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Multi-Modal Gastroprotective Effects of Dioscin in an Indomethacin-Induced Gastric Ulcer Model: Inflammation, Oxidative Stress, Nitric Oxide Pathways, Apoptosis, and Autophagy

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Article

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Abstract

Dioscin has shown protective effects in hepatic, renal, and pulmonary injury models. However, its multi-modal protective profile in NSAID-induced gastric ulceration has not been comprehensively evaluated. We investigated the gastroprotective effects of dioscin at two doses in a gastric ulcer model through biochemical, histopathological, and immunohistochemical alterations. 48 female Wistar albino rats were randomized into six groups: control, vehicle, omeprazole (30 mg/kg), indomethacin (30 mg/kg), dioscin 15 (15 mg/kg) and dioscin 30 (30 mg/kg). Blood and gastric tissues were collected 4 h after ulcer induction. IL-1β, TNF-α, IL-10, SOD, MDA, IMA, iNOS, and eNOS levels were measured by ELISA. Gastric lesions were graded histologically. Immunohistochemistry was performed for Bcl-2, p-Caspase-3, p-LC3B, and p-NFκB p65. Data were analyzed via one-way ANOVA ($p < 0.05$). Indomethacin markedly increased IL-1β, TNF-α, MDA, IMA, and iNOS while reducing SOD and disrupting mucosal architecture. 15 mg/kg dose of Dioscin produced the strongest suppression of IL-1β and TNF-α and reduced iNOS levels, while the 30 mg/kg dose of Dioscin maximally enhanced SOD activity and increased eNOS expression, indicating improved mucosal perfusion. Histopathology demonstrated dose-responsive restoration of epithelial integrity, with Dioscin (30 mg/kg) group exhibiting near-normal mucosal morphology comparable to omeprazole. Immunohistochemically, Dioscin increased Bcl-2 expression while reducing p-Caspase-3, p-LC3B, and p-NFκB p65, demonstrating amelioration of apoptosis, autophagy activation, and inflammatory signaling. Dioscin confers robust gastroprotection through coordinated anti-inflammatory, antioxidant, anti-apoptotic, and autophagy-modulating mechanisms. The 15 mg/kg dose predominantly attenuates inflammatory responses, whereas the 30 mg/kg dose provides additional mucosal repair via enhanced antioxidant activity and eNOS upregulation.

Highlights

Dioscin significantly protects gastric mucosa against indomethacin-induced gastric ulceration through multi-targeted mechanisms (anti-inflammatory, antioxidant, and anti-apoptotic effects).

15 mg/kg dioscin predominantly suppresses IL-1β, TNF-α and oxidative biomarkers (MDA, IMA), while 30 mg/kg enhances eNOS-mediated mucosal perfusion.

Dioscin reduces p-Caspase-3 and NF-κB p65 activation while restoring Bcl-2 expression and epithelial integrity.

Histopathological and immunohistochemical results show highly comparable recovery to omeprazole, suggesting strong therapeutic potential.

Dioscin represents a promising natural gastroprotective agent that may be applicable as an alternative or adjunct treatment to conventional anti-ulcer drugs.

INTRODUCTION

Peptic ulcer disease (PUD) represents a significant global health burden characterized by the breakdown of the gastrointestinal mucosal barrier due to an imbalance between mucosal defensive mechanisms and aggressive luminal factors. Clinically, gastric ulcers manifest with epigastric pain, bloating, nausea, dyspepsia, and may progress to bleeding and perforation. [1–3] The pathogenesis of PUD involves complex interactions among mucosal integrity, acid secretion, oxidative stress, and inflammatory responses.

Under physiological conditions, gastric mucosal defense is maintained by the mucus–bicarbonate barrier, continuous epithelial renewal, adequate mucosal microcirculation, and prostaglandin-mediated cytoprotection. [4, 5] Disruption of these mechanisms, combined with exposure to aggressive agents such as gastric acid, pepsin, bile reflux, and reactive oxygen species (ROS), forges a permissive environment for ulcer formation. [6, 7]

Helicobacter pylori infection and chronic nonsteroidal anti-inflammatory drugs (NSAIDs) usage are the two most prevalent etiological contributors to gastric ulceration. [8, 9] Among NSAIDs, indomethacin (Indo) is widely employed in experimental ulcer models due to its potent inhibition of COX-1 and COX-2, which results in diminished prostaglandin synthesis, impaired mucosal blood flow, and compromised epithelial defense. [10] Consequently, indomethacin-induced gastric injury is considered a reliable model for studying the mechanisms of mucosal damage.

ROS overproduction triggers lipid peroxidation, increases malondialdehyde (MDA) formation, and ultimately induces cell death. [11] Simultaneously, endogenous antioxidant defense systems—especially superoxide dismutase (SOD)—are downregulated, intensifying oxidative stress. [12, 13] Ischemia-modified albumin (IMA) is also a clinically relevant ischemia biomarker. [14, 15] Nitric oxide synthase isoforms (iNOS and eNOS) also play critical roles in vascular tone regulation and inflammatory signaling, with dysregulation contributing to mucosal injury. [16]

Indomethacin-induced gastric damage is accompanied by pronounced increases in pro-inflammatory cytokines, like IL-1β and TNF-α, which amplify mucosal inflammation.[17, 18] Conversely, IL-10 functions as a key anti-inflammatory cytokine; thus, reduced IL-10 levels have been associated with persistent mucosal injury and impaired resolution of inflammation.[19]

Apoptosis and autophagy pathways are also activated during gastric mucosal injury. Markers such as phosphorylated caspase-3, Bcl-2, phosphorylated NF-κB, and phosphorylated LC3B are essential indicators of cell death and survival Dynamics.[20–22] The central role of NF-κB in driving inflammatory gene expression further underscores its relevance as a therapeutic target in gastric injury.[23]

Although proton pump inhibitors (PPIs) such as omeprazole remain first-line therapy for acid suppression, long-term PPI use has been associated with potential adverse effects including micronutrient deficiencies and alterations in gut microbiota.[24, 25] This has intensified scientific interest in novel natural agents with potent antioxidant and anti-inflammatory profiles.

Dioscin, a steroidal saponin derived from Dioscorea species, has gained attention with its antioxidant, anti-inflammatory, antifibrotic, and cytoprotective activities.[26–28] Evidence has demonstrated dioscin's protective effects in models of hepatic, renal, and pulmonary injury [29–31], as well as in experimental gastric ulceration where it mitigates oxidative stress and inflammation. [32]

Despite these findings, there remains a lack of comprehensive studies evaluating dioscin's simultaneous effects on oxidative stress, inflammatory cytokines, nitric oxide pathways, apoptosis, and autophagy together in an indomethacin-induced gastric ulcer model.

Materials and Methods

Ethical Approval

and Experimental Setting

This study was approved by the Atatürk University Faculty of Medicine Local Ethics Committee for Animal Research (Decision No: 136, Date: 27/05/2024) and conducted at the Atatürk University Laboratory Animals Production and Research Center, the Department of Physiology, and the Department of Veterinary Pathology. All procedures strictly complied with the relevant guidelines and regulations. The animals were maintained in a regulated environment at 20–22°C with a 12-hour light–dark cycle and were provided unrestricted access to standard rat feed and tap water.

Animals and Experimental Design

A total of 48 female Wistar albino rats (10–12 weeks; 200–250 g) were obtained from the institutional animal facility. The rats were deprived of food for 24 hours while allowed unrestricted access to water, which was withdrawn one hour prior to the experiment. Gastric ulceration was induced via administration of indomethacin (30 mg/kg) by intragastric (i.g.) gavage. Four hours after ulcer induction, animals were euthanized and blood and stomach tissues were collected.

Indomethacin and omeprazole were dissolved in slightly acidic aqueous media and subsequently buffered with 5% sodium bicarbonate before administration. Each i.g. dosing volume was standardized to 1 mL. Animals were randomly allocated into six groups (n = 8 per group):

Control group (Control)

No intervention was performed to make a comparison.

Vehicle group (Vehicle)

5% $NaHCO_3$ i.g., followed by indomethacin after 1 h (33); euthanized 4 h post-indomethacin. This group was formed to measure solvent application effect on ulcer formation.

Omeprazole group (Omeorazole)

30 mg/kg Omeprazole i.g., followed by indomethacin after 1 h; euthanized 4 h later. This group was formed to demonstrate the effects of omeprazole, a drug with proven therapeutic effects on gastric ulcers in the literature, in our experiment and to compare these effects with those we obtained with dioscin.

Indomethacin group (Indo)

30 mg/kg indomethacin i.g.; euthanized 4 h post-treatment. This group was created to induce gastric ulcers.

Dioscin 15 mg/kg group (Dioscin 15)

15 mg/kg dioscin i.g., followed by indomethacin after 1 h; euthanized 4 h later. This group was formed to investigate the therapeutic effect of 15 mg/kg Dioscin pretreatment on gastric ulcer formation.

Dioscin 30 mg/kg group (Dioscin 30)

Same protocol as Dioscin 15 mg/kg group, but with a 30 mg/kg Dioscin dose. This group was formed to investigate the therapeutic effect of 30 mg/kg Dioscin pretreatment on gastric ulcer formation.

