ibuprofen [3]. Studies have shown that both the *Cucumis melo* pulp and its methanolic extract include substances such as polyphenols, terpenoids, cardiac glycosides, alkaloids, and steroids, which are elements that are likely conferring its antiulcer capacity on C. *melo* [21–23]. According to reports, *C. melo* is said to possess anti- ulcer properties due to its ability to reduce the lipid peroxidation, total stomach acidity, ulcer score, ulcer index, and H+/K+ ATPase activity, cucumis melo considerably preserves the integrity of the gastric mucosa during ibuprofen induced gastric ulceration [3]. However, there is unclear evidence about the effect of *C. melo* on inflammation during GIT ulceration. Therefore, this study investigated the impact of the methanolic extract of *Cucumis melo* (MECmS) on inflammation and ulcers in the gastrointestinal tract induced by indomethacin.

2. Materials and methods

2.1. Chemicals and reagents

Normal saline, Methanol, 30% Trichloroacetic acid (TCA), 10% formalin, 40mg/kg indomethacin stock solution, Phosphate buffer (0.01M, pH 7.0), Dichromate/acetic acid, 0.1M NaOH solution, NaH_2PO_4, Griess reagent, 0.75% Thiobarbituric acid (TBA), N-Hexane, Xylazine, 0.15M Tris-KCl buffer, ketamine, 0.3mM Adrenaline, Na_2HPO_4 Phenolphthalein, 5% $K_2Cr_2O_7$ (Dichromate Solution), NaOH, potassium iodide, $CuSO_4$. $5H_2O$, (BDH Chemicals, England), 0.05M Carbonate buffer (pH 10.2), KI (BDH Chemicals, England), 0.2M H_2O_2 (Hydrogen peroxide).

2.2. Collection, Authentication and Methanolic extraction of Cucumis melo L.

Cucumis melo was acquired from a fruit farm in Jos and authenticated at the Herbarium and Ethnobotany Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRID) by Akeem A. Lateef. A Specimen with voucher number: NIPRD/H/7216 was deposited at the Herbarium. The seeds were allowed to air dry, and pulverized using an electric blender. 200 g of the air-dried seeds were defatted with n-hexane using a Soxhlet extractor for 24 hours at a boiling point between 40 – 60 °C. After being defatted and dried, the marc was packed and extracted for 10 hours using methanol. In order to allow the methanol to fully escape, the concentrated methanol extract was exposed to air.

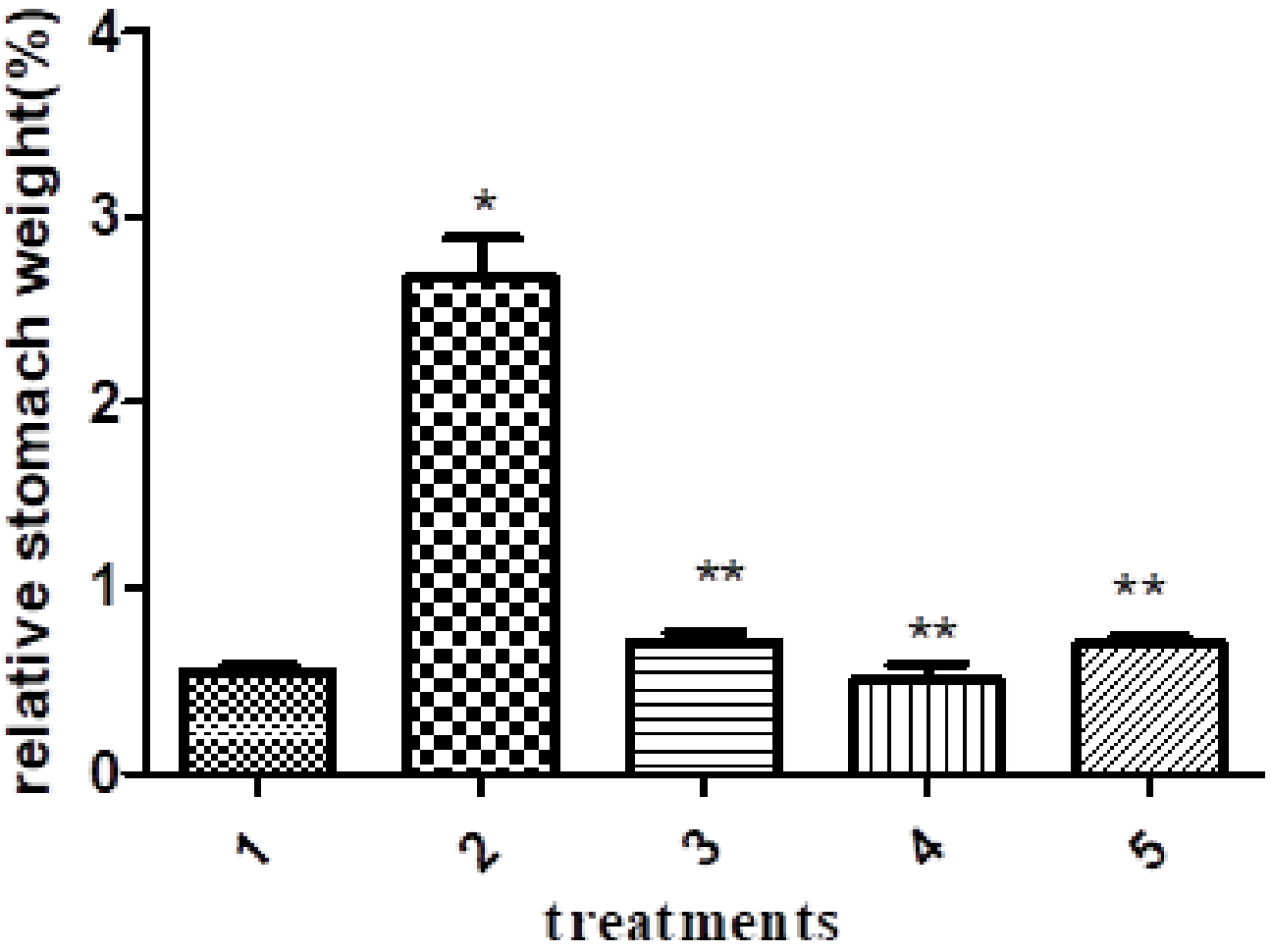

Figure 1. demonstrates the impact of MECmS on relative stomach weight change following ulcer induction. Bars are indicative of mean values ± SEM of group values. is used to express all data as mean SEM. *indicates a significant (p<0.05) difference from group 1. **Noteworthy in contrast to group 2.

2.3. Animals

Twenty-five male Wistar rats weighing between 130 and 150 grams were acquired from the Department of Physiology at Baze University College of Medicine in Abuja. The National Institutes of Health's guides for laboratory animal care, National Institutes of Publications No. 8023, revised 1978, was followed for both animal maintenance and experimental techniques. Animals in the control and experimental groups were housed separately in closely monitored environments in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals and EU Directive 2010/63/EU for animal research.

2.4. Animal grouping

1. water, rat pellets (Negative Control)
2. 40 mg/kg Indomethacin induced ulceration without treatment (Positive Control)
3. 50 mg/kg of methanolic *Cucumis melo* seed extract + Indomethacin; 40 mg/Kg
4. 100 mg/kg of methanolic *Cucumis melo* seed extract + Indomethacin; 40 mg/Kg
5. 200 mg/kg of methanolic *Cucumis melo* seed extract + Indomethacin; 40 mg/Kg

