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Antifibrotic effects of Berberine and N-acetylcysteine in a rat model of diabetic lung fibrosis

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Abstract

Objective: Diabetes mellitus (DM) is a metabolic condition characterized by chronically high blood glucose concentrations. DM affects many organs, including the liver, kidneys, and brain, and its association with lung damage, as manifested by pulmonary fibrosis, has been established. Chronic high blood sugar can also cause structural damage to the lungs, a condition known as diabetic lung fibrosis. Berberine (BBR) and N-acetylcysteine (NAC) exert a wide range of biological activities, including antioxidant, anti-inflammatory, and antifibrotic actions. The primary purpose of this research is to investigate the therapeutic potential of Berberine (BBR) and N-acetylcysteine (NAC) in the progression of lung fibrosis in rats with induced diabetes.

Methods: Thirty rats were included in the experiment and allocated into five groups (n = 6): a control; a diabetes (D) receiving streptozotocin (STZ, 45 mg/kg); a D+BBR group treated with STZ (45 mg/kg) and berberine (50 mg/kg/day); a D+NAC group administered STZ (45 mg/kg) and N-acetylcysteine (50 mg/kg/day); and a combined treatment group (D+NAC+BBR) given STZ (45 mg/kg) followed by NAC (50 mg/kg/day) and BBR (50 mg/kg/day). Lung tissues were evaluated by histopathological and immunohistochemical techniques.

Results: TGF-β1 and collagen expression significantly increased in group D (27,66) compared to the control group (2,16) ($p<0.001$). TGF-β1 expression was significantly reduced in both combined (9,83) and the individual treatment groups of D+BBR (17,51) ($p<0.01$) and D+NAC (11,16) ($p<0.001$). Similarly, both individual ($p<0.01$) and combined (9,51) ($p<0.001$) treatments of BBR (11,51) and NAC (13,33) significantly decreased collagen expression ($p<0.05$). Additionally, alveolar thickening and dense collagen deposition were observed in group D. These pathological changes were reduced with both combined and the individual treatments of BBR and NAC.

Conclusion: The results indicate that diabetes mellitus induces marked pathological alterations, including alveolar wall thickening, dense collagen deposition, and a significant upregulation of the pro-fibrotic cytokine TGF-β1. Based on the experimental findings, this study demonstrates that BBR and NAC possess significant antifibrotic properties in a rat model of diabetic lung fibrosis.

Keywords: Berberine, Diabetes mellitus, N-acetylcysteine, Pulmonary fibrosis, Rat

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Berberin ve N-asetilsisteinin diyabetik akciğer fibrozisi sıçan modelinde antifibrotik etkileri

Öz

Amaç: Diyabet mellitus (DM), kronik olarak yüksek kan şekeri konsantrasyonları ile karakterize edilen metabolik bir durumdur. DM, karaciğer, böbrekler ve beyin de dahil olmak üzere birçok organı etkiler ve akciğer fibrozisi ile kendini gösteren akciğer hasarıyla ilişkisi kanıtlanmıştır. Kronik yüksek kan şekeri ayrıca akciğerlerde yapısal hasara da neden olabilir; bu durum diyabetik akciğer fibrozisi olarak bilinir. Berberin (BBR) ve N-asetilsistein (NAC), antioksidan, antiinflamatuar ve antifibrotik etkiler de dahil olmak üzere geniş bir yelpazede biyolojik aktivite gösterir. Bu araştırmanın temel amacı, indüklenmiş diyabetli sıçanlarda akciğer fibrozisinin ilerlemesinde Berberin (BBR) ve N-asetilsisteinin (NAC) terapötik potansiyelini araştırmaktır.

Yöntemler: Deneyde otuz sıçan kullanıldı ve beş gruba ayrıldı (n = 6): bir kontrol grubu; tek doz streptozotosin (STZ, 45 mg/kg) alan bir diyabet (D) grubu; STZ (45 mg/kg) ve berberin (50 mg/kg/gün) ile tedavi edilen bir D+BBR grubu; STZ (45 mg/kg) ve N-asetilsistein (50 mg/kg/gün) uygulanan bir D+NAC grubu; ve STZ (45 mg/kg) ardından NAC (28 gün boyunca 50 mg/kg/gün) ve BBR (50 mg/kg/gün) verilen kombine bir tedavi grubu (D+NAC+BBR). Akciğer dokuları histopatolojik ve immünohistokimyasal tekniklerle değerlendirildi..

