ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Treadmill exercise promotes melatonin regulation of TXNIP/NLRP3 and inhibits pyroptosis-mediated osteoarthritis progression in a DMM rat model

Ruba Altahla[1,2†], Jamal Alshorman[1†], Li Chaoyi[1], Muhammad Umar[3], Mohd M. Hourani[4], and Yongping Wang[1*]

[1]Department of Orthopedics, The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China

[2]Department of Rehabilitation, The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China

[3]Research Center for Medical Artificial Intelligence, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China

[4]Head Health Centre, United Nations Relief and Works Agency, Zarqa, Jordan

[†]*These authors contributed equally to this work.*

***Corresponding author:**
Yongping Wang
(wangyp0312@126.com)

Citation: Altahla R, Alshorman J, Chaoyi L, Umar M, Hourani MM, Wang Y. Treadmill exercise promotes melatonin regulation of TXNIP/NLRP3 and inhibits pyroptosis-mediated osteoarthritis progression in a DMM rat model. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(4):73-84.
doi: 10.36922/ITPS025360046

Received: September 2, 2025

Revised: September 25, 2025

Accepted: October 10, 2025

Published online: November 18, 2025

Copyright: © 2025 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Osteoarthritis (OA) is characterized by the activation of nucleotide-binding and oligomerization domain-like receptor protein 3 (NLRP3) by thioredoxin-interacting protein (TXNIP), which promotes inflammation and pyroptosis. This study investigates whether treadmill exercise (TE) enhances melatonin-mediated regulation of the TXNIP/NLRP3 pathway and attenuates OA progression by modulating pyroptosis in a destabilization of the medial meniscus (DMM) rat model. A total of 32 male Sprague–Dawley rats (6 weeks old; 220 ± 20 g) were randomly assigned to four groups: Sham, DMM, DMM + melatonin, and DMM + melatonin + TE. The intervention lasted for 8 weeks. Morphological staining, immunofluorescence (IF), microcomputed tomography with three-dimensional reconstruction, Western blot, quantitative real-time polymerase chain reaction, and enzyme-linked immunosorbent assay were used to assess protein and gene expression. Compared with the DMM + melatonin group, the DMM + melatonin + TE group showed greater reductions in cartilage–subchondral bone damage and OA progression through modulation of the pyroptosis pathway. IF staining revealed that TXNIP protein expression was significantly reduced in the DMM + melatonin + TE group. Both the DMM + melatonin and combination treatment groups significantly regulated TXNIP/NLRP3 signaling and inhibited OA progression through pyroptosis. Furthermore, combining TE with melatonin significantly reduced the expression of pyroptotic cytokines compared with the DMM + melatonin group. This study suggests a potential therapeutic approach for managing OA by combining melatonin treatment with moderate TE.

Keywords: Osteoarthritis; Exercise; Melatonin; Thioredoxin-interacting protein; Nucleotide-binding and oligomerization domain-like receptor protein 3

1. Introduction

Osteoarthritis (OA) is a debilitating articular cartilage disease that significantly impairs lower limb function, exacerbating the social and monetary strain.[1] The key pathological features of OA include articular cartilage degeneration, subchondral bone damage, synovial inflammation, capsular damage, and associated musculature.[2] Traditional OA treatments provide only limited symptomatic relief, with potential adverse effects and risk of recurrence.

Thioredoxin-interacting protein (TXNIP) plays a crucial role in OA by modulating oxidative stress and inflammation.[3] Elevated TXNIP levels in OA patients enhance oxidative stress and trigger activation of the nucleotide-binding and oligomerization domain-like receptor protein 3 (NLRP3) inflammasome.[4] Therefore, targeting TXNIP and its related pathways represents a promising strategy for developing therapies that mitigate inflammation and slow OA progression. Pyroptosis is a form of cell death initiated by inflammasomes and serves as a key innate immune mechanism.[5] The NLRP3 inflammasome is a primary driver of pyroptosis—upon activation, it triggers caspase-1 enzymatic activity, leading to pyroptotic cell death.[6] Activated caspase-1 cleaves gasdermin D (GSDMD) and promotes the release of pyroptotic cytokines, including interleukin (IL)-1β and IL-18.[7] Studies have demonstrated increased levels of NLRP3, IL-1β, and IL-18 in the synovial layer of OA animal knee models, indicating that the inflammasome signaling pathway contributes to OA pathogenesis.[8] Although pyroptosis has been implicated in OA, the specific role of the TXNIP/NLRP3 pathway in mediating this process remains incompletely understood.

Melatonin, a hormone known for regulating sleep and circadian rhythms, also has emerging roles in managing OA.[9,10] By reducing oxidative damage and suppressing inflammatory responses, melatonin can potentially slow the progression of OA and alleviate symptoms, offering a promising adjunctive therapy or alternative to traditional treatments for this debilitating joint disease.[11]

Exercise therapy has emerged as an effective, reliable, and accessible long-term strategy for the prevention and management of OA,[12,13] and is closely associated with the regulation of pyroptosis.[14] Previous studies have demonstrated that exercise can reduce the expression of the NLRP3 inflammasome and significantly inhibit the activation of its downstream components, such as caspase-1, IL-1β, and IL-18.[14,15]

Melatonin and exercise both exert anti-pyroptotic effects. Melatonin inhibits NLRP3 inflammasome activation, while exercise downregulates NLRP3 and its downstream effectors. The combined modulation of pyroptosis pathways by these two interventions may provide additive or synergistic therapeutic benefits for pyroptosis-related diseases such as OA.

The objective of this study is to investigate how treadmill exercise (TE) enhances the therapeutic effects of melatonin. Specifically, the study aims to examine the regulation of the TXNIP/NLRP3 axis by the combined intervention of TE and melatonin treatment, and how this leads to the inhibition of OA progression.

2. Materials and methods

2.1. Experimental animals

A total of 32 6-week-old Sprague-Dawley male rats weighing approximately 220 ± 20 g were used in this study. The training protocol was administered at the Experimental Animal Centre of Tongji Medical University, where the experimental animals were kept in a specific pathogen-free environment. Two rats were housed per plastic cage under controlled conditions (temperature: 22 ± 2°C; humidity: 60 ± 5%) on a 12 h light/dark cycle. Animals had ad libitum access to food and water and unrestricted mobility in their cages.

2.2. OA model and experimental groups

The study protocols are illustrated in Figure 1. A total of 32 Sprague-Dawley rats underwent a 1-week acclimation period of TE training. The exercise protocol was performed using the Zhenghua Biological Instrument Equipment (China) with the following parameters: 10 min/day, 5 days per week, at a speed of 10 m/min.

