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Original Article

Platycodon grandiflorum Extracts Exhibits Anti-inflammatory Properties by Down-regulating MAPK Signaling Pathways Lipopolysaccharide-treated RAW264.7 Cells

Hyeon Jin Kim, Seong-Yun Jeong† and Jin-Kyung Kim†

Department of Biomedical Science, Catholic University of Daegu, Gyeongsan-Si 700-712, Korea

Platycodon grandiflorum is a medicinal herb that is used to treat pulmonary and respiratory allergic disorders. The objective of this study was to investigate the protective effects of ethyl acetate extract of *Platycodon grandiflorum* (PGEA) against inflammation and to discern the molecular mechanism of PGEA in lipopolysaccharide (LPS)-induced signal pathways in RAW264.7 macrophage cells. PGEA suppressed the generation of nitric oxide (NO) and the expression of inducible NO synthase induced by LPS in RAW264.7 cells, and inhibited the release of pro-inflammatory cytokines induced by LPS in RAW264.7 cells. Western blot analysis showed that PGEA suppressed LPS-induced phosphorylation of p38 and c-Jun N-terminal kinase (JNK) but not extracellular signal-regulated kinase and Iκ-Bα degradation. Inactivation of JNK and p38 was effectively alleviated by PGEA, which subsequently affected the activation of c-Jun and c-Fos, which are the essential components of the activator protein-1 (AP-1) transcription complex. Taken together, the results indicate PGEA suppress the activation of p38, JNK, and AP-1, thereby inhibiting the generation of NO and pro-inflammatory cytokines, which affect the regulation of inflammation. PGEA may be useful for the treatment of various inflammatory diseases.

Key Words: *Platycodon grandiflorum*, Inflammation, Macrophage, Cytokines, AP-1

INTRODUCTION

Many medicinal herbs have been shown to have therapeutic prowess in the control of various diseases. *Platycodon grandiflorum* A. DC (Campanulaceae) is a well-known perennial herb. It is also known as balloon flower or bell flower. The roots of this species have been used as a traditional Asian medicine for the treatment of pulmonary and respiratory disorders, such as bronchitis, tonsillitis, and asthma (Ozaki, 1995). Pharmacologic studies of *P. grandiflorum* root have demonstrated several biological activities, including chemopreventive (Kim et al., 2005; Lee et al., 2008; Shin et al., 2009), anti-oxidant (Lee et al., 2004), anti-hyperglycemic (Zheng et al., 2007), hepatoprotective, (Lee et al., 2008; Khanal et al., 2009), immunomodulatory (Han et al., 2009; Kim et al., 2011) and anti-obesity effects (Zheng et al., 2007; Noh et al., 2010). The main chemical constituents of *P. grandiflorum* identified to date include about 40 oleanane-type saponins; they are regarded as being responsible for these various biological effects.

Inflammation is an obligatory part of a body's reaction to infection, irritation, or other injury. The key features of inflammation are redness, warmth, swelling, and pain. Various mediators are the physiological messengers of the inflammatory response and some of the principal molecules involved are tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, and nitric oxide (NO). The main cells

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†Corresponding author: Jin-Kyung Kim. Department of Biomedical Science, College of Natural Science, Catholic University of Daegu, 330 Geumrak-Ri, Gyeoungsan-Si 700-712, Korea.
Tel: +82-53-850-3774, e-mail: toto0818@cu.ac.kr

†Corresponding author: Seong-Yun Jeong. Department of Biomedical Science, College of Natural Science, Catholic University of Daegu, 330 Geumrak-Ri, Gyeoungsan-Si 700-712, Korea.
Tel: +82-53-850-3772, e-mail: jsymicro@cu.ac.kr

involved in the inflammatory response are monocytes/ macrophages, polymorphonuclear leucocytes, and endothelial cells. When these cells become activated, they aggregate and infiltrate tissue where they undergo a respiratory burst, increasing their oxygen use and production of cytokines and other mediators of inflammation. Once the threat has been overcome, the inflammatory responses must end. If left uncontrolled, the responses can cause various diseases such as arthritis, asthma, allergies, skin diseases, and assorted autoimmune diseases. Various efforts to regulate inflammatory reactions have been explored. One avenue involves the discovery of medical herbs capable of suppressing inflammation (Setty and Sigal, 2005; Kaplan et al., 2006; Wu et al., 2010)