Euthanasia and Sample Collection

All rats were anesthetized with ketamine/xylazine (50/10 mg/kg intraperitoneally). The anesthetized rats were euthanized by drawing blood from their hearts, after which gastric tissue and blood were collected. Blood samples were collected into pre-cooled EDTA tubes, centrifuged (3000 g/5 min), and stored at −80°C. Stomachs were excised immediately below the gastroesophageal junction, opened along the greater curvature, rinsed in physiological saline, and photographed for macroscopic examination. Tissue weights were recorded, and gastric ulcers were quantified by counting mucosal petechiae to generate ulcer index scores.Each stomach was subsequently divided into three portions: [1] snap-frozen using liquid nitrogen for biochemical assays, [2] fixed in neutral buffered formalin (10%) for histopathology, and [3] reserved for immunohistochemistry.

Biochemical Analyses

Gastric mucosa samples were homogenized in cold 10 mM phosphate buffer (pH 7.4) and centrifuged (5000 rpm/10 min) at 4°C. Supernatants were analyzed using commercial rat-specific ELISA kits to quantify TNF-α, IL-1β, IL-10, eNOS, iNOS, SOD, ischemia-modified albumin (IMA), and malondialdehyde (MDA) according to manufacturers' protocols. The concentrations of TNF-α (YL biont, Cat No: YLA0118RA), iNOS (YL biont, Cat No: YLA0266RA), IL-10 (YL biont, Cat No: YLA0440RA), eNOS (YL biont, Cat No: YLA0533RA), IL-1β (BT LAB, Cat No: E0119Ra), SOD (BT LAB, Cat No: E0168Ra), IMA (BT LAB,

Cat No: EA0053Ra), and MDA (BT LAB, Cat No: E0156Ra) in the obtained serum samples were determined using commercially available ELISA kits. All analyses were performed following the protocols provided by the manufacturers.

Histopathological Evaluation

Formalin-fixed tissues were processed by routine paraffin embedding, sectioned at 5 μm thickness, and stained with hematoxylin & eosin. Ulcer severity was scored based on hemorrhagic lesions (0–4), mucosal edema (0–4), inflammatory cell infiltration (0–3), and epithelial loss (0–3), resulting in a total score range of 0–14. Microscopic assessments were conducted under a light microscope at 40× magnification in 10 randomly selected fields. Digital imaging and morphometric measurements were performed using ImageJ analysis software.

Immunohistochemistry

Sections mounted on charged slides were deparaffinized, rehydrated through graded ethanol series, and subjected to citrate buffer antigen retrieval via microwave heating. Endogenous peroxidase activity was blocked with 3% H_2O_2. The slides were left to incubate overnight at 4°C with primary antibodies targeting Bcl-2, phosphorylated Caspase-3, LC3B, and phosphorylated NF-κB p65, each applied at a 1:100 dilution. After washing, HRP-linked secondary antibodies and DAB chromogen were applied, followed by counterstaining with Mayer's hematoxylin. Five nonoverlapping fields per specimen were evaluated for staining intensity (0–3) and percentage of positive cells (0–3).

Statistical Analysis

Data were checked for missing values, outliers, and normality. Quantitative variables were presented as mean values with their corresponding standard deviations; categorical variables were summarized as frequency and percentage. Group comparisons were carried out using one-way ANOVA where normality and homogeneity assumptions were satisfied. Statistical significance was defined at a threshold of $p < 0.05$. All analyses were conducted using IBM SPSS Statistics (version 20.0).

RESULTS

Biochemical Findings

All biochemical results are presented in Table 1. Indomethacin administration resulted in a significant elevation of the pro-inflammatory cytokines IL-1β and TNF-α compared with the control group ($p < 0.05$), confirming successful inflamation induction.[5, 17] Dioscin treatment markedly attenuated this cytokine surge, with the 15 mg/kg dose demonstrating the most prominent suppression, whereas TNF-α levels exhibited a mild rebound at 30 mg/kg. Conversely, IL-10, an anti-inflammatory mediator known to

counterbalance inflammatory cascades[19], displayed dose-dependent increases in both dioscin-treated groups ($p < 0.05$).

Table 1
Biochemical Examination. ELISA Findings (IL-1 beta, TNF-alfa, IL-10, SOD, MDA, iNOS, eNOS)

Groups	IL-1 beta	TNF-alfa	IL-10	SOD	MDA	IMA	iNOS	eNOS
	ng/mL	ng/L	pg/mL	ng/mL	nmol/mL	U/mL	ng/mL	ng/mL
Control	14.1 ± 2.4^{a}	189.7 ± 22.8^{a}	62.1 ± 48.9^{a}	2.14 ± 0.38^{ab}	1.12 ± 0.24^{ab}	28.9 ± 14.2^{ab}	31.8 ± 12.4^{a}	22.1 ± 9.8^{a}
Vehicle	16.5 ± 2.9^{ab}	201.3 ± 24.1^{ab}	145.8 ± 112.4^{bc}	1.12 ± 0.41^{c}	0.98 ± 0.35^{bc}	35.8 ± 15.6^{ac}	65.4 ± 25.6^{b}	41.8 ± 15.2^{bc}
Omeprazol	15.3 ± 3.2^{a}	235.6 ± 28.4^{b}	98.2 ± 75.3^{ac}	1.25 ± 0.33^{c}	0.65 ± 0.19^{de}	24.1 ± 12.8^{bd}	58.9 ± 18.7^{bc}	36.4 ± 12.7^{ac}
Indo	38.2 ± 1.7^{c}	682.4 ± 45.2^{c}	192.4 ± 89.7^{bd}	2.01 ± 0.62^{b}	0.51 ± 0.12^{e}	62.4 ± 18.7^{e}	98.7 ± 19.2^{d}	65.2 ± 18.9^{d}
Dioscin 15	19.8 ± 3.8^{bd}	598.1 ± 32.9^{de}	387.9 ± 215.6^{de}	2.87 ± 0.71^{ad}	0.87 ± 0.28^{cd}	17.5 ± 13.4^{d}	47 ± 22.1^{c}	54.1 ± 20.6^{b}
Dioscin 30	23.4 ± 4.1^{d}	615.8 ± 38.7^{d}	412.5 ± 178.3^{e}	3.15 ± 0.54^{d}	1.42 ± 0.31^{a}	41.2 ± 22.3^{ce}	142 ± 28.4^{e}	89.7 ± 24.3^{e}

*Superscript letters (a, b, c, d, e) indicate the difference between groups.

Oxidative stress parameters revealed a similar pattern. SOD activity, a key component of endogenous antioxidant defense [7, 13], was highest in the higher dose dioscin group, indicating enhanced protection against reactive oxygen species. MDA levels, an index of lipid peroxidation [11], returned to near-control values in this group, reflecting reduced oxidative injury. Additionally, ischemia-modified albumin, which was markedly elevated in the indomethacin group, significantly decreased following 15 mg/kg dioscin treatment, indicating lower systemic oxidative damage. [14]

In the indomethacin group, iNOS expression, which drives nitric-oxide–mediated cytotoxicity [16], was significantly elevated. While 15 mg/kg dioscin reduced this rise, the 30 mg/kg dose further increased iNOS levels. However, this high dose simultaneously produced a notable upregulation of eNOS, a crucial mediator for mucosal perfusion and healing. [34] Seemingly lower-dose dioscin predominantly exerts anti-inflammatory effects, whereas the higher dose additionally promote angiogenic repair mechanisms.

Histopathological Findings

H&E-stained gastric tissues are shown in Fig. 9. Control and vehicle groups displayed normal gastric architecture with intact glands and no mucosal injury. In contrast, indomethacin caused extensive epithelial disruption, widespread ulceration, edema, and dense inflammatory cell infiltration, supporting severe mucosal damage. [4]

Histologically Dioscin treatment mitigated these alterations with a dose-responsive improvement. The 15 mg/kg dose of Dioscin produced moderate regeneration of mucosal integrity, while the 30 mg/kg dose restored near-normal epithelial continuity and substantially diminished ulcerated areas like those observed in the omeprazole-treated group, which served as a standard gastroprotective control.[35]

The histological recovery was similar to that seen in the omeprazole group, which was used as the reference gastroprotective control

Immunohistochemical Findings

Immunohistochemical images for Bcl-2, p-Caspase-3, p-LC3B, and p-NF-κB p65 are shown in Fig. 10. Compared with the indomethacin group, Bcl-2 immunoreactivity was markedly higher in both the dioscin and omeprazole groups. This increase in Bcl-2, an anti-apoptotic protein, indicates that apoptosis is suppressed/prevented in the tissue. [36] Conversely, p-Caspase-3 and p-NF-κB p65 expression, markers of apoptosis and active inflammatory transcription, respectively, were markedly reduced following dioscin treatment.[31, 37] Interestingly, p-LC3B, a key regulator of autophagy-associated cytoprotection[21], showed high expression in the indomethacin group, consistent with stress-induced autophagy. Dioscin reduced p-LC3B intensity, suggesting decreased cellular stress as mucosal recovery improved.[26] Together, these findings support that dioscin confers mucosal protection by reducing apoptosis and inflammatory signaling while restoring cellular homeostasis.

DISCUSSION

Dioscin, a naturally occurring steroidal saponin, were investigated in gastric ulcer model induced by indomethacin in our study. The results showed that dioscin has both anti-inflammatory and antioxidant effects, and a protective potential for the gastric mucosa. Our findings provide significant evidence that dioscin suppresses ulcer formation, reduces pro-inflammatory cytokines, supports antioxidant systems, and improves mucosal integrity.

