

Armeniaca semen Extract Suppresses Lipopolysaccharide-Induced Expressions of Cyclooxygenase-2 and Inducible Nitric Oxide Synthase in Mouse BV2 Microglial Cells

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Armeniaca semen is the seed of *Prunus armeniaca* L. var. *ansu* MAXIM which is classified into Rosaceae. In traditional oriental medicine, *Armeniaca semen* has been used for the treatment of pain and inflammatory diseases. In this study, the effect of *Armeniaca semen* extract on lipopolysaccharide-induced inflammation was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, reverse transcription-polymerase chain reaction (RT-PCR), Western blot, prostaglandin E₂ immunoassay, and nitric oxide detection on mouse BV2 microglial cells. In the present results, *Armeniaca semen* extract suppressed prostaglandin E₂ synthesis and nitric oxide production by inhibiting the lipopolysaccharide-stimulated enhancement of cyclooxygenase-2 and inducible nitric oxide synthase mRNA expression in BV2 cells. These results show that *Armeniaca semen* exerts anti-inflammatory and analgesic effects probably by suppression of cyclooxygenase-2 and inducible nitric oxide synthase expressions.

Key words *Armeniaca semen*; lipopolysaccharide; cyclooxygenase-2; prostaglandin E₂; inducible nitric oxide synthase; nitric oxide

Armeniaca semen is the seed of *Prunus armeniaca* L. var. *ansu* MAXIM, which has been classified into Rosaceae. *Armeniaca semen* is known to have many therapeutic effects such as relieving fever, stopping cough, quenching thirst, and so on. In traditional oriental medicine, *Armeniaca semen* has been used for the treatment of asthma, bronchitis, emphysema, constipation, nausea, leprosy, and leucoderma.^{1,2)} *Armeniaca semen* is divided into the outer husk and an inner part that contains glycoside, amygdaline, starch, and fatty acids. Among them, amygdalin is abundant in the seeds of the *Prunus* genus almond, apricots, and other rosaceous plants. Amygdalin is also known as vitamin B17, which had been used for the treatment of cancers, and vitamin B17 has been named as laetrile.³⁾ It has been reported that amygdalin is effective for the relief of the pain of cancer patients.^{4,5)}

Pain is first response to injury or infection. Injury and infection activates the immune system to produce an inflammatory responses.⁶⁾ Lipopolysaccharide (LPS) initiates a number of major cellular responses that play vital roles in the pathogenesis of inflammatory responses. LPS stimulates the production of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukins, prostaglandin E₂ (PGE₂), and leukotrienes.^{7,8)} Moreover, LPS enhances the pain response to various somatic stimuli.^{9,10)}

PGE₂ is a key inflammatory mediator and an increased level of PGE₂ mediates the cardinal features of inflammation such as pain, edema, and fever.^{11,12)} PGE₂ is converted from arachidonic acid by cyclooxygenase (COX). There are two isoforms of COX: COX-1 and COX-2. While COX-1 is a constitutively expressed form in normal physiologic functions, COX-2 is expressed only in response to such inflammatory signals such as cytokines and bacterial endotoxin, LPS. COX-2 produces large amount of PGE₂ that induces inflammation.^{13,14)}

NO is synthesized from L-arginine by nitric oxide synthase (NOS). NO plays an important role in the regulation of many

physiological processes.¹⁵⁾ There are three distinct isoforms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Of these, iNOS is an important enzyme involved in the regulation of inflammation.¹⁶⁾ It was reported that LPS upregulates iNOS expression in macrophages¹⁷⁾ and microglial cells.¹⁸⁾

In the present study, the effect of *Armeniaca semen* extract on the LPS-stimulated expression of COX-1, COX-2, and iNOS in mouse BV2 microglial cells was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, reverse transcription-polymerase chain reaction (RT-PCR), Western blot, PGE₂ immunoassay, and NO detection.

MATERIALS AND METHODS

Cell Culture Mouse BV2 microglial cells were cultured in Dulbecco's modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL) at 37 °C in 5% CO₂–95% O₂ in a humidified cell incubator. The cells were plated onto culture dishes at a density of 2 × 10⁴ cells/cm² 24 h prior to drug treatments.

