IJIE Int. J. Indust. Entomol. 45(2) 99-107 (2022)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2022.45.2.99

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)

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

Kamidi Rahul[1,2], HaeYong Kweon[1], Hun-bok Kim[1], and Ji Hae Lee[1,*]

[1]*Industrial Insect and Sericulture Division, National Institute of Agricultural Sciences, RDA, Wanju-gun 55365, Republic of Korea*
[2]*Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles: Govt. of India, Berhampore - 742101, Murshidabad, West Bengal, India*

Abstract

Silkworm pupal extracts (SPE) were prepared in different solvents (water, 30%, 50%, 70%, and 100% ethanol) and their anti-inflammatory effects were evaluated in the RAW264.7 cell line. The SPE composition was initially evaluated by determining the protein content and performing Fourier transform infrared (FTIR) analysis. The protein content of the different SPE ranged from 6.75-130.93 mg/g of extract. FTIR analysis exhibited distinguishable absorption peaks among the extracts and indicated the presence of lipids, proteins, carbohydrates, and nucleic acid moieties. The levels of released nitric oxide (NO) and interleukin-6 (IL-6) expression in lipopolysaccharide (LPS)-induced RAW264.7 cells were only attenuated by 100% ethanolic SPE to 19.44% and 16.77%, respectively. The other solvent extracts were ineffective. Hence, further studies were conducted with 100% ethanolic SPE from three distinct stages of male and female silkworm pupae belonging to four silkworm varieties (Baegokjam; B, GoldenSilk; G, Juhwangjam; J, and YeonNokjam; Y). The best reduction in NO release and interleukin-1β (IL-1β) expression levels was achieved by the SPE of early female pupae belonging to the Baegokjam variety (32.72%) and those of early female pupae belonging to the Baegokjam and GoldenSilk (59.93%) varieties, respectively. The best reduction in IL-6 expression by 49.70% was achieved by SPE from female pupae of the mid-pupal stage belonging to the Baegokjam variety.

© 2022 The Korean Society of Sericultural Sciences
Int. J. Indust. Entomol. 45(2), 99-107 (2022)

Received : 25 Oct 2022
Revised : 24 Nov 2022
Accepted : 12 Dec 2022

Keywords:
Silkworm pupae,
Anti-inflammatory,
Interleukin-1β,
Interleukin-6,
Tumor necrosis factor-α

Introduction

Silkworm pupae, once considered a by-product in the silk textile industry, are now considered more valuable as dietary sources/supplements due to reports of their diverse nutritional components and physiological activity. Silkworm pupa has a protein content in the range of 45-64% and fat content in the range of 25-45%. In particular, it contains eight essential amino acids (Met, Thr, Val, Ile, Leu, Phe, Lys, Trp) and has a high content of unsaturated fatty acids (linolenic acid, oleic acid) (Lee *et al*., 2021a).

Inflammation refers to the immune system response to diverse

***Corresponding author.**
Ji Hae Lee, Ph.D.
Industrial Insect and Sericulture Division, National Institute of Agricultural Sciences, RDA, Wanju-gun 55365, Republic of Korea
Tel: +82-63-238-2844 / FAX: +82-63-238-3833
E-mail: jihae@korea.kr

© 2022 The Korean Society of Sericultural Sciences

stimuli, including infectious (bacteria, fungi, protozoa, viruses) as well as non-infectious factors (exposure to chemical irritants, tissue damage), and is one of the body's immediate defense mechanisms (Azab *et al*., 2016). Sensation of heat, appearance of redness, swelling, pain, and impairment of function at the affected region are typical indicators of inflammation (Bennett *et al*., 2018; Scott *et al*., 2004). Depending on the nature of the stimulus and how efficiently the body responds to eliminate it or the damaged tissues, inflammation may be acute or chronic (Chen *et al*., 2017). The aforementioned stimuli trigger a series of chemical reactions that activate leukocytes, which subsequently secrete inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). These cytokines activate diverse transcription factors involved in specific antimicrobial defense and tissue repair. Thus, acute inflammation acts as a defense mechanism by eliminating the harmful stimuli and initiating the rehabilitation process of the damaged tissue (Kiss, 2022).

On the other hand, a chronic inflammatory state is associated with a number of disorders, and especially with cardiovascular anomalies, cancer, and neurological disorders (Hussain *et al*., 2020). Currently, the most widely used therapeutic approaches for inflammation are steroidal medications (glucocorticoids) and non-steroidal anti-inflammatory drugs, which have anti-inflammatory, analgesic, and other curative properties. Nevertheless, the prolonged use of the aforesaid medications may result in adverse side effects, including gastrointestinal disorders, hepatic and renal malfunction, and skin diseases (Deng *et al*., 2022). Therefore, there is an urgent need to identify natural products that possess anti-inflammatory properties and can serve as a substitute to conventional anti-inflammatory medications.

