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Punicalagin Inhibits Inflammation in LPS-Induced RAW264.7 Macrophages via the Suppression of TLR4-Mediated MAPKs and NF-κB Activation

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Abstract—Punicalagin (2,3,hexahydroxydiphenoyl-gallagyl-D-glucose and referred to as PUN) is a bioactive ellagitannin isolated from pomegranate, which is widely used for the treatment of inflammatory bowel disease (IBD), diarrhea, and ulcers in Chinese traditional medicine. In this study, we detected the anti-inflammation potentials of PUN in lipopolysaccharide (LPS)-induced macrophages and tried to uncover the underlying mechanism. Results demonstrated that PUN (25, 50, or 100 μM) treatment could significantly decrease the LPS-induced production of nitric oxide), prostaglandin E_2 (PGE_2), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in RAW264.7 cells. Molecular research showed that PUN inhibited the activation of upstream mediator nuclear factor-κB by suppressing the phosphorylation of IκBα and p65. Results also indicated that PUN could suppress the phosphorylation of mitogen-activated protein kinase including p38, c-Jun N-terminal kinase, and extracellular signal-regulated kinase. In conclusion, we observed that PUN could inhibit LPS-induced inflammation, and it may be a potential choice for the treatment of inflammation diseases.

KEY WORDS: punicalagin; anti-inflammation; proinflammatory cytokines; MAPKs; NF-κB.

INTRODUCTION

Inflammation is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischaemic, toxic, or autoimmune injury [1]. Appropriate inflammatory response is a crucial protective attempt of the host defense to remove the injurious stimuli and to initiate the healing process, but acute inflammation has been considered to be one of major causes leading to tissue damage, sepsis, cancer and shock [2-5]. When exposed to proinflammatory factors and metabolic stimuli, monocytes transfer to extravascular tissues and differentiate into macrophages. Once activated, macrophages are the main source of nitric oxide (NO), prostaglandin E (PGE_2) and cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, whose abundant expression may cause deep influence on endothelial, epithelial, and mesenchymal cells in the local microenvironment and contribute to host defence, tissue remodeling, and repair [6].

Lipopolysaccharide (LPS) is an endotoxin released by Gram-negative bacteria that can be transferred to cluster of differentiation (CD) 14 by LPS-binding protein (LBP) and recognized by Toll-like receptor 4 (TLR4) on the cellular surface of macrophages [7, 8]. This interaction results in the activation of intracellular signaling through MyD88 and TRIF pathways leading to the activation of major MAP kinase cascades and translocation of regulator nuclear factor-κB (NF-κB), which is involved in expression of inducible NO synthase (iNOS), COX-2, IL-1β, IL-6, and TNF-α genes [9].

Punicalagin (2,3,hexahydroxydiphenoyl-gallagyl-D-glucose, referred to as PUN here), an ellagitannin isolated

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from pomegranate polyphenols, is abundant in the fruit husk and juice in significant quantities reaching levels of >2 g/l juice [10]. Previous research reported that the water-soluble ellagitanin PUN is toxic to cattle, but repeated oral administration of high doses of PUN to rats for 37 days is not toxic [11]. Other studies showed that PUN has considerable antioxidant activity and anti-proliferative activity [12, 13]. However, there is no investigation focusing on the inhibitory effect of PUN on LPS-induced inflammation in RAW 264.7 macrophages.

In this study, we performed a preliminary assessment on cytotoxicity of PUN in macrophages and investigated the anti-inflammation potentials of PUN on expression of proinflammatory factors and cytokines including NO, PGE_2, IL-1β, IL-6, and TNF-α in LPS-stimulated RAW264.7 macrophages. We also tried to uncover the mechanisms of anti-inflammation through modulating mitogen-activated protein kinases (MAPKs) and NF-κB signaling pathways.

