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Silkworm pupal extracts attenuate interleukin-1β-induced expression of matrix metalloproteinases and inflammatory mediators in the SW1353 human chondrosarcoma cell line

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Abstract

Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases and is more common in older and obese individuals. Silkworm male pupae exerts tonic effects by increasing testosterone secretion and the forced swimming time and muscle ratio increased in mice consuming silkworm pupae, which may be beneficial to the older population. Therefore, it will be beneficial to investigate the effects of silkworm pupal extracts (SPE) on OA. To confirm this effect, we prepared SPE in different solvents, and their ability to attenuate matrix metalloproteinases (MMPs) and inflammatory mediators (interleukin-6 [IL-6], interleukin-8 [IL-8] and tumor necrosis factor-α [TNF-α]) were evaluated in an interleukin-1β (IL-1β)-induced SW1353 human chondrosarcoma cell line. 70% ethanolic SPE outperformed the other solvents, reducing MMP-1 and MMP-3 expression by up to 53% and 13%, respectively. Further experiments were performed using 70% ethanolic SPE from three distinct pupation stages in males and females. SPE treatment alleviated MMP-1 expression (43.9–47.4%) regardless of pupation stage and sex. Among the inflammatory mediators, 70% ethanolic SPE alleviated IL-6 and TNF-α levels, and the concentrations thereof were lowest in the early-stage male SPE-treated group (43.15% and 56.74%, respectively). In conclusion, 70% ethanolic SPE may prevent IL-1β-induced osteoarthritis by inhibiting MMPs and inflammatory cytokines. Therefore, SPE is a potential therapeutic agent for the treatment of OA.

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Introduction

Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases, affecting more than 25% of the population worldwide (Zhang *et al.,* 2018), and is more common in older and obese individuals (Chien *et al.*, 2020). Generally, the fundamental feature of OA is the deterioration in structure and function of the articular cartilage (Jia *et al.*, 2020). OA is characterized by joint swelling, pain, dysfunction, and disability (Lu *et al.,* 2018).

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Fig. 1. Classification of silkworm pupae according to pupation days and sex. Silkworm pupae were divided into three groups according to the pupation duration. The early-, mid-, and late-pupal stages were collected on days 6–7, 8–10, and 11–13, respectively. The pupae were divided by sex (male and female). Females are larger than males, and wrinkles become deeper and clearer in the later stages.

Although the underlying mechanism of OA remain largely unknown, inflammatory responses are hypothesized to play a substantial role in its onset and progression (Jia *et al.,* 2020). Inflammatory cytokines–most importantly, interleukin-1β (IL-1β)–have been reported to play substantial roles in the pathogenesis of OA by elevating the release of inflammatory mediators and upregulating the expression of matrix metalloproteinases (MMPs), resulting in the disintegration of the extracellular matrix (ECM) (Park *et al.,* 2018). Currently, the most commonly recommended medications for OA include nonsteroidal anti-inflammatory drugs, analgesics, and glucocorticoids. It is important to note that neither of the aforementioned therapies can stop OA from worsening, nor can they undo the harm caused by the disease. Moreover, medical practitioners recommend surgical treatment when OA progresses and has a substantial impact on the patient's quality of life, (Rao *et al.,* 2018).

Silkworm pupae are by-products of the silk textile industry and are consumed in many Asian countries (He *et al.,* 2021). Per gram, silkworm pupae contain 450–650 mg of protein (*w/w*, dry weight) and 250–450 mg of fat (*w/w*, dry weight) and has a high proportion of essential amino acids, linolenic acid, and oleic acid. Interestingly, it was confirmed that there is a difference in nutrient composition of the silkworm pupae depending on the sex and harvest time of the silkworm pupae (Lee *et al.,* 2021a). Therefore, the expected bioactivity may differ. Male silkworm pupae have been reported to exert tonic effects by increasing testosterone secretion (Ryu *et al.,* 2002). In addition, the forced swimming time and muscle ratio increased in mice consuming silkworm pupae, which could be valuable for the elderly (Lee *et al.,* 2019).

