Accepted manuscript

The protective effects of Gamma-linolenic acid against indomethacin-induced gastric ulcer in rats

Kaveh Rahimi[1*], Masoumeh Ezzati Givi[1], Anahita Rezaie[2], Mohammad Hekmatmanesh[1], Ysamin Shaker Ardakani[1]

[1]Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

***Corresponding author:** Kaveh Rahimi, Assistant Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran. Tell: 98613330073 Fax: 98613360807 E-mail: k.rahimi@scu.ac.ir. Kaveh_rahimi66a@yahoo.com ORCID ID: 0000-0002-8674-3993

Running title: Gamma-linolenic acid and gastric ulcer

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114524000382
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

Abstract

The primary goal of the investigation was to analyze the anti-inflammatory, and antioxidant properties of Gamma-linolenic acid (GLA) on rats with Indomethacin (IND)-induced gastric ulcers. Thirty rats were divided into five groups: Control, IND (50 mg/kg, p.o.), IND pretreated with GLA 100 mg/kg (p.o. for 14 days), IND pretreated with GLA 150 mg/kg (p.o. for 14 days), and IND pretreated with omeprazole (20 mg/kg, p.o. for 14 days). The stomach tissues were examined to calculate the ulcer index, and pH, and analyze biochemical markers (PGE2, COX1, TNF-1, IL-6, and ICAM1), and oxidative stress parameters (MDA, SOD, GSH, and CAT) as well as undergo histopathological assessment. GLA 100 and 150 mg/kg showed a protective effect against IND-induced gastric damage. It reduced levels of COX1, TNF-1, IL-6, and ICAM and increased PGE2 levels. GLA also normalized antioxidant function by modulating MDA, SOD, GSH, and CAT. GLA intervention protects against IND-induced Gastric ulcers (GU) by restoring oxidant/antioxidant balance and reducing inflammation.

Keywords: Gamma-linolenic acid, indomethacin, gastric ulcer, rat.

Abbreviations:

Gamma-linolenic acid **GLA**

Indomethacin **IND**

Prostaglandin E2 **PGE2**

Cyclooxygenase 1 **COX-1**

Tumour necrosis factor α **TNFα**

Interleukin 6 **IL-6**

Intercellular adhesion molecule-1 **ICAM-1**

Malondialdehyde **MDA**

Superoxide dismutase **SOD**

Glutathione **GSH**

Catalase **CAT**

1.Introduction

Gastric ulcers (GU) are a prevalent digestive disorder that affects individuals globally [1]. Psychological stress, Helicobacter pylori infection, alcohol consumption, tobacco smoking, and nonsteroidal anti-inflammatory drugs (NSAID) are all contributing factors to GU formation. NSAIDs alone contribute to 25% of incidence [2]. Indomethacin (IND) mainly treats inflammatory diseases such as rheumatoid arthritis, osteoarthritis, and tendonitis. IND is also widely used for experimental induction of GU due to its higher ulcerogenic potency in comparison to other NSAIDs [3, 4]. Several mechanisms are proposed to explain the harmful effects of IND on the stomach. These include inhibition of the cyclooxygenase enzyme, reduced synthesis of mucus and bicarbonate, impaired release of the gastroprotective prostaglandin E2, enhancement of acid production, and oxidative stress [5]. Recent studies have found a correlation between autophagy and apoptosis with mucosal erosions and ulcerations caused by IND [6].

Certain natural products possess antioxidant and anti-inflammatory properties that may protect against gastrointestinal inflammation [7-9]. Gamma-linolenic acid (GLA), also known as cis 6, cis 9, cis 12-octadecatrienoic acid, is a type of fatty acid belonging to the n-6 family. Although the body can produce it from the essential fatty acid linoleic acid, it is conditionally essential. Only trace amounts of GLA can be found in many plants, and it is not typically present in most commercial vegetable seed oils. However, certain species in families such as Saxifragaceae, Aceraceae, Boraginaceae, Cannabinaceae, Onagraceae, Liliaceae, Ranunculaceae, and Scrophulariaceae contain GLA. Few plant sources have been used for commercial GLA production, mainly in health food, pharmaceutical, pet food, and cosmetic industries. These sources include oils from borage, evening primrose, black currant, and more recently, hemp [10]. The GLA treatment effectively inhibits the growth of gastric cancer cells in hypoxia. It achieves this by reducing cell viability and colony formation while increasing apoptosis. Additionally, GLA treatment regulates the expression of epithelial and stromal marker proteins and inhibits both cell migration and invasion. Furthermore, it reduces the expression of b-catenin in the Wnt/b-catenin pathway [11]. A study compared GLA and linoleic acid effects on aspirin-induced gastric bleeding in rats. The mice that were fed with a GLA-enriched diet did not experience bleeding, whereas the mice that were fed with a linoleic acid-enriched diet did. This study suggests that GLA may protect the gastric mucosa from aspirin-induced damage by bypassing

the decrease in delta-6 saturation and providing precursors for the synthesis of arachidonic acid and prostaglandins [12].

Although GLA has been shown to have beneficial effects on the gastrointestinal tract, there is still incomplete information regarding its protective effect on gastric ulcers. Our objective is to investigate the impact of GLA on gastric ulcers caused by it and to identify its potential mechanisms.

2.Methods

2.1. Animals

Male Wistar rats (250-260 g) were obtained and allowed one week for adaptation. The rats were kept under a 12-hour light/dark cycle with a temperature of 22 ± 2°C. They were fed standard chow pellets and had unlimited access to water. The study was conducted following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and ARRIVE guidelines for reporting in-vivo experiments [13]. The experimental protocol was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran (Protocol No. EE/1401.2.24226203/scu.ac.ir).

2.2. Study design

Thirty rats were randomly divided into five groups, with each group comprising six rats. The control and IND groups were administered sunflower oil through oral gavage for 14 consecutive days. The rats in GLA 100 and 150 mg/kg groups were orally administered GLA (100 or 150 mg/kg) (12) dissolved in sunflower oil (15% saturated, 85% unsaturated fatty acid and consisting of 14–43% oleic and 44–75% linoleic acids [14]) for 14 successive days. The rats in the omeprazole 20 mg/kg group were orally administered omeprazole 20 [15] for 14 successive days. On day 14 of the experiment, all rats except those in the control group were given a single oral dose of IND (50 mg/kg) [16]. The rats were not allowed to eat but had access to water for 24 hours before receiving the IND. Rats were anesthetized with thiopental sodium (50 mg/kg, i.p.) and euthanized by decapitation 4 hours after IND administration. The stomachs from each group were then isolated. GLA was obtained from evening primrose (*Oenothera biennis* L.) oil manufactured by Barij Essence Pharmaceutical Company in Iran.

2.3. Assessment of gastric pH

The gastric pylorus was tied off and a section of the stomach was removed through the greater curvature. The stomach contents were collected immediately and transferred to a centrifuge tube. The tube was then centrifuged for 5 minutes at 2500× g to remove any debris before measuring the amount of supernatants. The pH of the gastric juice was measured using a digital pH meter.

2.4. Assessment of ulcer index and inhibition index

After removing the stomachs, they were sliced along the greater curvature and rinsed with 5 mL of normal saline. A blinded evaluation of gross lesions was performed. The mean number of ulcers per stomach for each rat was calculated using an ulcer scoring system for each group. 0 means no ulcer, 1 means pinpoint ulcers and superficial mucosal changes, 2 means ulcers smaller than 1mm, 3 means ulcers between 1mm and 2mm, and 4 means ulcers larger than 2mm or a perforated ulcer.

[(UIcontrol -UItreated)/UIcontrol]×100, where UI represents ulcer index.

2.5. Histopathological examination

Stomachs were fixed in 10% formaldehyde solution then dehydrated, embed them in paraffin, and finally, cut them into 4 μm sections using a microtome. These sections were then stained with hematoxylin and eosin (H&E) for histopathological examination.

2.6. Biochemical measurements

Enzyme-linked immunosorbent assay (ELISA) methods were utilized to determine various biochemical and antioxidant parameters in the gastric tissues. The parameters included Prostaglandin E2 (PGE2), Cyclooxygenase 1 (COX-1), Tumour necrosis factor α (TNFα), Interleukin 6 (IL-6), Intercellular adhesion molecule-1 (ICAM-1), malondialdehyde (MDA), as well as antioxidant parameters such as superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). All samples were tested for total protein content by the Bradford method. The test kits used for PGE2, COX-1, and ICAM-1 were purchased from MyBioSource in the USA, while those for TNF-α, IL-6, MDA, SOD, GSH, and CAT were obtained from Kiazist in Hamedan, Iran.

2.7. Statistical analysis

We used SPSS software version 26 to analyze the data. First, we conducted a normalization test on the data to ensure that it was normally distributed and had homogeneity of variances, using the Kolmogorov-Smirnov test on SPSS. As the data passed the normality test, we then performed a one-way analysis of variance (ANOVA) to compare the groups. We conducted post-hoc analyses utilizing Tukey tests. Ulcer index data were analyzed by the Kruskal–Wallis test. We considered a significance level of $p<0.05$.

