Bone and Arthrosurgery Science, 2026, Volume 4, Issue 2
http://ojs.bbwpublisher.com/index.php/BAS
Online ISSN: 2981-8222
Print ISSN: 3083-4856

Effects of Grape Seed Extract on NF-κB Expression in Synoviocytes of Mice with Rheumatoid Arthritis

Fan Li, Shun Yuan, Qun Kong, Jingzheng Wang, Zhuoran Li, Chanjuan Liu, Yue Wang, Qingjun Ding*

Qilu Institute of Technology, Jinan 250200, Shandong, China

Author to whom correspondence should be addressed.

Abstract: *Objective:* To investigate the effects of grape seed extract (GSE) on NF-κB expression and inflammatory injury in synoviocytes of mice with rheumatoid arthritis (RA), so as to provide experimental evidence for the natural drug therapy of RA. *Methods:* RA mouse model was established by collagen induction. The mice were randomly divided into a normal control group, a model group, and low-/medium-/high-dose GSE groups, and received intragastric intervention for 48 hours. Synoviocytes were isolated and cultured *in vitro*. The mRNA and protein expressions of NF-κB/p65 were detected by qRT-PCR and Western blot, respectively. The secretion levels of TNF-α, IL-6, and IL-1β were detected by ELISA, and the pathological changes of synovial tissue were observed by HE staining. *Results:* Compared with the model group, the expression of NF-κB/p65 in each GSE dose group decreased in a dose-dependent manner ($P < 0.05$ or $P < 0.01$). The expression and secretion of downstream inflammatory factors were significantly down-regulated, and the pathological injury of synovial tissue was significantly improved, with the most significant effect in the high-dose group. *Conclusion:* GSE can inhibit the NF-κB signaling pathway, down-regulate the release of inflammatory factors, and alleviate synovial inflammation and pathological injury in RA mice. It is expected to be a potential natural drug for the treatment of RA.

Keywords: Grape seed extract; Rheumatoid arthritis; Synoviocytes; Nuclear factor-κB; Inflammatory factors

Online publication: May 14, 2026

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and hyperplasia of joint synovium. The global prevalence rate is 0.5–1%, and it mostly occurs in middle-aged women. Its pathological process is accompanied by abnormal proliferation of synoviocytes and massive release of inflammatory factors, which eventually lead to the destruction of articular cartilage and bone, causing joint

deformity and even disability [1]. At present, the clinical treatment of RA mainly relies on disease-modifying antirheumatic drugs, biological agents, glucocorticoids, etc. However, such drugs have problems such as obvious side effects, high treatment costs, and easy drug resistance. Therefore, searching for safe, efficient, and economical natural therapeutic drugs has become a research hotspot in this field [2].

As a core transcription factor regulating inflammation, nuclear factor-kappa B (NF-κB) plays a key role in the pathological process of RA. In RA synovial tissue, abnormal activation of NF-κB can trigger the cascade release of downstream inflammatory factors such as TNF-α, IL-6, and IL-1β, forming a vicious inflammatory cycle, promoting synovial hyperplasia and joint structure destruction [3]. Inhibiting the activation of the NF-κB signaling pathway has become an important target for the research and development of new therapeutic drugs for RA.

Grape seed extract (GSE) is a natural polyphenol extract from grape seeds, and its main active component is proanthocyanidins, which have significant antioxidant, anti-inflammatory, and immunomodulatory properties [4]. In recent years, studies have found that GSE can alleviate the pathological injury of various inflammatory diseases such as cardiovascular diseases and nervous system diseases by inhibiting the activation of NF-κB [5]. However, the regulatory effect and molecular mechanism of GSE on NF-κB expression in synoviocytes of RA mice are still not fully understood [6].

In this study, RA mouse model was used as the research object to investigate the effects of different concentrations of GSE on NF-κB expression in synoviocytes and the release of downstream inflammatory factors, aiming to provide experimental evidence for the clinical application of GSE in the treatment of RA and open up a new direction for the research and development of natural drugs for RA.

2. Materials and methods

2.1. Experimental animals

Sixty SPF-grade C57BL/6 female mice, aged 6–8 weeks, weighing 18–22 g, were selected. The mice were raised in the animal room of the School of Medicine, Qilu Institute of Technology, under constant temperature and humidity, with a 12-hour light-dark cycle, free access to food and water, and adaptive feeding for 1 week before the experiment.