The prevalence of OA is increasing globally, necessitating the development of innovative treatments and prevention methods. Considering the pivotal role played by IL-1β in the progression of OA, a prospective therapeutic that may inhibit IL-1β-induced inflammation may work well as a new OA treatment strategy. Numerous natural products, particularly those derived from plants, have been shown to possess these properties. This study aimed to evaluate the potential of silkworm pupal extracts (SPE) for the attenuation of the IL-1β-induced expression of MMPs and inflammatory mediators (IL-6, IL-8, and tumor necrosis factor-α [TNF-α]) in the SW1353 human chondrosarcoma cell line, given that silkworm pupae, in addition to being known for their high nutritional content, exhibit a variety of biological activities, including antiapoptotic, antimicrobial, antitumor, antidiabetic, and cardio- and hepato-protective effects (Zhou *et al.,* 2022).

Materials and Methods

Silkworm pupae and extract preparation

Pupae of the Korean Baegokjam silkworm variety obtained from the Uljin Silk Farm (Uljin, Korea) were separated according to pupation stage (early, mid, and late) and sex (female and male; Fig. 1). Pupae were stored at –80 °C overnight before being freeze-dried in a lyophilizer (IlShinBioBase, Gyeonggi-do, Korea). Thereafter, the pupae were powdered in a grinder, mixed with 20 times the volume of different extraction solvents (distilled water, 30%, 50%, 70%, and 100% ethanol, and hexane), and stirred at room temperature for 24 h. The extracts were then filtered through a Miracloth (Merck) and centrifuged at 11 200 × g for 10 min. The supernatants were freeze-dried, powdered, and stored in a refrigerator until further use (Rahul *et al.,* 2022b).

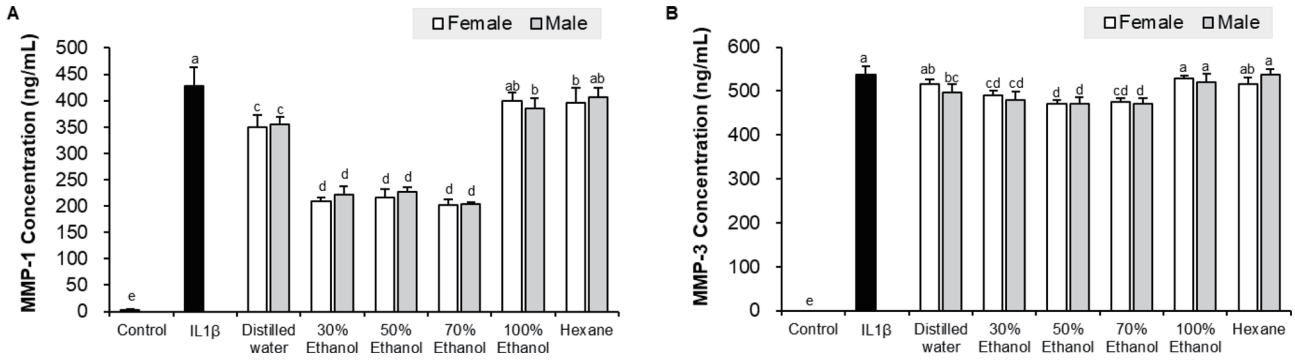

Fig. 2. Comparison of the effects of silkworm pupal extracts according to solvents on the expression of MMP-1 and MMP-3 in IL-β-induced chondrocytes. Extracts were prepared using distilled water, 30%, 50%, 70%, and 100% ethanol, and hexane. Silkworm variety: Baegokjam; Pupation time: mid-stage (8–10 days); sex: male and female. SW1353 cells were pre-treated with 100 g/mL of the extracts and stimulated with IL-1β (40 ng/mL) for 24 h. Expression levels of MMP-1 (A) and MMP-3 (B) were measured using ELISA. Error lines represent the standard deviation of the mean (n = 3). Different lowercase letters above the columns indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$).