3.Results

3.1.Effect of GLA pre-treatment on macroscopic inspection of gastric mucosa

According to the results of this study, it was found that the groups treated with IND, GLA 100 mg/kg, and GLA 150 mg/kg had a significantly higher average ulcer score compared to the control group ($p<0.001$, $p<0.05$, and $p<0.05$, respectively). However, there was no significant difference in the mean ulcer score between the omeprazole group and the control group. Conversely, the groups treated with GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole had a significantly lower average ulcer score compared to the IND group ($p<0.01$). This can be seen in Figures 2 A and D.

The study found that the control group had a significantly higher percentage of ulcer inhibition than the groups given IND, GLA 100 mg/kg, GLA 150 mg/kg, and omeprazole 20 mg/kg ($p<0.001$, $p<0.05$, $p<0.05$, and $p<0.05$, respectively). On the other hand, the groups given GLA 100 mg/kg, GLA 150 mg/kg, and omeprazole 20 mg/kg had a significantly higher percentage of ulcer inhibition compared to the IND group ($p<0.001$) (Figures 2 B and d).

3.2.Effect of GLA pre-treatment on gastric pH

The study revealed that groups treated with IND and GLA 100 mg/kg had a lower pH level of gastric juice compared to the control group ($p<0.001$ and $p<0.01$, respectively). However, there was no significant difference in the pH level of gastric juice between the GLA 150 mg/kg and omeprazole 20 mg/kg groups and the control group. On the other hand, the groups treated with GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole had a higher gastric juice pH level

than the IND group ($p<0.01$, $p<0.01$ and $p<0.001$, respectively). Figure 2 C depicts this information.

3.3.Effect of GLA on histopathological assessment

Microscopic examination of the stomach sections in the control sunflower group showed the absence of extensive changes in the mucosal surface. In most cases, cylindrical mucous cells with clear cytoplasm were visible. In some cases, hyperemia was also seen in the parenic capillaries. In the microscopic examination of stomach sections in the group receiving indomethacin, multiple, wide and deep wounds were observed on the mucosal surface. In these foci, gastric mucous cells, main and borderline, were necrotic, which were characterized by more colorful cytoplasm and compact and dark-colored nuclei. Also, in some parts, the mucous cells were removed. This tearing continued to the deep parts of the stomach pits as well (Figure 2E).

In the GLA50 treatment groups, wounds were still seen on the surface of the mucosa. In these foci, similar to the previous group, necrosis of mucous cells, main and border cells were seen. In the GLA100 treatment group, a large part of the mucosa was healthy the mucosal tissue had complete integrity, and only small foci of mucosal cell necrosis were seen. In the group receiving indomethacin and omeprazole, as in the previous group, a wide range of gastric mucosa was healthy, and small foci of surface necrosis were observed (Figure 2E).

3.4.Effect of GLA pre-treatment on the levels of gastric PGE2, COX-1, TNF-a, IL-6, and ICAM-1 Content

The PGE2 levels were significantly higher in the groups that received GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole compared to the IND group. The p-values for this difference were also less than 0.001. Conversely, the groups that received IND, GLA 100 mg/kg, and GLA 150 mg/kg, showed significantly lower levels of PGE2 than the control group ($p<0.001$, $p<0.05$, and $p<0.05$, respectively). These findings are presented in Figure 3A.

The groups given GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole had significantly higher COX-1 levels compared to the group given IND ($p<0.05$, $p<0.01$, and $p<0.05$, respectively). However, the groups given IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole showed significantly lower levels of COX-1 than the control group ($P<0.001$) (Figure 3 B).

The TNF-α levels in the GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole groups are significantly decreased compared to the IND group ($p<0.001$). Additionally, the levels of TNF-α are significantly higher in the groups given IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole, compared to the control group ($p<0.001$), as shown in Figure 3 C.

The IL-6 levels in the GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole groups are significantly decreased compared to the IND group ($p<0.001$). Furthermore, the groups given IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole have significantly higher levels of IL-6 compared to the control group ($p<0.001$, $p<0.001$, $p<0.01$, and $p<0.001$, respectively), as shown in Figure 3 D.

The ICAM-1 levels in the groups that received GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole were significantly decreased compared to the IND group ($p<0.05$, $p<0.01$, and $p<0.01$, respectively). The group that received IND showed significantly higher levels of ICAM-1 compared to the control group ($p<0.001$). However, there was no significant difference in the levels of ICAM-1 between the groups that received GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole and the control group (Figure 3 E).

3.5.Effect of GLA pre-treatment on the oxidative stress markers

The study found that IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole increased MDA levels compared to the control group ($p<0.001$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively). However, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg omeprazole reduced MDA levels compared to IND ($p<0.01$) (Fig 4 A).

The results showed that the groups treated with IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole experienced a significant decrease in SOD levels compared to the control group ($p<0.001$). However, SOD levels in the groups treated with GLA 100 mg/kg, GLA 150 mg/kg, and omeprazole 20 mg/kg were found to be significantly higher than the IND group ($p<0.001$) (Fig 4 B).

The groups that received IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole demonstrated a significant decline in GSH levels when compared to the control group ($p<0.001$). However, the groups that were treated with GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of

omeprazole had significantly higher GSH levels than the IND group ($p<0.05$, $p<0.01$, and $p<0.01$, respectively), as depicted in Fig 4 C.

According to the findings, the groups that received IND, GLA 100 mg/kg, GLA 150 mg/kg, and 20 mg/kg of omeprazole had notably lower CAT levels compared to the control group ($p<0.001$). However, the CAT levels in the groups that were administered GLA 100 mg/kg, GLA 150 mg/kg, and omeprazole 20 mg/kg were significantly higher than the IND group ($p<0.001$) (as shown in Fig. 4 D).

4.Discussion

Polyunsaturated fatty acids (PUFAs) such as α-linolenic acid (ALA, 18:3n-3) and γ-linolenic acid (GLA, 18:3n-6) are beneficial for human health [17]. In some cases, GLA had a better effect, while in others, GLA and ALA had similar effects [17, 18]. In a related study, researchers suggest that GLA treatment may protect the gastric mucosa from aspirin-induced damage by providing a precursor for the synthesis of arachidonic acid (AA) and prostaglandins, bypassing the decrease in delta-6 desaturation [12]. Supplementing with GLA, EPA, and DHA has been shown to effectively increase DGLA, AA, EPA, and DHA levels in equine plasma and RBCs, which may prevent or resolve severe stomach ulcers [19].

After being produced in the body, GLA rapidly elongates into DGLA which can be converted into either anti-inflammatory (1-series prostaglandins) or pro-inflammatory products (2-series prostaglandins and 4-series leukotrienes) [20, 21]. When there is limited activity of Δ5-desaturase, DGLA is changed to AA. However, when dietary GLA supplementation occurs, DGLA can accumulate instead of ARA in many cell types. DGLA has two pathways of metabolism - the cyclooxygenase (COX-1 and COX-2) pathway and the 15-lipoxygenase (LOX) pathway. In the COX pathway, DGLA turns into 1-series prostaglandins (PGs), especially PGE1. While in the LOX pathway, DGLA gets converted to 15-(S)-hydroxy-8,11,13-eicosatrienoic acid (15-HETrE). Both metabolites have been shown to have several benefits, such as reducing inflammation, promoting vasodilation, preventing smooth muscle cell proliferation, decreasing blood pressure, and having anti-cancer effects [20]. In our study, pretreatment with GLA increased both COX-1 and PGE-2 in the IND-induced gastric ulcer model, while IND decreased them. According to Henry and Robertson (1993), among NSAIDs, IND is the most ulcerogenic to humans [22]. It has been observed to induce a range of pro-inflammatory mediators and inhibit the

gastroprotective COX-1 and angiogenesis in gastric tissue [23]. Additionally, NSAIDs contribute to gastric mucosal damage by causing oxidative stress and generating reactive oxygen species (ROS) [24]. Blocking COX enzymes also inhibits the protective effects of PGE2, which include increased mucus and bicarbonate secretion, as well as an increase in gastric blood flow [25].

IND can cause oxidative stress which leads to inflammation and the production of pro-inflammatory mediators such as IL-6 and TNF-α [26]. The oxidative stress brought about by IND may also cause mitochondrial respiration uncoupling, resulting in the production of pro-inflammatory cytokines like TNF-α and IL-6. These cytokines can promote the expression of adhesion molecules such as ICAM-1 which play a significant role in the development and advancement of injury and inflammation in the gastric tissue [27]. ICAM-1 is responsible for the adhesion of leukocytes and endothelial cells after an injury [28]. In this study, pre-treatment with GLA significantly reduced the levels of IL-6, TNF-α, and ICAM-1 in rats exposed to IND, effectively mitigating inflammation. These findings align with previous research which demonstrated that GLA can decrease the production of inflammatory cytokines [29].