Natural compounds such as dioscin, have emerged as promising gastroprotective agents. Dioscin exhibits anti-oxidant, anti-inflammatory, and anti-apoptotic properties by suppressing NF-κB signaling, reducing TNF-α, IL-1β, and IL-6 levels, modulating iNOS and eNOS balance, enhancing Nrf2-mediated antioxidant pathways, and inhibiting caspase-3–dependent apoptosis.[29, 31] Dioscin also regulates autophagy through modulation of LC3B and related signaling pathways, restoring cellular homeostasis under stress conditions.[21]

In our study, indomethacin caused severe mucosal damage, leading to widespread epithelial damage, extensive ulceration, edema, and intense inflammatory cell infiltration. Furthermore, a significant increase in IL-1β and TNF-α levels was observed after indomethacin administration. This supports the finding that indomethacin exacerbates inflammatory responses in the gastric mucosa and disrupts epithelial integrity. Lower dose Dioscin (15 mg/kg) administration, significantly suppressed these pro-inflammatory mediators, demonstrating the drug's effectiveness in modulating inflammation. Danisman et al. also reported significantly higher IL-1β levels compared to the control group in their study whose model is the same with ours.[18] TNF-α plays a critical role in indomethacin-induced gastric mucosal injuries, as evidenced by the parallel increase in TNF-α levels with mucosal damage.[17] Accordingly, our results demonstrate that dioscin suppresses inflammation by preventing TNF-α increase and protects the gastric mucosa.

Inflammation is tightly linked to oxidative injury in NSAID-induced gastric damage. Indomethacin significantly increases TNF-α, IL-1β, and IL-6 levels, which promote neutrophil infiltration, endothelial dysfunction, and epithelial apoptosis.[17, 38] TNF-α plays a pivotal role by facilitating neutrophil adherence to the gastric microcirculation by increasing adhesion molecules like ICAM-1, thus aggravates mucosal injury.[39, 40]

These inflammatory events are orchestrated by nuclear factor-kappa B (NF-κB) activation, a master regulator of immune and inflammatory responses. NF-κB activation increases transcription of TNF-α, IL-1β, IL-6, iNOS, and other pro-inflammatory mediators, thereby amplifying gastric injury.[41, 42] NF-κB also contributes to apoptosis by inducing DNA damage and activating caspase-3 signaling.[43]

Collectively, the literature demonstrates that indomethacin-induced gastric injury is a multifactorial process involving prostaglandin depletion, oxidative stress, inflammation, apoptosis, autophagy dysregulation, and impaired nitric oxide signaling. Therapeutic agents such as omeprazole exert gastroprotective effects by targeting these interconnected pathways, highlighting their potential value againts NSAID-induced gastric ulceration. Caspase-3, the central executioner of apoptosis, is markedly upregulated following indomethacin administration, contributing to epithelial cell loss and ulcer progression.[44, 45]

The indomethacin effect on apoptosis was measured by Caspase-3, its effect on autophagy by LC3B, and its effect on inflammation by Nf-kB in our study. Immunoreactivity of p-Caspase-3, p-LC3B, and p-Nf-kB-p65 antibodies were significantly higher in Indo group compared to control and both Dioscin groups, and a significant increase in IL-1β and TNF-α levels was observed after indomethacin administration. Considering the effect of Nf-kB on the inflammatory pathway mentioned in the literature, we can assume that dioscin reduces IL-1β and TNF-α by inhibiting NF-κB pathways, thus demonstrating its anti-inflammatory effect. Immunohistochemical analyses showed significant positivity for P-Caspase-3 (indicating apoptosis), P-LC3B (indicating autophagy), and P-NF-κB p65 (indicating inflammation) in the indomethacin group, while this reactivity was significantly reduced in the dioscin groups. These findings

indicate that indomethacin induces apoptosis, autophagy, and inflammation in the gastric mucosa, while dioscin suppresses these mechanisms, contributing to the preservation of mucosal integrity.

Dioscin significantly upregulates IL-10, a key regulator that suppresses excessive immune responses by its anti-inflammatory effect, and promotes tissue repair.[19] IL-10 inhibits pro-inflammatory cytokine production and contributes to resolution of inflammation and restoration of gastric mucosal integrity. The IL-10 levels showed a dose-dependent increase in the dioscin groups compared to the control. Likewise, a study by Almasaudi et al revealed significantly the increase in IL-10 levels in the treatment groups againts gastric ulcers in rat model compared to control.[46] Similarly, in their study of indomethacin-induced gastric ulcers in rats, Harakeh et al. observed an increase in IL-10 levels in the treatment groups compared to the control.[47]

Indomethacin markedly increases MDA, an indicator of lipid peroxidation, while significantly reducing antioxidant defenses such as SOD. Excessive ROS production disrupts cellular proteins, lipids, and nucleic acids, impairs mitochondrial function, and activates apoptotic cascades through caspase-3 activation.[35, 48] Thus, oxidative stress represents a central mechanism in indomethacin-induced gastric injury.

NSAIDs are reported to decrease in SOD activity. For example, in a study by Antonisamy et al. on indomethacin-induced gastric ulcers in rats, it was observed that SOD levels in the indomethacin group were reduced compared to the control group.[48] Previous studies have also reported similar protective effects of dioscin in liver[29], kidney[30], and lung[31] damage associated with oxidative stress. Dioscin has been shown to reduce cellular damage by suppressing oxidative stress and inflammation in a gastric ulcer model.[32] In our study, we found that levels of the antioxidant marker SOD were significantly increased in the high-dose (30 mg/kg) dioscin group, while oxidative stress markers such as MDA and IMA were decreased. These results indicate that dioscin has the capacity to neutralize reactive oxygen species in gastric tissue as well, supporting the idea that it is effective against oxidative damage in the gastric mucosa.

The Bcl-2 protein family raises the apoptosis threshold of cells by controlling cytochrome c release via the mitochondrial pathway; increased Bcl-2 in epithelial cells during ongoing inflammation prevents the normal elimination of damaged cells, contributing to the chronicity of the lesion.[36] An increase in Bcl-2 in the gastric mucosa indicates that programmed cell clearance is hampered, and apoptosis is suppressed/prevented in the tissue.[36] The higher immunoreactivity of Bcl-2 in the dioscin groups supports the idea that apoptotic signaling is suppressed by dioscin. These results suggest that dioscin also reduces gastric damage through intracellular anti-apoptotic signals. Similarly to our findings, Balaha et al. detected apoptosis in the gastric mucosa in response to indomethacin in their indomethacin-induced gastric ulcer experiments in rats, and a significant decrease in Bcl-2 levels was found in the Indo group compared to the control.[49]

Playing a critical role in cellular autophagy and various physiological processes, p-LC3B, is an RNA-binding protein that initiates mRNA degradation to accelerate autophagy. Indomethacin activates autophagy, as evidenced by increased expression LC3B, a critical regulator of autophagosome formation.[21] While basal autophagy maintains cellular homeostasis, excessive autophagy under oxidative and inflammatory stress may exacerbate mucosal damage. In our study, we observed statistically significant decreases in p-LC3B levels, which were found to be high in the indomethacin group, in the dioscin treatment groups. This suggests that dioscin may function as an autophagy inhibitor.

Our histopathological findings also support our biochemical analyses. Dioscin treatment alleviated histopathological changes with a dose-related improvement. The 15 mg/kg dose provided moderate restoration of mucosal integrity, while the 30 mg/kg dose restored almost normal epithelial continuity and significantly reduced ulcerated areas. While inflammatory cells, intense ulceration and edema at mucosa were observed in the indomethacin group, higher dose dioscin group (30 mg/kg), in particular, largely preserved the histological integrity of the gastric mucosa. This finding demonstrates that high-dose dioscin has positive effects on mucosal regeneration and can reverse ulcerative changes. Likewise, dioscin has been reported as protective at the histological level in gastric ischemia/reperfusion injury.[31, 32]

Under physiological conditions, endothelial nitric oxide synthase (eNOS)-derived NO maintains mucosal blood flow, inhibits platelet aggregation, enhances mucus secretion, and suppresses acid secretion.[4, 50] Conversely, excessive inducible nitric oxide synthase (iNOS)-derived NO contributes to nitrosative stress, DNA damage, and apoptosis.[16, 51] In our study, 15 mg/kg dioscin reduced the increase in iNOS, while a dose of 30 mg/kg further increased iNOS levels. Furthermore, the significant increase in eNOS levels in the dioscin groups, according to the literature[34], suggests improved blood flow in the gastric mucosa and support for epithelial healing. On the other hand, the observed increase in iNOS levels with high-dose dioscin should be carefully considered in terms of potential nitric oxide-induced prooxidative effects. However, generally, considering the balance in the eNOS/iNOS ratio, it can be said that an effect profile favoring vascular protection is formed.

Nitric oxide production, particularly in the gastrointestinal mucosa, plays a critical role not only in cytotoxicity but also in vasodilation and maintaining microcirculation.[4, 16] Increased iNOS expression after tissue damage is considered an adaptive response that contributes to the healing process by increasing blood flow in some conditions.[38] Furthermore, when evaluated together with eNOS, the increase in total nitric oxide bioavailability has been shown to strengthen mucosal defense and support epithelial healing.[50] In this context, it can be said that the net effect of increased iNOS levels with high-dose dioscin, when evaluated together with increased eNOS, is in the direction of vascular protection and mucosal healing.