MATERIALS AND METHODS

Reagents

PUN [>98% high-performance liquid chromatography (HPLC) purity] was purchased from YingZeNaXin (Beijing, China). LPS (*Escherichia coli* 055:B5) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-dipheny l tetrazolium bromide were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), antibiotic–antimycotic, and TRIzol® reagent were purchased from Gibco (Grand Island, NY, USA). Bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA). Antibodies for GAPDH, p38, p-p38, extracellular signal-regulated kinase (ERK), P-ERK, c-Jun N-terminal kinase (JNK), p-JNK, p-IκBα, and p-p65 were purchased from Cell Signaling Technology (Danvers, MA, USA). The goat anti-mouse antibody was purchased from Li-cdr Odyssye® (Lincoln, NE, USA). Enzyme-linked immunosorbent assay (ELISA) kits for PGE_2, IL-1β, IL-6, and TNFα were obtained from R&D Systems (Minneapolis, MN, USA). The nitrate assay kit was purchased from Beyotime (Haimen, China).

Cell Line

RAW264.7 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in DMEM medium supplemented with 10 % FBS and antibiotics (100 U/ml penicillin and 100 U/ml streptomycin) at 37 % in a humidified incubator with 5 % CO_2.

MTT Assay for Cell Viability

Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reduction assay. In brief, RAW264.7 cells were preincubated overnight in 96-well plates at a density of 4×10^4 cells per well. After 24 h, cells were treated with various concentrations of PUN (5, 25, 50, 100, 200, or 400 μM) for another 24 h. The mediums then were removed, and solution containing 10 % MTT and DMEM was added to each well. The cells were incubated for 4 h at 37 °C, the supernatants were removed, and dimethyl sulfoxide was added to each well. The optical density was measured at 570 nm using a microplate reader (Bio-Rad, USA).

NO Assay

The nitrite accumulated in the culture medium was measured as an indicator of NO production based on the Griess reaction. RAW264.7 cells were treated with or without LPS (1 μg/ml) in the presence of various concentrations of PUN (25, 50, or 100 μM, 1 h prior to LPS treatment). After 24 h, culture supernatants were mixed with Griess reagent [equal volumes of 1 % (w/v) sulfanilamide in 5 % (v/v) phosphoric acid and 0.1 % (w/v) naphtylethylenediamine-HCL], and incubated at room temperature for 10 min. Absorbance values were detected at 550 nm and NO concentration was calculated with reference to standard curve of sodium nitrite.

Enzyme-Linked Immunosorbent Assay

To detect the effect of PUN on pro-inflammatory cytokines generation from LPS-stimulated cells, RAW264.7 cells were preincubated on 24-well plates (4×10^5) and were pretreated with PUN (25, 50, or 100 μM) 1 h prior to LPS (1 μg/ml) treatment in a 37 °C, 5 % CO_2 incubator for 24 h. The supernatants were then collected and assayed immediately. PGE_2, IL-1β, IL-6, and TNF-α concentration were measured using ELISA kits according to the manufacturer's instructions.

Quantitative RT-PCR Analysis

To detect the effect of PUN on gene expression in LPS-stimulated cells, RAW264.7 cells were preincubated on six-well plates (1×10^6) and were pretreated with PUN (25, 50, or 100 μM) 1 h prior to LPS (1 μg/ml) treatment in

a 37 °C, 5 % CO_2 incubator for 12 h. Total RNA from RAW264.7 cells were extracted using TRIzol® reagent as described before [14]. The concentration and integrity of total RNA were measured at a 260/280 nm ration. Quantitative polymerase chain reaction (PCR) analysis was carried out using the DNA Engine Mx3000P® (Agilent, Santa Clara, CA, USA) fluorescence detection system against a double-stranded DNA-specific fluorescent dye (Stratagene, La Jolla, CA, USA) according to optimized PCR protocols. β-actin was amplified in parallel with the target genes and used as a normalization control. The cycling conditions were as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 60 s. Expression levels were determined using the relative threshold cycle (CT) method as described by the manufacturer (Stratagene). The PCR reaction system (25 μl in total) contained 12.5 μl of SYBR Green PCR mix (Stratagene), 0.375 μl of reference dye, 1 μl of each primer (both 10 μmol/l), 1 μl of cDNA template, and 9.125 up of DEPC-treated water. Table 1 lists seven gene-specific oligonucleotide primers used for RT-PCR.

