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Potential Role of Laurus nobilis Essential Oil in Reducing Indomethacin-Induced Gastric Ulcer in Rats

Laurus nobilis Esansiyel Yağının Sıçanlarda İndometasin Kaynaklı Mide Ülserinin Azaltılmasında Potansiyel Rolü

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ABSTRACT

Objective: This study aimed to investigate the gastroprotective effects of Laurus nobilis leaves essential oil (LANO) against indomethacin (INDO)-induced gastric ulcers in rats.

Materials and Methods: In this study, an indomethacin-induced gastric ulcer model was employed. 30 Sprague-Dawley rats were divided into five groups (n=6): Control, LANO, INDO, INDO with famotidine (FAM), and INDO with LANO. Indomethacin (25 mg/kg) induced ulcers, while LANO and FAM were administered by oral gavage at 200 mg/kg and 40 mg/kg, respectively. Gastric tissues underwent histopathological examination for ulceration, and biochemical assays measured total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and nitric oxide (NO) levels.

Results: Compared to the INDO group, treatment with LANO significantly decreased the number of gastric ulcer foci. Biochemically, LANO moderated TOS and OSI levels and preserved TAS, indicating reduced oxidative stress. Additionally, LANO appeared to stabilize NO levels. These biochemical findings were corroborated by histopathological examination.

Conclusions: The study's results indicate that LANO may be beneficial in protecting against NSAID-induced gastric damage. LANO's observed modulation of oxidative stress markers and NO levels suggests its potential role in managing gastric ulcers.

Keywords: Gastric ulcer, indomethacin, Laurus nobilis, nitric oxide, oxidative stress

ÖZ

Amaç: Bu çalışmanın amacı, sıçanlarda indometasin (INDO) ile indüklenen gastrik ülserlere karşı Laurus nobilis yaprakları uçucu yağının (LANO) gastroprotektif etkilerini araştırmaktır.

Materyal ve Metot: Bu çalışmada, INDO ile indüklenen gastrik ülser modeli kullanıldı. 30 adet Sprague-Dawley sıçan beş gruba ayrıldı (n=6): Kontrol, LANO, INDO, famotidin (FAM) ile INDO, LANO ile INDO. INDO (25 mg/kg) ülserleri indüklerken, LANO ve FAM oral gavaj yoluyla sırasıyla 200 mg/kg ve 40 mg/kg olarak uygulandı. Gastrik dokular ülserasyon açısından histopatolojik incelemeye tabi tutuldu ve biyokimyasal analizlerle toplam oksidan durum (TOS), toplam antioksidan durum (TAS), oksidatif stres indeksi (OSI) ve nitrik oksit (NO) seviyeleri ölçüldü.

Bulgular: İndometasin grubuyla karşılaştırıldığında, LANO ile tedavi gastrik ülser odaklarının sayısını önemli ölçüde azalttı. Biyokimyasal olarak, LANO uygulaması TOS ve OSI seviyelerini düşürdü ve TAS seviyesini korudu, bu da oksidatif stresin azaldığını göstermektedir. Ayrıca, LANO'nun NO seviyelerini stabilize ettiği görüldü. Bu biyokimyasal bulgular histopatolojik inceleme ile desteklendi.

Sonuç: Çalışmanın sonuçları, LANO'nun INDO kaynaklı mide hasarına karşı korunmada faydalı olabileceğini göstermektedir. LANO'nun oksidatif stres belirteçleri ve NO seviyelerinde gözlenen modülasyonu, mide ülserlerinin yönetiminde potansiyel rolünü ortaya koymaktadır.