After the 1st week of TE training for acclimation, the rats were randomly divided into two experimental cohorts: Sham (n = 8) and destabilization of the medial meniscus (DMM; n = 24). Regarding anesthesia, isoflurane was administered throughout all surgical procedures. Briefly, the DMM group underwent surgical interventions on the right knee joint, which included anterior cruciate ligament transection and DMM surgery. The sham group served as the control group and underwent an incision of the skin and medial capsule only.

Four weeks post-surgery, eight animals from each group were randomly selected and euthanized to assess the success of the DMM model. Four weeks after surgery, the rats in the DMM group were randomly allocated into three groups: DMM (n = 8), DMM + melatonin (n = 8), and DMM + melatonin + TE groups, while the sham group (n = 8) included all sham animals in the study. The exercise training protocol was adapted from a previously published study.[16]

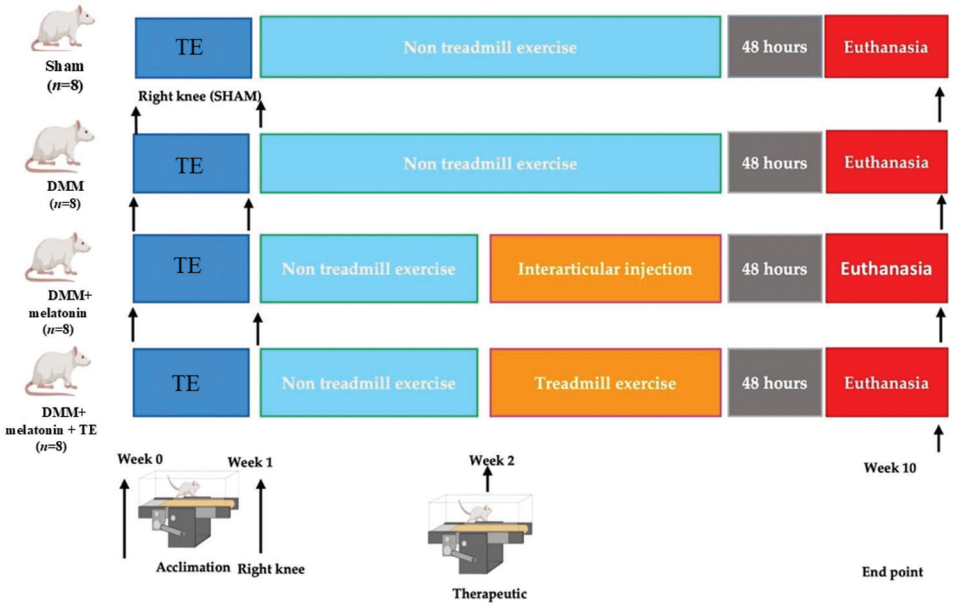


Figure 1. A total of 32 Sprague-Dawley rats underwent a 1-week acclimation training on a treadmill. Following this period, the rats were randomly assigned to four groups: sham, DMM, DMM + melatonin, and DMM + melatonin + TE. The DMM + melatonin group received an intra-articular injection of 10 μL melatonin solution. The DMM + melatonin + TE group received a combination of intra-articular melatonin injection (10 μL) and TE for 8 weeks. All rats were euthanized within 48 h after the final TE session.
Abbreviations: DMM: Destabilization of the medial meniscus; TE: Treadmill exercise.

2.3. Intervention treatment

In the experiment, melatonin was initially solubilized in 100% ethanol and subsequently diluted in saline (0.9% sodium chloride) to achieve a final concentration of 10 mg/mL.[17]

Following induction of the DMM model, rats in the DMM + melatonin and DMM + melatonin + TE groups received intra-articular injections of 10 μL of melatonin solution via the patellar tendon twice weekly for 6 weeks. The sham and DMM groups received an equal volume of normal saline. The DMM + melatonin + TE group underwent moderate TE for 8 weeks at a speed of 15 m/min for 30 min/day, 5 days per week. Within 48 h after the final TE session, all rats were euthanized. Blood plasma was collected via cardiac puncture, and tissue samples were harvested for subsequent analysis (Figure A1).

2.4. Microcomputed tomography with three-dimensional reconstruction

After euthanasia, samples of the rats' right knee joints were collected and scanned using a microcomputed tomography (micro-CT) (50 Scanco Medical, Switzerland) with a voxel size of 10.5 μm, 100 kV voltage, and 98 μA current. The integrated evaluation system generated three-dimensional (3D) images and quantitative data, including bone volume/tissue volume fraction (BV/TV), trabecular thickness (Tb. Th), trabecular number (Tb. N), and trabecular separation (Tb. Sp).

2.5. Histological staining

Samples of the rats' knee joints underwent decalcification in a 10% ethylenediaminetetraacetic acid solution. Following dehydration, the samples were embedded in paraffin. Sagittal sections (4 μm) were prepared for histological examination. Hematoxylin-eosin and safranin O/fast green staining were conducted to analyze the articular cartilage microscopically. Knee joint injury was evaluated using the OA Research Society International scoring system.[18]

2.6. Immunohistochemistry staining

The levels of tissue proteins were assessed using immunohistochemistry staining for caspase-1 (rabbit; 1:200; ER1905–47, Hua'an Biotechnology Co., LTD., China), GSDMD (GSDMD; rabbit; 1:200; ER1901–37, Hua'an Biotechnology Co., LTD., China), and NLRP3 (rabbit; 1:200; ET1610–93, Hua'an Biotechnology Co., LTD., China).

2.7. Immunofluorescence staining

Paraffin-embedded samples were processed following standard protocols and incubated with TXNIP (rabbit; 1:200, ET1705–72, Hua'an Biotechnology Co., LTD., China). Subsequently, slides were incubated with secondary

antibodies (Bosterbio, China) for 1 h. The nuclei were stained for 10 min with 4',6-diamidino-2-phenylindole (Bosterbio, China). Finally, images were captured using the EVOS FL Auto Imaging System (Hua'an Biotechnology Co., LTD., China).

2.8. Quantitative real-time polymerase chain reaction

Total RNA was extracted from articular cartilage using RNA Trizol (Powerful Biotechnology, China). Complementary DNA was synthesized using Hifair® III 1st Strand Synthesis Super Mix (Hua'an Biotechnology Co., LTD., China). The complementary DNA served as a template for PCR, with glyceraldehyde 3-phosphate dehydrogenase used as the internal reference. mRNA levels were quantified using SYBR Green Master Mix (Hua'an Biotechnology Co., LTD., China) and the $2^{-\Delta\Delta CT}$ method. Primer sequences, listed in Table 1, were obtained from GeneBank and synthesized by Bao Biomedical Technology Co., Ltd., China. All reactions were performed in triplicate, and fluorescence signals were detected using the ABI QuantStudio™ 1 System (Hua'an Biotechnology Co., LTD., China).