Dioscin's dose-dependent effects reveal a pharmacodynamic profile that differs not only quantitatively but also functionally. While significant anti-inflammatory suppression is observed at low doses (15 mg/kg), antioxidant capacity and mucosal perfusion become more dominant at high doses (30 mg/kg), consistent with the "dose-dependent pathway selectivity" of natural compounds.[29; 31] Therefore, dioscin's ability to control inflammation at low doses and support tissue repair and microcirculation at high doses demonstrates a therapeutically optimized "dual-action profile".

CONCLUSION

This study evaluates dioscin in an experimental gastric ulcer model induced by indomethacin. Our findings indicated that dioscin could be a potential protective agent in reducing damage to the gastric mucosa. Light microscopy images showed severe ulceration, marked epithelial cell detachment, and inflammatory cell infiltration in the indomethacin group, while dose-dependent improvements were observed in the treatment groups. In particular, higher dose dioscin significantly reduced the ulcerative appearance, with the mucosa almost returning to a normal histological structure. Biochemical analysis results revealed that dioscin suppressed pro-inflammatory cytokine (IL-1β, TNF-α) levels while enhancing the anti-inflammatory (IL-10) response. Furthermore, dioscin increased SOD levels, decreased IMA levels, and increased MDA levels in a dose-dependent manner. The Dioscin treatment decreased iNOS and eNOS levels at lower dose, while it increased them at higher dose. Immunohistochemical findings revealed that dioscin administration decreased levels of p-NFκB, an inflammation marker, and p-LC3B, an autophagy biomarker. Furthermore, a decreasing effect was observed on p-Caspase-3 levels, an apoptosis biomarker, in 30 mg/kg dioscin group, while levels of Bcl-2, another apoptosis biomarker, increased at both doses.

Overall, these results demonstrate that dioscinin can exert multifaceted effects on the inflammatory and oxidative mechanisms involved in ulcer formation (Fig. 11). In light of these data, further detailed investigation of dioscin's pharmacological profile in future studies may allow for its clinical evaluation as a potential agent in exhibiting protective effects on the gastric mucosa. Seemingly dioscin can protect the gastric mucosa by positively influencing both inflammation and oxidative stress in a gastric ulcer model. However, detailed investigation of the long-term effects of different dosages and the mechanistic pathways is crucial for future clinical applications.

The findings of this study demonstrate that dioscin is not only an effective agent at the experimental level, but also a multi-targeted therapeutic candidate with translational potential for clinical application. Dioscin's ability to simultaneously regulate inflammation, oxidative stress, apoptosis, and nitric oxide balance could make it an alternative or complementary agent to classical proton pump inhibitors. However, long-term safety, dose optimization, and human studies are needed before these experimental findings can be translated into clinical practice. In this respect, dioscin can be considered a promising candidate for translational pharmacology in the future.

Abbreviations

COX-1
Cyclooxygenase-1
COX-2
Cyclooxygenase-2
Dioscin 15
Dioscin 15 mg/kg group
Dioscin 30
Dioscin 30 mg/kg group
eNOS
Endothelial nitric oxide synthase
IL-10
Interleukin-10
IL-1β
Interleukin-1 beta
IMA
Ischemia-modified albumin
Indo
Indomethacin group
i.g.
Intragastric
iNOS
Inducible nitric oxide synthase
MDA
Malondialdehyde
NSAID
Nonsteroidal anti-inflammatory drugs
p-LC3B
Phosphorylated LC3B
p-NFκB p65
Phosphorylated nuclear factor-kappa B
PPIs
Proton pump inhibitors
PUD
Peptic ulcer disease
ROS
Reactive oxygen species
SOD
Superoxide dismutase
TNF-α
Tumor Necrosis Factor-alpha

Declarations

Data availability:

All data supporting the findings of this study are included in this manuscript. Additional data are available from the corresponding author upon reasonable request at baylan@sakarya.edu.tr

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Author Contributions

T.B.: Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Visualization, Supervision. **A.T.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Visualization, Supervision. **H.B.:** Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision. **D.G.E.:** Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision. **D.Ç.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Visualization, Supervision. **Ö.Ç.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Visualization, Supervision. **E.E.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Supervision. **S.B.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Supervision. **A.Ö.B.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Supervision. **M.F.:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Data curation, Supervision.

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Institutional Review Board Statement

All animal procedures were approved by the Atatürk University Faculty of Medicine Local Ethics Committee for Animal Research (Decision No: 136, Date: 27/05/2024) and and conducted at the Atatürk University Laboratory Animals Production and Research Center, the Department of Physiology, and the Department of Veterinary Pathology. All procedures strictly complied with the relevant guidelines and regulations.

Competing Interest

The authors declare no competing interests.

