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Wedelolactone mitigates mitochondrial dysfunction to enhance food-related biochemical interventions in disc degeneration

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ORIGINAL ARTICLE

Abstract

Intervertebral disc degeneration (IVDD), a prevalent age-related musculoskeletal disorder, poses a significant global health burden. While understanding that its pathogenesis and identifying its effective therapeutic compounds remain essential, increasing attention has been directed toward bioactive compounds derived from food sources for their potential role in health management. Wedelolactone, a phytochemical with diverse biological activities, has emerged as a promising candidate. This study examines its effects in an IVDD cell model, where nucleus pulposus (NP) cells isolated from Sprague Dawley rat intervertebral discs were exposed to 200 μM H_2O_2 for 24 hours to induce oxidative stress. Wedelolactone significantly reduced H_2O_2-induced reactive oxygen species (ROS) production and alleviated mitochondrial dysfunction in NP cells. It also inhibited cellular senescence and apoptosis while regulating the expression of genes involved in extracellular matrix synthesis and degradation. Mechanistic investigations revealed that Wedelolactone exerted its protective effects through the AMPK/SIRT1/PGC1α (adenosine monophosphate-activated protein kinase/silent information regulator 1/peroxisome proliferator-activated receptor coactivator-1 alpha) axis, highlighting its role in mitochondrial homeostasis. These findings suggest that Wedelolactone, a potential food-derived compound, could be a promising candidate for functional food development aimed at mitigating IVDD and associated mitochondrial dysfunction.

Keywords: disc degeneration (IVDD); Wedelolactone; ROS; ECM; AMPK/SIRT1/PGC1α axis

Introduction

Intervertebral disc degeneration (IVDD) is a common age-related musculoskeletal disorder that significantly reduces quality of life and imposes a substantial clinical and socioeconomic burden worldwide (Kang *et al.*, 2023; Yang *et al.*, 2020). It is known to occur from a complex interplay of pathological factors, with more than 40% of lower back pain cases being attributed to IVDD progression (Ashinsky *et al.*, 2020). The disease is characterized by degradation of the nucleus pulposus (NP) matrix, reduced proteoglycan content, diminished hydration, and shrinkage of the NP (Zhao *et al.*, 2020). In IVDD, the annulus fibrosus becomes thickened and disorganized, often developing fissures, while the cartilage endplate progressively thins and undergoes calcification; these pathophysiological alterations are closely linked to age-related cellular and tissue dysfunction.

Despite extensive research in this field and studies revealing several factors implicated in IVDD pathogenesis, such as oxidative stress, mechanical load, immune inflammation, apoptosis, mitochondrial dysfunction and stem cell decline (Kučírková *et al*., 2018), the precise mechanisms underlying IVDD remain unclear, complicating the development of effective prevention and treatment strategies. Oxidative stress-induced reactive oxygen species (ROS) contribute to NP cell apoptosis and senescence as it can disrupt the balance between anabolic and catabolic processes in the extracellular matrix (ECM) (Kučírková *et al*., 2018; Bortolini *et al*., 2022). Therefore, a deeper understanding of IVDD pathogenesis is essential for developing novel therapeutic strategies.

Mitochondria, as the primary intracellular targets of ROS, play a central role in maintaining cellular metabolic balance. Dysfunctional mitochondria impairs ATP (adenosine triphosphate) production, essential for cellular function, and promotes inflammatory degeneration (Zhang *et al*., 2023, Ren *et al*., 2019). Mitochondrial damage has been closely linked to the progression of degenerative diseases, emphasizing its importance in cellular homeostasis (Zhang *et al*., 2023).

The relationship between oxidative stress and mitochondrial dysfunction is bidirectional. Oxidative stress disrupts mitochondrial function by altering membrane potential, calcium homeostasis, and respiratory chain activity, further exacerbating cellular damage (Zhang *et al*., 2023). In turn, mitochondria, as the primary metabolic centers of the cell, are highly susceptible to oxidative insults and environmental toxins. Mitochondrial damage caused by oxidative stress can trigger the release of inflammatory mediators, such as cytokines, thereby amplifying inflammatory responses and accelerating IVDD progression (Ren *et al*., 2019).

Adenosine monophosphate-activated protein kinase (AMPK) is a key metabolic sensor that detects cellular energy deficits by monitoring AMP (adenosine monophosphate-activated protein) and ADP (adenosine diphosphate) levels (Ren *et al*., 2023). Its activation represents an essential defense mechanism against degenerative diseases, as it enhances mitochondrial homeostasis through the upregulation of silent information regulator 1 (SIRT1) (Luan *et al*., 2023). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α), a major downstream effector of AMPK, plays an essential role in mitochondrial biogenesis, oxidative phosphorylation, and ATP synthesis, collectively mitigating mitochondrial dysfunction (Zhao & Jing, 2023; Trefts & Shaw, 2021).

Wedelolactone, a naturally occurring phytochemical, has gained attention for its diverse biological activities (Malik *et al*., 2023). Traditionally, it is derived from *Eclipta prostrata L.*, an herb widely used in Chinese medicine to support liver and kidney function and has also been employed in treating osteoporosis and other bone-related conditions (Rubalcava-Gracia *et al*., 2023). Beyond its traditional applications, it has demonstrated anti-inflammatory and antifibrotic effects in various disease models. In collagen-induced arthritis, Wedelolactone alleviates synovial inflammation and cardiac complications by inhibiting NF-κB/NLRP3 (nuclear factor-kappa B/nucleotide-binding domain, leucine-rich repeat, and pyrin domain containing 3) inflammasome activation. Additionally, it can mitigate pulmonary fibrosis by activating AMPK and modulating the RAF-MAPK (rapidly accelerated fibrosarcoma-mitogen-activated protein kinase) signaling pathway (Kučírková *et al*., 2018). Despite these promising effects, its potential role in IVDD and the underlying mechanisms remains unexplored.

