

Protective Effects of Diosmin on Inflammatory and Antioxidant Profiles in Carrageenan-Induced Paw Edema

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Abstract

Objectives: This study aimed to evaluate the protective effects of DIO against carrageenan (Carr)-induced paw edema in rats.

Method: Forty-two male Wistar rats were randomly divided into six experimental groups. Indomethacin (5 mg/kg) and different doses of DIO (25, 50, and 100 mg/kg) were administered intraperitoneally 30 minutes before the intra-plantar injection of 1% carrageenan (0.1 mL/paw). Paw volume was measured before and at 0.5, 1, 2, 3, 4, and 5 hours after carrageenan injection. Five hours post-injection, the rats were euthanized, and the inflamed paw tissues were collected for biochemical analysis of inflammatory markers [nitric oxide (NO), tumor necrosis factor-alpha (TNF- α), and cyclooxygenase-2 (COX-2)] and oxidative stress parameters [glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA)].

Findings: Carrageenan injection caused a significant increase in paw edema, MDA, NO, TNF- α , and COX-2 levels, along with a marked decrease in GSH content and the activities of CAT and GPx. Pretreatment with DIO significantly attenuated these carrageenan-induced alterations in a dose-dependent manner ($P < 0.05$), comparable to the reference drug indomethacin.

Conclusion: The findings of this study demonstrate the potent anti-inflammatory and antioxidant effects of diosmin in carrageenan-induced paw edema in rats, suggesting its potential therapeutic value in acute inflammatory conditions.

Keywords: Inflammation, diosmin, antioxidant, carrageenan, rat

Introduction

Inflammation is a multistep immune response to internal or external harmful factors. This process involves cellular, molecular, and vascular changes. In the early stages, increased vascular permeability leads to plasma leakage and fluid accumulation in the tissue. Immune cells such as neutrophils and macrophages migrate to the site of injury and enhance the defense response by secreting inflammatory mediators. Clinically, these reactions manifest as the classical signs of inflammation, including redness, swelling, pain, and warmth of the site.^{1,2}

Biologically, inflammation is divided into three principal stages: 1) acute inflammation: Initiated immediately after tissue injury and is accompanied by the secretion of mediators such as histamine and prostaglandins, which cause vasodilation and increase vascular permeability. 2) Immune response: Subsequently, both innate and adaptive immune systems are activated, and cytokines such as IL-1, IL-2, and TNF- α are secreted, attracting leukocytes, activating defense pathways, and facilitating tissue repair. 3) Chronic inflammation: If the injurious stimulus persists, inflammation progresses to a chronic phase, which is accompanied by the infiltration of immune cells, granuloma formation, and gradual tissue destruction, which can ultimately lead to fibrosis and dysfunction.³⁻⁵ Cyclooxygenases (COX) are key enzymes in the production of prostaglandins, which play an important role in inflammation, pain, and fever. Induction of their expression in pathological conditions exacerbates inflammatory responses in body tissues.⁶

The treatment of inflammation has two main goals: pain relief and prevention of tissue damage. Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their effects by inhibiting cyclooxygenase (COX) and reducing the synthesis of

prostaglandins. However, they can cause side effects, such as gastrointestinal problems, bleeding, and renal dysfunction.⁷ Corticosteroids also have a stronger effect due to their broad inhibition of inflammatory pathways, but their long-term use is associated with serious side effects such as osteoporosis, hypertension, thrombosis, and psychiatric disorders.⁸

Due to the limitations of conventional drugs, recent research has focused on discovering novel anti-inflammatory compounds with natural origin. Medicinal plants are considered valuable sources for the production of new drugs due to their availability, low cost, and social acceptance. A detailed study of these phytochemicals and their anti-inflammatory mechanisms could pave the way for the development of safer and more effective drugs.^{9,10} Polyphenols are among the most common antioxidants in the human diet. Diosmin (DIO) is a natural flavonoid substance found in the outer shell of citrus fruits and olive leaves. Pharmacokinetic studies have shown that diosmin (DIO) is rapidly metabolized by the intestinal flora into its aglycone, diosmetin, which is then rapidly absorbed into the plasma and distributed throughout the body. It has a half-life of 26–23 hours and is excreted in the urine.¹¹ Literature reviews of previous studies have shown that DIO exerts notable anti-inflammatory effects, primarily through inhibition of key inflammatory mediators such as NF- κ B, IL-6, IL- β 1, TNF- α , and COX-2.^{12,13} In addition to its anti-inflammatory properties, DIO also exhibits significant antioxidant activity by scavenging free radicals and enhancing endogenous antioxidant defense systems. This dual action is important for reducing oxidative stress and improving tissue damage.¹⁴ Moreover, DIO is a vasoprotective agent that can improve blood circulation and lymphatic flow.^{11,15}

To investigate the mechanisms of inflammation, various animal models are used, among which the carrageenan (Carr)-induced paw edema model is one of the most widely used.