Preparation of *Armeniaca semen* *Armeniaca semen* used in this experiment was obtained from the Kyungdong market (Seoul, Korea). After immersing in 0.1% citric acid for 1 min, *Armeniaca semen* was rinsed and dried in room temperature for 24 h. Then, it was pulverized by crusher (Hanil, Seoul, Korea) and the fine powder was sifted from the coarse particles by a mesh screen with a pore diameter of 2 mm. In order to obtain the aqueous extract of *Armeniaca semen*, the fine powder was subsequently heat-extracted by distilled water, pressure-filtered, and concentrated with a rotary evaporator (Eyela, Tokyo, Japan). The resulting 34.48 g powder (yield of 6.88%) was obtained from 500 g of *Armeniaca semen* through lyophilization by a drying machine

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(Ilsin, Kyungkido, Korea) for 24 h.

MTT Cytotoxicity Assay Mouse BV2 microglial cells were grown in a final volume of 100 μ l culture medium per well in a 96-well plates. In order to determine the cytotoxicity of *Armeniacae semen*, the cells were treated with *Armeniacae semen* extract at concentrations of 10^{-3} mg/ml, 10^{-2} mg/ml, 10^{-1} mg/ml, 1 mg/ml, and 10 mg/ml for 24 h. Cultures of the cells of the control group were left untreated. After adding 10 μ l of the MTT labeling reagent containing 5 mg/ml 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide in phosphate-buffered saline to each well, the plates were incubated for 4 h. Solubilization solution 100 μ l containing 10% sodium dodecyl sulfate in 0.01 M hydrochloric acid was added to each well, and the cells were incubated for another 12 h. The absorbance was then measured with a microtiter plate reader (Bio-Tek, Winooski, VT, U.S.A.) at a test wavelength of 595 nm with a reference wavelength of 690 nm. The optical density (O.D.) was calculated as the difference between the absorbance at the reference wavelength and that observed at the test wavelength. Percent viability was calculated as (O.D. of drug-treated sample/control O.D.) \times 100.

RNA Isolation and RT-PCR To identify the expression of COX-1, COX-2, and iNOS mRNA, RT-PCR was performed. The total RNA was isolated from BV2 cells using RNazo1TMB (TEL-TEST, Friendswood, TX, U.S.A.). Two micrograms of RNA and 2 μ l of random hexamers (Promega, Madison, WI, U.S.A.) were added together, and the mixture was heated at 65 °C for 10 min. One microliter of AMV reverse transcriptase (Promega), 5 μ l of 10 mM dNTP (Promega), 1 μ l of RNasin (Promega), and 5 μ l of 10 \times AMV RT buffer (Promega) were then added to the mixture, and the final volume was brought up to 50 μ l volume with diethylpyrocarbonate (DEPC)-treated water. The reaction mixture was then incubated at 42 °C for 1 h.

PCR amplification was performed in a reaction volume of 40 μ l containing 1 μ l of the appropriate cDNA, 1 μ l of each set of primers at a concentration of 10 pM, 4 μ l of 10 \times RT buffer, 1 μ l of 2.5 mM dNTP, and 2 units of Taq DNA polymerase (Takara, Shiga, Japan). For mouse COX-1, the primer sequences were 5'-AGTGCGGTCCAACCTTATCC-3' (a 20-mer sense oligonucleotide) and 5'-CCGCAGGTGAT-ACTGTGCTT-3' (a 20-mer anti-sense oligonucleotide). For mouse COX-2, the primer sequences were 5'-CCAGATGC-TATCTTTGGGAGAC-3' (a 23-mer sense oligonucleotide) and 5'-CTTGCATTGATGGTGGCTG-3' (a 19-mer anti-sense oligonucleotide). For mouse iNOS, the primer sequences were 5'-ATGAGGTACTCAGCGTGCTCCAC-3' (a 23-mer sense oligonucleotide) and 5'-CCACAATAGTACAATACTACTTGG-3' (a 24-mer anti-sense oligonucleotide). For cyclophilin, the internal control used in the study, the primer sequences were 5'-ACCCACCGTGTCTT-CGAC-3' (a 20-mer sense oligonucleotide) and 5'-CATTTGCCATGGACAAGATG-3' (a 20-mer anti-sense oligonucleotide). The expected size of the PCR product was 381 bp for COX-1, 249 bp for COX-2, 395 bp for iNOS, and 299 bp for cyclophilin.