2.9. Western blot

Cartilage tissue samples were washed with phosphate-buffered saline and mechanically disrupted through grinding and sonication. Radioimmunoprecipitation buffer containing enzyme inhibitors was used for cell lysis. After centrifugation, the protein-containing supernatant was collected. Protein concentration was assessed using a BCA kit (BF0026, Bolff Biotechnology Co., LTD., China), and samples were denatured by heating at 100°C for 5 min in sample buffer.

Table 1. Forward and reverse primer sequences used for quantitative real-time polymerase chain reaction of articular cartilage samples (*n*=8)

Gene	RNA sequence (5' to 3')
R *GAPDH* F	ACTCTACCCACGGCAAGTTC
R *GAPDH* R	TGGGTTTCCCGTTGATGACC
R *CASP1* F	GACCGAGTGGTTCCCTCAAG
R *CASP1* R	GACGTGTACGAGTGGGTGTT
R *GSDMD* F	GATGCCTGCTTGTTGAGTTGG
R *GSDMD* R	AGAATTCCGAAGGCAGTCCA
R *TXNIP* F	TCTGCCTCACGGAGAGACTT
R *TXNIP* R	CATGATTGAGCCGAGTGGGT
R *NLRP3* F	CTGCATGCCGTATCTGGTTG
R *NLRP3* R	GGTACCCCATAGACTGGCAC

Abbreviations: F: Forward; R: Reverse.

The membranes were blocked with 5% milk solution to minimize non-specific binding. Primary antibodies—caspase-1 (1:1000), GSDMD (1:1000), NLRP3 (1:1000), TXNIP (1:2000), and glyceraldehyde 3-phosphate dehydrogenase (1:5000)—were incubated overnight at 4°C. After washing, membranes were incubated with secondary antibodies conjugated to fluorescent tags for 1 h at 4°C. Protein bands were detected using an infrared imaging system (software, Wuhan Guide Infrared Co., Ltd., China), and band intensities were quantified using ImageJ software (Laboratory for Optical and Computational Instrumentation, USA).

2.10. Enzyme-linked immunosorbent assay

Rats were anesthetized with isoflurane, and blood plasma was collected via cardiac puncture and stored at −80°C for subsequent assessment of inflammatory factors. The levels of IL-18 (FY-EH-6593, Feiyue Biotechnology, China) and IL-1β (FY-EH6675, Feiyue Biotechnology, China) were measured using enzyme-linked immunosorbent assay kits (A-A04, Qianbaidu Biotechnology Co., LTD., China) according to the manufacturer's instructions.

2.11. Statistical analyses

Data are presented as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism version 10 (Dotmatics, US). Comparisons among multiple groups were conducted using two-way analysis of variance, followed by Tukey's *post hoc* test for pairwise comparisons. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Intervention treatment and body weight

As depicted in Figure 2, over the course of the intervention period, the body weights of rats in all groups showed an increasing trend, indicating normal growth and development.

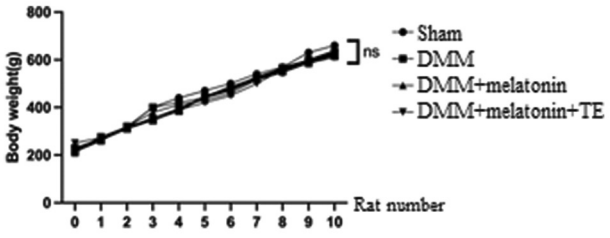


Figure 2. Intervention treatment and body weight of the rats at the study endpoint.
Notes: Data Data are presented as mean ± standard deviation. "ns" indicates no statistically significant difference.
Abbreviations: DMM: Destabilization of the medial meniscus; TE: Treadmill exercise.

3.2. Melatonin and TE treatment mitigate DMM-associated characteristics of the joint

To assess the impact of TE on enhancing melatonin's effects on the knee joint in the DMM model, microCT–3D analysis was performed to evaluate damage in the tibial trabecular bone (Figure 3A).

The combination of exercise and intra-articular melatonin injections significantly improved subchondral bone integrity and restored microstructural changes in the tibial bone following injury, compared to the DMM group that received only intra-articular melatonin injections.

The findings revealed that BV/TV, Tb. Th, and Tb. N values increased in the DMM + melatonin + TE group, whereas Tb. Sp decreased (Figure 3B).

The OA Research Society International score in the DMM group was significantly higher than that in the sham group. In contrast, the DMM group receiving intra-articular melatonin and TE exhibited reduced damage severity, as evidenced by less safranin O/fast green loss and improved surface regularity (Figure 4A and B). These findings indicate that combined TE and melatonin treatment effectively inhibits cartilage lesion progression and maintains structural integrity compared to the DMM + melatonin group.

In addition, immunohistochemistry analysis was performed to assess pyroptosis-related biomarkers and investigate the effects of TE combined with melatonin on the pyroptosis pathway. Rats in the DMM-only group showed elevated levels of NLRP3, caspase-1, and GSDMD compared to the DMM + melatonin group (Figure 4C and D). In contrast, TE combined with intra-articular melatonin significantly reduced the number of NLRP3-, caspase-1–, and GSDMD-positive cells in rat cartilage tissue (Figure 4C and D). These findings demonstrate that TE enhances the effects of melatonin in suppressing OA progression and reduces the expression of NLRP3, caspase-1, and GSDMD.

3.3. Effects of TE and melatonin on TXNIP expression levels

Immunofluorescence staining was used to assess TXNIP protein expression in cartilage tissue. The levels of TXNIP protein in the cartilage of the DMM group were significantly higher than those in the sham group ($p<0.0001$). In contrast, TXNIP protein expression in the DMM + melatonin group was significantly higher than that in the DMM + melatonin + TE group ($p<0.05$; Figure 5A and B).