Herein, this study investigates whether Wedelolactone can mitigate IVDD by targeting mitochondrial dysfunction and related cellular processes in an in vitro IVDD model.

Materials and Methods

Isolation of rat NP cells

Rats were humanely euthanized following ethical guidelines and their lumbar spine aseptically excised. The intervertebral discs were dissected under a dissecting microscope to minimize damage to the NP tissue. Then the NP tissue was carefully isolated using fine-tipped forceps and enzymatically digested in 0.2% collagenase type II (C6885; Sigma-Aldrich, St. Louis, MO, USA) dissolved in Dulbecco's Modified Eagle Medium (DMEM) (Gibco 11965-092; Thermo Fisher Scientific, Waltham, MA, USA). Digestion was performed for 4 hours at 37°C in a humidified atmosphere with 5% CO_2 (carbon dioxide), and the resulting suspension was filtered through a strainer (352350; BD Biosciences, Franklin Lakes, NJ, USA) to remove undigested tissue and debris. The filtered cells were washed twice with phosphate-buffered saline (PBS) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco 10437-028; Thermo Fisher Scientific, Waltham, MA, USA). These cells were maintained at 37°C in a humidified 5% CO_2 atmosphere; the culture medium was replaced every two days until confluence was achieved. Ethical approval for animal use was granted by the Ethics Committee of the First Affiliated Hospital of Wannan Medical College (Yijishan Hospital) (Approval No. 2020-26).

Construction of IVDD cell model

An in vitro IVDD (intervertebral disc disease) cell model was constructed based on a previously described method.

NP cells were treated with 200 μM hydrogen peroxide (H_2O_2) (Sigma-Aldrich, H1009, USA) for 24 hours to induce oxidative stress and cellular damage, mimicking degenerative conditions. Following H_2O_2 treatment, cells were exposed to Wedelolactone (Sigma-Aldrich, W4014, USA) at concentrations of 1.25, 2.5, 5, 10, and 20 μM for an additional 24 hours to evaluate its therapeutic effects. For subsequent experiments, cells were divided into five groups: (1) blank control (untreated cells); (2) H_2O_2-treated control group (cells without Wedelolactone); (3) H_2O_2-treated Wedelolactone group (1.25 μM); (4) H_2O_2-treated Wedelolactone group (2.5 μM); and (5) H_2O_2-treated Wedelolactone group (5 μM).

Cell viability assay

NP cells were seeded into 96-well plates and treated (described in Section 2.2). Cell viability was measured using the Cell Counting Kit-8 (CCK-8) (C0038; Beyotime, Beijing, China). Briefly, the cells were incubated with CCK-8 solution for 4 hours and the optical density (OD) at 450 nm was recorded to assess cell viability.

DCF staining for ROS detection

To evaluate intracellular ROS levels, NP cells were fixed following treatment and blocked with goat serum for 1 hour. ROS detection was performed using a DCF (dichlorofluorescein)/ROS detection kit (ab238535; Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. After staining, cells were washed with PBS and fluorescence images were captured to visualize intracellular ROS levels.

Cell apoptosis assay

The cells were divided into five groups: (1) blank control (untreated cells); (2) H_2O_2-treated control group (cells without Wedelolactone); (3) H_2O_2-treated Wedelolactone group (1.25 μM); (4) H_2O_2-treated Wedelolactone group (2.5 μM); and (5) H_2O_2-treated Wedelolactone group (5 μM). Cell apoptosis was assessed using a FITC (fluorescein isothiocyanate) Annexin V/Propidium Iodide (PI) Apoptosis Detection Kit (Beyotime, Beijing, China). Briefly, the NP cells were fixed with 70% ethanol for 2 hours, stained with Annexin V-FITC and PI at 4°C, and analyzed for apoptotic levels using fluorescence microscopy.

JC-1 staining for mitochondrial membrane potential

To assess mitochondrial membrane potential, NP cells were incubated with 2 μM JC-1 (fluorescent dye) (Beyotime, Beijing, China) for 15 minutes at 37°C in the dark following PBS rinsing. and then, the fluorescence signals were observed under a fluorescence microscope to evaluate mitochondrial integrity.

MitoSOX detection for mitochondrial superoxide

Mitochondrial superoxide production was detected using the MitoSOX Red mitochondrial superoxide indicator (M36008; Thermo Fisher Scientific, Inc., Waltham, MA, USA). NP cells were incubated with 5 μM MitoSOX Red reagent for 10 minutes at 37°C in the dark, followed by fluorescence imaging to assess mitochondrial oxidative stress.

SA-β-gal staining for cellular senescence

To evaluate cellular senescence, the NP cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature and stained with SA-β-gal solution (#9860; Cell Signaling Technology, Danvers, MA, USA) at pH (potential of hydrogen) 6.0 for 12 hours. SA-β-gal-positive cells were quantified under a microscope to determine the extent of senescence.