Western Blotting Analysis

The RAW264.7 cells (1×10^6), cultured in tissue culture flasks for 24 h, were pretreated with PUN (25, 50, or 100 μM) 1 h prior to treatment with LPS (1 mg/l) for 30 min in a 37 °C, 5 % CO_2 incubator. Cells were then harvested on ice, washed twice using ice-cold PBS, and suspended in 500 μl lysis buffer supplemented with protease inhibitor. After incubating on ice for 30 min, cell extracts were subjected to centrifugation (12,000×*g*) at 4 °C for 15 min to get cell protein and quantified using a BCA protein assay kit. Proteins were separated by SDS-PAGE and electro-transferred to nitrocellulose membranes (Pierce, USA), then hybridized with the specific antibodies. Blots were normalized by use of GAPDH to correct for differences in loading of the proteins. Densitometric values of immunoblot signals were obtained from three separate experiments using Image J (National Institutes of Health, USA).

Statistical Analysis

The results were expressed as means ± S.E.M and differences between mean values of normally distributed data were assessed by the one-way analysis of variance (ANOVA) multiple comparisons. *P* value of 0.05 or less was considered as significant.

RESULTS

Effect of PUN on Cell Viability *In Vitro*

In order to exam the cytotoxic of various concentrations (0–400 μM) of PUN on RAW264.7 cells, cell viability was determined using the MTT assay. Results showed that PUN, in concentrations from 0–400 μM, had no cytotoxic effect on RAW264.7 cells (Fig. 1), suggesting that PUN inhibitory effects on LPS-induced inflammation were not as a result of cytotoxicity caused cell viability reduction.

Table 1. Gene-Specific Oligonucleotide Primers Used for RT-PCR

Description	Accession number[1]	Primer sequence (5′→3′)	Product (bp)
β-actin	NM_173979.3	F CCTGCGGCATTCACGAAACTAC R ACTCCTGCTTGCTGATCCACATC	273
TNF-α	NM_173966.3	F ACGGGCTTTACCTCATCTACTC R GGCTCTTGATGGCAGACAGG	141
IL-1β	NM_174093.1	F GGCAACCGTACCTGAACCCA R CCACGATGACCGACACCACC	206
IL-6	NM_173923.2	F CCTTCACTCCATTCGCTGTCT R TCCTGATTTCCCTCATACTCG	391
COX-2	NM_011198.3	F TTGCTGTACAAGCAGTGGCAAAGG R AGGACAAACACCGGAGGGAATCTT	688
iNOS	NM_010927.3	F CAGATCGAGCCCTGGAAGAC R CTGGTCCATGCAGACAACCT	249
TLR4	NM_021297.2	F TCTGGGGAGGCACATCTTCT R CAGGTCCAAGTTGCCGTTTC	111

[1] Primers were designed from the published sequences in the GenBank database under the indicated accession numbers
F forward primer, *R* reverse primer

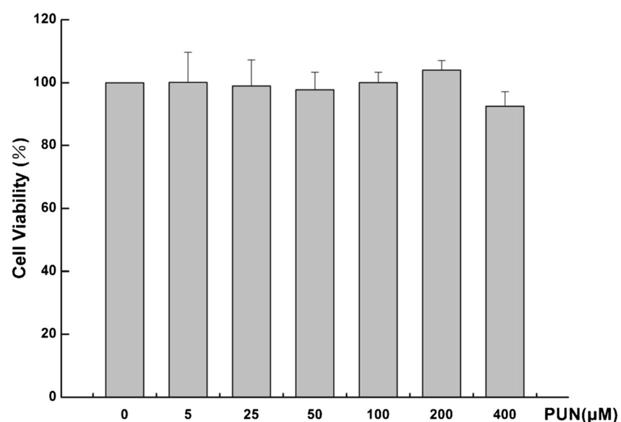

Fig. 1. Cytotoxicity of PUN on RAW264.7 cells. Effect of PUN on the viability of RAW264.7 cells was measured using MTT assay. Cells were incubated with PUN (0–400 μM) for 24 h. Data represent the mean ± S-.E.M of three independent experiments and differences between mean v-alues were assessed by one-way ANOVA.