2.2. Experimental reagents and instruments

Grape seed extract; complete Freund's adjuvant, incomplete Freund's adjuvant; primers for NF-κB/p65, TNF-α, IL-6, IL-1β; qRT-PCR kit, RNA extraction kit; Western blot-related antibodies, HRP-labeled secondary antibody; ELISA detection kit; ultra-low temperature refrigerator; real-time fluorescent quantitative PCR instrument; protein electrophoresis instrument, membrane transfer instrument; microplate reader; inverted microscope; high-speed refrigerated centrifuge.

2.3. Experimental methods

2.3.1. Establishment and grouping of RA mouse model

Sixty mice were randomly divided into a normal control group (n = 10) and a model establishment group (n = 50). The RA model was established in the model establishment group by collagen induction: type II collagen was mixed with complete Freund's adjuvant in equal volume, and after full emulsification, it was injected intradermally at the base of the mouse tail, with the initial immunization dose of 100 μg per mouse; on day

21, booster immunization was performed with the emulsion of type II collagen and incomplete Freund's adjuvant at a dose of 50 μg per mouse. The normal control group was injected with the same volume of normal saline. After booster immunization, the joint symptoms of mice were observed weekly, and the modeling effect was evaluated according to the arthritis index (AI) scoring standard. A score ≥4 was judged as successful modeling [7].

After successful modeling, the modeled mice were randomly divided into RA model group, low-dose GSE group, medium-dose GSE group, and high-dose GSE group, with 10 mice in each group.

The normal control group and RA model group were given the same volume of normal saline by gavage, and each GSE group was given 10, 50, 100 μg/mL GSE solution by gavage, respectively, with a gavage volume of 0.2 mL/10 g body weight, once a day for 48 consecutive hours.

2.3.2. Isolation and culture of synoviocytes

Twenty-four hours after the last administration, the mice were sacrificed by cervical dislocation. The ankle synovial tissues of mice were separated under sterile conditions, rinsed 3 times with phosphate buffer, cut into pieces of about 1 mm^3, digested with 0.25% trypsin in a 37°C incubator for 30 minutes, and then the digestion was terminated with DMEM medium containing 10% fetal bovine serum. The mixture was filtered through a 200-mesh sieve, and the filtrate was collected and centrifuged at 1,000 r/min for 5 minutes.

The supernatant was discarded, and the cells were resuspended in DMEM complete medium. The cell concentration was adjusted to 1×10^6 cells/mL, inoculated into 6-well culture plates, and cultured in a 37°C, 5% CO_2 incubator. Subsequent experiments were performed when the cells adhered and fused to 70–80%.

2.3.3. Detection of mRNA expression of NF-κB/p65 and inflammatory factors by qRT-PCR

Synoviocytes cultured in each group were collected, and total cellular RNA was extracted using an RNA extraction kit. The purity and concentration of RNA were detected by a nucleic acid protein detector [8]. RNA was reverse-transcribed into cDNA according to the instructions of the qRT-PCR kit, and real-time fluorescent quantitative PCR amplification was performed using cDNA as a template [9].

PCR reaction system: 2×SYBR Green Mix 10 μL, upstream and downstream primers 0.5 μL each, cDNA template 2 μL, RNase-free H_2O 7 μL, total system 20 μL. Reaction conditions: pre-denaturation at 95°C for 30 s; denaturation at 95°C for 5 s, annealing at 60°C for 30 s, 40 cycles; final extension at 72°C for 5 min. Using β-actin as the internal reference gene, the relative expression of the target gene was calculated by the 2^(-ΔΔCt) method. The primer sequences of each gene are shown in **Table 1**.

Table 1. Primer sequences

Gene name	Forward primer (5'→3')	Reverse primer (5'→3')	Product length (bp)
NF-κB/p65	GAGCAAGAGCAAGAAGATGG	GCTGGTGATGGTGATGGTAA	215
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG	198
IL-6	CCAGAGTCATTCAGAGCAATG	TTGGATGGTCTTGGTCCTTA	203
IL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG	220
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	189

2.3.4. Detection of NF-κB/p65 protein expression by Western blot

Synoviocytes in each group were collected, lysed on ice for 30 minutes with RIPA lysis buffer (containing

1% protease inhibitor), centrifuged at 12,000 r/min, 4°C for 15 minutes, and the supernatant was collected. Protein concentration was determined by BCA method. Equal amounts of protein (50 μg/well) were subjected to SDS-PAGE electrophoresis.