Injection of Carr into the mouse hind paw causes edema and acute peripheral inflammation, accompanied by increased production of reactive oxygen species (ROS).^{16,17} This condition is associated with increased arachidonic acid metabolism and elevated levels of prostaglandin E₂, nitric oxide, and malondialdehyde, while glutathione content and antioxidant enzyme activities such as superoxide dismutase (SOD) are reduced. Free radicals such as superoxide anion (O₂⁻), peroxynitrite, and hydroxyl radicals (•OH) also play critical roles in the development of Carr-induced inflammatory pain and edema.¹⁷

Therefore, the present study was conducted to examine the protective role of DIO on Carr -Induced Paw Edema in rats.

Materials and Methods

Drugs and Chemicals

DIO, λ-Carr, reduced glutathione (GSH), 1,1,3,3-tetraethoxypropane (TEP), trichloroacetic acid (TCA), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Indomethacin was generously provided by Iran Daru Pharmaceutical Co. (Tehran, Iran). All other chemicals were of analytical grade.

COX-2 ELISA kit was sourced from Abcam Biochemical (Cambridge, UK) and Rat TNF-α ELISA kit was purchased from BioSource International, Inc. (Camarillo, CA, USA).

Animals

Forty-two male Wistar rats weighing 180–220 g were obtained from the Laboratory Animal Breeding and Maintenance Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The animals were kept in polypropylene cages in the animal room of the Faculty of Pharmacy under controlled temperature conditions (23 ± 2°C) with a 12-hour light-dark cycle. They were provided with a standard rat diet and water ad libitum.

This study was also performed according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethic code: IR.AJUMS.ABHC.REC.1397.073).

Experimental Procedures

λ-Carr was dissolved in normal saline. Indomethacin was prepared in normal saline containing 10% dimethyl sulfoxide (DMSO). All drugs were administered intraperitoneally (i.p.) in a constant volume of 5 ml/kg body weight. Carr was injected subcutaneously (SC) into the plantar surface of the right hind paw at a volume of 0.1 ml per paw. The animals were randomly divided into six equal groups ($n = 7$ per group) as follows:

Group 1 (Normal saline group): Received an intraperitoneal injection of normal saline, followed by a subcutaneous injection of 100 μl normal saline into the plantar surface of the right hind paw after 30 minutes.

Group 2 (Carr group): Received intraperitoneal normal saline, followed by subcutaneous injection of 100 μl of a 1%

Carr solution into the plantar surface of the right hind paw after 30 minutes.¹⁸

Group 3 (Indomethacin group): Received indomethacin (5 mg/kg, i.p.), followed by a subcutaneous injection of 100 μl of 1% Carr solution into the plantar surface of the right hind paw after 30 minutes.¹⁹

Groups 4–6 (DIO-treated groups): Received DIO at doses of 25, 50, and 100 mg/kg (i.p.), respectively, followed by a subcutaneous injection of 100 μl of 1% Carr solution into the plantar surface of the right hind paw after 30 minutes.^{20,21}

Carrageenan-Induced Paw Edema

The induction of paw edema due to Carr is a biphasic phenomenon. Paw edema was induced in the right paw of rats by intra-plantar injection (i.pl) of 100 μl of a 1% Carr solution. The level of inflammation was measured by assessing the change in paw volume immediately before and at 0.5, 1, 2, 3, 4, and 5 hours after the Carr injection using a Plethysmometer (Ugo Basile, Italy).

In this study, because at time zero the paw volumes of the rats were not identical, the relative paw edema percentage was calculated using the following formula:

$$\% \text{ Relative Paw Edema} = ((V_2 - V_1) / V_1) * 100$$

where V_1 is the paw volume before carrageenan injection, and V_2 is the paw volume measured 0.5 to 5 hours after the carrageenan injection.

Sample Collection

Five hours after Carr injection, animals were anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg).¹⁸ The inflamed paw pad was cut and removed for the measurement of tissue factors and histopathological examination. A portion of the tissue was stored at -70°C for subsequent biochemical analysis, while the remaining specimen was fixed in 10% formalin solution.