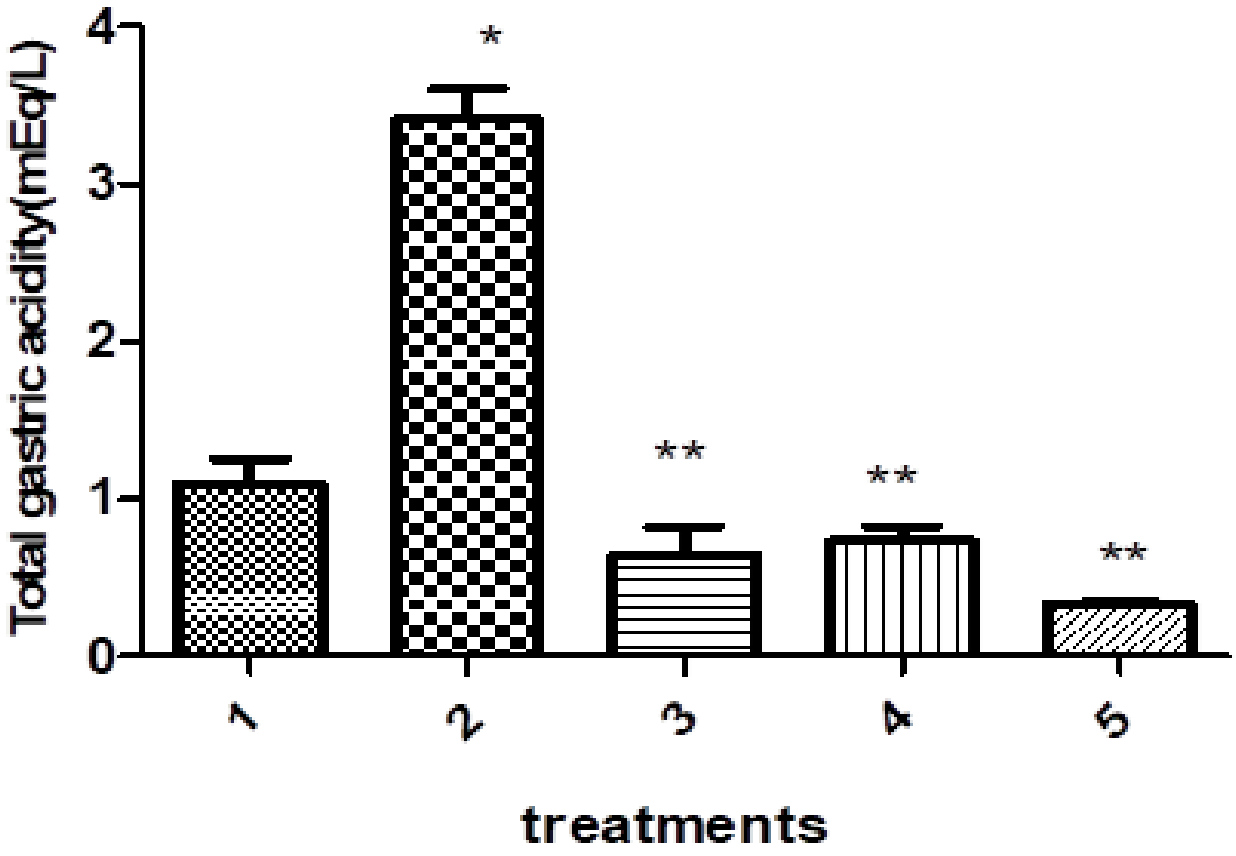

Figure 2. Impact of *Cucumis melo* seeds methanolic extract on stomach acidity following ulcer induction. The mean SEM, $p<0.05$, is used to express all values. * Significant in relation to group 1; ** Significant in relation to group 2.

2.5. Pretreatment with MECmS and Induction of Gastric Ulcer

MECmS were administered orally for a duration of 14 days prior to ulcer induction. Gastric ulcer was induced with 40 mg/Kg of Indomethacin after a 24-hours fast (rats had unlimited access to water during the fast). Rats were euthanized after 6 hours of receiving indomethacin. The blood was collected and organs of interest were harvested and stored for further analysis [24].

2.6. Assessment of gastric ulceration by scoring

The ulcer index was used to grade and express gastric lesions in the fundic stomach, following the method outlined by Adinortey *et al*. [25]. The total sum of all scores divided by the overall number of animals produces the mean ulcer index.

zero = no damage; one = blood at the lumen; two = pinpoint erosions; three = one to five small erosions <2 mm; four = more than five tiny erosions <2 mm; five = one to three big erosions >2 mm; and six = more than three large erosions >2 mm.

2.7. Measurement of gastric acid secretion

A 10 ml beaker was filled with 1 ml of gastric content, diluted with 0.01M sodium hydroxide (NaOH) and 2-3 drops of phenolphthalein until the solution took on a pink hue. It was noted and read how much of NaOH had been added. Equation (1) was used to determine the gastric acidity of the stomach contents:

$$Acidity = \frac{Volume\ \ of\ \ NaOH\ \ x\ \ NaOH\ \ Normalcy\ \ x\ \ 100g}{0.1}. \quad (1)$$

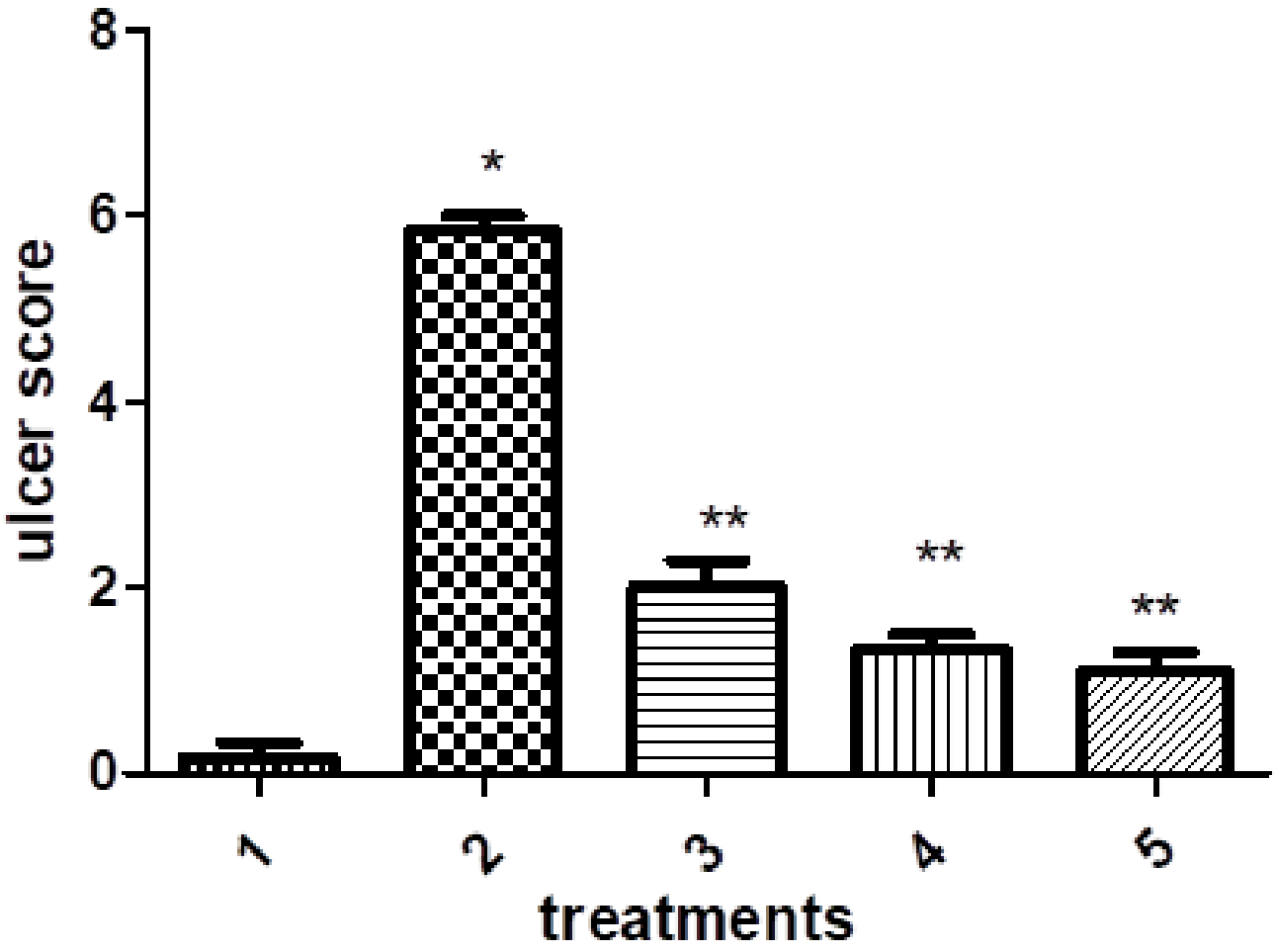

Figure 3. The impact of *Cucumis melo* seeds methanolic extract on ulcer score following ulcer induction. Every value is given as mean SEM, $p<0.05$. * as significant as group 1; ** as significant as group 2.

2.8. Measurement of relative stomach weights

The relative weighted stomach was evaluated using equation (2).

$$Relative\ \ weight\ \ of\ \ Stomachs\ \ = \frac{A}{B}\ \ x\ \ 100, \tag{2}$$

where A = weight of stomach; b = weight of animal.

2.9. Measurement of hematological parameters

The hematological parameters; PCV, red blood cell (RBC), white blood cell (WBC), neutrophil, monocyte, lymphocyte, and eosinophil were measured using an auto-analyzer instrument (SFRI blood cell Counter, H18 light, France).

2.10. Assessment of Biochemical parameters

A section of the stomach tissue was excised to be used for histological analysis, while the remaining stomach tissue was homogenized in phosphate buffer and centrifuged in a cold centrifuge. The supernatant fraction was obtained and stored in the freezer.

