Original article

Red jasmine rice extract suppresses genes associated with cartilage degradation in IL-1β-stimulated chondrosarcoma (SW1353) via blocking NF-κB pathway

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Objective This study aimed to evaluate the chondroprotective potential of red jasmine rice crude extract (RRE) against an expression of genes associated with cartilage degradation stimulated by IL-1β in a human chondrosarcoma cell line, SW1353, culture model.

Methods SW1353 cells were pre-incubated with 1 ng/mL of recombinant human interleukin-1β (IL-1β) followed by the addition of RRE in a range of concentrations from of 0 to 100 μg/mL. After 24 hours of incubation, cells were harvested and expression of genes associated with cartilage degradation was measured using real-time RT-PCR. The culture media were evaluated for MMP-13 levels using an ELISA kit. Upregulation of the signaling pathway which promotes the inflammatory cascade, nuclear factor kappa B (NF-κB), was determined by Western blot analysis.

Results Cytokine IL-1β significantly upregulated the expression of the proinflammatory mediators IL-1β, IL-6, TNF-α, and COX-2 as well as enzymes involved in osteoarthritis such as MMP-13, ADAMTS-4, and ZIP-8. The results showed that RRE at a concentration of 100 μg/mL significantly suppressed IL-1β-induced expression of these genes. Moreover, the effect of IL-1β on NF-κB signaling pathway activation was suppressed by RRE via decreased phosphorylated form of IKKα/β, IκBα, and NF-κB p65.

Conclusion RRE has chondroprotective potential through reduction of the gene upregulating effect of IL-1β which involve cartilage degradation via suppression of the NF-κB signaling pathway. Development of RRE has for alternative use potential in treating degenerative joint diseases. **Chiang Mai Medical Journal 2018;57(2):71-82.**

Keywords: red jasmine rice crude extract, cartilage degradation, chondroprotection

Introduction

Osteoarthritis (OA) is the most common joint disorder associated with cartilage destruction. It has a multi-factor etiology including genetics, gender, trauma, ageing and overweight (1). The major characteristic of osteoarthritis is articular cartilage breakdown. Pathogenesis of the disease is mainly caused by excessive production of proinflammatory cytokines, leading to an extensive destruction of extracellular matrix (ECM) biomolecules of the tissue (2).

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Proinflammatory mediators such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2) have been reported to be involved in the pathogenesis of OA (3). IL-1β has been reported to be one of the key activators of the disease (4). This cytokine downregulates the expression of ECM synthesis such as collagen type II and aggrecan in parallel with upregulatation of the expression of catabolic enzymes such as matrix metalloproteinase-13 (MMP-13) and aggrecanase-1 (ADAMTS-4) (5). These events lead to progressive loss of cartilage matrix biomolecules. It has been reported that the signal transduction of this cytokine is also associated with the activation of several pathways, especially nuclear factor κB (NF-κB) signaling (4).

MMP-13 is one of the key enzymes responsible for cartilage degradation in osteoarthritis (6). This enzyme belongs to the matrix metalloproteinases (MMPs) family which are Zn-dependent endopeptidases (7). Zinc transporter protein-8 (ZIP-8) mediates Zn^{2+} influx into chondrocytes, leading to the upregulation of matrix-degrading enzymes expression including MMP-3, MMP-9, MMP-12, and MMP-13 (8).

Inhibition of the arthritic mechanisms associated with inflammation and cartilage degradation are the main target of osteoarthritis therapy. Treatments of osteoarthritis are classified as non-pharmacological, pharmacological, complementary and alternative medicine, and surgical (9). Nonsteroidal anti-inflammatory drugs (NSAIDs), which are commonly used for OA treatment, cause dangerous side effects including gastrointestinal bleeding, renal dysfunction, and blood pressure elevation (10). Alternative treatments for OA therapy have become increasingly favored in recent years. This increase in the use of alternative treatments has led to an rise in the number of scientific reports on natural products and herbal plants which exert anti-arthritic properties. For example, it has recently been reported that total flavonoids from *Juniperus sabina* L. extracts inhibit arthritis (11) and taraxasterol from *Taraxacum mongolicum* Hand.-Mazz. and *Taraxacum sinicum* Kitag. has been found to contain anti-inflammation and anti-arthritis (12).

There have been reported that the bioactivities of pigmented rice extracts offer many health benefits. Red and black rice contain a source of antioxidants in functional foods. Several studies have stated that red rice has a higher antioxidant capacity than white rice extract (13,14). Antioxidant compounds in red jasmine rice have been found to include phenolic compounds, flavonoids, vitamin E derivatives, γ-oryzanol, and proanthocyanidin (15). It has been reported that proanthocyanidin from grape seed extract exhibits anti-inflammatory (16) and anti-arthritic properties (17). Red jasmine rice (*Oryza sativa* L.) has been found to contain many active compounds, in particular, proanthocyanidin. Crude extract of red jasmine rice (RRE) has been shown to have antioxidant and anti-inflammatory properties (18). To investigate further, we used a SW1353 cell culture model to determine whether RRE exhibits anti-arthritic activities against IL-1β-induced expression of genes associated with osteoarthritis.