Cell culture and SPE treatment

Human chondrosarcoma SW1353 cells (American Type Culture Collection, Virginia, United States) were cultured in Dulbecco's modified Eagle's medium (DMEM; Caisson, Smithfield, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gendepot, Barker, TX, USA) and 1% penicillin-streptomycin (Caisson). Cells were seeded at a density of 3×10^4 cells/well in a 12-well plate and incubated in a humidified incubator with 5% CO_2 at 37 °C for 24 h. The cells were treated with SPE (100 g/mL), followed by incubation with IL-1β (40 ng/mL) for 24 h. Cells treated with the medium or IL-1β alone served as controls. Supernatants from the treated as well as control groups were collected and stored at –80 °C until the following assays were performed.

Determination of MMP and pro-inflammatory cytokine expression

MMP (MMP-1 and MMP-3) and pro-inflammatory cytokine (IL-6, IL-8, and TNF-α) expression levels in the supernatants were determined using standard enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK).

Statistical analysis

The above mentioned assays were performed in triplicate, and the results are represented as the mean ± standard deviation. Data were analyzed using a one-way analysis of variance (ANOVA). Significant differences among mean values were determined by Duncan's multiple range test using the SAS 7.1 software package (SAS Institute Inc., Cary, NC, USA). P-values < 0.05 were considered statistically significant.

Results and Discussion

Effect of SPE with different extraction solvents on IL-1β-induced MMP-1 and MMP-3 expression

The effects of various solvent extracts (distilled water, 30%, 50%, 70%, and 100% ethanol, and hexane) of silkworm pupae on MMP-1 and MMP-3 expression levels in IL-1β-induced SW1353 cells were determined by ELISA. Significant increases in MMP-1 and MMP-3 expression were observed in IL-1β-induced SW1353 cells compared to untreated cells. Pretreatment with distilled water and SPE from both male and female pupae with 30, 50, and 70% ethanol significantly reduced the expression of MMP-1 and MMP-3 ($p < 0.05$ vs. IL-1β). Variations in the reduction of MMP-1 expression by distilled water (17.1–18.4%), 30% ethanol (48.3–51.1%), 50% ethanol (46.8–49.3%), 70% ethanol (52.4–53%), 100% ethanol (6.4–9.7%) and hexane (4.7–7.4%) extracts were observed (Fig. 2A), with 70% ethanolic extracts faring best. MMP-3 expression was attenuated by the 50% (12.7%) and 70% ethanol extracts (12.4%; Fig. 2B). Further experiments were performed using 70% ethanolic SPE to delineate the different silkworm extract varieties, pupation times, and sex-induced variations in the reduction of MMPs and cytokine expression in IL-1β-stimulated SW1353 cells.