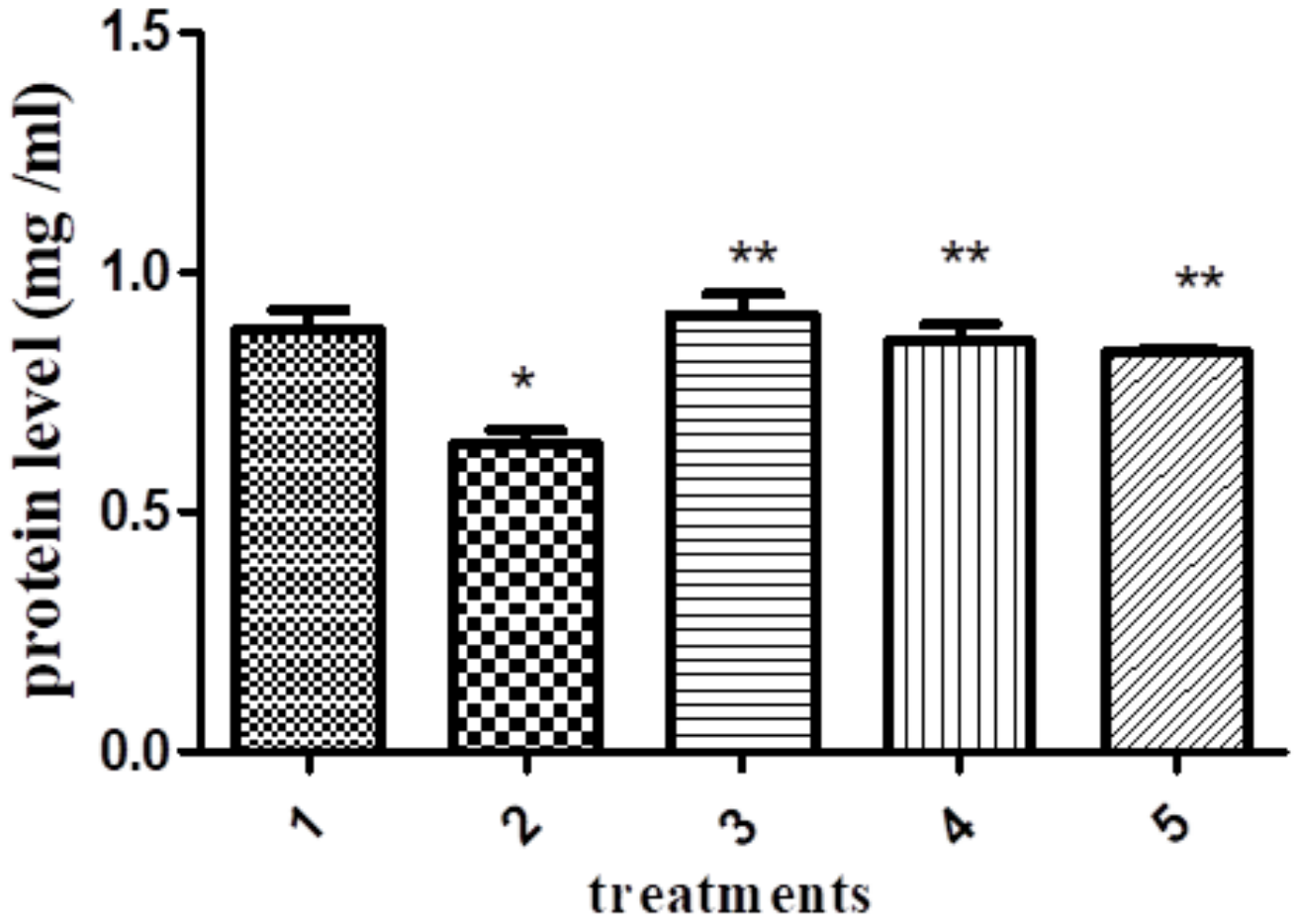

Figure 4. demonstrates how the MECmS affects the quantity of protein following the formation of ulcers. Every value is presented as mean SEM, with $p<0.05$. * Significant compared to group 1; ** significant compared to group 2.

2.11. Lipid peroxidation

Lipid peroxidation was assessed by measuring the malonaldehyde (MDA); a product of lipid peroxidation in the stomach tissue. This was carried out in accordance with Varshney and Kale's work [26]. The protein content of the stomach tissue was evaluated using the Biuret method [27]. Catalase was assessed utilizing Clabiorne's technique.

2.12. Tumor Necrosis Factor Alpha Concentration (TNF-α)

Colorimetric assay using Cloud-clone SEA133RA 96 Test kits. ELIZA kits were utilized to test the levels of tumor necrosis factor alpha (TNF-α).

2.13. Nitrite level

Evaluation of the nitrite level was done according to the modified method of Ignarro *et al.* [28], which was first described by Griess in 1879.

2.14. Histological evaluation

Portions of stomach's tissues which were excised for histological examination were preserved in 10% buffered formalin. Tissues were dehydrated and embedded in paraffin wax and sectioned at 5μm using a LICA microtome. The sections were mounted, deparaffinized by immersion in xylene, and rehydrated through a series of graded alcohols, then water. Thereafter, the slides were stained with standard hematoxlin and eosin (H&E) dyes and examined under a light microscope. Lesion scores were compared using the following semi-quantification technique.

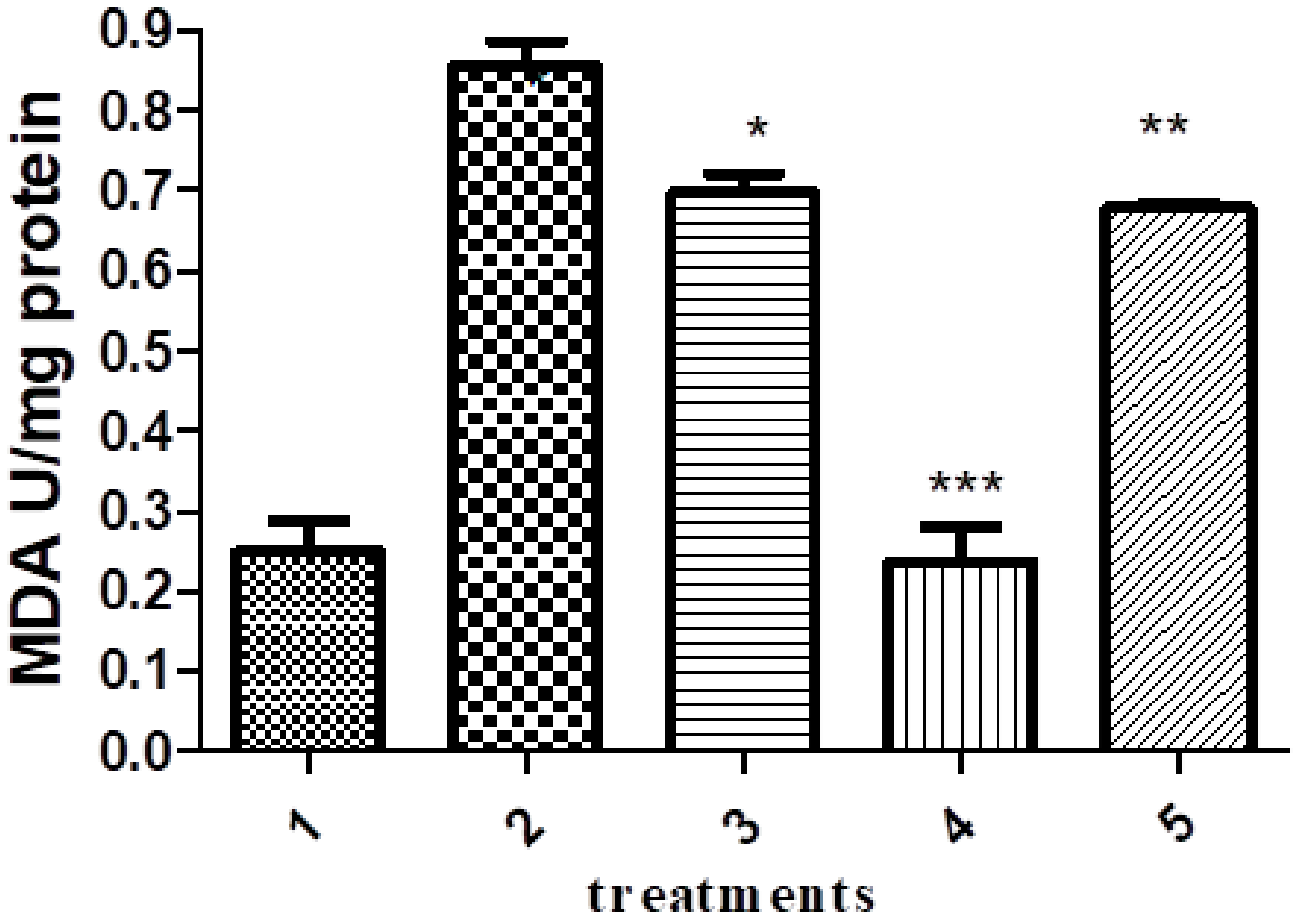

Figure 5. demonstrates how a methanolic extract from *Cucumis melo* seeds affects catalase activity following ulcer induction. The mean ±SEM, p<0.05, is used to express all values. Significant differences between groups 1 and 2 are indicated by an asterisk (**).

3. Statistical analysis

Every piece of data is displayed as mean ± standard error of mean (SEM). The means were compared using one-way ANOVA while Bonferroni Post Hoc analysis was used for pair-wise comparisons. Differences (*F* values) were considered to be significant when p value is less than 0.05; $p<0.05$. All statistical analysis were carried out using Graph Pad Prism version 5.0.

