Chul Hwan Kim, Buyng Su Hwang, Yong Hwang, Young Taek Oh and Jin-Woo Jeong*

Evaluation of antioxidant and antiinflammatory activity of ethanolic extracts of *Polygonum senticosum* in lipopolysaccharide-induced RAW 264.7 macrophages

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Abstract

Objectives: This study aimed to examine the antioxidant activity and antiinflammatory effects of ethanol extract of *Polygonum senticosum* (EPS) on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.

Methods: Antioxidant activity of EPS was assessed by radical-scavenging effects on ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Proinflammatory markers produced by LPS-induced RAW 264.7 macrophages were quantified to assess the antiinflammatory activity of EPS.

Results: Our results showed that EPS significantly increased FRAP and DPPH radical-scavenging activity. Additionally, EPS reduced LPS-induced proinflammatory mediators, such as nitric oxide (NO) and prostaglandin E₂ (PGE₂), along with proinflammatory cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , without significant cytotoxicity. EPS significantly downregulated the expression of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), TNF- α , and IL-1 β in LPS-stimulated RAW 264.7 macrophages. Positive correlations were noted between FRAP and DPPH radical-scavenging activity and antiinflammatory capacity.

Conclusions: Our results indicate that EPS downregulates the expression of pro-inflammatory mediators such as NO, PGE₂, and cytokines (IL-1 β and TNF- α) in LPS-stimulated RAW 264.7 macrophage cells. Further research is needed for its use as a treatment for inflammation and related diseases.

Introduction

Reactive oxygen species (ROS) are commonly produced during the normal metabolic processes and are known to play an important role in cellular signaling and homeostasis [1]. Free radicals elevated by oxidative stress, such as ultraviolet light, environmental stress, diseases, etc., damage cells and are harmful to the body [2]. Oxidative stress causes chronic fatigue, arteriosclerosis, heart disease, peripheral vascular disease, allergic dermatitis, cancer, aging, and kidney disease, and exacerbates existing diseases [2].

To combat ROS overproduction, localized inflammation is induced [3]. Inflammation is a biological defense mechanism that attempts to localize its effects by removing harmful factors (pathogens, toxic compounds, damaged cells, irritants, etc.) [4], suppressing cell damage at an early stage, removing destroyed tissue and necrotic cells in the wound, and regenerating tissue at the same time [3, 5, 6]. Inflammation itself is not a disease but rather a defense system necessary for life [6]. However, it is known that if the inflammatory response is poorly controlled, leading to chronic inflammation, it causes disease and promotes aging [7]. Many studies have been conducted to prevent inflammation of cells with foods rich in antioxidants [3, 8]. Fruits and vegetables contain physiologically active substances, including vitamins, minerals, antioxidants like polyphenols (stilbenes, phenolic acids, benzoic acid, etc.), flavonoids (flavonols, flavanols, anthocyanins, etc.), etc., which are known to prevent aging and diseases [8, 9].

Complementary and alternative medicines (CAM) is known as “traditional oriental medicine” and is best known as natural health practice that uses natural sources such as plants and minerals to treat diseases. CAM has been widely used in general hospitals to overcome illnesses such as infections and complications (cellulitis, acute pneumonia, rheumatoid arthritis, atherosclerosis,

*Corresponding author: Jin-Woo Jeong, Nakdonggang National Institute of Biological Resources, 137, Donam 2-gil, Sangju-si, Gyeongsangbuk-do 37242, Republic of Korea, Phone: +82 54 530 0881; E-mail: jwjeong@nnibr.re.kr

Chul Hwan Kim, Buyng Su Hwang, Yong Hwang and Young Taek Oh, Nakdonggang National Institute of Biological Resources, Sangju-si, Gyeongsangbuk-do, Republic of Korea

periodontitis, etc.) as well as to maintain patients' health [10]. *Polygonum senticosum* (Meisn.) Franch. & Sav. (common *Persicaria senticosa*) is a plant belonging to *Polygonum*, which is known as the largest genus in the Polygonaceae family, and is widely distributed in East Asian countries such as Korea, China, and Japan [11, 12]. The plants have been used locally for numerous ailments, such as inflammatory, eczema, hemorrhoids, circulating blood, and removing blood clots [13, 14]. Its medicinal usage is well-recognized in Asian traditional medicines. *P. senticosum* contains bioactive substances with a diverse range of pharmacological functions (for e.g., treating psoriasis) [13, 14].

