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Protective effects of *Centaurium minus* oil against gastric ischemia-reperfusion injury via oxidative stress and TLR4/NF-κB modulation

Efectos protectores del aceite de *Centaurium minus* frente a la lesión por isquemia-reperfusión gástrica mediante la modulación del estrés oxidativo y la vía de señalización TLR4/NF-κB

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ABSTRACT

Gastric ischemia-reperfusion (GI/R) injury was established by 30 min of celiac artery occlusion followed by 60 min of reperfusion. Gastric tissues were evaluated biochemically for superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA), as well as histopathological and immunohistochemically for transforming growth factor-beta (TGF-β), toll-like receptor 4 (TLR4), and nuclear factor kappa B (NF-κB) expression. GI/R markedly increased MDA levels while reducing SOD and CAT activities compared with the control group. Histopathological examination showed epithelial cell loss, mucosal edema, hemorrhagic damage, and inflammatory cell infiltration in the groups subjected to GI/R injury. Immunoreactivities of TGF-β, TLR4, and NF-κB were significantly increased after GI/R injury. Pre-treatment with *Centaurium minus* oil significantly improved activities of antioxidant enzymes, reduced lipid peroxidation, attenuated histopathological damage, and suppressed expression of TGF-β, TLR4, and NF-κB. These findings indicate that *C. minus* oil exerts gastroprotective effects against GI/R injury by enhancing antioxidant defenses and attenuating TLR4/NF-κB-mediated inflammatory responses. Therefore, *C. minus* oil may represent a promising natural therapeutic agent for the prevention of gastric ischemia-reperfusion injury.

Key words: *Centaurium minus*; gastric ischemia-reperfusion; oxidative stress; TLR4; NF-κB

RESUMEN

La lesión por isquemia-reperfusión gástrica (I/R) se estableció mediante 30 min de oclusión de la arteria celíaca seguidos de 60 min de reperfusión. Los tejidos gástricos se evaluaron bioquímicamente para superóxido dismutasa (SOD), catalasa (CAT) y malondialdehído (MDA), así como histopatológicamente e inmunohistoquímicamente para la expresión del factor de crecimiento transformante beta (TGF-β), el receptor tipo Toll 4 (TLR4) y el factor nuclear kappa B (NF-κB). La I/R aumentó marcadamente los niveles de MDA mientras redujo las actividades de SOD y CAT en comparación con el grupo control. El examen histopatológico mostró pérdida de células epiteliales, edema de la mucosa, daño hemorrágico e infiltración de células inflamatorias en los grupos sometidos a lesión por GI/R. Las inmunorreactividades de TGF-β, TLR4 y NF-κB aumentaron significativamente después de la lesión por I/R. El pretratamiento con aceite de *Centaurium minus* mejoró significativamente la actividad de las enzimas antioxidantes, redujo la peroxidación lipídica, atenuó el daño histopatológico y suprimió la expresión de TGF-β, TLR4 y NF-κB. Estos hallazgos indican que el aceite de *C. minus* ejerce efectos gastroprotectores contra la lesión por isquemia-reperfusión gastrica (I/R) al potenciar las defensas antioxidantes y atenuar las respuestas inflamatorias mediadas por TLR4/NF-κB. Por lo tanto, el aceite de *C. minus* podría representar un agente terapéutico natural prometedor para la prevención de la lesión por isquemia-reperfusión gástrica.

Palabras clave: *Centaurium minus*; isquemia-reperfusión gástrica; estrés oxidativo; TLR4; NF-κB

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INTRODUCTION

The celiac artery, which arises as the first major branch of the abdominal aorta, is a major source of arterial blood supply to the stomach [1]. Ligation or occlusion of this artery can result in severe gastric perfusion disturbances and subsequent ischemia-reperfusion (I/R) injury [1, 2, 3].

Gastric ischemia-reperfusion (GI/R) injury is a severe pathological condition that can happen during hemorrhagic shock, peptic ulcer bleeding, microvascular circulatory disorders, and various gastrointestinal diseases [3, 4]. This condition leads to severe morbidity and mortality due to tissue integrity loss and organ function compromise [1, 2, 4].

Despite advances in modern medical technology, the in-hospital mortality rate related to I/R injury in the digestive system has been reported to range between 60 and 80% [5].

Although various pharmacological and natural agents have been evaluated in experimental and clinical research for the treatment of GI/R injury, there is currently no effective approach recognized as a standard of care [4, 5, 6]. Therefore, there is a need to investigate new and alternative treatment approaches that target mechanisms such as inflammation, apoptosis, and oxidative stress, which play a role in the pathogenesis of GI/R injury.

Different experimental models of ischemia were developed to study these pathophysiological processes. Of these, celiac artery ligation is the most common surgical model used to induce ischemia in upper abdominal organs and study the pathophysiological changes that occur after reperfusion [6, 7, 8].