The present study found that IND can induce oxidative stress, as shown by decreased GSH content and SOD and CAT activity in gastric tissue and a significant increase in MDA concentration. These results are consistent with previous studies, which suggest that IND is involved in the production of ROS and associated gastric mucosal apoptosis [30-33]. It's interesting to note that gastric ulcers occur due to a high concentration of ROS, including hydroxyl radicals, hydrogen peroxide, and superoxide anions. These ROS cause oxidative stress in the gastric tissue, which leads to gastric bleeding and ulcer development, according to Repetto and Llesuy 2002 [9]. However, intracellular antioxidant enzymes like catalase can counteract the harmful effects of ROS. Also, GSH can prevent tissue damage by neutralizing ROS. Oxidative stress can increase lipid peroxidation and produce MDA, which is commonly used as a marker for lipid peroxidation [30, 34]. In our study, GLA increased the levels of SOD, GSH, and catalase and decreased MDA in gastric ulcers induced by IND compared to the model group.

5.Conclusion

The administration of GLA at doses of 100 and 150 mg/kg was found to decrease oxidative stress markers. Additionally, the gastroprotective effect of GLA at these doses could be attributed to the reduction of TNF-1, IL-6, and ICAM levels, as well as the increase of PGE2 and COX1 levels. It seems that GLA strengthens the defense barrier against indomethacin damage by boosting antioxidant levels, reducing oxidant reduction, and decreasing inflammatory factors.

Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

None

Authorship

K.R conceptualized and designed the study. K.R, M.E.G, A.R, M.H, and Y.SH.A analyzed and interpreted the data; K.R supervised the project; all authors have approved the final version of the manuscript.

Acknowledgment

We are grateful to the Research Council of Shahid Chamran University of Ahvaz (GN: SCU.VB1402.50857).

References

1. Ford AC, Gurusamy KS, Delaney B, et al.. Eradication therapy for peptic ulcer disease in Helicobacter pylori-positive people. The Cochrane database of systematic reviews. 2016;4(4):Cd003840.

2. Adhikary B, Yadav SK, Roy K, et al.. Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action. Evidence-based complementary and alternative medicine : eCAM. 2011;2011.

3. Lucas S. The Pharmacology of Indomethacin. Headache. 2016;56(2):436-46.

4. Suleyman H, Albayrak A, Bilici M, et al.. Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers. Inflammation. 2010;33(4):224-34.

5. Takeuchi K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. World journal of gastroenterology. 2012;18(18):2147-60.

6. Gebril SM, Ito Y, Shibata MA, et al. Indomethacin can induce cell death in rat gastric parietal cells through alteration of some apoptosis- and autophagy-associated molecules. International journal of experimental pathology. 2020;101(6):230-47.

7. Hassan TKM, Kamal AM, Nassar MM, et al.. Phenolic contents of Gleditsia triacanthos leaves and evaluation of its anti-inflammatory, analgesic, hepatoprotective and antimicrobial activities. Life Science Journal. 2013;10:3445-66.

8. El Morsy EM, Ahmed MAE. Carvedilol attenuates l-arginine induced acute pancreatitis in rats through modulation of oxidative stress and inflammatory mediators. Chemico-biological interactions. 2020;327:109181.

9. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 2002;35(5):523-34.

10. Kapoor R, Nair H. Gamma Linolenic Acid: Sources and Functions. Bailey's Industrial Oil and Fat Products. p. 1-45.

11. Wang Y, Shi J, Gong L. Gamma linolenic acid suppresses hypoxia-induced gastric cancer cell growth and epithelial-mesenchymal transition by inhibiting the Wnt/b-catenin signaling pathway. Folia histochemica et cytobiologica. 2020;58(2):117-26.

12. Huang YS, Drummond R, Horrobin DF. Protective effect of gamma-linolenic acid on aspirin-induced gastric hemorrhage in rats. Digestion. 1987;36(1):36-41.

13. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS biology. 2020;18(7):e3000410.

14. Flagella Z, Rotunno T, Tarantino E, et al.. Changes in seed yield and oil fatty acid composition of high oleic sunflower (Helianthus annuus L.) hybrids in relation to the sowing date and the water regime. European journal of agronomy. 2002;17(3):221-30.

15. Rahimi K, Shirvani N, Sanaie P, et al.. The effects of alpha-pinene on the Nrf2-HO1 signaling pathway in gastric damage in rats. Molecular biology reports. 2023;50(10):8615-22.

16. Shaik RA, Eid BG. Piceatannol Affects Gastric Ulcers Induced by Indomethacin: Association of Antioxidant, Anti-Inflammatory, and Angiogenesis Mechanisms in Rats. Life (Basel, Switzerland). 2022;12(3).

17. González-Fernández MJ, Ortea I, Guil-Guerrero JL. α-Linolenic and γ-linolenic acids exercise differential antitumor effects on HT-29 human colorectal cancer cells. Toxicology research. 2020;9(4):474-83.

18. Yadav S, Tiwari V, Singh M, et al. Comparative efficacy of alpha-linolenic acid and gamma-linolenic acid to attenuate valproic acid-induced autism-like features. Journal of Physiology and Biochemistry. 2017;73(2):187-98.

19. Pagan JD, Hauss AA, Pagan EC, et al.. Long-chain polyunsaturated fatty acid supplementation increases levels in red blood cells and reduces the prevalence and severity of squamous gastric ulcers in exercised Thoroughbreds. Journal of the American Veterinary Medical Association. 2022;260(S3):S121-S8.

20. Mustonen AM, Nieminen P. Dihomo-γ-Linolenic Acid (20:3n-6)-Metabolism, Derivatives, and Potential Significance in Chronic Inflammation. International journal of molecular sciences. 2023;24(3).

21. Fan YY, Chapkin RS. Importance of dietary gamma-linolenic acid in human health and nutrition. The Journal of nutrition. 1998;128(9):1411-4.

22. Henry D, Robertson J. Nonsteroidal anti-inflammatory drugs and peptic ulcer hospitalization rates in New South Wales. Gastroenterology. 1993;104(4):1083-91.

23. Yadav SK, Adhikary B, Chand S, et al.. Molecular mechanism of indomethacin-induced gastropathy. Free radical biology & medicine. 2012;52(7):1175-87.

24. Utsumi H, Yasukawa K, Soeda T, et al. Noninvasive mapping of reactive oxygen species by in vivo electron spin resonance spectroscopy in indomethacin-induced gastric ulcers in rats. The Journal of pharmacology and experimental therapeutics. 2006;317(1):228-35.

25. Asako H, Kubes P, Wallace J, et al.. Indomethacin-induced leukocyte adhesion in mesenteric venules: role of lipoxygenase products. The American journal of physiology. 1992;262(5 Pt 1):G903-8.

26. Rahman I. Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. Biochemical pharmacology. 2002;64(5-6):935-42.

27. Bindu S, Mazumder S, Dey S, et al. Nonsteroidal anti-inflammatory drug induces proinflammatory damage in gastric mucosa through NF-κB activation and neutrophil infiltration: anti-inflammatory role of heme oxygenase-1 against nonsteroidal anti-inflammatory drug. Free radical biology & medicine. 2013;65:456-67.

28. Bella J, Kolatkar PR, Marlor CW, et al.. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(8):4140-5.

29. Chang CS, Sun HL, Lii CK, et al.. Gamma-linolenic acid inhibits inflammatory responses by regulating NF-kappaB and AP-1 activation in lipopolysaccharide-induced RAW 264.7 macrophages. Inflammation. 2010;33(1):46-57.

30. Dursun H, Bilici M, Albayrak F, et al. Antiulcer activity of fluvoxamine in rats and its effect on oxidant and antioxidant parameters in stomach tissue. BMC gastroenterology. 2009;9:36.

31. Kim JH, Kim BW, Kwon HJ, et al.. Curative effect of selenium against indomethacin-induced gastric ulcers in rats. Journal of microbiology and biotechnology. 2011;21(4):400-4.

32. Olaleye SB, Farombi EO. Attenuation of indomethacin- and HCl/ethanol-induced oxidative gastric mucosa damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seed. Phytotherapy research : PTR. 2006;20(1):14-20.

33. Chung YM, Bae YS, Lee SY. Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. Free Radical Biology and Medicine. 2003;34(4):434-42.

34. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, et al. In vivo antioxidant and antiulcer activity of Parkia speciosa ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PloS one. 2013;8(5):e64751.