Inhibition of LPS-Induced NO and PGE_2 Production by PUN

To investigate the potential anti-inflammatory property of PUN, we detected its effect on LPS-induced NO production by measuring the accumulated nitrite in the culture medium as estimated by Griess reaction. Results demonstrated that LPS challenge significantly increased the production of NO in RAW264.7 cells compared with control group, pretreatment with PUN (25, 50, and 100 μM) significantly decreased NO concentration in a concentration-dependent manner (Fig. 2a). PGE_2 detection was performed by measuring the concentration in the culture medium using an ELISA kit. Results showed PUN also notably decreased LPS-induced PGE_2 overexpression in RAW264.7 cells in a concentration-dependent manner (Fig. 2b).

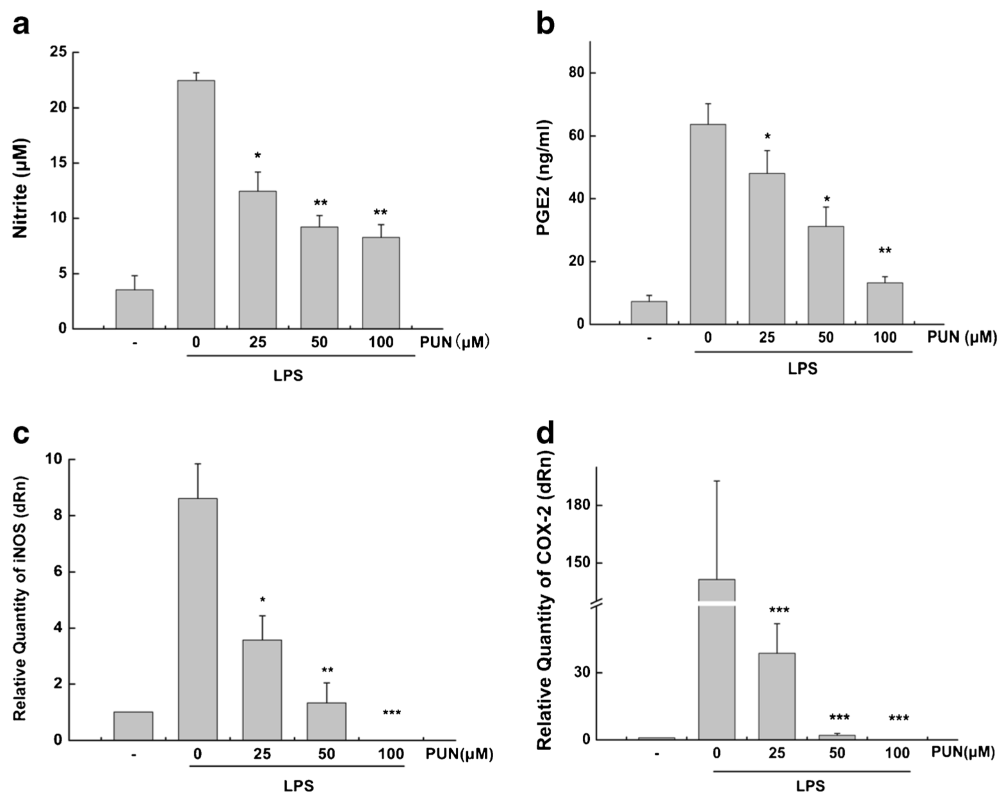

Fig. 2. Effect of PUN on LPS-induced NO and PGE2 expression. **a** and **b** Effect of PUN in different concentrations on LPS-induced NO and PGE_2 production. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 24 h, NO production in the supernatant was measured using Griess reaction, and PGE_2 production was detected using an ELISA kit. **c** and **d** Effect of PUN in different concentrations on LPS-induced iNOS and COX-2 mRNA expression. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 12 h, iNOS and COX-2 mRNA expression were detected using RT-PCR. Data represent the mean ± S.E.M of three independent experiments and differences between mean values were assessed by one-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ indicate significant differences compared with the LPS-treated group.

Inhibition of LPS-Induced iNOS and COX-2 Expression by PUN

iNOS and COX-2 are important upstream regulators for NO and PGE_2 expression, respectively. To detect the inhibitory effect of PUN on LPS-induced iNOS and COX-2 expression in macrophages, total RNA was extracted and performed real time (RT)-PCR research. LPS challenge significantly increased iNOS and PGE_2 messenger RNA (mRNA) expression in RAW264.7 cells; however, PUN pretreatment notably attenuated iNOS and COX-2 gene overexpression in a concentration-dependent manner (Fig. 2c, d).

Inhibition of LPS-Induced Proinflammatory Cytokines Production by PUN

Concentrations of IL-1β, IL-6, and TNF-α in the culture medium of RAW264.7 were measured using ELISA kits. LPS exposure activated RAW264.7 cells inflammation reflection, IL-1β, IL-6, and TNF-α secretion in the supernatants significantly enhanced after LPS stimulation for 24 h, and pretreatment with PUN (25, 50, or 100 μM) in prior to LPS challenge notably attenuated the enhancement of these cytokines secretion. Results showed that for IL-1β and TNF-α generation, PUN exerted an apparently inhibitory effect at concentrations from 25 to 100 μM (Fig. 3a, c); for IL-6 generation, PUN did not show significant inhibitory effect till concentration was higher than 50 μM (Fig. 3b). Furthermore, we detected the suppressive effect of PUN on LPS-induced proinflammatory cytokines at the mRNA level. Results indicated that PUN (25, 50, and 100 μM) had strong effect on reducing LPS-induced expression of IL-1β, IL-6, and TNF-α mRNA (Fig. 3d, e, f).