4. Results

4.1. Cucumis melo seeds decreased organ toxicity in indomethacin-induced ulcers

A significant ($p<0.05$) increase in the relative stomach weight of rats in group 2 (untreated ulcer group) was observed in comparison to group 1 (normal rats) as shown in Figure 1. Pretreatment with MECmS led to a significant decline in the relative stomach weight as compared to group 2, and this was observed in all the doses of MECmS administered. Also, induction of ulcer with Indomethacin led to a significant rise in the total gastric acidity of rats in group 2, which was not observed in groups pretreated with MECmS when compared with the normal rats (Figure 2). Furthermore, the significant increase observed in the ulcer score of group 2 following indomethacin administration, was not seen in groups pretreated with MECmS.

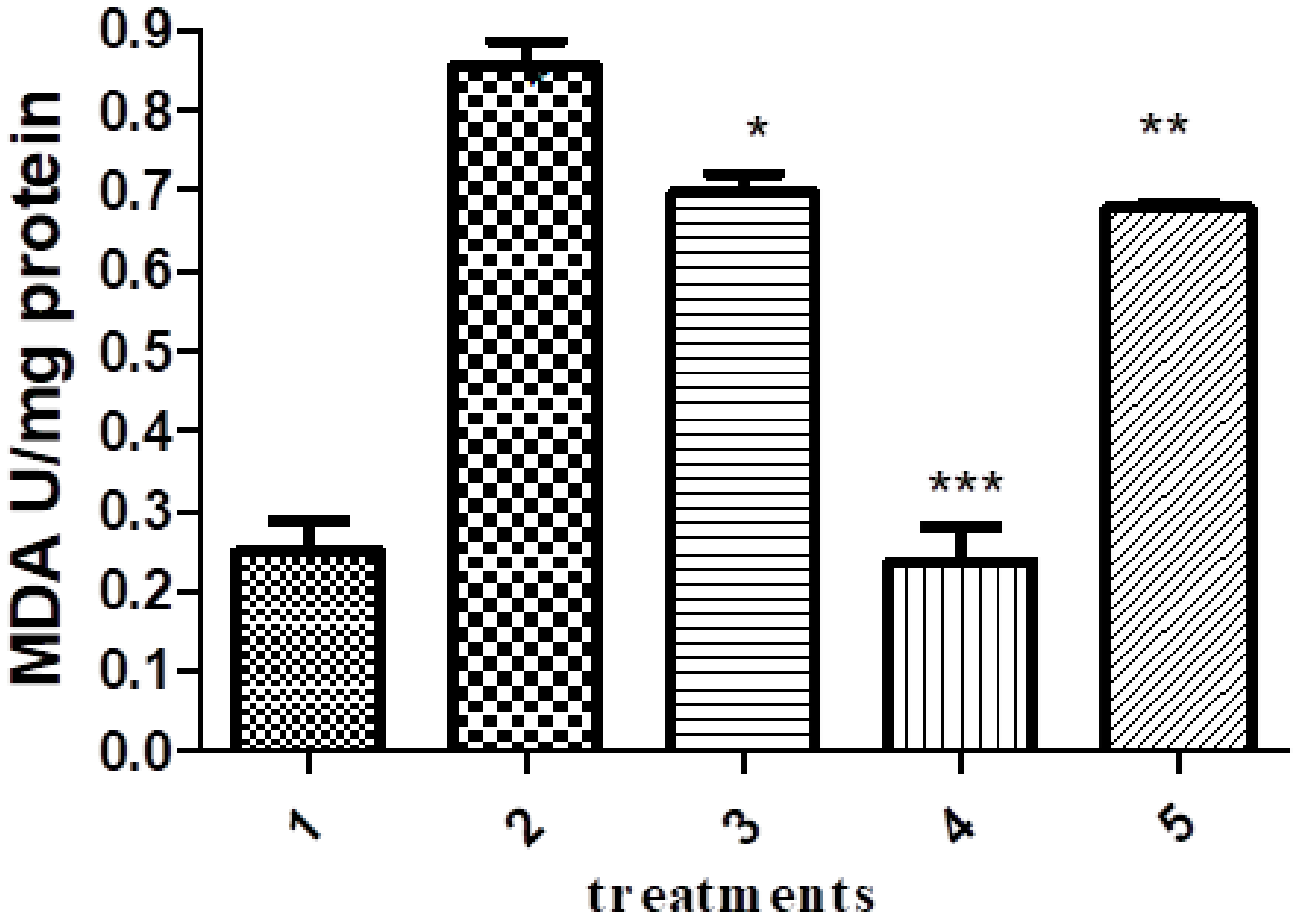

Figure 6. Impact of MECmS extract on MDA protein expression following ulcer induction. Every value is given as mean± SEM, p<0.05. * as significant as group 1; ** as significant as group 2.

4.2. The impact of Cucumis melo seeds methanolic extract on ulcer score

According to Figure 3, group 2's ulcer score increased significantly (p<0.05) when compared to group 1. When compared to group 2, the administration of a MECnS significantly reduced the stomach ulcer score in groups 3, 4, and 5. p<0.05.

4.3. Impact of Cucumis melo seeds on hematological parameters in indomethacin-induced ulcers

The effect of *Cucumis melo* methanolic extract on hematological biomarkers is displayed in Table 1. There was a significant decrease in the hemoglobin and red blood cells after inducing ulcers while white blood cells (WBC), neutrophils, lymphocytes, and N/L ratio significantly increased when compared to the normal rats. Supplementation with 100mg/Kg MECmS prior to indomethacin administration, resulted in a significant decrease in WBC, neutrophils, lymphocytes, and N/L ratio in comparison with the untreated, ulcer control group. In addition, 100mg/Kg MECmS, significantly raised the PCV and hemoglobin levels when compared with untreated ulcer groups. 50mg/kg MECmS also modulated hematological parameters when compared to the untreated, but not significantly, except for N/L ratio which was significantly abated. Similarly, 200mg/kg MECmS modulated hematological parameters when compared to the untreated, but not significantly, except for PCV that was significantly elevated.

4.4. Cucumis melo seeds enhanced antioxidant status and prevent oxidative stress in indomethacin-induced ulcers

The effect of *Cucumis melo* seeds on antioxidant enzyme; catalase (CAT), total protein levels and lipid peroxidation marker (MDA) is showcased in Figures 4, 5 & 6. Induction of ulcer using indomethacin caused a significant decrease in CAT activity, total

Table 1. Effect of pretreatment with *Cucumis melo* seeds on hematological parameters in indomethacin-induced ulcer.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
PCV	55.13±2.81	40.33±4.18*	43.63±1.73*	62.00±6.35**	47.00±1.73**
Hemoglobin	18.38±0.94	13.44±1.39*	14.54±0.58	20.67±2.18**	15.67±0.58
RBC	9.65±0.58	6.20±0.55*	6.90±0.55	10.16±0.84**	6.95±0.14
WBC	3.08±0.34	6.40±0.17	6.20±2.07	4.34±0.64*	6.03±0.33
Neutrophils(N)	0.00±0.00	36.67±3.33*	32.00±0.00	3.85±0.20**	34.33±4.33
Lymphocytes(L)	0.00±0.00	57.33±3.93*	58.00±0.00	86.90±0.64**	58.33±4.41
N/L Ratio	0.00±0.00	0.625±0.0.96*	0.55±0.00**	0.44±0.0027**	0.61±0.13

All values are presented as Mean ±SEM, P<0.05; * significant as compared to group 1, ** significant compared to group 2. PCV- pack cell volume, RBC- red blood cells, WBC- white blood cells, N/L ratio- Neutrophils-Lymphocytes ratio.

protein concentration and a significant rise in MDA levels when compared to normal rats. Administration of all doses of MECmS prevented the significant decrease in protein and CAT activity while significantly inhibiting lipid peroxidation (MDA levels) in comparison with untreated ulcer group. It is noteworthy that 100 mg/Kg MECmS significantly inhibited rise in MDA levels in pretreated rats as comparable to normal rats.

4.5. Cucumis melo seeds inhibits inflammation and protects the GIT lining in indomethacin induced gastric ulceration

Induction of gastric ulcer with indomethacin triggered significant increase in the TNF-α concentration and significant decrease in nitrite levels in untreated group. Intervention with MECmS significantly impeded the elevation of TNF-α levels while enhancing nitrite oxide concentration in treated ulcer groups as shown in Figures 7 & 8.

4.6. Cucumis melo attenuates visible ulceration of the gastric mucosa in indomethacin-induced gastric ulceration

. Sections of the stomach displayed in Figure 9, an intact gastric mucosa in A(group 1:normal rats), while erosion of the gastric mucosa with the presence of more than 10 petechial hemorrhages was observed in untreated ulcer groups(B:group 2). In C- E (group 3-5), the groups pretreated with varying doses of MECmS, show a significant decrease in peechail hemorrhage and erosion in the gastric mucosa of the rats.

Figure 9a shows Group 1 (normal rats) with intact gastric mucosa, Figure 9b also displayed Group 2 (untreated ulcer group) and the group had petechial hemorrhages with severely eroded gastric mucosa, Figure 9c shows Group 3 pretreated with 50mg/kg MCmS + indomethacin and it presented some ulceration with moderately eroded mucosa, Figure 9d shows Group 4 (100 mg/kg MCmS + indomethacin) wih mildly eroded mucosa with little ulceration, and Figure 9e shows Group 5 (200mg/kg + indomethacin) displayed no visible ulceration with light erosion of the gastric mucosa.