For COX-1 and iNOS, the PCR procedures were carried out using a GeneAmp 9600 PCR system (Perkin Elmer, Norwalk, CT, U.S.A.) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 amplification

cycles, each consisting of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, and extension at 72 °C for 60 s, with an additional extension step at the end of the procedure at 72 °C for 10 min. For COX-2, the PCR procedures was under the following conditions: initial denaturation at 94 °C for 5 min, followed by 30 amplification cycles, each consisting of denaturation at 94 °C for 30 s, annealing at 30 °C for 30 s, and extension at 72 °C for 60 s, with an additional extension step at the end of the procedure at 72 °C for 10 min. For cyclophilin, the PCR procedure was under the following conditions: initial denaturation at 94 °C for 5 min, followed by 25 amplification cycles, each consisting of denaturation at 94 °C for 30 s, annealing at 55 °C, and extension at 72 °C for 45 s, with an additional extension step at the end of the procedure at 72 °C for 10 min. The final amount of RT-PCR product for each of the mRNA species was calculated densitometrically using Molecular AnalystTM version 1.4.1 (Bio-Rad, Hercules, CA, U.S.A.).

Western Blot Analysis Mouse BV2 microglial cells were lysed in a ice-cold whole cell lysate buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahydrate, 1 mM ethyleneglycol-bis-(β -aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μ g/ml leupeptin, 1 μ g/ml pepstatin, 1 mM sodium *ortho* vanadate, and 100 mM sodium fluoride, and the mixture was incubated 30 min at 4 °C. Cell debris was removed by microcentrifugation, followed by quick freezing of the supernatant. The protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, U.S.A.). Protein of 30 μ g was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). Goat COX-1 antibody, goat COX-2 antibody (1 : 2000; Santa Cruz Biotech, CA, U.S.A.), and rabbit iNOS antibody (1 : 500; Santa Cruz Biotech) were used as a primary antibody. Horseradish peroxidase-conjugated anti-goat antibody (1 : 2000; Santa Cruz Biotech) was used to probe for COX-1 and COX-2, and anti-rabbit antibody (1 : 2000; Santa Cruz Biotech) for iNOS was used as a secondary antibody. Band detection was performed using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biothech GmbH, Freiburg, Germany).

Measurement of Prostaglandin E₂ Synthesis Assessment of PGE₂ synthesis was performed using a commercially available PGE₂ competitive enzyme immunoassay kit (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, U.S.A.). Supernatant 100 μ l from the culture medium and the standards were put into different wells on the goat anti-mouse IgG-coated microtiter plate provided in the kit. Mouse anti-PGE₂ antibody and peroxidase-conjugated PGE₂ were added to each well, and the plate was incubated at room temperature with shaking for 1 h. The wells were drained and washed, and 3,3',5,5'-tetramethylbenzidine/hydrogen peroxide solution was then added. The plate was incubated at room temperature with shaking, and the reaction was stopped after 30 min through the addition of H₂SO₄. The absorbance of the content of each well was then measured at a wavelength of 450 nm.

Determination of Nitric Oxide Synthesis In order to determine the effect of *Armeniacae semen* extract on NO

synthesis, the amount of nitrite in the supernatant was measured, based on the Griess reaction, as an indicator of NO production. After collection of 100 μ l of cell culture medium, 50 μ l of 1% sulfanilamide was added to each well, and the plate was incubated at room temperature for 10 min. 0.1% naphthylethylenediamine containing 5% phosphoric acid was then added, and the plate was incubated at room temperature for 10 min. The absorbance of the content of each well was measured at a wavelength of 450 nm. The nitrite concentration was calculated from a nitrite standard curve generated by mixing 0 to 200 μ M sodium nitrite solutions with the Griess reagent. The standard curve was typically linear between 0 and 200 μ M of sodium nitrite.