This study aimed to investigate the effect of antioxidant and antiinflammatory activities of the ethanol extract of *P. senticosum* (EPS) in lipopolysaccharide (LPS)-induced RAW 264.7 cells.

Materials and methods

Sample preparation

The specimen of *P. senticosum* was collected in Seomchon-ri, Irwol-myeon, Yeongyang-gun, Gyeongsangbuk-do, Republic of Korea. A voucher specimen (NNIBRVP60040) was deposited at the Library of Nakdonggang National Institute of Biological Resources (NNIBR). Aerial parts of *P. senticosum* were air-dried at room temperature and ground to powder using a mechanical grinder. The powdered *P. senticosum* (10 g) were extracted successively with 70% EtOH (600 mL) at room temperature and filtered (Whatman No. 2, Advantec Co., Tokyo, Japan). The filtrate was evaporated (Tokyo Rikakikai Co., Tokyo, Japan) under vacuum to obtain an ethanol extract (205 mg, 2%). The extract was then dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) to obtain a 25 mg/mL stock solution and stored in Freshwater Bioresources Culture Collection (FBCC; FBCC-EP117). This solution was diluted to the desired concentration with physiological saline prior to use.

Reducing power and scavenging activity of the ethanol extract of *P. senticosum*

The reducing power of EPS was determined using the previously described method [15]. EPS extract (0–100 µg/mL) was dissolved in phosphate buffer (0.1 M, pH 6.6) and added to 1% potassium ferricyanide (50 mL, 0.5 g). After the incubation at 50 °C for 30 min, 10% trichloroacetic acid (50 mL, 5 g) was added. 100 µL supernatant was mixed with distilled water (100 µL) and 0.1% ferric chloride (10 mL, 0.01 g). The absorbance was measured at 700 nm with a Cytation-3 microplate reader (Biotek, Shoreline, WA, USA). The result was converted to 100 µg/mL of ascorbic acid equivalent based on the standard curve for ascorbic acid.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined using the method described previously by Cheel et al. [16]. A 100 µL aliquot of the diluted EPS extracts (0–100 µg/mL)

was added to a methanolic solution (100 µL) of DPPH radical (final concentration, 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance was then measured spectrophotometrically at 517 nm. The tests were run in duplicate, and all samples were analyzed in triplicate.

Cell viability

The effect of EPS on the growth of RAW 264.7 cells was determined by an MTT assay. RAW 264.7 cells purchased from the American Type Culture Collection (Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin in a humidified incubator containing 5% CO₂ at 37 °C. RAW 264.7 cells were seeded into 96-well plates at a density of 4×10^3 cells/well and maintained at 37 °C for 24 h. The cells were exposed to various concentrations of EPS (0, 25, 50, and 100 µg/mL) for 1 h and stimulated with LPS (500 ng/mL). After 24 h of incubation, the MTT (0.5 mg/mL in phosphate-buffered saline, PBS) solution was added to each well and incubated for another 3 h. The formazan crystals were dissolved in 200 µL of DMSO, and the cell viability was determined by measuring the absorbance at a wavelength of 540 nm with a microplate reader (Dynatech MR-7000; Dynatech Laboratories Inc., Chantilly, VA, USA).

Measurement of nitric oxide and prostaglandin E₂ production

The nitrite concentration in the medium was measured according to the Griess reaction and the calculated concentration was taken as an indicator of nitric oxide (NO) production. The supernatants of cell cultures were mixed with an equal volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water). The optical density at 540 nm was measured and calculated against a sodium nitrite standard curve. The accumulated prostaglandin E₂ (PGE₂) in the culture medium was measured using a PGE₂ enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions.