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Figures

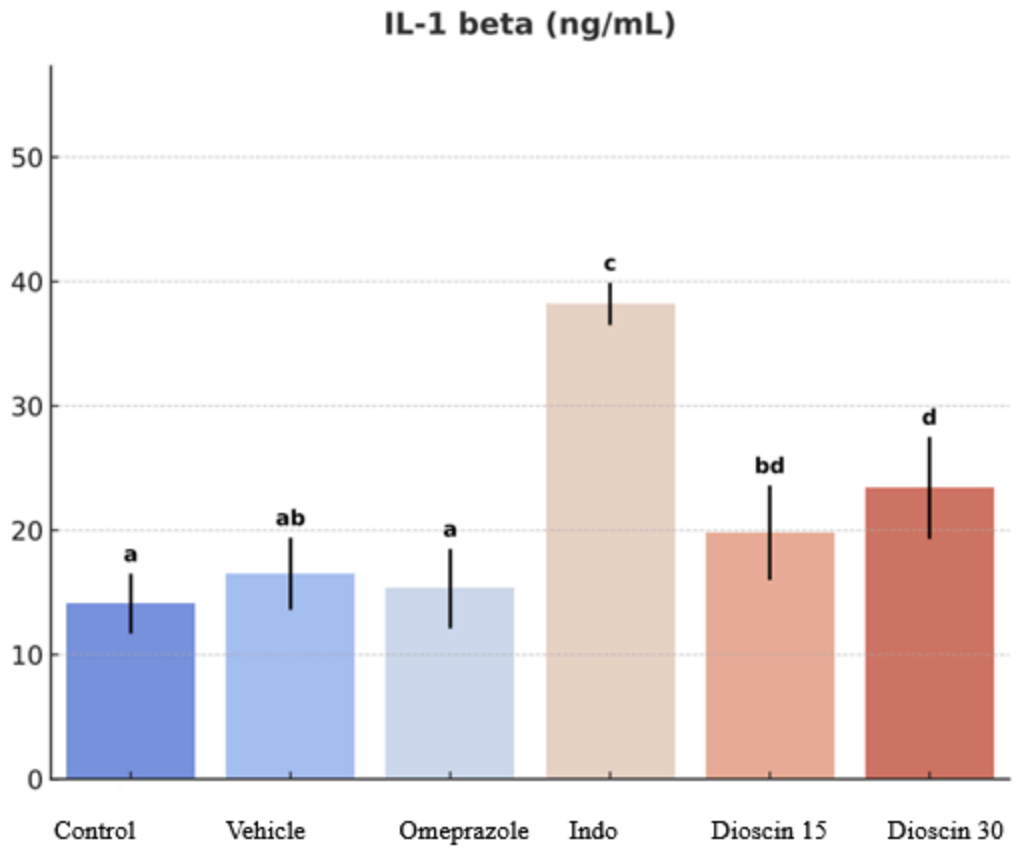


Figure 1

IL-1 beta findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

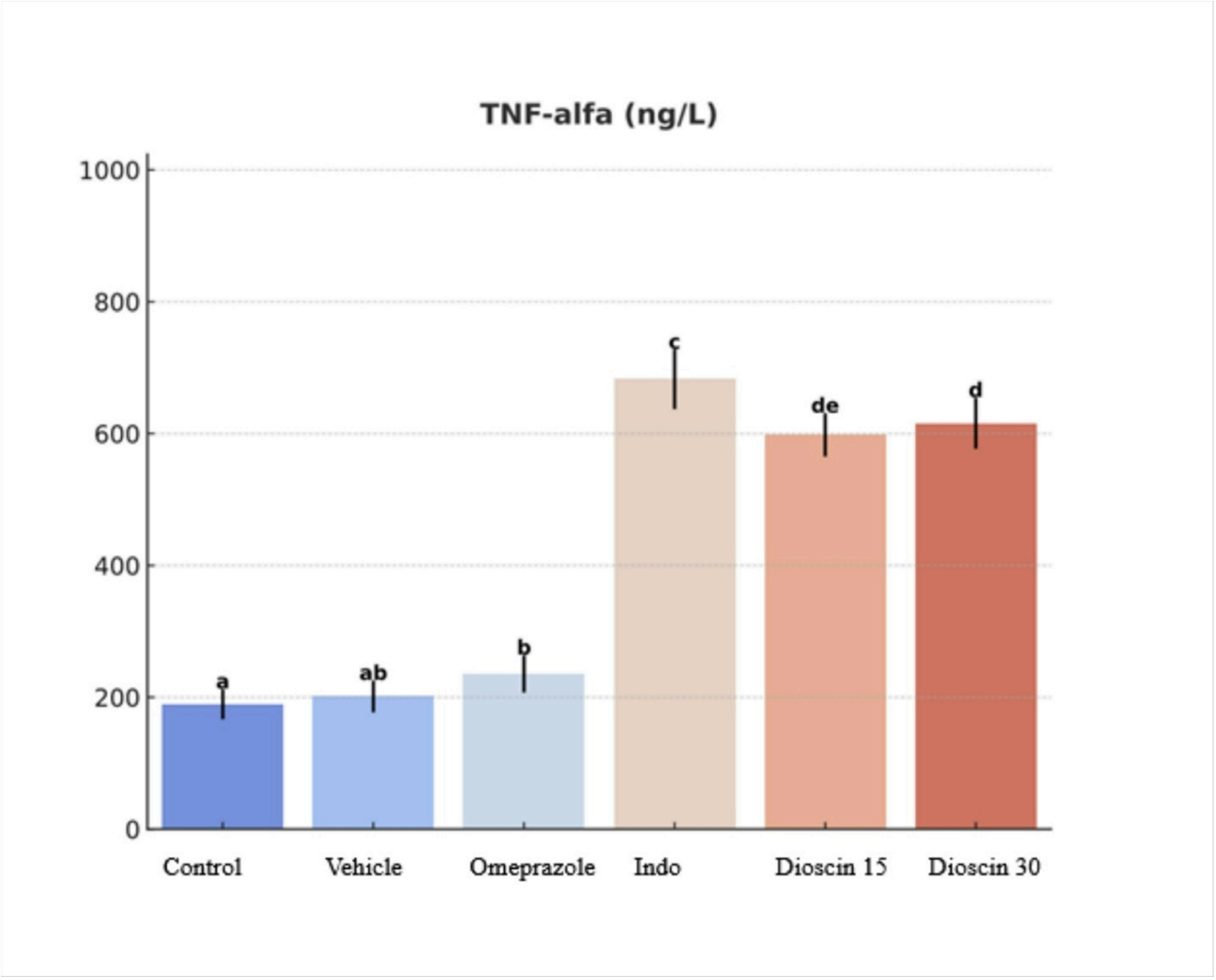


Figure 2

TNF-alfa findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

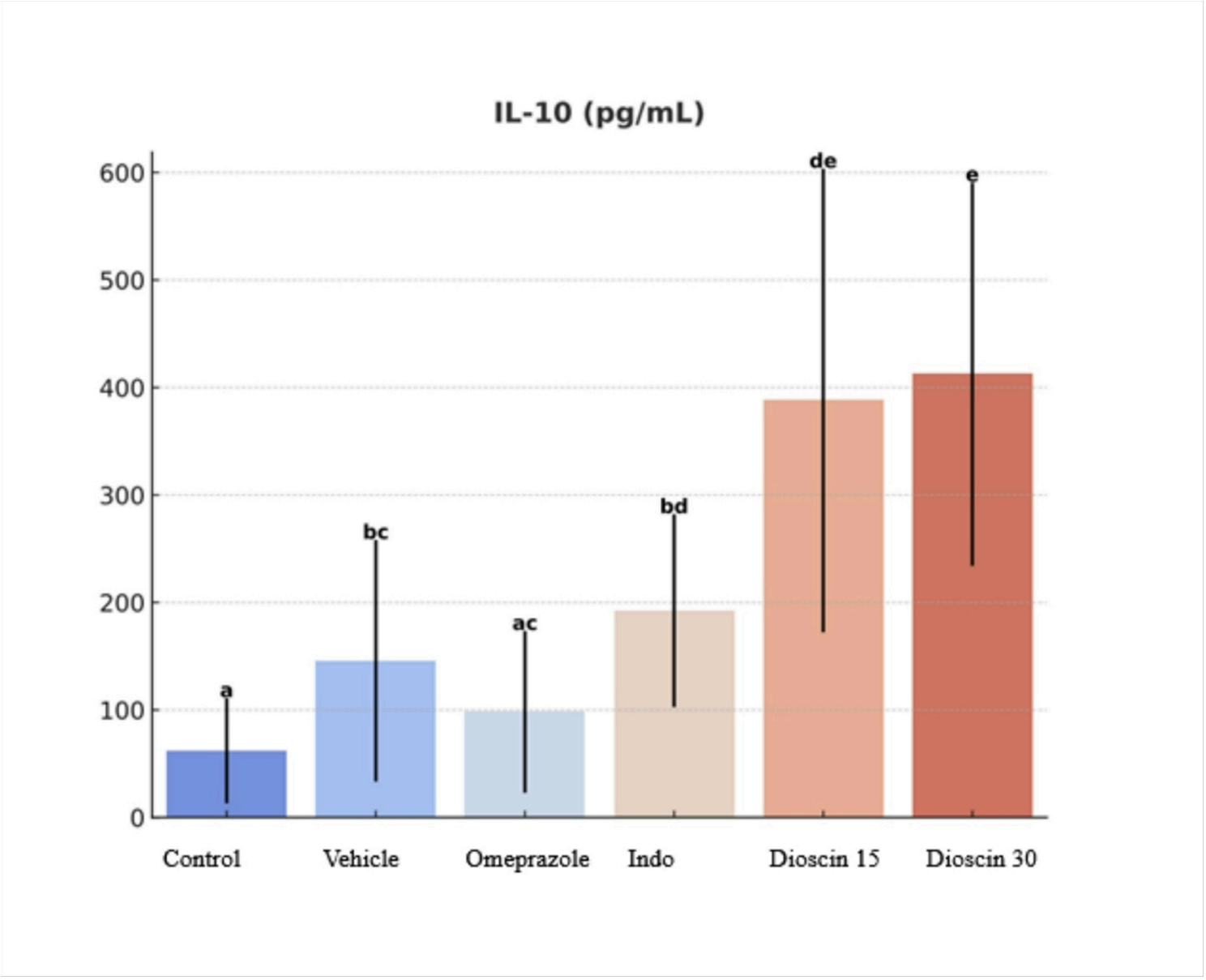


Figure 3

IL-10 findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

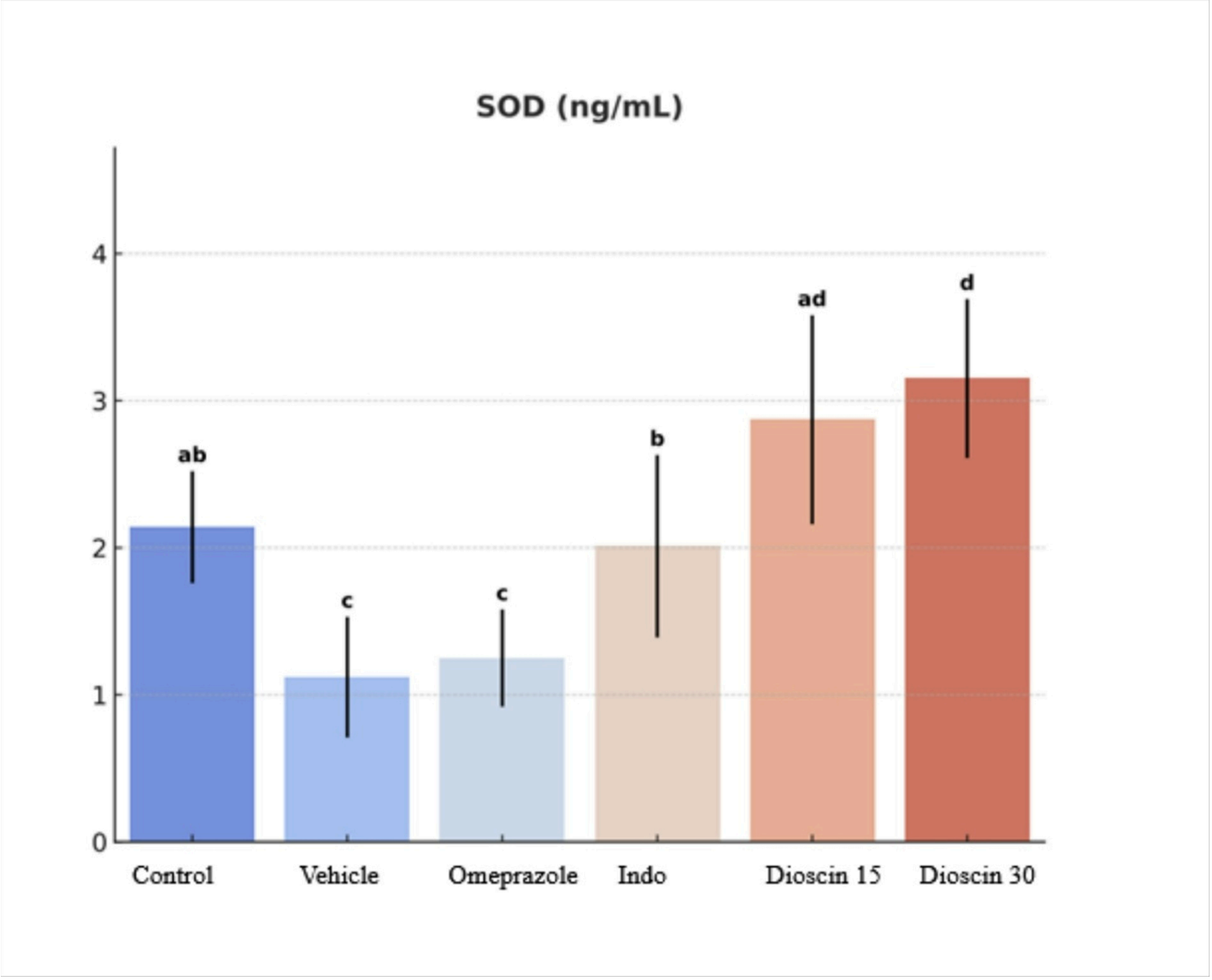


Figure 4