Anahtar Kelimeler: İndometasin, Laurus nobilis, mide ülseri, nitrik oksit, oksidatif stres

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INTRODUCTION

Gastric ulcers, common in peptic ulcer disease, occur due to an imbalance between mucosal defense and aggressive factors like acid and pepsin and are caused by various factors, including *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs (NSAIDs), stress, smoking, and genetics.[1] The pathogenesis of gastric ulcers involves disrupted mucosal defense, increased gastric acid secretion, and impaired epithelial repair mechanisms.[2]

Indomethacin (INDO), an NSAID, is known to inflict more significant damage on the gastric mucosa in rats than other similar medications. This damage includes vascular harm, ulcerations, and cell necrosis within the gastric lining. The INDO-induced gastric ulcer model serves as a key tool for exploring the mechanisms of gastric ulcer development and testing new treatments. Central to the damage caused by INDO is the increased production of reactive oxygen species, leading to oxidative stress. This process depletes key antioxidants like superoxide dismutase and glutathione in the stomach's defense system, resulting in harmful oxidation products that damage the gastric mucosa, underscoring the need for targeted therapeutic strategies to mitigate these effects[3].

Laurus nobilis (LANO), an evergreen tree native to the Mediterranean region, has been used traditionally for its medicinal properties. The leaves and fruits of LANO are known for their antibacterial, antioxidant, and gastroprotective properties.[4,5] These effects are primarily attributed to their rich composition of bioactive compounds such as flavonoids, tannins, and essential oils.[6] LANO oil's therapeutic potential in gastric ulcers is hypothesized to stem from its ability to enhance mucosal defense, reduce oxidative stress in gastric tissues, and possibly modulate gastric acid secretion.[7] The antioxidant properties of LANO, containing numerous compounds such as eucalyptol, α-terpinyl acetate, sabinene, α-pinene, and α-terpineol, may play a vital role in neutralizing free radicals, thus protecting the gastric mucosa from oxidative damage.[8,9] Additionally, the anti-inflammatory properties of LANO can mitigate inflammation, which is a key component in the pathogenesis of gastric ulcers.[10]

The study aimed to investigate the gastroprotective effects of LANO in treating gastric ulcers, focusing on the possible underlying mechanisms.

MATERIALS AND METHODS

Experimental Animals and Ethics

Ethics Committee Approval: The study was sanctioned by the Erzincan Binali Yıldırım University's Experimental Animals Local Ethics Committee (Date: 28.12.2023, decision no: 322655). The study strictly adhered to the ARRIVE guidelines and was conducted following the national regulations for the ethical use and care of laboratory animals.

Animals: For this research, we procured thirty Sprague–Dawley female rats, each weighing between 200-250 grams and aged around 10 to 12 weeks, from the Experimental Research and Application Center of Erzincan Binali Yıldırım University. During the experiment, the rats were allowed to consume standard pellet food and water without any restrictions. They were accommodated in an environment with controlled humidity and ambient temperature suitable for their well-being.

Experimental Design Models and Groups: In the study, thirty rats were divided into five groups (n=6). The groups were as follows: Control, *Laurus nobilis* (LANO), indomethacin (INDO), INDO+Famotidine (FAM), and INDO+LANO. The rat received oral administration of INDO at 25 mg/kg,[11] FAM at 40 mg/kg,[11] and LANO at 200 mg/kg[7].

LANO was administered prophylactically via gavage to the rats for seven days before the ulcer induction[7]. After this period, the rats fasted for 24 hours. On the seventh day, targeted groups received LANO and FAM. One hour after this administration, a 25 mg/kg dose of INDO was given to all groups except the Control and LANO groups to induce ulcers. Six hours after the INDO administration[11], rats were euthanized using an intraperitoneal injection of thiopental at 50 mg/kg, and their stomachs were excised for macroscopic examination and photography of ulcers.

Plant Materials and Essential Oil Extraction: LANO leaves from Hatay-Türkiye were cleaned and dried at 40 °C in a vacuum oven. Then, 5 grams were extracted using an ultrasonic bath at 18 kHz with 50 mL of 50:50 ethanol-water for 20 minutes at 40 °C, as per Tometri et al. The extract was filtered and concentrated using a rotary evaporator, and the essential oil was stored at −18 °C for experimental use.[12]