Quantitative real-time PCR

Total RNA (ribonucleic acid) was extracted from NP cells using Trizol reagent (TaKaRa, Japan) and reverse-transcribed into cDNA (complementary deoxyribonucleic acid) using the RT Reagent Kit (TaKaRa, Japan). Quantitative polymerase chain reaction (qPCR) was performed using SYBR Ex Taq™ (fluorescent dye) II (TaKaRa, Japan) to quantify gene expression levels, with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) serving as an internal reference. Relative gene expression was calculated using the 2^(-ΔΔCt) method. The primer sequences used were:

Col2α: Forward 5'-GGCAATAGCAGGTTCAC GTACA-3', Reverse 5'-CGATAACAGTCTTGCC CCACTT-3'

Sox9: Forward 5'-GGAGCGCAGCAAGGTGAGT-3', Reverse 5'-GGGCTTGTAGGTGATGAGGG-3'

Acan: Forward 5'-CGAGGAGGCGAGAGAGG ACT-3', Reverse 5'-CGTTTGCAGGAGTCCAG TGT-3'

Mmp3: Forward 5'-ACACGACATCAAGGAGT GGCT-3', Reverse 5'-TTCCCATCATCATCGGG TAACT-3'

Mmp13: Forward 5’-CAGTGGTTTGCCCTGAAA CT-3’, Reverse 5’-AGCAGCATCGTCCACATA GTT-3’

Adamts5: Forward 5’-TGGAGGACGGTGAGG ATGAT-3’, Reverse 5’-TGGGTTTGATCTCGTGT AGG-3’.

Immunoblot analysis

Total protein was extracted from NP cells separated via SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred onto PVDF (polyvinylidene fluoride) membranes. After blocking with 5% milk in TBST (Tris Buffered Saline with Tween 20), membranes were incubated with primary antibodies (1:500–1:3000; Abcam, Cambridge, MA, USA) targeting Col2α, MMP13, Adamts5, AMPK, p-AMPK, SIRT1, PGC1α, and GAPDH. After incubation with secondary antibodies, protein signals were detected using a chemiluminescence system.

Immunofluorescent staining

NP cells were fixed with 4% paraformaldehyde, blocked with 5% BSA (bovine serum albumin), and incubated with a primary antibody against PGC1α (ab176328; Abcam, Cambridge, MA, USA). After washing, cells were incubated with Alexa Fluor 488-conjugated secondary antibodies (invitrogen) and counterstained with DAPI (4’,6-diamidino-2-phenylindole). Fluorescence images were captured using a fluorescence microscope.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 8.0. Independent t-tests were applied for comparisons between two groups when data were normally distributed and the Mann-Whitney U test was used for non-normally distributed data. For multiple-group comparisons, one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test was performed. Statistical significance was set at $P < 0.05$ and all experiments were conducted in triplicate.

Results

Wedelolactone alleviates H_2O_2-induced ROS production in NP cells

To assess the therapeutic potential of Wedelolactone in IVDD, an in vitro model was established using H_2O_2-treated NP cells. CCK-8 assays indicated that Wedelolactone at low concentrations (1.25, 2.5, and 5 μM) had minimal effect on NP cell viability, whereas higher concentrations (10 and 20 μM) significantly reduced viability ($P < 0.01$). Consequently, low concentrations were selected for subsequent experiments (Figure 1A). H_2O_2 treatment inhibited NP cell growth, but Wedelolactone reversed this effect, enhancing cell viability (Figure 1B).

The antioxidative effects of Wedelolactone were further examined using DCF staining and flow cytometry. H_2O_2 treatment significantly increased intracellular ROS levels, indicating oxidative stress. However, Wedelolactone reduced ROS intensity and decreased the percentage of FITC-A-positive cells, demonstrating its capacity to mitigate oxidative damage (Figure 1C–F). These findings suggest that the antioxidative properties of Wedelolactone may be attributed to its phytochemical composition.

Wedelolactone alleviates mitochondrial dysfunction in H_2O_2-treated NP cells

Mitochondrial integrity is essential for cellular metabolism and energy production. In this study, mitochondrial dysfunction, a key feature of IVDD, was evaluated using MitoSOX and JC-1 staining. H_2O_2 treatment induced mitochondrial ROS production, which was significantly reduced following Wedelolactone treatment (Figure 2B). JC-1 staining further revealed that H_2O_2 disrupted the mitochondrial membrane potential, increasing the proportion of JC-1 monomers while decreasing JC-1 aggregates. Wedelolactone restored mitochondrial membrane potential, indicating its protective effect in preserving mitochondrial function (Figure 2A). These findings highlight the potential of plant-derived compounds such as Wedelolactone that could be sourced from functional foods or dietary supplements to support mitochondrial health.

Wedelolactone blocks senescence and apoptosis in H_2O_2-induced NP cells

The effects of Wedelolactone on apoptosis and cellular senescence were evaluated to determine its potential in counteracting IVDD progression. Flow cytometry analysis showed that H_2O_2 treatment significantly increased apoptotic cell percentages, whereas Wedelolactone treatment effectively reduced apoptosis in NP cells ($P < 0.05$, Figure 3A and B). Similarly, SA-β-gal staining demonstrated that H_2O_2 exposure increased the proportion of senescent cells, a hallmark of IVDD. Wedelolactone treatment decreased cellular senescence, enhancing NP cell resilience under oxidative stress ($P < 0.05$) (Figure 3C,D). Together, these findings align with growing evidence supporting the antiapoptotic and anti-senescence properties of certain food-derived phytochemicals.