Paw Homogenate Preparation

The paw tissue samples were homogenized in 0.1 M phosphate buffer (pH of 7.4) at a concentration of 10% using an FSH-2A homogenizer. The homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C using a refrigerated centrifuge (Hettich Zentrifugen, Germany) to eliminate cellular debris. The protein content of the tissue homogenate was assessed according to the Bradford method.²² The tissue homogenate was subsequently used for measurement of oxidative stress and inflammatory biomarkers, including malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), catalase (CAT), glutathione peroxidase (GPx), cyclooxygenase-2 (COX-2), and tumor necrosis factor-α (TNFα).

Biochemical Assays

Evaluation of GSH Level

GSH content was measured based on the Ellman method.²³ Briefly, 200 μL of tissue supernatant was added to 200 μl of phosphate buffer, followed by the addition of 40 μL of DTNB

reagent (10 mM). After 20 minutes of incubation, the yellow color complex was read at 412 nm using a spectrophotometer (UV-1650 PC, Shimadzu, Japan). The glutathione standard was used to draw the calibration curve. Results were expressed as nmol/mg protein.

Evaluation of GPx and CAT Activities

The activity of CAT and GPx enzymes were determined using calorimetric enzymatic assay kits from ZellBio (GmbH, Ulm, Germany). Enzyme activities were determined as units per mg of protein.

Evaluation of Malondialdehyde (MDA)

MDA levels were measured according to the method of Buege and Aust.²⁴ Briefly, 150 μ L of 10% trichloroacetic acid was added to 50 μ L of tissue supernatant, and then centrifuged at 4000 $\times$ g for 10 minutes. The supernatant was separated and mixed with 200 μ L of 0.67% thiobarbituric acid solution, then incubated in boiling water for 30 minutes. Absorbance of the resulting pink complex was recorded at 532 nm using a spectrophotometer (UV-1650 PC, Shimadzu, Japan). The concentration of MDA was determined using tetraethoxypropane as the standard and the results were reported in nmol/mg of protein.

Evaluation of COX-2 Protein Levels by ELISA in Paw Tissue

Paw tissue levels of COX-2 protein were determined by an enzyme immunoassay kit. COX-2 ELISA kit was provided by Abcam Biochemical Co. (Cambridge, UK), and assays were performed according to the manufacturer's instructions. Absorbance of the produced color was measured spectrophotometrically at 450 nm using a microplate reader. The limit of detection was 0.026 ng/mL.

Evaluation of TNF- α by ELISA in Paw Tissue

TNF- α levels were determined by enzyme immunoassay kits. Rat TNF- α kit was purchased from eBiosource

International, Inc. (Camarillo, CA) and assays were performed according to the manufacturer's guidelines (eBioscience, Vienna, Austria). Absorbance was measured at 450 nm using a microplate reader. The limit of detection for TNF- α was 11 pg/mL.

Histopathological Examination

After fixation of the paw tissues in formalin solution, they were dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. The specimens were cut into 5- μ m sections using a microtome and then stained with hematoxylin and eosin (H&E). After that, a histologist examined the slides with a light microscope (Olympus, Japan). The severity of inflammation was graded on a 5-point scale according to the Coura et al., study as follows: 0: none; 1: mild; 2: mild/moderate; 3: moderate; 4: moderate/severe; and 5: severe inflammation.²⁵

Statistical Analysis

Data analysis was conducted using the GraphPad Prism 8.0 statistical software (San Diego, CA, USA). The findings were expressed as means $\pm$ standard deviation (SD). A one-way ANOVA test was utilized for comparing the results, followed by Tukey's post hoc test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Effects of DIO on Right Hind Paw Inflammation

The results showed that administration of different doses of DIO, dose-dependently reduced right hind paw inflammation compared to the Carr group (Fig. 1A). Chart 1-B illustrates the anti-inflammatory effects of various doses of DIO at different time points (0.5, 1, 2, 3, 4, and 5 hours). DIO at doses of 25, 50, and 100 mg/kg, as well as indomethacin, significantly reduced right hind paw inflammation compared to the Carr group (*P* < 0.05). This reduction at doses of 50 and 100 mg/