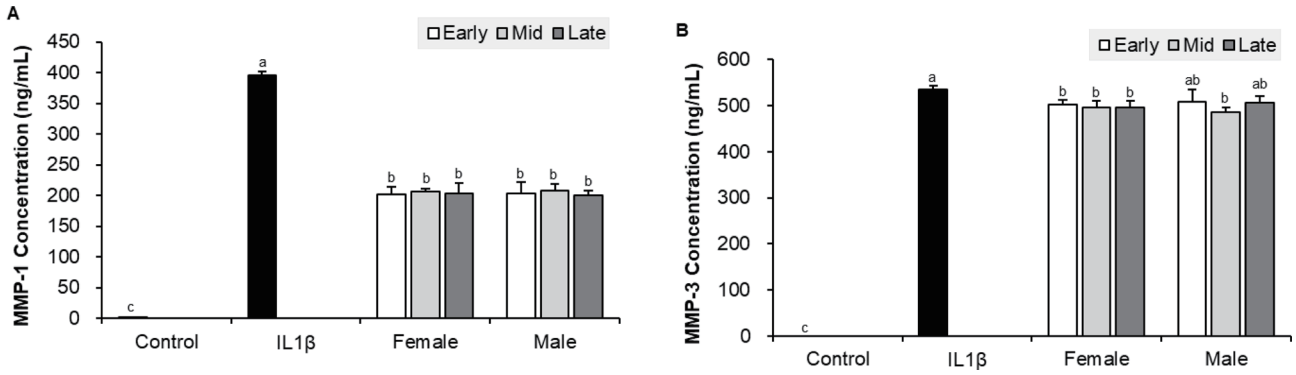

Fig. 3. Comparison of the effects of 70% ethanolic silkworm pupal extracts according to pupation time and sex on the expression of MMPs in IL-β-induced chondrocytes. Extracts were prepared in 70% ethanol. Silkworm variety: Baegokjam; Pupation stage: early stage (6–7 days), mid stage (8–10 days), and late stage (11–13 days); sex: male and female. SW1353 cells were pre-treated with 100 g/mL of the extracts and stimulated with IL-1β (40 ng/mL) for 24 h. Expression levels of MMP-1 (A) and MMP-3 (B) were measured using ELISA. Error lines represent the standard deviation of the mean (n = 3). Different lowercase letters above the columns indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$).

SW1353 cells are widely used as an *in vitro* model in research related to delineating the underlying mechanisms involved in OA progression, owing to their resemblance to actual OA chondrocytes. These cells have also been employed to evaluate the effectiveness of and screen various pharmacological agents involved in the treatment of OA (Pang *et al.,* 2021; Liu *et al.,* 2017). Chondrocytes are the only type of cells that constitute cartilage, and are involved in the synthesis and turnover of ECM constituents, including collagen, glycoproteins, hyaluronan, and proteoglycans (Archer and Francis-West, 2003). The anabolism of ECM constituents is complemented by catabolic turnover in healthy cartilage to maintain joint homeostasis. In the case of OA, any alteration in the intracellular environment of the joint, either by injury, aging, or any other factor, may tilt the balance in favor of degradation, with metalloproteinases breaking down collagen and aggrecan and causing gradual joint deterioration (McClurg *et al.,* 2021).

Variations in the effects of SPE with different solvents were observed, in which the 70% ethanolic extract had the greatest OA-protective effect. Ethanol-water solvent systems performed better than aqueous extracts, whereas 100% ethanolic extracts and hexane extracts exhibited negligible or meager mitigation potential. This indicates that the bioactive components involved in OA prevention were best extracted from silkworm pupae in ethanol-water solvent systems. Our previous studies have also indicated the potential of 70% ethanolic SPE to exhibit anti-collagenase, anti-elastase, and anti-tyrosinase activities (Rahul *et al.,* 2022b). In another preliminary study, the effects of SPE on antioxidant and anti-inflammatory activities were highest using 30% and 100% ethanolic extracts. (Lee *et al.,* 2021b, Rahul *et al.,* 2022a). These results suggest that the effective substances in silkworm pupae adapt differently according to their physiological activity; the active substances in silkworm pupae include trace chemicals, peptides, and fatty acids.

Effect of 70% ethanolic SPE on IL-1β-induced MMPs

The MMP-1 and MMP-3 levels in untreated and non-induced cells were negligible, whereas these levels augmented to 395.3 ng/mL and 534.4 ng/mL, respectively, in their IL-1β-treated counterparts. Pretreatment with 70% ethanolic SPE, irrespective of pupation time or sex, significantly reduced the expression levels of MMP-1 (47.37–49.19%, Fig. 3A). There were no significant differences in the reduction of MMP-1 among the 70% ethanolic SPE of different silkworm pupation times or sexes. Except for extracts from the early and late stages of male pupae, the other extracts attenuated the levels of MMP-3 (9–6%, Fig. 3B).