After electrophoresis, the protein was transferred to a PVDF membrane, blocked with 5% skimmed milk at room temperature for 2 hours, and incubated with primary antibodies (NF-κB/p65 1:1,000, β-actin 1:2,000) overnight at 4°C. The membrane was washed 3 times with TBST for 10 minutes each, incubated with HRP-labeled secondary antibody (1:5,000) at room temperature for 1 hour, and washed 3 times with TBST again.

Development was performed using an ECL chemiluminescence kit, and images were taken with a gel imaging system. Using β-actin as an internal reference, the gray value of the target protein band was analyzed with ImageJ software, and the relative expression was calculated [10].

2.3.5. Detection of inflammatory factor secretion by ELISA

The culture supernatant of synoviocytes in each group was collected, and the operation was performed according to the instructions of the TNF-α, IL-6, and IL-1β ELISA detection kits. Standard wells and sample wells were set up, and the absorbance (OD value) at 450 nm was measured on a microplate reader after adding the corresponding reagents. The concentration of each inflammatory factor was calculated according to the standard curve.

2.3.6. Observation of synovial tissue pathological morphology by HE staining

The ankle synovial tissues of mice in each group were taken, fixed with 4% paraformaldehyde for 24 hours, routinely dehydrated, cleared, waxed, embedded, and made into 5 μm-thick paraffin sections. HE staining was performed, and neutral gum was used for sealing. The pathological morphology of synovial tissues was observed under an inverted microscope, including synoviocyte proliferation, inflammatory cell infiltration, tissue edema, etc., and pathological scoring was performed [11].

2.4. Statistical analysis

SPSS 26.0 statistical software was used for data analysis. Measurement data were expressed as mean ± standard deviation (SD). One-way ANOVA was used for comparison among multiple groups, and LSD-t test was used for pairwise comparison between groups. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely statistically significant.

3. Results

3.1. Effects of GSE on arthritis index in RA mice

Mice in the normal control group had no joint swelling or deformity, and the AI score was 0. Mice in the model group began to show symptoms such as joint swelling and limited activity 2–3 weeks after booster immunization, and the AI score gradually increased. Four weeks after modeling, the AI score was ≥4, and the modeling success rate was 84% (42/50). A total of 50 mice were finally included in the experiment (10 in the normal control group, 10 in the model group and each GSE dose group).

Compared with the RA model group, the joint swelling symptoms of mice in each GSE intervention group were significantly relieved, and the AI score was significantly reduced ($P < 0.01$), and the AI score of the high-dose GSE group was the lowest ($P < 0.01$), as shown in **Table 2**.

Table 2. Comparison of arthritis index scores in mice of each group (mean ± SD, n = 10, score)

Group	AI score
Normal control group	0.00 ± 0.00
RA model group	6.23 ± 0.58**
Low-dose GSE group	4.15 ± 0.42*#
Medium-dose GSE group	2.87 ± 0.35**#
High-dose GSE group	1.52 ± 0.28**# △

Note: Compared with the normal control group, $P < 0.01$; compared with the RA model group, *$P < 0.05$, **$P < 0.01$; compared with the low-dose GSE group, △ $P < 0.01$; compared with the medium-dose GSE group, ▲$P < 0.01$ (the same below).

3.2. Effects of GSE on NF-κB/p65 mRNA and protein expression in synoviocytes of RA mice

qRT-PCR results showed that compared with the normal control group, the relative expression of NF-κB/p65 mRNA in synoviocytes of mice in the RA model group was significantly increased ($P < 0.01$); compared with the RA model group, the relative expression of NF-κB/p65 mRNA in each GSE intervention group was significantly decreased ($P < 0.01$), and showed a gradual downward trend with the increase of GSE concentration. The down-regulation effect of the high-dose GSE group was the most significant ($P < 0.01$).

Western blot results were consistent with qRT-PCR results. Compared with the normal control group, the relative expression of NF-κB/p65 protein in synoviocytes of mice in the RA model group was extremely significantly increased ($P < 0.01$); the expression level of NF-κB/p65 protein in each GSE intervention group was significantly lower than that in the RA model group ($P < 0.01$) in a dose-dependent manner, and the effect of the high-dose GSE group was the best ($P < 0.01$).