Bulgular: D grubunda (27,66) kontrol grubuna (2,16) kıyasla TGF-β1 ve kollajen ekspresyonu anlamlı derecede artmıştır ($p<0.001$). TGF-β1 ekspresyonu, hem kombine (9,83) hem de D+BBR (17,51) ($p<0.01$) ve D+NAC (11,16) ($p<0.001$) gruplarının bireysel tedavilerinde anlamlı derecede azalmıştır. Benzer şekilde, BBR (11,51) ve NAC (13,33)'nin hem bireysel ($p<0.01$) hem de kombine (9,51) ($p<0.001$) tedavileri kollajen ekspresyonunu anlamlı derecede azaltmıştır ($p<0.05$). Ek olarak, D grubunda alveol kalınlaşması ve yoğun kollajen birikimi gözlemlenmiştir. Bu patolojik değişiklikler, BBR ve NAC'nin hem kombine hem de bireysel tedavileriyle azalmıştır.

Sonuç: Sonuçlar, diyabetin alveol duvarı kalınlaşması, yoğun kolajen birikimi ve pro-fibrotik sitokin TGF-β1'in önemli ölçüde artışı da dahil olmak üzere belirgin patolojik değişikliklere neden olduğunu göstermektedir. Deneysel bulgulara dayanarak, bu çalışma BBR ve NAC'nin diyabetik akciğer fibrozisi sıçan modelinde önemli antifibrotik özelliklere sahip olduğunu göstermektedir.

Anahtar kelimeler: Berberin, Diyabetes mellitus, N-asetilsistein, Pulmoner fibroz, Sıçan.

INTRODUCTION

Diabetes mellitus (DM) is a systemic disorder marked by persistently elevated blood glucose levels and is associated with many micro and macrovascular complications[1]. DM leads to a range of complications, including microvascular damage such as nephropathy and retinopathy, macrovascular disorders like coronary artery disease, cerebrovascular events, and peripheral arterial disease. As well as pulmonary manifestations, including impaired lung function and the development of pulmonary fibrosis[2]. Idiopathic pulmonary fibrosis (IPF), a progressive and fatal lung disease, is characterized by the proliferation and differentiation of fibroblasts in the lungs. In recent years, the frequent diagnosis of DM in IPF patients has drawn attention to the relationship between these two diseases[27]. Although there are not enough experimental studies to fully explain the relationship between diabetes and pulmonary fibrosis, many clinical studies have reported a very high prevalence of diabetes in IPF patients[28,29].

The presence of an extensive and intricate alveolar–capillary network renders the lung particularly susceptible to damage associated with diabetes[3]. Previous studies have shown that individuals with diabetes have a higher susceptibility to respiratory disorders, including asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis, and pneumonia. These disorders may be accompanied by a decline in lung function[4,5]. Pulmonary fibrosis represents an important cause of early mortality in affected individuals. Current therapeutic approaches largely focus on controlling inflammation through

corticosteroids and immunosuppressive agents, along with anti-cytokine strategies and antifibrotic drugs. However, clinical outcomes remain limited. Consequently, clarifying the contribution of diabetes to the onset of fibrotic changes in the lungs and elucidating the underlying mechanisms by which diabetes promotes lung fibrogenesis are essential for the identification of more effective treatment options[5].

Berberine (BBR) is a naturally occurring alkaloid derived from several medicinal plants[6]. It has been reported that berberine displays a wide spectrum of biological activities, encompassing anti-inflammatory, antioxidant, antidiabetic, and immune-regulating effects[7-9]. It has also been demonstrated that berberine may have an antifibrotic effect in bleomycin-induced lung fibrosis by increasing A2aR expression and attenuating the SDF-1/CXCR4-related pathway[10]. Similarly, N-acetylcysteine (NAC) has been reported to possess multiple biological effects, including antioxidative and anti-inflammatory activities, along with antidiabetic potential[11-14]. Furthermore, NAC has been reported to exhibit antifibrotic effects by reducing inflammatory responses and collagen accumulation in a bleomycin-induced lung fibrosis model in mice[15].