SOD findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

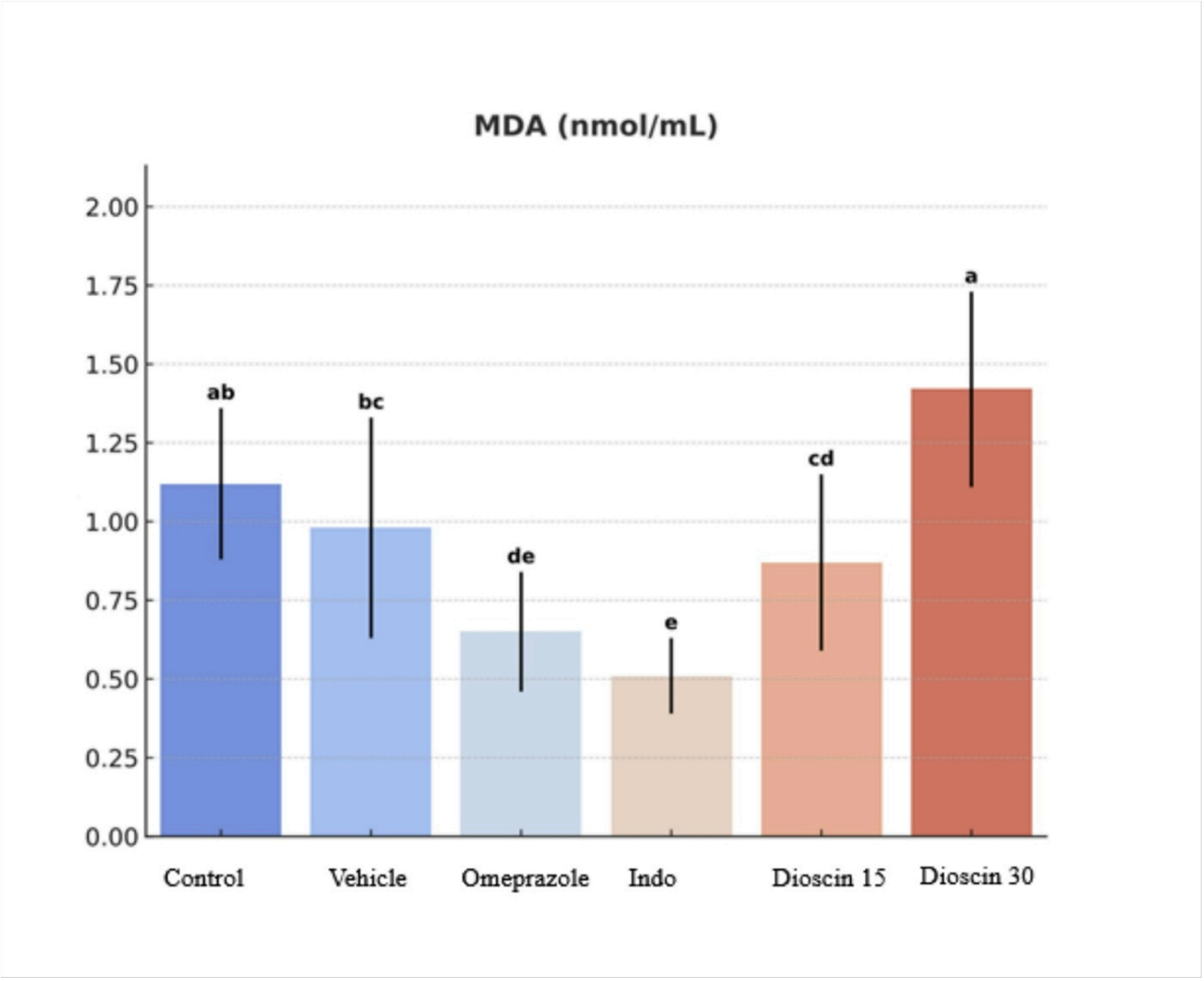


Figure 5

MDA findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

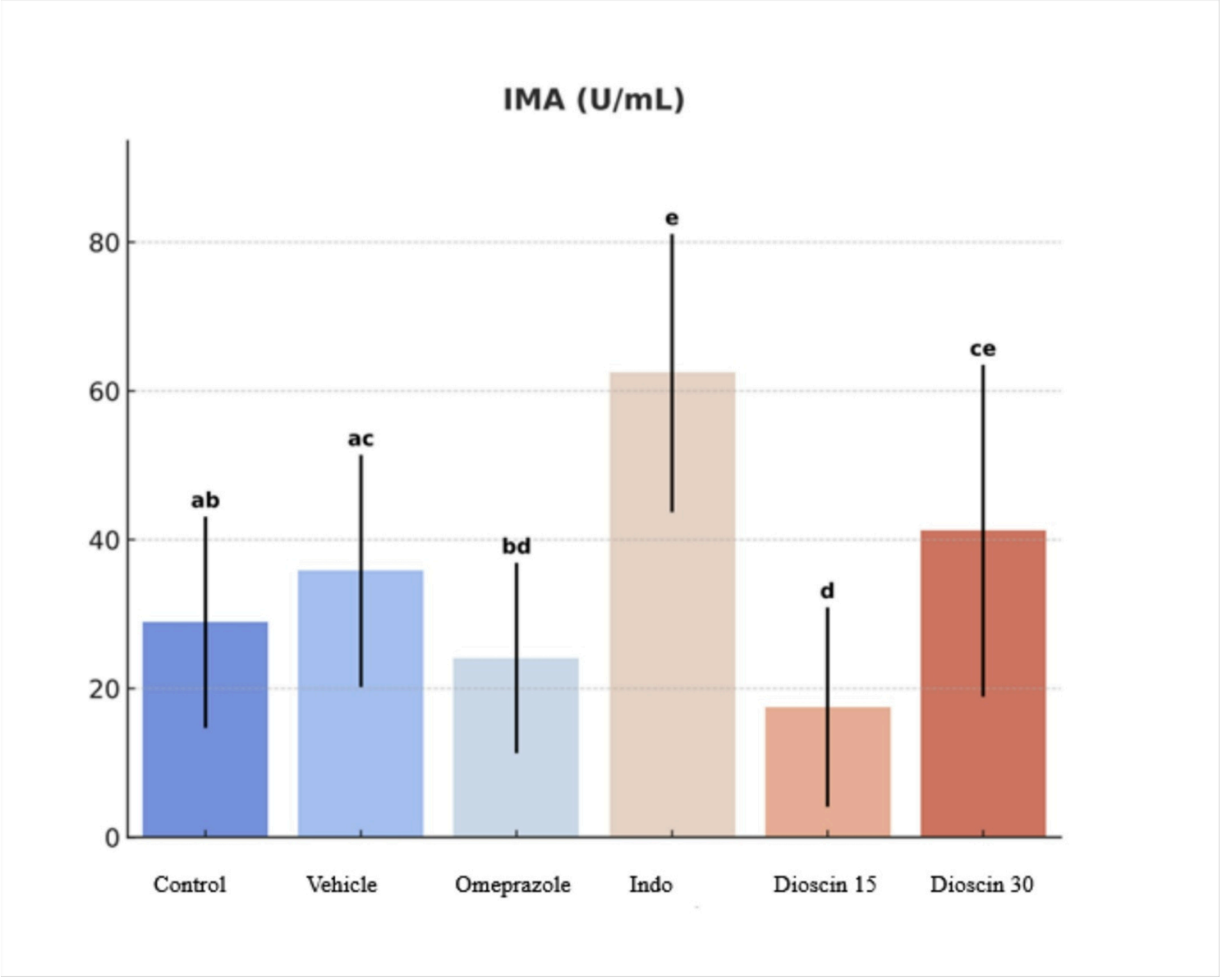


Figure 6

IMA findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

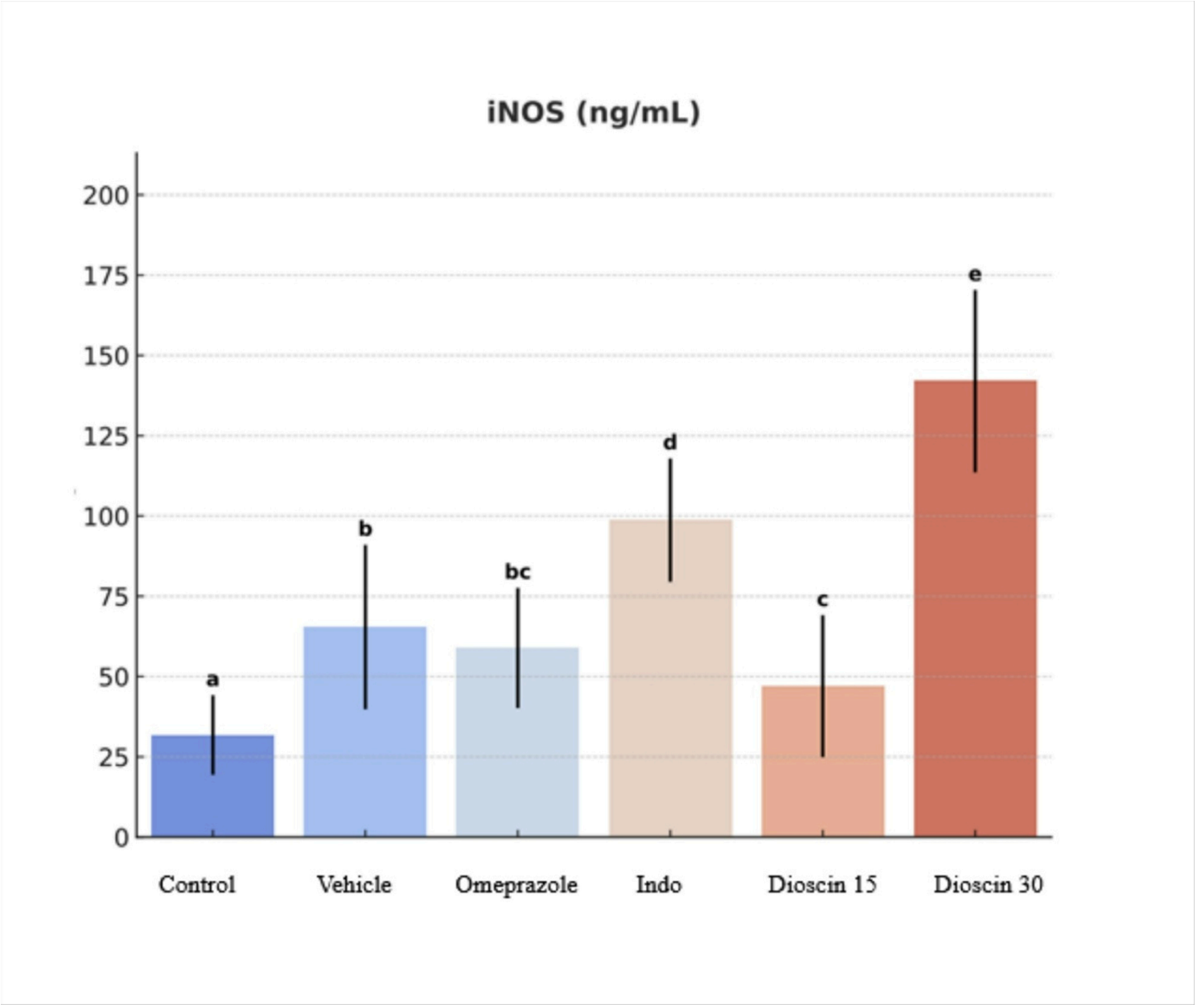


Figure 7

iNOS findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

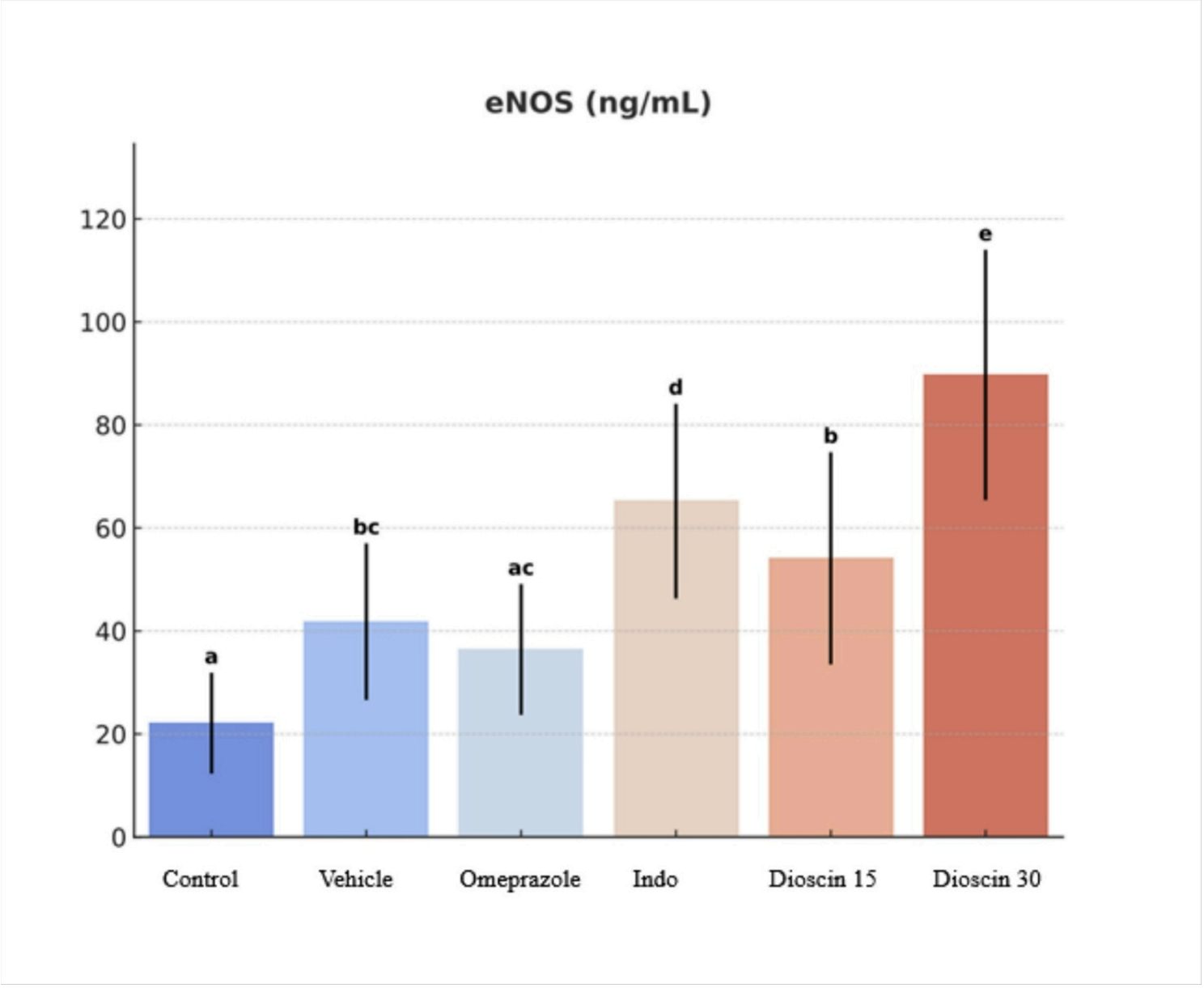


Figure 8