4.7. MECmS repairs the gastric mucosa of the indomethacin-induced gastric ulceration

Findings from the histological examination of the stomach tissues are revealed in Figure 10. The micrograph section of A(Group 1) shows normal histo-architecture, the gastric mucosa is thick (black star). There is mild localized loss of the mucosa epithelium (thin, black arrow). The mucous (red arrow) and parietal (green arrow) cells in the stomach glands appear normal. The B(Group 2) shows notable vascular alterations, including a wide-area of severe, tunic mucosal necrosis (thick black arrow) and the formation of modest numbers of inflammatory cells (An arrow should describe this on the H&E micrograph).

In C(Group 3) contains large areas of moderate epithelial loss (thick black arrow). A significant proliferation of the stomach's mucosal glandular cells was noticed (red arrow). The tunic submucosa (black star) appears modestly enlarged due to oedema fluid and the deposition of mononuclear cellular aggregates. The stomach mucosa in D(group 4), is thick (black star), with few regenerative glands with flattened epithelium and dilated lumens (thin black arrow). The parietal (green arrow) and mucous (red arrow) cells of the stomach glands exhibit various degeneration. Blood vessels are moderately congested (thick black arrow). E(Group 5) shows focus of necrosis as well as loss of gastric mucosa tips (arrow to indicate this). There are regenerated gastric glands with dilated lumens (red arrows). There is a slight buildup of inflammatory cells near the base of the tunic mucosa (thin black arrow) as shown in Figure 10.

5. Discussion

In this study, the anti-ulcer potential of methanolic extract obtained from *Cucumis melo* on indomethacin-induced ulcers in the stomach of male Wistar rats was successfully investigated. Indomethacin, an NSAID, is used to treat pain, inflammation, and hyperpyrexia in newborns, as well as patent ductus arteriosus [29]. Indomethacin is also said to have some anticancer properties

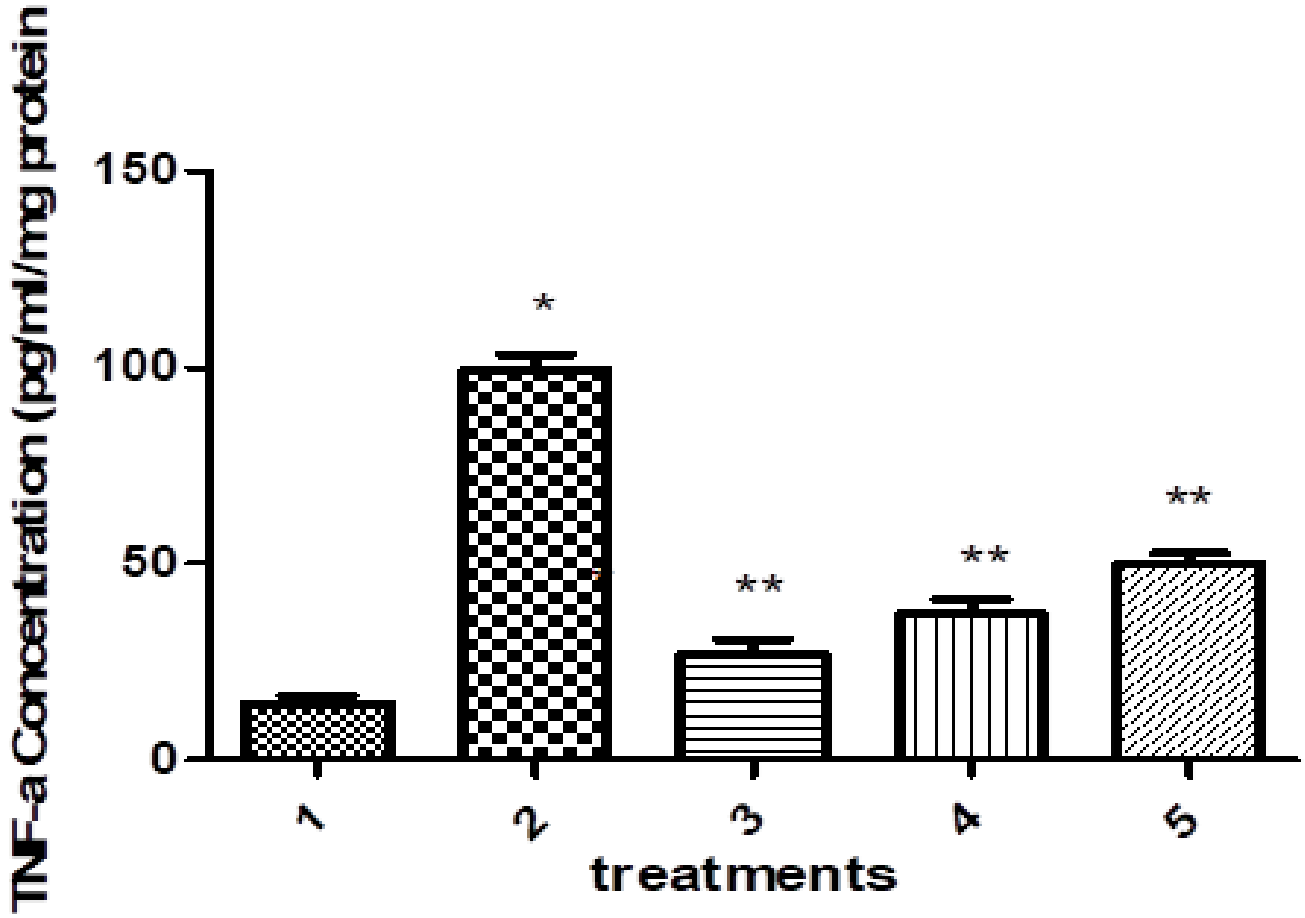

Figure 7. MECmS on TNF-α levels in indomethacin-induced stomach ulcers; all results are mean± SEM, p<0.05. * -significant as compared to group 1, ** significant compared to group 2.

[30, 31]. Despite its multiple benefits, Indomethacin produces upper gastrointestinal discomfort, which can lead to ulcers, preventing its use [32, 33].

The increase in the stomach relative weight of animals treated with indomethacin is most likely due to bleeding, edema, necrosis, and inflammation caused by indomethacin-induced damage to the stomach mucosa. Pretreatment with MECmS dramatically reduces stomach weight. The findings from this study revealed that the methanolic extract of *Cucumis melo* increases blood flow by increasing RBC, PCV, and haemoglobin levels (Table 1). Increased blood flow aids in the flow of nutrients and oxygen to the site of injury, improving hemodynamics is important in the healing process, and this helps to increase the rate of healing in the stomach mucosa [34]. The increased neutrophils, lymphocytes, and white blood cells upon induction with indomethacin is indicative of gastric tissue inflammation. This could have led to the recruitment of the body's cellular defense mechanism and infiltration of inflammatory cells at the site of injury as shown in Figure 10 [35]. The administration of MECmS decreased the WBC, lymphocytes, and neutrophils, especially the 100 mg/Kg, thereby significantly abating inflammation.The increased gastric acidity and ulcer score as shown in Figures 2 and 3 are abated by MECmS may be attributed to its ability to increase the proliferation of the mucosal glandular cells. The gastric mucosal glands play important roles in protecting the stomach lining as they secret mucus and bicarbonate, hydrochloric acid, intrinsic factor, pepsinogen, and various hormones which work together to break down food, absorb nutrients, and protect the stomach's lining [36]. In the pathophysiology of stomach ulceration, a rise in total gastric acidity has been identified as the primary cause of gastrointestinal damage. Gastric acidity is linked to increased H^+ concentration and low pH. It is reported that an imbalance in gastric acidity termed the "offensive and defensive" factors results in ulceration [37]. In this study, indomethacin increased the total gastric acidity probably through its ability to alter defensive factors such as blood flow (decreasing the RBC, HB, PCV), and mucosal