Tissue Preparation and Histopathological Examination: Gastric tissue samples were divided for analyses: one-third for biochemical assays and two-thirds fixed in 10% formalin for 24 hours. The fixed tissues were sectioned into 2 mm strips and processed with the Sakura Tissue-Tek VIP 6 AI system. Post-processing, they were embedded in paraffin, cut into 3 micrometer sections, and stained using hematoxylin and eosin for microscopic analysis, focusing on ulceration or necrosis. Histopathological examination was performed using an Olympus B70 light microscope. Tissue areas (mm^2) were calculated by measuring the size of the examined areas under the light microscope, employing standardized methods. In histopathological evaluation, ulceration/

necrotic foci were evaluated, and the number of ulcerated/necrotic foci was determined for each case. The Lesion Index was determined for each parameter to quantify the extent of histopathological changes. As defined by Natale et al., it was calculated using the formula: Lesion Index=[Length of the damaged area/total mucosal length]×100, expressing the percentage of mucosal damage.[13]Additionally, the number of foci exhibiting histopathological changes was recorded. To standardize the assessment, the focus score was calculated as the number of foci with changes per square centimeter of the sampling area (Focus Score=[Number of foci with change/ Sampling area mm^2]×100).

Biochemical Analyses: To determine oxidant and antioxidant parameters (TAS, TOS, OSI, and NO), a 100 mg sample of gastric tissue was weighed for each rat. These samples were homogenized on ice using a tissue homogenizer in pH 7.4 phosphate buffer.

Total Antioxidant Status (TAS) Analysis: The assessment of TAS in gastric tissues was performed following the established Erel method.[14] TAS in tissue was measured with a Rel Assay Diagnostics kit, converting ABTS radical to colorless, indicating antioxidant capacity, at 660 nm. Calibration used a Trolox equivalent, ensuring assay accuracy. CV% was <10%, standard range of 1.20-1.50 mmol/L. Results expressed in mmol/g protein.

Total Oxidant Status (TOS) Analysis: To quantify the TOS in gastric tissue, a methodology was developed by Erel[15] utilizing a kit from Rel Assay Diagnostics in Gaziantep, Türkiye. The method oxidized the iron ion-o-dianisidine complex to ferric form using tissue oxidants, enhanced by glycerol and measured under acidic conditions with xylenol orange by spectrophotometry, indicating oxidant levels. CV% was <10% for 4-6 µmol/L, calibrated with hydrogen peroxide, with results in µmol H2O2 equivalent per gram of protein.

Oxidative Stress Index (OSI) Calculation: OSI was computed using the formula OSI = (TOS/TAS*100).

Evaluation of Nitrate and Nitrite Concentrations: In the study, the measurement of nitrate (NO_3^-) and nitrite (NO_2^-) concentrations was crucial due to the transient nature of NO. NO quickly reacts within biological systems, forming NO_2, which equilibrates to N_2O_4 and subsequently yields NO_2^- and NO_3^- alongside water. These reactions culminate in the formation of N_2O_3, which also produces NO_2^- and water. Recognizing the variability of NO2 and NO3 levels, we assessed the total NO production by measuring both compounds' concentrations using the Cayman Nitrate/Nitrite Colorimetric Assay Kit (Item No: 780001). This kit converts NO_3^- to NO_2^- through nitrate reductase, allowing for comprehensive NO_2^- analysis, with a noted precision in its coefficient of variation (CV) at 2.7% for intra-assay and 3.4% for inter-assay variability.

Statistical Analysis: The normality assumption of the parameters was examined by the Shapiro-Wilk test, and the homogeneity of group variances was checked by Levene's test. The distribution of all parameters in the groups met the normality assumption. One-way ANOVA analysis was used to compare the measurement results of the parameters between the control and experimental groups. After one-way ANOVA analysis, pairwise differences between subgroups were analyzed by Tukey HSD (when the population variances of the groups were equal) or Games-Howell (when the population variances of the groups were not equal) post-hoc tests. The distribution of parameters in the groups was summarized with box-plot graphs. Box-plot graphs were drawn considering minimum, first quartile, median, third quartile and maximum values. SPSS (version 26.0, SPSS Inc., Chicago) package program was used for statistical data analysis. $p<0.05$ was considered statistically significant.