TGF-β1, an important profibrotic cytokine, is an important inducer of the pathogenesis of pulmonary fibrosis[16]. In pulmonary fibrosis, fibroblasts in the lung mesenchyme show elimination of collagen secretion, which is the main component of the extracellular matrix (ECM). A central mediator in this process is TGF-β1, a potent pro-fibrotic cytokine that promotes the transition of fibroblasts to myofibroblasts and increases collagen production. As current therapeutic options for pulmonary fibrosis remain limited, there is a rising focus on naturally derived substances that exhibit antioxidant activity[17].

The relationship between DM and pulmonary fibrosis, and the role of BBR and NAC in this mechanism, has not been fully elucidated. There are insufficient studies on this subject. Therefore, this study aims to evaluate the individual and combined antifibrotic effects of BBR and NAC in a streptozotocin-induced diabetic rat model, focusing on their ability to modulate TGF-β1 and collagen expression and preserve lung architecture.

METHODS

Experimental Animals

In this study, thirty Wistar albino rats with body weights ranging from 200 to 250 g were utilized. The rats were kept in cages made of polycarbonate material at a room temperature of 21-24 °C, under ambient conditions and a 12-hour light/dark photoperiod. The rats were housed under standard conditions and provided with commercial pellet feed and tap water freely throughout the study. Ethics permission for the study was obtained from Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Approval no: 2025/10-11).

Experimental Protocol

Rats were randomly assigned to five groups (n = 6). Diabetes was induced by a single intraperitoneal (IP) injection of streptozotocin (STZ; 45 mg/kg, sc-200719, Santa Cruz). Blood glucose levels were assessed 72 hours after induction to confirm the diabetic state. The control group received only water via intragastric (IG) administration for 28 days. The diabetes (D) group was treated with a single dose of STZ (45 mg/kg). In the D+BBR group, rats received STZ (45 mg/kg, single dose) followed by daily berberine (BBR; 50 mg/kg/day, CAS: 633-65-8, Sigma-Aldrich) for 28 days. The D+NAC group was administered STZ (45 mg/kg, single dose, IP) along with N-acetylcysteine (NAC; 50 mg/kg/day, CAS: 616-91-1, Sigma-Aldrich) for 28 days. In the

D+NAC+BBR group, animals received STZ (45 mg/kg, single dose) in combination with NAC (50 mg/kg/day) and BBR (50 mg/kg/day) for 28 days. Both BBR and NAC were delivered via the intragastric route. At the end of the experimental period, the rats were anesthetized with ketamine (50 mg/kg, IP; Ketalar) and xylazine (10 mg/kg, IP), after which lung tissues were excised and fixed in 10% formaldehyde for subsequent histological and immunohistochemical analyses.

Histological examination protocol

At the conclusion of the experiment, lung specimens were fixed in 10% neutral buffered formalin at room temperature for 48 hours. Following fixation, the samples were rinsed under running tap water to eliminate residual fixative. Standard tissue processing protocols were then performed prior to paraffin embedding. Briefly, the tissues were dehydrated through a graded ethanol series (70%, 80%, 96%, and 100%). Subsequently, clearing was carried out using xylene to remove alcohol and facilitate paraffin infiltration. The specimens were then infiltrated with molten paraffin at 58°C and embedded to obtain properly oriented paraffin blocks. Sections with a thickness of 5 μm were cut from the blocks using a microtome. For histological evaluation, routine hematoxylin and eosin (H&E) staining was performed. After deparaffinization and rehydration, the sections were sequentially stained with hematoxylin and eosin. Finally, the slides were dehydrated through graded alcohols, cleared in xylene, and mounted using Entellan. The preparations, examined under a light microscope (Olympus BX53, Tokyo, Japan), were evaluated for inflammation, collagen deposition, and alveolar septum thickening. Histopathological examinations and photography were performed by a blinded specialist. Histopathological findings were evaluated according to: (−) normal, (+) mild, (++) moderate, and (+++) severe.