Methods

Reagents

Cell culture reagents were purchased from Gibco BRL, Life Technologies (Grand Island, NY, USA). Recombinant human interleukin-1β (IL-1β) was purchased from ProSpec Protein Specialist (Ness-Ziona, Israel). The ELISA kits (MMP-13) were purchased from Elabscience Biotechnology Co., Ltd (Wuhan, China). The illustra RNAspin Mini Kit was purchased from GE Healthcare (Buckinghamshire, UK). BAY 11-7082 (BAY) was purchased from Calbiochem (Merck Millipore, Darmstadt, Germany). DMSO and ethanol were purchased from Sigma-Aldrich (Darmstadt, Germany).

Plant material

The crude ethanolic extract of red jasmine rice (*Oryza sativa* L.) was a gift from Prof.Dr. Pornngarm Dejkriengkraikul, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand. It was prepared as described in previous publications (18,19). The stock solution of crude ethanolic extract (RRE) was prepared in 0.01% DMSO and diluted to final concentrations ranging from 25 to 100 μg/mL in the culture media.

Cell line and culture conditions

The human chondrosarcoma cell line, SW1353 (HTB-94, ATCC), was authenticated on May 2015, by DiagCor Bioscience Incorporation Limited (Kowloon Bay, Hong Kong). SW1353 was maintained in serum-free Dulbecco's modified Eagle's media (DMEM) with 200 U/mL penicillin and 200 µg/mL streptomycin plus 10% FBS at 37°C in a 5% CO_2 atmosphere. SW1353 was cultured at a density of 1×10^5 cells per well at 37°C and 5% CO_2 for 2 days prior to inducing the stress condition by recombinant human IL-1β 1 ng/mL for 2 hours followed by co-incubation with various concentrations of RRE. BAY 11-7082 at a concentration of 2 µM (NF-κB inhibitor) was used as positive control. After 24 hours of incubation, the cells were harvested. The culture media were collected and stored at -20°C for further analytical assay.

Cell viability assay

SW1353 was seeded in a 96-well plate (2×10^4 cells/well) and incubated overnight. Cells were treated with various concentrations of red jasmine rice extract. DMSO was used as the control. After 24 hours, the culture media were discarded and replaced with new culture media composed of 5 mg/mL MTT (3,[4,4-dimethy thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) dye (Sigma-Aldrich, St. Louis, MO, USA). After 4 hours, all the media was discarded, then 0.2 mL of dimethyl sulfoxide (DMSO) per well was added to solubilize the formazan crystals. Absorbance was measured at 540 nm using a microplate reader (2). The cell viability percentage was calculated as follows:

$$\text{Cell viability percentage} = \frac{\text{Absorbance at 540 nm of sample}}{\text{Absorbance at 540 nm of control}} \times 100$$

Gene expression analysis

The mRNA expression of MMP-13, ZIP-8, ADAMTS-4, IL-1β, IL-6, TNF-α, ICE (interleukin-1 converting enzyme), and COX-2 were determined by real time quantitative reverse transcription-polymerase chain reaction (real time RT-PCR) as previously described (20). The total RNA from the harvested cells was extracted using an illustra RNAspin Mini Kit. The evaluation of RNA quality and integrity was conducted as previously described (21). The RNA was converted to cDNA using a BIOLINE SensiFAST™ cDNA synthesis kit, (London, United Kingdom) following the mixer and reaction protocol as previously described (22). The cDNA samples were analyzed by real-time PCR using specific primers and a SensiFAST™ SYBR® No-ROX kit (Bioline, Luckenwalde, Germany) and were processed with the Chromo4™ Four-Color Real-Time detector (Bio-Rad, Hercules, CA, USA). The mixer for the real-time PCR reaction solution consisted of a SensiFAST™ SYBR® No-ROX kit 10 µL, Nuclease-free water 3 µL, cDNA 5 µL, and primer 2 µL. The real-time PCR condition consisted of polymerase activation at 95°C for 2 min, denaturation at 95°C for 5 sec, and annealing/extension at 60°C for 30 sec. The sequences of these primers was received from the primer design of GenBank by blasting GenBank accession numbers NM_002427.3 (MMP-13), NM_022154.5 (ZIP-8), NM_005099.4 (ADAMTS-4), NM_000576.2 (IL-1β), NM_000600.4 (IL-6), NM_000594.3 (TNF-α), NM_001257118.2 (ICE), and NM_000963.3 (COX-2). Gene expression analysis was conducted using real time RT-PCR. Relative gene expression was normalized by GAPDH gene and calculated using the $2^{-\Delta\Delta CT}$ method (23).

ELISA assay for the MMP-13 enzyme

The levels of matrix metalloproteinase-13 (MMP-13) in the supernatant of the culture media were determined using a commercial sandwich-based MMP-13 ELISA test kit as following the manufacturer's instructions (Elabscience Biotechnology).

