International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.1098.1111

Research Article
Resveratrol Extract on the Protein Expression of Inflammatory Factors in Osteoarthritis Chondrocytes

Hougen Lu and Jian Xiong

Department of Orthopedics, Jingzhou Central Hospital, Jingzhou 434020, Hubei, China

Abstract

Background and Objective: Osteoarthritis is mainly the degenerative destruction of articular cartilage and joint inflammatory response. This research aimed to discuss the outcome of resveratrol (RES) extract on the protein expression of inflammatory factors in osteoarthritis chondrocytes. **Materials and Methods:** Human osteoarthritis cartilage cell line (HC-OA) was used as the research material and HC-OA were induced by Interleukin-1β (IL-1β) to simulate the joint inflammatory environment. The cell viability was tested by Cell Counting Kit-8 (CCK-8) method. The expression of type collagen in the cells was tested by immunocytochemical staining of type collagen. The cell proliferation cycle was tested by flow cytometry (FC). The protein and mRNA levels of Toll-Like Receptor 4 (TLR4) and Nuclear Factor-κB (NF-κB) at were tested by western blot (WB) and Real-Time Fluorescent Quantitative PCR (RT-PCR). Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) were tested by Enzyme-Linked Immunosorbent Assay (ELISA) to evaluate the improvement effect of RES on the protein expression of inflammatory factors. **Results:** As against the IL-1β, the activity of HC-OA in the IL-1β+RES was obviously enhanced and the proportion of HC-OA in G0/G1 phase was obviously raised. The TLR4 mRNA, NF-κB mRNA, TLR4 and NF-κB protein in HC-OA of IL-1β+RES had an obvious decrease. The protein expression of IL-6 and TNF-α in chondrocytes of IL-1β+RES group was obviously decreased and the protein expression of IL-10 had a clear increase. **Conclusion:** The RES could clearly improve the protein expression of inflammatory factors in osteoarthritis chondrocytes, indicating that RES can be adopted as a possible therapy drug for osteoarthritis and provide a new idea for clinical treatment.

Key words: RES, osteoarthritis, HC-OA, inflammatory factors, TLR4, NF-κB, cell viability

Citation: Lu, H. and J. Xiong, 2024. Resveratrol extract on the protein expression of inflammatory factors in osteoarthritis chondrocytes. Int. J. Pharmacol., 20: 1098-1111.

Corresponding Author: Jian Xiong, Department of Orthopedics, Jingzhou Central Hospital, Jingzhou 434020, Hubei, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Osteoarthritis is an age-related degenerative joint disease characterized by degenerative destruction of articular cartilage and joint inflammatory response. The clinical symptoms are joint pain, inflammation and dysfunction and it is one of the most common chronic diseases in the world[1,2]. The factors leading to the occurrence of osteoarthritis include osteoporosis, overuse of joints or injury, metabolic disorders and abnormalities of the immune system[3-6]. At present, the treatment methods for osteoarthritis mainly include drug therapy, physical therapy, rehabilitation exercise and surgery, which can relieve pain and improve joint function.

In recent years, more and more studies have shown that plant extracts have potential benefits for the treatment of patients with osteoarthritis. The RES is a compound naturally present in a variety of plants. As a natural anti-inflammatory agent and antioxidant, it is thought to reduce the inflammatory response of osteoarthritis patients and protect articular cartilage from oxidative stress damage. Animal studies have found that RES extract can inhibit the pathological changes of arthritis in arthritis model animals, reduce inflammatory cell infiltration and cartilage degradation[7]. In addition, RES extract may also exert its therapeutic outcome by regulating a variety of signaling pathways and gene expression, which can suppress the production of inflammatory mediators, regulate inflammation-related genes and promote the survival and proliferation of articular chondrocytes[8,9]. It has also been found that after the treatment of chondrocytes with RES extract, the levels of Chemokine (C-X-C motif) Ligand 1 (CXCL1), Hypoxia-Inducible Factor-1α (HIF-1α), IL-6, Matrix Metalloproteinase 3 (MMP3) and NADPH-oxidase 4 (NOX4) in chondrocytes were detected and Prostaglandin-Endoperoxide Synthase 2 (PTGS2) were clearly decreased, but HIF-α was clearly increased. It is revealed that CXCL1, HIF-1α, IL-6, MMP3, NOX4 and PTGS2 are the targets of RES treatment in arthritis[10]. Studies have proposed that RES extract has the effects of scavenging free radicals *in vivo* and *in vitro*, anti-inflammatory, anti- apoptosis, anti-aging and anti-oxidation and in the study of Huo *et al.*[11], it has the ability to delay the progression of intervertebral disc (IVD) degeneration. Some studies have proposed that RES extract can regulate the TLR4 pathway and reduce inflammatory response, which reduces the expression of TLR4 by regulating microRNA and antioxidant pathways, thereby inhibiting inflammatory response[12]. The TLR4 and NF- κB are closely related to inflammation and TLR4 can recognize and respond to the Pathogen-Associated Molecular Patterns (PAMPs) and other microorganisms. When TLR4 binds to its ligands, it activates the NF-κB signal transduction pathway in cells. The activated NF-κB can enter the nucleus and bind to DNA, to promote the transcription and production of IL-6 and TNF-α. These inflammatory factors further trigger the inflammatory response and participate in the regulation and development of inflammation. Nevertheless, people have little understanding of the outcomes of RES on inflammatory factors in osteoarthritis chondrocytes, so it is necessary to further explore its mechanism of action and potential therapeutic outcomes.

Therefore, this research explored the changes of cellular inflammatory factors in terms of TLR4 and NF-κB, reveals whether RES can suppress the overexpressed inflammatory factors in osteoarthritis chondrocytes and evaluates the degree of its anti-inflammatory outcome. It proposes more ideas for the RES in osteoarthritis, providing an important basis for further research on its mechanism and development of related drugs.

MATERIALS AND METHODS

Study area: The research was performed in Jingzhou Central Hospital from December, 2022 to December, 2023.

Cell culture and identification: Human articular chondrocytes (HC-OA) (Merck Biological, Germany) from T25 (Beijing Solarbio Technology Co. Ltd., China) culture flasks were collected, transferred to Eppendorf (EP) tubes (Beijing Solarbio Technology Co. Ltd., China), centrifuged at 1,700 r/10 min and then the upper liquid was discarded. They were suspended using RPMI-1640 medium (Beijing Solarbio Technology Co. Ltd., China) having 10% FBS (Beijing Solarbio Technology Co. Ltd., China), which was then changed into a new T25 culture flask for culture. They were then placed in a cell incubator (Thermo Fisher, USA) at 37°C in 5% CO_2. When they were grown to 80%, passage was carried out, which can generally be passaged twice a week. The phenotype of HC-OA was identified by immunocytochemical staining of type collagen. During subculture, some cells were placed on slides and cultured for 24 hrs. They were rinsed to the third time applying PBS at 25°C, fixation applying 4% paraformaldehyde for 15 min, rinsed with deionized water and allowed to dry. The streptavidin peroxidase conjugated method (SP) immunohistochemical kit (Beijing Solarbio Technology Co. Ltd., China) was used to detect HC-OA, which was carried out strictly based on the introduction. The objects were then subjected to observation using a microscope (Wuxi Jiebo Instrument Technology Co. Ltd., China).

Groups treatment: The HC-OA in logarithmic phase in the plate having 96 wells (Beijing Solarbio Technology Co. Ltd., China) according to the density of 10^3-10^4 cells/well were divided into 5 groups. Control group (Ctrl): Normal culture medium; Negative control group (NC): Conventional medium containing 0.1% Dimethyl Sulfoxide (DMSO); RES group: 2.25, 4.5, 9, 18, 32, 64 μmol/L RES culture medium; IL-1β group: Conventional medium with 10 ng/mL IL-1β and 0.1% DMSO; IL-1β+RES group: Conventional medium having 10 ng/mL IL-1β and 2.25, 4.5, 9, 18, 32 and 64 μmol/L RES; Each had 5 replicates.