eNOS findings. Values are given as mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Tukey's test. Letters a, b, c, d, e indicate statistically significant differences between groups ($p < 0.05$).

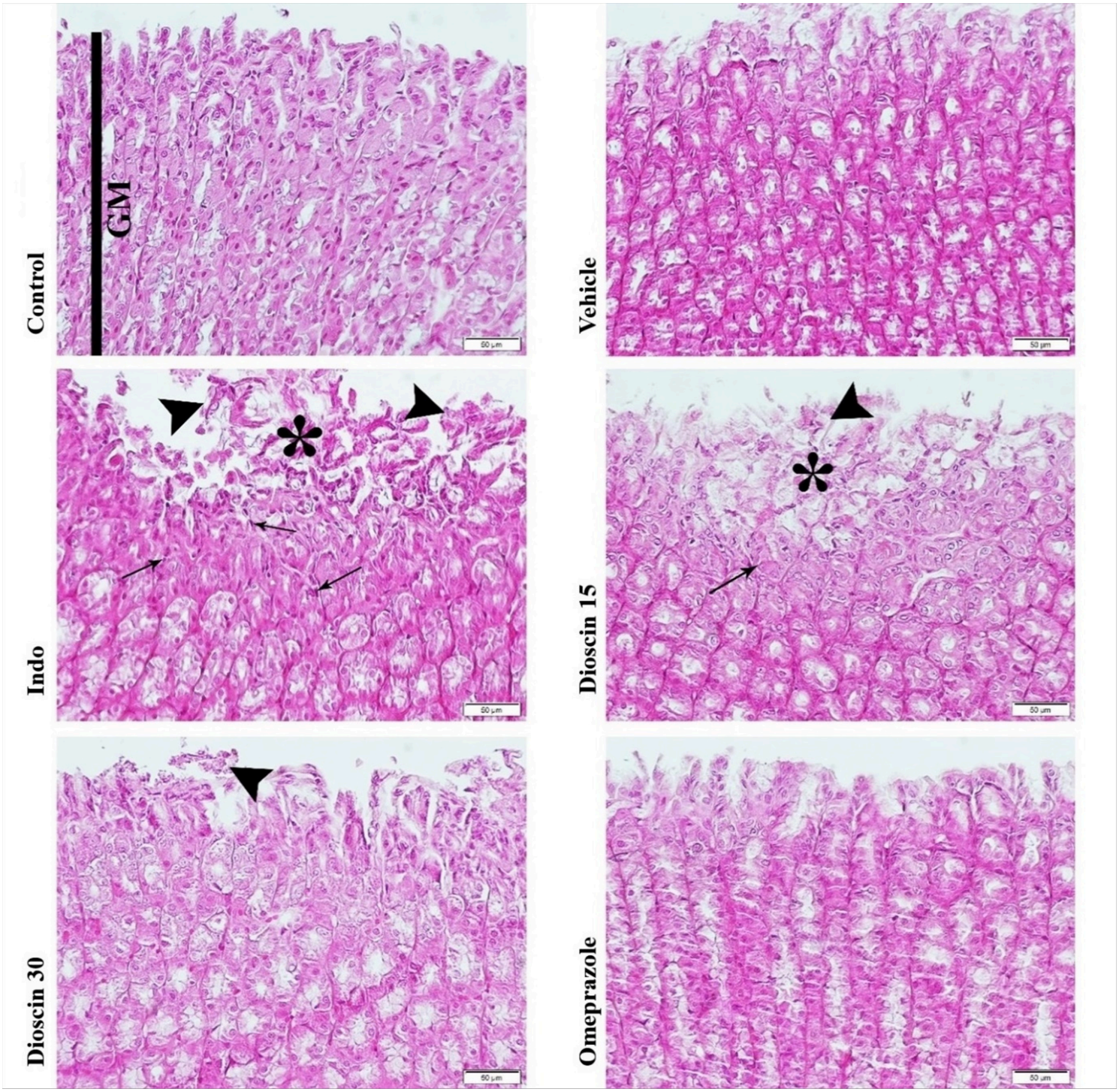


Figure 9

Histopathological Examination. Photomicrographs of gastric mucosa in different groups. Gastric mucosa (GM), epithelial cell detachment (arrowhead), ulcerative area and edema (star), inflammatory cell (arrow). Staining: H&E, magnification: 200X.