A number of studies have demonstrated that natural products, including alkaloids, flavonoids, pigments, polyphenols, polysaccharides, quinones, terpenes and volatile oils from plants, possess anti-inflammatory properties (Wu *et al*., 2021). In addition to the similar therapeutic potential, the relatively low toxicity of natural products, in comparison to that of conventional drugs, is an added advantage (Deng *et al*., 2022).

In the present study, we evaluated the silkworm pupal extract (SPE)-induced attenuation of NO production and IL-1β, IL-6, and TNF-α levels in lipopolysaccharide (LPS)-induced inflammation in the murine macrophage cell line RAW264.7. Variations in the above parameters according to the extraction solvent (water, 30%, 50%, 70%, and 100% ethanol), silkworm variety (Baegokjam, B; GoldenSilk, G; Juhwangjam, J; and YeonNokjam, Y), pupation time (7, 9-10, and 11 days) and sex (female; F and male; M) were also determined.

Materials and Methods

Preparation of SPE

Silkworm pupae belonging to four varieties (B, G, J, and Y) obtained from Uljin Silk Farm (Uljin, Korea) were segregated according to pupation day (7, 9-10, and 11 days) and sex (F and M). Pupae were stored at -80 °C overnight and subjected to freeze drying in a lyophilizer (IlShinBioBase, Gyeonggi-do, Korea). Then, they were coarsely pulverized in a domestic mixer grinder, mixed with 20 times the volume of the extraction solvent (distilled water, 30%, 50%, 70%, and 100% ethanol) and subjected to stirring for 24 h at room temperature. Subsequently, the extracts were filtered using a Miracloth (Merck), and centrifuged at 11200 × g for 10 min. The resultant supernatant was lyophilized, powdered, and refrigerated until use (Rahul *et al*., 2022; Lee *et al*., 2021b).

Analysis of SPE components

SPE protein concentrations were measured using the Bradford assay. Briefly, 10 μL of samples were allowed to react with 200 μL of Bradford reagent (Sigma Aldrich, St. Louis, MO, USA). Samples were shaken for 30 s and absorbance was measured at 595 nm using a spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). Bovine serum albumin was used as a standard. The extract composition was analyzed using Fourier transform infrared (FTIR) spectroscopy (S100, Perkin Elmer, Waltham, MA, USA). Data are expressed as transmittance in the wavenumber range of 800-4000 cm^{-1}. Thirty-two scans were averaged with a resolution of 4 cm^{-1} (Lee *et al*., 2020).

Cell culture and SPE treatment

Murine macrophage Raw264.7 (Korean Cell Line Bank, Seoul, Korea) cells were maintained in Dulbecco's modified Eagle's medium (Caisson, Smithfield, UT, USA) supplemented with 10% fetal bovine serum (Gendepot, Barker, TX, USA) and 1% penicillin-streptomycin (Caisson). Cells were seeded at a density of 2.5×10^5 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 then treated with SPE (100 μg/mL) followed by incubation