Western blot analysis

SW1353 was pretreated with various concentrations of either RRE or NF-κB inhibitor (BAY) 20 µM. BAY 11-7082 (NF-κB inhibitor) was used as positive control. After 2 hours of incubation, IL-1β at a concentration of 2 ng/mL was added. Thirty minutes later, the cell lysates of SW1353 were collected by cell scraper and put into radioimmunoprecipitation assay (RIPA) buffer. Protein samples were subjected to 10% sodium dodecyl sulfate (SDS)-PAGE under a reducing condition. After electrophoresis, the separated proteins were transferred to a nitrocellulose membrane (GE Healthcare Life Sciences, Chalfont, UK). The membrane was blocked with 5% skim milk in tris-buffered saline (TBS) containing 0.1% (v/v) Tween® for 1 hour. The membrane was probed with the primary antibodies such as phospho-specific IKKα/β, IκBα, or NF-κB p65 for the NF-κB pathway (Cell Signaling Technology, MA, USA) at 4°C overnight. The membrane was washed and a secondary antibody conjugated with horseradish peroxidase (HRP) was added and detected using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare). The intensity level of each band was analyzed using Image J software (1.50i, freeware).

Statistical analysis

All of the experiments were performed in triplicate. Quantifications are reported as mean±SD of the three independent experiments. Statistically significance was calcluated using one-way analysis of variance

(ANOVA) followed by Post Hoc multiple comparison. P-values less than 0.05 were considered statistically significant.

Results

Effect of red jasmine rice extract on viability of SW1353 culture

The cytotoxic effect of RRE on the human chondrosarcoma cell line (SW1353) was evaluated by MTT assay. As shown in Figure 1, various concentrations of up to 125 µg/mL of crude extract had no effect on SW1353 cell viability. Non-toxic concentrations of RRE, i.e., concentrations between 25 and 100 µg/mL, were used throughout the study.

Effect of red jasmine rice extract on suppression of IL-1β-induced an expression of cartilage degrading factors

The chondroprotective potential of red jasmine rice crude extract (RRE) against IL-1β-induced expression of enzymes and cytokines which are associated with osteoarthritis was investigated in SW1353 culture model. It was found that IL-1β highly upregulated the mRNA expression of key cartilage degradation enzymes such as MMP-13 (Figure 2A) and AD-AMTS-4 (Figure 3B). The zinc transporter protein, ZIP-8 (Figure 3A), was also activated by IL-1β. The crude extract at a concentration of 100 µg/mL clearly suppressed MMP-13, AD-AMTS-4, and ZIP-8 mRNA expression (Figures 2 and 3). Figure 2B illustrates that MMP-13 protein levels increased in the culture media of the IL-1β-treated group. These were significantly reduced when the culture media were co-treated with RRE at a concentration of 100 µg/mL. To confirm the regulatory effects of NF-κB signaling pathways in IL-1β-induced cartilage degradation enzyme expression, we examined the effect of BAY, an NF-κB inhibitor, on the expression of those genes. As shown in Figures 2 and 3, treatment of the cells with BAY reduced the expression of cartilage degradation enzymes.

Effect of red jasmine rice extract on reduction of IL-1β-induced an expression of proinflammatory mediators

In order to investigate the effect of RRE on IL-1β-induced proinflammatory cytokines which is involved in cartilage degradation in SW1353 cells, the mRNA levels of IL-1β, IL-6, TNF-α, and ICE were determined. As shown in Figures 4 and 5, IL1-β-induced mRNA expression of IL-1β, ICE (IL-1β converting enzyme), IL-6, TNF-α, and COX-2 in SW1353. Moreover, the effect of IL-1β on the expression of these genes was suppressed by BAY to a compatible degree with groups co-treated with RRE at a concentration of 100 µg/mL.

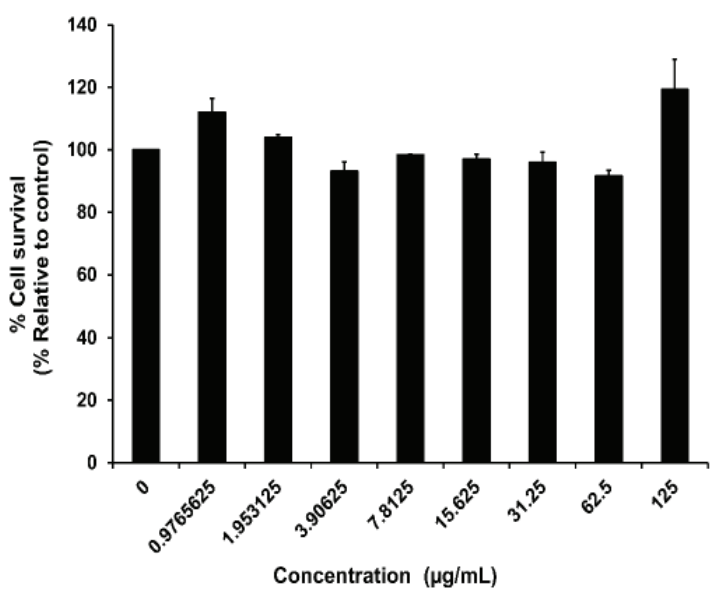


Figure 1. Effect of red jasmine rice crude extract (RRE) on cell viability in the human chondrosarcoma cell line (SW1353). Cells were seeded into 96-well plates and treated with RRE (0–125 µg/mL) for 24 hours. Cell viability was determined by MTT assay. The percentage of cell viability was related to control (0 µg/mL). Data are expressed as mean±SD from three independent experiments.

Effect of red jasmine rice extract on suppression of IL-1β-induced phosphorylation of the NF-κB signaling pathway

The mechanism underlying protective the potential of the crude extract was further investigated via phosphorylation of IKKα/β, IκBα, and NF-κB p65 of the NF-κB signaling pathway using the Western blotting technique (Figure 6). It was found that RRE clearly suppressed this pathway by reducing the IL-1β-activated phosphorylation of IKKα/β, IκBα, and NF-κB p65 (Figures 6A and 6B).