Although *P. grandiflorum* has potent biological and physiological functions, its' involvement in the regulation of inflammatory reactions remains unknown. In this study, we examined the effects of the extracts of *P. grandiflorum* on lipopolysaccharide (LPS)-induced inflammatory responses in murine RAW264.7 macrophages. *P. grandiflorum* ethyl acetate extract (PGEA) inhibited LPS-induced phosphorylation of p38 and c-Jun N-terminal kinase (JNK) as well as activator protein-1 (AP-1), thereby inhibiting the expression of inflammatory mediators such as NO and pro-inflammatory cytokines.

MATERIALS AND METHODS

Chemicals and reagents

LPS derived from *Escherichia coli* (0111:B4) and dimethylsulfoxide (DMSO) was obtained from Sigma-Aldrich (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Hyclone (Logan, UT, USA). The final concentrations of DMSO never exceeded 0.1%, which did not affect the assay systems. Rabbit polyclonal antibodies to inducible NO synthase (iNOS), c-Jun, phospho-c-Jun, c-Fos, phospho-c-Fos, JNK, phospho-JNK, extracellular signal-regulated kinase (ERK) 1/2, phospho-ERK1/2, p-38, phospho-p38, and poly-ADP-ribose polymerase (PARP) were obtained from Cell Signaling Technology (Danvers, MA, USA). Monoclonal Ab (mAb) to inhibitor of nuclear factor-kappa B (NF-κB) (IκBα) was obtained from Cell Signaling Technology. mAb to β-actin was obtained from Sigma-Aldrich.

Preparation of plant material

P. grandiflorum roots were purchased from Sam-Hong medicinal herb store (Seoul, Korea) in 2011. A voucher specimen (number PG-11-1) has been deposited at the Department of Biomedical Science, Catholic University of Daegu, Gyeongsan, Korea. In order to get water extract (PGW), the dried roots (100 g) were put into herbal bags and were extracted with distilled water at 100℃ for 6 h. PGW was filtered through a 0.2 μm pore-size filter, freeze-dried (yield, 44.3 g), and kept at -20℃ until use. Other dried roots (100 g) were ground into powder, and extracted with methanol (2 L) by shaking for 72 h at room temperature. After the powder particles had settled, the clear yellow supernatant was filtered with a 0.2 μm pore-size polytetrafluoroethylene filter (Millipore, Billerica, MA, USA), and the methanol extract of *P. grandiflorum* (PGM) was concentrated (21.5 g, dry weight) by a rotary vacuum evaporator (Eyela, Tokyo, Japan). To localize the active fractions, PGM was subsequently partitioned using ethyl acetate. The ethyl acetate solvent was removed from by rotating vacuum evaporation. The residual *P. grandiflorum* ethyl acetate (PGEA) layer (724 mg dry weight) was dissolved in methanol as a stock solution at 10 mg/mL concentration, and stored in aliquots at -20℃ until used.

Cells and cell viability assay

RAW264.7 murine macrophages obtained from the Korean Cell Bank (Seoul, Korea) were cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37℃ in a 5% CO_2 atmosphere. The effects of various PGEA preparations on cell viability were tested using the CellTiter 96® AQ_{ueous} One Solution Assay of cell proliferation (Promega, Madison, WI, USA). RAW264.7 cells were plated at a density of 2×10^4 cells in a 96-well flat-bottom plate, and vehicle (0.01% DMSO) or PGEA was added to each plate at the indicated concentrations. After 24 h incubation, the number of viable cells was counted according to the manufacturer's instructions. This

assay is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to formazan, which has an optimum absorption at 490 nm. The quantity of the product in the cell culture is indicated by the optical density of formazan at 490 nm, which is directly proportional to the number of living cells.