RESULTS

According to the histopathological analysis, as illustrated in Figure 1, the number of ulcerative lesions across the different treatment groups showed marked variation. The control group had no lesions, serving as a baseline. The INDO group showed significantly more lesions, indicating greater gastric damage. The INDO+FAM group had fewer lesions, suggesting famotidine's protective effect with INDO. The LANO group's lesion count was similar to the control, highlighting LANO's effectiveness. The INDO+LANO group had fewer lesions than INDO alone, but more than INDO+FAM.

The macroscopic and histopathological appearance of the gastric resection material of one case from each group is presented in Figure 2.

A comparison between LANO and INDO groups shows marked differences in ulcerative/necrotic foci burden and variations in lesion index and focus scores (Table 1). Notably, the INDO+LANO group exhibited significantly lower values, suggesting a potentially attenuated severity of lesions.

In the biochemical analyses, oxidative stress parameters and nitrate+nitrite values, highlighted in Figure

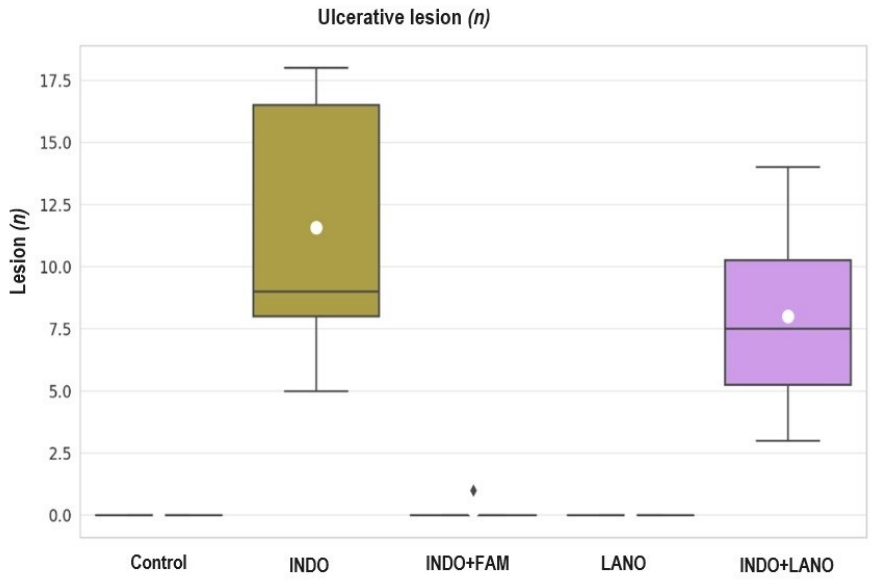

Figure 1. Comparing the number of lesions across various treatment groups. The box plots illustrate the distribution of lesion counts in the groups.