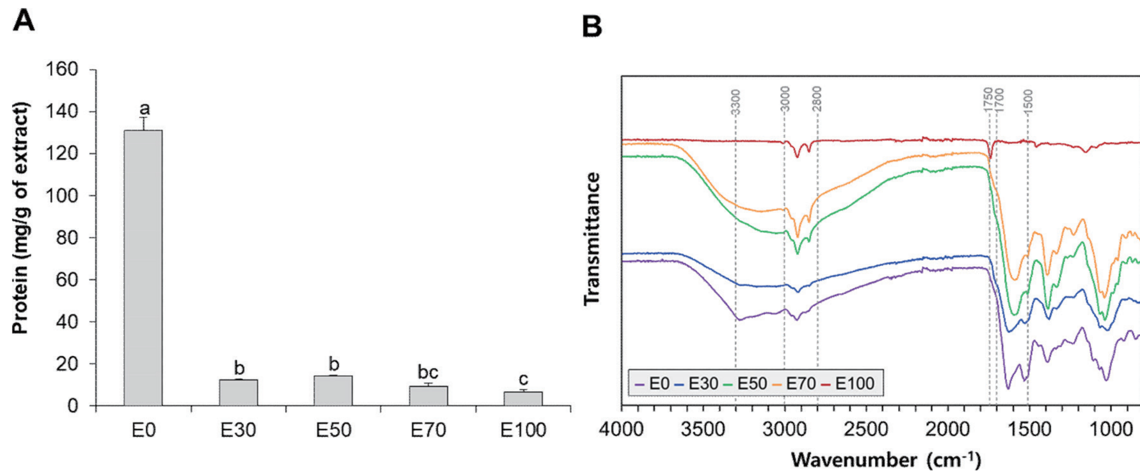

Fig. 1. Nutritional composition of silkworm pupal extracts. (A) Protein content in various solvent extracts of silkworm pupae. (B) Fourier transform infrared (FTIR) chromatogram of various solvent extracts of silkworm pupae. The extracts were prepared using distilled water (E0), 30% ethanol (E30), 50% ethanol (E50), 70% ethanol (E70), and 100% ethanol (E100). Silkworm variety: GoldenSilk; Pupation time: seven days; Sex: Female. Error bars represent standard deviation from measurements in triplicate. Data were analyzed using one way analysis of variance. Lowercase letters above the columns indicate significant differences among the treatments according to Duncan's multiple range test (p <0.05).

with LPS (1 μg/mL) for 24 h to induce acute inflammatory responses. The cells that were only treated with medium and LPS alone served as controls. Supernatants from treated and control cells were collected and further analyzed.

Determination of NO and pro-inflammatory cytokine levels

The Griess Reagent System (Promega Corporation, Madison, WI, USA) was used to determine NO production in the test samples by measuring its stable end product nitrite. According to the manufacturer instructions, media samples (50 μL) were dispensed into a 96-well plate followed by the addition of 50 μL of sulfanilamide solution. Upon incubation for 10 min, N-1-napthylethylenediamine dihydrochloride solution was added and the mixture was allowed to stand for another 10 min. The incubation steps were carried out in the dark. Absorbance was measured at 530 nm using a microplate reader and a standard nitrite curve was used to estimate the concentrations of nitrite in the test samples. The expression of pro-inflammatory cytokines (IL-1β, IL6, and TNF-α) in the cell culture supernatants was determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Abcam, Cambridge, UK).

Statistical analysis

The results from the aforementioned assays performed in triplicates are presented as means ± standard deviation. One way analysis of variance (ANOVA) was employed for data analysis. Duncan's multiple range tests were adapted to detect significant differences among mean values. P values of <0.05 were considered statistically significant. Wherever necessary, for the comparison of mean values between two groups, the Student's t-test was used (SAS Enterprise Guide 7.1, SAS Institute Inc., Cary, NC, USA).

Results and Discussion

SPE composition according to solvent

The protein content of various solvent extracts (distilled water, 30% ethanol, 50% ethanol, 70% ethanol, and 100% ethanol) of silkworm pupae (Variety - G, Pupation time - seven days, Sex - F) differed significantly with the exception of 30% and 50% ethanolic SPE ranging from 6.75-130.93 mg/g of extract. The best and worst extraction rates were observed with distilled water and 100% ethanol, respectively (Fig. 1A). The spectra from FTIR analysis of different solvent SPE were distinguishable, depicting absorption peaks at approximately 300-2800 cm^{-1}: lipids, 1700-1500 cm^{-1}: proteins, and 1200-900 cm^{-1}: nucleic acids and carbohydrates. Aqueous extracts exhibited strong peaks at approximately 1500-1700 cm^{-1} and 3300 cm^{-1}, whereas 100% ethanol