Measurement of NO and inflammatory cytokines

The amount of nitrite, IL-6 and TNF-α produced by the mouse macrophages was measured in RAW264.7 cell culture supernatant. RAW264.7 cells were plated at a density of 2.5×10^5 cells in a 48-well cell culture plate with 500 μL of culture medium and incubated for 12 h. They were then treated with the indicated concentrations of PGEA plus LPS (100 ng/mL) and incubated for another 24 h. NO secreted from cells is rapidly oxidized to nitrite in the culture medium, therefore determinations of nitrite concentrations were used as a measurement of NO production. Fifty microliters of culture supernatant were mixed with 50 μL of Greiss reagent (Promega) in triplicate in 96-well plates. After incubation for 10 min at room temperature, absorbance was read at 550 nm and the level of NO was calculated using a standard curve with known sodium nitrite concentrations. IL-6 and TNF-α were measured using an enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

Western blotting analysis

Whole cell lysates were extracted by Pro-prep protein extraction solution (iNtRON Biotechnology, Seongnam-Si, Korea) according to the manufacturer's instructions. Meanwhile, each nuclear and cytosol extract was isolated using a NE-PER nuclear and cytoplasmic extraction reagent kit (Pierce, Rockford, IL, USA). Whole cell lysates and nuclear fractions were estimated with a dye reagent concentrate (Bio-Rad Laboratories, Hercules, CA, USA), then resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were electrophoretically transferred to nitrocellulose membranes. Immunoreactive bands were detected by incubating the samples with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using a WEST-ZOL plus Western Blot Detection System (iNtRON Biotechnology). Since PARP is found in the cell nucleus it was used as a loading control for nuclear protein.

Table 1. Effect of *Platycodon grandiflorum* extracts on NO release in LPS-stimulated RAW264.7 cells[1]

Platycodon grandiflorum extracts	Concentration (μg/mL)	Inhibition of NO release (%)
Water	50	7.15 ± 0.99
	500	4.94 ± 4.24
Methanol	50	8.43 ± 4.20
	500	7.15 ± 0.99
Ethyl acetate	50	46.81 ± 1.59***
	500	82.68 ± 0.04***

[1]LPS-stimulated RAW264.7 cells were cultured with indicated concentration of PG extracts for 24 h and NO production was measure. Statistical significance is based on the difference when compared with LPS-stimulated cells (***$P < 0.001$).

Statistical analysis

The data are depicted as the mean ± SEM. Student *t*-test was performed using the GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, USA) and $P < 0.05$ was considered as statistically significant.

RESULTS

Effects of PGEA on the release of NO and expression of iNOS

Murine RAW264.7 macrophages were chosen for use in an investigation of the anti-inflammatory effects of the extracts of *P. grandiflorum*. Three different extracts of *P. grandiflorum* were prepared: water (PGW), methanol (PGM), and ethyl acetate (PGEA). The extracts were screened for their inhibition of NO release in LPS-stimulated RAW264.7 cells. As shown in Table 1, PGEA strongly inhibited LPS-induced NO production among extracts at 50 and 500 μg/mL. In contrast, PGW and PGM did not inhibit LPS-induced NO production.

Since PGEA blocked NO secretion in LPS-simulated RAW264.7 cells, the next set of experiments investigated the anti-inflammatory effect of PGEA. The first experiment

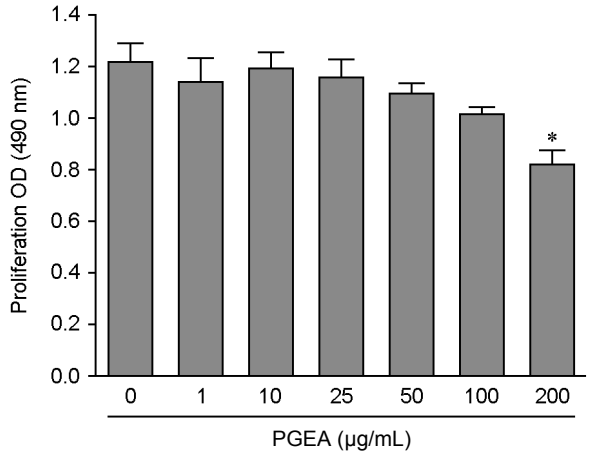

Fig. 1. Effects of PGEA on murine macrophage viability. RAW264.7 cells were treated with the indicated concentrations of PGEA for 24 h, and cell proliferation was determined. The results are reported as mean ± SEM of three independent experiments in triplicate.