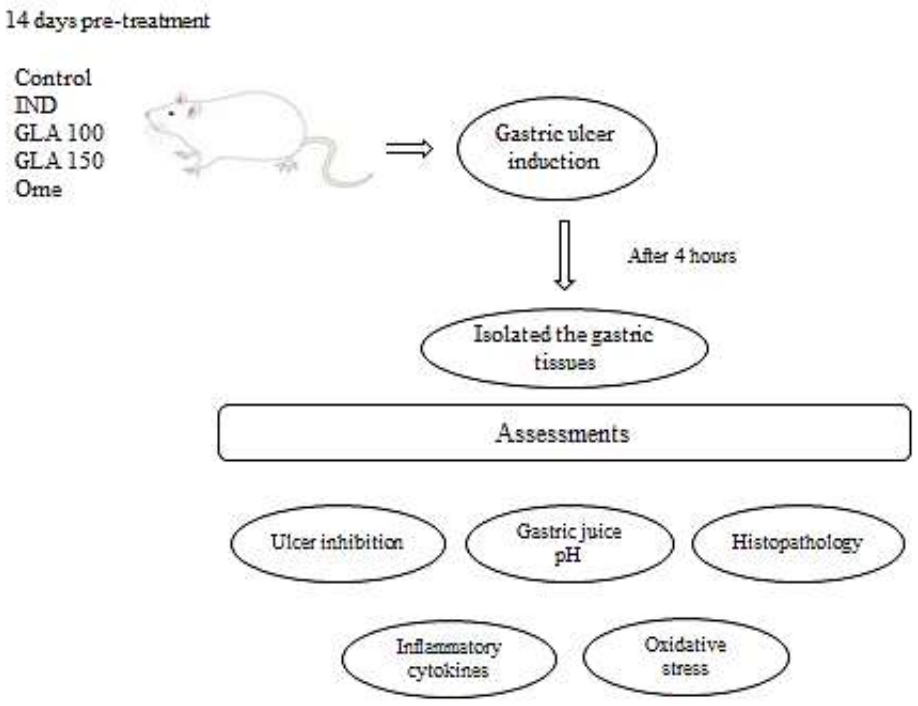
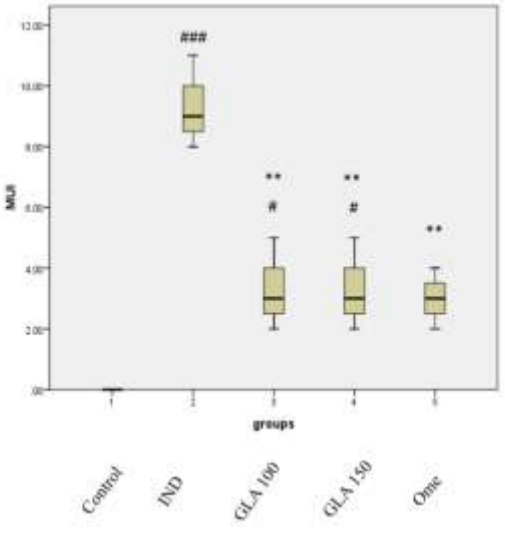
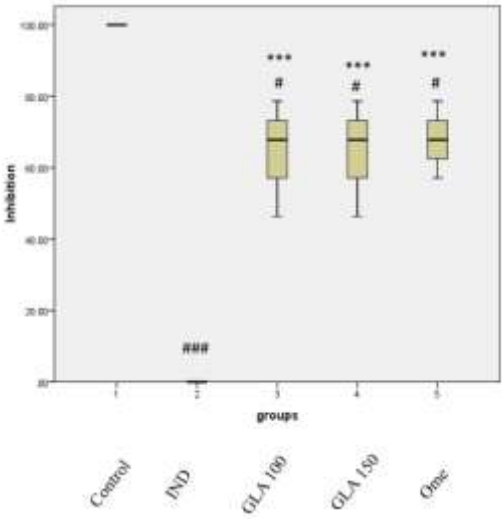


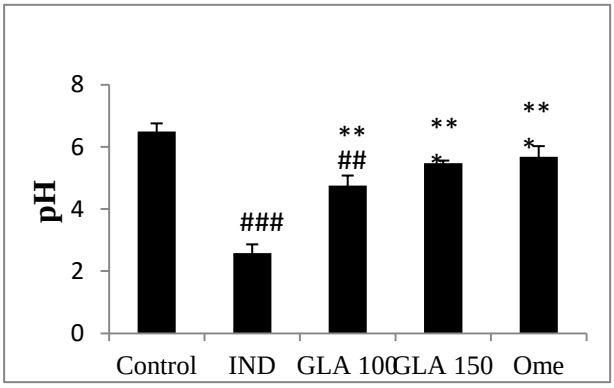
Figure 1. Schematic diagram.