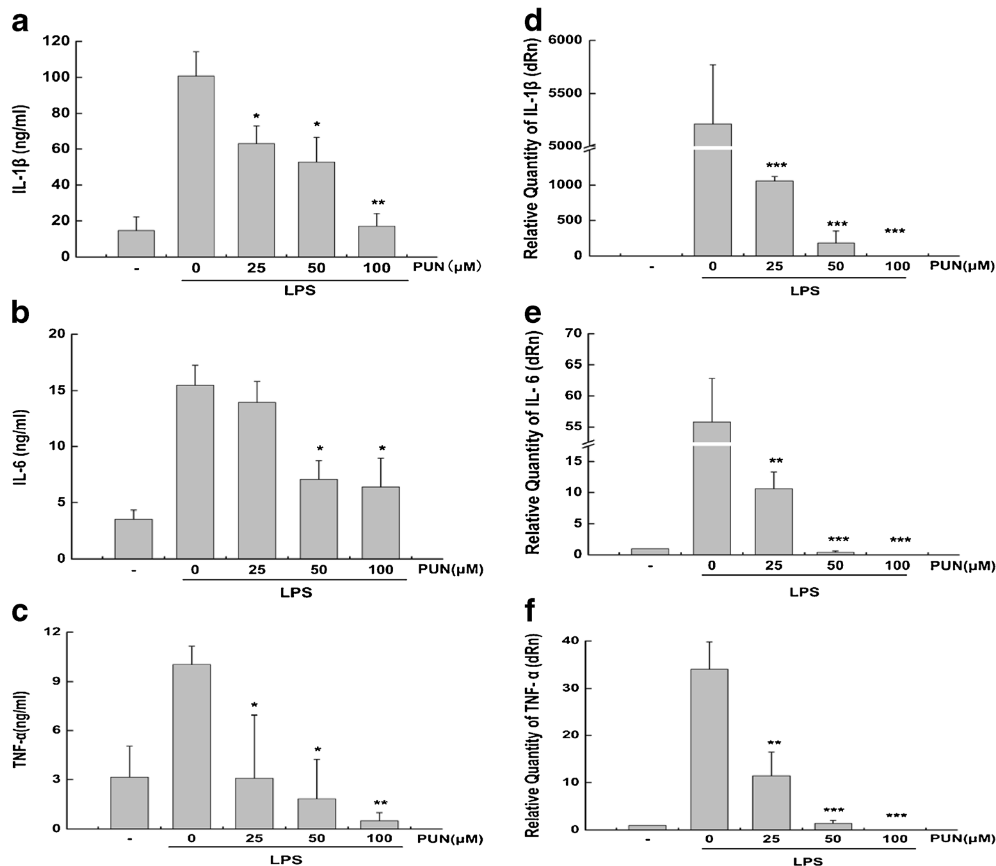

Fig. 3. Effect of PUN on LPS-induced proinflammatory factors and cytokines. **a**, **b**, and **c** Effect of PUN in different concentrations on LPS-induced IL-1β, IL-6, and TNF-α production in RAW264.7 cells. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 24 h, IL-1β, IL-6, and TNF-α production in the supernatant was measured using ELISA kits. **d**, **e**, and **f** Effect of PUN in different concentration on LPS-induced IL-1β, IL-6, and TNF-α mRNA expression in RAW264.7 cells. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 12 h, IL-1β, IL-6, and TNF-α mRNA expression were detected using RT-PCR. Data represent the mean±S.E.M of three independent experiments and differences between mean values were assessed by one-way ANOVA. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ indicate significant differences compared with the LPS-treated group.

Inhibition of LPS-Induced MAPK Pathway Activation by PUN

To further expound the mechanism of inhibition effect on LPS-induced proinflammatory cytokines expression by PUN, we then investigated the intervention of PUN on LPS-induced activation of MAPKs signaling pathway. Cell proteins were extracted for Western blotting analysis. We assessed the effect of PUN on LPS-induced phosphorylation of p38, JNK, and ERK using three different phospho-specific antibodies. Results indicated that as important signaling pathways in growth and proliferation, p38, JNK, and ERK showed slight phosphorylation in cells of control group. LPS