3.3. Effects of GSE on mRNA expression of downstream inflammatory factors in synoviocytes of RA mice

Compared with the normal control group, the relative mRNA expressions of TNF-α, IL-6, and IL-1β in synoviocytes of mice in the RA model group were extremely significantly increased ($P < 0.01$); compared with the RA model group, the mRNA expression levels of the above inflammatory factors in each GSE intervention group were significantly down-regulated ($P < 0.05$ or $P < 0.01$). The down-regulation effect of the high-dose GSE group was significantly better than that of the low- and medium-dose groups ($P < 0.01$), and the medium-dose group was better than the low-dose group ($P < 0.01$).

3.4. Effects of GSE on the secretion of inflammatory factors in the culture supernatant of synoviocytes of RA mice

ELISA results showed that the concentrations of TNF-α, IL-6, and IL-1β in the culture supernatant of synoviocytes of mice in the normal control group were very low; compared with the normal control group, the concentrations of each inflammatory factor in the RA model group were extremely significantly increased ($P < 0.01$); compared with the RA model group, the concentrations of inflammatory factors in each GSE intervention group were significantly decreased ($P < 0.05$ or $P < 0.01$), and gradually decreased with the increase of GSE concentration, showing an obvious dose-effect relationship ($P < 0.01$).

3.5. Effects of GSE on the pathological morphology of synovial tissue of RA mice

Compared with the RA model group, the pathological injury of synovial tissue in each GSE intervention

group was significantly improved, the degree of synoviocyte proliferation was reduced, inflammatory cell infiltration was decreased, tissue edema was subsided, and the pathological score was significantly reduced ($P < 0.01$); among them, the synovial tissue morphology of the high-dose GSE group was close to normal, with only a small amount of inflammatory cell infiltration, and the pathological score was the lowest ($P < 0.01$). See **Table 3**.

Table 3. Comparison of synovial tissue pathological scores in mice of each group (mean ± SD, $n = 10$, score)

Group	Pathological score
Normal control group	0.32 ± 0.11
RA model group	7.85 ± 0.62**
Low-dose GSE group	5.12 ± 0.45*#
Medium-dose GSE group	3.05 ± 0.38**#
High-dose GSE group	1.26 ± 0.21**# △ ▲

4. Discussion

Rheumatoid arthritis is characterized by chronic inflammation and abnormal proliferation of synovium. Abnormal activation of the NF-κB signaling pathway is a key molecular mechanism mediating synovial inflammation [12]. Activation of this pathway can significantly promote the massive expression and release of pro-inflammatory factors such as TNF-α, IL-6, and IL-1β, triggering an inflammatory cascade, aggravating synovial hyperplasia and destruction of articular cartilage and bone. Targeted inhibition of the NF-κB pathway has become an important research direction for the anti-inflammatory treatment of RA [13].

The main active component of GSE is proanthocyanidins, which have strong antioxidant and anti-inflammatory activities. Previous studies have confirmed that GSE can reduce the injury of various inflammatory diseases by regulating the NF-κB pathway, but its regulatory effect and dose-effect on NF-κB in RA synoviocytes remain unclear [14].

The results of this study showed that GSE could down-regulate the mRNA and protein expression of NF-κB/p65 in synoviocytes of RA mice in a dose-dependent manner, and the high-dose inhibition effect was the most significant, which could directly block the activation of the NF-κB pathway. At the same time, GSE could significantly reduce the transcription and secretion levels of downstream inflammatory factors, alleviate the pathological injury of synovial tissue, reduce the arthritis index, and improve joint swelling symptoms. Compared with traditional antirheumatic drugs, GSE has the advantages of high safety, wide sources, and small side effects, and has the potential to be developed as an adjuvant natural drug for RA [15].

5. Conclusion

In conclusion, grape seed extract can inhibit the expression of NF-κB in synoviocytes of RA mice in a dose-dependent manner, thereby down-regulating the release of its downstream inflammatory factors TNF-α, IL-6, and IL-1β, reducing the inflammatory response and pathological injury of synovial tissue, and improving the joint symptoms of mice. Its mechanism may be related to the regulation of the NF-κB signaling pathway. Grape seed extract is expected to be a potential natural drug for the treatment of RA. This study provides new experimental evidence and research ideas for the natural drug therapy of RA, and also lays a foundation for

the clinical application and high-value utilization of grape seed extract.