3.4. Comparative impact of TE and melatonin on pyroptosis markers, TXNIP in cartilage tissue, and pyroptotic cytokine levels

The expression of pyroptosis biomarkers and TXNIP in cartilage tissues was compared between the DMM + melatonin group and the DMM + melatonin + TE groups (Figure 6). The mRNA (Figure 6A-D) and protein levels (Figure 6E and F) of caspase-1, GSDMD, NLRP3, and TXNIP in the DMM group were significantly higher than those in the DMM + melatonin and DMM + melatonin + TE groups ($p<0.01$). Additionally, the DMM + melatonin group exhibited elevated mRNA ($p<0.001$) and protein ($p<0.01$) levels compared to the DMM + melatonin + TE group.

Blood samples collected 48 h post-intervention were analyzed for serum inflammatory factor levels to assess the effects of exercise on inflammation during OA progression. Results indicated that serum inflammatory factor levels in the DMM group were significantly higher than those in the DMM + melatonin and DMM + melatonin + TE groups ($p<0.05$). Furthermore, the DMM + melatonin

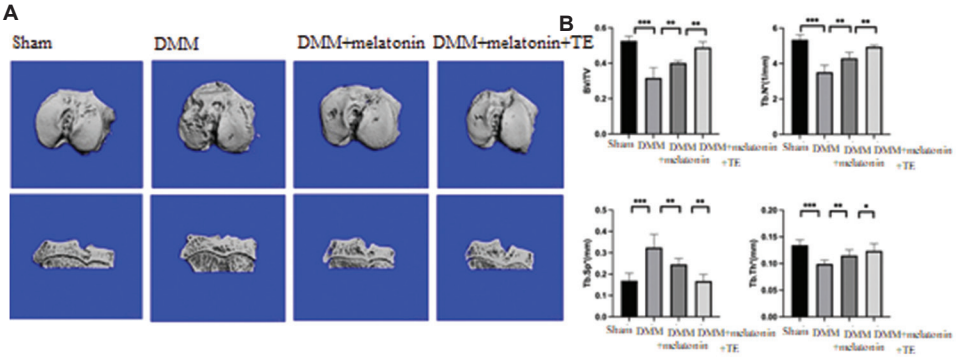


Figure 3. Microcomputed tomography and three-dimensional reconstruction images. (A) Representative microcomputed tomography and three-dimensional reconstruction images of the sham, DMM, DMM + melatonin, and DMM + melatonin + TE groups (scale bar: 1 mm). (B) Quantitative analysis of bone parameters. Note: Data are presented as mean ± standard deviation. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ ($n = 8$).
Abbreviations: BV/TV: Bone volume/tissue volume; DMM: Destabilization of the medial meniscus; Tb. N: Trabecular number; Tb. Sp: Trabecular separation; Tb. Th: Trabecular thickness; TE: Treadmill exercise.

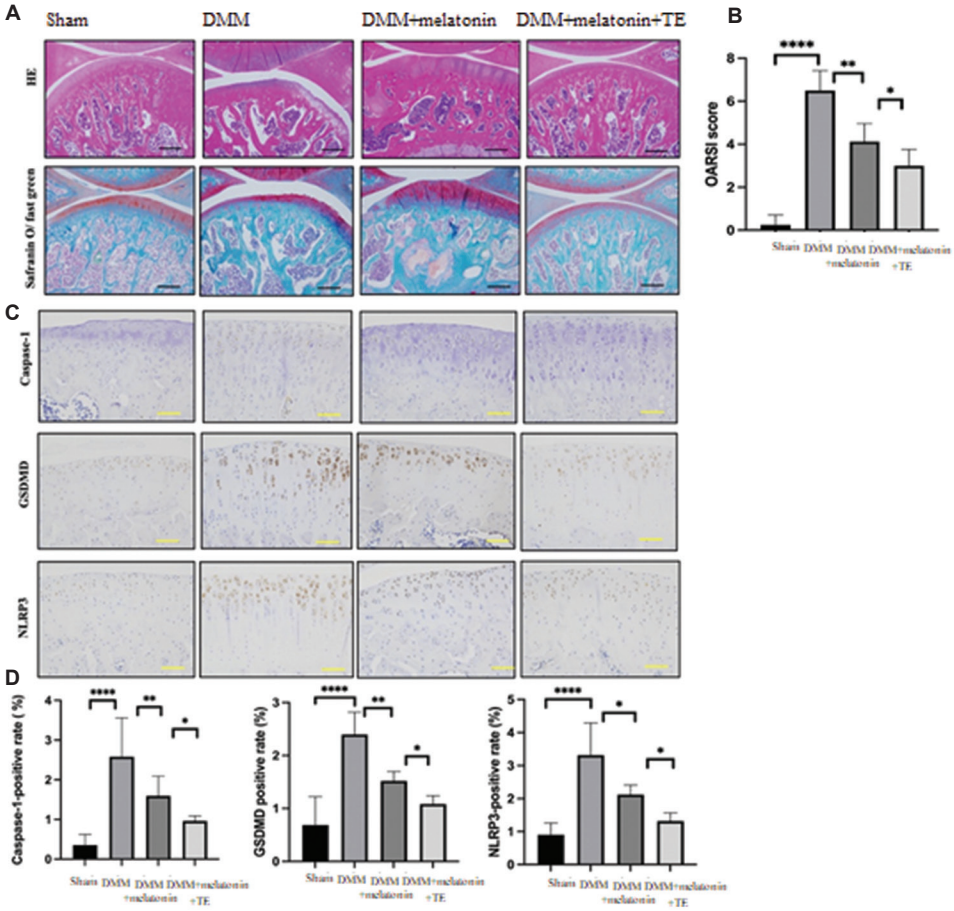


Figure 4. Histological and immunohistochemical analysis. (A) Representative HE and safranin O/fast green staining images (scale bar: 1,000 μm; magnification: ×100μm). (B) Osteoarthritis Research Society International score. (C) Representative immunohistochemical staining images of caspase-1, GSDMD, and NLRP3 (scale bar: 200 μm; magnification: ×200μm). (D) Quantification of the percentage of caspase-1–, GSDMD-, and NLRP3-positive cells. Note: Data are presented as mean ± standard deviation. *p<0.05, **p<0.01, and ****p<0.0001 (n = 8).
Abbreviations: DMM: Destabilization of the medial meniscus; GSDMD: Gasdermin D; HE: Hematoxylin–eosin; NLRP3: Nucleotide-binding and oligomerization domain-like receptor protein 3; TE: Treadmill exercise.

+ TE group showed lower serum levels compared to the DMM + melatonin group (p<0.05). These findings suggest that TE enhances the effects of melatonin by reducing inflammation in OA (Figure 7).