Measurement of proinflammatory cytokine production

The inhibitory effect of EPS on the production of proinflammatory cytokines—tumor necrosis factor (TNF)-α and IL-1β—from LPS-treated RAW 264.7 cells was determined using a mouse ELISA kit (R&D Systems, Minneapolis, MN, USA), as described previously [17].

RNA isolation and reverse transcriptase polymerase chain reaction assay

After the removal of supernatants from cells cultured in the presence of EPS alone or in combination with LPS for 24 h, total RNA was isolated using TRIzol reagent (Invitrogen Co., Carlsbad, CA, USA) according to the manufacturer's instructions. From each sample, 2 µg of total RNA was reverse transcribed to single-stranded cDNA by M-MLV reverse transcriptase (Promega, Madison, WI, USA). Then polymerase chain reaction (PCR) analyses were performed on the

aliquots of the cDNA preparations to detect cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS), TNF- α , and IL-1 β gene expression. The iNOS, COX-2, IL-1 β , and TNF- α genes were amplified from the cDNA using PCR. The PCR primers were as follows: mouse iNOS (5'-ATG TCC GAA GCA AAC ATC AC-3' and 5'-TAA TGT CCA GGA AGT AGG TG-3'), COX-2 (5'-CAG CAA ATC CTT GCT GTT CC-3' and 5'-TGG GCA AAG AAT GCA AAC ATC-3'), IL-1 β (5'-ATG GCA ACT GTT CCT GAA CTC AAC T-3' and 5'-TTT CCT TTC TTA GAT ATG GAC AGG AC-3'), and TNF- α (5'-ATG AGC ACA GAA AGC ATG ATC-3' and 5'-TAC AGG CTT GTC ACT CGA ATT-3'). After amplification, the PCR products were electrophoresed in 1% agarose gels and visualized by ethidium bromide (EtBr) staining and ultraviolet irradiation. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Protein extraction and Western blot analysis

After 24 h of EPS treatment, the total proteins of RAW 264.7 cells were directly prepared using a lysis buffer (0.5% Triton, 50 mM β -glycerophosphate [pH 7.2], 0.1 mM sodium vanadate, 2 mM MgCl₂, 1 mM EGTA, 1 mM dithiothreitol, 2 μ g/mL leupeptin, 0.1 mM phenylmethylsulfonyl urea, and 4 μ g/mL aprotinin). The protein concentration in the cell lysate was determined using detergent-compatible protein assay from Bio-Rad (Hercules, CA, USA). Equal amount of protein was resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH, USA). Subsequently, the membranes were blocked in Tris-buffered saline (10 mM Tris-Cl, pH 7.4) containing 0.5% Tween 20 and 5% nonfat dry milk for 1 h. After incubation with the appropriate primary antibodies for 1 h, the membranes were incubated for 1 h at room temperature with secondary antibodies conjugated to horseradish peroxidase. The protein bands were detected by ECL solution.

Statistical analysis

All experiments were performed at least three times. Data was analyzed using the GraphPad Prism software (version 5.03; GraphPad Software, Inc., La Jolla, CA, USA) and expressed as the mean $\pm$ standard deviation (SD). Differences between groups were assessed using analysis of variance followed by analysis of variance (ANOVA)-

Tukey's posthoc test, and $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Antioxidant activity of EPS

The presence of antioxidants causes the Fe³⁺/ferric cyanide complex to be reduced to the ferrous form, the concentration of which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. We assessed the ability of EPS to reduce Fe³⁺ to Fe²⁺ using the method mentioned previously [18], using ascorbic acid as a positive control. EPS exhibited a dose-dependent reducing power across the measured concentrations (0, 25, 50, and 100 μ g/mL), with 100 μ g/mL of EPS having the highest reducing power (%) (Figure 1A). The reducing power was greater than the positive control. Next, we performed the DPPH radical-scavenging assay using different concentrations of the EPS extract. We observed that the antioxidant activity of EPS increased with increasing concentrations (Figure 1B).