In recent years, interest in essential oils from medicinal plants for use in alternative medicine has been steadily increasing [9]. The literature reports that essential oils possess antioxidant, antiapoptotic, antidiabetic, and organ-protective effects against damage in various organs [9, 10, 11, 12].

Centaurium minus Moench (syn. *Centaurium erythraea*), is a biennial herbaceous plant belonging to the Gentianaceae family and is widely distributed in Europe, the Mediterranean basin, North Africa, and Western Asia [13]. *Centaurium* species are of pharmaceutical interest due to the active secoiridoid compounds they contain [13, 14]. It was also reported that certain secoiridoids isolated from the plant have therapeutic potential, especially for liver diseases [15]. Other studies reported that extracts and essential oils derived from *Centaurium* species have potent anti-inflammatory and antioxidant activities [13, 16].

Experimental investigations have shown that the plant's extract reduces the formation of reactive oxygen species (ROS), inhibits lipid peroxidation, and enhances endogenous antioxidant defense systems such as SOD, CAT, and GPx [13, 16, 17]. Additionally, it has been reported that the plant's essential oils exhibit anti-inflammatory properties in various experimental models [9, 18].

Given the regulatory effects of oxidative stress and inflammation on the underlying pathogenic mechanisms of GI/R injury, this suggests that *C. minus* oil may possess a protective potential against GI/R injury. To the best of our knowledge, there is no research in the literature that has evaluated the protective effects of *C. minus* oil in an experimental gastric ischemia-reperfusion model at the biochemical, histopathological, and immunohistochemical levels.

The aim of this research was to investigate the potential protective effects of *C. minus* oil in an experimental GI/R model from biochemical, histopathological, and immunohistochemical perspectives.

MATERIAL AND METHODS

Oil chromatograms

The oil preparation of *C. minus* used in this study was supplied by Smart Kimya Inc. (Tito, Smart Kimya Inc., Türkiye). A commercially available *C. minus* maceration oil (70% *C. minus*, 30% olive oil) produced by olive-oil maceration followed by cold pressing was used in this study. The product was manufactured under registered food production standards (TR-35-K-047442) and stored under cool and dry conditions until experimental use.

Experimental animal and surgery

This experimental study was conducted after receiving approval No. 2025/10–06 from the Local Ethics Committee of Hatay Mustafa Kemal University (HMKU), with animal welfare taken into consideration. All rats (*Rattus norvegicus*) were housed in individual cages in rooms maintained at an ambient temperature of 24 ± 2°C and humidity of 60%, under a 12:12-hour light-dark cycle, with *ad libitum* access to water and food. A total of 35 male Wistar Albino rats weighing between 250 and 270 g, were divided into the following groups: Control (C), Control+GI/R (C+GI/R), Vehicle+GI/R (VHC+GI/R), *C. minus* oil+GI/R (CM+GI/R), and Lansoprazole+GI/R (LAN+GI/R).

Experimental groups

- Group C: The healthy control group, which was given physiological saline (2 mL·kg^{-1}) 30 min before the procedure and underwent laparotomy only,
- Group C+GI/R: The ischemic control group, which was given physiological saline (2 mL·kg^{-1}) 30 min before the procedure and underwent GI/R injury,
- Group VHC+GI/R: A carrier group that was administered olive oil (2 mL·kg^{-1}) 30 min before the procedure and in which GI/R injury was induced,
- Group CM+GI/R: A treatment group that was administered *C. minus* oil (2 mL·kg^{-1}) 30 min before the procedure and in which GI/R injury was induced,
- Group LAN+GI/R: The treatment group administered lansoprazole (30 mg·kg^{-1}) 30 min before the procedure and subjected to GI/R injury.

The treatment protocols for the experimental groups were administered orally via gavage for 7 days (d). On the final day the GI/R procedure was performed, the final dose was administered 30 min before ischemia induction. The selected doses of *C. minus* oil (2 mL·kg^{-1}) was determined with reference to previous studies investigating the biological effects and safety of *Centaurium species*

[19, 20], as well as studies reporting the gastroprotective effects of orally administered plant oils [21]. In these studies [19, 20], it was reported that there were no adverse effects even at high levels of exposure to the agent.

Gastric-ischemia reperfusion model

The GI/R model was established following the methods described in previously reported studies [6, 7, 8]. Prior to the experiment, all rats were anesthetized with intramuscular (i.m.) doses of Xylazine (Xylazinbio 2%, İnterhas, Türkiye), (10 mg·kg^{-1}) and Ketamine (Keta-control, Doğa İlaç, Türkiye), (80 mg·kg^{-1}). Following anesthesia, the abdominal area was shaved and aseptically prepared with povidone-iodine (Batticon, Adeka, Türkiye), after which a midline laparotomy was performed. The celiac artery was carefully dissected and exposed, and a 30 min ischemia followed by 1 h of reperfusion was induced using an atraumatic Yaşargil aneurysm clip (Aesculap, FE693K, Germany). In the control group, the same surgical procedure was carried out without ligation of the celiac artery.