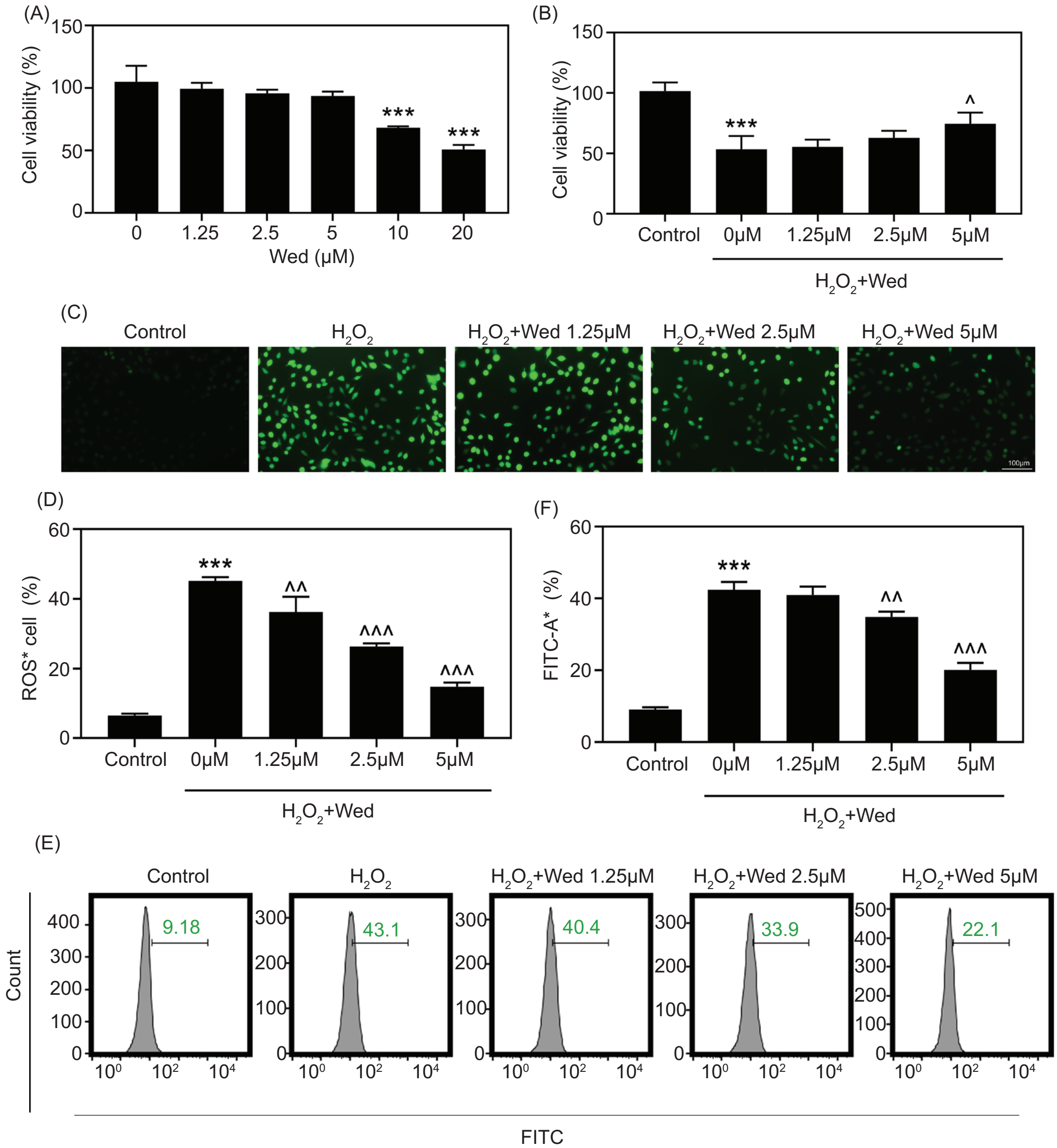

Figure 1. Wedelolactone alleviates H_2O_2-induced ROS production in NP cells. (A) CCK-8 assay showing NP cell viability following treatment with Wedelolactone (0, 1.25, 2.5, 5, 10, and 20 μM) for 24 hours. OD_{450} values were measured and compared. ****P* < 0.001, Wedelolactone versus control. (B) CCK-8 assay showing NP cell viability after treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. OD_{450} values were measured and compared. (C) DCF staining illustrating ROS levels in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. Green fluorescence represents ROS. Scale bar, 100 μm. (D) Quantification of panel C showing the percentage of ROS-positive cells. (E) Flow cytometry analysis of ROS intensity in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (F) Quantification of panel E showing the percentage of FITC-A-positive cells. ****P* < 0.001, H_2O_2 versus control; ^^*P* < 0.01, ^^^*P* < 0.001, H_2O_2 + Wedelolactone versus H_2O_2. Wed: Wedelolactone.

Wedelolactone modulates ECM synthesis and degradation in H_2O_2-induced NP cells

The ECM is essential for maintaining the structural integrity of intervertebral discs. Disruption of ECM homeostasis contributes to IVDD progression. H_2O_2 treatment significantly decreased the expression of ECM synthesis markers, including Col2α, Sox9, and Acan, while increasing the expression of degradation markers such as Mmp3, Mmp13, and Adamts5 at both mRNA and protein levels ($P < 0.05$) (Figure 4A). Wedelolactone treatment restored