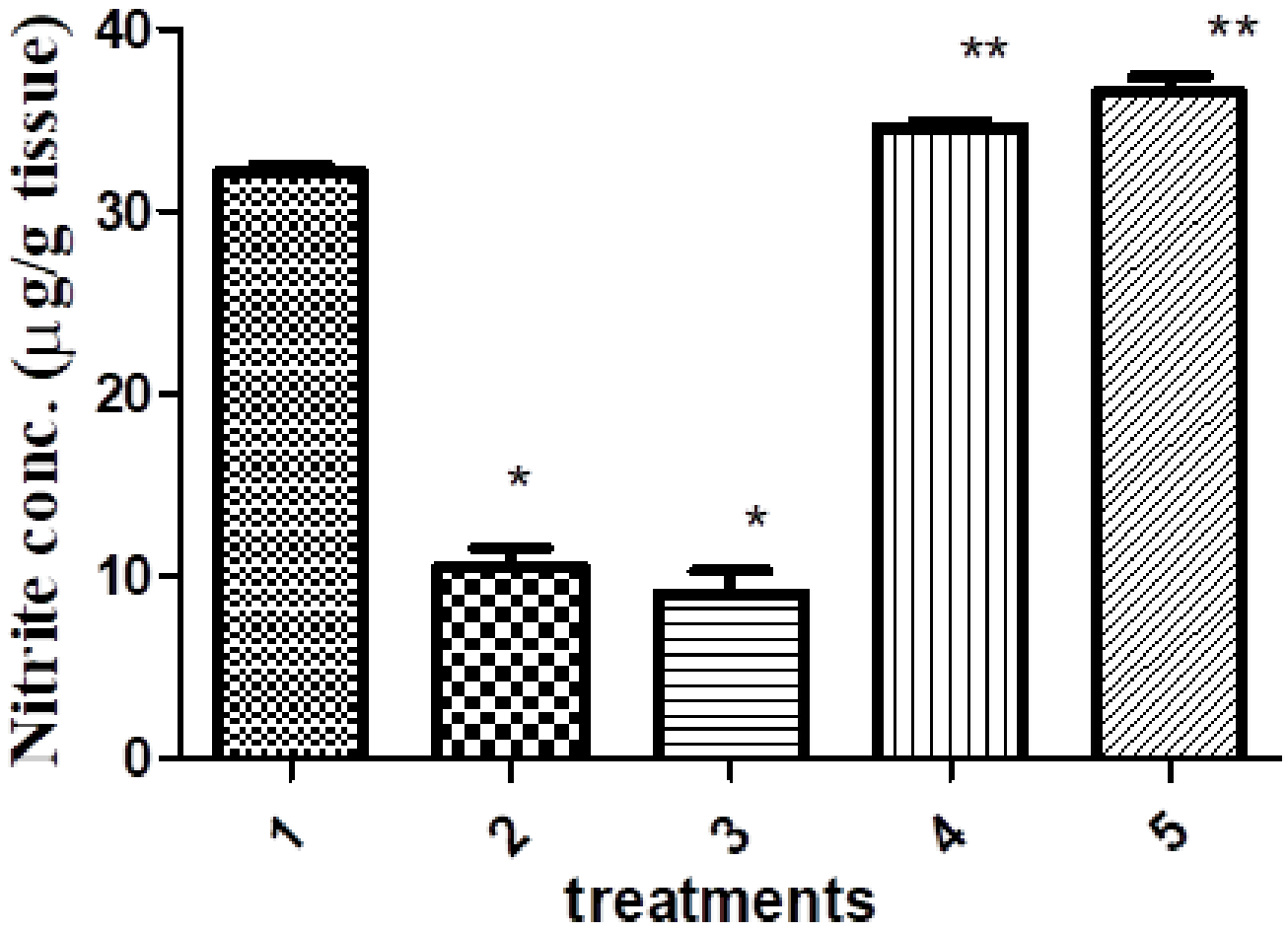

Figure 8. *Cucumis melo* methanolic extract on the nitrite level in stomach ulcers caused by indomethacin. Every value is given as mean SEM, $p<0.05$. * -significant as compared to group 1, ** significant compared to group 2.

glandular cells (responsible for mucosal bicarbonate secretion). This is in consonance with previous researches we conducted [3, 37]. Pretreatment with MECmS before gastric ulcerative induction using indomethacin, showed that it was able to reduce total gastric acidity by enhancing the proliferation of mucosal glandular cells and increasing blood flow (Figure 10 and Table 1).

Nitric oxide has a dual effect on the gastric mucosa by either enhancing the gastric mucosa defence or increasing reactive oxygen species which damages the gastric mucosa. In this study, nitrite level was used to quantify the nitric oxide level because of its instability. The nitrite level in indomethacin-induced group (group II) was significantly decreased, while it was enhanced by the administration of MECmS in all the treatment groups. It is suggested that increase in nitrite level may have led to an increased nitric oxide levels, which possibly conferred protection of the gastric mucosa by triggering blood flow in the gastrointestinal tract.

Increased MDA content in the stomach of indomethacin-ulcerated rats indicates increased lipid peroxidation and overproduction of free radicals, which causes mucosal injury. Indomethacin increased gastric oxidative stress, by increasing MDA, and decreasing antioxidant enzymatic activity (CAT) in the stomach, an important factor in the mechanism of indomethacin-induced toxicity [38]. These findings are in harmony with the reports by *Ajeigbe et al.* [39]. The administration of MECmS prevented lipid peroxidation by increasing antioxidant activities such as catalase and protein levels, which is indicative of its gastro-protective properties *Adebayo-Gege et al.* [3, 37] revealed that *Cucumis melo* contains phytochemicals such as terpenoids, cardiac glycosides, steroids, phenols, flavonoids, and alkaloids. Phenolic acids are said to be strong antioxidant and beneficial to general health, with a alkaloids, tannins and flavonoid contents that are active in mopping and chelating reactive oxygen species [3, 37]. These bioactive compounds present in *Cucumis melo* is suggestive of its strong antioxidant properties.

Indomethacin increased TNF-α level in group 2 compared to other groups, indicating cytokine invasion and stimulation of gastric

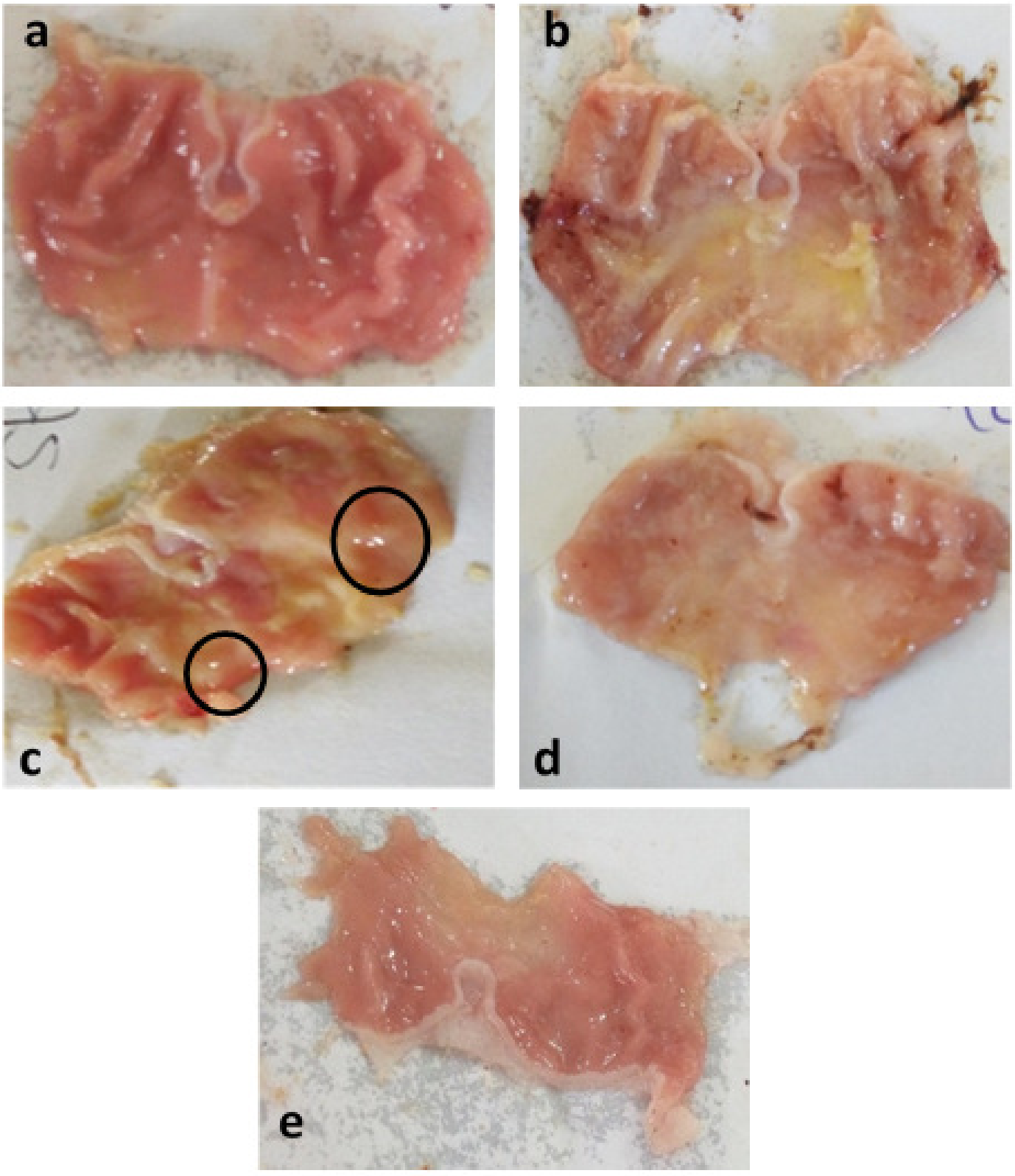

Figure 9. Gross morphology showing effect of MCmS on the stomach mucosa of the indomethacin-induced gastric ulceration.