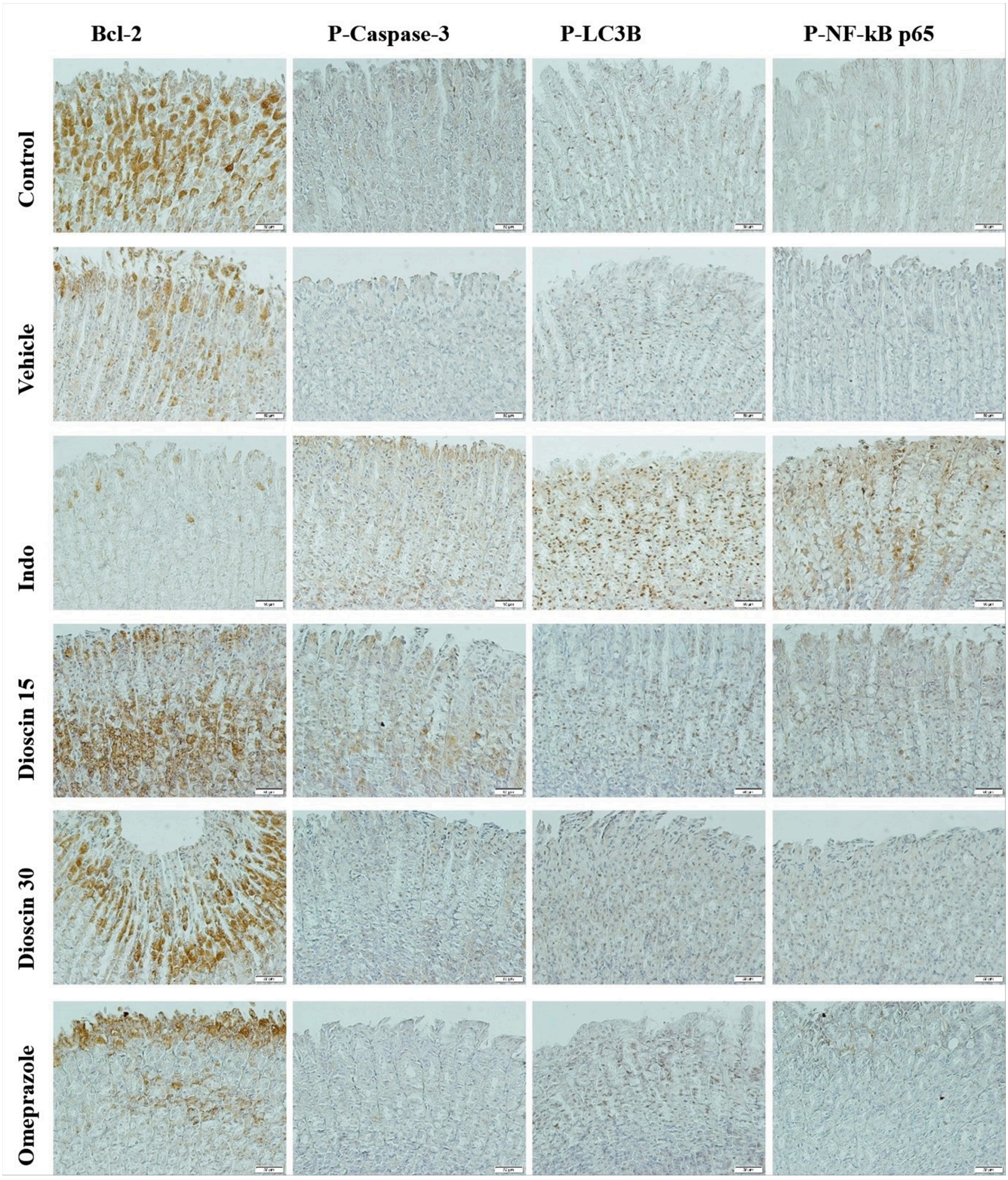


Figure 10

Immunohistochemical Examination. Immunoreactivities of Bcl-2, p-Caspase-3, p-LC3B and p-NF-kB p65 antibodies in all groups. Staining method: Streptavidin-Biotin Peroxidase Method. Magnification: 200X.

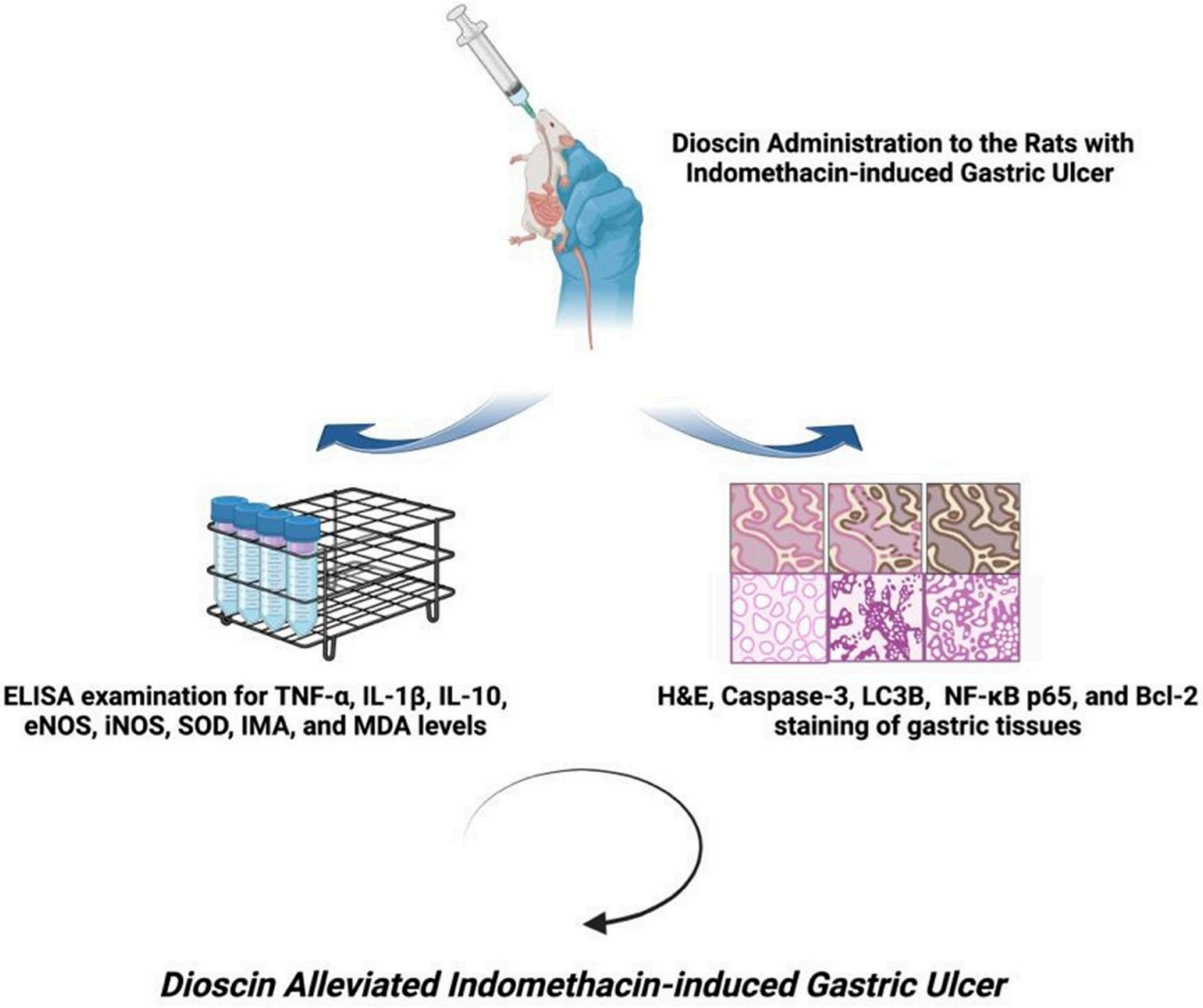


Figure 11

A summary of our study