4. Discussion

The findings revealed that intra-articular injection of melatonin attenuates OA progression by modulating pyroptosis. TE further enhances melatonin's effects, leading to a greater reduction in OA progression through this pathway. In addition, TE amplifies melatonin's regulation of the TXNIP/NLRP3 signaling pathway, thereby inhibiting OA progression mediated by pyroptosis. These findings provide valuable insights into the molecular mechanisms through which TE contributes to the suppression of OA progression.

Exercise has been extensively employed in the management and treatment of numerous chronic conditions, helping patients alleviate symptoms, restore function, and improve overall well-being.[19-21] Previous studies have shown that moderate exercise can preserve joint integrity and mitigate disorder in DMM rats.[22,23] Exercise also exerts a notable anti-inflammatory effect associated with the regulation of pyroptosis.[24] It is well established that exercise can reduce chronic inflammation and effectively suppress inflammatory factor levels, thereby promoting the secretion of anti-inflammatory cytokines. Furthermore, studies have demonstrated that physical exercise can downregulate NLRP3 expression and significantly suppress the activation of apoptosis-associated speck-like protein containing a caspase recruitment domain, caspase-1, IL-1β, and IL-18.[10,24,25]

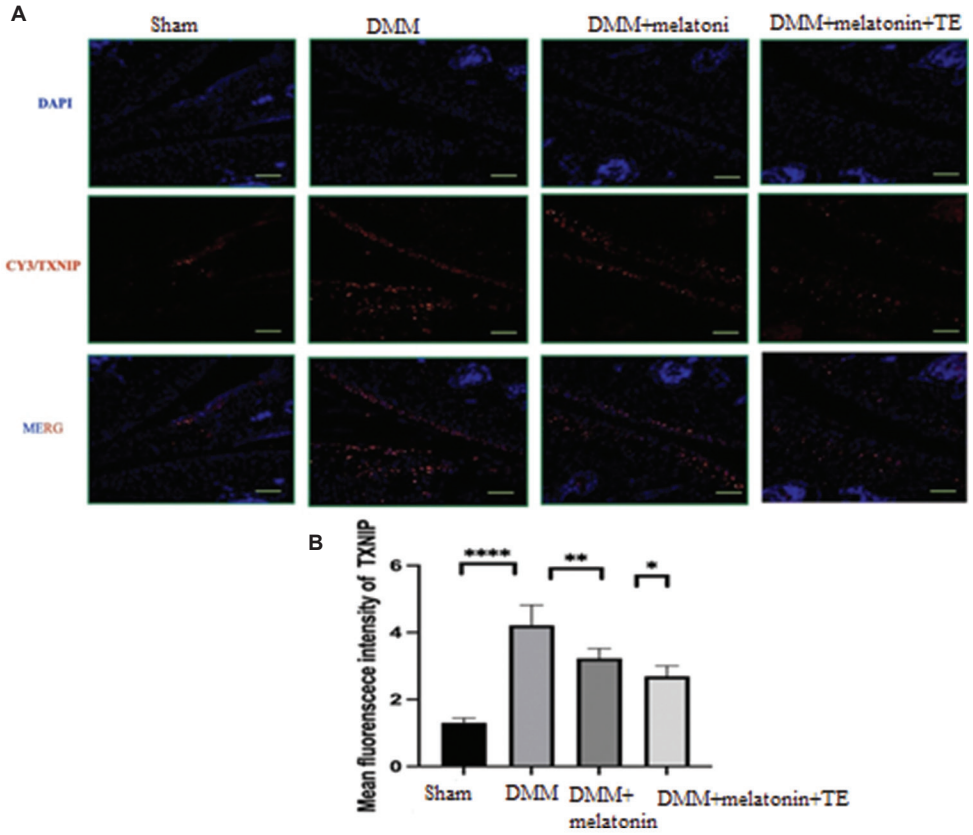


Figure 5. Effects of TE and melatonin on TXNIP expression. (A) Representative immunofluorescence staining images of TXNIP (scale bar: 200 μm; magnification: ×200μm). (B) Quantification of mean fluorescence intensity of TXNIP in cartilage.
Note: Data are presented as mean ± standard deviation. *p<0.05, **p<0.01, and ****p<0.0001 (n = 8).
Abbreviations: CV3: Caveolin-3; DAPI: 4',6-diamidino-2-phenylindole; DMM: Destabilization of the medial meniscus; MERG: DAPI with CV3/TXNIP; TE: Treadmill exercise; TXNIP: Thioredoxin-interacting protein

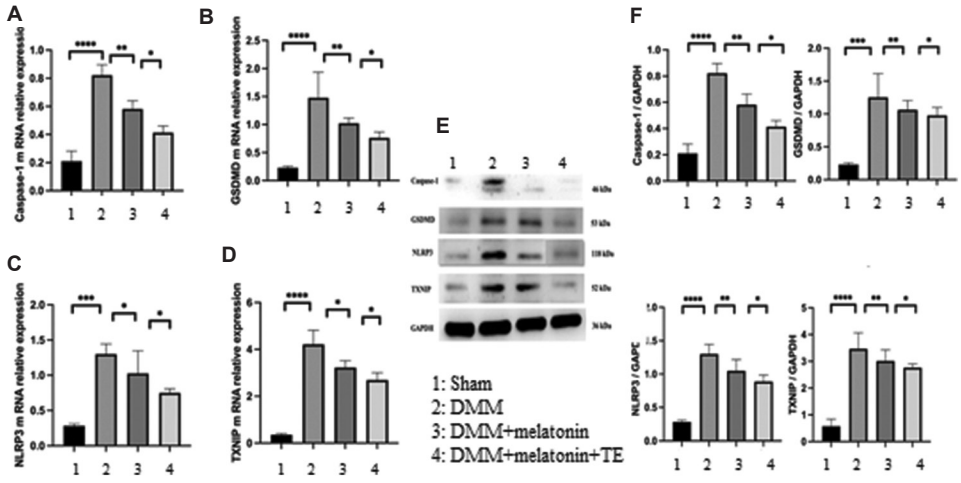


Figure 6. Comparative effects of TE and melatonin on pyroptosis markers and TXNIP. (A) Caspase-1 mRNA levels. (B) GSDMD mRNA levels. (C) NLRP3 mRNA levels. (D) TXNIP mRNA levels. (E) Protein expression of caspase-1, GSDMD, NLRP3, and TXNIP in cartilage detected by Western blot. (F) Quantification of protein expression levels.
Note: Data are expressed as mean ± standard deviation of two independent experiments. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 (n = 8).
Abbreviations: DMM: Destabilization of the medial meniscus; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GSDMD: Gasdermin D; NLRP3: Nucleotide-binding and oligomerization domain-like receptor protein 3; TE: Treadmill exercise; TXNIP: Thioredoxin-interacting protein.