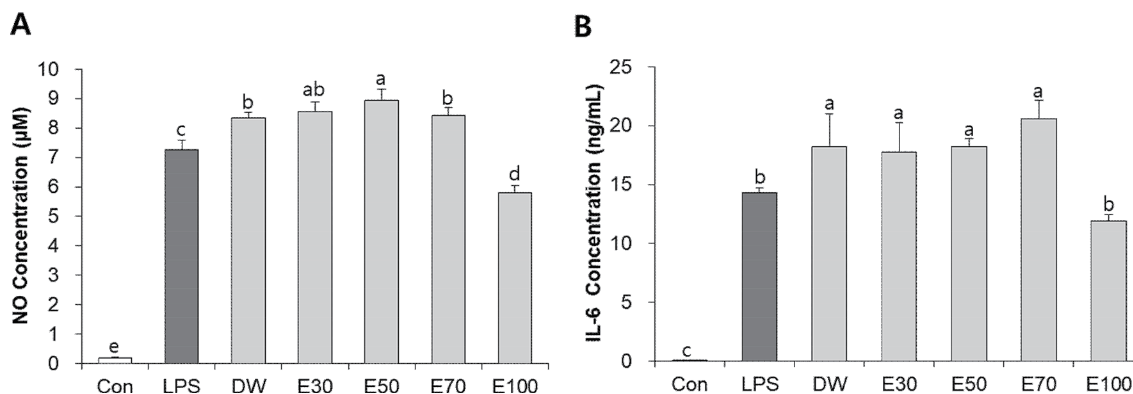

Fig. 2. Comparison of the anti-inflammatory effects of silkworm pupal extracts according to solvents. (A) Effect of various solvent extracts of silkworm pupae on nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. (B) Effect of various solvent extracts of silkworm pupae on interleukin-6 (IL-6) expression in LPS-stimulated RAW264.7 cells. The extracts were prepared using distilled water (E0), 30% ethanol (E30), 50% ethanol (E50), 70% ethanol (E70), and 100% ethanol (E100). Silkworm variety: GoldenSilk; Pupation time: seven days; Sex: Female. Error bars represent standard deviation from measurements in triplicate. Data were analyzed using one way analysis of variance. Lowercase letters above the columns indicate significant differences among the treatments according to Duncan's multiple range test (p <0.05).

extracts showed characteristic absorption peaks at approximately 2800-3000 cm^{-1} and 1750 cm^{-1} (Fig. 1B).

Silkworm pupae proteins are a potential source of bioactive peptides with therapeutic potential (Kumar *et al*., 2015). In the present study, the protein content was better extracted in water rather than in the ethanol-water solvent system. The processing conditions, particularly the solvent of choice, play an important role in protein recovery. Aqueous extractions are mostly preferred and useful in view of the high protein stability and solubility. However, for proteins that contain non-polar, hydrophobic and/or aromatic amino acid residues, organic solvents, such as acetone, butanol, and ethanol are the most preferred (Franca-Oliveira *et al*., 2021). Silkworm pupae are marginally rich in polar amino acids (Tomotake *et al*., 2010). The nutritional composition of silkworm pupae belonging to the four silkworm varieties used in this study was deduced earlier and it was found that they are rich in proteins, unsaturated fatty acids, and polyphenols. The aforesaid constituents along with the fat, ash, fiber, and mineral contents of silkworm pupae varied according to the silkworm variety, pupation time, and sex (Lee *et al*., 2021a). The role of the extraction solvent is clearly demonstrated by the FTIR results wherein different solvent extracts exhibited distinguishable absorption peaks indicating differences in the extract composition.

Effect of SPE with different extraction solvent on LPS-stimulated NO production and IL-6 expression

The effects of various solvent extracts (distilled water, 30% ethanol, 50% ethanol, 70% ethanol, and 100% ethanol) of silkworm pupae (Variety - G, Pupation time - 7 days, Sex - F) on NO production and IL-6 expression levels in LPS-stimulated RAW264.7 cells were determined. The purpose of this preliminary experiment was to screen different solvent extracts and select the one with the strongest anti-inflammatory activity irrespective of the SPE composition for subsequent experiments. The selected solvent was used in further experiments to prepare extracts from distinct stages of male and female silkworm pupae of the four silkworm varieties used in this study and screen them for anti-inflammatory potential.

Significant increases in NO production and IL-6 expression were observed in LPS-induced cells in comparison to untreated cells. Pretreatment with SPE extracted in distilled water, 30% ethanol, 50% ethanol, and 70% ethanol significantly increased the levels of NO. In contrast, the 100% ethanolic SPE significantly reduced the NO levels to 19.44%. The levels of IL-6 expression were also most effectively decreased by the 100% ethanolic SPE by 16.77% (Fig. 2A and 2B). Additional experiments were performed using 100% ethanolic SPE to delineate the different silkworm extract variety-, pupation time-, and sex-induced variations in NO release reduction and cytokine expression in LPS-stimulated macrophage cells (RAW264.7). The results are depicted below.

LPS, an essential lipid component of the outer membrane of gram-negative bacteria, has the potential to elicit an immune response in the host (Noailles *et al*., 2018). When LPS stimulates