It is well-demonstrated that IL-1β plays a key role in inflammation as well as deterioration of connective tissue in osteo- and rheumatoid arthritis (Vincenti and Brinckerhoff, 2001). Elevated IL-1β levels were detected in patients suffering from OA compared to normal individuals (Lu *et al.,* 2018). When chondrocytes are stimulated with IL-1β, numerous intracellular signaling pathways are activated, which cause the expression of genes related to inflammation and MMPs, subsequently leading

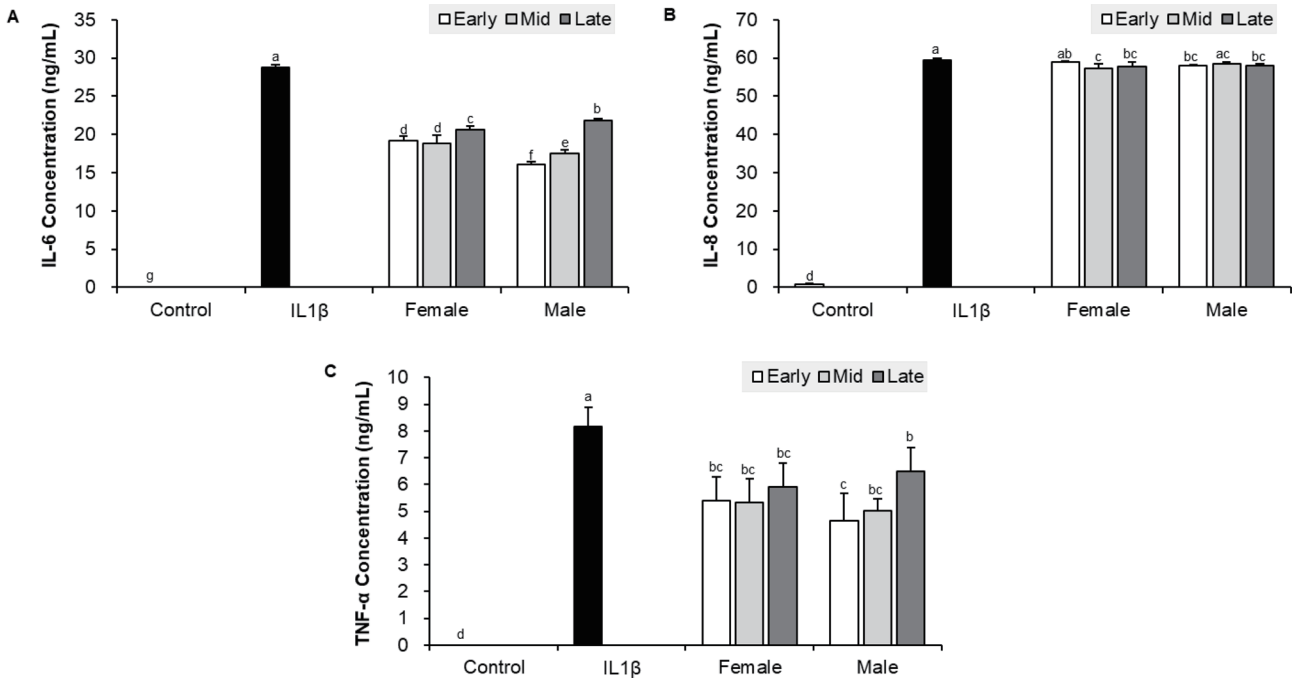

Fig. 4. Comparison of the effects of 70% ethanolic silkworm pupal extracts according to pupation time and sex on pro-inflammatory cytokines in IL-β-induced chondrocytes. Extracts were prepared in 70% ethanol. Silkworm variety: Baegokjam; Pupation stage: early stage (6–7 days), mid stage (8–10 days), and late stage (11–13 days); sex: male and female. SW1353 cells were pre-treated with 100 g/mL of the extracts and stimulated with IL-1β (40 ng/mL) for 24 h. The levels of IL-6 (A), IL-8 (B), and TNF-α (C) in the culture media were measured using ELISA. Error lines represent the standard deviation of the mean (n = 3). Different lowercase letters above the columns indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$).

to cartilage degradation (Choi, 2020).