treatment significant increased activation of MAPKs by strengthening phosphorylation of p38, JNK, and ERK; however, the phosphorylation levels were attenuated to some degree in PUN-pretreated cells compared with LPS-treated cells. No significant changes were found in the total protein level of MAPKs (Fig. 4).

Inhibition of LPS-Induced NF-κB Activation by PUN

NF-κB is an important upstream transcription factor inducing expression of iNOS, COX-2, and cytokines mRNA after stimulation of LPS. Therefore, we detected the phosphorylation of p65, a functional subunit of NF-κB complex. Challenge with LPS for 30 min significantly enhanced the phosphorylation of p65 in RAW264.7 cells; however, pretreatment with PUN notably inhibited this excessive phosphorylation. During this process, phosphorylation of IκBα is essential to release NF-κB from NF-κB/IκBα complex. We further investigated the effect of PUN on LPS-induced phosphorylation of IκBα. Results showed that PUN strongly suppressed phosphorylation of IκBα, suggesting that PUN inhibited NF-κB activation and phosphorylation of p65 by reducing phosphorylation of IκBα (Fig. 5).

Inhibition of LPS-Induced TLR4 mRNA Expression by PUN

To detect the inhibitory effect of PUN on LPS-induced TLR4 expression in macrophages, total RNA was extracted and performed RT-PCR research. Results demonstrated that when exposed to LPS for 12 h, TLR4 mRNA level were upregulated. PUN could significantly inhibit LPS-induced TLR4 mRNA expression enhancement in a concentration-dependent manner (Fig. 6).