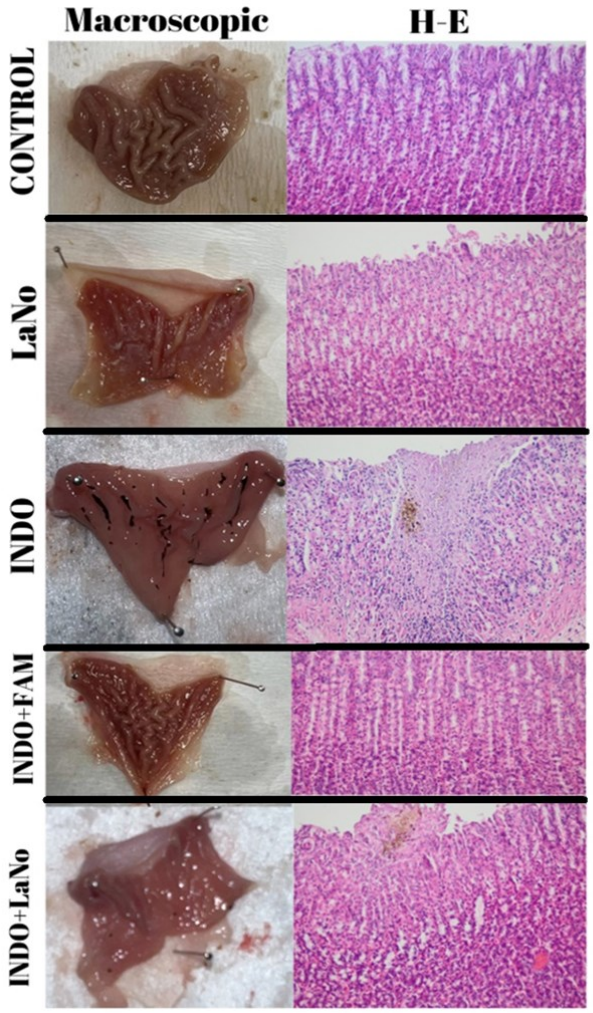

Figure 2. Macroscopic views and H&E staining findings in stomach tissue. Normal-appearing gastric mucosa with regular folds seen in Control, LANO and INDO+FAM groups. Extensive ulcerated areas of hemorrhagic gastric mucosa seen in INDO group. Focally ulcerated gastric mucosa seen in LANO group. Normal epithelial lining and underlying lamina propria seen in Control, LANO and INDO+FAM groups (H&E x200). Superficial mucosal ulcers in INDO+LANO groups (H&E x200). Deep and broad-based ulcer in INDO group (H&E x200).

Table 1. Ulcerative/Necrotic Focus, Lesion Index, and Focus Score in experimental groups.

Groups	Ulcerative/Necrotic Focus (n)	Lesion Index	Focus Score
Control	0	0	0
LANO	0	0	0
INDO	11.57	40	3.8
INDO+FAM	0.2	0.5	0.09
INDO+LANO	8	21	2.7

3, illustrate notable differences across the experimental groups. As shown in Figure 3A, TOS levels were significantly elevated in the INDO group compared to the control ($p<0.01$). However, the INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) groups demonstrated a marked reduction in TOS, suggesting the mitigating effects of famotidine and LANO when administered with indomethacin. Notably, there was no significant difference between the INDO+FAM and INDO+LANO groups ($p>0.05$). The LANO group showed TOS levels comparable to the control group, indicating no significant oxidative stress increase. Conversely, TAS experienced a significant decrease in the INDO group, indicative of depleted antioxidant reserves due to oxidative stress. Treatments with INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) presented a partial restoration of TAS levels, with no significant difference between them ($p>0.05$). The LANO group's TAS levels remained on par with the control group, highlighting its antioxidative potential (Figure 3B). For OSI, which represents the ratio of TOS to TAS, a substantially higher level was observed in the INDO group ($p<0.01$). This level significantly decreased in the INDO+FAM ($p<0.01$) and INDO+LANO ($p<0.01$) groups, with no significant difference between them ($p>0.05$), underscoring an improved oxidative stress regulation with these combination therapies. The OSI of the LANO group was similar to that of the control, further reinforcing its role in