Discussion

Osteoarthritis (OA) is one of the joint degenerative disorders associated with cartilage destruction (4). Proinflammatory cytokines, especially IL-1β, are the critical mediators in the pathogenesis of osteoarthritis. This cytokine is the key proinflammatory cytokine involved in the pathophysiology of OA. It stimulates cartilage degradation by upregulating the expression of catabolic enzymes such as MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and ADAMTS-4 (24,25). IL-1β also stimulates the

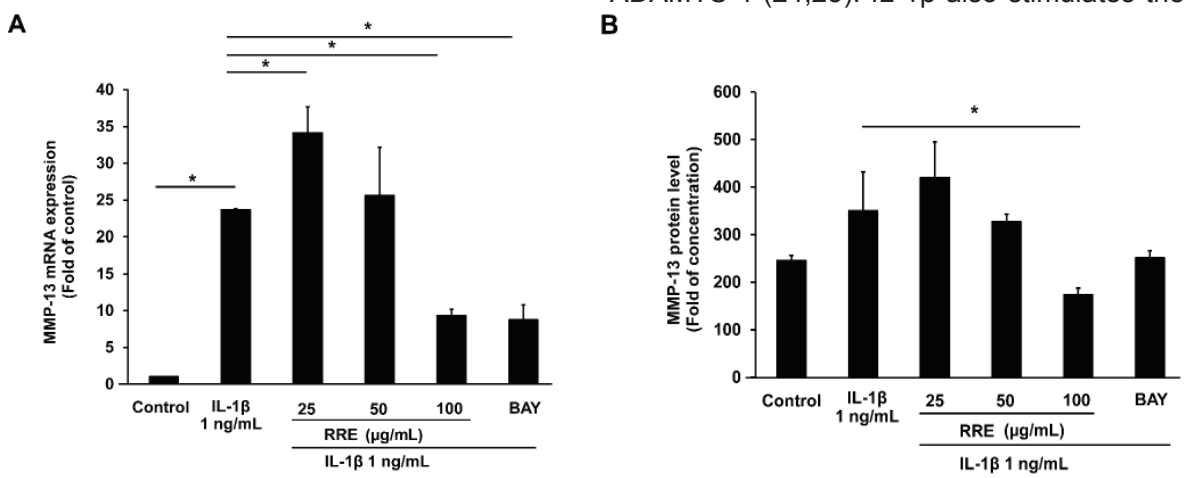


Figure 2. Effect of red jasmine rice crude extract (RRE) on MMP-13 mRNA expression (A) and MMP-13 protein levels (B). SW1353 was pretreated with 1 ng/mL IL-1β for 2 hours followed by the addition of various concentrations of the RRE (25, 50, and 100 μg/mL). After 24 hours of incubation, cells were harvested for mRNA expression analysis by real-time RT-PCR. MMP-13 protein levels were determined using an ELISA kit. Data are expressed as mean ± SD of three independent experiments. BAY (2 μM) performed as NF-κB inhibitor (positive control). Note: * = p <0.05

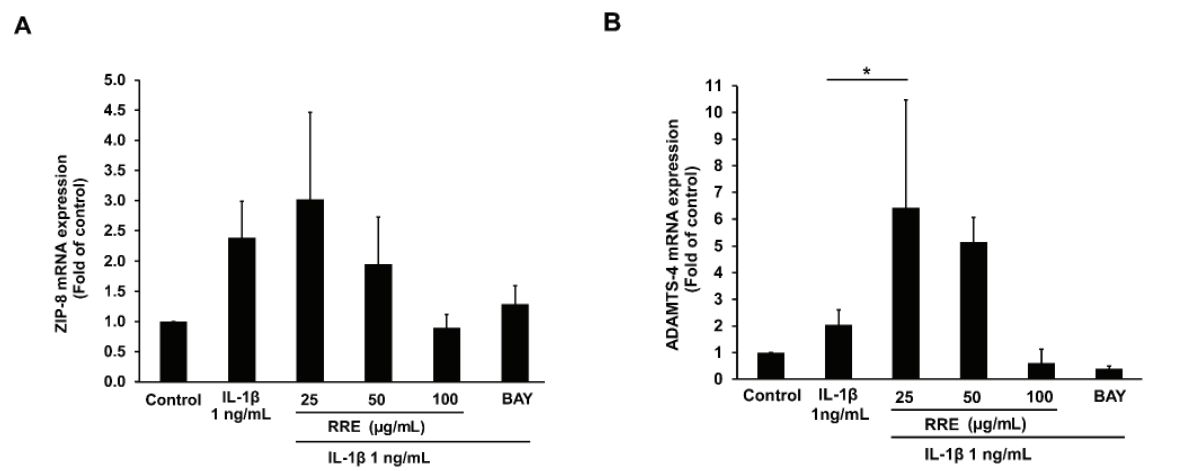


Figure 3. Effect of red jasmine rice crude extract (RRE) on mRNA expressions of ZIP-8 (A) and ADAMTS-4 (B). SW1353 was pretreated with 1 ng/mL IL-1β for 2 hours followed by the addition of various concentrations of the RRE (25, 50, and 100 μg/mL). After 24 hours of incubation, cells were harvested for mRNA expression analysis by real-time RT-PCR. Data are expressed as mean±SD of the three independent experiments. BAY (2 μM) performed as NF-κB inhibitor (positive control). Note: * = p <0.05