Immunohistochemical Method

The expression levels of TGF-β1 and collagen were investigated in paraffin sections using immunohistochemical staining. All procedures were performed according to standard procedures optimized to prevent antigen loss and minimize non-specific staining. Paraffin-embedded tissue samples were cut into 5 μm sections and placed on poly-L-lysine–coated slides. The sections were then deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide (H_2O_2). To unmask nuclear antigens, antigen retrieval was performed by heating the slides in citrate buffer (pH 6.1) in a microwave oven for two 5-minute cycles. Nonspecific background staining was minimized by incubating the sections with Ultra V Block for 10 minutes at room temperature. Sections were incubated with TGF-β1 (Bioss, bs-4538R, 1:200) and collagen (Bioss, bs-10423r, 1:200) primary antibodies at + 4 ^{o}C in a humidity chamber overnight. After washing the sections with PBS, they were sequentially incubated with Biotinylated Goat Anti-Polyvalent and Streptavidin–peroxidase conjugate, each for 10 minutes. Diaminobenzidine (DAB) was used as the chromogen to visualize staining, and the slides were then counterstained with Mayer's hematoxylin. It was then viewed under a light microscope (Olympus BX53, Japan), and the images were analyzed and photographed using Olympus Cellsens Software. For immunohistochemical analysis, 15–20 regions per animal were randomly selected and evaluated. Staining intensity was quantified using the H-score method based on the brown color density in the chosen areas. Immunohistochemical staining was evaluated using a four-tier scoring system: 0 indicated no staining, 1+ represented weak staining observable only at high magnification, 2+ indicated moderate staining, and 3+

corresponded to strong staining visible even at low magnification. The proportion of cells at each staining intensity was estimated visually, and the overall score was calculated using the formula: 1 × (% of 1+) + 2 × (% of 2+) + 3 × (% of 3+)[32].

Statistical Analysis

Statistical analyses of the immunohistochemistry data were conducted using IBM SPSS Statistics 20.0. Comparisons between independent groups were made using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test for variables with normal distribution. Results are expressed as mean ± standard deviation (mean ± SD), and p-values below 0.05 were considered statistically significant.

RESULTS

Histopathological findings of the lungs in diabetic rats

Histopathological examination of the lung showed that diabetes causes fibrosis-associated lung damage. Lung sections of the control group had normal architecture. On the other hand, pathological findings primarily associated with fibrosis in the lungs, such as inflammatory cell infiltration, collagen deposition, and alveolar septum thickening, were observed in group D. However, both BBR and NAC, when given alone or in combination, attenuated fibrotic alterations such as diabetes-related inflammatory cell infiltration, collagen deposition, and alveolar septum thickening. Additionally, no notable difference was found between combined and alone treatments of BBR and NAC. Combination therapies were particularly more effective (Figure 1 and Table 1).

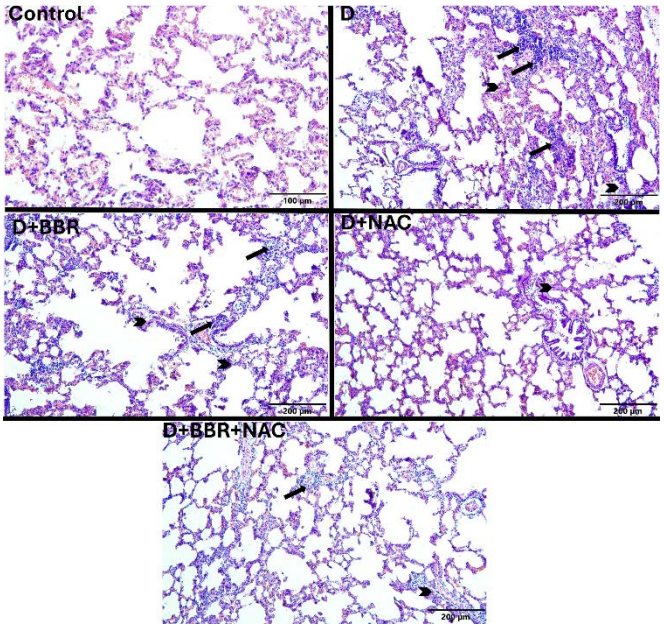

Figure 1. Light microscopic images of lung tissues from the rats. Inflammation (arrow) and collagen deposition (arrowhead) are observed in group D. These pathological changes are observed with both individual and combined treatments of BBR and NAC. H&E. Bar: 200µm.