After the reperfusion period was complete, all animals were euthanized under Xylazine and Ketamine anesthesia. Gastric tissues were rapidly excised, rinsed with saline, and photographed using an iPhone 16 (Apple Inc., Cupertino, CA, USA) for macroscopic examination. While some tissue samples were stored at −80°C (UniFreez U series, DAIHAN Scientific, Korea) for biochemical analysis, others were fixed in 10% neutral buffered formalin (Merck, Darmstadt, Germany) for histopathological and immunohistochemical examinations.

Histopathological and immunohistochemical evaluation

Following euthanasia under Xylazine and Ketamine anesthesia, the stomach tissues were rapidly excised, rinsed with physiological saline, and immediately fixed in 10% neutral buffered formalin for 48 h for histopathological and immunohistochemical evaluation. Subsequently, the formaldehyde was removed by washing the tissues in tap water for 12 h, and they underwent routine tissue processing. These procedures consisted of a series of 70%, 80%, 90% and absolute alcohol and xylene solutions, after which the tissues were embedded in paraffin. 5 μm (Leica RM 2125RT, Nussloch, Germany) sections cut from the paraffin blocks were stained with hematoxylin-eosin (H-E) and were evaluated under a light microscope (Olympus BX51, Tokyo, Japan).

The histopathological assessment between the experimental groups was performed semi-quantitatively by an experienced pathologist who was unaware of the research. In this context, the samples were evaluated for epithelial cell loss (score: 0–3), mucosal edema (score: 0–4), hemorrhagic damage (score: 0–4), and inflammatory cell infiltration (score: 0–3) [22].

Sections taken from paraffin blocks were mounted on slides and incubated (Dedeoğlu, İstanbul, Türkiye) at 60°C for 15–20 min. Immunohistochemical staining was carried out using a commercially available kit according to the manufacturer's protocol, as previously described [23]. TGF-β (Affinity Biosciences, AF1027, USA, 1:200 dilution), NF-κB (Affinity Biosciences, AF5006, USA, 1:200 dilution), and TLR4 (Affinity Biosciences, AF7017, USA, 1:200 dilution) primary antibodies were used in the study.

In addition, the negative control groups were treated with PBS instead of antibodies. Diaminobenzidine (DAB) served as the chromogenic substrate, and the sections were counterstained with Mayer's hematoxylin. Immunohistochemical assessment was conducted semi-quantitatively by an experienced pathologist who was unaware of the research (0: absent; 1: mild; 2: moderate; 3: severe; 4: very severe) [23].

Biochemical analysis

Gastric tissues were washed in cold saline. Approximately 100 mg of tissue sample was taken for homogenization of tissue samples to be used in biochemical analyses and homogenized in 1 mL of phosphate-buffered saline (PBS) using a tissue homogenizer (DLAB D-160 Homogenizer, China). The homogenates were subjected to centrifugation (Hettich Mikro 200 (2400), Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 2376 × g for 5 min at +4°C, and the resulting supernatants were collected. The obtained supernatants were analyzed using the ELISA. MDA (E0156Ra/BT LAB, China) levels, SOD (E0168Ra/BT LAB, China), and CAT (E0869Ra/BT LAB, China) activities were determined using an ELISA reader device following the manufacturer's instructions.

Statistical analysis

The results obtained from the experimental groups were assessed using SPSS 25.0. Biochemical findings were compared using ANOVA with Duncan's *post hoc* test; pathological findings were compared using the Kruskal-Wallis test and the Mann-Whitney U test between groups. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Fatty acid analysis results

The fatty acid chromatograms for olive oil (OO) and *Centaurium minus oil* (CMO) are shown in FIGURE 1, and their fatty acid compositions are presented in TABLE I. The fatty acid profile of *C. minus* oil was characterized predominantly by oleic acid (67.00%), followed by palmitic acid (13.17%) and linoleic acid (9.46%). The overall fatty acid composition was broadly similar to that of olive oil, although minor differences were observed in the proportions of individual fatty acids.

The fatty acid profile of *C. minus oil* is relevant to interpreting the biological effects observed in this study. In both *C. minus oil* and *olive oil*, oleic acid was the main fatty acid, while the proportions of the other major fatty acids differed only slightly. The gastroprotective effects observed, therefore, cannot be explained solely by differences in the oils' overall fatty acid composition. Bioactive phytochemicals derived from *C. minus* may also contribute to the activity of its oil, particularly in light of the reported antioxidant and anti-inflammatory properties of *Centaurium* species [9, 13]. Still, because the present study did not isolate or assess individual fatty acids or phytochemical constituents separately, the specific contribution of each to the observed effects remains undetermined.