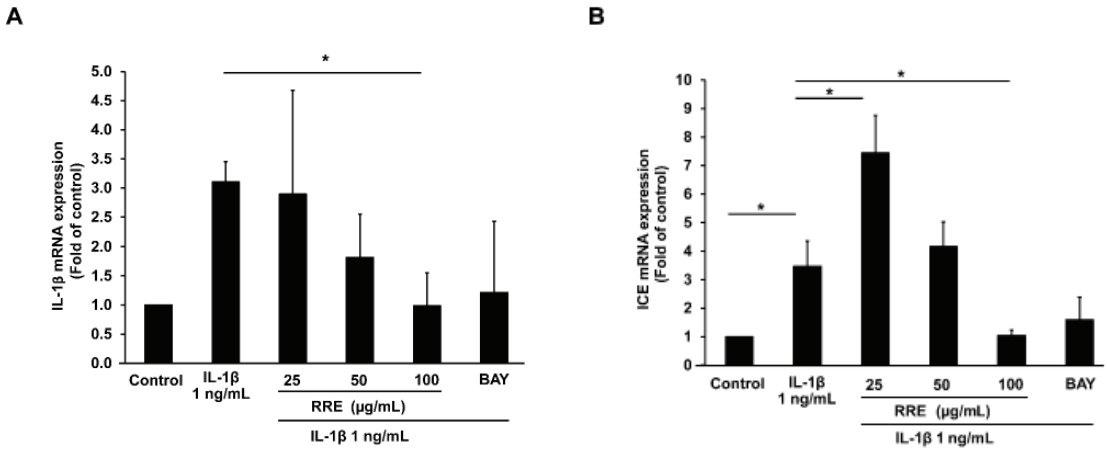


Figure 4. Effect of red jasmine rice crude extract (RRE) on mRNA expression of IL-1β (A) and interleukin-1 converting enzyme (ICE) (B). SW1353 was pretreated with 1 ng/mL IL-1β for 2 hours followed by the addition of various concentrations of RRE (25, 50, and 100 μg/mL). After 24 hours of incubation, cells were harvested for mRNA expression analysis by real-time RT-PCR. Data are expressed as mean±SD of the three independent experiments. BAY (2 μM) performed as NF-κB inhibitor (positive control). Note: * = p <0.05