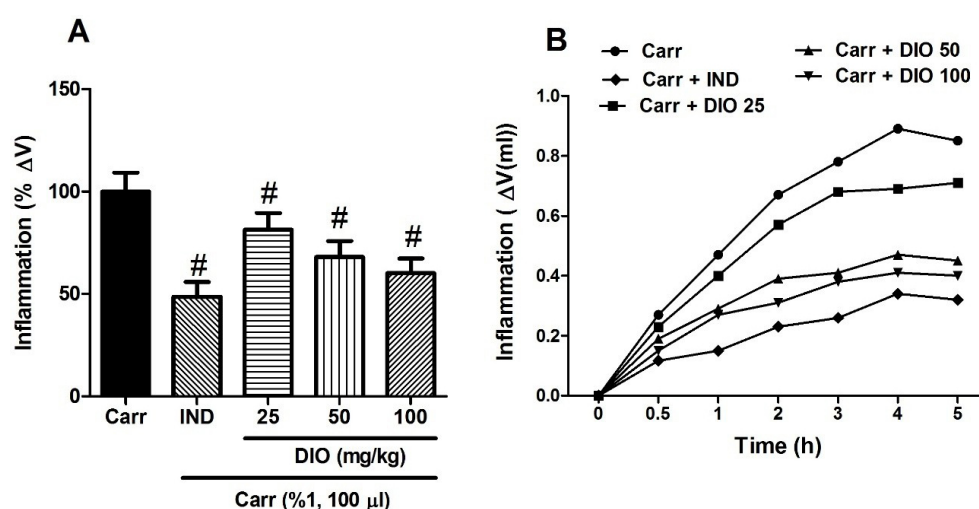


Fig. 1 Effect of DIO on carrageenan-induced paw edema. The medications were administered 30 minutes before carrageenan injection. Data are presented as Mean $\pm$ SD (*n* = 7). # indicates a significant difference compared with the carrageenan group (*P* < 0.05). Abbreviations: Carr, carrageenan; IND, indomethacin; DIO, diosmin.

kg was significantly different from that at 25 mg/kg, but there was no significant difference between the doses of 50 and 100 mg/kg (Fig. 1).

Effects of DIO on Paw Oxidative Stress Parameters

Figure 2 illustrates the impact of DIO on oxidative stress parameters, including GSH and MDA levels, as well as the activities of antioxidant enzymes GPx and CAT, in paw tissues. In the Carr group, there was a significant reduction in GSH levels, as well as in the activities of GPx and CAT, when compared to the NS group ($P < 0.05$). Additionally, the MDA levels in the Carr group were significantly elevated in comparison to the NS group ($P < 0.05$). Pretreatment with DIO at doses of 100 mg/kg led to a significant enhancement in GSH content and CAT activity compared to the Carr group ($P < 0.05$). DIO pretreatment at both 50 and 100 mg/kg also significantly boosted GPx activity compared to the Carr group ($P < 0.05$). All doses of DIO pretreatment significantly lowered MDA levels compared to the Carr group ($P < 0.05$). Furthermore, the ameliorative effects of DIO were found to be dose-dependent.