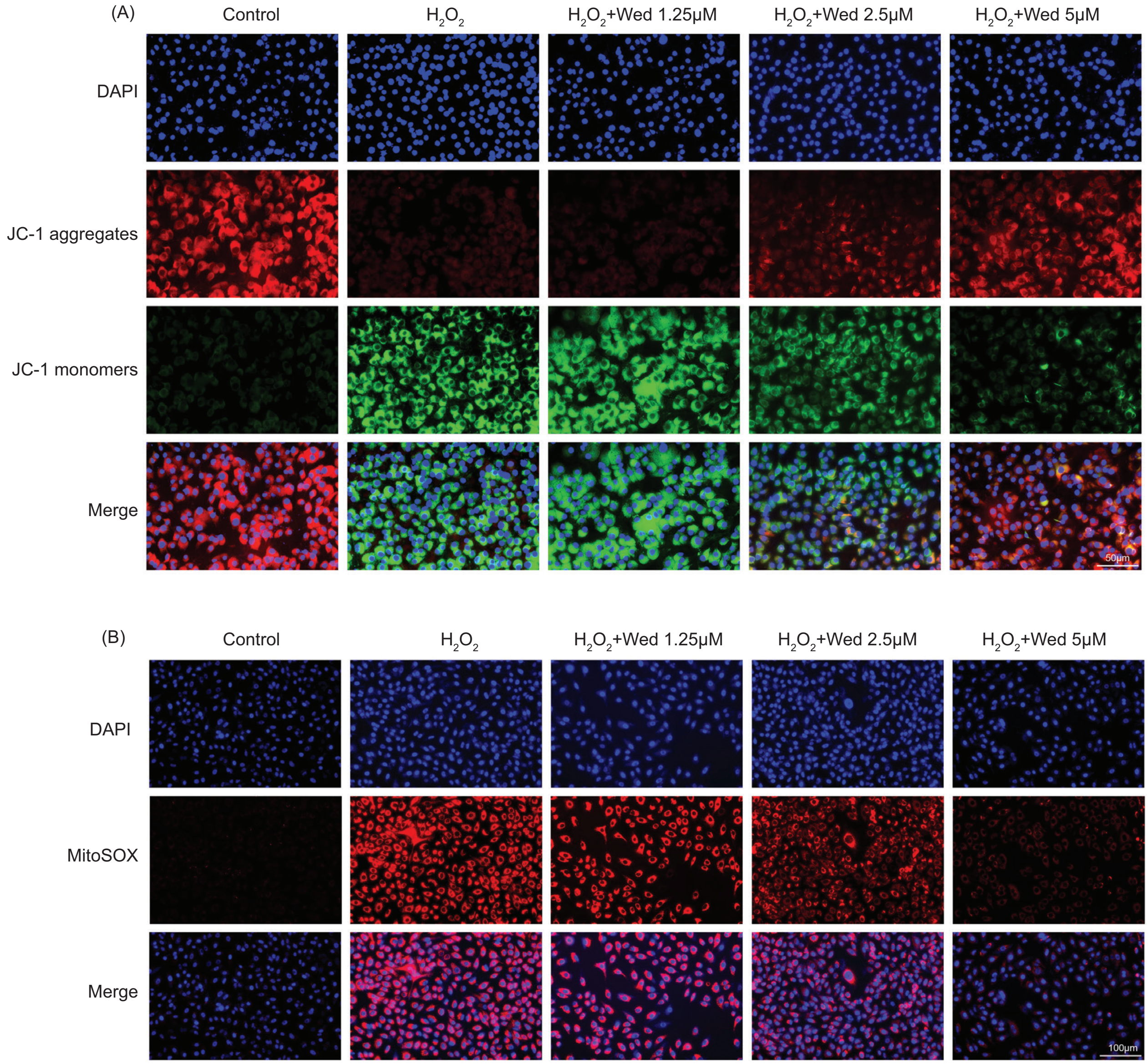

Figure 2. Wedelolactone alleviates mitochondrial dysfunction of NP cells induced by H_2O_2. (A) Immunostaining showing mitochondrial ROS levels in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours, where red fluorescence represents mitoSOX staining. Scale bar, 100 μm. (B) JC-1 staining of NP cells treated with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours, where red fluorescence indicates JC-1 aggregates while green fluorescence represents JC-1 monomers. Scale bar, 100 μm. Wed Wedelolactone.

ECM balance by upregulating synthesis markers and downregulating degradation markers, as confirmed by immunoblotting ($P < 0.05$) (Figure 4B,C). These findings indicate that Wedelolactone helps preserve ECM integrity, an essential factor in preventing IVDD progression.

Wedelolactone mediates the AMPK/SIRT1/PGC1*α* axis in H_2O_2-induced NP cells

To elucidate the molecular mechanisms underlying Wedelolactone's protective effects, the AMPK/SIRT1/PGC1α signaling pathway, a key regulator of cellular metabolism and stress responses, was examined. H_2O_2 treatment reduced AMPK phosphorylation and downregulated SIRT1 and PGC1α expression, leading to impaired mitochondrial function and metabolic imbalance. Wedelolactone reversed these effects by restoring AMPK activation and increasing SIRT1 and PGC1α levels ($P < 0.05$) (Figure 5A,B). Immunofluorescent staining further confirmed an increase in PGC1α intensity in Wedelolactone-treated NP cells (Figure 5C). Collectively, these findings suggest that Wedelolactone exerts its protective effects against oxidative stress, mitochondrial