Cell proliferation activity detection: Cell Counting Kit-8 (CCK-8) (Beijing Solarbio Technology Co. Ltd., China) was used to detect the activity of HC-OA in Ctrl, NC, RES, IL-1β and IL-1β+RES following 24 hrs of treatment. As 10 μL CCK-8 solution was applied to each well. It was mixed by oscillator, incubated in cell culture and detected after 4 hrs:

$$\text{Cell viability}(\%) = \frac{A_{\text{Dosing}} - A_{\text{Blank}}}{A_0 - A_{\text{Blank}}} \times 100$$

where, A_{Dosing} is the absorbance (OD) with cells, CCK-8 and drug in the well; A_0 is the OD value with cells, CCK-8 and no drug in the well; A_{Blank} is the OD without cells in the well.

Detecting the cell cycle: The FC (Beckman Coulter Company, Germany) was used to detect the HC-OA cycle and cells in subsequent experiments were treated using the set of RES concentrations with the best activity detection. The Ctrl, IL-1β, IL-1β+0.1% DMSO and IL-1β+RES were selected. Cells were collected after 0.25% trypsin digestion and centrifuged at 1,700 r/10 min. The upper liquid was discarded and the cell precipitation was washed with PBS. The objects were in the incubator for 1 hr and then the machine was started.

Fluorescence quantitative PCR detection: The TLR4 mRNA and NF-κB were subjected to detection using RT-PCR. Total RNA was subjected to extraction using TRIZOL method and the concentration and concentration of the extracted RNA were detected. Then, the extracted RNA was reverse transcribed into cDNA to detect the mRNA levels of related proteins and the operation was carried out strictly according to the instructions of the kit (Thermo Fisher, USA). The target gene was amplified using a T100 fluorescence quantitative PCR instrument (Thermo Fisher, USA). The reaction conditions were denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec, with β-actin as the internal control. As 40 cycles were carried out, followed by extension at 72°C for 5 min. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Quantitative primer information is as follows:

- TLR4 F: 5'-AGGACTGGGTAAGGAATGAGC-3'
- R: 5'-ATCACCTTTCGGCTTTTATGG-3'
- NF-κB F: 5'-AGGAGAGGATGAAGGAGTTGTG-3'
- R: 5'-CCAGAGTAGCCCAGTTTTTGTC-3'
- β-actin F: 5'-CACACTGTGCCCATCTACGA-3'
- R: 5'-CTCAGTGAGGATCTTCATGAGGTAGT- 3'

Detection of protein expression: The cells were collected into EP tubes and placed in an ice box and then they were lysed with tissue lysate (RIPA). After centrifugation at 12,000 r/10 min, the upper liquid was collected and used as the samples for WB. Bicinchoninic acid (BCA) (Thermo Fisher, USA) assay was used to detect the concentration of the collected upper liquid. The DYCP-44P electrophoresis instrument (Beijing Liuyi Biotechnology Co. Ltd., China) was used for electrophoresis. Through SDS-PAGE gel electrophoresis and the protein sample was loaded according to the detected concentration, with no less than 20 μg per well. After the gel electrophoresis was completed, the membrane was transferred, blocking with 5% skim milk powder. The primary antibody (1: 200) (anti-TLR4 antibody (MA5-16216) (Thermo Fisher, USA), anti-NF-κB antibody (9936T) (Cell Signaling Technology, USA), p-NF-κB (3031S) (Cell Signaling Technology, USA), ERK1/2 (13-6200) (Thermo Fisher, USA), p-ERK1/2 (44- 680G) (Thermo Fisher, USA) and anti-GAPDH antibody (MA1-16757) (Thermo Fisher, USA) were put, overnight at 4°C. The membranes were subjected to washing with Tris- HCl+Tween the next day, incubation with secondary antibodies (G21234 and A10648) with horseradish peroxidase (HRP) for 2 hrs and washing again with TBST. The OD of the bands was detected using WD-9413 gel imager (Beijing Liuyi Biotechnology Co. Ltd., China) analysis software and the ratio of protein to GAPDH was used as the expression level.

Detection of TNF-α, IL-6 and IL-10: The TNF-α, IL-6 and IL-10 in the supernatant were detected by ELISA. As 200 μL diluted TNF-α, IL-6 and IL-10 antigens to each well was incubated at 37°C for 1 hr, overnight at 4°C. Following washing the plate, 200 μL blocking solution was applied and placed at 37°C for

1 hr before washing. After dilution of serum, 200 µL was put to each well, at 37°C for 2 hrs and rinsing. As 200 µL of HRP labeled IgG antibody was put, at 37°C for 1 hr and rinsing. As 200 µL of O-phenylene diamine solution was applied, in a dark place at 25°C for 10 min. As 50 µL termination solution to each well, the OD of each well was detected at 490 nm by microplate reader.

Statistical analysis: All data were processed by SPSS22.0 software and the data following normal distribution were presented as Mean±SD ($\bar{\chi}\pm$sec). One-way ANOVA and least significant difference-t (LSD-t) test were adopted for comparison. The $p<0.05$ meant there was an obvious difference.

RESULTS

Phenotypic identification of HC-OA: Following immunocytochemical staining of HC- OA cells with type II collagen, cultured for 24 hrs, it was observed under a microscope that the cytoplasm of HC-OA cells appeared uniformly brownish-yellow (Fig. 1).

Cell activity at different concentrations of RES: The activity of cells in each group under different drug concentrations was assessed and the results were depicted in Fig. 2(a-b) (b1-b8). There was no significant difference in the activity of HC-OA cells between the control group and the negative control group ($p>0.05$). Following treatment with varying

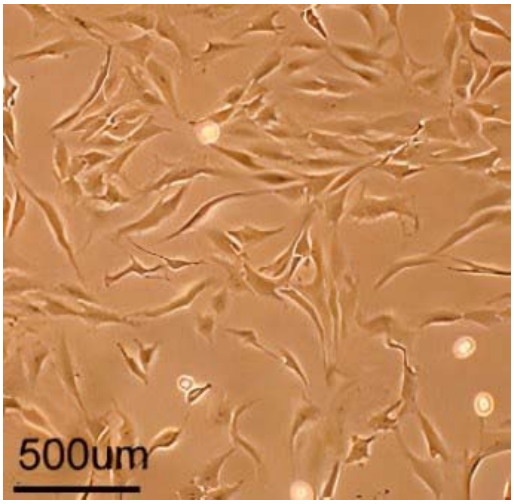

Fig. 1: Immunocytochemical staining of HC-OA at passage 3 (×200)

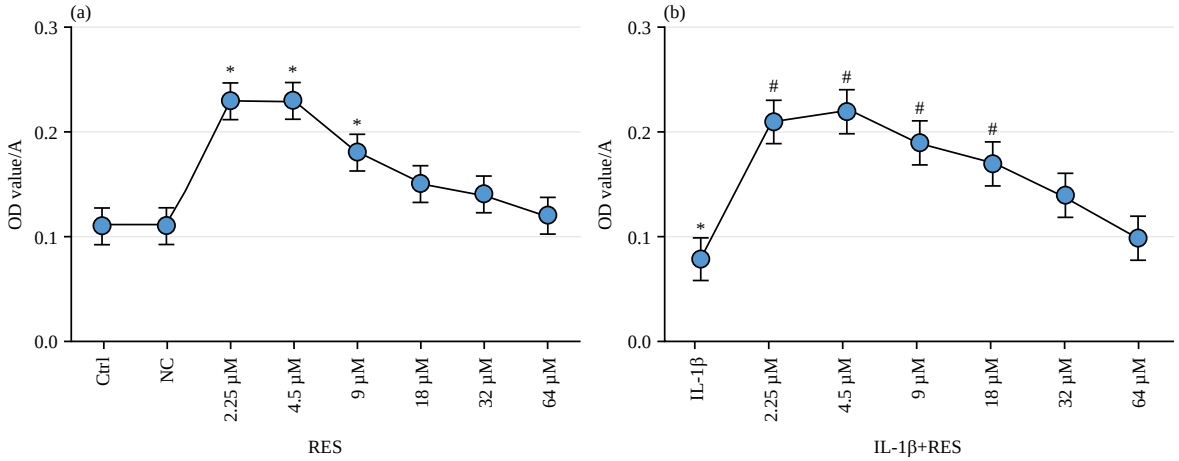

Fig. 2(a-b): Cell activity at different concentrations of RES, (a) Ctrl, NC and RES groups and (b) IL- 1β and IL-1β+RES groups