DISCUSSION

As a traditional medicine used for clinical therapy for decades, pomegranate products showed significant efficacy for the treatment of IBD, diarrhea and ulcers, cancer, diabetes mellitus [15-17]. Because of the wide and excellent traditional ethno-medical use of pomegranate, researchers, in the past decades, paid more attention to investigating the mechanism underlying cure effect of ingredients in pomegranate products. Antioxidant and anti-inflammatory activity of pomegranate polyphenols may contribute to pharmacological effects [11, 18, 19]. PUN, one of the most abundant of these polyphenols, is a kind of ellagitannin possessing more than 50% antioxidant activity of pomegranate juice [20]. Previous study showed that PUN may be toxic to cattle, but nontoxic to rat. In our study, MTT assay showed PUN did not have cytotoxic effect in concentrations from 0 to 400 μM when treating RAW264.7 cells, indicating that PUN showed inhibitory effect not due to decreasing cells viability. Recent researches also showed that pomegranate extract and Pun have certain effect on anti-inflammatory effect and immune-suppressive activity [21, 22], but no systematic research was carried out to uncover the mechanism underlying inhibition effect of Pun on LPS-induced macrophages inflammation.

Macrophages, as innate immune cells, initiate inflammation and immune response [23]. When challenged with LPS, macrophages are activated and release various proinflammatory factors and cytokines, whose excessive release may result in extensive tissue damage and pathological changes. NO, an important cellular signaling molecule and proinflammatory mediator produced by iNOS, plays a key role in the pathogenesis of inflammation due to overproduction in abnormal situations [24]. When stimulated by LPS, TNF-α, or IL-1, iNOS expression increased and macrophages released NO in high concentrations [25]. PGE_2 is another important proinflammatory factor regulated by COX-2, whose expression is elevated in LPS-induced macrophages [26]. PGE_2 is associated with many chronic inflammatory diseases including cardiovascular diseases, arthritis, IBD, angiogenesis, and chronic gastric ulcer [27-29]. In the present study, PUN was proved to significantly inhibit overexpression of iNOS and COX-2 mRNA and thus decreased the concentration of NO and PGE_2 in the supernatant in LPS-induced RAW264.7 cells. Upon activation, macrophages also release other proinflammatory factors and cytokines including IL-1β, IL-6 and TNF-α used to evaluate the potential anti-inflammation property against LPS-induced macrophages activation