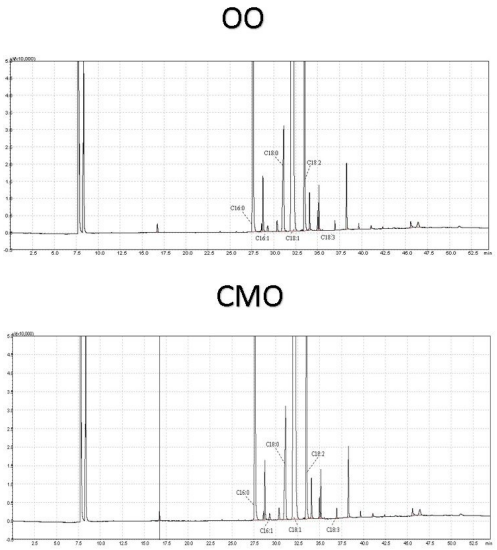


FIGURE 1. Chromatograms of OO (olive oil) and CMO (*Centaurium minus* oil) showing their fatty acid profiles. (GC-FID analysis)

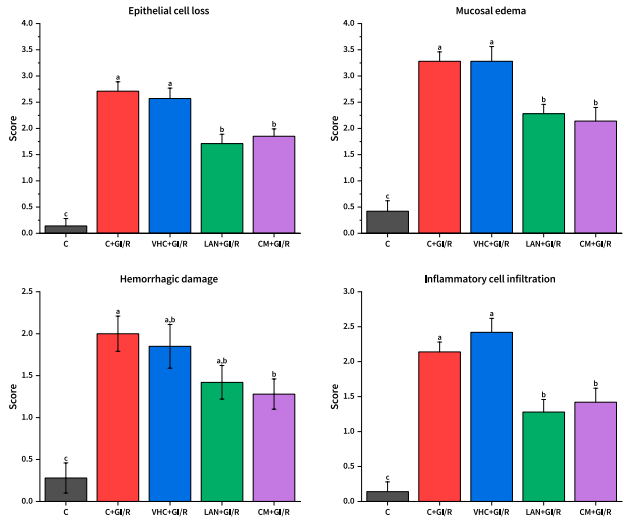


FIGURE 2. Histopathological scores of epithelial cell loss, mucosal edema, hemorrhagic damage, and inflammatory cell infiltration in the experimental groups. Data are presented as mean ± SE. Different superscript letters (a-c) within the same row denote statistically significant differences among the groups (P<0.05). C: Control; C+GI/R: Control + gastric ischemia-reperfusion; VHC+GI/R: Vehicle + gastric ischemia-reperfusion; LAN+GI/R: Lansoprazole + gastric ischemia-reperfusion; CM+GI/R: *Centaurium minus* oil + gastric ischemia-reperfusion

TABLE I
Fatty acid composition (%) of *Centaurium minus* oil and Olive oil determined by GC-FID analysis

%	*C. minus* Oil	Olive Oil
Oleic Acid (C18:1)	67.00	67.66
Palmitic Acid (C16:0)	13.17	12.59
Linoleic Acid (C18:2)	9.46	8.18
Stearic Acid (C18:0)	2.88	3.27
Palmitoleic Acid (C16:1)	0.87	0.63
Linolenic Acid (C18:3)	0.14	0.11
Others	6.48	7.56

Histopathological and immunohistochemical findings

The histopathological findings are presented in FIG. 2 and FIG. 3, while the immunohistochemical findings are presented in FIG. 4 and FIG. 5. Histopathologically, it was observed that the epithelial layer of Group C animals was well-organized and that the integrity of the glandular structure was preserved (FIG. 3-A). On the other hand, histopathological findings such as epithelial cell loss, mucosal edema, hemorrhagic damage, and inflammatory cell infiltration were observed in the groups with induced GI/R injury (FIG. 3B-E). It was determined that the CM+GI/R and LAN+GI/R groups had significantly lower histopathological damage scores for epithelial cell loss, mucosal edema, and inflammatory cell infiltration relative to the C+GI/R and VHC+GI/R groups.

In the present study, histopathological findings in groups subjected to GI/R injury included epithelial cell loss, mucosal edema, hemorrhagic damage, and infiltration of inflammatory cells. These findings are consistent with those reported in other studies [6, 7, 24, 25] and were considered evidence of the oxidative stress associated with GI/R injury. Indeed, a considerable body of recent evidence has closely associated I/R injury in various tissues with oxidative stress and histopathological damage [26, 27, 28]. Due to the presence of oxygen during reperfusion, reactive oxygen species can rapidly increase and damage cell membranes and other cellular structures [29, 30]. Oxidative stress can weaken the epithelial barrier and increase the permeability of blood vessels, leading to mucosal edema and hemorrhagic changes. Reperfusion also activates inflammatory pathways that recruit inflammatory cells to the damaged mucosa and exacerbate tissue injury [29, 30]. Therefore, the histopathological findings in this study may reflect the combined effects of oxidative stress, inflammation, and cellular damage associated with GI/R.