*As against NC, #as against IL-1β group, all $p<0.05$

concentrations of RES, it was observed that at concentrations of 2.25, 4.5 and 9 μM, the activity of HC-OA cells significantly increased compared to the negative control group ($p<0.05$). However, at concentrations of 32 and 64 μM, the activity of HC-OA cells did not significantly differ from that of the negative control group ($p>0.05$). Pre-treatment of cells in an IL-1β environment resulted in a significant decrease in the activity of HC-OA cells compared to the negative control group ($p<0.05$). Following treatment with different concentrations of RES, it was observed that the activity of HC-OA cells at concentrations ranging from 2.25 to 18 μM significantly increased compared to the IL-1β group ($p<0.05$).

Impacts of RES densities on cell cycle: The primary function of chondrocytes is to synthesize and secrete collagen proteins, thus under normal conditions, they remain in the G0/G1 phase of the cell cycle. The results of cell cycle analysis in this study, as depicted in Fig. 3, showed a significant decrease in the proportion of HC-OA cells in the G0/G1 phase in the IL-1β group compared to the control group ($p<0.05$). Following treatment with different concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9 and 18 μM RES, the proportion of cells in the G0/G1 phase significantly increased compared to the IL-1β group ($p<0.05$), while the proportions of cells in the S phase and G2/M phase significantly decreased ($p<0.05$). However, with increasing concentrations of RES, the proportion of cells entering the S phase and G2/M phase gradually increased. When the RES concentration reached 32 and 64 μM, compared to the IL-1β group, there was a significant decrease in the proportion of cells in the G0/G1 phase, while the proportions of cells in the S phase and G2/M phase significantly increased.