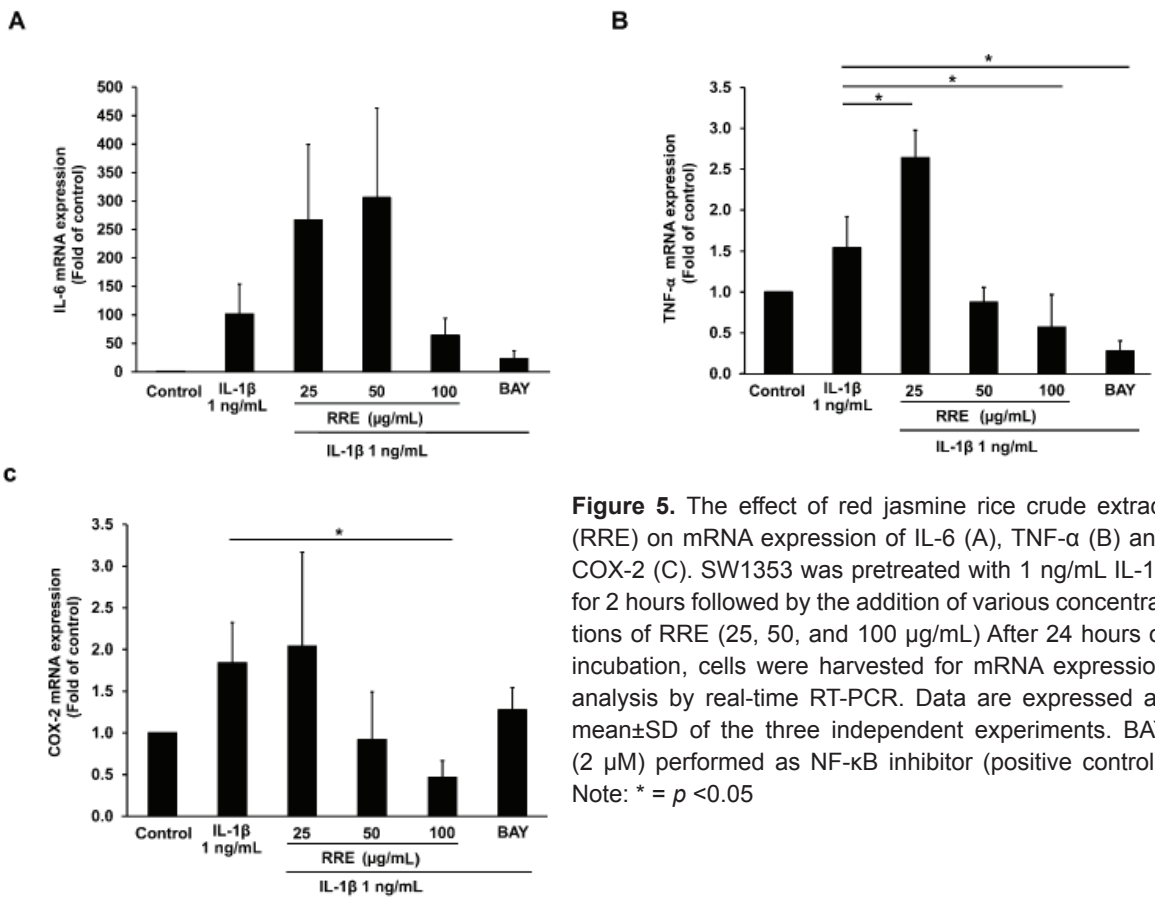


Figure 5. The effect of red jasmine rice crude extract (RRE) on mRNA expression of IL-6 (A), TNF-α (B) and COX-2 (C). SW1353 was pretreated with 1 ng/mL IL-1β for 2 hours followed by the addition of various concentrations of RRE (25, 50, and 100 μg/mL) After 24 hours of incubation, cells were harvested for mRNA expression analysis by real-time RT-PCR. Data are expressed as mean±SD of the three independent experiments. BAY (2 μM) performed as NF-κB inhibitor (positive control). Note: * = p <0.05

production of IL-6, TNF-α, and IL-8 in chondrocytes which amplify the inflammatory processes (26). A recent study demonstrated that the elevation of endogenous expression of IL-1β causes induction of COX-2 expression in the human OA joint (27). Oxidative stress and inflammation have been found to be associated with OA and to lead to activation of the

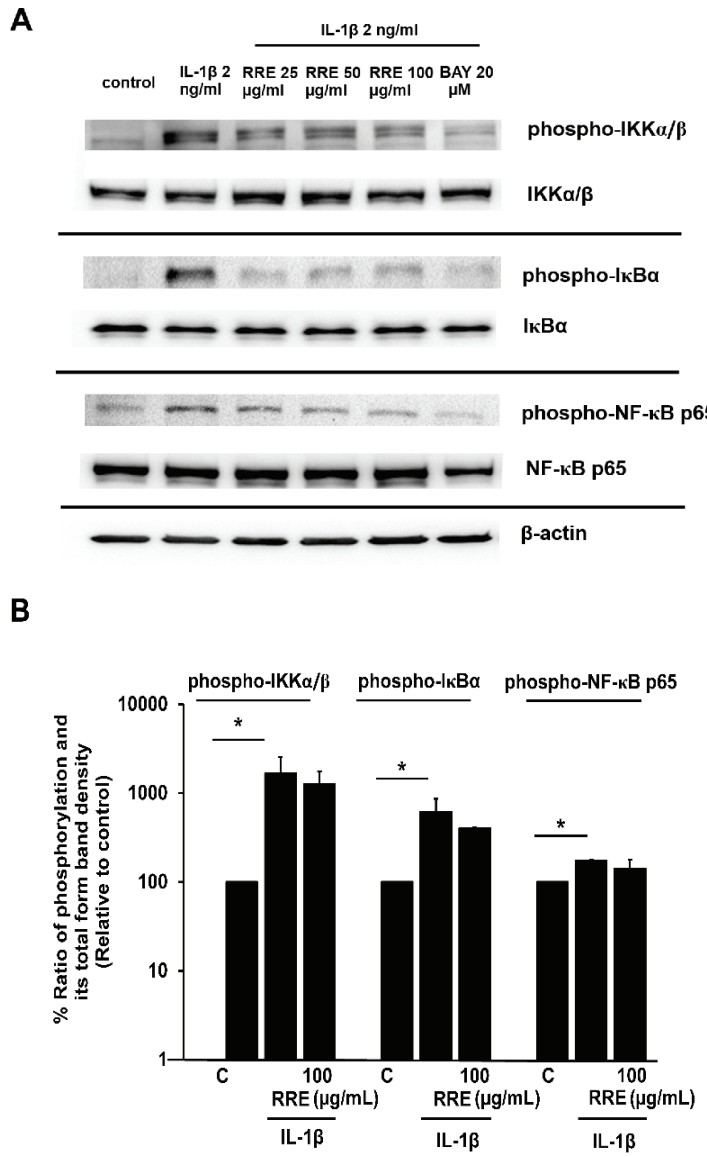


Figure 6. The effect of red jasmine rice crude extract (RRE) on IL-1β-induced phosphorylation of NF-κB protein families in SW1353. Representative immunoblot effect on NF-κB signaling molecules is shown in A. The data from the triplicate experiments are expressed as mean±SD of proportion between the band density of phosphorylated- and total-forms of IKKα/β, IκBα, and NF-κB p65 in each conditions are represented by bar graph (B). BAY (20 µM) performed as NF-κB inhibitor (positive control). Note: * = p <0.05

catabolic cascades, resulting in degradation of cartilage tissue. Several anti-osteoarthritic drugs have been reported to counteract these processes (1,28).