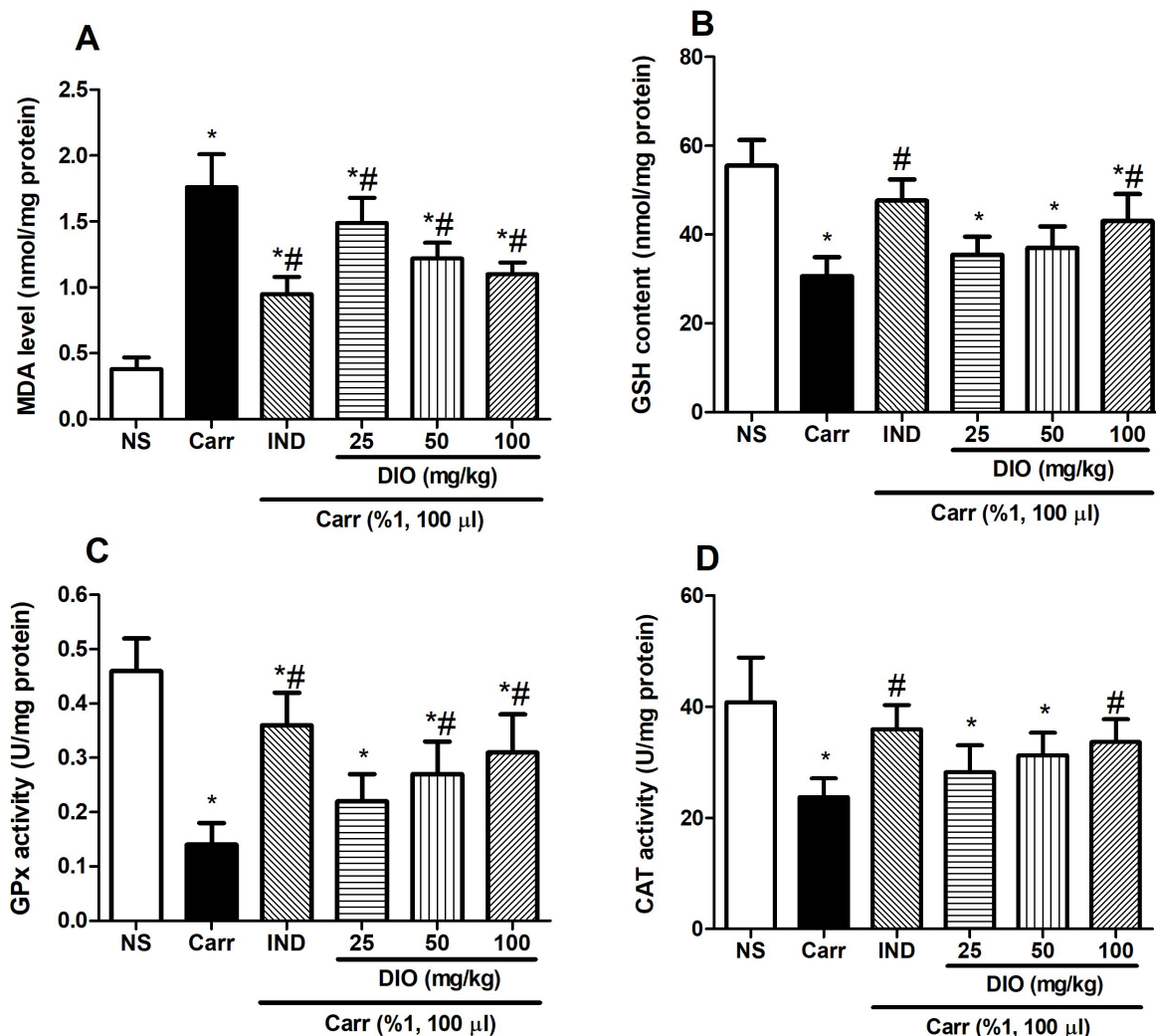


Fig. 2 Effect of DIO on paw tissue GPx and CAT activities and MDA and GSH levels. Drugs were administered 30 min before Carr injection. Data are represented as Mean $\pm$ SD ($n = 7$). # indicates a significant difference compared with the carrageenan group ($P < 0.05$). Abbreviations: NS, normal saline; Carr, carrageenan; IND, indomethacin; DIO, diosmin.

Effects of DIO on Paw Inflammatory Parameters

Figure 3 illustrates that Carr treatment significantly elevated the levels of TNF- α , COX-2, and NO as inflammatory markers compared to the NS group ($P < 0.05$). The findings showed that pretreatment with all doses of DIO (25, 50, and 100 mg/kg) resulted in a significant decrease in COX-2 compared to the Carr group ($P < 0.05$). Regarding the TNF- α and NO, pretreatment with doses of 50 and 100 mg/kg of DIO led to a significant reduction in these inflammatory markers when compared to the Carr group ($P < 0.05$).

The Impact of DIO on Histopathologic Parameters

Microscopic observations of the skin tissue in the control group showed normal histological architecture. Compared with the control group, the Carr-treated group exhibited mild dermal edema characterized by focal widening of the interstitial spaces, mild infiltration of inflammatory cells into the dermis, and marked vascular congestion and focal hemorrhage. In addition, the epidermis appeared thinner than that of the control group, whereas no marked disorganization of dermal collagen fibers was observed. In contrast, the

indomethacin-treated group showed a nearly normal epidermal thickness and dermal architecture. In the Carr + 25 mg/kg DIO group and the Carr + 50 mg/kg DIO group, dermal edema, vascular congestion, and focal hemorrhage were

partially attenuated, although epidermal thinning persisted. Treatment with DIO at 100 mg/kg markedly ameliorated the histopathological alterations and improved tissue architecture, indicating a dose-dependent protective effect (Figure 4).