Effects of the ethanol extract of *P. senticosum* on lipopolysaccharide-induced cytotoxicity

Effect of EPS on the viability was investigated on LPS-induced RAW 264.7 cells using an MTT assay and cell morphological change, respectively. EPS alone did not show any obvious cytotoxic effect at the concentrations of 25–100 μ g/mL. However, a statistically significant level of cytotoxicity was found at concentrations of ≥ 200 μ g/mL EPS (Figure 2A, C). Moreover, LPS (500 ng/mL) treatment along with 25, 50, and 100 μ g/mL concentrations of EPS did

Figure 1: Antioxidant (Reducing power, DPPH) activities of ethanol extract of *P. senticosum* (EPS).

(A) Reducing power and (B) DPPH free radical-scavenging activity of EPS obtained with bioprotease in an *in vitro* cell-free experiments. Each value is presented as the mean $\pm$ SD and is representative of the results obtained from three independent experiments. The significance was determined by the Student's t-test (* $p < 0.05$, ** $p < 0.01$, compared with control).

not affect the cell viability (Figure 2B, D). Therefore, a concentration of EPS within this range was applied in the remaining experiments.

Effect of the ethanol extract of *P. senticosum* on nitric oxide and prostaglandin E₂ production in lipopolysaccharide-stimulated RAW 264.7 cells

Proinflammatory mediators such as NO and PGE₂ are important in the inflammatory response. To determine the level of NO production, nitrite released into the culture medium was measured using Griess reagent. As shown in Figure 3A, LPS alone (μ M) markedly induced NO production in the cells compared with that in the control. However, pretreatment with EPS (up to 100 μ g/mL) significantly reduced the levels of NO production in LPS-stimulated RAW 264.7 cells in a concentration-dependent manner

(Figure 3A). The effects of EPS on the production of PGE₂, another important inflammatory mediator, were also investigated in LPS-stimulated RAW 264.7 cells. As shown in Figure 3B, treatment of RAW 264.7 cells with LPS resulted in a marked increase in PGE₂ release (pg/mL) compared with that in the untreated control (pg/mL) after 24 h of exposure to LPS. However, after 25, 50, and 100 μ g/mL of EPS treatment, the PGE₂ levels were lowered to and pg/mL, respectively. Thus, EPS reduced LPS-mediated PGE₂ production in a concentration-dependent manner. These results suggest that pretreatment with EPS results in significant reduction in the levels of LPS-mediated proinflammatory mediators.

Effect of EPS on LPS-stimulated iNOS and COX-2 expression

To elucidate the mechanism involved in the down regulation of NO and PGE₂ by EPS in LPS-stimulated RAW 264.7

Figure 2: Effect of EPS on the viability of RAW 264.7 cells.

The cells were treated with the indicated concentrations of EPS alone (A and C) or in combination with LPS (500 ng/mL) (B and D) for 24 h. Cell viability was assessed using the MTT assay, and the results are expressed as the percentage of surviving cells over control cells (no addition of EPS). Each value is presented as the mean $\pm$ SD and is representative of the results obtained from three independent experiments. The significance was determined by the Student's t-test (* p <0.05, compared with control group; NS, not significant). The cell morphology was visualized with an inverted microscope (magnification, $\times$ 200), respectively.

Figure 3: Down regulation of nitric oxide (NO) and prostaglandin E_2 (PGE_2) production by EPS in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.

(A) Cells were pretreated with various concentrations of EPS (25, 50, and 100 $\mu\text{g}/\text{mL}$) for 1 h prior to incubation with LPS (500 ng/mL) for 24 h, and nitrite content was measured using the Griess reaction. (B) The PGE_2 concentration was measured in culture media using a commercial enzyme-linked immunosorbent assay kit. Each value is presented as the mean $\pm$ SD and is representative of the results obtained from three independent experiments. The significance was determined by the Student's t-test (* $p < 0.05$, compared with control group; # $p < 0.05$ LPS groups vs. LPS + EPS group).

cells, the effect of EPS on iNOS and COX-2 protein and gene expression levels was investigated by Western blot and reverse transcriptase PCR (RT-PCR) analyses (Figure 4). iNOS and COX-2 protein and mRNA expression in unstimulated RAW 264.7 cells was hardly detectable. However, iNOS and COX-2 mRNA expression significantly increased in response to LPS. EPS significantly reduced the iNOS and COX-2 mRNA levels in a concentration-dependent manner (Figure 4A). Under the similar conditions, iNOS and COX-2 mRNA levels were correlated with their protein levels (Figure 4B). iNOS and COX-2 protein expressions were downregulated in a concentration-dependent manner after the EPS pretreatment in LPS-induced RAW 264.7 cells. These results indicated that reduced mRNA and protein

expression of iNOS and COX-2 by EPS was responsible for reducing NO and PGE_2 production.