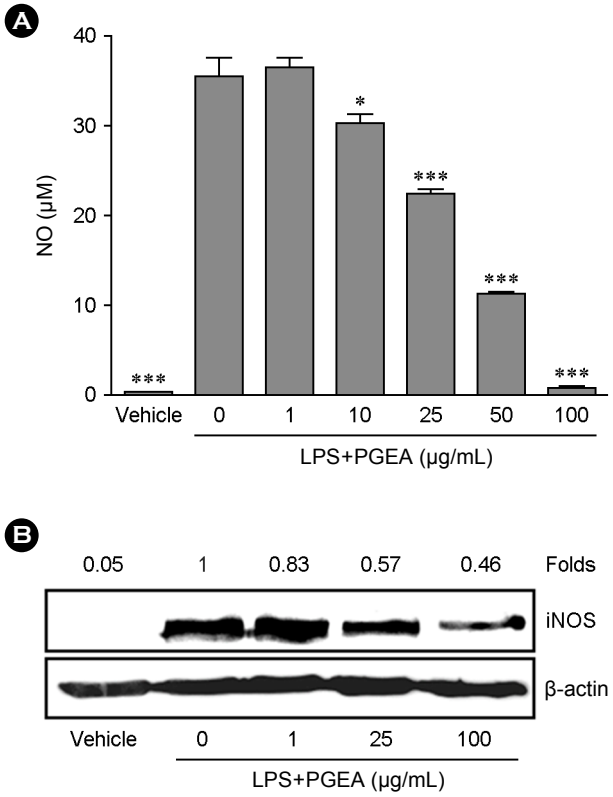

Fig. 2. Effects of PGEA on LPS-induced NO release and iNOS expression. (A) RAW264.7 cells were treated with the indicated concentrations of PGEA in the presence of 100 ng/mL of LPS or with LPS alone for 24 h, and NO release was determined. The results are reported as mean ± SEM of three independent experiments in triplicate. Statistical significance is based on the difference when compared with LPS-stimulated cells ($^{*}P < 0.05$, $^{***}P < 0.001$). (B) Thirty micrograms of protein obtained from each cell lysate was resolved by 10% SDS-PAGE for iNOS determination. β-actin expression is shown as a loading control. The bands were quantified using NIH image analysis software and their relative intensity was expressed as fold-change against the image of the LPS-stimulated RAW264.7 cells.

examined whether PGEA was cytotoxic to RAW264.7 cells. No notable cytotoxicity was observed when RAW264.7 macrophages were exposed to up to 100 μg/mL of PGEA for 24 h (Fig. 1). Thereafter, 100 μg/mL PGEA was used. We first sought to determine the effect of PGEA on the LPS-induced release of NO by RAW264.7 cells. As shown in Fig. 2A, PGEA inhibited LPS-induced NO release in a dose-dependent manner. The nitrite concentrations in LPS-stimulated cells and cells exposed to 100 μg/mL of PGEA were 35.06 ± 2.02 μM and 0.61 ± 0.16 μM, respectively. To determine the mechanism by which PGEA reduced LPS-induced NO, the effect of PGEA on iNOS protein expression in RAW264.7 cells was studied using Western blot analysis, since those enzymes catalyze the reaction for NO. Consistent with the findings related to NO production, the protein expression of iNOS induced by LPS in RAW-264.7 cells was also reduced by PGEA treatment (Fig. 2B).

Effects of PGEA on the release of pro-inflammatory cytokines

We next examined if PGEA reduced the release of pro-inflammatory cytokines in LPS-stimulated RAW264.7 cells. Although the concentrations of IL-6 and TNF-α were not detected in vehicle-treated RAW264.7 cells, LPS treatment elevated the levels of IL-6 (11.04 ± 0.80 ng/mL) and TNF-α (18.85 ± 0.90 ng/mL) in LPS-treated RAW264.7 cells. PGEA induced a marked suppression of increases induced by LPS in these cytokines (Fig. 3). LPS-treated RAW264.7 cells exposed to PGEA at concentrations of 1, 25, and 100 μg/mL displayed a dose-dependent inhibited production of IL-6 (9.82 ± 0.89 ng/mL, 8.06 ± 0.07 ng/mL, and 1.02 8.06 ± 0.09 ng/mL, respectively) and TNF-α production (17.70 ± 1.28 ng/mL, 15.63 ± 2.05 ng/mL, and 12.92 ± 0.66 ng/mL, respectively).