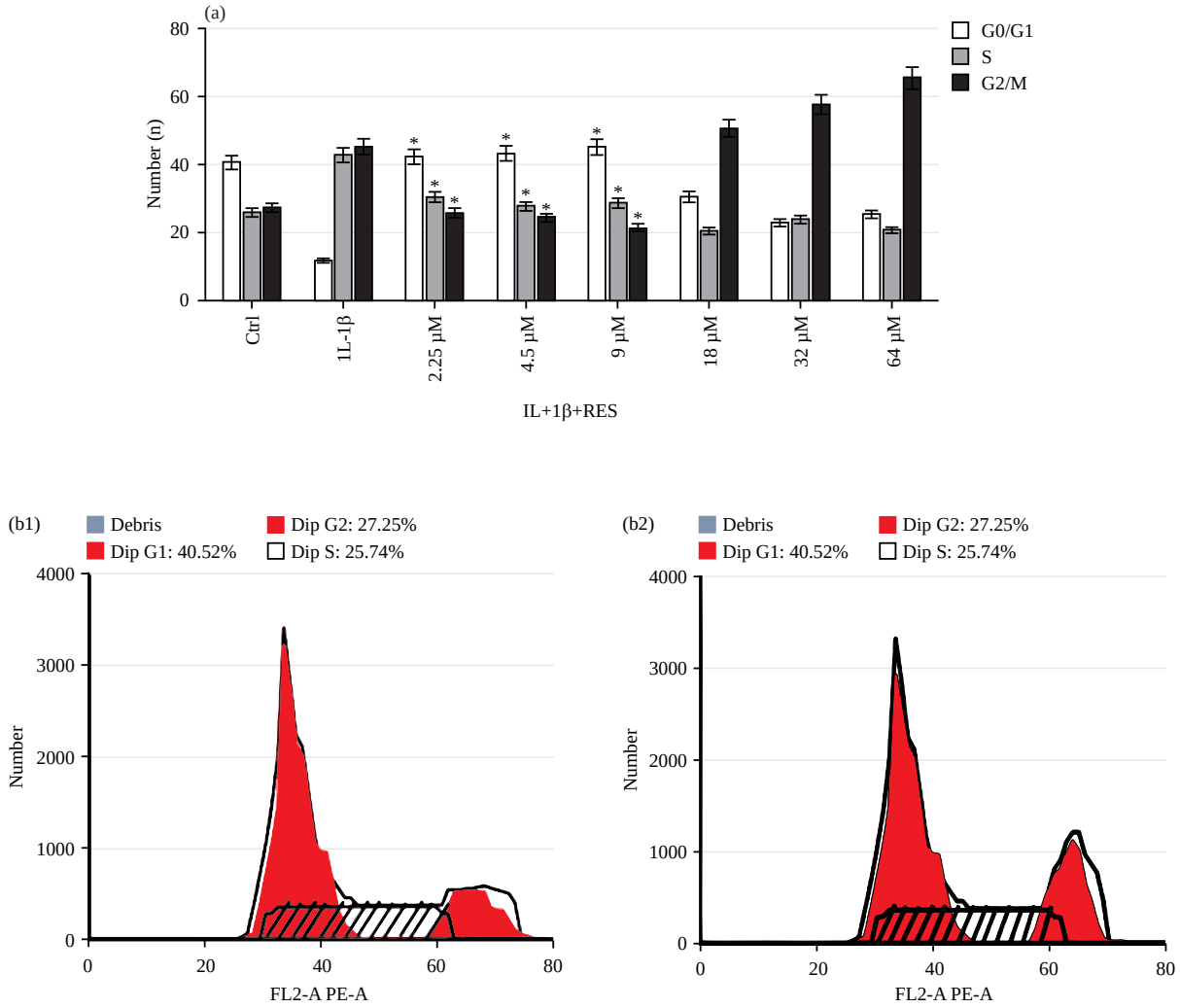

Fig. 3(a, b1-b8): Continue

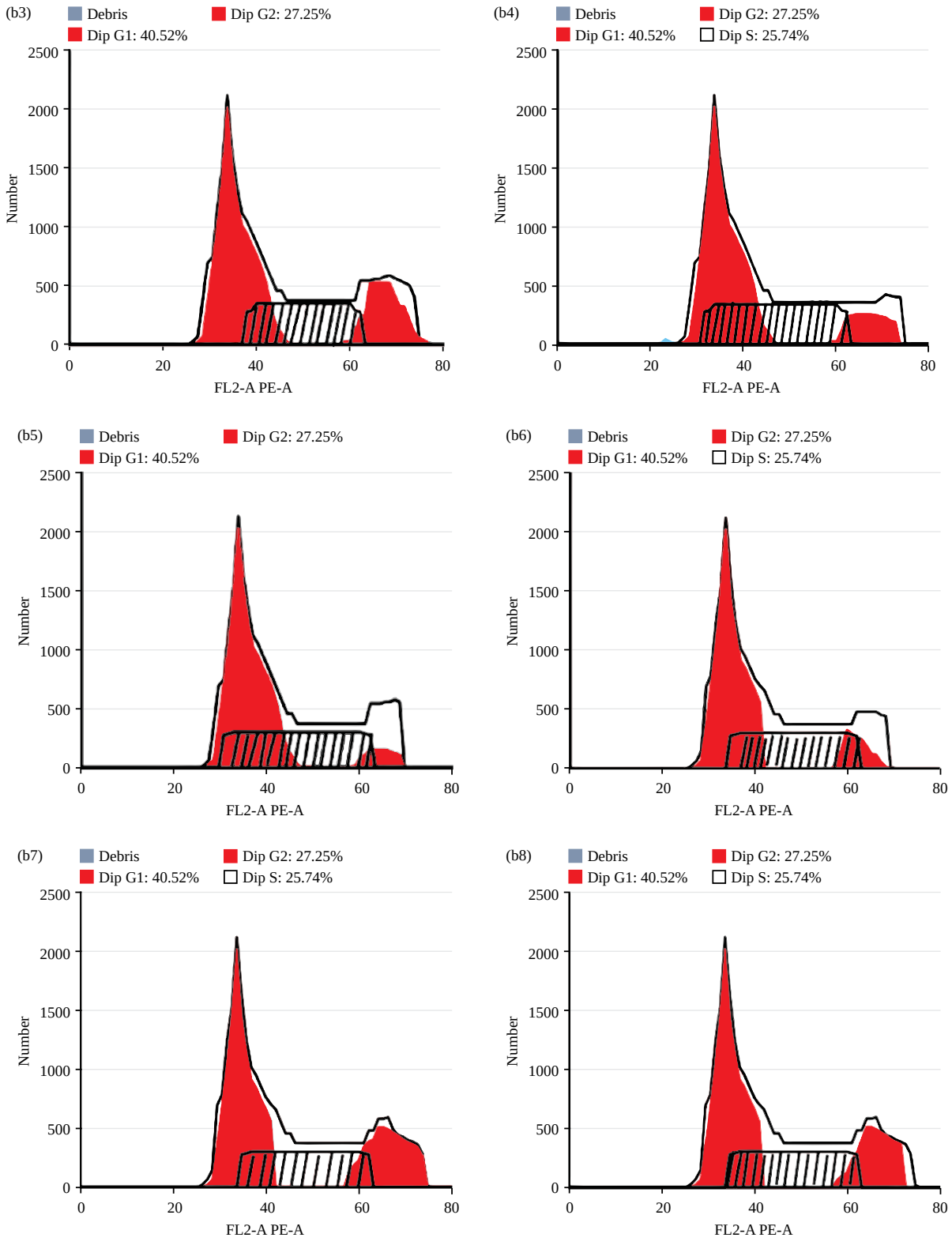

Fig. 3(a, b1-b8): Impact of RES densities on the cell cycle, (a) Cell cycle for the impact of RES densities on cell cycle, (b) FC of the impacts of RES densities on cell cycle, b1-b8: Ctrl, IL-1β, IL-1β+RES groups, 2.25, 4.5, 9, 18, 32 and 64 μM

*As against IL-1β group and p<0.05

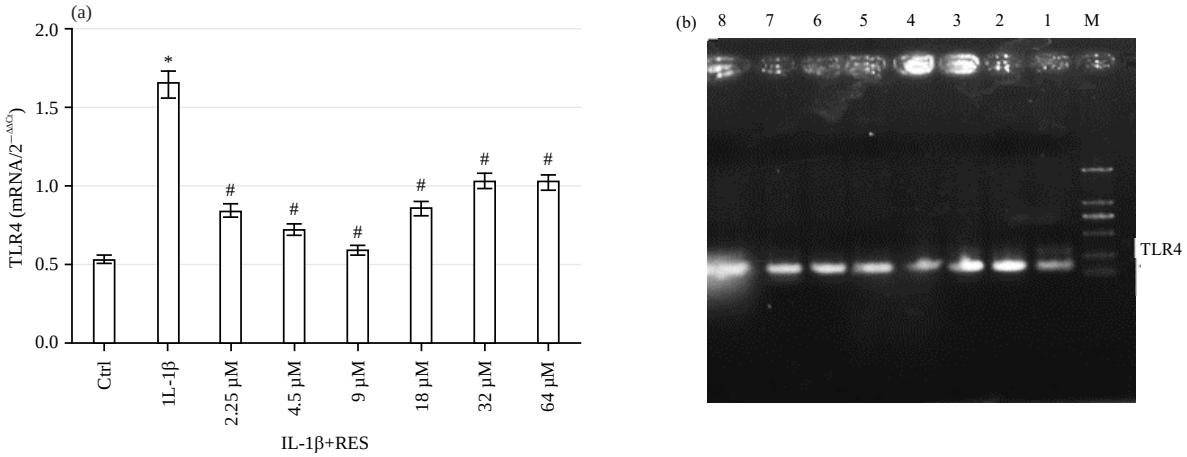

Fig. 4(a-b): Expression levels of TLR4 mRNA in HC-OA treated with various densities of RES, (a) TLR4 mRNA expression and (b) Gel diagram of TLR4 mRNA expression (M is Marker, 1-8 are: DMSO, DMSO+IL-1β, 2.25, 4.5, 9, 18, 32 and 64 μM RES)

*As against Ctrl, #as against IL-1β group and all $p<0.05$

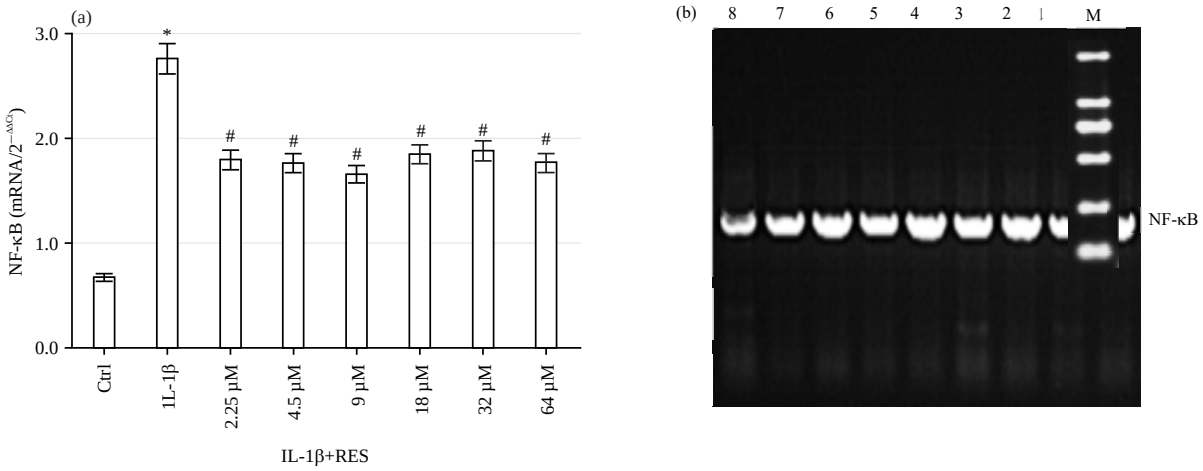

Fig. 5(a-b): Expression levels of NF-κB mRNA in HC-OA adopting various degrees of RES, (a) NF-κB mRNA expression levels and (b) Gel plot of NF-κB mRNA expression (M is Marker, 1-8 are: DMSO, DMSO+IL-1β, 2.25, 4.5, 9, 18, 32 and 64 μM RES)

*As against Ctrl, #as against IL-1β group and all $p<0.05$

TLR4 mRNA expression in HC-OA treated with various densities of RES: Treatment of HC-OA cells with different drug concentrations yielded results as illustrated in Fig. 4(a-b). The expression levels of TLR4 mRNA in HC-OA cells of the IL-1β group were significantly elevated compared to the control group ($p<0.05$), demonstrating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the expression levels of TLR4 mRNA in HC-OA cells were significantly reduced compared to the IL-1β group ($p<0.05$), indicating a significant difference.