Effect of the ethanol extract of *P. senticosum* on proinflammatory cytokines production in lipopolysaccharide-stimulated RAW 264.7 cells

Since EPS was revealed as a potent reducer of the proinflammatory mediators, we further investigated its effect on proinflammatory cytokine release by ELISA. The results obtained showed that treatment of RAW 264.7 cells with LPS alone (pg/mL) resulted in a significant increase in

Figure 4: Down regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression by EPS in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.

RAW 264.7 macrophage cells were pretreated with EPS (25, 50, and 100 $\mu\text{g}/\text{mL}$) 1 h prior to incubation with LPS (500 ng/mL) for 24 h. (A) Total RNA was prepared for reverse transcriptase PCR (RT-PCR) analysis of iNOS and COX-2 gene expression in LPS-stimulated RAW 264.7 cells. (B) Cell lysates were then prepared and Western blot analysis was performed using antibodies specific for murine iNOS and COX-2. The experiment was repeated three times. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and actin and were used as internal controls for the RT-PCR and Western blot analyses, respectively.

Figure 5: Effect of EPS on lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-1β production in RAW 264.7 cells. Cells were pretreated with the indicated concentrations of EPS (25, 50, and 100 μg/mL) for 1 h prior to incubation with LPS (500 ng/mL). After incubation for 24 h, the levels of IL-1β (A) and TNF-α (B) present in the supernatants were measured using ELISA kits. Each value is presented as the mean ± SD and is representative of the results obtained from three independent experiments. The significance was determined by the Student's t-test (*p<0.05, compared with control group; #p<0.05 LPS groups vs. LPS + EPS group).

production of IL-1β levels compared with that generated under control conditions (pg/mL) (Figure 5A). However, pretreatment with EPS considerably reduced IL-1β levels to and pg/mL (after 25, 50, and 100 μg/mL EPS treatment, respectively) in a concentration-dependent manner. Under these conditions, pretreatment with EPS also reduced TNF-α production dramatically to and pg/mL at 25, 50, and 100 μg/mL of EPS concentration, respectively (Figure 5B). We performed Western blot analysis and RT-PCR to detect of protein and mRNA levels to examine whether reducing IL-1β and TNF-α production by EPS was associated with decreased levels of IL-1β and TNF-α at the transcriptional and translational level. As shown in Figure 6A, B, treatment of RAW 264.7 cells with EPS before LPS treatment resulted in a concentration-dependent decrease in the levels of mRNA and protein of IL-1β and TNF-α cytokines,

suggesting that EPS-mediated reduction of these cytokines may also be regulated at the transcriptional and translational level.

Discussion

Although the *P. senticosum* is widely used in traditional oriental medicine owing to its multiple beneficial potentials, the molecular targets and mechanisms underlying its antiinflammatory activities are still unclear. Inhibitors of proinflammatory mediators and cytokines have been considered as candidates for antiinflammatory agents. In this study, we have demonstrated that EPS reduced LPS-induced proinflammatory mediators (NO, PGE₂) and cytokines (IL-1β and TNF-α) production in RAW 264.7

Figure 6: Effect of EPS on lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-1β expression in RAW 264.7 cells. (A) RAW 264.7 macrophage cells were pretreated with EPS (25, 50, and 100 μg/mL) 1 h prior to incubation with LPS (500 ng/mL) for 24 h. (A) Total RNA was prepared for reverse transcriptase PCR (RT-PCR) analysis of IL-1β and TNF-α gene expression in LPS-stimulated RAW 264.7 cells. (B) Cell lysates were then prepared and Western blot analysis was performed using antibodies specific for murine IL-1β and TNF-α. The experiment was repeated three times. GAPDH and actin and were used as internal controls for the RT-PCR and Western blot analyses, respectively.

macrophages through down regulation of respective protein and mRNA expression.