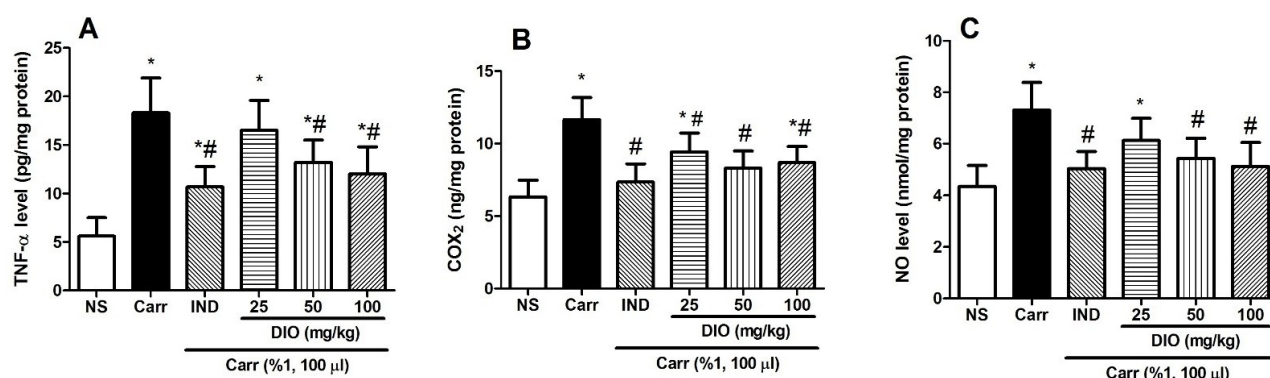


Fig.3 Effect of DIO on TNF- α , COX-2 and NO levels in paw tissue. Drugs were administrated 30 min prior to Carr injection. Data are represented as Mean $\pm$ SD ($n = 7$). # indicates a significant difference compared with the carrageenan group ($P < 0.05$). Abbreviations: Carr, carrageenan; IND, indomethacin; DIO, diosmin.