NF-κB mRNA in HC-OA with various densities of RES: Treatment of HC-OA cells with different drug concentrations yielded results as illustrated in Fig. 5(a-b). The expression levels of NF-κB mRNA in HC-OA cells of the IL-1β group were significantly elevated compared to the control group ($p<0.05$), indicating a significant difference. Following

treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the expression levels of NF-κB mRNA in HC-OA cells were significantly reduced compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

TLR4 protein in HC-OA adopting various degrees of RES: Treatment of HC-OA cells with different drug concentrations resulted in the outcomes depicted in Fig. 6(a-b). The expression levels of TLR4 protein in HC-OA cells of the IL-1β group were significantly elevated compared to the control group ($p<0.05$), indicating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the expression levels of TLR4 protein in HC-OA cells were significantly reduced compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

Protein expression of NF-κB, p-NF-κB, ERK1/2 and p-ERK1/2 in HC-OA adopting various degrees of RES: Treatment of HC-OA cells with different drug concentrations yielded results as depicted in Fig. 7(a-e). The expression levels of NF-κB, p-NF-κB, ERK1/2 and p-ERK1/2 in HC-OA cells of the IL-1β group were significantly elevated compared to the control group ($p<0.05$), indicating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the expression levels of NF-κB, p-NF-κB, ERK1/2 and p-ERK1/2 in HC-OA cells were significantly reduced compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

Secretion of TNF-α, IL-6 and IL-10 in HC-OA adopting various degrees of RES: Treatment of HC-OA cells with different drug concentrations resulted in outcomes as shown in Fig. 8a. The level of TNF-α in the culture supernatant of the IL-1β group was significantly elevated compared to the control group ($p<0.05$), indicating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the level of TNF-α in the culture supernatant was significantly reduced compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

Treatment of HC-OA cells with different drug concentrations resulted in outcomes as shown in Fig. 8b. The level of IL-6 in the culture supernatant of the IL- 1β group was significantly elevated compared to the control group ($p<0.05$),

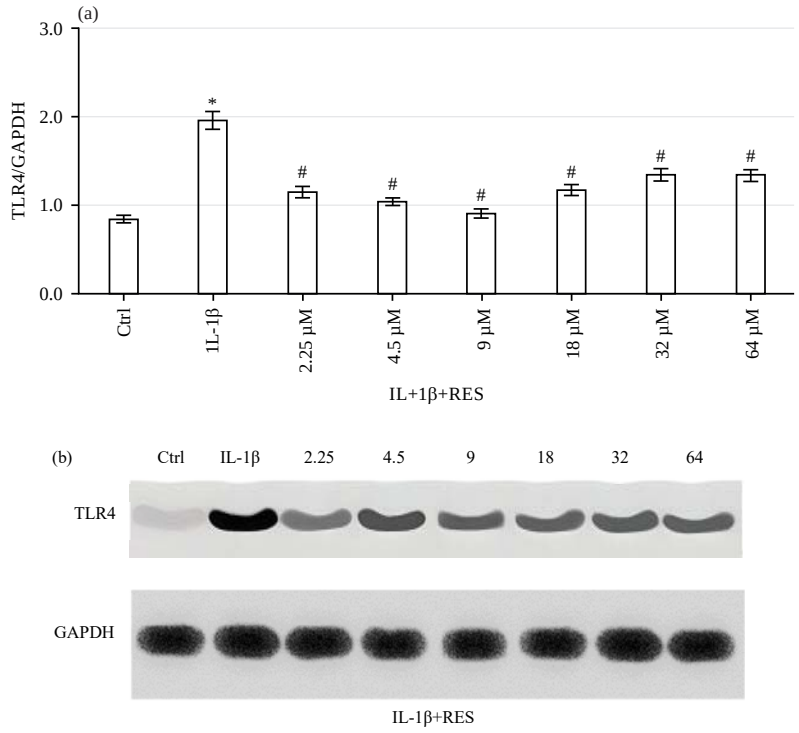

Fig. 6(a-b): Expression levels of TLR4 protein in HC-OA adopting various degrees of RES, (a) TLR4 protein expression level and (b) WB gel plot of TLR4 protein

*Relative to Ctrl, #as against IL-1β group and all $p<0.05$

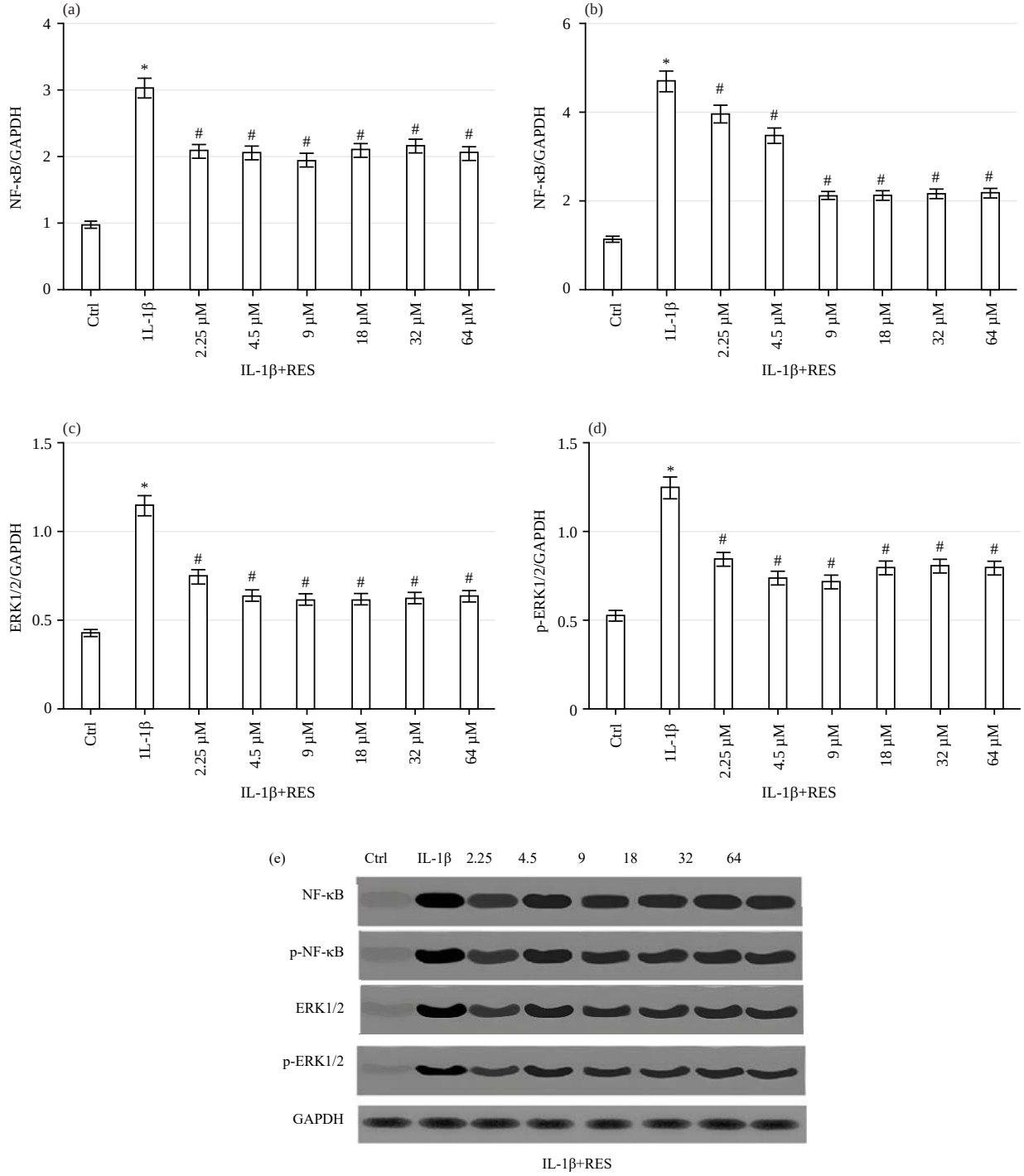

Fig. 7(a-e): Protein expression of NF-κB, p-NF-κB, ERK1/2 and p-ERK1/2 in HC-OA adopting various degrees of RES, (a) NF-κB, (b) p-NF-κB, (c) ERK1/2, (d) p-ERK1/2 and (e) WB gel plot of their protein expression