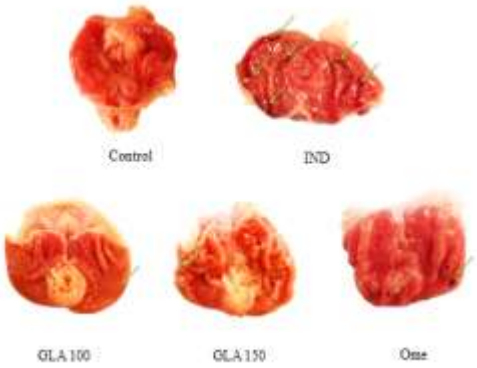
A



B



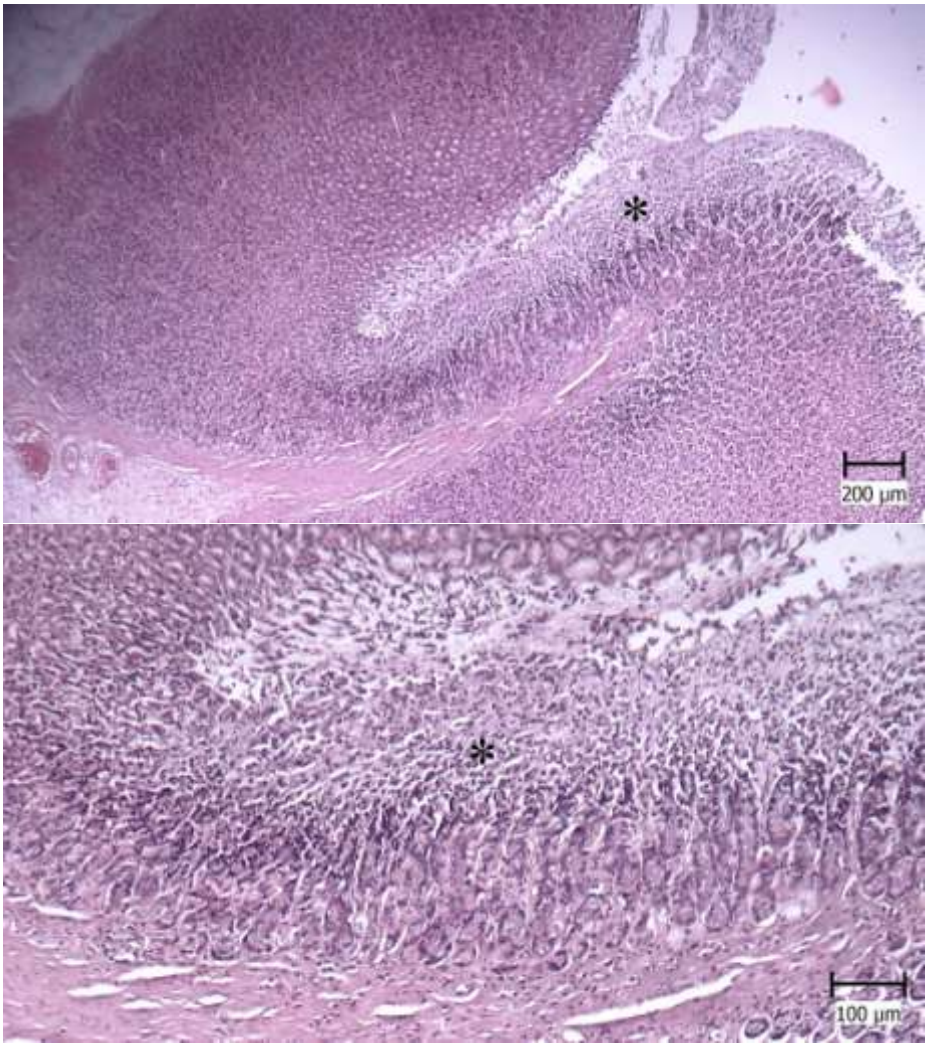
C

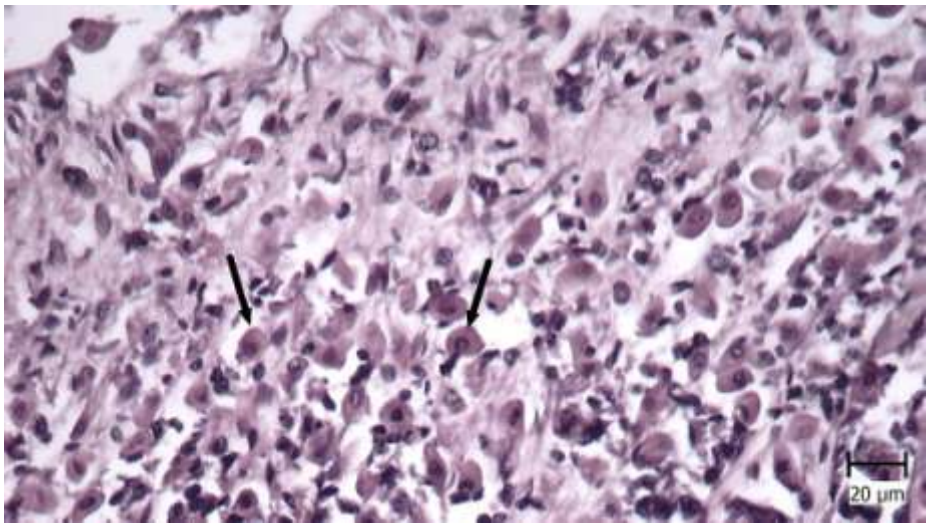


D

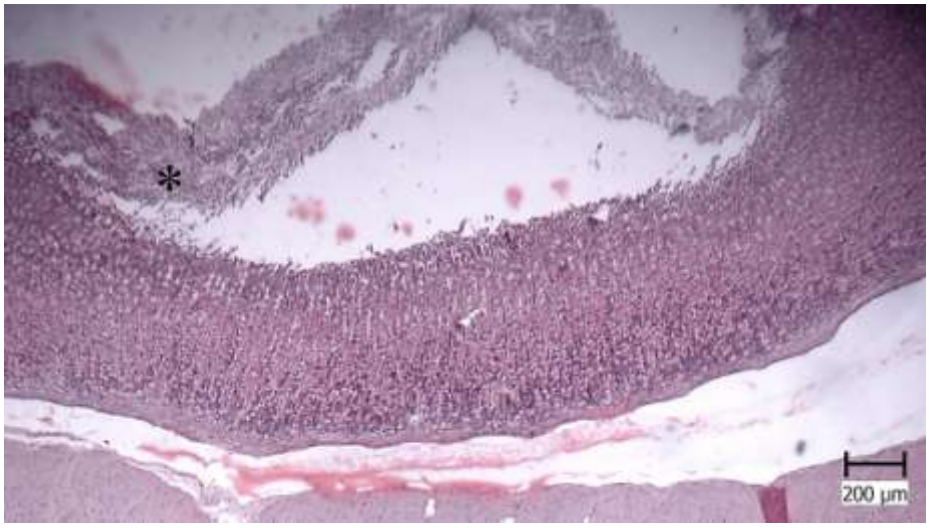
E

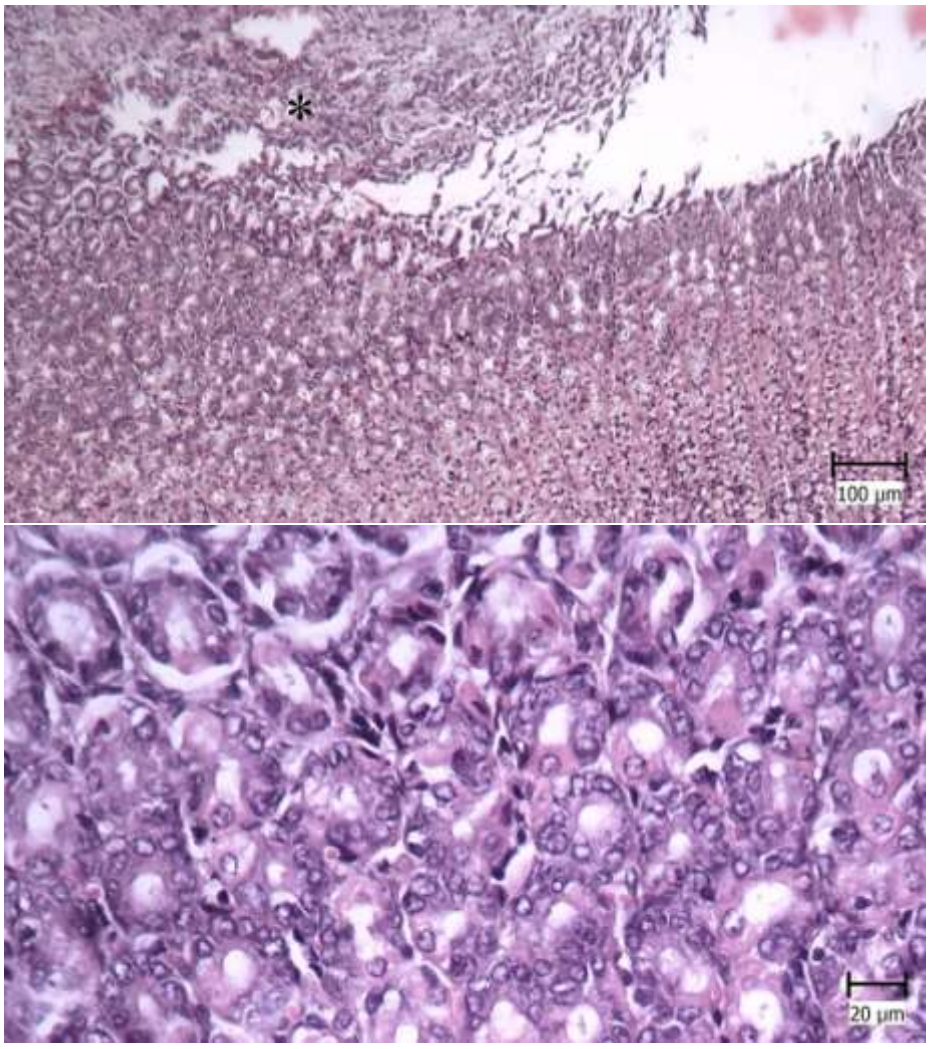
IND



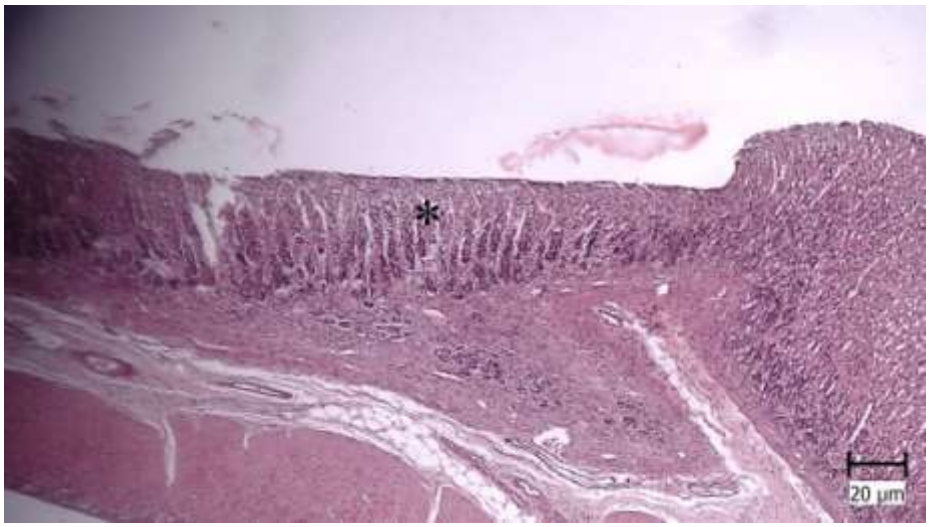


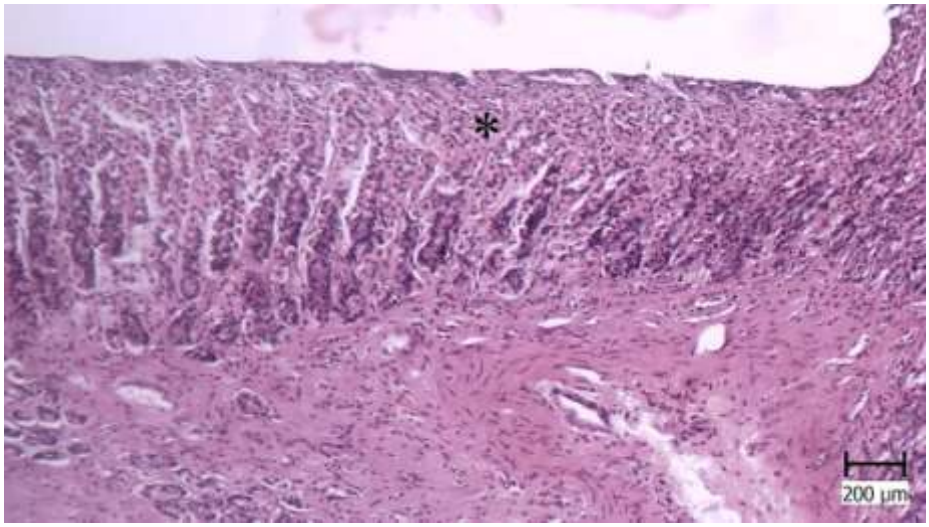
IND +Omeprazole

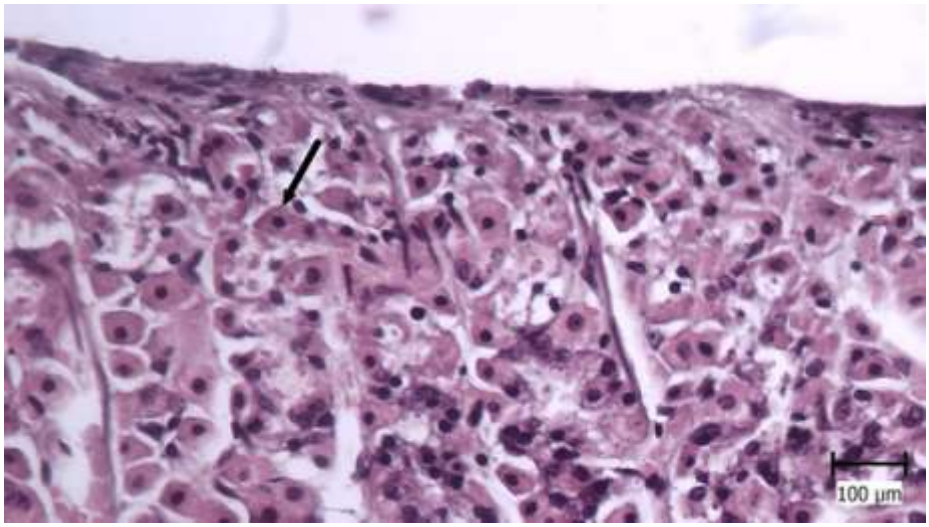




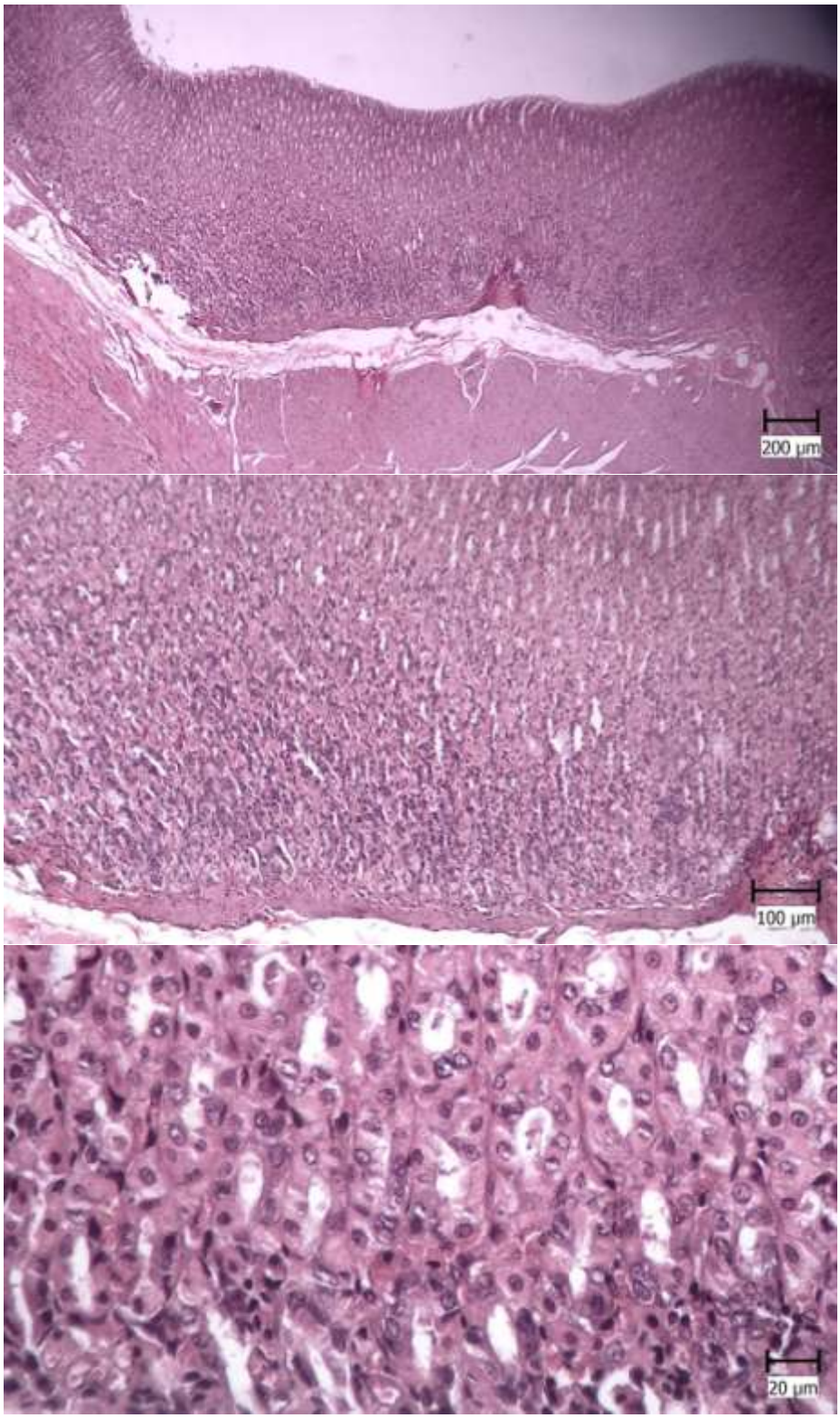
GLA 50



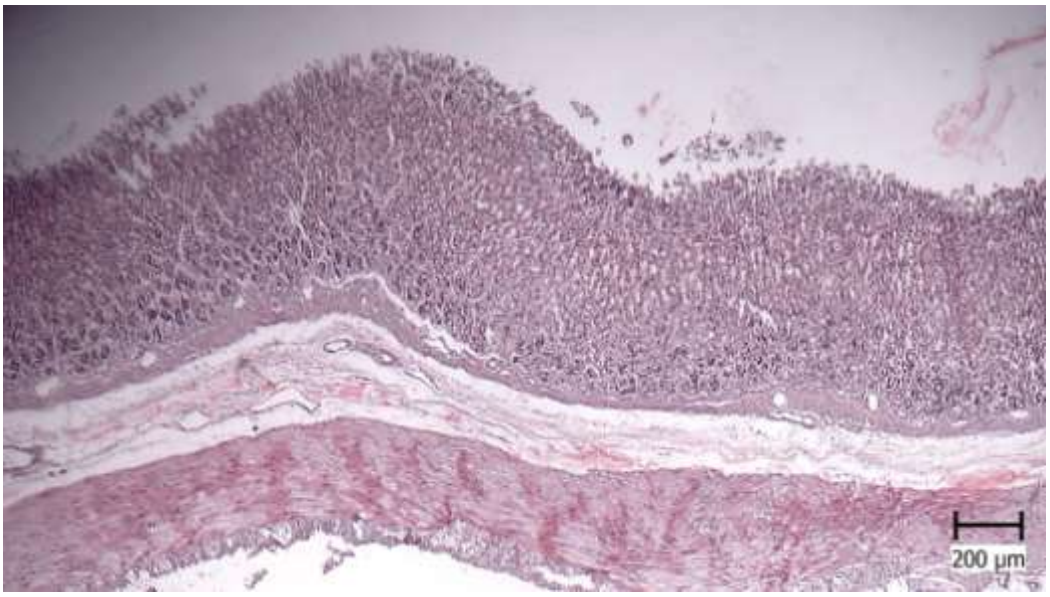