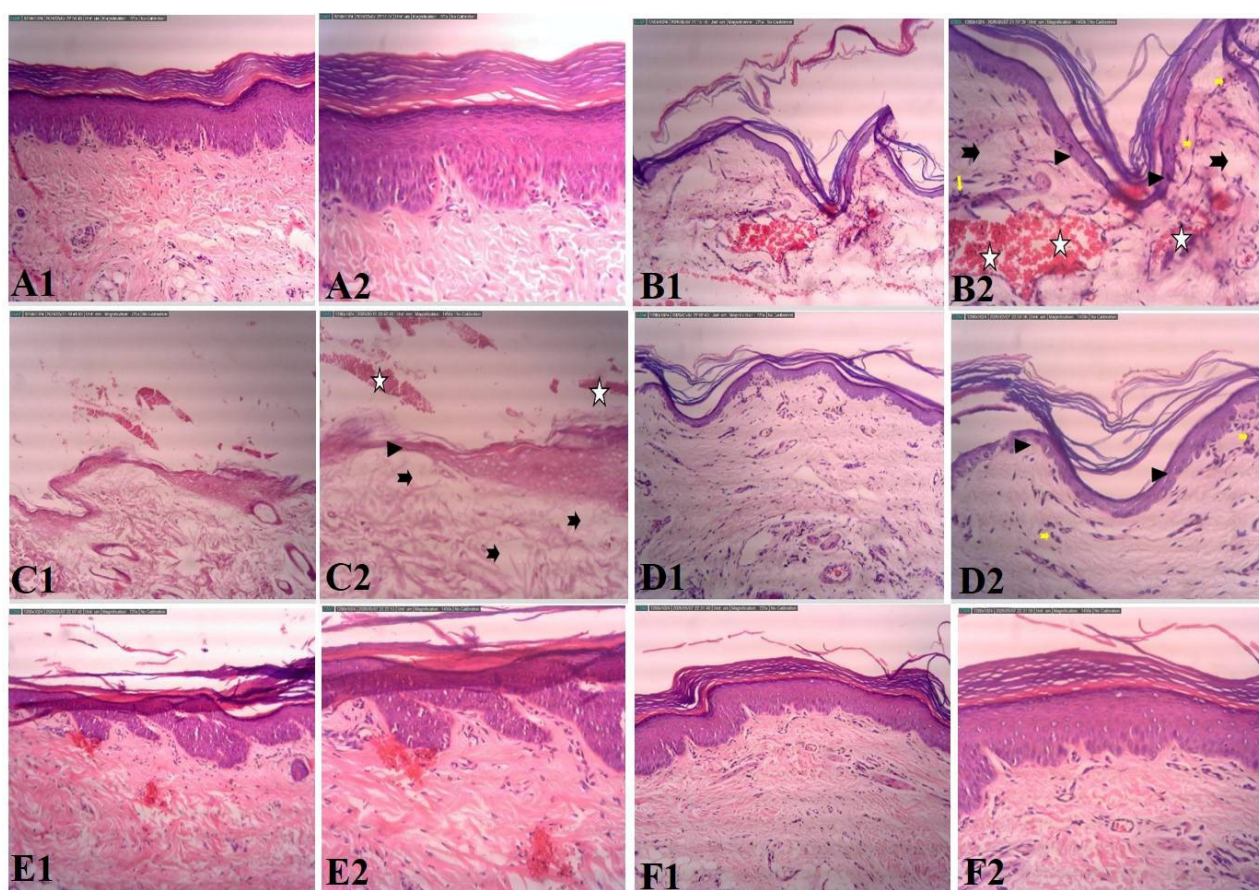


Fig. 4 Histopathological observation of skin tissue across the study groups (stained with H&E; magnification: 100 $\times$ and 200 $\times$). (A1) Control group, 100 $\times$; (A2) Control group, 200 $\times$; (B1) Carr group, 100 $\times$; (B2) Carr group, 200 $\times$; (C1) Carr + 25 mg/kg DIO, 100 $\times$; (C2) Carr + 25 mg/kg DIO, 200 $\times$; (D1) Carr + 50 mg/kg DIO, 100 $\times$; (D2) Carr + 50 mg/kg DIO, 200 $\times$; (E1) Carr + 100 mg/kg DIO, 100 $\times$; (E2) Carr + 100 mg/kg DIO, 200 $\times$; (F1) Carr + Indomethacin, 100 $\times$; (F2) Carr + Indomethacin, 200 $\times$. Edema (Black arrow), infiltration of inflammatory cells (Yellow arrow), vascular congestion and focal hemorrhage (star sign) thinning of the epidermis (arrowhead).

Discussion

The results of the present study showed that pretreatment with DIO significantly reduced paw inflammation and oxidative stress in a dose-dependent manner. This was evident by a significant reduction in paw edema, improvement in oxidative stress factors and inflammatory mediators and histopathological amelioration.

Carr administration causes the bradykinin release, which stimulates the production of prostaglandins and other auto-coids, resulting in inflammatory reaction and paw edema.^{26,27} In the present study, intraplantar injection of Carr produced marked paw edema compared with the control group. Conversely, pretreatment with DIO at doses of 25, 50, and 100 mg/kg significantly reduced paw swelling in a dose-dependent manner.

DIO prevents tissue edema by protecting vascular endothelial cells, reducing capillary permeability, and inhibiting the accumulation of neutrophils and macrophages at the site of inflammation.²⁸ This effect is directly observable in the Carr model through a reduction in paw swelling. Moreover, DIO decreases the synthesis of prostaglandins and leukotrienes by inhibiting the enzymes COX-2 and lipoxygenase (LOX). These mediators play a major role in the development of swelling, pain, and redness; therefore, their reduction leads to a noticeable decrease in tissue inflammation.²⁹

Oxidative stress plays an important role in the pathogenesis of Carr-induced edema. Carr, as an inflammatory agent, activates oxidative pathways, leading to excessive production of free radicals and ROS. These radicals can cause damage to cells and tissues and further amplify inflammatory responses.³⁰ Considering that Carr induces oxidative stress in paw tissue, the levels of MDA, GSH, GPX, and CAT were measured to investigate lipid peroxidation and antioxidant defense status.

MDA is the end product of lipid peroxidation and a key indicator for assessing the severity of membrane oxidative damage.³¹ Glutathione is a major intracellular antioxidant compound that protects cells against oxidative damage, free radicals, and peroxides. The antioxidant enzymes CAT and GPX also play essential roles in preventing oxidative damage in cells.³²

In the present study, Carr administration significantly increased MDA levels while reducing GSH content and GPX and CAT activities, indicating an increase in oxidative stress. These findings are also consistent with the studies of other researchers. For example, studies conducted by Cordaro et al., in 2020 and Albarakati et al., in 2022 found that Carr injection into the paw tissue of rats increased oxidative stress markers, including MDA and NO, while decreased GSH and antioxidant defenses in inflamed paw tissue.^{33,34}

Pretreatment with DIO reduced Carr-induced oxidative stress in a dose-dependent manner. One of the key findings was the marked reduction of MDA levels in the tissue. The reduction of MDA in the presence of DIO suggests that this compound was able to inhibit the production of free radicals and their destructive effects. Additionally, DIO increased GSH levels and restored GPX and CAT activities, which indicate DIO not only prevented oxidative damage but also enhanced the endogenous antioxidant defenses. This dual effect, including reduction of lipid peroxidation and increasing

antioxidant capacity, plays a key role in mitigating Carr-induced inflammation.