Regarding the *in vitro* model of osteoarthritis using primary chondrocyte culture, there are several limitations including the proliferative rate and chondrogenic properties (29). The present study adopted a culture model using SW1353, a human chondrosarcoma cell line, for investigation of the anti-arthritic potential of red jasmine rice crude extract. This cell line has been used as an *in vitro* model to study the effect of cytokine on induction of the expression of cartilage-degrading enzymes (30). It has been shown to contribute to catabolic factors of cartilage degradation when activated by IL-1β, similar to the primary chondrocytes

(31). It has been reported that the expression of 312 genes of primary human chondrocytes were similar to those in SW1353 (29). The present study demonstrated the strong induction effects of IL-1β on expression of genes which are associated with cartilage degeneration such as IL-1β, IL-6, TNF-α, MMP-13, and COX-2 when compared to the untreated control. The expression of these genes was suppressed by BAY11-7082, a NF-κB inhibitor. These results were confirmed by the Western blot assays which clearly showed that BAY successfully suppressed IL-1β-induced phosphorylation of IKK, IκBα, and NF-kB p65, finding similar to previous reports (32). This agent is an IKK inhibitor which has pharmacological properties such as inhibition of inflammatory cytokines including IL-1β (33,34). These properties suggest that SW1353 culture model is appropriate as an *in vitro* model of OA.

Red jasmine rice extract has been reported to contain bioactive constituents such as γ-tocotrienol, γ-oryzanol, α-tocotrienol, catechin, and proanthocyanidin. One of those, the crude ethanolic extract of red jasmine rice, contains predominantly proanthocyanidin and γ-oryzanol (18). The γ-oryzanol has been shown to provide anti-oxidation and anti-inflammation effects of in cell culture models (35,36). Several studies of proanthocyanidin demonstrated its potent anti-oxidation, anti-inflammation and anti-arthritis effects (16,17). In the present study, ethanolic extract of red jasmine rice, which contains proanthocyanidin as a major constituent, clearly demonstrated protective activity against IL-1β-induced upregulation of genes associated with OA pathogenesis. This seems to suggest that proanthocyanidin in crude extract of red jasmine rice plays as anti-arthritis role. The specific active components of red jasmine rice which act in response to stimulate the protective activities should be identified.

In OA pathogenesis, the breakdown of cartilage tissue is mainly mediated by the MMPs and the ADAMTS families which are activated by proinflammatory cytokines (37). MMP-13 is one of the key associated enzyme which is activated by proinflammatory cytokines, especially IL-1β, causing the loss of biomolecules from the cartilage tissue (24). In the present study, red jasmine rice crude extract demonstrated the ability to suppress MMP-13 mRNA expression consistently through reduction of MMP-13 protein levels in the SW1353 culture model. These results are similar to a previous report that demonstrated the inhibitory effect of red jasmine rice extract on MMPs activity in cancer cell lines (18).

MMPs are a large family of proteinases which are highly conserved Zn-dependent endopeptidases that use Zn ion as cofactor (7). ZIP-8 mediates Zn^{2+} influx to upregulate the expression of matrix-degrading enzymes such as MMP-3, MMP-9, MMP-12, and MMP-13 in chondrocytes (8). The previous report has been showed that IL-1β induces the expression of ADAMTS-4, MMP-1, MMP-3, MMP-9, and MMP-13, leading to acceleration of aggrecan and collagen in cartilage tissue (38). The present study demonstrated that red jasmine rice crude extract effectively suppressed IL-1β-induced ZIP-8 and ADAMTS-4 mRNA expression. That implies that the crude extract may provide chondroprotective activities by decreasing synthesis of several enzymes associated with cartilage degradation including the MMPs and ADAMTS families.

IL-1β is the main proinflammatory cytokine associated with osteoarthritis. After translation, the pro-form of IL-1β is inactive. It can be cleaved to generate the active form by ICE (39). The results of this study showed that external IL-1β was able to upregulate mRNA expression of IL-1β in parallel with ICE but that upregulation was reduced by red jasmine rice crude extract. In addition, the influence of IL-1β-induced expression of several proinflammatory mediators involved in OA pathogenesis, such as COX-2, TNF-α, and IL-6, were downregulated by red jasmine rice crude extract. These results agree with a previous report on proanthocyanidin from grape seed extract which was found to exert anti-inflammatory properties by downregulating chemokines and cytokines such as IL-1β, IL-6, and TNF-α (40). It may be postulated that red jasmine rice crude extract containing proanthocyanidin

may effectively diminish the inflammatory cascades leading to downregulation of cartilage-degrading enzymes to deceleration of the progression of cartilage loss in OA. However, the present study was conducted in a model of cell line monolayer culture which included some factors which limit the extent of interpretations and conclusions. Further studies should be conducted using a 3D culture model and an in vivo experimental design.

The cellular mechanisms of the crude extract against IL-1β-induced inflammation via activation of the NF-κB signaling pathway were further investigated in the present study (41). The activation of this signaling pathway strongly induces MMPs and ADAMTS-4 expression in various cells including human chondrocytes (42,43). The proinflammatory cytokines, especially IL-1β, activate the NF-κB dimers by triggering the NF-κB signaling pathway that leads to the phosphorylation of the IκB kinase (IKK) complex. The IKK complex consists of IKKα and IKKβ which activate the IκBα protein by phosphorylation. Next, the NF-κB dimers, p65/RelA proteins, translocate from the cytoplasm to the nucleus in order to the promoter regions of target genes (42). The present study demonstrated that the crude extract inhibited IL-1β-induced NF-κB activation by diminishing of phosphorylation of IKKα/β, IκBα, and NF-κB p65, leading to the report finding that proanthocyanidin-rich red rice extract suppresses the NF-κB signaling pathways in RAW 264.7 cell culture which downregulate the DNA binding activity of NF-κB (44).