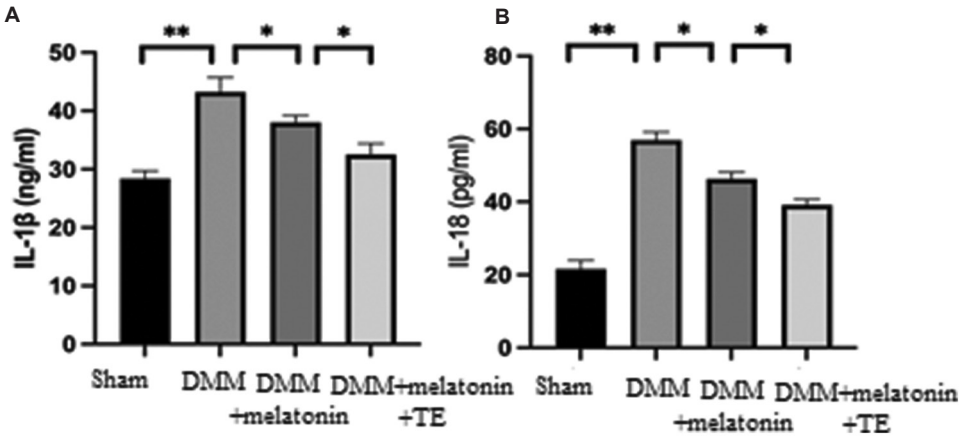


Figure 7. Quantification of serum inflammatory cytokines: (A) IL-1β and (B) IL-18.
Note: Data are expressed as mean ± standard deviation. $p<0.05$ and $^{**}p<0.01$ ($n = 8$).
Abbreviations: DMM: Destabilization of the medial meniscus; IL: Interleukin; TE: Treadmill exercise.

The present study investigated how TE enhances the effects of melatonin treatment in mitigating cartilage degradation in OA through the pyroptosis pathway. Using the DMM model, we found that both melatonin treatment and its combination with exercise significantly reduced inflammation and cartilage damage in the knee joints of DMM rats. Multiple analytical methods, including micro-CT and histological staining, were used to assess OA progression. The DMM + melatonin + TE group showed significantly greater protective effects on cartilage and reduced subchondral bone damage compared to the DMM + melatonin group. In addition, moderate TE enhanced melatonin's inhibitory effects on the expression of pyrotosis-related proteins, which are associated with OA. Furthermore, serum analysis showed a significant reduction in the levels of IL-1β and IL-18. These findings are consistent with previous studies.[24,26-28]

This study further demonstrated that TE enhances the effects of melatonin treatment, leading to a more pronounced attenuation of OA progression through the pyroptosis pathway. TXNIP plays a key role in this process, as it regulates cellular redox homeostasis and activates NLRP3, a crucial component of the pyroptosis-mediated inflammatory response.[29] Melatonin, with its potent antioxidant and anti-inflammatory properties, is known to inhibit the TXNIP/NLRP3 signaling axis, thereby suppressing pyroptosis and the associated inflammation.[30] The findings suggest that the combination of TE and melatonin treatment enhances this regulatory mechanism, resulting in a more significant reduction in OA progression compared to melatonin treatment alone. By elucidating the interplay between TXNIP, melatonin, and NLRP3 within the pyroptosis pathway, this study highlights the potential therapeutic benefits of this combined approach in mitigating OA progression.

Immunofluorescence staining showed that treatment with melatonin significantly reduced TXNIP protein expression in the DMM + melatonin group compared to the untreated DMM group. This finding is consistent with previous studies that investigated the effects of melatonin on TXNIP expression.[27,31-35] In addition, exercise notably enhanced the effect of melatonin treatment by further reducing TXNIP protein expression. This finding is consistent with previous studies conducted on different diseases.[36-38]

In addition, quantitative real-time polymerase chain reaction and Western blot analyses demonstrated that rats in the DMM + melatonin group showed significantly reduced mRNA and protein levels of pyroptosis-related proteins and TXNIP. Furthermore, combining melatonin with TE led to an even greater decrease in these markers compared to the DMM + melatonin group. These findings provide strong evidence that melatonin treatment, especially in combination with TE, effectively reduces the expression of key inflammatory and apoptotic markers in the DMM model. Overall, these findings suggest that melatonin, particularly when paired with exercise, could alleviate inflammation and pyroptosis, highlighting the potential of combined therapeutic strategies for managing inflammatory conditions.

This study has several limitations that should be considered when interpreting the findings. First, the short experimental duration limits the ability to assess the long-term effects of OA progression. Second, the small sample size may reduce the statistical power and generalizability of the results. Third, the study focused primarily on advanced OA stages, which may not fully reflect the potential benefits during earlier phases of the

disease. Fourth, functional assessments (e.g., gait analysis and weight-bearing tests) were not performed, thereby limiting our understanding of the clinical relevance of the observed molecular and structural changes. Future research should evaluate the effects of moderate TE in larger cohorts, including early OA stages, and assess its impact on cartilage-subchondral bone interactions and the pyroptosis pathway. Furthermore, investigating the long-term effects of different training durations and varied doses or delivery methods of melatonin would enhance our understanding of their therapeutic potential in OA management.

5. Conclusion

TE significantly enhances melatonin-mediated regulation of the TXNIP/NLRP3 pathway and inhibits pyroptosis-driven OA in the DMM rat model. These findings suggest that combining melatonin treatment with moderate TE may offer a promising therapeutic strategy for managing OA by effectively reducing pyroptotic cell death.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Yongping Wang, Li Chaoyi
Formal analysis: Jamal Alshorman
Investigation: Yongping Wang
Methodology: Ruba Altahla
Writing – original draft: Ruba Altahla, Jamal Alshorman
Writing – review & editing: Ruba Altahla, Jamal Alshorman

Ethics approval and consent to participate

All subjects involved in this study were treated in accordance with the ethical principles outlined in the Declaration of Helsinki, and the study was approved by the ethics committee (No. 2025-M20-21).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