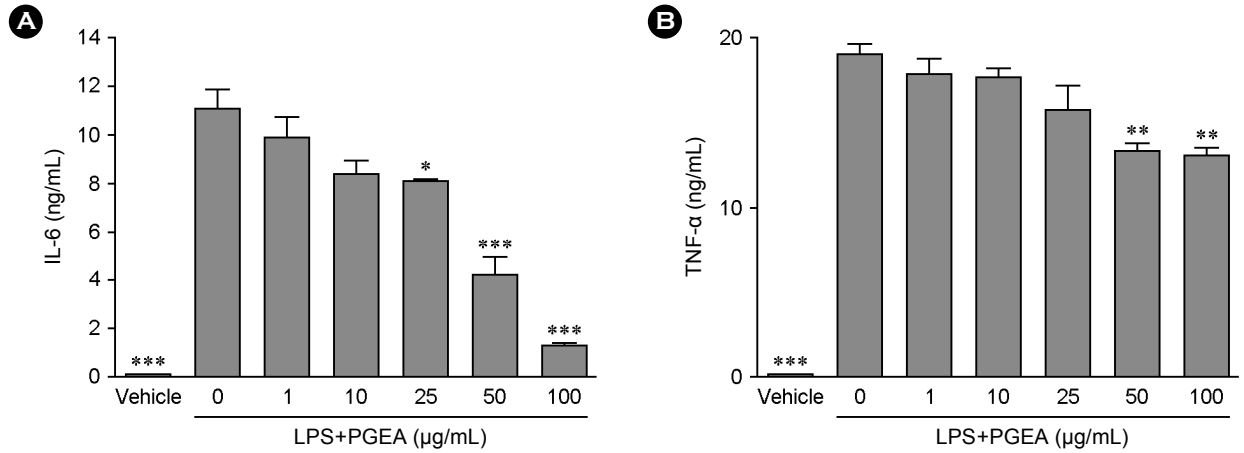

Fig. 3. Effects of PGEA on LPS-induced inflammatory cytokine production in murine macrophages. RAW264.7 cells were treated with the indicated concentrations of PGEA in the presence of 100 ng/mL LPS or with LPS alone for 24 h. The cell culture media were then collected, and the amount of (A) IL-6 and (B) TNF-α released was measured. The results are reported as mean ± SEM of three independent experiments in triplicate. Statistical significance is based on the difference when compared with LPS-stimulated cells ($^{*}P < 0.05$, $^{***}P < 0.01$, $^{***}P < 0.001$).

Effect of PGEA on mitogen-activated protein kinases (MAPKs) and AP-1 activation

Since NF-κB signals regulate the transcription of various genes, including inflammatory mediators, RAW264.7 macrophages were treated with LPS in the presence or absence of PGEA and the degradation of Iκ-Bα, an important biochemical event for the nuclear translocation of NF-κB, was determined. We initially determined the time of LPS treatment that cause Iκ-B degradation. Since Iκ-B degradation was induced at 20~30 min after LPS treatment, we treated LPS for 20 min (data not shown). As shown in Fig. 4A, PGEA treatment had no effect of the degradation of Iκ-Bα induced by LPS stimulation. In addition to NF-κB, MAPK pathways are also involved in the regulation of pro-inflammatory mediator expression (Kaminska et al., 2005; Ashwell et al., 2006). Treatment with LPS for 20 min resulted in a significant increase in the phosphorylation of p38, JNK, and ERK compared to the vehicle-treated group (Fig. 4A). PGEA markedly prevented LPS-induced increase of p38 and JNK phosphorylation in a concentration-dependent manner, but not phosphorylation of ERK (Fig. 4A).

Since AP-1, a heterodimeric protein that comprises c-Jun and c-Fos, regulates several genes involved in inflammation and is mainly activated through MAPKs (Kaminska et al., 2005), we determined the nuclear translocation of c-Jun and c-Fos. The nuclear levels of c-Jun and c-Fos were significantly increased after LPS stimulation (Fig. 4B). PGEA treatment reduced the amount of c-Fos, but not c-Jun, in a dose-dependent manner. These results indicated that the inhibitory effect of PGEA on LPS-activated macrophages may occur through both the AP-1 signaling pathways. Consistent with the findings related to Iκ-Bα degradation, the nuclear levels of p65 was not changed by PGEA in LPS-stimulated RAW264.7 cells (Fig. 4B), indicating anti-inflammatory effects of PGEA may not involved in NF-κB activation.