ROS are known as signaling molecules related to any host-defense response [19]. ROS are required for important physiological functions and behaviors. However, they act as inflammatory mediators in the progression of inflammatory disorders. Moreover, excessive production of ROS and related species has harmful effects on cell homeostasis, structures, and functions and results in oxidative stress [20–23]. Oxidative stress is highly implicated in various noncommunicable diseases such as inflammation, cancer, chronic fatigue, arteriosclerosis, heart disease, peripheral vascular disease, allergic dermatitis, aging, kidney disease, and cardiovascular disease [18, 24–26]. ROS, associated with the inflammatory response and oxidative stress, is removed by antioxidants in healthy physiological conditions [27–29]. To investigate the antioxidant activity of EPS, we evaluated its reducing power and DPPH radical-scavenging activity. Antioxidant activity of EPS was increased in a concentration-dependent manner (Figure 1A, B).

Among the proinflammatory mediators released by macrophages, NO and PGE₂ have been implicated as the main cytotoxic mediators participating in the innate response in mammals. Although NO generation by macrophages is a part of the human immune response, activated macrophages following bacterial infection or inflammatory responses generate a large amount of NO from arginine and oxygen through transcriptional activation of iNOS. Furthermore, excessive release of NO by activated macrophages is correlated with the progression of inflammation-associated disorders [30, 31]. Many studies have reported that the expression of COX-2, a key enzyme involved in the conversion of arachidonic acid to PGE₂, is upregulated in activated macrophages. In addition, numerous reports demonstrated that the inhibition of COX-2 induction and/or activity reduces the progression of inflammatory disorders [32, 33]. Thus, inhibitors of these proinflammatory mediators have been considered as potential candidates for antiinflammatory agents to alleviate the progression of inflammatory diseases such as atherosclerosis, chronic hepatitis, pulmonary fibrosis, and rheumatoid arthritis caused by the activation of macrophages. Our results clearly demonstrated that EPS reduced NO and PGE₂ production via the suppression of iNOS and COX-2 mRNA and protein expression, respectively, which appears to be due to the transcriptional suppression of these genes (Figures 3, 4).

The release of proinflammatory cytokines is elevated predominantly by activated macrophages during pathogen

infection, and they are involved in the upregulation of inflammatory reactions. Of these cytokines, IL-1 β is a potent activator of immune responses directed against bacterial infections. Abnormal IL-1 β release changes the conditions toward a pathological microenvironment, resulting either in chronic inflammation or in the absence of a proper immune surveillance against infections [34, 35]. In addition, inflammation is often associated with the overexpression of TNF- α , which is a multifunctional cytokine involved in the signaling pathways implicated in inflammation, immunity, cell survival, and even tumorigenesis. This cytokine exhibits its proinflammatory activity by regulating several intercellular and vascular cell-adhesion molecules, resulting in the recruitment of leukocytes to sites of inflammation [36, 37]. Moreover, the production of TNF- α and IL-1 β is crucially required for the synergistic induction of NO and PGE₂ production in LPS-stimulated macrophages [38, 39]. These findings suggest that the inhibition of these cytokines' production could be a useful approach as a treatment strategy for various inflammatory diseases. In the current investigation, the concentrations of TNF- α and IL-1 β were markedly increased after treatment with LPS in RAW 264.7 macrophages, whereas pretreatment with EPS significantly reduced this effect in a concentration-dependent manner (Figure 5). We also found that the suppressive effect of EPS on LPS-induced production of TNF- α and IL-1 β was mediated at the transcription level (Figure 6). These findings provide evidence that EPS possesses potentially useful antiinflammatory capacity.

In conclusion, the results of this study have demonstrated that EPS treatment results in a decrease in proinflammatory mediators following LPS stimulation in RAW 264.7 cells. EPS also significantly reduced the release of IL-1 β and TNF- α and decreased their mRNA and protein expression levels in a concentration-dependent manner. Therefore, further investigation of antiinflammatory effects of EPS on molecular level in detail is warranted.

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