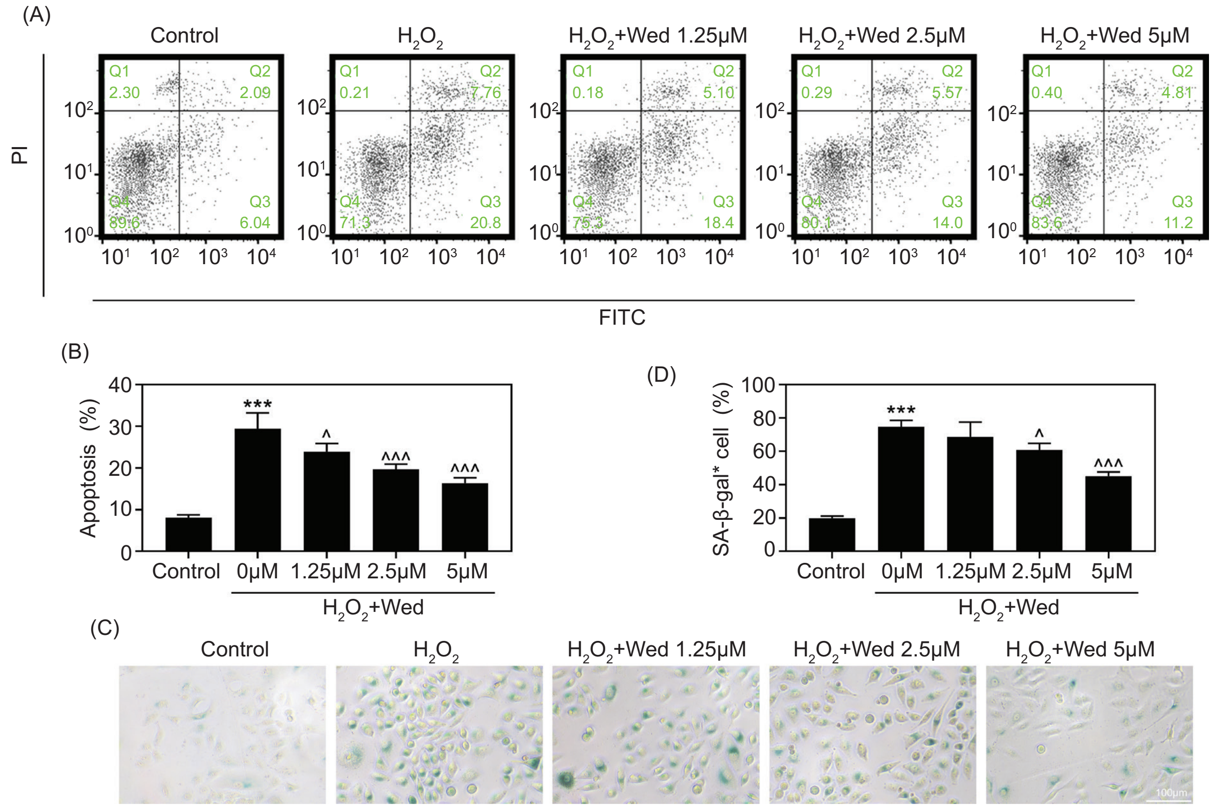

Figure 3. Wedelolactone blocks senescence and apoptosis of H_2O_2-induced NP cells. (A) Flow cytometry analysis of apoptosis in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (B) Quantification of panel A showing the percentage of apoptotic cells. (C) SA-β-gal staining illustrating senescence in NP cells treated with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (D) Quantification of panel C showing the percentage of SA-β-gal-positive cells. ****P* < 0.001, H_2O_2 versus control; ^*P* < 0.05, ^^^*P* < 0.001, H_2O_2 + Wedelolactone versus H_2O_2. Wed: Wedelolactone.

dysfunction, and ECM degradation by activating the AMPK/SIRT1/PGC1α axis.

Discussion

IVDD is a common age-related musculoskeletal disorder driven by multifactorial pathological processes (Kang *et al.*, 2023). Excessive production of ROS and mitochondrial dysfunction play critical roles in IVDD progression (Bortolini *et al.*, 2022), highlighting the need for therapeutic strategies that target these mechanisms to slow disease progression.

This study demonstrates the therapeutic potential of Wedelolactone, a phytochemical with antioxidative and anti-inflammatory properties, in alleviating IVDD-related pathology. By reducing ROS levels, restoring mitochondrial function, and activating the AMPK/SIRT1/PGC1α pathway, Wedelolactone could effectively counteract oxidative stress-induced damage in NP cells. Overall, these findings contribute to a growing body of evidence supporting the use of bioactive compounds, including those derived from functional foods, as potential therapeutic agents for degenerative diseases such as IVDD.

The ability of Wedelolactone to attenuate ROS production and preserve mitochondrial function suggests its potential as a therapeutic candidate for IVDD. ROS-induced oxidative stress disrupts mitochondrial homeostasis, a key factor in cellular aging and apoptosis. In this study, H_2O_2-induced oxidative stress significantly increased ROS levels, impaired mitochondrial function, and promoted NP cell senescence and apoptosis. However, Wedelolactone treatment could mitigate ROS accumulation, preserve mitochondrial membrane potential, reduce senescence markers, and inhibit apoptosis, thereby interrupting the cycle of oxidative damage and ECM degradation.

The effects of Wedelolactone on mitochondrial health are consistent with its reported efficacy in other disease models, including arthritis and pulmonary fibrosis. These