Funding

This paper was the research result of the 2024 University-level Science and Technology Project of Qilu Institute of Technology (Project Name: Experimental Study on the Effects of Grape Seed Extract on NF-κB Expression in Synoviocytes of RA Mice; Project No.: QIT24NN0724).

Disclosure statement

The authors declare no conflict of interest.

References

[1] Xie Y, Wang N, Cao NN, et al., 2025, Grape Seed Extract Inhibits Chondrocyte Apoptosis by Regulating miR-539-5p in Rat Growth Plate. Chinese Journal of Integrated Traditional and Western Medicine, 45(04): 447–453.

[2] Sun Z, Liu YQ, Dong H, et al., 2023, Effects of Grape Seed Extract on Endocrine Metabolism in Menopausal Model Mice. Journal of Medical College of Yanbian University, 46(04): 252–254.

[3] Li D, Xu SL, Wang Y, et al., 2023, Protective Mechanism of Grape Seed Extract on Cerebral Ischemia-Reperfusion Injury in Rats through NF-κB/TLR4 Signaling Pathway. Chinese Journal of Integrative Medicine on Cardio-Cerebrovascular Disease, 21(19): 3527–3531.

[4] Lei JQ, Tian YX, Li ZG, et al., 2023, Molecular Mechanism of Grape Seed Extract Proanthocyanidins Inhibiting Proliferation, Migration and Invasion of Hepatocellular Carcinoma Hep3B Cells by Regulating EMT via LncRNA NBR2/miR-650. Chinese Journal of Gerontology, 43(17): 4307–4312.

[5] Zhang SS, Wang N, Li YJ, 2024, Grape Seed Extract Inhibits Apoptosis of Rat Growth Plate Chondrocytes and Promotes Tibial Growth. Chinese Journal of Tissue Engineering Research, 28(16): 2494–2499.

[6] Zhang ZX, Guo JQ, Zhu CH, 2023, Study on Antioxidant Effect of Grape Seed and Vitamin C on D-Galactose Model Mice. China Food Additives, 34(01): 208–213.

[7] Sun J, Zou YJ, Sun X, et al., 2024, Study on Anti-*Helicobacter pylori* Effect and Mechanism of Grape Seed Extract and Fucoidan Composition. Food and Fermentation Industries, 50(18): 279–283.

[8] Ding QJ, Niu JP, Ding HL, et al., 2025, Analysis of Molecular Action Path of Grape Seed Extract Regulating NF-κB. Sino-Foreign Food Industry, (21): 79–81.

[9] Feng SL, Tan XG, 2025, Effects of Red Bean Coix Seed Fulxia Dampness-Removing and Detoxification Tea Combined with Grape Seed Extract on People with Phlegm-Dampness Constitution. Western Journal of Traditional Chinese Medicine, 38(12): 126–128.

[10] Jia SZ, 2025, Evaluation Test of Antioxidant Function of Only 100® Grape Seed Extract Tablets in Human Trial. Shanghai Light Industry, (01): 61–63.

[11] Ma J, Li T, Lin L, et al., 2024, Biological Function of Grape Seed Extract and Its Application in Livestock and Poultry Production. Chinese Journal of Animal Nutrition, 36(09): 5441–5450.

[12] Li JF, Diao JJ, Chen ZH, et al., 2024, Analysis of Chemical Constituents of Grape Seed Extract and Determination of Multi-Component Content Based on UHPLC-MS/MS. Food and Fermentation Industries, 50(20): 303–312.

[13] Ding QJ, Lv JW, Ding HL, et al., 2024, Experimental Study on Inhibitory Effect of Grape Seed Extract on

Bacterial Biofilm. Sino-Foreign Food Industry, (11): 49–51.

[14] Geng Z, Fu JL, Guo WT, 2023, Preparation and Antibacterial Property of Grape Seed Extract Composite Film. Hubei Agricultural Sciences, 62(11): 118–124 + 130.

[15] Zhou XH, 2023, Research Progress on Nutritional Composition and Biological Function of Grape Seed Extract. Food and Machinery, 39(07): 228–233 + 240.