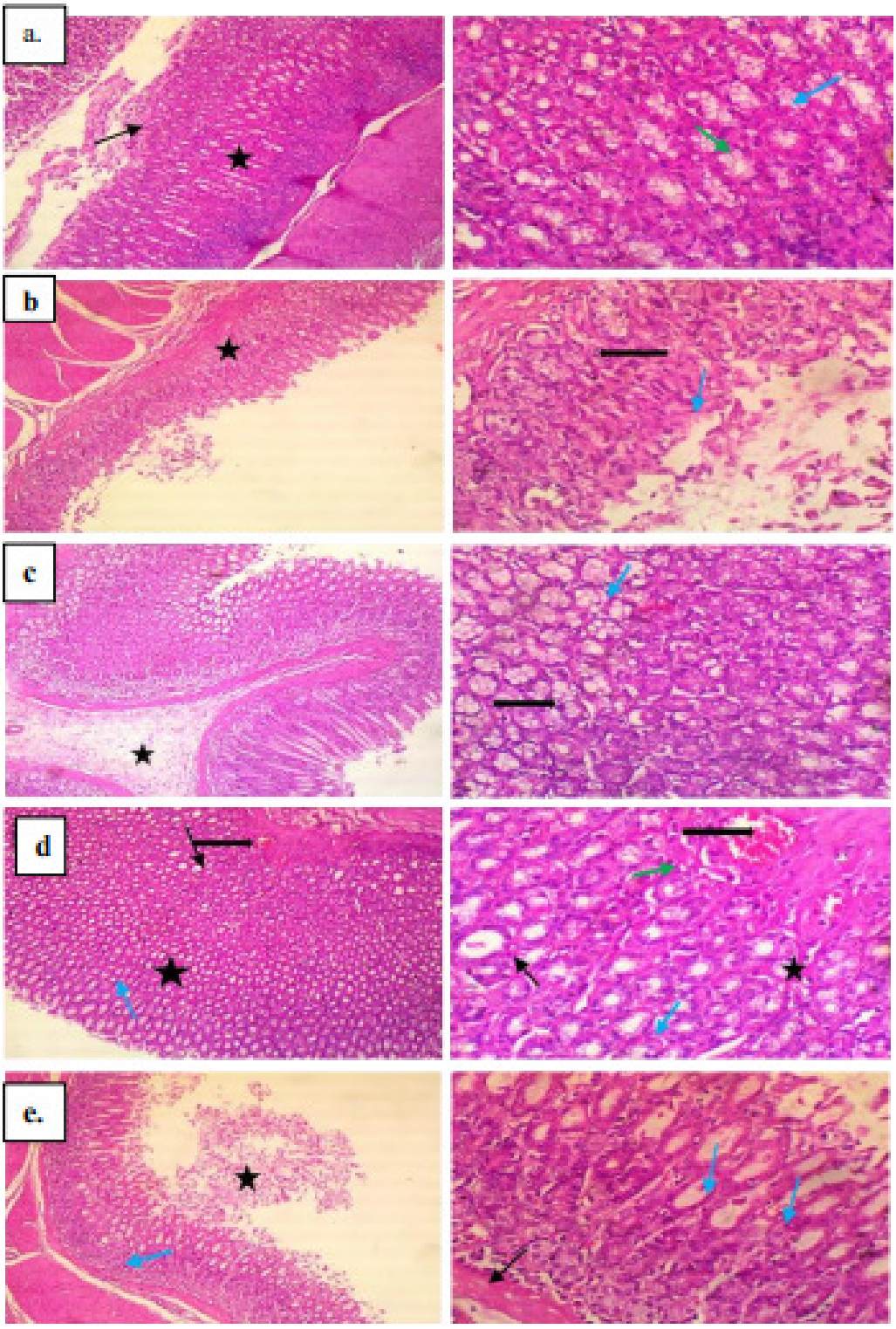

Figure 10. Gross morphology showing effect of MCmS on the stomach mucosa of the indomethacin-induced gastric ulceration.

mucosal inflammatory pathways, which is consistent with previous studies [40]. Treatment with MECmS inhibited indomethacin-stimulated gastric mucosal inflammation by decreasing the TNF-α level. This can also be attributed to anti-inflammatory phytochemicals in the seeds such as amentoflavone and Gallic acid [41, 42]. The gross morphology of the gastric mucosa of the groups pretreated with *Cucumis melo,* showed a decrease in the hemorrhage streaks and erosion compared to the indomethacin-induced group, which was confirmed by the histological examination. The histological analysis affirmed that indomethacin disrupts the gas-

tric mucosa by generating a locally broad focus of severe tunic mucosal necrosis (Figure 10), and the buildup of moderate numbers of inflammatory cells. However, treatment with MECmS promoted gland regeneration, reduction in inflammatory cells, and epithelial layer degradation. This was validated by the gross morphology results, which displayed significant healing of the ulceration in MECmS-treated groups.

6. Conclusion

Findings from this study show that methanolic extracts of *Cucumis melo* seeds possess antiulcer properties due to their ability to reduce total gastric acidity, pro-inflammatory cytokine (TNF-α), ulcer score, and lipid peroxidation. Furthermore, this suggested that *Cucumis melo* seeds have both anti-secretory and anti-inflammatory properties, which showcases it as a therapeutic agent that can be explored against gastric ulceration.

Data availability

The data underlying the findings of this study can be obtained upon request from the corresponding author.

References

[1] D. Y. Graham, "History of helicobacter pylori, duodenal ulcer, gastric ulcer and gastric cancer", World Journal of Gastroenterology **20** (2014) 5191. https://dx.doi.org/10.3748/wjg.v20.i18.5191.

[2] L. X. Jin, Y. P. Fang, C. M. Xia, T. W. Cai, Q. Q. Li, Y. Y. Wang, H. F. Yan & X. Chen, "Helicobacter pylori infection alters gastric microbiota structure and biological functions in patients with gastric ulcer or duodenal ulcer", World Journal of Gastroenterology **30** (2024) 3076. https://doi.org/10.3748/wjg.v30.i24.3076.

[3] G. I. Adebayo-Gege, Z. S. Uthman, M. D. Adams, F. Tarfa, U. H. Danazumi, M. A. Ngabea, J. L. Hamidu, Q. Ozegbe, C. Onwuchekwa, A. Meraiyebu, K. Ojo, "Molecular docking and anti-ulcerative potential of *Cucumis (L. Inodorous)* on ibuprofen induced gastric ulceration in male wistar animals", Biomedicine & Pharmacotherapy **161** (2023) 114531. https://doi.org/10.1016/j.biopha.2023.114531.

[4] G. I. Adebayo-Gege, B. J. Okoli, P. O. Oluwayinka, A. F. Ajayi, F. Mtunzi, "Antiulcer and cluster of differentiation-31 properties of cucumis melo l. on indomethacin-induced gastric ulceration in male wistar rats", in *Chemistry for a Clean and Healthy Planet*, P. Ramasami (eds.), Springer Nature, Switzerland, 2019, pp. 501–516. https://doi.org/10.1007/978-3-030-20283-5_29.

[5] O. C. Rogoveanu, C. T. Streba, C. C. Vere, L. Petrescu & R. Traistaru, "Superior digestive tract side effects after prolonged treatment with NSAIDs in patients with osteoarthritis", Journal of Medicine and Life **8** (2015) 458. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4656952/.

[6] F. Y. Zaman, S. G. Orchard, A. VHaydon, J. R. Zalcberg "Non-aspirin non-steroidal anti-inflammatory drugs in colorectal cancer: a review of clinical studies", British Journal of Cancer **127** (2022) 1735. https://doi.org/10.1038/s41416-022-01882-8.

[7] G. Rashid, N. A. Khan, D. Elsori, A. Rehman, A. Tanzeelah, H. Ahmad, H. Maryam, A. Rais, M. S. Usmani, A. M. Babker & M. A. Kamal, "Non-steroidal anti-inflammatory drugs and biomarkers: A new paradigm in colorectal cancer", Frontiers in Medicine **10** (2023) 1130710. https://doi.org/10.3389%2Ffmed.2023.1130710.

[8] T. Hamoya, G. Fujii, S. Miyamoto, M. Takahashi, Y. Totsuka, K. Wakabayashi, J. Toshima & M. Mutoh, "Effects of NSAIDs on the risk factors of colorectal cancer: a mini review", Genes and Environment **36** (2016) https://doi.org/10.1186/s41021-016-0033-0.

[9] P. M. Rothwell, M. Wilson, C. E. Elwin, B. Norrving, A. Algra, C. P. Warlow, T. W. Meade, "Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomized trials", Lancet **376** (2010) 1741. https://doi.org/10.1016/s0140-6736(10)61543-7.

[10] L. Melcarne, P. García-Iglesias & X. Calvet, "Management of NSAID-associated peptic ulcer disease", Expert Review of Gastroenterology and Hepatology **10** (2016) 723. https://doi.org/10.1586/17474124.2016.1142872.

[11] O. M. Ighodaro & O. A. Akinloye, "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid", Alexandria Journal of Medicine **54** (2018) 287. https://doi.org/10.1016/j.ajme.2017.09.001.

[12] A. I. Elshamy, A. R. H. Farrag, I. M. Ayoub, K. A. Mahdy, R. F. Taher, A. E. Gendy, T. A. Mohamed, S. S. Al-Rejaie, Y. A. EI-Amier, A. M. Abd-EIGawad & A.F.Mohammed, "UPLC-qTOF-MS phytochemical profile and antiulcer potential of *Cyperus conglomeratus* Rottb. alcoholic extract", Molecules **25** (2020) 4234. https://doi.org/10.3390/molecules25184234.