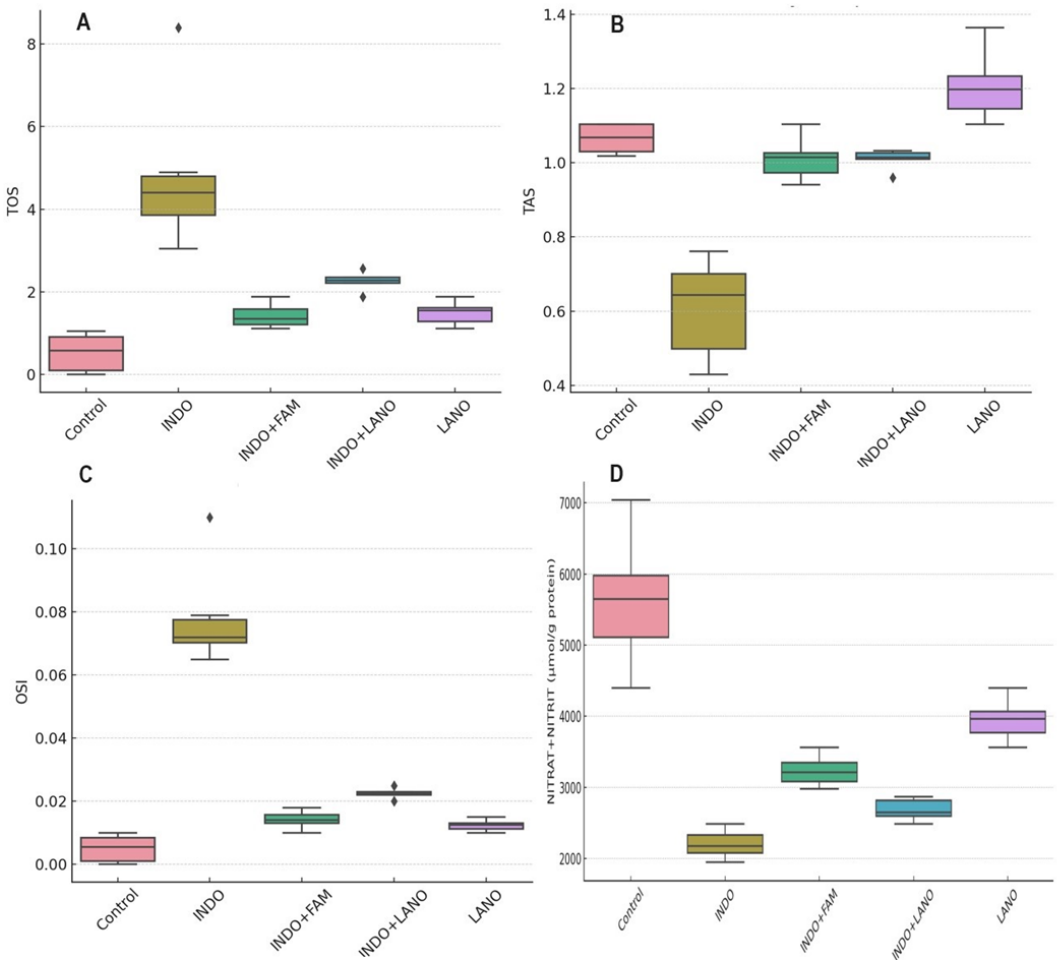

Figure 3. Oxidative stress parameters and Nitrat+Nitrit (NO) values across treatment groups. The box plots display the distribution of Total Oxidant Status (TOS), Total Antioxidant Status (TAS), and Oxidative Stress Index (OSI) among the groups.

protecting against oxidative stress (Figure 3C). Figure 3D delineates the nitrate and nitrite concentrations among the different treatment groups. In the INDO group, a significant decrease in median values of these markers was observed ($p<0.01$ for INDO vs. INDO+FAM; $p<0.05$ for INDO vs. INDO+LANO), suggesting a potential suppression in the synthesis or an increased turnover of nitrate and nitrite due to INDO treatment. However, the treatment groups receiving INDO+FAM and INDO+LANO presented nitrate and nitrite levels with median values approaching those of the control group. Specifically, the INDO+FAM group exhibited nitrate and nitrite levels notably closer to the control group's median ($p<0.01$ compared to the INDO group), indicating a significant mitigation of indomethacin's effect. No substantial difference in nitrate and nitrite levels was observed between the INDO+FAM and INDO+LANO groups ($p>0.05$), suggesting comparable efficacy in normalizing these parameters.

DISCUSSION AND CONCLUSION

In this study, the anti-ulcerative potential of LANO was assessed in a rat model with INDO-induced gastric ulcers. Through histopathological and biochemical methodologies, the study scrutinized LANO's therapeutic impact.

Stress-induced gastric ulceration involves complex etiopathological factors, leading to inconsistent pharmacotherapy and exploring new strategies.[16] Gastric distress and ulceration result from dietary imbalances, NSAID misuse like INDO, and altered gastric acid. Gastrointestinal damage stemming from inflammatory, oxidant, and cytotoxic activities overpowers mucosal defenses. It's linked to disrupted gastric defenses like acid/enzyme secretion, tissue integrity, and prostaglandin-aided mucosal protection.[17,18] In experimental gastroenterology, the INDO-induced stress ulcer model is commonly used to mimic NSAID-related ulceration for research.