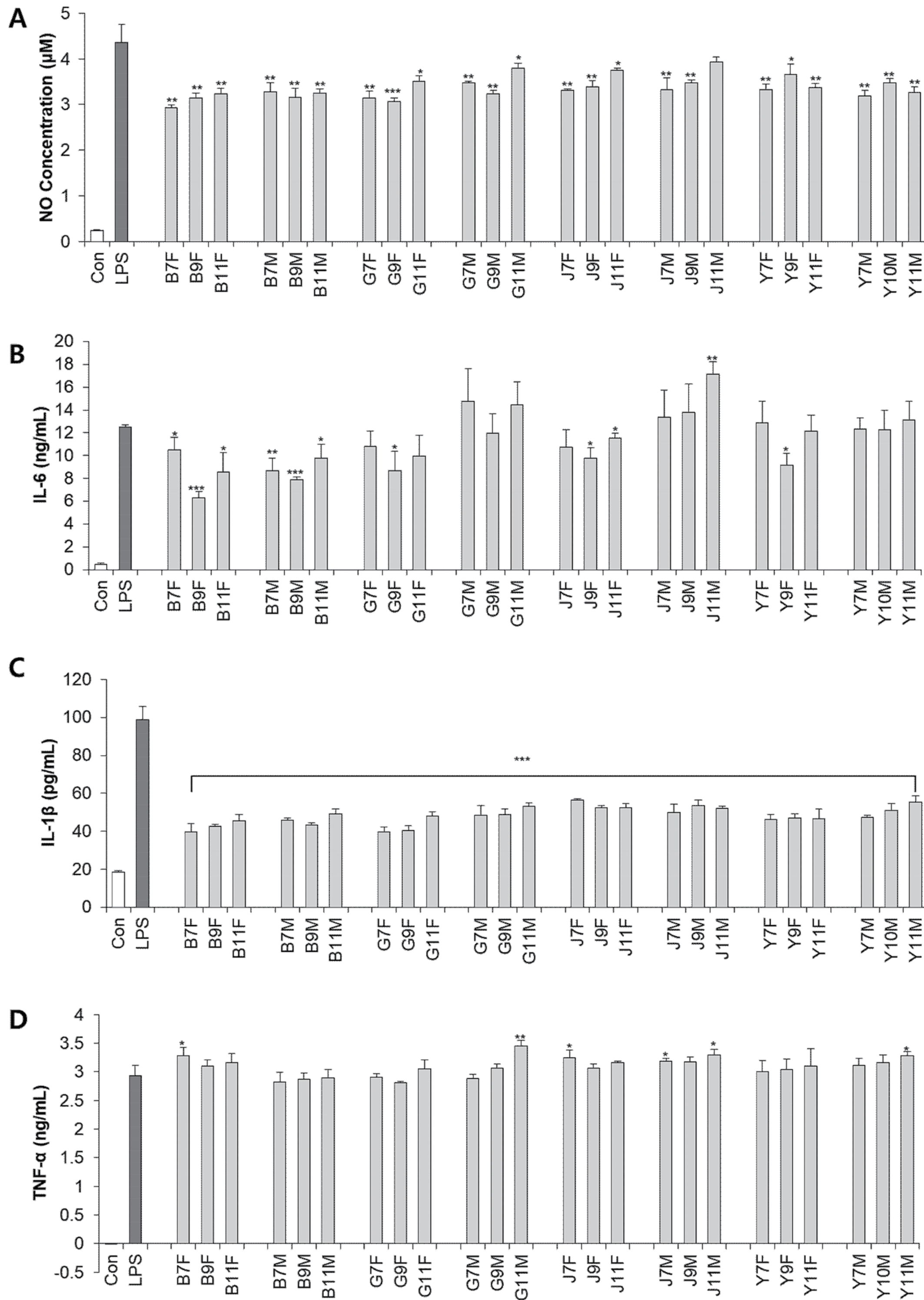

Fig. 3. Comparison of the anti-inflammatory effects of silkworm pupal extracts according to variety, pupation time, and sex. (A) Effect of 100% ethanol extracts of silkworm pupae on nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. (B) Effect of 100% ethanol extracts of silkworm pupae on interleukin-6 (IL-6) expression in LPS-stimulated RAW264.7 cells. (C) Effect of 100% ethanol extracts of silkworm pupae on IL-1β expression in LPS-stimulated RAW264.7 cells. (D) Effect of 100% ethanol extracts of silkworm pupae on tumor necrosis factor-α (TNF-α) expression in LPS-stimulated RAW264.7 cells. Silkworm variety (Baegokjam; B, GoldenSilk; G, Juhwangjam; J, and YeonNokjam; Y), pupation time (7, 9-10, 11 days) and sex (Female; F and male; M). Error bars represent standard deviation from measurements in triplicate. Data were analyzed using the Student's *t*-test. Significant differences compared to the LPS-treated group are indicated by **p*<0.05, ***p* <0.01 and ****p* <0.001, respectively.

the host's macrophages during a microbial infection, the immune system secretes cytokines and chemokines, followed by a variety of inflammatory responses. Therefore, preventing the LPS-induced macrophage activation is a key goal of therapeutic approaches for the management of inflammatory disorders (Baek *et al*., 2020). LPS-based macrophage stimulation and studying the levels of chemokines (interleukin-8, CC-chemokine ligand 5, eotaxin), cytokines (TNF-α, IL-1α, IL-1β, IL-6, granulocyte-macrophage colony-stimulating factor) and pro-inflammatory mediators (NO, inducible nitric oxide synthase [iNOS], cyclooxygenase-2 [COX-2]) are employed to screen anti-inflammatory compounds (Liu *et al*., 2022). In the present study, the variation in the reduction/increase of NO release and IL-6 expression among the different solvent SPE might be attributable to the compositional differences among them. The bioactive components responsible for alleviation of NO release and IL-6 expression might be best extracted in 100% ethanolic extracts. FTIR analysis also indicated that the 100% ethanolic SPE exhibited characteristic absorption peaks at approximately 2800-3000 cm^{-1} and 1750 cm^{-1} which are distinguishable from those of the other solvent extracts. However, the compounds contributing to the anti-inflammatory activities of the extracts were not delineated in the present study.