Statistical Analysis The results are presented as the mean \pm standard error mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Duncan's *post-hoc* test using SPSS. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of *Armeniacae semen* Extract on Viability of BV2 Cells

In order to assess the cytotoxic effect of *Armeniacae semen* extract on the mouse microglial cells, BV2 cells were cultured with *Armeniacae semen* at final concentrations of 10⁻³ mg/ml, 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 10 mg/ml for 24 h, and MTT assays was then carried out. Cells cultured in *Armeniacae semen*-free media was used as the control. The viability of cells incubated with *Armeniacae semen* extract at concentrations of 10⁻³ mg/ml, 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 10 mg/ml for 24 h was 102.30 \pm 1.57%, 97.05 \pm 0.61%, 98.12 \pm 4.03%, 94.50 \pm 2.48%, and 53.63 \pm 1.11% of the control value, respectively (Fig. 1).

The present results show that *Armeniacae semen* extract exerted no significant cytotoxicity until it was at a concentration of 10 mg/ml.

Effect of *Armeniacae semen* Extract on mRNA Expression of COX-1, COX-2, and iNOS RT-PCR analysis of the mRNA level of COX-1, COX-2 and iNOS was performed in order to provide an estimate of the relative level of expression of these genes. In the present study, the mRNA level of COX-1, COX-2 and iNOS in the control cells was set as 1.00.

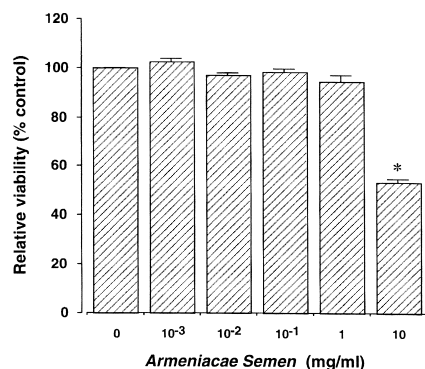


Fig. 1. Cytotoxic Effect of *Armeniacae semen* on BV2 Microglial Cells

BV2 cells were incubated in the presence of the indicated concentrations of *Armeniacae semen* (AS) for 24 h. Cells were stained MTT and analyzed by enzyme-linked immunosorbent assay (ELISA). The experiments were repeated four times. The results are presented as the mean \pm standard errors mean (S.E.M.). * $p < 0.05$ compared to the control.

The level of COX-1 mRNA following treatment with 2 μ g/ml LPS for 24 h was 0.96 \pm 0.05. The level of COX-1 mRNA when the cells were treated with *Armeniacae semen* extract at concentrations of 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M acetylsalicylic acid (ASA) was 0.88 \pm 0.02, 0.79 \pm 0.01, 0.82 \pm 0.04, and 0.33 \pm 0.04, respectively. The level of COX-2 mRNA was markedly increased to 10.07 \pm 1.26 following a treatment with 2 μ g/ml LPS for 24 h. The level of COX-2 mRNA was decreased to 6.62 \pm 0.86, 3.49 \pm 0.44, 1.85 \pm 0.23, and 2.17 \pm 0.252 in cells treated *Armeniacae semen* extract at 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M ASA, respectively. The level of iNOS mRNA following a treatment with 2 μ g/ml LPS for 24 h was markedly increased to 8.17 \pm 1.48. The level of iNOS mRNA was decreased to 6.14 \pm 1.00, 4.01 \pm 0.73, 3.03 \pm 0.91, and 3.48 \pm 0.70 in cells treated with *Armeniacae semen* extract at 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M ASA, respectively (Fig. 2).

The present results show that LPS enhanced COX-2 and iNOS mRNA expression in BV2 cells and *Armeniacae semen* extract suppressed LPS-induced COX-2 and iNOS mRNA expression. However, LPS and *Armeniacae semen* extract slightly decreased COX-1 mRNA expression.

Western Blot Analysis of COX-1, COX-2, and iNOS Protein

LPS treatment did not enhance the expression of COX-1 protein. Treatment with 10⁻² mg/ml, 10⁻¹ mg/ml, and 1 mg/ml of *Armeniacae semen* slightly decreased COX-1 (72 kDa) protein expression. Treatment with 2 μ g/ml LPS for 24 h increased the level of COX-2 protein (74 kDa) and iNOS protein (130 kDa) in BV2 cells. Treatment with *Armeniacae semen* at 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M ASA, however, decreased the LPS-induced expression of COX-2 protein and iNOS protein (Fig. 3).