Table 1: Histopathological findings of rat lung tissues

Groups	Inflammation	Collagen accumulation	Alveolar septum thickening
Control	-	-	-
D	+++	++	++
D+BBR	++	+	+
D+NAC	+	+	+
D+BBR+NAC	+	+	+

Immunohistochemical findings of TGF-β1 and collagen expression in diabetic rats

TGF-β1 and collagen are important molecular parameters used to elucidate the mechanism of fibrosis. Therefore, in our study, immunohistochemical analysis of TGF-β1 and collagen expression was performed to evaluate pulmonary fibrosis. TGF-β1 and Collagen expression was substantially upregulated in the D group versus the control group ($p<0.001$).

This is an important finding proving that diabetes induces pulmonary fibrosis. This indicates a strong upregulation of pro-fibrotic factors and extracellular matrix deposition. TGF-β1 expression was significantly reduced in both the alone treatment groups D+BBR ($p<0.01$) and D+NAC ($p<0.001$), and the combined treatment group D+BBR+NAC ($p<0.001$). In particular, it was observed that NAC alone and BBR + NAC combined treatments were more effective in reducing TGF-β1 expression. Similarly, while combined treatments of BBR and NAC were more effective, both their individual ($p<0.01$) and combined ($p<0.001$) applications significantly decreased collagen expression (Figure 2).

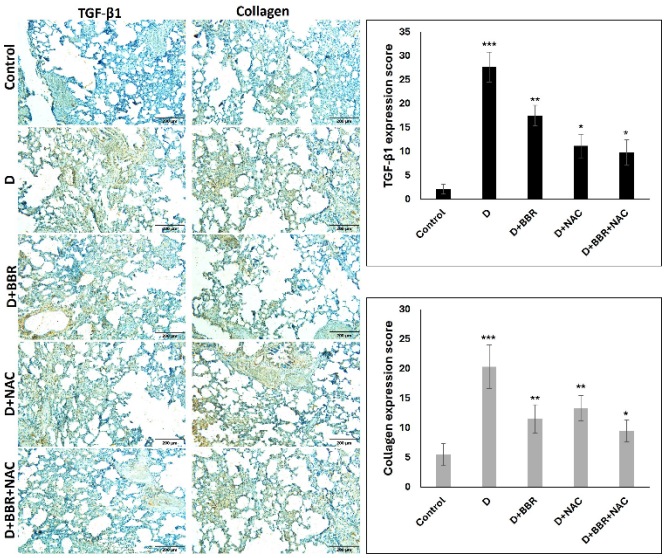

Figure 2. Microscopic images of TGF-β1 and collagen expression in rat lung tissues. Brown areas indicate the degree of positivity of expression. Bar: 200µm.

DISCUSSION

In this study, we focused on the impact of diabetes on the lung and the potential activity of BBR and NAC. The findings of our study showed that fibrotic changes occur in the diabetic lung, but BBR and NAC exhibit a protective effect against pulmonary fibrosis, inhibiting TGF-β1 and collagen expressions. The lung is affected by diabetic microangiopathy in diabetic humans and experimental animals. It has been proven that pathological changes such as basal lamina thickening, alveolar epithelium, pulmonary capillary basal lamina, centrilobular emphysema and fibrosis occur in the diabetic lung[18]. Furthermore, it has been reported that diseases such as diabetes mellitus, metabolic syndrome, obesity, and cardiovascular disease may contribute to the development of pulmonary fibrosis[19].

Previous studies have demonstrated that respiratory dysfunction, preserved Forced expiratory volume in first second (FEV1)/Forced vital capacity (FVC)% ratio, and reduced carbon monoxide diffusing capacity (DLCO) in diabetic patients cause restrictive lung defects. Furthermore, microangiopathic changes seen in diabetic lungs, such as fibrosis and basal lamina thickening, have been reported to be associated with restrictive lung defects[20]. Similarly, Yeh et al. reported that FEV1 and FVC decreased in patients with diabetes and that this was associated with pulmonary dysfunction[21]. Previous clinical investigations have asserted that the lungs may be one of the target organs of diabetes due to decreased vital and total lung capacity in diabetic patients. The presence of fibrotic streaks and increased TGF-β1 and other inflammatory cytokines in the BAL fluid of diabetic patients without a history of chronic obstructive pulmonary disease (COPD) on High-resolution computed tomography (HRCT) images suggests that the lung may be a direct target of diabetes. In the same study, it was reported that cell infiltration and collagen accumulation in the lung tissues of diabetic rats were compared to the control group, as well as increased collagen, fibronectin, and α-SMA expressions in the lung tissue in WB analyses[22]. TGF-β1 is widely recognized as the master regulator of profibrotic responses. In the diabetic state, high glucose levels stimulate the expression of TGF-β1, which in turn promotes the differentiation of fibroblasts into