DISCUSSION

PGEA inhibits the oxidative stress-induced death of human hepatic HepG2 cells and the levels of superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, and metallothionein messenger RNA are increased in PGEA-treated HepG2 cells (Kim et al., 2008). In addition, a recent study demonstrated the beneficial effects of PGEA on hyperlipidemia in high-fat diet-induced obese mice *via* the up-regulation of anti-oxidant proteins (Chung et al., 2012). These previous results prompted the present examination of the anti-inflammatory effect of PGEA, since various natural products that have anti-oxidant effects also

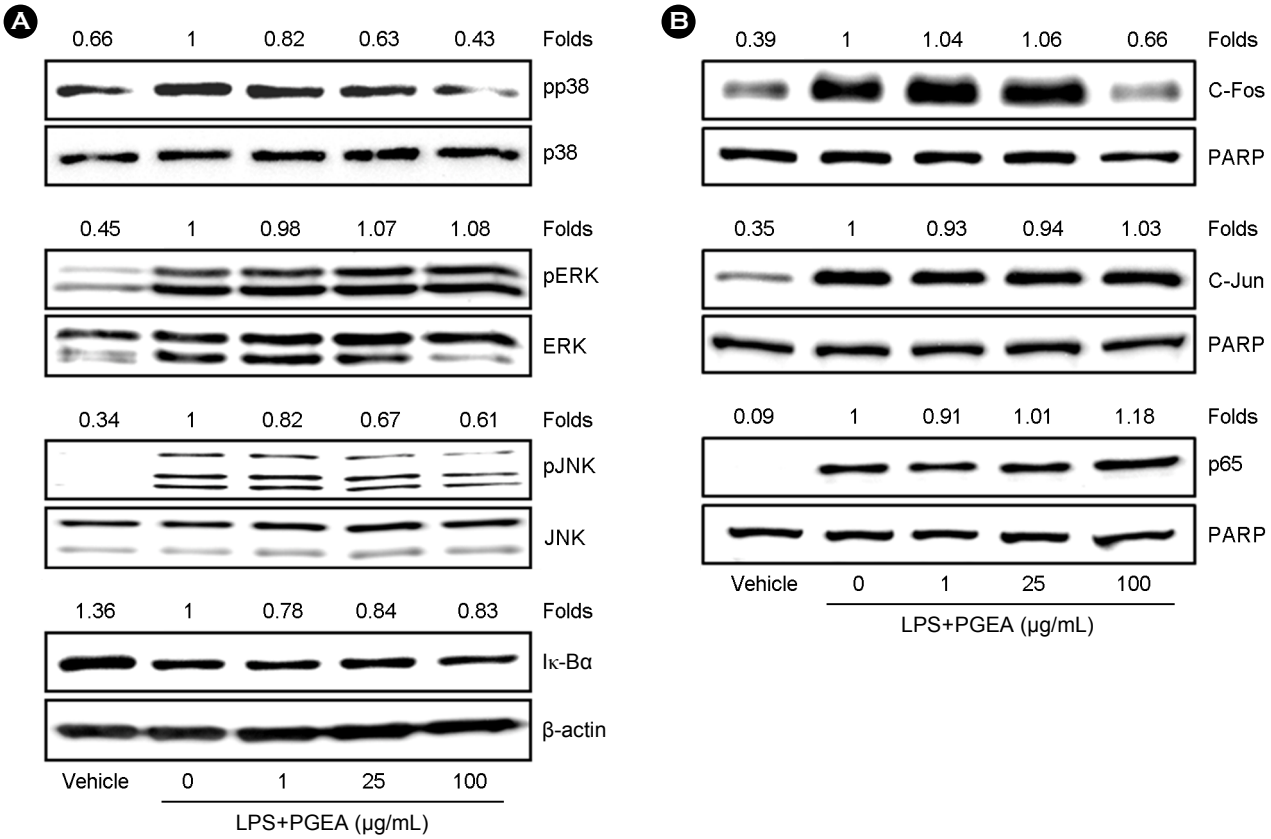

Fig. 4. Effect of PGEA on LPS-induced MAPK and AP-1 activation. RAW264.7 cells were plated in 100 mm-diameter dishes. After 12 h of seeding, cells were treated with different doses of PGEA for 1 h, followed by stimulation with 100 ng/mL of LPS for 20 min. (A) Whole cell extracts and (B) nuclear proteins were immunoblotted with the indicated Abs. β-actin and poly ADP ribose polymerase were used as a control. The bands were quantified using NIH image analysis software and their relative intensity was expressed as fold-change against the image of the LPS-stimulated RAW264.7 cells.

possess anti-inflammatory activity.

The main aim of the present study was to evaluate the therapeutic potential of *P. grandiflorum* for the regulation of inflammation. First, we tested three different extracts isolated from *P. grandiflorum* on LPS-induced NO release in RAW264.7 cells. The increase in NO release caused by LPS was significantly reduced with PGEA treatment at concentrations of 50 and 500 μg/mL, but not by PGW and PGM. Additionally, experiments explored possible mechanisms involved in the cellular action of PGEA on anti-inflammatory efficacy. The effect of PGEA on the phosphorylation of MAPKs as well as degradation of Iκ-B in LPS-stmulated RAW264.7 cells were examined using Western blotting. PGEA did not affect the degradation of Iκ-B, but suppressed the phosphorylation of p38 and JNK, as well as nuclear translocation of c-Fos, which were induced by LPS stimulation. Concerning the signaling generated by LPS in macrophages, the activation of transcription factors results in the production of both pro- and anti-inflammatory mediators. The binding of LPS to TLR-4 leads to activation of transcription factor NF-κB and AP-1, which regulates innate immune responses (Zhong et al., 2006). Activation of NF-κB and AP-1 induces the expression of several inflammatory