The present results show that LPS enhanced COX-2 and iNOS protein expressions in BV2 cells and *Armeniacae semen* extract suppressed LPS-induced COX-2 protein and iNOS protein expression. However, LPS and *Armeniacae semen* extract slightly decreased COX-1 protein expression.

Effect of *Armeniacae semen* Extract on PGE₂ Synthesis

From PGE₂ immunoassay, the amount of PGE₂ from the culture medium was increased from 49.23 \pm 1.03 pg/ml to 227.25 \pm 11.89 pg/ml after 24 h of exposure to LPS. PGE₂ synthesis was decreased to 114.92 \pm 5.67 pg/ml, 77.25 \pm 8.90 pg/ml, 63.79 \pm 10.67 pg/ml, and 71.67 \pm 8.23 pg/ml by the treatment with *Armeniacae semen* extract at 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M ASA, respectively (Fig. 4).

The present results show that LPS enhanced PGE₂ synthesis in BV2 cells and *Armeniacae semen* extract suppressed LPS-induced PGE₂ synthesis.

Effect of *Armeniacae semen* Extract on NO Synthesis

From the NO detection assay, the amount of nitrite was increased from 1.10 \pm 0.14 to 7.67 \pm 0.67 μ M after 24 h of exposure to LPS. NO synthesis was decreased to 5.65 \pm 0.36 μ M, 4.65 \pm 0.23 μ M, 1.03 \pm 0.02 μ M, and 2.58 \pm 0.34 μ M by treatment with *Armeniacae semen* extract at 10⁻² mg/ml, 10⁻¹ mg/ml, 1 mg/ml, and 100 μ M ASA, respectively (Fig. 5).

The present results show that LPS enhanced NO synthesis in BV2 cells and *Armeniacae semen* extract suppressed LPS-induced NO synthesis.

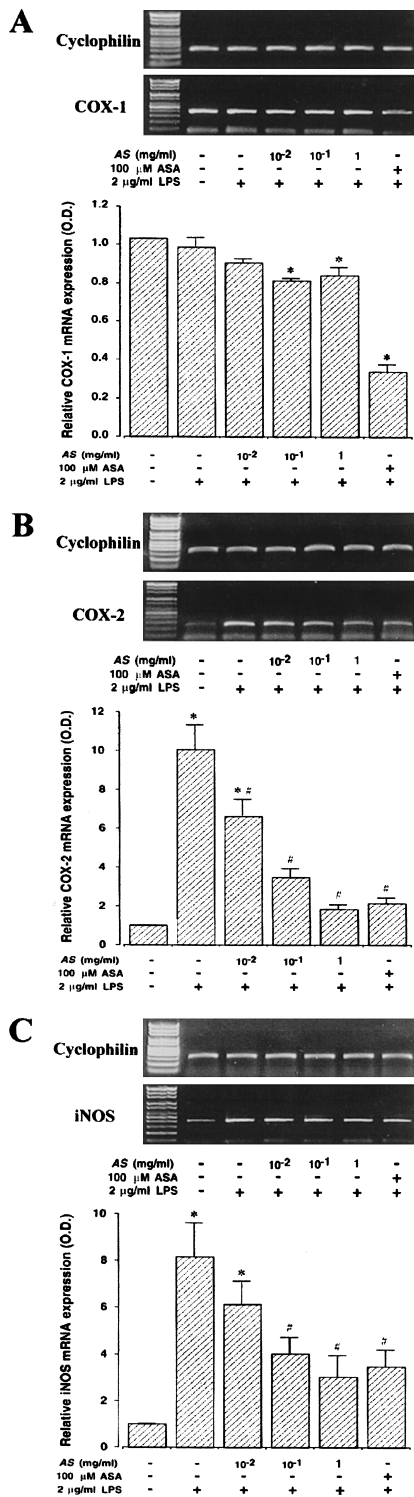


Fig. 2. Results of RT-PCR Analysis of the mRNAs Level of COX-1, COX-2, and iNOS

BV2 cells were incubated in the presence of the indicated concentrations of *Armenicae semen* (AS) or acetylsalicylic acid (ASA) for 24 h in the absence (-) or in the presence (+) of lipopolysaccharide (LPS). RNA were extracted and subjected to RT-PCR analysis of COX-1 (A), COX-2 (B), and iNOS (C). The experiments were repeated four times. Cyclophilin mRNA was used as the internal control. * $p < 0.05$ compared to the control. # $p < 0.05$ compared to the LPS-treated group.