References

1. Katz JN, Arant KR, Loeser RF. Diagnosis and treatment of hip and knee osteoarthritis: A review. *JAMA*. 2021;325(6):568-578.
   doi: 10.1001/jama.2020.22171
2. Żylińska B, Sobczyńska-Rak A, Lisiecka U, Stodolak-Zych E, Jarosz Ł, Szponder T. Structure and pathologies of articular cartilage. *In Vivo*. 2021;35(3):1355-1363.
   doi: 10.21873/invivo.12388
3. Gu Y, Ren K, Jiang C, Wang L, Yao Q. Regulation of cartilage damage caused by lack of Klotho with thioredoxin/peroxiredoxin (Trx/Prx) system and succedent NLRP3 activation in osteoarthritis mice. *Am J Transl Res*. 2019;11(12):7338-7350.
4. Sui J, Dai F, Shi J, Zhou C. Ubiquitin-specific peptidase 25 exacerbated osteoarthritis progression through facilitating TXNIP ubiquitination and NLRP3 inflammasome activation. *J Orthop Surg Res*. 2023;18(1):762.
   doi: 10.1186/s13018-023-04083-y
5. Kovacs SB, Miao EA. Gasdermins: Effectors of pyroptosis. *Trends Cell Biol*. 2017;27(9):673-684.
   doi: 10.1016/j.tcb.2017.05.005
6. Tang R, Xu J, Zhang B, *et al*. Ferroptosis, necroptosis, and pyroptosis in anticancer immunity. *J Hematol Oncol*. 2020;13(1):110.
   doi: 10.1186/s13045-020-00946-7
7. Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010;140(6):821-832.
   doi: 10.1016/j.cell.2010.01.040
8. Zhou J, Zhao Y, Wu G, Lin B, Li Z, Liu X. Differential miRNAomics of the synovial membrane in knee osteoarthritis induced by bilateral anterior cruciate ligament transection in rats. *Mol Med Rep.* 2018;18(4):4051-4057.
   doi: 10.3892/mmr.2018.9385
9. Lu KH, Lu PW, Lu EW, *et al*. The potential remedy of melatonin on osteoarthritis. *J Pineal Res*. 2021;71(3):e12762.
   doi: 10.1111/jpi.12762
10. Kaur T, Shyu BC. Melatonin: A new-generation therapy for reducing chronic pain and improving sleep disorder-related pain. *Adv Exp Med Biol*. 2018;1099:229-251.
    doi: 10.1007/978-981-13-1756-9_19
11. Kohler LJ, Schneider EA. *The Effect of Melatonin Administration on Perioperative Cortisol Levels and Opioid Consumption in Total Knee Arthroplasty Patients*. United States: Webster University; 2020.
12. Skou ST, Pedersen BK, Abbott JH, Patterson B, Barton C. Physical activity and exercise therapy benefit more than just symptoms and impairments in people with hip and knee

osteoarthritis. *J Orthop Sports Phys Ther*. 2018;48(6):439-447.

doi: 10.2519/jospt.2018.7877

13. Shi J, Hu ZY, Wen YR, *et al*. Optimal modes of mind-body exercise for treating chronic non-specific low back pain: Systematic review and network meta-analysis. *Front Neurosci*. 2022;16:1046518.

doi: 10.3389/fnins.2022.1046518

14. Kar S, Shahshahan HR, Hackfort BT, *et al.* Exercise training promotes cardiac hydrogen sulfide biosynthesis and mitigates pyroptosis to prevent high-fat diet-induced diabetic cardiomyopathy. *Antioxidants (Basel)*. 2019;8(12):638.

doi: 10.3390/antiox8120638

15. Hu S, Wan X, Li X, Wang X. Aerobic exercise alleviates pyroptosis-related diseases by regulating NLRP3 inflammasome. *Front Physiol*. 2022;13:965366.

doi: 10.3389/fphys.2022.965366

16. Shang X, Hao X, Hou W, *et al*. Exercise-induced modulation of myokine irisin on muscle-bone unit in the rat model of post-traumatic osteoarthritis. *J Orthop Surg Res*. 2024;19(1):49.

doi: 10.1186/s13018-024-04532-2.

17. Zhang Y, Lin J, Zhou X, *et al.* Melatonin prevents osteoarthritismyokine irisin on muscle-bone unit in the rat model of post*Oxidat Med Cell Longev*. 2019;2019(1):9705929.

doi: 10.1155/2019/9705929

18. Glasson S, Chambers M, Van Den Berg W, Little C. The OARSI histopathology initiative-recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010;18 Suppl: S17-S23.

doi: 10.1016/j.joca.2010.05.025

19. Pedersen BK, Saltin B. Exercise as medicine-evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports*. 2015;25 Suppl:1-72.

doi: 10.1111/sms.12581

20. Uchiyama K, Adachi K, Muraoka K, *et al*. Homema K, Adachi K, Muraoka K, as therapy in 26 different chronic nic kidney disease: A randomized controlled trial. *J Cachexia Sarcopenia Muscle*. 2021;12(6):1789-1802.

doi: 10.1002/jcsm.12775

21. Tucker WJ, Fegers-Wustrow I, Halle M, Haykowsky MJ, Chung EH, Kovacic JC. Exercise for primary and secondary prevention of cardiovascular disease: JACC focus seminar 1/4. *J Am Coll Cardiol*. 2022;80(11):1091-1106.

doi: 10.1016/j.jacc.2022.07.004

22. Hao X, Wang S, Zhang J, Xu T. Effects of body weight-supported treadmill training on cartilage-subchondral bone unit in the rat model of posttraumatic osteoarthritis. *J Orthop Res*. 2021;39(6):1227-1235.

doi: 10.1002/jor.24791

23. Hao X, Zhang J, Shang X, *et al*. Exercise modifies the disease-relevant gut microbial shifts in post-traumatic osteoarthritis rats. *Bone Joint Res*. 2022;11(4):214-225.

doi: 10.1302/2046-3758.114.BJR-2021-0192.R1

24. Lee J, Hong J, Umetani M, Lavoy EC, Kim JH, Park Y. Vascular protection by exercise in obesity: Inflammasome-associated mechanisms. *Med Sci Sports Exerc*. 2020;52(12):2538-2545.

doi: 10.1249/MSS.0000000000002419

25. Zhang W, Wang X, Tang Y, Huang C. Melatonin alleviates doxorubicin-induced cardiotoxicity via inhibiting oxidative stress, pyroptosis and apoptosis by activating Sirt1/Nrf2 pathway. *Biomed Pharmacother*. 2023;162:114591.

doi: 10.1016/j.biopha.2023.114591

26. Wei XB, Fu YB, Xu J, *et al*. Melatonin alleviates lipopolysaccharide-induced myocardial injury by inhibiting inflammation and pyroptosis in cardiomyocytes. *Ann Transl Med*. 2021;9(5):413.