On the other hand, a significant finding of the current study was that the CM+GI/R group exhibited lower scores for epithelial cell loss, mucosal edema, and inflammatory cell infiltration compared to the C+GI/R and VHC+GI/R groups, demonstrating a significant attenuation of histopathological changes. These results suggest that *C. minus* oil exerts a protective effect on gastric tissue in the CM+GI/R group. A similar protective profile was also observed in the LAN+GI/R group. The histopathological findings in the CM+GI/R group are further supported by biochemical results. Rats in the CM+GI/R group, pre-treated with *C. minus* oil, exhibited significantly higher SOD and CAT activities compared to the untreated GI/R groups. These results may reflect the oil's ability to reduce oxidative stress during reperfusion. The protective effect observed in the CM+GI/R group may be related to the previously reported anti-inflammatory and tissue-healing properties of *C. minus* oil [13, 18, 19]. These biological effects have been associated, at least in part, with the flavonoid and phenolic compounds present in *Centaurium* species [9, 13, 16, 17, 18].

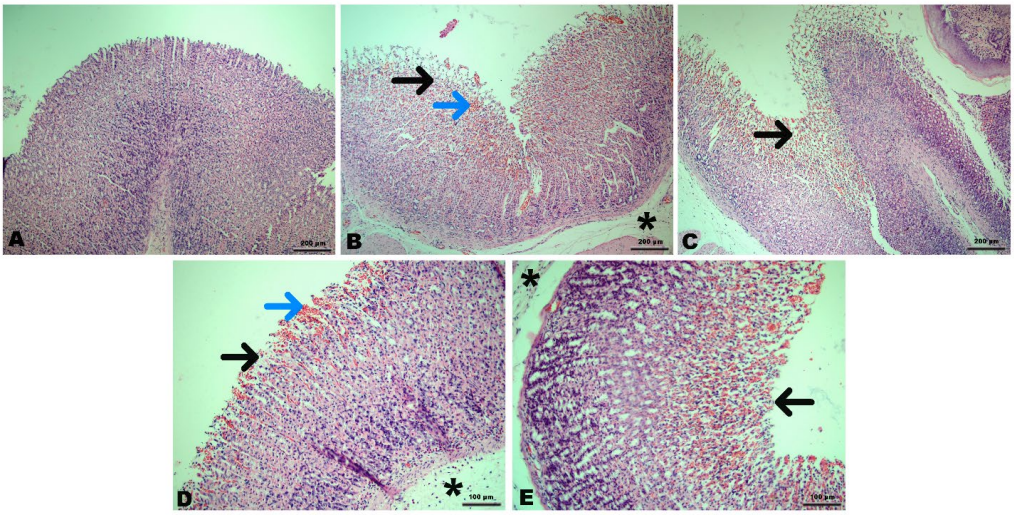


FIGURE 3. Micrographs of groups A (C), B (C+GI/R), C (VHC+GI/R), D (LAN+GI/R), and E (CM+GI/R) stained using the hematoxylin-eosin method. The black arrow indicates epithelial cell loss; the blue arrow indicates hemorrhagic damage; the asterisk indicates mucosal edema

Immunohistochemically, TGF-β showed mild immunoreactivity in Group C animals and was observed in superficial and glandular epithelial cells of the mucosa. On the other hand, TGF-β immunoreactivity increased significantly in the presence of GI/R injury relative to Group C, and immunoreactivity was particularly detected in the cytoplasm of epithelial cells in areas of epithelial cell loss, and glandular epithelial cells (FIG. 4). It was observed that the CM+GI/R and LAN+GI/R groups had significantly lower immunohistochemical scores compared to the other GI/R groups (C+GI/R and VHC+GI/R) (FIG. 5).

TGF-β is known to be a multifunctional cytokine that controls adhesion, cellular differentiation, and motility for many cells. It is also reported that TGF-β plays important roles in the wound healing process and in maintaining tissue homeostasis [31, 32]. In this study, TGF-β immunohistochemical levels in gastric tissue were significantly higher in the groups with GI/R injury (C+GI/R and VHC+GI/R) than in the C group, a finding consistent with results from studies on I/R injury in different tissues, such as cardiac [33] and cerebral [34] tissues.