*As against Ctrl, #as against IL-1β group and all $p<0.05$

indicating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the level of IL-6 in the culture supernatant was significantly reduced compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

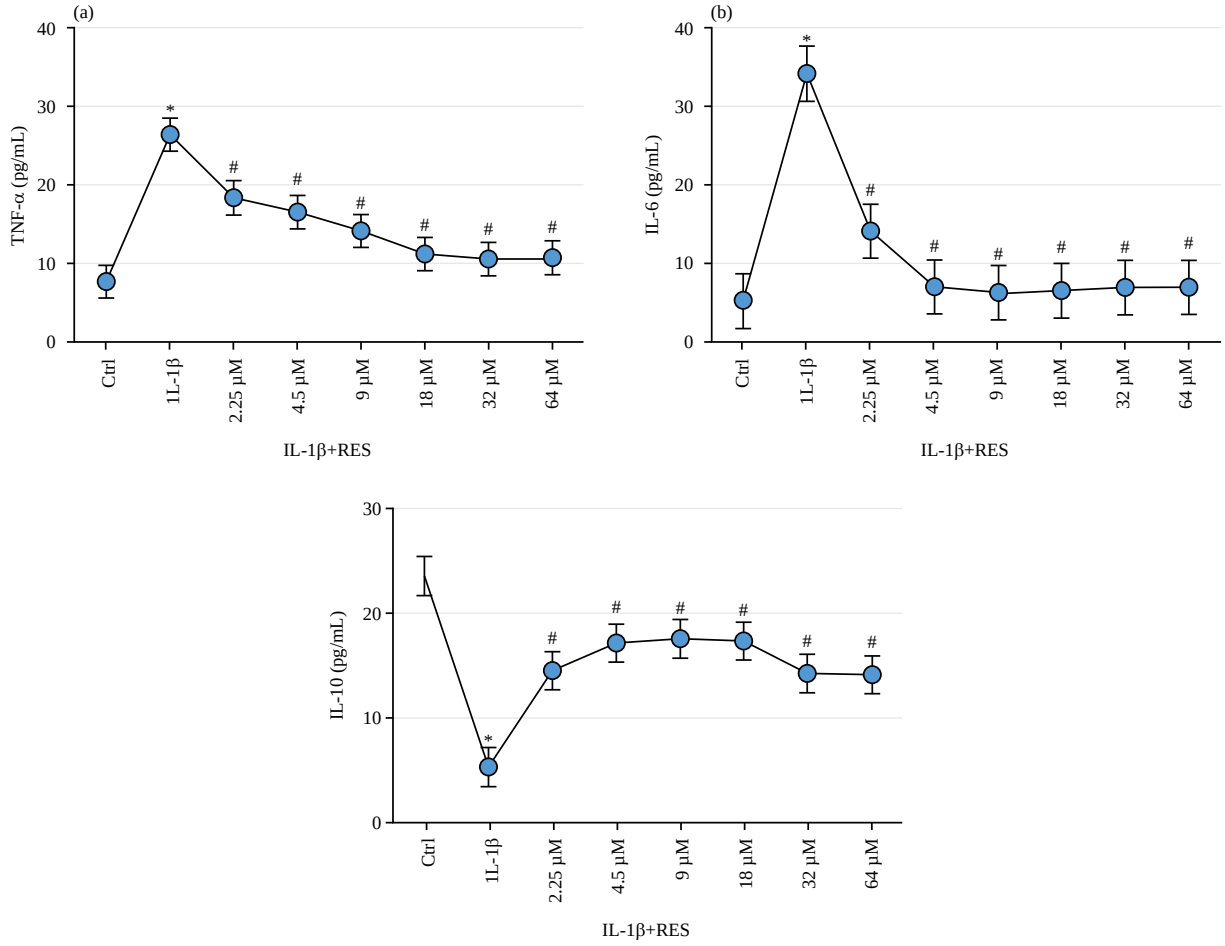

Fig. 8(a-c): Levels of TNF-α, IL-6 and IL-10 secretion in HC-OA applied with various degrees of RES, (a) TNF-α secretion, (b) IL-6 secretion and (c) IL-10 secretion

*As against Ctrl, #as against IL-1β group and all $p<0.05$

Treatment of HC-OA cells with different drug concentrations resulted in outcomes as shown in Fig. 8c. The level of IL-10 in the culture supernatant of the IL-1β group was significantly reduced compared to the control group ($p<0.05$), indicating a significant difference. Following treatment with varying concentrations of RES, it was observed that at concentrations of 2.25, 4.5, 9, 18, 32 and 64 μM RES, the level of IL-10 in the culture supernatant was significantly elevated compared to the IL-1β group ($p<0.05$), demonstrating a significant difference.

DISCUSSION

Osteoarthritis is a common disease among the elderly population, characterized by unbearable joint pain due to degeneration of the bone joints, resulting in difficulty in mobility for patients. The occurrence of osteoarthritis is associated with various factors such as age, gender, obesity and joint injuries[13,14]. As researchers delve deeper into the mechanisms underlying osteoarthritis, inflammation, cellular apoptosis and autoimmune attacks have been identified as important factors triggering the condition[15-17]. The levels of cytokines and inflammatory factors associated with inflammation, autoimmunity and cellular apoptosis serve as crucial indicators for observing improvements in osteoarthritis. Inflammation is a significant pathological process in osteoarthritis, capable of causing swelling, erythema and pain in the surrounding joint tissues[18]. During the inflammatory process, various cytokines and inflammatory mediators such as TNF-α and IL-1β are activated and released. These cytokines not only increase intra-articular inflammation but also contribute to the destruction and damage of joint cartilage. Besides inflammation, attacks from the autoimmune system are also implicated in the onset of osteoarthritis. In

osteoarthritis patients, the immune system may become activated and mistakenly attack the body's own tissues, including joint cartilage. Monitoring changes in the levels of cytokines and inflammatory factors can provide a better understanding of the pathological process of osteoarthritis and serve as a basis for formulating treatment plans to improve the quality of life for patients[19].

In this study, type II collagen in HC-OA cells was examined, revealing a brownish- yellow coloration upon collagen immunocytochemical staining, indicating the presence of type II collagen in the cellular fluid. This result suggests that HC-OA cells possess the capability to synthesize and secrete collagen proteins. Since chondrocytes are the primary site to produce type II collagen protein, the detection of type II collagen in these cells confirms that the cells utilized in the study are indeed chondrocytes[20].

The IL-1β is a key inflammatory factor in osteoarthritic cartilage. Robinson *et al.*[21] employed IL-1β-induced chondrocyte inflammation models to investigate the therapeutic mechanisms of osteoarthritis. In this study, a 10 ng/mL IL-1β concentration was utilized to create an inflammatory microenvironment for culturing HC-OA cells. The RES extract, a non-flavonoid polyphenolic compound, possesses anti-inflammatory, free radical scavenging, anti-apoptotic and anti-aging properties[22]. In this study, HC-OA cells were treated with different concentrations of RES extract, followed by assessment of cell viability using the CCK-8 assay. The results revealed no significant inhibition of HC-OA cell activity by RES extract and notably, enhanced cell activity was observed under treatment with lower concentrations of RES extract (2.25, 4.5 and 9 μM). In the presence of 10 ng/mL IL-1β, the activity of HC-OA cells was significantly decreased. Upon treatment with different concentrations of RES extract, it was found that RES extract could inhibit the IL-1β-induced decrease in cell activity. Consistent with the findings of Woodell-May and Sommerfeld[23], who demonstrated that treatment with 6, 12 and 24 μM RES increased cell activity in IL-1β-induced human OA chondrocytes, the results of this study indicated that 10 ng/mL IL-1β exerted an inhibitory effect on the proliferation of HC-OA cells, while RES extract promoted the proliferative activity of HC-OA cells.