doi: 10.21037/atm-20-8196

27. Li XH, Liu LZ, Chen L, *et al*. Aerobic exercise regulates FGF21 and NLRP3 inflammasome-mediated pyroptosis and inhibits atherosclerosis in mice. *PLoS One*. 2022;17(8):e0273527.

doi: 10.1371/journal.pone.0273527

28. Ding R, Ou W, Chen C, *et al.* Endoplasmic reticulum stress and oxidative stress contribute to neuronal pyroptosis caused by cerebral venous sinus thrombosis in rats: Involvement of TXNIP/peroxynitrite-NLRP3 inflammasome activation. *Neurochem Int*. 2020;141:104856.

doi: 10.1016/j.neuint.2020.104856

29. Arioz BI, Tarakcioglu E, Olcum M, Genc S. The role of melatonin on NLRP3 inflammasome activation in diseases. *Antioxidants (Basel)*. 2021;10(7):1020.

doi: 10.3390/antiox10071020

30. Cao Z, Fang Y, Lu Y, *et al.* Melatonin alleviates cadmiumum M, Genc S. The role of melatonin on NLRP3 inflammasome activati *J Pineal Res*. 2017;62(3):e12389.

doi: 10.1111/jpi.12389

31. Gao H, Hu R, Yin L, *et al*. Knowledge, attitudes and practices of the Chinese public with respect to coronavirus disease (COVID-19): An online cross-sectional survey. *BMC Public Health*. 2020;20(1):1816.

doi: 10.1186/s12889-020-09961-2

32. Huang J, Li Z, Hu Y, *et al*. Melatonin, an endogenous hormone, modulates Th17 cells via the reactive-oxygen species/TXNIP/HIF-1α axis to alleviate autoimmune uveitis. *J Neuroinflamm*. 2022;19(1):124.

doi: 10.1186/s12974-022-02477-z

33. Miao Z, Miao Z, Teng X, Xu S. Melatonin alleviates lead-induced intestinal epithelial cell pyroptosis in the common carps (*Cyprinus carpio*) via miR-17-5p/TXNIP axis. *Fish Shellfish Immunol*. 2022;131:127-136.

doi: 10.1016/j.fsi.2022.09.071

34. Lim JO, Lee SJ, Kim WI, *et al*. Melatonin alleviates silica nanoparticle-induced lung inflammation via thioredoxin-interacting protein downregulation. *Antioxidants (Basel)*. 2021;10(11):1765.

doi: 10.3390/antiox10111765

35. Li Y, Xu P, Wang Y, *et al*. Different intensity exercise preconditions affect cardiac function of exhausted rats through regulating TXNIP/TRX/NF-κBp65/NLRP3 inflammatory pathways. *Evid Based Complement Alternat Med*. 2020;2020(1):5809298.

doi: 10.1155/2020/5809298

36. Chaves AB, Miranda ER, Mey JT, *et al*. Exercise reduces the protein abundance of TXNIP and its interacting partner REDD1 in skeletal muscle: Potential role for a PKA-mediated mechanism. *J Appl Physiol (1995)*. 2022;132(2):357-366.

doi: 10.1152/japplphysiol.00229.2021

37. Ra SG, Kawamoto E, Koshinaka K, *et al.* Acute bout of exercise downregulates thioredoxinand eracting protein expression in rat contracting skeletal muscles. *Physiol Rep.* 2020;8(11):e14388.

doi: 10.14814/phy2.14388

38. Weng Y, Zhang Y, Wang D, *et al*. Exercise-induced irisin improves follicular dysfunction by inhibiting IRE1α-TXNIP/ROS-NLRP3 pathway in PCOS. *J Ovarian Res*. 2023;16(1):151.

doi: 10.1186/s13048-023-01242-x

Appendix

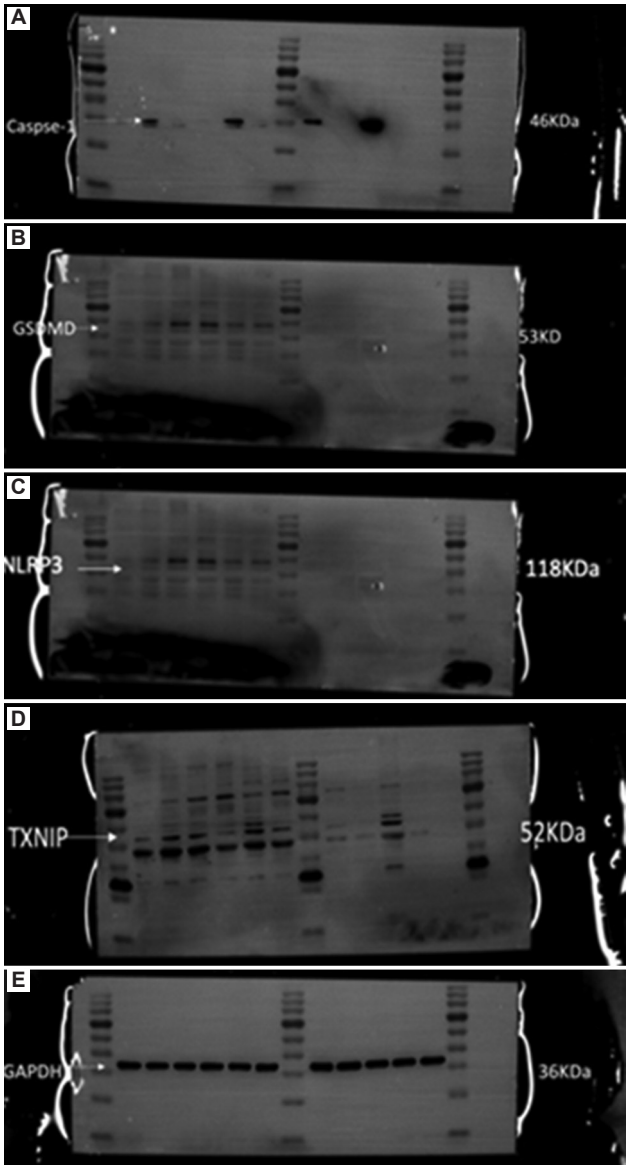


Figure A1. Original Western blot images of (A) caspase-1, (B) GSDMD, (C) NLRP3, (D) TXNIP, and (E) GAPDH in rat cartilage
Abbreviations: GSDMD: Gasdermin D; NLRP3: Nucleotide-binding and oligomerization domain-like receptor protein 3; TXNIP: Thioredoxin-interacting protein; GAPDH: Glyeceraldehyde-3-phosphate dehydrogenase.