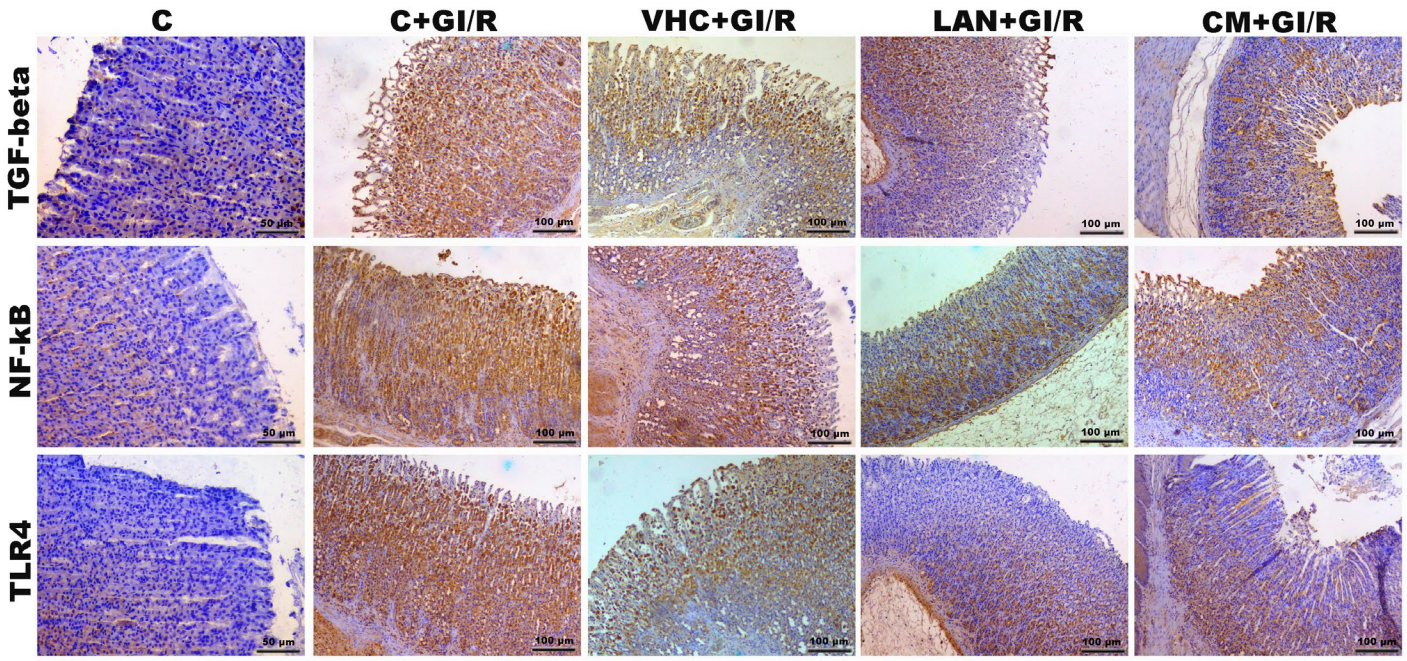


FIGURE 4. Microscopic images of the experimental groups obtained using immunohistochemical staining for TGF-β, NF-κB, and TLR4. C: Control; C+GI/R: Control + gastric ischemia-reperfusion; VHC+GI/R: Vehicle + gastric ischemia-reperfusion; LAN+GI/R: Lansoprazole + gastric ischemia-reperfusion; CM+GI/R: *Centaurium minus* oil + gastric ischemia-reperfusion

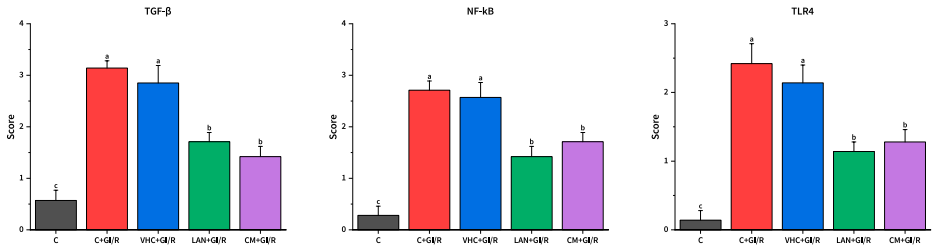


FIGURE 5. Immunohistochemical scores for TGF-β, NF-κB, and TLR4 in the experimental groups. Data are presented as mean ± SE. Different superscript letters (a-c) within the same row denote statistically significant differences among the groups (*P*<0.05). TGF-β: Transforming growth factor-beta; TLR4: Toll-like receptor 4; NF-κB: Nuclear factor kappa B; C: Control; C+GI/R: Control + gastric ischemia-reperfusion; VHC+GI/R: Vehicle + gastric ischemia-reperfusion; LAN+GI/R: Lansoprazole + gastric ischemia-reperfusion; CM+GI/R: *Centaurium minus* oil + gastric ischemia-reperfusion

It was hypothesized that the increased TGF-β immunoreactivity in gastric tissue in the C+GI/R and VHC+GI/R groups might stem from the response to tissue damage and inflammation. On the other hand, it was found that pre-treatment with LAN and *C. minus* oil particularly *C. minus* oil reduced gastric tissue TGF-β immunoreactivity compared to the C+GI/R and VHC+GI/R groups, indicating that these treatments suppressed the gastric tissue TGF-β response.