DISCUSSION

Microglia are the macrophage-like cells of the central nervous system (CNS), and they generally considered as im-

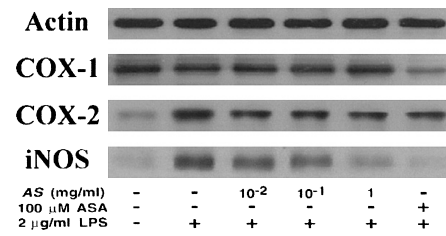


Fig. 3. Results of Western Blot Analysis of the Protein Levels of COX-1, COX-2, and iNOS

BV2 cells were incubated in the presence of the indicated concentrations of *Armenicae semen* (AS) or acetylsalicylic acid (ASA) for 24 h in the absence (-) or in the presence (+) of lipopolysaccharide (LPS). Protein were extracted and subjected to Western blot analysis of COX-1, COX-2, and iNOS. Actin was used as the internal control (46 kDa). The experiments were repeated four times.

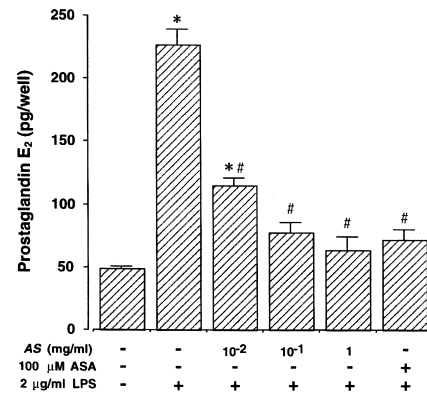


Fig. 4. Measurement of PGE₂ in BV2 Microglial Cells

BV2 cells were incubated in the presence of the indicated concentrations of *Armenicae semen* (AS) or acetylsalicylic acid (ASA) for 24 h in the absence (-) or in the presence (+) of lipopolysaccharide (LPS). Total PGE₂ were detected and analyzed by enzyme-linked immunosorbent assay (ELISA). The experiments were repeated four times. * $p < 0.05$ compared to the control. # $p < 0.05$ compared to the LPS-treated group.

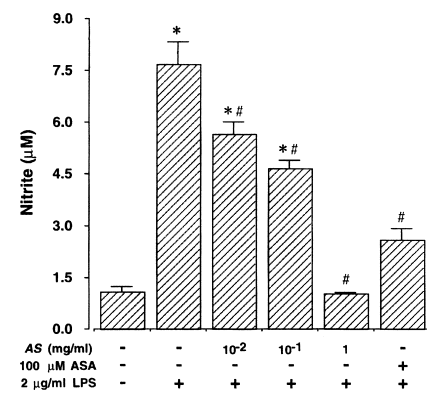


Fig. 5. Measurement of NO Production in Mouse BV2 Microglial Cells

BV2 cells were incubated in the presence of the indicated concentrations of *Armenicae semen* (AS) or acetylsalicylic acid (ASA) for 24 h in the absence (-) or in the presence (+) of lipopolysaccharide (LPS). From the supernatant, NO was detected and analyzed by enzyme-linked immunosorbent assay (ELISA). The experiments were repeated four times. * $p < 0.05$ compared to the control. # $p < 0.05$ compared to the LPS-treated group.

munologically quiescent under normal conditions. Exaggerated pain is induced by the activation of astrocytes and microglia. Astrocytes and microglia are activated by pain-inducing neurotransmitters including substance P, glutamate, and fractalkine. These neurotransmitters are known to excite the pain-responsive neurons, resulting in the production of reactive oxygen species (ROS), NO, prostaglandins (PGs),

and growth factors.¹⁹⁾ Microglia activation induced by CNS injury or infection is associated with neurodegenerative disorders and the release of proinflammatory cytokines.²⁰⁾ These cytokines are the critical mediators of exaggerated pain.