In the present study, we observed that the expression levels of MMPs (MMP-1 and MMP-3) were increased multiple fold in IL-1β stimulated chondrocytes compared to their untreated counterparts. Strong mitigation of MMP-1 by SPE was observed, while this effect was not significantly altered by sex and pupation time. It was assumed that the bioactive compounds involved in MMP regulation are stable compounds that are not affected by pupa sex or growth stage. In addition, MMP3 expression was only slightly regulated by SPE treatment. As MMP-1 belongs to a class of collagenases and MMP-3 acts as a matrix lyase, which plays different roles in OA (Wang and He, 2018), SPE is hypothesized to inhibit the collagen damage caused by inflammation.

Effect of 70% ethanolic SPE on IL-1β-induced pro-inflammatory cytokine expression

The expression levels of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) were significantly augmented in IL-1β-stimulated cells compared to the unstimulated chondrocytes (Fig. 4). Regardless of pupation stage or sex, all the 70% ethanolic SPEs were able to mitigate the expression levels of IL-6 (24.23–43.16%, $p < 0.05$). however, early-stage male extracts performed better than the other extracts (Fig. 4A). The 70% ethanolic SPE did not alleviate the levels of IL-8 (1–3.5%, Fig. 4B). However, TNF-α levels were significantly reduced by SPE (20.5–43.3%, $p < 0.05$). The greatest reduction was observed in the male early-stage extract group (Fig. 4C). Irrespective of sex, early pupation stage extracts outperformed their later stage counterparts.

The effects of the SPEs on inflammatory mediators (IL-6 and TNF-α) were tested in SW1353 cells, and the results showed significant differences between sexes and pupation stages. This may be due to metabolic differences between male and female pupae and pupation duration; there are often variations in the accumulation of bioactive components, which have been reflected in biological activity in previous studies (Rahmathulla *et al.,* 2005; Lee *et al.,* 2021a; Lee *et al.,* 2021b; Rahul *et al.,* 2022b). However, this study did not outline the bioactive constituents of SPE or the pathways involved in the attenuation of the MMPs and inflammatory mediators. Delineating the above

can shed light on the differences in the mitigation patterns of MMPs and inflammatory mediators using extracts belonging to different silkworm sexes and pupation times.

In conclusion, we demonstrated that 70% ethanolic SPE can effectively alleviate the expression of key players in the progression of OA, i.e., MMP-1, MMP-3, IL-6, and TNF-α expression in IL-1β-stimulated SW1353 human chondrosarcoma cells. These properties indicate the potential of SPE as a therapeutic agent for the treatment of OA. Investigation of its anti-inflammatory efficacy in the early stages is expected to shorten the harvest time for pupae. In addition, this study suggests the possibility of its development as a material for joint improvement. Along with its tonic and muscle strengthening effects, in societies where the elderly population is increasing, this may contribute to increasing the value of pupae.

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References

Archer CW, Francis-West P (2003) The chondrocyte. Int J Biochem Cell Biol 35, 401-404.

Chien SY, Tsai CH, Liu SC, Huang CC, Lin TH, Yang YZ, Tang CH (2020) Noggin inhibits IL-1β and BMP-2 expression, and attenuates cartilage degeneration and subchondral bone destruction in experimental osteoarthritis. Cells 9,927.

Choi YH (2020) *Carpomitra costata* extract suppresses Interleukin-1β-induced inflammatory response in SW1353 human chondrocytes through suppressing NF-κB signaling pathway. J Mar Biosci Biotechnol 12(2),99-107.

He W, He K, Sun F, Mu L, Liao S, Li Q, *et al.* (2021) Effect of heat, enzymatic hydrolysis and acid-alkali treatment on the allergenicity of silkworm pupa protein extract. Food Chem 343,128461.

Jia Y, He W, Zhang H, He L, Wang Y, Zhang T, *et al.* (2020) Morusin ameliorates IL-1β-induced chondrocyte inflammation and osteoarthritis via NF-κB signal pathway. Drug Des Devel Ther 14,1227-1240.