It has been reported that *Centaurium* plant species exhibit potent antioxidant and anti-inflammatory properties owing to flavonoids, phenolic compounds, and other bioactive phytochemicals present [18, 35]. In this context, it is suggested that the reduced TGF-β immunoreactivity in gastric tissue after pre-treatment with *C. minus* oil in GI/R injury may indicate lower levels of tissue damage and inflammatory response in the gastric mucosa.

NF-κB and TLR4 immunoreactivity was mild and/or negligible in animals in the C group. In the groups with induced GI/R injury, TLR4 and NF-κB immunoreactivity showed an important increase relative to the C group. In the groups with induced GI/R injury, immunoreactivity was cytoplasmic in areas of epithelial cell loss, inflammatory cell infiltrates, and glandular epithelial cells (FIG.5). It was found that the CM+GI/R and LAN+GI/R groups had significantly lower immunohistochemical scores compared to the C+GI/R and VHC+GI/R groups (FIG. 4).

TLR4 belongs to the family of cell surface pattern recognition receptors and serves as a key mediator of innate immunity. On the other hand, NF-κB, recognized as an important transcription factor closely associated with TLR4, regulates the transcription of numerous pro-inflammatory genes, including TNF-α, IL-1β, and IL-6. Once TLR4 is activated, it can activate NF-κB via adaptor proteins such as MyD88 and TRIF [36, 37].

Furthermore, the TLR4/NF-κB signaling pathway not only increases the production of proinflammatory cytokines but can also influence the expression of growth factors such as TGF-β [38]. Oxidative stress and certain cellular damage products (HMGB1, HSP70, mtDNA, etc.) that arise alongside ischemia-reperfusion (I/R) injury can activate TLR4, thereby leading to increased NF-κB activation [39, 40].

This causes an elevation in the severity of inflammation and tissue damage. Various studies on tissue ischemia-reperfusion (I/R) have reported that TLR4 and NF-κB levels rise in the I/R group, resulting in more severe tissue damage [27, 28].

In the present study, significant increases were observed in the C+GI/R and VHC+GI/R groups relative to the C group. Furthermore, it was found that pre-treatment with *C. minus* oil reduced TLR4 and NF-κB immunoreactivity relative to the C+GI/R and VHC+GI/R groups. This suggests that pre-treatment with *C. minus* oil may reduce the inflammatory response and exert gastroprotective effects by suppressing NF-κB activation via the TLR4 pathway. However, the decrease in TGF-β levels may be an indirect effect of inhibiting the TLR4/NF-κB signaling pathway.

Biochemical findings

Intergroup SOD, MDA, and CAT levels are presented in FIG. 6. It was determined that gastric tissue MDA levels in the groups with induced GI/R damage C+GI/R, VHC+GI/R, LAN+GI/R, and CM+GI/R were higher than those in the C group. However, gastric tissue SOD and CAT activities in the C+GI/R and VHC+GI/R groups were found to be lower than those in the C group. MDA levels and SOD and CAT activities in the CM+GI/R and LAN+GI/R groups were at similar levels, and the SOD and CAT activities in these groups were found to be higher than those in the C+GI/R group. It was determined that MDA levels in the CM+GI/R and LAN+GI/R groups were lower than those in the C+GI/R group.

GI/R injury is defined as a pathological process that develops when gastric tissue is deprived of blood flow for a certain period of time, followed by reperfusion of the gastric tissue [1, 4, 29]. This process represents a paradox: in the initial phase, cellular energy reserves are depleted due to oxygen and nutrient deprivation, while in the second phase, ROS and the inflammatory response lead to further exacerbation of tissue damage.

Excessive amounts of ROS generated during the reperfusion phase can easily damage lipids, proteins, and DNA in cell membranes [29, 30]. The literature reports that endogenous antioxidant systems are insufficient to protect against the oxidative stress caused by GI/R, and studies continue to investigate the use