Chondrocytes are the primary cells responsible for synthesizing type II collagen and proteins, thus most cartilage cells remain in a prolonged G0/G1 phase[24]. In this study, treatment of HC-OA cells with IL-1β revealed a significant promotion of cells remaining in the G2/M phase for an extended period, whereas upon administration of RES extract, an increased number of cells were observed in the G0/G1 phase, accompanied by a significant decrease in cells in the G2/M phase. Treatment with low concentrations of RES extract did not show significant changes in the number of cells in the S phase, while treatment with 18, 32 and 64 μM RES extract resulted in a notable decrease in the number of cells in the S phase. This suggests that RES extract may possess the ability to inhibit the cell cycle, particularly by blocking progression into the G2/M phase, thereby causing cells to remain in the G0/G1 phase for an extended duration. Additionally, it was found that an appropriate concentration of RES can promote HC-OA cells to enter the G0/G1 phase while inhibiting cell division in the S and G2/M phases. However, excessively high concentrations of RES may lead to cell cycle dysregulation. Previous studies have also shown a significant increase in the number of cells in the G0/G1 phase when treated with 30 μM in nucleus pulposus (NP) cells[25]. This consistency in results validates the findings of this study, suggesting that RES extract can impede cells from entering the G2/M phase.

The TNF-α and IL-6 are common inflammatory factors closely associated with the development of osteoarthritis due to their induction of pro-inflammatory cascades[26]. Studies have found that RES can interact with various molecular targets (NF-κB, TNF-α), thereby inhibiting inflammatory reactions[27]. In this study, treatment with different concentrations of RES extract was able to reduce the protein expression levels of IL-6 and TNF-α, while promoting the expression levels of IL-10, thereby alleviating arthritis inflammation. This indicates that RES can significantly improve the protein expression of inflammatory factors in osteoarthritis chondrocytes. The IL-10 is an anti-inflammatory cytokine with roles in suppressing inflammatory responses and regulating immune responses. In a study using an arthritis rat model, it was found that RES could increase IL-10 levels, thereby reducing the severity of arthritis in rats[28]. This is consistent with the results of this study, demonstrating an improvement in the levels of inflammatory factors and alleviation of arthritis damage.

The TLR4 is a transmembrane receptor protein in the immune system capable of recognizing and binding to specific molecular patterns (PAMPs) of microorganisms such as bacteria and fungi, thereby activating immune responses and promoting the secretion of inflammatory factors[29]. Studies suggested that the NF-κB signaling pathway mediated by TLR4 is implicated in the pathogenesis of osteoarthritis. In animal experiments, it was found that the expression levels of TLR4 were significantly decreased in an OA mouse model, along with a notable decrease in the levels of p-NF-κB[30].

Furthermore, an *in vitro* study revealed that RES inhibits IL-1β-mediated inflammation by suppressing TLR4. Additionally, when TLR4 was silenced in this experiment, a significant reduction in NF-κB activity was observed[31]. In this study, it was observed that treatment of HC-OA cells with IL-1β resulted in a significant decrease in the expression levels of TLR4 mRNA, NF-κB mRNA, TLR4 and NF-κB. However, after administering different concentrations of RES, there was a significant increase in the expression levels of TLR4 mRNA, NF-κB mRNA, TLR4 and NF-κB. This indicates that RES can alleviate the inflammatory response of HC-OA cells, promote the expression of TLR4 mRNA, NF-κB mRNA, TLR4 and NF-κB and effectively reverse the IL-1β-induced inflammatory response. These findings are consistent with the results of other studies.

The ERK1/2 is a relevant protein in the NF-κB pathway and its levels increase during inflammatory responses. Upon activation by phosphorylation, p-ERK1/2 translocates into the cell nucleus and phosphorylates downstream target proteins, thereby regulating the progression of inflammatory responses. When inflammation occurs, the levels of p-ERK1/2 typically increase, reflecting an increase in ERK1/2 activity[32]. Studies found that early-stage osteoarthritis can stimulate an elevation in ERK1/2 and p-ERK1/2 levels in chondrocytes, while RES can inhibit a series of inflammatory reactions induced by inflammation, reducing the levels of ERK1/2 and p-ERK1/2[33]. In this study, examination of cells treated with IL-1β revealed a significant increase in ERK1/2 and p-ERK1/2 levels, indicating that the cells were in an inflammatory state. After treatment with different concentrations of RES, the levels of ERK1/2 and p-ERK1/2 were significantly reduced. This suggests that RES extract has an inhibitory effect on the inflammatory response induced by IL-1β, reducing ERK1/2 activation and thereby alleviating the inflammatory response.

In summary, the research findings demonstrate that IL-1β induces a decrease in the activity of HC-OA cells and triggers an inflammatory response, promoting the expression of TLR4, as well as phosphorylation of NF-κB and ERK1/2 proteins, leading to a series of inflammatory cascades and enhancing the expression of TNF-α and IL-6. After treatment with different concentrations of RES, it was observed that RES significantly increases the activity of HC-OA cells. Furthermore, it markedly increases the proportion of HC-OA cells in the G0/G1 phase while reducing the proportion of cells in the S and G2/M phases. However, as the concentration of RES increases, the proportion of cells in the S and G2/M phases gradually rises. The RES also significantly reduces the expression levels of TLR4 mRNA and NF-κB mRNA, as well as the expression levels of TLR4 and NF-κB proteins in HC-OA cells, inhibits the secretion of IL-6 and TNF-α and increases the secretion of IL-10 in HC-OA cells, thereby alleviating the inflammatory response.

CONCLUSION

The IL-1β induced a decrease in the activity of HC-OA and triggered an inflammatory response in HC-OA. It promoted the expression of TLR4 and the phosphorylation of NF-κB and ERK1/2 proteins and triggered a series of inflammatory cascade reactions to promote the expression of TNF-α and IL-6. The RES could markedly improve the activity of HC-OA. Moreover, RES could markedly increase the ratio of HC-OA in G0/G1 stage and reduce the ratio of cells in S and G2/M stages. However, with the increase of RES concentration, the ratio gradually raised in S and G2/M stages. The RES can suppress the secretion of IL-6 and TNF-α and increase the secretion of IL-10 in HC-OA, thereby alleviating the inflammatory response. The RES may further alleviate the symptoms of arthritis by suppressing inflammatory response and RES may be adopted as a potential therapeutic drug for the remedy of osteoarthritis, which provides a new idea for clinical remedy. However, further studies should be carried out to explore the mechanism of action and clinical application prospects of RES.

SIGNIFICANCE STATEMENT

Osteoarthritis is a degenerative joint disease associated with aging, characterized primarily by degenerative destruction of articular cartilage and inflammatory responses within the joints. This study aimed to investigate whether resveratrol can inhibit the excessive expression of inflammatory factors in osteoarthritic chondrocytes and assess the extent of its anti-inflammatory effects. The results indicated that resveratrol enhanced the activity of osteoarthritic chondrocytes, increased the number of cells in the G0/G1 phase, decreased the expression levels of TLR4, NF-κB, IL-6 and TNF-α proteins and elevated the level of IL-10, significantly ameliorating the protein expression of inflammatory factors in osteoarthritic chondrocytes. This suggests that resveratrol may serve as a potential therapeutic agent for osteoarthritis, offering new insights for clinical treatment.