[13] M. Shahnaz, P. Kaur & D. N. Prasad, "Gastroprotective NSAIDs", Journal of Drug Delivery and therapeutics **8** (2018) 141. https://doi.org/10.22270/jddt.v8i5.1905.

[14] J. L. Wallace & M. J. Miller,"Nitric oxide in mucosal defense: a little goes a long way", Gastroenterology **119** (2000) 512. https://doi.org/10.1053/gast.2000.9304.

[15] C. H. Cho,"Current roles of nitric oxide in gastrointestinal disorders", Journal of Physiology **95** (2001) 253. https://doi.org/10.1016/s0928-4257(01)00034-1.

[16] T. K. Motawi, H. M. Abd Elgawad & N. N.Shahin, "Modulation of indomethacin-induced gastric injury by spermine and taurine in rats", Journal of Biochemical and Molecular Toxicology **21** (2007) 280. https://doi.org/10.1002/jbt.20194.

[17] S. M. Andrabi, N. S. Sharma, A. Karan, S. M. Shatil Shahriar, B. Cordon, B. Ma & J. Xie, "Nitric oxide: physiological functions, delivery, and biomedical applications", Advanced Science **10** (2023) 2303259. https://doi.org/10.1002/advs.202303259.

[18] S. Okabe & K. Amagase, "An overview of acetic acid ulcer models–the history and state of the art of peptic ulcer research", Biol Pharm Bull. **28** (2005) 1321. https://doi.org/10.1248/bpb.28.1321.

[19] C. Hawkins & G. W. Hanks, "The gastroduodenal toxicity of nonsteroidal anti-inflammatory drugs: A review of the literature", The Journal of Pain and Symptom Management **20** (2000) 140. https://doi.org/10.1016/s0885-3924(00)00175-5.

[20] A. O. Ayoka, R. O. Akomolafe, O. S. Akinsomisoye & O. E. Ukponmwan, "Medicinal and economic value of Spondiasmombin", African Journal of biomedical Research **11** (2008) 129. https://doi.org/10.4314/ajbr.v11i2.50714.

[21] M. A. Silva, T. G. Albuquerque, R. C. Alves, M. B. P. Oliveira & H. S. Costa, "Melon (Cucumis melo L.) by-products: Potential food ingredients for novel functional foods?", Trends in Food Science & Technology **98**, 181. https://doi.org/10.1016/j.tifs.2018.07.005.

[22] S. K. Prasad & M. K. Singh, "Horse gram- an underutilized nutraceutical pulse crop: a review", J Food Sci Technol. **52** (2015) 2489. https://doi.org/10.1007/s13197-014-1312-z.

[23] N. Jorge, A. C. da Silva & C. M. Veronezi, "Antioxidant and pharmacological activity of Cucumis melo var. cantaloupe", *Multiple Biological Activities of Unconventional Seed Oils*, Abdalbasit Adam Mariod (Eds.), Academic Press, 2022, pp. 147–170. https://doi.org/10.1016/B978-0-12-824135-6.00001-5.
[24] G. I. Adebayo- Gege, A. T. Salami, A. O. Odukanmi, I. O. Omotosho & S. B. Olaleye, "Pro-ulcerogenic activity of sodium arsenite in the gastric mucosa of male wistar rats", Journal of African Association of Physiological Scences **6** (2018) 95. https://www.ajol.info/index.php/jaaps/article/view/181995.
[25] M. B. Adinortey, C. Ansah, N. A. Galyuonm & Nyarko, "In vivo models used for evaluation of potential antigastroduodenal ulcer agents", Hindawi Publishing Corporation Ulcers **2013** (2013) 12. http://dx.doi.org/10.1155/2013/796405.
[26] R. Varshney & R. K. Kale, "Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsomes", International Journal of Radiation Biology **58** (1990) 733. https://doi.org/10.1080/09553009014552121.
[27] A. C. Gornall, C. J. Bardawill & M. M. David, "Determination of serum protein by means of Biuret reaction", Journal of Biological Chemistry **177** (1949) 751. https://doi.org/10.1016/S0021-9258(18)57021-6.
[28] L. J. Ignarro, G. M. Buga, K. S.Wood, R. E. Byrns & G. Chaudhuri, "Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide", Proc Natl Acad Sci U S A. **84** (1987) 9265. https://doi.org/10.1073/pnas.84.24.9265.
[29] G. M. Pacifici, "Clinical pharmacology of indomethacin in preterm infants: implications in patent ductus arteriosus closure", Pediatric Drugs **15** (2013) 363. https://doi.org/10.1007/s40272-013-0031-7.
[30] M. A. Hull, S. H. Gardner & G. Hawcroft, "Activity of the non-steroidal anti-inflammatory drug indomethacin against colorectal cancer", Cancer Treatment Reviews **29** (2003) 309. https://doi.org/10.1016/s0305-7372(03)00014-8.
[31] T. M. Okda, S. K. Abd-Elghaffar, M. A. Katary & M. M Abd-Alhaseeb, "Chemopreventive and anticancer activities of indomethacin and vitamin D combination on colorectal cancer induced by 1,2 dimethylhydrazine", Biomedical Reports **14** (2021) 27. https://doi.org/10.3892/br.2020.1403.
[32] M. A. Morsy & M. A. El-Moselhy, "Mechanisms of the protective effects of curcumin against indomethacin-induced gastric ulcer in rats", Pharmacology **91** (2013) 267. https://doi.org/10.1159/000350190.
[33] M. A. El-Moselhy, N. M. Abdel-Hamid & S. Abdel-Raheim, "Gastro-protective effect of nicorandil in indomethacin and alcohol-induced acute ulcers", Applied Biochemical and Biotechnology **152** (2009) 449. https://doi.org/10.1007/s12010-008-8384-z.
[34] B. Danisman, B. Cicek, S. Yildirim, I Bolat, D. Kantar, K. S. Golokhvast, D. Nikitovic, A.Tsatsakis & A. Taghizadehghalehjoughi, "Carnosic acid ameliorates indomethacin-induced gastric ulceration in rats by alleviating oxidative stress and inflammation", Biomedicines **11** (2023) 829. https://doi.org/10.3390/biomedicines11030829.
[35] H. Yandrapu & J. Sarosiek, "Protective factors of the gastric and duodenal mucosa: an overview", Current gastroenterology reports **17** (2015) 1. https://doi.org/10.1007/s11894-015-0452-2.
[36] M. Magierowski, K. Magierowska, S. Kwiecien & T.Brzozowski, "Gaseous mediators nitric oxide and hydrogen sulfide in the mechanism of gastrointestinal integrity, protection and ulcerhealing", Molecules **20** (2015) 9099. https://doi.org/10.3390/molecules20059099.
[37] G. Adebayo-Gege, V. Alicha, T. O. Omayone, S. C. Nzekwe, C. A. Irozuoke, O. A. Ojo & A. F. Ajayi, "Anti-atherogenic and cardio-protective properties of Cucumis melo (Cucumis melo. L. Inodorus) seed extract on high fat diet induced obesity in male wistar rats", BMC Complementary Medicine and Therapies **22** (2022) 334. https://doi.org/10.1186/s12906-022-03793-w.
[38] G. I. Adebayo-Gege, D. A. Adetomiwa, T. P. Omayone, C. O. Akintayo, Q. B. Ozegbe, D. I. Yusuff & O. A. Ojo, "Anti-Ulcer potentials of aqueous extract of triticum aestivum on delayed healing of experimentally induced/gastric ulcer", Nigerian Journal of Experimental and Clinical Biosciences **10** (2022) 90. http://dx.doi.org/10.4103/njecp.njecp_13_22.
[39] K. Ajeigbe, K. Aibangbee, S. Saeed & O. Ajeigbe, A. Onifade, "Folic acid protects and heals gastric mucosa: role of acid output, inflammatory cytokines, angiogenic and growth factors", The Journal of Basic and Applied Zoology **83** (2022) 15. https://doi.org/10.1186/s41936-022-00280-z.
[40] S. Sabiu, T. Garuba, T. Sunmonu, E. Ajani, A. Sulyman, I.Nurain & A. Balogun, "Indomethacin-induced gastric ulceration in rats: protective roles of spondias mombin and ficus exasperate", Toxicology Reports **2** (2015) 261. https://doi.org/10.1016/j.toxrep.2015.01.002.
[41] I. O. Olubunmi, J. A. Bamidele & F. Omolara, "Phytochemical composition and in vitro antioxidant activity of golden melon (Cucumis melo L.) seeds for functional food application", International Journal of Biochemistry Research & Review **25** (2019) 1. https://doi.org/10.9734/ijbcrr/2019/v25i230070.
[42] A. M. Abbas & H. F. Sakr, "Effect of selenium and grape seed extract on indomethacin-induced gastric ulcers in rats", Journal of Physiology and Biochemistry **69** (2013) 527. https://doi.org/10.1007/s13105-013-0241-z.