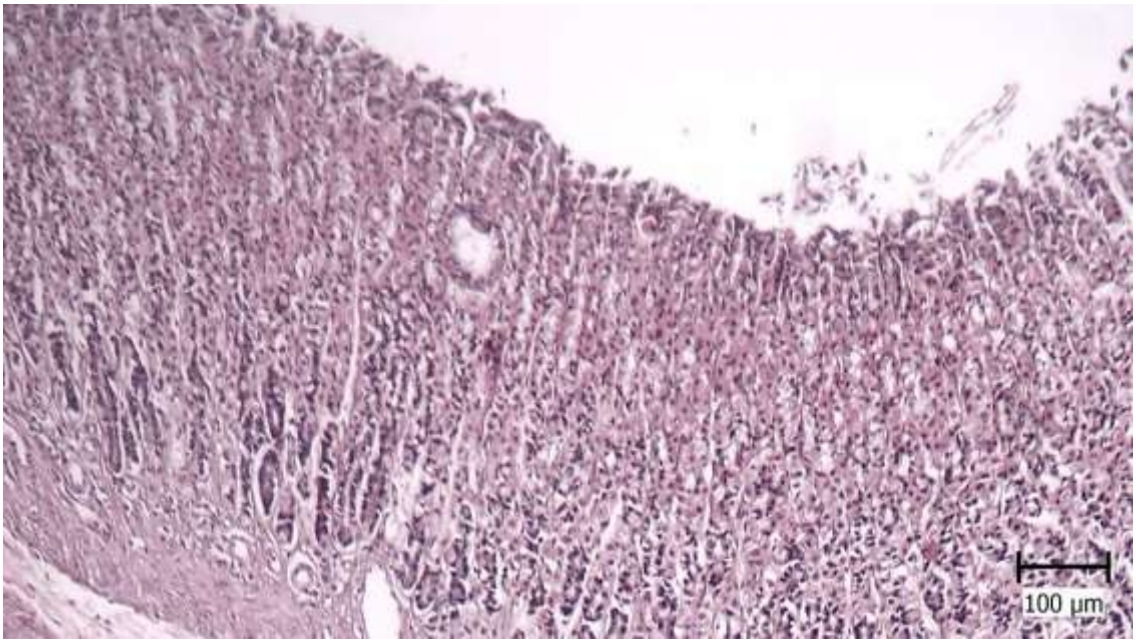


GLA100



Control





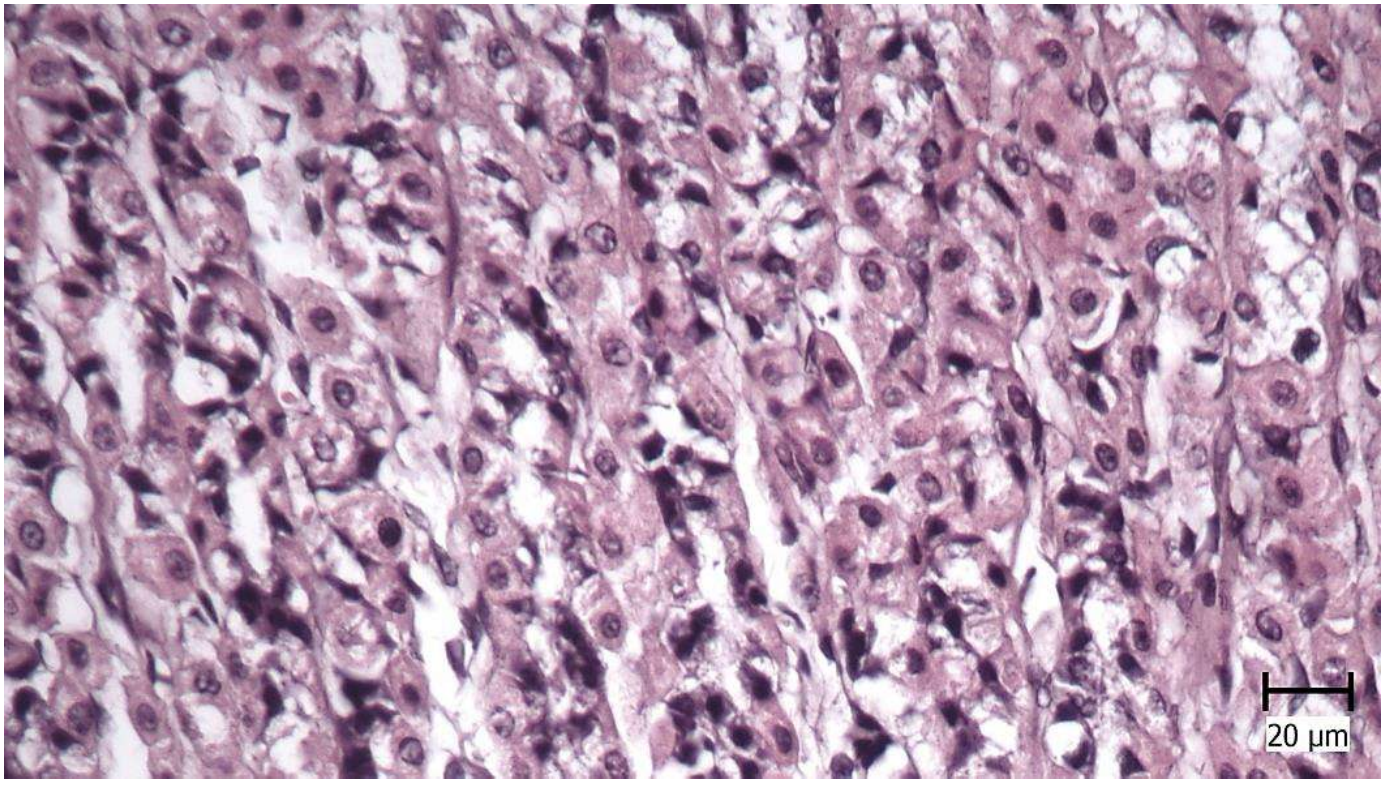
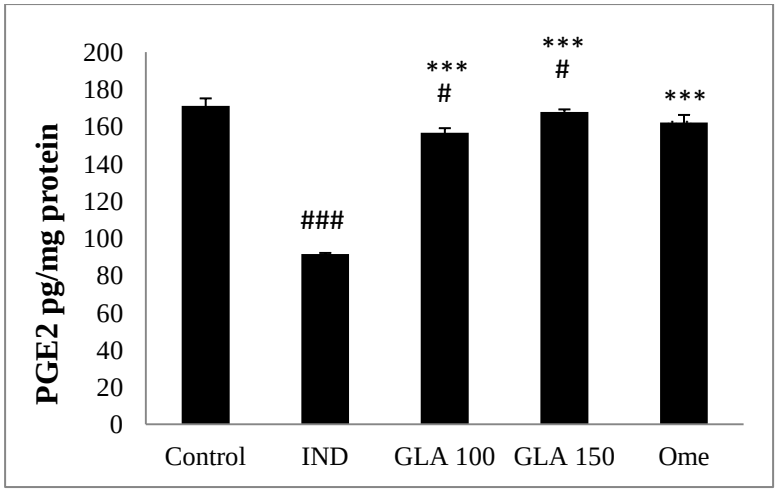
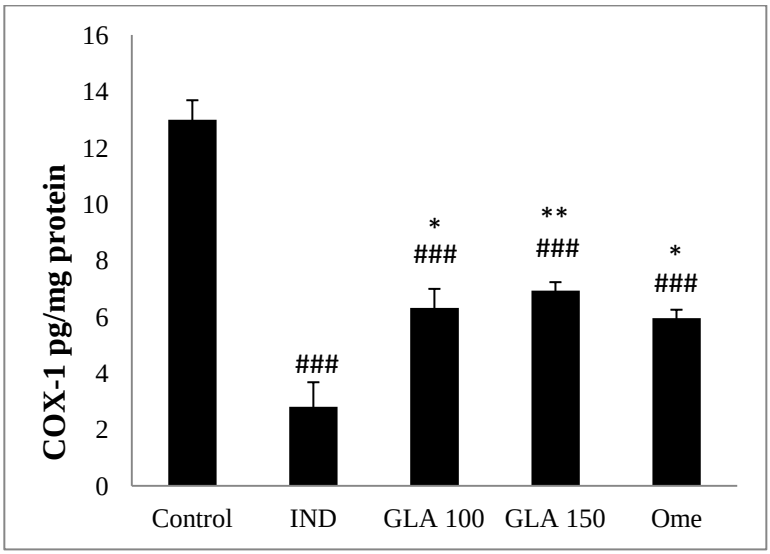


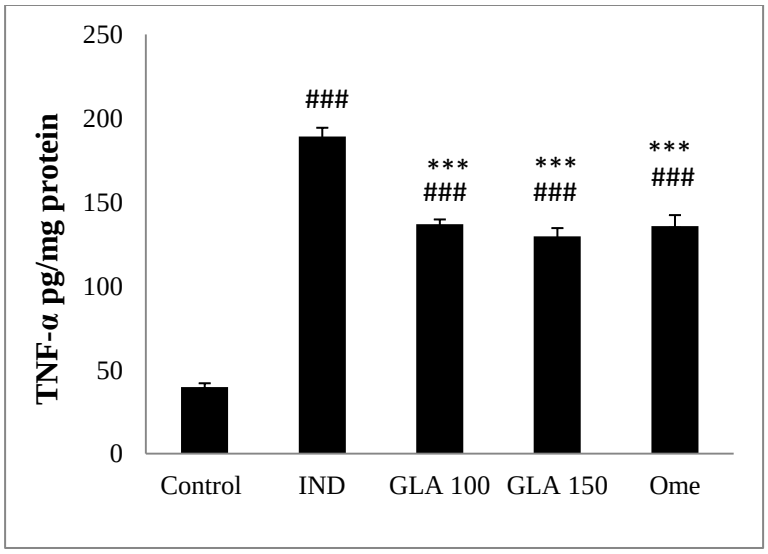
Figure 2. Effects of GLA on (**A**) Ulcer index, (**B**) % Inhibition ulcer, (**C**) pH, and (**D**) Macroscopic photographs of rat's Stomachs. (**E**) Histological examination. Pay attention to the necrosis of gastric mucosa (star), degenerate cells with dark and compact nuclei, and colorful cytoplasm (arrow) (hematoxylin and eosin staining). Data are presented as Mean ± SD (n = 6). Control, Indomethacin (IND), GLA 100 and 150 mg/kg groups + IND, and Omeprazole 20 mg/kg group (Ome) with a dose of 20 mg/kg + IND. #p< 0.05, ##p< 0.01, and ###p< 0.001 show a significant level difference compared to the control group. *p< 0.05, **p< 0.01, and ***p< 0.001 show a significant level difference compared to the IND group.