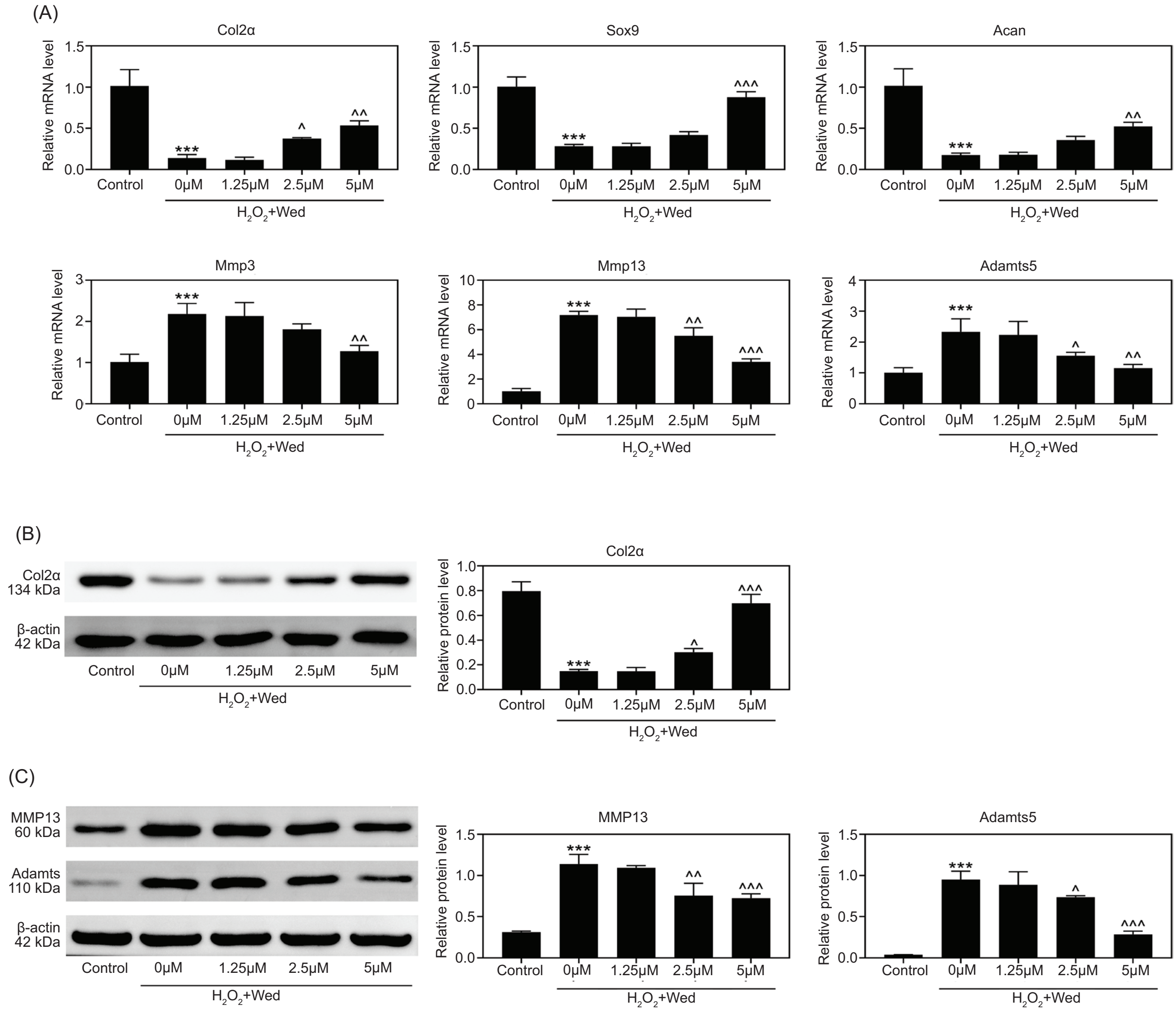

Figure 4. Wedelolactone regulates ECM synthesis and degradation in H_2O_2-induced NP cells. (A) qPCR analysis of Col2α, Sox9, Acan, Mmp3, Mmp13, and Adamts5 mRNA levels in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (B) Immunoblot analysis of Col2α protein expression in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (C) Immunoblot analysis of Mmp13 and Adamts5 protein expression in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. ******P* < 0.001, H_2O_2 versus control; ^*P* < 0.05, ^^*P* < 0.01, ^^^*P* < 0.001, H_2O_2 + Wedelolactone versus H_2O_2. Wed: Wedelolactone.

protective effects are largely mediated through its regulation of key signaling pathways, such as NF-κB/NLRP3 inflammasome inhibition, RAF-MAPK signaling modulation, and AMPK activation (Nottingham *et al.*, 2014; Mohd Isa *et al.*, 2022). The findings from this study reinforce the broad therapeutic potential of Wedelolactone and support its suitability as a dietary intervention for conditions associated with oxidative stress and mitochondrial dysfunction.

The AMPK/SIRT1/PGC1α axis plays a central role in maintaining cellular energy homeostasis, regulating mitochondrial function, and protecting against oxidative damage (Dai *et al.*, 2023; Yang *et al.*, 2019). As a key sensor of cellular energy status, AMPK activation enhances SIRT1 expression, which, in turn, promotes PGC1α activity. PGC1α, a master regulator of mitochondrial biogenesis and oxidative defense, is essential for maintaining cellular redox balance and mitigating oxidative stress (Kang *et al.*, 2020; Zhang *et al.*, 2022; Nottingham *et al.*, 2014).

In this study, Wedelolactone-mediated activation of the AMPK/SIRT1/PGC1α axis in H_2O_2-treated NP cells highlights its mechanism in alleviating IVDD-associated cellular damage. Activation of this pathway restored mitochondrial function, reduced ROS levels, and improved ECM homeostasis, demonstrating its ability to counteract key pathological features of IVDD.