Effect of 100% ethanolic SPE on LPS-stimulated NO production

The effect of the 100% ethanolic SPE on LPS-stimulated NO release was determined employing the Griess reagent system. The LPS-treated cells exhibited an 18.35-fold increase in NO production in comparison to untreated cells. The same was significantly reduced by pretreatment with the 100% ethanolic SPE irrespective of pupation time, sex, or silkworm variety except for the extracts from the late male pupae of the J variety. The levels of NO were most effectively reduced by extracts of the B variety (24.83-32.72%) followed by those of the G (12.91-29.59%), Y (16.04-26.76%), and J (9.63-24.08%) varieties. The extracts of the B variety regardless of pupation time or sex did not differ significantly from one another in the mitigation of NO levels, unlike the extracts belonging to the other three silkworm varieties. The best reduction in NO levels was achieved by the extracts of the early female pupae belonging to the B variety (32.72%) (Fig. 3A).

NO is an important molecule that plays a significant role in the pathophysiology of inflammation. Under typical physiological conditions, NO has an anti-inflammatory action. However, when overproduced under abnormal circumstances, NO is regarded a pro-inflammatory mediator which causes inflammation (Sharma *et al*., 2007). When NO levels are elevated, they combine with superoxide anion to form peroxynitrite. Peroxynitrite is toxic and associated with a number of disorders including cancers, cardiovascular anomalies, and neurodegenerative disorders (Lee *et al*., 2019). LPS stimulation of macrophage cells favors the release of increased NO levels through the breakdown of L-arginine. NO production in the mammalian immune system is modulated by iNOS in macrophages. Inflammatory stimuli, such as LPS, trigger the upregulation of iNOS via the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways and subsequent NO production (Pansanit *et al*., 2013). Hence, in contemporary studies, iNOS is one of the major targets in anti-inflammation research (Hsieh *et al*., 2011).

Effect of 100% ethanolic SPE on LPS-induced pro-inflammatory cytokine secretion

The expression levels of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in LPS-stimulated RAW264.7 cells were determined using ELISA kits. The expression levels of IL-6 and IL-1β increased by 26.02- and 5.33-fold, respectively in LPS-treated cells in comparison to their untreated counterparts. Irrespective of pupation time or sex, pretreatment with 100% ethanolic SPE of the B variety significantly attenuated the expression levels of IL-6. The B variety extracts lowered IL-6 expression by 15.92-49.70% in comparison to their LPS-treated counterparts. The best reduction was achieved by extracts from female pupae of the mid-pupal stage (49.70%). The extracts of other silkworm varieties except those from female G pupae of the mid-pupal stage, mid- and late-pupal stage female J pupae and mid-pupal stage female Y pupae were not successful in the alleviation of IL-6 expression in LPS-stimulated murine macrophage cells (Fig. 3B).

The expression levels of IL-1β were significantly attenuated in 100% ethanolic SPE-pretreated cells. Regardless of the silkworm variety, pupal stage, or gender of the silkworm pupae, all extracts were effective in suppressing the LPS-induced IL-1β expression in murine macrophage cells (RAW264.7). The expression levels were most effectively reduced by extracts of the B variety (50.17-59.93%) followed by those of the G (45.99-59.93%), Y (43.80-53.16%), and J (42.81-49.58%) varieties. The extracts from early female pupae of the B and G varieties most effectively lowered the IL-1β expression levels by 59.93% (Fig. 3C). TNF-α ex-

pression levels were negligible in control cells, while they were increased to 2.93 ± 0.18 ng/mL in LPS-stimulated RAW264.7 cells. Pretreatment with 100% ethanolic SPE irrespective of silkworm variety, pupation time, or sex could not reduce the levels of TNF-α, whereas after a few treatments the TNF-α levels were significantly increased (Fig. 3D).

Macrophages, operating as sensing and reacting elements to control inflammatory and immunological responses, constitute the most efficient cells of the adaptive immune system during an inflammatory response. The association of LPS and toll-like receptor 4 (TLR4) activates intracellular signaling via myeloid differentiation factor 88 (MyD88) pathways, leading to the activation and translocation of NF-κB and its upstream regulators (MAPKs including p38, extracellular signal-regulated kinase 1/2 [ERK1/2], and c-Jun N-terminal kinase [JNK]) (Cao *et al*., 2019).