REFERENCES

1. Motta, F., E. Barone, A. Sica and C. Selmi, 2023. Inflammaging and osteoarthritis. Clin. Rev. Allergy Immunol., 64: 222-238.
2. Mandl, L.A., 2019. Osteoarthritis year in review 2018: Clinical. Osteoarthritis Cartilage, 27: 359-364.
3. Kim, D., A.A. Pirshahid, Y. Li, T. Varghese and J.E. Pope, 2022. Prevalence of osteoporosis in osteoarthritis: A systematic review and meta-analysis. Osteoporosis Int., 33: 1687-1693.
4. Palazzo, C., C. Nguyen, M.M. Lefevre-Colau, F. Rannou and S. Poiraudeau, 2016. Risk factors and burden of osteoarthritis. Ann. Phys. Rehabil. Med., 59: 134-138.
5. O'Neill, T.W., P.S. McCabe and J. McBeth, 2018. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. Best Pract. Res. Clin. Rheumatol., 32: 312-326.
6. Mondadori, C., S. Palombella, S. Salehi, G. Talò and R. Visone *et al*., 2021. Recapitulating monocyte extravasation to the synovium in an organotypic microfluidic model of the articular joint. Biofabrication, Vol. 13. 10.1088/1758-5090/ac0c5e.
7. Long, Z., W. Xiang, J. Li, T. Yang and G. Yu, 2021. Exploring the mechanism of resveratrol in reducing the soft tissue damage of osteoarthritis based on network pharmacology and experimental pharmacology. Evidence-Based Complementary Altern. Med, Vol. 2021. 10.1155/2021/9931957.
8. He, Y., Q. Ding, W. Chen, C. Lin and L. Ge *et al*., 2022. LONP1 downregulation with ageing contributes to osteoarthritis via mitochondrial dysfunction. Free Radical Biol. Med., 191: 176-190.
9. Deng, Z., Y. Li, H. Liu, S. Xiao and L. Li *et al*., 2019. The role of sirtuin 1 and its activator, resveratrol in osteoarthritis. Biosci. Rep., Vol. 39. 10.1042/BSR20190189.
10. Zhou, M., D. Wang and J. Tang, 2021. Identification of the resveratrol potential targets in the treatment of osteoarthritis. Evidence-Based Complementary Altern. Med., Vol. 2021. 10.1155/2021/9911286.
11. Huo, Y., D. Yang, K. Lai, J. Tu, Y. Zhu, W. Ding and S. Yang, 2022. Antioxidant effects of resveratrol in intervertebral disk. J. Invest. Surg., 35: 1135-1144.
12. Yang, Y., W. Weng, J. Peng, L. Hong and L. Yang *et al*., 2017. *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-κB, and up-regulating expression of microRNA-21. Gastroenterology, 152: 851-866.E24.
13. Georgiev, T. and A.K. Angelov, 2019. Modifiable risk factors in knee osteoarthritis: Treatment implications. Rheumatol. Int., 39: 1145-1157.
14. Knapik, J.J., R. Pope, R. Orr and B. Schram, 2018. Osteoarthritis: Pathophysiology, prevalence, risk factors, and exercise for reducing pain and disability. J. Spec. Oper. Med., 18: 94-102.
15. Ao, Y., W. Tang, H. Tan, J. Li, F. Wang and L. Yang, 2022. Hydrogel composed of type II collagen, chondroitin sulfate and hyaluronic acid for cartilage tissue engineering. Bio-Med. Mater. Eng., 33: 515-523.
16. Lu, J., Z. Miao, Y. Jiang, W. Xia and X. Wang *et al*., 2023. Chrysophanol prevents IL-1β-Induced inflammation and ECM degradation in osteoarthritis via the Sirt6/NF-κB and Nrf2/NF-κB axis. Biochem. Pharmacol., Vol. 208. 10.1016/j.bcp.2022.115402.
17. Galiniak, S., D. Aebisher and D. Bartusik-Aebisher, 2019. Health benefits of resveratrol administration. Acta Biochim. Pol., 66: 13-21.
18. Yi, H., W. Zhang, Z.M. Cui, S.Y. Cui, J.B. Fan, X.H. Zhu and W. Liu, 2020. Resveratrol alleviates the interleukin-1β-induced chondrocytes injury through the NF-κB signaling pathway. J. Orthop. Surg. Res., Vol. 15. 10.1186/s13018-020-01944-8.
19. Xue, J.F., Z.M. Shi, J. Zou and X.L. Li, 2017. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. Biomed. Pharmacother., 89: 1252-1261.
20. Jiang, Y., G. Dong and Y. Song, 2018. Nucleus pulposus cell senescence is alleviated by resveratrol through regulating the ROS/NF-κB pathway under high-magnitude compression. Biosci. Rep., Vol. 38. 10.1042/BSR20180670.
21. Robinson, W.H., C.M. Lepus, Q. Wang, H. Raghu, R. Mao, T.M. Lindstrom and J. Sokolove, 2016. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat. Rev. Rheumatol., 12: 580-592.
22. Bao, J., Z. Chen, L. Xu, L. Wu and Y. Xiong, 2020. Rapamycin protects chondrocytes against IL-18-induced apoptosis and ameliorates rat osteoarthritis. Aging, 12: 5152-5167.
23. Woodell-May, J.E. and S.D. Sommerfeld, 2020. Role of inflammation and the immune system in the progression of osteoarthritis. J. Orthop. Res., 38: 253-257.
24. Heine, H. and A. Zamyatina, 2023. Therapeutic targeting of TLR4 for inflammation, infection, and cancer: A perspective for disaccharide lipid a mimetics. Pharmaceuticals, Vol. 16. 10.3390/ph16010023.
25. Karimy, J.K., B.C. Reeves and K.T. Kahle, 2020. Targeting TLR4-dependent inflammation in post-hemorrhagic brain injury. Expert Opin. Ther. Targets, 24: 525-533.
26. Liu, L., H. Gu, H. Liu, Y. Jiao and K. Li *et al*., 2014. Protective effect of resveratrol against IL-1β-induced inflammatory response on human osteoarthritic chondrocytes partly via the TLR4/MyD88/NF-κB signaling pathway: An "*in vitro* study". Int. J. Mol. Sci., 15: 6925-6940.
27. Abekura, F., J. Park, H. Lim, H.D. Kim, H. Choi, M.J. Lee and C.H. Kim, 2022. *Mycobacterium tuberculosis* glycolipoprotein LprG inhibits inflammation through NF-κB signaling of ERK1/2 and JNK in LPS-induced murine macrophage cells. J. Cell. Biochem., 123: 772-781.

28. Zhang, T., H. Ouyang, X. Mei, B. Lu and Z. Yu *et al*., 2019. Erianin alleviates diabetic retinopathy by reducing retinal inflammation initiated by microglial cells *via* inhibiting hyperglycemia-mediated ERK1/2-NF-κB signaling pathway. FASEB J., 33: 11776-11790.
29. Wang, L. and C. He, 2022. Nrf2-mediated anti-inflammatory polarization of macrophages as therapeutic targets for osteoarthritis. Front. Immunol., Vol. 13. 10.3389/fimmu.2022.967193.
30. Frostegård, J., 2023. Antibodies against phosphorylcholine-implications for chronic inflammatory diseases. Metabolites, Vol. 13. 10.3390/metabo13060720.
31. Zhang, J., Y. Rong, C. Luo and W. Cui, 2020. Bone marrow mesenchymal stem cell-derived exosomes prevent osteoarthritis by regulating synovial macrophage polarization. Aging, 12: 25138-25152.
32. Wang, D.D., C.Y. He, Y.J. Wu, L. Xu and C. Shi *et al*., 2022. AMPK/SIRT1 deficiency drives adjuvant-induced arthritis in rats by promoting glycolysis-mediated monocytes inflammatory polarization. J. Inflammation Res., 15: 4663-4675.
33. Knights, A.J., S.J. Redding and T. Maerz, 2023. Inflammation in osteoarthritis: The latest progress and ongoing challenges. Curr. Opin. Rheumatol., 35: 128-134.