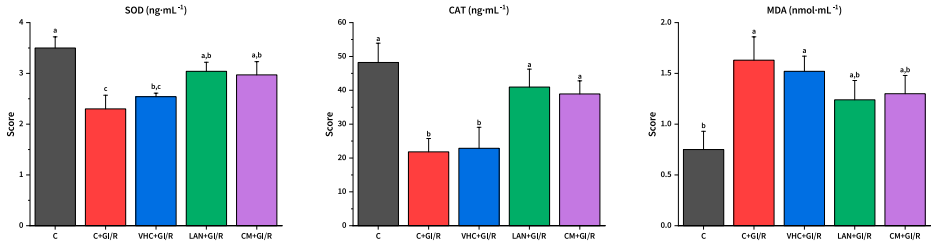


FIGURE 6. Effects of *Centaurium minus* oil on MDA, SOD, and CAT levels in gastric tissue of rats with GI/R injury induced by celiac artery ligation. Group means are presented as Mean ± SE. Different superscript letters (a-c) within the same row denote statistically significant differences among the groups (*P*<0.05). C: Control; C+GI/R: Control + gastric ischemia-reperfusion; VHC+GI/R: Vehicle + gastric ischemia-reperfusion; LAN+GI/R: Lansoprazole + gastric ischemia-reperfusion; CM+GI/R: *Centaurium minus* oil + gastric ischemia-reperfusion

of exogenous antioxidants and anti-inflammatory agents against GI/R-induced damage [24, 29, 41, 42, 43, 44]. In this context, this study aimed to evaluate the efficacy of *C. minus* oil on GI/R injury.

However, the literature on the protective effects of *C. minus* on the stomach is quite limited. A study demonstrated the gastroprotective effect of *C. minus* extract in an aspirin-induced gastric injury model through antioxidant mechanisms (MDA, GSH, and CAT). In contrast, the present study utilized a gastric ischemia-reperfusion model; *C. minus* oil was administered, and its protective effects were examined not only through oxidative stress markers but also histopathologically and immunohistochemically. Oxidative stress induced by GI/R was assessed using oxidant (MDA) and antioxidant (SOD and CAT) parameters, and the inflammatory response was evaluated based on tissue TGF-β, TLR4, and NF-κB immunoreactivity.

It has been stated that GI/R injury is a complex pathological process that begins with oxygen deprivation in the tissue, continues with a shift in the oxidant-antioxidant balance in favor of oxidants, and is exacerbated by an inflammatory response [24, 25, 41, 44]. Recent studies have shown that gastric MDA levels increase in the pathogenesis of gastric ischemia-reperfusion injury induced by celiac artery ligation in rats [24, 25, 43, 44], along with SOD [24, 25, 43], glutathione peroxidase (GPx) [44], and total glutathione (tGSH) [24, 25, 44].

In line with previous studies, this study also demonstrated that in rats with induced GI/R damage (in the C+GI/R and VHC+GI/R groups), gastric tissue MDA levels were importantly higher relative to healthy rats (in the C group), while gastric tissue SOD and CAT activities were diminished.

In addition, in the present study, it was found that rats pretreated with lansoprazole and *C. minus* oil (LAN+GI/R and CM+GI/R, respectively) had gastric MDA levels that were lower though not significantly so compared to rats that underwent GI/R injury alone (C+GI/R), and that SOD and CAT activities in gastric tissue were significantly higher compared to these groups. In the literature [24, 25, 29, 42, 43, 44], it has been reported that endogenous antioxidant systems are insufficient to protect against oxidative stress caused by GI/R injury, and that protection against GI/R injury can be achieved by pre-treatment with exogenous antioxidant and anti-inflammatory agents, which support an increase in the production of endogenous antioxidants (SOD, CAT, GPx, tGSH).

In agreement with previous studies, this study also demonstrates that pre-treatment with *C. minus* oil alleviates oxidative stress by increasing gastric antioxidant parameters (SOD and CAT) and may have a protective effect against GI/R injury. In addition to the oxidative stress associated with GI/R injury, molecular and biochemical changes such as mitochondrial dysfunction, cell death mechanisms (necrosis/apoptosis), and inflammation manifest as various histopathological lesions [4, 6, 26].

This study has some limitations. Specific SOD isoforms, the GSH/GSSG ratio, and nitric oxide levels were not measured. Assessment of these parameters could have provided further information on the redox balance and nitrosative stress associated with gastric ischemia-reperfusion injury and could have helped to clarify the mechanisms involved in the protective effect of *C. minus* oil. Further studies including these parameters may provide a more detailed understanding of this protective effect.

CONCLUSION

This study demonstrated, on the one hand, that the GI/R process leads to oxidative damage and inflammation in gastric tissue, as evidenced by biochemical, histopathological, and immunohistochemical findings. On the other hand, it was found that *C. minus* oil can attenuate oxidative damage in the stomach by enhancing antioxidant activity (SOD and CAT).

Furthermore, the study demonstrated that pre-treatment with *C. minus* oil can reduce the inflammatory response during the GI/R process by reducing the expression levels of TGF-β, NF-κB, and TLR4. These results suggest that pre-treatment with *C. minus* oil may be beneficial in preventing GI/R-induced gastric damage and may have a protective effect on the stomach.

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Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no commercial or financial interests that could be considered a potential conflict of interest.

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