The imperative signaling molecules in the TLR pathway are NF-κB and MAPK (Kim *et al*., 2013). When not stimulated, NF-κB (p50/p65) heterodimers are in an inactive state in the cytoplasm owing to the binding of inhibitor of κB (IκB) protein. When stimulated by LPS, NF-κB (p50/p65) is released via IκB phosphorylation and degradation. Consequently, NF-κB p65, which is deemed to play a substantial role in inflammation, enters the nucleus and induces the expression of diverse cytokines and chemokines (Dong *et al*., 2018).

MAPKs constitute a group of serine/threonine kinases that are involved in a variety of physiological reactions to external stimuli. The well-recognized MAPKs, comprise ERK1/2, JNK1 to 3, p38 (α, β, γ, and δ) and ERK5 families (Cargnello and Roux, 2011). In a manner similar to NF-κB, LPS-induced iNOS and COX-2 production in activated macrophages is mediated via MAPK signaling pathways. Moreover, MAPKs are involved in the activation of NF-κB. Hence, a potential combination treatment for inflammatory disorders could involve the inhibition of NF-κB and MAPK pathways (Li *et al*., 2018; Dong *et al*., 2018).

To summarize, it is evident that the silkworm pupae extracted with 100% ethanol in this study exhibited anti-inflammatory properties, which significantly varied across varieties, pupation times, and between the sexes. Notably, the protein content extracted from silkworm pupae was the lowest in 100% ethanolic extracts. Among the silkworm varieties tested, the SPE of the B variety showed better anti-inflammatory effects compared to those of the G, Y, and J varieties. In addition, with a few notable exceptions, female pupae extracts outperformed their male counterparts. Mid/early-pupal stage SPE performed better than late-pupal stage SPE when the pupation stage was considered. This can be attributed to the metabolic differences across silkworm varieties. Furthermore, previous reports indicated that the food utilization indices across different silkworm varieties differ significantly (Rahmathulla *et al*., 2005). Moreover, variations in the levels of various biomolecules/bioactive components in the different silkworm developmental stages have been previously reported. It is observed that the protein levels are similar in the late-larval stage and early-pupal stage but the same declined rapidly during the late-pupal stage since a lot of protein is utilized for the development of adult structures. Furthermore, the levels of carbohydrates, lipids, and amino acid contents varied between the silkworm sexes across developmental stages (Gilbert and Schneiderman, 1961). This subsequently results in disparities in qualitative and quantitative accumulation of bioactive components, explaining the variations in the anti-inflammatory activities of SPE of different varieties, sexes, and developmental stages. Our previous studies also indicated the same differences in various biomolecules and bioactive components across different developmental stages, sexes, and experimented silkworm varieties (Lee *et al*., 2021a; Lee *et al*., 2021b).

Although the present study demonstrated the SPE-induced decrease of NO, IL-6, and IL-1β levels in LPS-stimulated RAW264.7 cells, the exact underlying mechanism and the bioactive components of SPE were not deciphered.

Conclusion

In the present study, we showed that 100% ethanolic SPE can effectively reduce NO release and IL-1β and IL-6 expression in LPS-stimulated murine macrophage cells indicating its anti-inflammatory potential. The above properties also differed across the silkworm variety, pupation time, and sex of silkworm pupae. This study is one of the comprehensive reports on the potential of SPE as an anti-inflammatory agent. These findings provide preliminary data on the potential of SPE as a valuable component in the treatment of inflammation.

Acknowledgments

This research activity was supported by the ‘Research Pro-

gram for Agriculture Science and Technology Development' (Grant No. PJ01502202), National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

References

Azab A, Nassar A, Azab AN (2016) Anti-Inflammatory activity of natural products. Molecules 21, 1321.

Baek SH, Park T, Kang MG, Park D (2020) Anti-inflammatory activity and ROS regulation effect of sinapaldehyde in LPS-stimulated RAW264.7 macrophages. Molecules 25(18), 4089.

Bennett JM, Reeves G, Billman GE, Sturmberg JP (2018) Inflammation-Nature's way to efficiently respond to all types of challenges: implications for understanding and managing "the epidemic" of chronic diseases. Front Med (Lausanne) 5, 316.

Cao Y, Chen J, Ren G, Zhang Y, Tan X, Yang L (2019) Punicalagin prevents inflammation in LPS-induced RAW264.7 macrophages by inhibiting FoxO3a/Autophagy signaling pathway. Nutrients 11(11), 2794.