Taken together, the results of the present study suggest that red jasmine rice crude extract may exert chondroprotective potential by suppressing the activation of the NF-κB signaling pathway, resulting in reduction of proinflammatory cascades and thus the production of cartilage-degrading enzymes which are involved in the pathogenesis of degenerative joint diseases. These results firstly contribute to the scientific evidence of the antiarthritic properties of red jasmine rice extract which may lead to development of the alternative treatments of arthritis. Nevertheless, the safety and efficacy of the extract should be further investigated in animal models prior to application for treatment arthritis in humans.

Acknowledgements

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Conflicts of Interest

We have no conflicts of interest.

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สารสกัดข้าวหอมมะลิแดงยับยั้งยีนซึ่งเกี่ยวข้องกับการสลายกระดูกอ่อนที่กระตุ้นโดยอินเตอร์ลิวคิน-1บีตา ผ่านการยับยั้งวิถี NF-κB ในเซลล์ SW1353 เพาะเลี้ยง

สุพิชชา ธงห้อย, รุ่งนรี ใจธรรม, ปฏิวัติ ก๋องแดง, ศุภชัย ยอดคีรี, ศิริวรรณ องค์ไชย และ พรงาม ลิ้มตระกูล
ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

วัตถุประสงค์ เพื่อประเมินศักยภาพการปกป้องกระดูกอ่อนของสารสกัดข้าวหอมมะลิแดง ในโมเดลเซลล์มะเร็งกระดูกอ่อนมนุษย์เพาะเลี้ยง SW1353 ที่ถูกกระตุ้นให้อยู่ในภาวะเพิ่มการแสดงออกของยีนที่เกี่ยวข้องกับการสลายกระดูกอ่อนด้วยสารไซโตไคน์ก่อการอักเสบ IL-1β

วิธีการศึกษา เซลล์ SW1353 ถูกกระตุ้นด้วย IL-1β ที่ความเข้มข้น 1 นก./มล. จากนั้นจึงเติมสารสกัดข้าวหอมมะลิแดง (crude extract) ที่ความเข้มข้น 0-100 มคก./มล. และบ่มไว้จนครบเวลา 24 ชั่วโมง จากนั้นจึงเก็บเซลล์เพื่อสกัด RNA และวิเคราะห์การแสดงออกของยีนด้วยวิธี real-time RT-PCR ทำการตรวจวัดระดับ MMP-13 ในน้ำเลี้ยงเซลล์ด้วย ELISA kit ส่วนวิถีการส่งสัญญาณภายในเซลล์ที่ส่งเสริมกระบวนการอักเสบ (NF-κB) ถูกวิเคราะห์ด้วยวิธี Western blot analysis

ผลการศึกษา พบว่า IL-1β ซึ่งเป็นสารไซโตไคน์ก่อการอักเสบเกี่ยวข้องกับภาวะสลายกระดูกอ่อนสามารถกระตุ้นการแสดงออกของยีนต่าง ๆ ที่เป็นสาเหตุของโรคข้อเสื่อม ได้แก่ กลุ่มสารสื่อการอักเสบเช่น IL-1β, IL-6, TNF-α รวมทั้ง COX-2 และเอ็นไซม์ที่เกี่ยวข้องกับโรคข้อเสื่อมเช่น MMP-13, ADAMTS-4 และ ZIP-8 ผลการทดลองแสดงให้เห็นว่า สารสกัดข้าวหอมมะลิแดงที่ความเข้มข้น 100 มคก./มล. สามารถกดการแสดงออกของยีนดังกล่าวที่ถูกกระตุ้นโดย IL-1β ได้อย่างมีนัยสำคัญ โดยพบว่า สารสกัดข้าวหอมมะลิแดงสามารถลดฤทธิ์ของ IL-1β ผ่านการยับยั้งการส่งสัญญาณภายในเซลล์ในวิถี NF-κB โดยลดการเติมฟอสเฟตให้กับ IKKα/β, IκBα and NF-κB p65

สรุปผลการศึกษา สารสกัดข้าวหอมมะลิแดงมีศักยภาพในการปกป้องการสลายกระดูกอ่อน โดยสามารถต้านฤทธิ์ IL-1β ที่กระตุ้นการแสดงออกของยีนซึ่งเกี่ยวข้องกับการสลายกระดูกอ่อนผ่านการยับยั้งวิถีส่งสัญญาณภายในเซลล์แบบ NF-κB ผลการทดลองครั้งนี้จึงอาจมีประโยชน์ในการพัฒนาสารสกัดข้าวหอมมะลิแดงเพื่อเป็นทางเลือกสำหรับการใช้ในโรคข้อเสื่อมต่อไป **เชียงใหม่เวชสาร 2561;57(2):71-82.**

คำสำคัญ: สารสกัดหอมมะลิข้าวแดง การเสื่อมสลายของกระดูกอ่อน การปกป้องกระดูกอ่อน