myofibroblasts. These myofibroblasts are responsible for the excessive synthesis and deposition of collagen, as seen in the high expression scores of group D in our study. The thickening of the alveolar septum observed in the histopathological examination is a direct physical manifestation of this molecular upregulation, which ultimately impairs gas exchange and lung compliance.

The signals that TGF-β1 sends into the cell through Smad proteins increase the production of secondary fibrotic cytokines. These cytokines cause extracellular matrix accumulation, resulting in pulmonary fibrosis[23]. Previous studies reported that expression of TGF-β1 and collagen-III significantly increased, which are associated with pulmonary fibrosis, in the lungs of diabetic rats[30]. Similarly, Aisanjiang et al. reported that histopathological evaluation of the lungs of diabetic mice revealed alveolar structural distortion and thickening of the alveolar septum. In the same study, Masson Trichrome staining results showed collagen fiber accumulation around the alveolar septum, which they reported as an important criterion in explaining the pathogenesis of pulmonary fibrosis[31]. Lung fibrosis is fueled by a breakdown in autophagy across alveolar cells, fibroblasts, and macrophages, triggering a cascade of inflammation and disordered collagen production[33]. Our study's histopathological findings reveal the occurrence of pathological changes such as inflammation, collagen deposition, and alveolar septum thickening in diabetic rats. Thus, our findings are consistent with the literature and appear to contribute to elucidating the pathogenesis of diabetic pulmonary fibrosis.

Chitra et al. demonstrated that berberine attenuates experimental pulmonary fibrosis through Smad pathway signaling pathway inhibition[16]. It has also been reported that BBR reduces experimental pulmonary fibrosis by preventing oxidative stress and inflammation[24]. Pulmonary fibrosis develops as a result of COPD, which is associated with smoking. Small airway epithelial-mesenchymal transformation (EMT) is a major cause of COPD and pulmonary fibrosis[25]. Previous studies have reported that NAC improves pulmonary fibrosis in COPD by inhibiting EMT[26]. Zhao et al. reported that BBR suppresses pulmonary fibrosis by reducing TGFBR2 expression, thereby weakening TGF-β/Smad2/3 signaling, while simultaneously promoting autophagy via activation of the PI3K/AKT/mTOR pathway through phosphorylation[34]. In our study, increased TGF-β1 and collagen expression, which are associated with the pathogenesis of pulmonary fibrosis in diabetic rats, and the fibrotic changes observed in our histopathological findings, suggest that diabetes may induce pulmonary fibrosis. The most notable finding of this study is the significant reduction in fibrotic markers after treatment with BBR and NAC. In particular, combined treatment with these two molecules had a more potent antifibrotic effect. This suggests that the combined application of these two molecules may create a synergistic effect.

The results of this study demonstrate that diabetes induces significant structural and molecular alterations in the lung, characterized by inflammatory cell infiltration, alveolar septum thickening, and marked fibrosis. These findings align with the emerging concept of diabetic lung fibrosis, where chronic hyperglycemia triggers a cascade of oxidative stress and inflammatory responses, leading to the remodeling of the pulmonary extracellular matrix (ECM). Our immunohistochemical analysis specifically highlights the upregulation of TGF-β1 and Collagen as central drivers of this pathology. Our study findings suggest that diabetes may cause pulmonary fibrosis, and that BBR and NAC, especially their combined treatment, may exhibit antifibrotic effects by suppressing TGF-β1 and collagen expression.

Data Availability Statement: The data supporting the findings of this study are available from the authors upon reasonable request.

Ethics Committee Approval: Ethics permission for the study was obtained from Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Approval no: 2025/10-11).

Conflict of Interest: The author(s) declare that there is no financial conflict of interest related to this article.

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