mediators such as iNOS, cyclooxygenase-2, IL-1β, and IL-6, along with many other genes (Guha and Mackman, 2001; Ono, 2008). The degradation and phosphorylation of Iκ-B are necessary to release NF-κB from the cytoplasmic NF-κB/Iκ-B complex and allow its subsequent translocation to the cell nucleus. AP-1 is composed of proteins belonging to the Jun and Fos families, and c-Jun and c-Fos are immediate-early genes (Vesely et al., 2009). MAPK signaling pathways regulate

AP-1 activity by increasing transcription and by the phosphorylation of AP-1 proteins. These results suggest that the inhibition of NO and pro-inflammatory cytokines in PGEA treated RAW264.7 cells is indeed caused by the down-regulation of AP-1 transcription factors through the inhibition of the MAPK signaling pathway.

Due to their effectiveness and relatively low toxicity, herbal medicines have drawn increasing attention during the past decades. With the growing use of herbal products, quality and safety become especially important to guarantee the safety and efficacy of the utilization of herbal medicines. Unlike synthetic drugs, herbal medicines have complex compositions. The effectiveness of herbal medicines may be attributed to the overall effect of all the components rather than a single component.

Two limitations of this study are that we used PGEA extracts that included several compounds and the use of an *in vitro* model. Further studies that focus on the purification and structural identification of the individual anti-inflammatory compounds in the PGEA extract are needed. In addition, a further challenge is to delineate *in vivo* actions of PGEA to provide a better understanding of the health-promoting effects of PGEA that is widely consumed globally.

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REFERENCES

Ashwell, JD. The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol. 2006. 6: 532-540.

Chung MJ, Kim SH, Park JW, Lee YJ, Ham SS. *Platycodon grandiflorum* root attenuates vascular endothelial cell injury by oxidized low-density lipoprotein and prevents high-fat diet-induced dyslipidemia in mice by up-regulating antioxidant proteins. Nutr Res. 2012. 32: 365-373.

Guha M, Mackman N. LPS induction of gene expression in human monocytes. Cell Signal. 2001. 13: 85-94.

Han EH, Park JH, Kim JY, Chung YC, Jeong HG. Inhibitory mechanism of saponins derived from roots of *Platycodon grandiflorum* on anaphylactic reaction and IgE-mediated allergic response in mast cells. Food Chem Toxicol. 2009. 47: 1069-1075.

Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. Biochim Biophys Acta. 2005. 1754: 253 -262.

Kaplan M, Mutlu EA, Benson M, Fields JZ, Banan A, Keshavarzian A. Use of herbal preparations in the treatment of oxidant-mediated inflammatory disorders. Complement Ther Med. 2006. 15: 207-216.

Khanal T, Choi JH, Hwang YP, Chung YC, Jeong HG. Saponins isolated from the root of *Platycodon grandiflorum* protect against acute ethanol-induced hepatotoxicity in mice. Food Chem Toxicol. 2009. 47: 530-535.

Kim MS, Hur YG, Kim WG, Park BW, Ahn KS, Kim JJ, Bae H. Inhibitory effect of *Platycodon grandiflorum* on T(H)1 and T(H)2 immune responses in a murine model of 2,4-dinitrofluorobenzene-induced atopic dermatitis-like skin lesions. Ann Allergy Asthma Immunol. 2011. 106: 54-61.

Kim SH, Choi HJ, Oh HT, Chung MJ, Cui CB, Ham SS. Cytoprotective effect by antioxidant activity of Codonopsis lancelata and *Platycodon grandiflorum* ethyl acetate fraction in human HepG2 cells. Korean J Food Sci Technol. 2008. 40: 696-701.

Kim YS, Kim JS, Choi SU, Kim JS, Lee HS, Roh SH, Jeong YC, Kim YK, Ryu SY. Isolation of a new saponin and cytotoxic effect of saponins from the root of *Platycodon grandiflorum* on human tumor cell lines. Planta Med. 2005. 71: 566-568.

Lee JY, Yoon JW, Kim CT, Lim ST. Antioxidant activity of phenylpropanoid esters isolated and identified from *Platycodon grandiflorum* A. DC. Phytochemistry. 2004. 65: 3033-3039.

Lee KJ, Hwang SJ, Choi JH, Jeong HG. Saponins derived from the roots of *Platycodon grandiflorum* inhibit HT-1080 cell invasion and MMPs activities: regulation of NF-kappaB activation via ROS signal pathway. Cancer Lett. 2008. 268: 233-243.

Lee KJ, Choi JH, Kim HG, Han EH, Hwang YP, Lee YC, Chung YC, Jeong HG. Protective effect of saponins derived from the roots of *Platycodon grandiflorum* against carbon tetrachloride induced hepatotoxicity in mice. Food Chem Toxicol. 2008. 46: 1778-1785.

Noh JR, Kim YH, Gang GT, Yang KJ, Kim SK, Ryu SY, Kim

YS, Lee CH, Lee HS. Preventative effects of *Platycodon grandiflorum* treatment on hepatic steatosis in high fat diet-fed C57BL/6 mice. Biol Pharm Bull. 2010. 33: 450-454.

Ono M. Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci. 2008. 99: 1501-1506.

Ozaki Y. Studies on antiinflammatory effect of Japanese Oriental medicines (kampo medicines) used to treat inflammatory diseases. Biol Pharm Bull. 1995. 18: 559-562.

Setty AR, Sigal LH. Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. Semin Arthritis Rheum. 2005. 34: 773-784.

Shin DY, Kim GY, Li W, Choi BT, Kim ND, Kang HS, Choi YH. Implication of intracellular ROS formation, caspase-3 activation and Egr-1 induction in platycodon D-induced apoptosis of U937 human leukemia cells. Biomed Pharmacother. 2009. 63: 86-94.

Vesely PW, Staber PB, Hoefler G, Kenner L. Translational regulation mechanisms of AP-1 proteins. Mutat Res. 2009. 682: 7-12.

Wu PF, Zhang Z, Wang F, Chen JG. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. Acta Pharmacol Sin. 2010. 31: 1523-1531.

Zheng J, He J, Ji B, Li Y, Zhang X. Antihyperglycemic effects of *Platycodon grandiflorum* (Jacq.) A. DC. extract on streptozotocin-induced diabetic mice. Plant Foods Hum Nutr. 2007. 62:7-11.

Zhong B, Tien P, Shu HB. Innate immune responses: crosstalk of signaling and regulation of gene transcription. Virology. 2006. 352: 14-21.