Previous studies also indicated that the antioxidant mechanism of DIO is primarily exert through the direct scavenging of free radicals, particularly ROS, and the upregulation of endogenous antioxidant defenses. This is achieved through the enhanced activity of key enzymes, such as superoxide dismutase (SOD), CAT, GPx, and glutathione S-transferase (GST). By attenuating lipid peroxidation and preserving cellular membrane integrity, DIO mitigates oxidative stress across various tissues and confers protection to endothelial cells. Such effects have been extensively documented in both *in vitro* and *in vivo* models, including those of diabetes, ischemia-reperfusion, and toxin-induced injury.^{35,36}

The inflammatory response to Carr is biphasic. The initial phase, which occurs within the first two hours, is characterized by the infiltration of neutrophils and the release of mediators such as histamine, serotonin, and bradykinin. This is followed by a secondary phase involving the synthesis of prostaglandins via the COX-2 pathway and the release of nitric oxide (NO) and TNF- α . In addition, MDA levels may increase as a result of free radicals and contribute to the exacerbation of inflammation.^{37,38} In this study, the administration of Carr into the paw pad resulted in a significant increase in the levels of TNF- α , COX-2, and NO, indicating an elevated inflammatory response induced by this substance. These cytokines are key factors in the exacerbation of acute inflammation, and their increase in the Carr model is well-documented.³³

On the other hand, the dose-dependent reduction of these inflammatory cytokines in the groups receiving DIO suggests that this compound is capable of modulating inflammatory responses. The inhibition of these molecules by DIO may be attributed to its effects on signaling pathways such as NF- κ B or MAPK, which have also been discussed in previous studies.^{13,39}

The activation of NF- κ B in response to inflammatory stimuli increases the expression of the genes TNF- α , IL-1 β , IL-6, and COX-2. DIO inhibits the activation of NF- κ B, resulting in lower levels of these cytokines and consequently reducing both systemic and local inflammation.^{29,40}

Therefore, the reduction of inflammatory cytokines observed in the present study may be considered one of the main mechanisms underlying the anti-inflammatory effect of DIO.

Moreover, in inflammation, excessive production of nitric oxide (NO) by iNOS leads to tissue damage. DIO balances NO levels by reducing iNOS expression, thereby preventing tissue injury.²⁹

These results are consistent with previous reports supporting the capability of DIO to hinder tissue damage by enhancing antioxidant defense and reducing inflammatory responses. For example, the results of Zhao et al. study demonstrated that DIO inhibits NF- κ B p65 signaling pathway through the activation of SIRT3, thereby reducing the expression of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α .¹³

Similarly, AlAsmari et al. reported the beneficial effects of DIO against doxorubicin-induced hepatotoxicity. Their results showed that DIO exerted its effects through the restoration of hepatic antioxidant factors (SOD, CAT, GSH, and MDA) and

the reduction of inflammatory biomarkers (NF- κ B, TNF- α , IL-1 β , and IL-6).³⁹

In other study, Wang et al., demonstrated the beneficial effects of DIO in the treatment of Alzheimer's disease. Their findings revealed that DIO reduced the levels of IL-1 β , IL-6, and TNF- α , indicating its anti-inflammatory effects.⁴¹

Histological observation further confirmed the biochemical findings. The severe disruption of epidermal and dermal structures, accompanied by extensive infiltration of inflammatory cells in the Carr-treated group, was dose-dependently attenuated by DIO, indicating that DIO not only inhibits the inflammatory cascade, but also enhances tissue integrity and accelerates repair mechanisms.

Conclusion

Our findings demonstrated the potential ameliorative effects of DIO on Carr-induced paw edema in rats. This effect was dose-dependent, and the highest doses of DIO showed better outcomes. DIO exerts this effect through its antioxidant and anti-inflammatory properties. Ultimately, although indomethacin exhibited superior effects compared to DIO, it is important to note that the side effects of herbal compounds may be less severe in the long term compared to those of synthetic medications.

Limitation

Although this study showed the anti-inflammatory and antioxidant potential of DIO in a Carr-induced model, but there's some limitations. The carrageenan-induced paw edema model is an acute inflammatory model. Therefore, the results of this model are not always fully generalizable to chronic inflammatory diseases in humans. This study only used male mice, and given that hormonal fluctuations in the female sex can affect inflammatory responses, this limits the generalizability of your findings to the entire population. This study focused solely on the "pre-treatment" effect of DIO, and its therapeutic effect was not investigated.

Conflict of Interest

All authors declare no conflict of interest related to the present work.

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