Lee JH, Jo YY, Ju WT, Kim KY, Kweon HY (2019) Effects of silkworm and its by-products on muscle mass and exercise performance in ICR mice. Int J Indust Entomol 39(1),34-38.

Lee JH, Jo YY, Kim SW, Kweon HY (2021a) Analysis of nutrient composition of silkworm pupae in Baegokjam, Goldensilk, Juhwangjam, and YeonNokjam varieties. Int J Indust Entomol 43(2),85-93.

Lee JH, Jo YY, Kim SW, Kweon HY (2021b) Antioxidant capacity of silkworm pupa according to extraction condition, variety, pupation time, and sex. Int J Indust Entomol 43(2),59-66.

Liu CC, Zhang Y, Dai BL, Ma YJ, Zhang Q, Wang Y, Yang H (2017) Chlorogenic acid prevents inflammatory responses in IL-1β-stimulated human SW-1353 chondrocytes, a model for osteoarthritis. Mol Med Rep 16(2),1369-1375.

Lu C, Li Y, Hu S, Cai Y, Yang Z, Peng K (2018) Scoparone prevents IL-1β-induced inflammatory response in human osteoarthritis chondrocytes through the PI3K/Akt/NF-κB pathway. Biomed Pharmacother 106,1169-1174.

McClurg O, Tinson R, Troeberg L (2021) Targeting cartilage degradation in osteoarthritis. Pharmaceuticals (Basel). 14(2),126.

Pang KL, Chow YY, Leong LM, Law JX, Ghafar NA, Soelaiman IN, Chin KY (2021) Establishing SW1353 chondrocytes as a cellular model of chondrolysis. Life (Basel). 11(4),272.

Park EH, Kim JS, Lee JS, Lee YJ, Song YW, Lee EY (2018) Compound K inhibits Interleukin-1β-induced expression of inflammatory mediators and matrix metalloproteinases by inhibiting mitogen-activated protein kinase activation in chondrocytes. J Rheum Dis 25(3),188-196.

Rahmathulla VK, Rufaie SZH, Himantharaj MT, Vindya GS, Rajan RK (2005) Food ingestion, assimilation and conversion efficiency of mulberry silkworm *Bombyx mori*, L. Int J Indust Entomol 11(1),1-12.

Rahul K, Kweon HY, Kim HB, Lee JH (2022a) Evaluation of the anti-inflammatory effects of silkworm (*Bombyx mori* L.) pupal extracts against lipopolysaccharide-induced inflammation in the murine macrophage cell line RAW264.7. Int J Indust Entomol 45(2):99-107.

Rahul K, Kweon HY, Kim HB, Lee JH (2022b) *In vitro* screening of anti-skin aging and antioxidant properties of aqueous/solvent extracts from distinctive stages of silkworm (*Bombyx mori* L.) pupae. Int J Indust Entomol 45(1),1-11.

Rao Z, Wang S, Wang J (2018) Protective effects of psoralidin on IL-1β-induced chondrocyte apoptosis. Mol Med Rep 17(2):3418-3424.

Ryu KS, Ahn MY, Lee HS, Kim I, Kim JW, Kim SH, Park JH (2002) The tonic effect of the extract from male silkworm (*Bombyx mori* L.)

pupae on rats. Int J Indust Entomol 5(1),123-126.

Vincenti MP, Brinckerhoff CE (2001) Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1beta. Arthritis Res. 3(6),381-388.

Wang T, He C (2018) Pro-inflammatory cytokines: The link between obesity and osteoarthritis. Cytokine Growth Factor Rev 44,38-50.

Zhang L, Ma S, Su H, Cheng J. (2018) Isoliquiritigenin inhibits IL-1β-induced production of matrix metalloproteinase in articular chondrocytes. Mol Ther MethodsClin Dev 9,153-159.

Zhou Y, Zhou S, Duan H, Wang J, Yan W. (2022) Silkworm pupae: A functional food with health benefits for humans. Foods. 11,1594.