In the present study, LPS treatment enhanced the expression of COX-2 and iNOS, and it increased the synthesis of PGE₂ and NO in mouse BV2 microglial cells. PGE₂ and NO are known to be involved in the processes of inflammation and carcinogenesis. Inducible COX-2 and iNOS are mainly responsible for the production of PGE₂ and NO.^{21,22)} Immunosuppressive drugs such as cyclosporine, tacrolimus, methotrexate, and azathioprine are known to usually down-regulate the functions of T-cell and they suppress cytokines production and release. As these pro-inflammatory cytokines are known to activate the transcription of COX-2 and iNOS genes, immunosuppressive drugs may also reduce PGs and NO production.^{23,24)}

In the present study, *Armeniacae semen* extract inhibited LPS-stimulated enhancement of COX-2 enzyme activity and PGE₂ production in the mouse BV2 microglial cells. Elevation of COX-2 activity is closely associated with the occurrence of cancers, arthritis, and several types of neurodegenerative disorders. PGE₂, a major metabolite of the COX-2 pathway, has emerged as an important lipid mediator of inflammatory and immunoregulatory processes. PGE₂ has been implicated in the pathogenesis of acute and chronic inflammatory disease states.²⁵⁾ Specific COX-2 inhibitors are also known to attenuate the symptoms of inflammation.^{14,26)}

In the present study, *Armeniacae semen* extract inhibited LPS-stimulated enhancement of iNOS enzyme activity and NO production in the mouse BV2 microglial cells. COX activity and the subsequent production of PGE₂ are closely related to the generation of the NO radical. It was reported that NO modulates the activity of COX-2 in a cGMP-independent manner, and NO plays a critical role in the release of PGE₂ by the direct activation of COX-2.²⁷⁾ NO produced by the constitutive isoform of NOS is a key regulator of homeostasis and an important mediator of inflammation in several animal models.²⁸⁾ Especially, the generation of NO by iNOS plays an important role in inflammation, host-defense responses, and tissue repair.²⁹⁾ After exposure to endogenous and exogenous stimulators such as LPS and viral infections, iNOS is quantitatively induced in various cells, and it triggers several deleterious cellular responses inducing inflammation, sepsis, and stroke.^{16,30)} Inhibition of the iNOS expression in murine macrophages has been suggested as another possible mechanism of non-steroidal anti-inflammatory drugs.³¹⁾

In the present study, *Armeniacae semen* extract suppressed PGE₂ and NO production by inhibiting LPS-stimulated enhancement of COX-2 enzyme activity and iNOS expression in the BV2 microglial cells. *Armeniacae semen* is known to have antidiarrheic, antipyretic, antiemetic, and anthelmintic effects.^{1,2)} The anti-inflammatory and analgesic activities of *Armeniacae semen* have also been previously reported.^{2,32)} *Armeniacae semen* has traditionally been used as an ingredient of some oriental medicine prescriptions for relieving of pain and inflammation. Xiao Qing Long Tang and Xing Su San are traditional medicines that have *Armeniacae semen* in their formulation, and these medicines are used for the treatment of bronchitis, asthma with fever, headache and pain in

limbs. *Armeniacae semen* is also used in the Sang Ju Yin medication which is the prescription for the treatment of influenza, pneumonia, and acute tonsillitis.

Armeniacae semen possesses glycoside, amygdaline, starch, fatty acids and etc. Of these, amygdalin is known to be abundant in the *Armeniacae semen*. The content of amygdalin in the *Armeniacae semen* used in this study was 11.00%. Amygdalin has been used as anti-cancer drug, and it was also reported that amygdalin is effective for the relief of pain.³⁻⁵⁾ Based on the results, the suppressive effect of *Armeniacae semen* on COX-2 and iNOS presented in this study can be ascribed to the action of amygdalin. Our present study has shown that *Armeniacae semen* exerts anti-inflammatory and analgesic effects probably by suppressing COX-2 and iNOS expressions, and resulting in the inhibition of PGE₂ and NO synthesis.

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