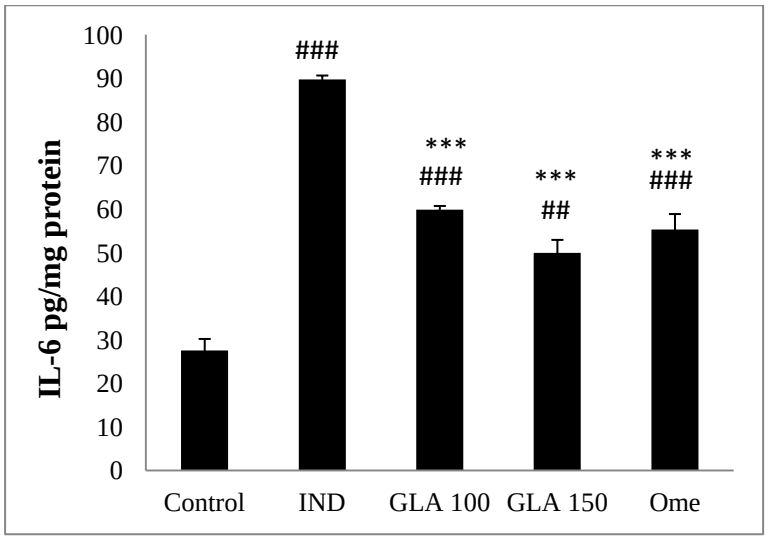
A



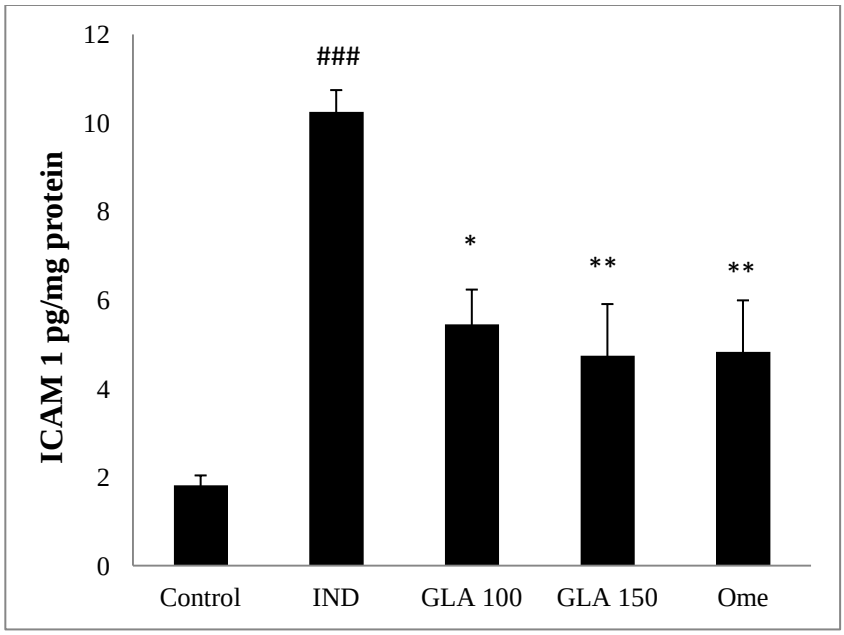
B



C

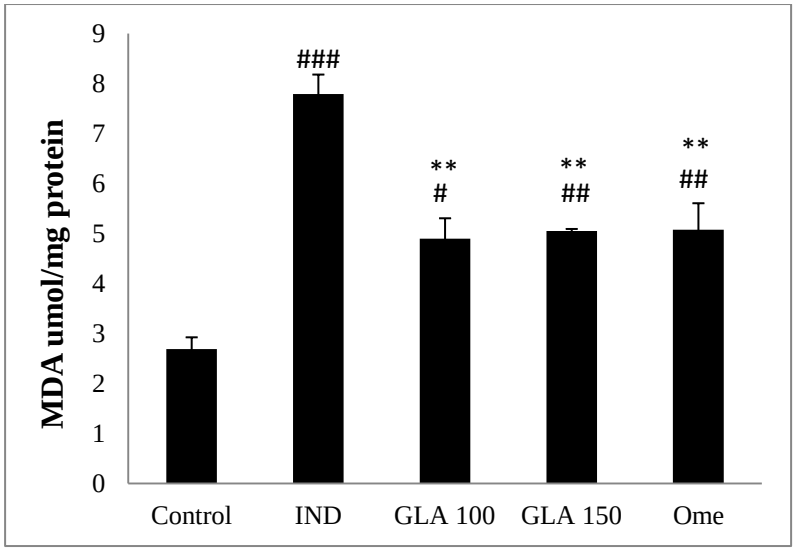


D

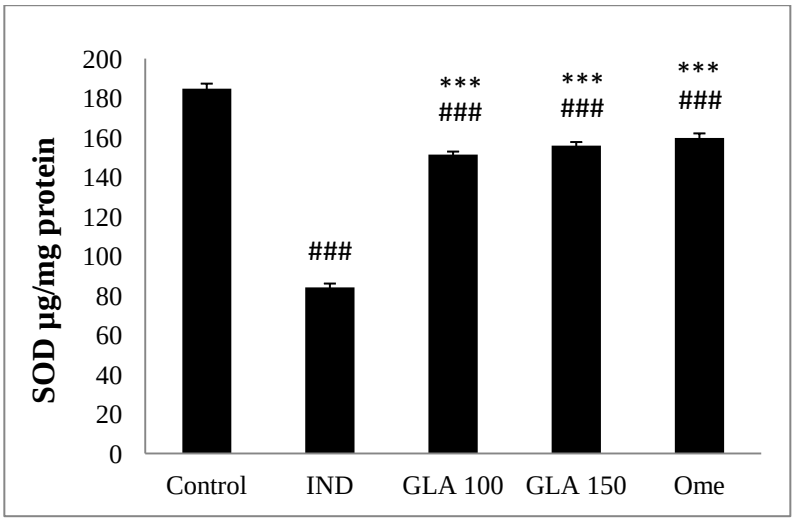


E

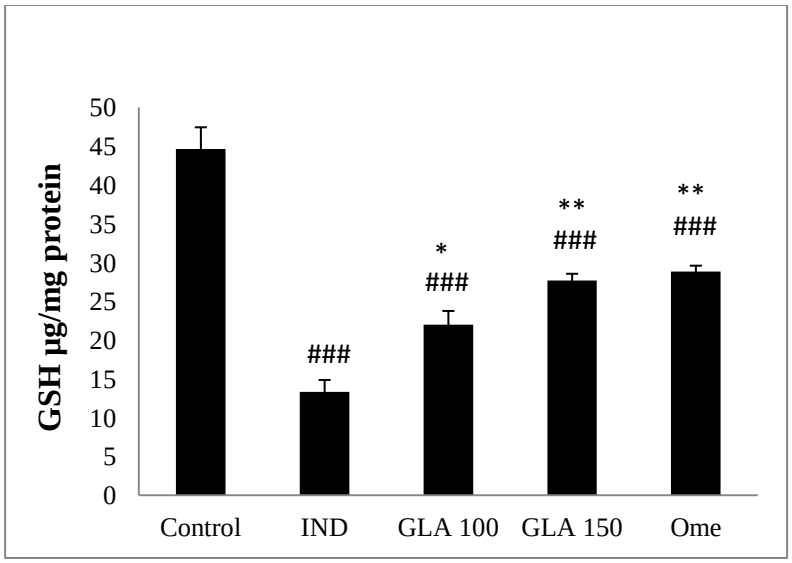
Figure 3. Effects of GLA on (**A**) PGE2, (**B**) COX-1, (**C**) TNF-α, (**D**) IL-6, and (**E**) ICAM-1. Data are presented as Mean ± SD (n = 6). Control, Indomethacin (IND), GLA 100 and 150 mg/kg groups + IND, and Omeprazole 20 mg/kg group (Ome) with a dose of 20 mg/kg + IND. #p< 0.05, ##p< 0.01, and ###p< 0.001 show a significant level difference compared to the control group. *p< 0.05, **p< 0.01, and ***p< 0.001 show a significant level difference compared to the IND group.



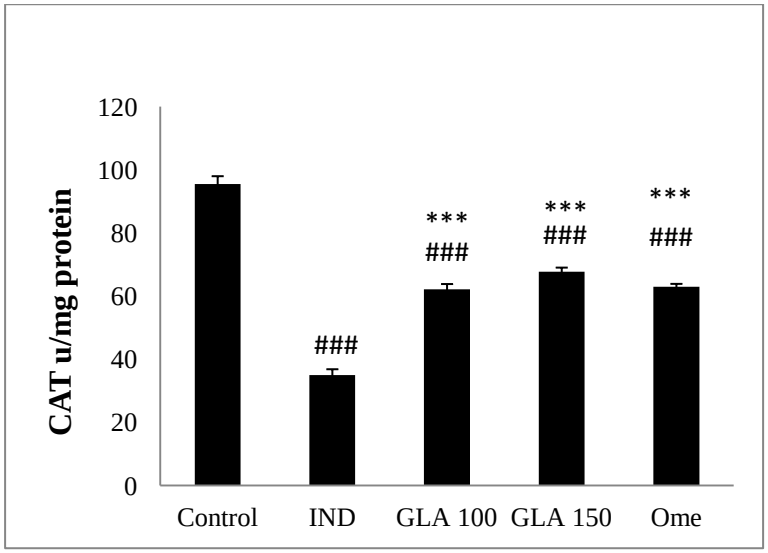
A



B



C



D

Figure 4. Effects of GLA on (**A**) MDA, (**B**) SOD, (**C**) GSH, (**D**) CAT. Data are presented as Mean ± SD (n = 6). Control, Indomethacin (IND), GLA 100 and 150 mg/kg groups + IND, and Omeprazole 20 mg/kg group (Ome) with a dose of 20 mg/kg + IND. #p< 0.05, ##p< 0.01, and ###p< 0.001 show a significant level difference compared to the control group. *p< 0.05, **p< 0.01, and ***p< 0.001 show a significant level difference compared to the IND group.