Nitric oxide, a widely distributed signalling molecule and free radical, is naturally produced in the body, and its release from certain non-steroidal anti-inflammatory drugs helps mitigate gastrointestinal toxicity linked to traditional NSAID use.[19] Gastric ulcers are related to disrupted NO synthesis and activity, exacerbated by INDO's inhibition of prostaglandin synthesis, crucial for gastric lining protection.[19,20] Prostaglandins and NO are considered to work synergistically to maintain gastric integrity. Indeed, in our study, we observed that INDO administration significantly down-regulated nitric oxide levels, which aligns with the suppression of endogenous prostaglandin production.[21-23] This finding mirrors the literature, where reduced NO availability is closely associated with increased gastric vulnerability and ulceration.[21,24] Our findings indicate LANO application may counteract indomethacin's negative effects, supporting gastric protection via the NO pathway. This is evidenced by reduced lesion formation, highlighting a potential therapeutic strategy for NSAID-induced gastropathy.

The hallmark of INDO-induced gastric ulceration is the presence of macroscopic ulcerative foci within the gastric mucosa, a phenomenon well-documented in the literature.[11,25] Histopathological analysis showed significant ulcer foci in the gastric tissues of the INDO group rats, but treatments with famotidine and LANO notably reduced these ulcers. Additionally, a study by Stefanova et al. found that LANO essential oil demonstrated antimicrobial activity against pathogenic and spoilage microorganisms, potentially relevant to its therapeutic effects.[26] Furthermore, in a study, Yazıcı et al. observed that 0.3% laurel oil in the diet of Nile tilapia didn't cause histopathological changes but rather improved liver and intestinal tissues.[27] The reduction in ulceration with LANO treatment may be attributable to its anti-inflammatory constituents,[7] which could exert protective effects similar to those of prostaglandin-mediated mucosal defense mechanisms disrupted by INDO.

The intricate relationship between oxidative stress and the pathophysiology of gastric ulcers has been increasingly recognized in gastroenterological research.[11] Oxidants, such as reactive oxygen species (ROS), are pivotal in exacerbating gastric mucosal damage, often overwhelming the natural antioxidant defenses and leading to cellular injury and ulcer formation.[27,28] Our study revealed that INDO administration significantly increased TOS and OSI while decreasing TAS, thereby indicating elevated oxidative stress in the gastric environment. This aligns with previous studies that have established the role of oxidants in developing NSAID-induced gastric ulcers.[22,28] LANO showed promising effects with diverse phytochemicals. It displayed significant antioxidant, anti-diabetic, and antimicrobial effects at low concentrations, therapeutic potential.[29] The LANO-treated groups showed decreased TOS and preserved TAS, resulting in a lower OSI than the INDO group. This suggests LANO's potential to enhance mucosal defense by boosting antioxidant capacity or scavenging ROS, thereby mitigating oxidative damage in gastric tissue. This is corroborated by a study by Mssillou et al., which found that essential oil from LANO flowers exhibited significant antifungal and antioxidant activities, attributed to high levels of 1,8-cineole, further underscoring the importance of antioxidants in managing gastric ulcers.[30]

In conclusion, our study revealed that LANO mitigates INDO-induced gastric ulcers, reducing ulcer foci, normalizing TOS, TAS, and OSI levels, and stabilizing NO dynamics, suggesting its potential for

clinical use. However, further research is needed to understand its mechanisms.

Ethics Committee Approval: The work described in this article has been carried out by the Erzincan Binali Yıldırım University's Experimental Animals Local Ethics Committee (Date: 28.12.2023, decision no: 322655).

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept - HÜ, FDK; Supervision – HÜ, GI; Materials- HÜ, NK, SK; Data Collection and/or Processing- HÜ, NGT, NK, FDK; Analysis and/or Interpretation - NK, GI, SK; Writing - HÜ, GI.

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