Cargnello M, Roux PP (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 75(1), 50–83.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. (2017) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9(6), 7204–7218.

Deng W, Du H, Liu D, Ma Z (2022) Editorial: The role of natural products in chronic inflammation. Front Pharmacol 13, 901538.

Dong J, Li J, Cui L, Wang Y, Lin J, Qu Y, *et al*. (2018) Cortisol modulates inflammatory responses in LPS-stimulated RAW264.7 cells via the NF-κB and MAPK pathways. BMC Vet Res 14(1), 30.

Franca-Oliveira G, Fornari T, Hernández-Ledesma B (2021) A review on the extraction and processing of natural source-derived proteins through eco-innovative approaches. Processes 9(9), 1626.

Gilbert LI, Schneiderman HA (1961) Some biochemical aspects of insect metamorphosis. Am Zool 1, 11–51.

Hsieh IN, Chang ASY, Teng CM, Chen CC, Yang CR (2011) Aciculatin inhibits lipopolysaccharide-mediated inducible nitric oxide synthase and cyclooxygenase-2 expression via suppressing NF-κB and JNK/p38 MAPK activation pathways. J Biomed Sci 18, 28.

Hussain T, Murtaza G, Yang H, Kalhoro MS, Kalhoro DH (2020) Exploiting anti-inflammation effects of flavonoids in chronic inflammatory diseases. Curr Pharm Des 26, 2610–2619.

Kim KS, Cui X, Lee DS, Sohn JH, Yim JH, Kim YC, *et al*. (2013) Anti-inflammatory effect of neoechinulin a from the marine fungus *Eurotium* sp. SF-5989 through the suppression of NF-κB and p38 MAPK pathways in lipopolysaccharide-stimulated RAW264.7 macrophages. Molecules 18(11), 13245–13259.

Kiss AL (2022) Inflammation in focus: The beginning and the end. Pathol Oncol Res 27, 1610136.

Kumar D, Dev P, Kumar RV (2015) Biomedical applications of silkworm pupae proteins; in Biomedical applications of natural proteins. Kumar D, Kundapur RR (eds.), pp. 41–49, Springer, India.

Lee AY, Choi JW, Yokozawa T, Cho EJ (2019) Preventive effect of oligonol on nitric oxide and reactive oxygen species production through regulation of nuclear factor kappa B signaling pathway in RAW 264.7 macrophage cells against sodium nitroprusside. RSC Adv 9, 3987–3993.

Lee S, Kim SH, Jo YY, Kim SW, Kim HB, Kweon HY, *et al*. (2020) Characterization of mulberry root bark extracts (*Morus alba* L.) based on the extraction temperature and solvent. Int J Indust Entomol 41(2), 36–44.

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, 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, 59–66.

Li H, Zhang Q, Jin X, Zou X, Wang Y, Hao D, *et al*. (2018) Dysifragilone A inhibits LPS-induced RAW264.7 macrophage activation by blocking the p38 MAPK signaling pathway. Mol Med Rep 17, 674–682.

Liu Y, Chen W, Zheng F, Yu H, Wei K (2022) Xanthatin alleviates LPS-induced inflammatory response in RAW264.7 macrophages by inhibiting NF-κB, MAPK and STATs activation. Molecules 27(14), 4603.

Noailles A, Maneu V, Campello L, Lax P, Cuenca N (2018) Systemic inflammation induced by lipopolysaccharide aggravates inherited retinal dystrophy. Cell Death Dis 9, 1–18.

Pansanit A, Park EJ, Kondratyuk TP, Pezzuto JM, Lirdprapamongkol K, Kittakoop P (2013) Vermelhotin, an anti-inflammatory agent, suppresses nitric oxide production in RAW 264.7 cells via p38 inhibition. J Nat Prod 76, 1824–1827.

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, 1–12.

Rahul K, Kweon HY, Kim HB, Lee JH (2022) *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.

Scott A, Khan KM, Cook JL, Duronio V (2004) What is “inflammation”? Are we ready to move beyond Celsus? Br J Sports Med 38(3), 248–249.

Sharma JN, Al-Omran A, Parvathy SS (2007) Role of nitric oxide in inflammatory diseases. Inflammopharmacology 15(6), 252–259.

Tomotake H, Katagiri M, Yamato M (2010) Silkworm pupae (*Bombyx mori*) are new sources of high quality protein and lipid. J Nutr Sci Vitaminol 56, 446–448

Wu S, Pang Y, He Y, Zhang X, Peng L, Guo J, *et al*. (2021). A comprehensive review of natural products against atopic dermatitis: Flavonoids, alkaloids, terpenes, glycosides and other compounds. Biomed Pharmacother 140, 111741.