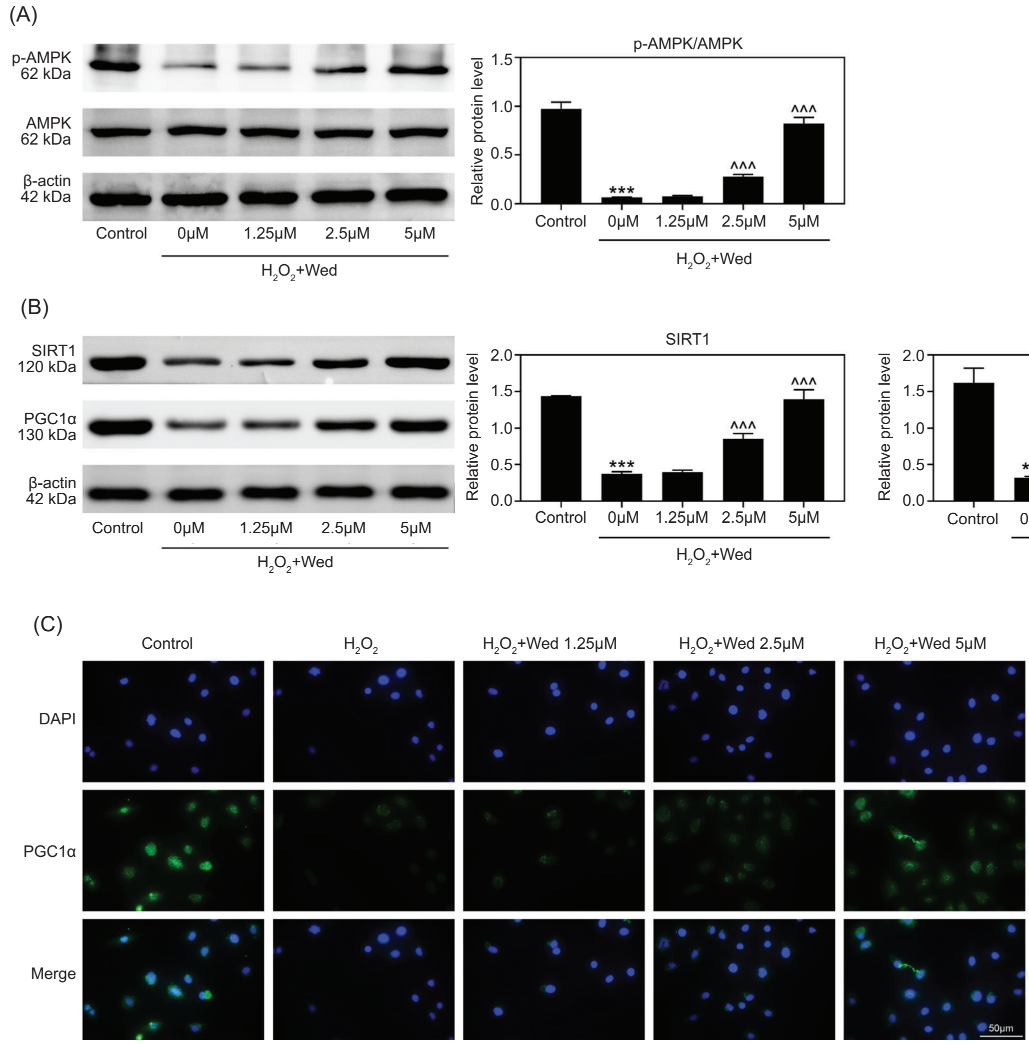

Figure 5. Wedelolactone mediates the AMPK/SIRT1/ PGC1α axis in H_2O_2-induced NP cells. (A) Immunoblot analysis of AMPK expression and phosphorylation levels in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (B) Immunoblot analysis of SIRT1 and PGC1α expression levels in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. (C) Immunofluorescent staining of PGC1α expression in NP cells following treatment with H_2O_2 (200 μM) and Wedelolactone (0, 1.25, 2.5, and 5 μM) for 24 hours. Green fluorescence represents PGC1α. Scale bar, 100 μm. *$P < 0.001$, H_2O_2 versus control; ^$P < 0.05$, ^^^$P < 0.001$, H_2O_2 + Wedelolactone versus H_2O_2. Wed: Wedelolactone.

The primary limitation of this study is the absence of in vivo and clinical investigations. While these findings provide valuable mechanistic insights, further validation using animal models and clinical samples is necessary. Additionally, rescue experiments and pathway-specific inhibitors could strengthen the causal link between AMPK/SIRT1/PGC1α activation and Wedelolactone's protective effects in IVDD.

The promising effects of Wedelolactone in IVDD underscore the potential of food-derived bioactive compounds in treating degenerative diseases. As a phytochemical (derived from plants commonly used in traditional medicine), Wedelolactone exemplifies the intersection of food science and therapeutic research. Functional foods enriched with bioactive compounds such as Wedelolactone could serve as preventative or complementary interventions for oxidative stress-related conditions, including IVDD.

Future research should focus on improving Wedelolactone's bioavailability through optimized food matrices and delivery systems. Additionally, long-term safety and efficacy studies in clinical settings are essential to assess its potential for incorporation into nutraceuticals or functional foods. Such approaches could provide a noninvasive and sustainable strategy for managing musculoskeletal health, particularly in populations at risk of IVDD.

Conclusion

This study provides compelling evidence supporting Wedelolactone as possibly a therapeutic agent for IVDD. By targeting key pathological processes, including oxidative stress, mitochondrial dysfunction, and ECM dysregulation, Wedelolactone offers a multifaceted approach to mitigating IVDD progression. Its ability to activate the AMPK/SIRT1/PGC1α pathway further highlights its broad therapeutic potential. Beyond its pharmacological effects, Wedelolactone represents a promising candidate for functional food and nutraceutical development aimed at managing degenerative diseases. Future research should focus on optimizing its delivery, improving bioavailability, and validating its efficacy in vivo, paving the way for its integration into clinical and dietary strategies to support musculoskeletal health.

Acknowledgments

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request. Data sharing does not apply to this article as no new data were created or analyzed in this study.

Ethics Approval

All animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Wannan Medical College (Yijishan Hospital) to use animals and conducted per the National Institutes of Health Laboratory Animal Care and Use Guidelines (Approval No. 2020-26).

Author Contributions

Min Zhang designed the study, completed the experiment, and supervised the data collection. Bin Hu analyzed and interpreted the data. Liangye Sun prepared the manuscript for publication and reviewed the draft. All authors have read and approved the manuscript.

Conflicts of Interests

The authors state that there are no conflicts of interest to disclose.

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