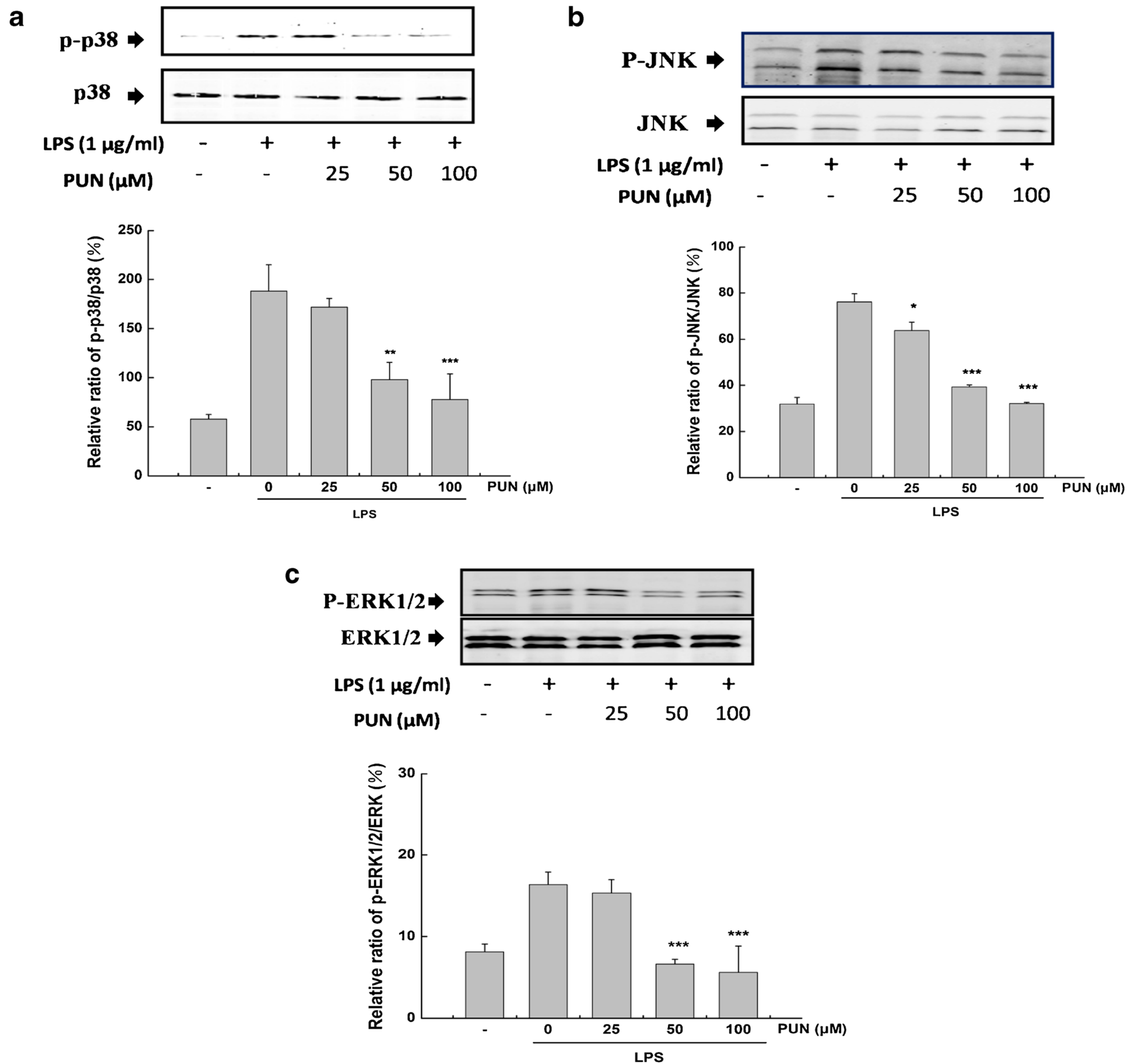

Fig. 4. Effect of PUN on LPS-induced MAPKs activation in RAW264.7 cells. Effect of PUN in different concentrations on LPS-induced MAPKs activation in RAW264.7 cells. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 30 min, protein samples were analyzed by Western blot. Phosphorylation of p38 (**a**), JNK (**b**), and ERK1/2 (**c**) were analyzed using phospho-specific anti-p38, phospho-specific anti-pJNK, and phospho-specific anti-ERK1/2 antibodies. Data represent the mean ± S.E.M of three independent experiments, and differences between mean values were assessed by one-way ANOVA. **p*<0.05, ***p*<0.01, and ****p*<0.001 indicate significant differences compared with the LPS-treated group.

[30]. This study indicated that PUN could prevent and reduce inflammatory responses by inhibiting IL-1β, IL-6, TNF-α mRNA expression as well as their secretion.

NF-κB plays a central role in regulating immune and inflammatory processes and thus becomes target for developing novel treatments for inflammatory diseases [31-33]. Before activation, NF-κB is combined with IκBα, an inhibitory protein keeping NF-κB in an inactive state in the cytoplasm. Induced by various stimuli, such as LPS and proinflammatory factors, IκBα is phosphorylated and degenerates apart from NF-κB. The free NF-κB translocates from cytoplasm into the nucleus, where the

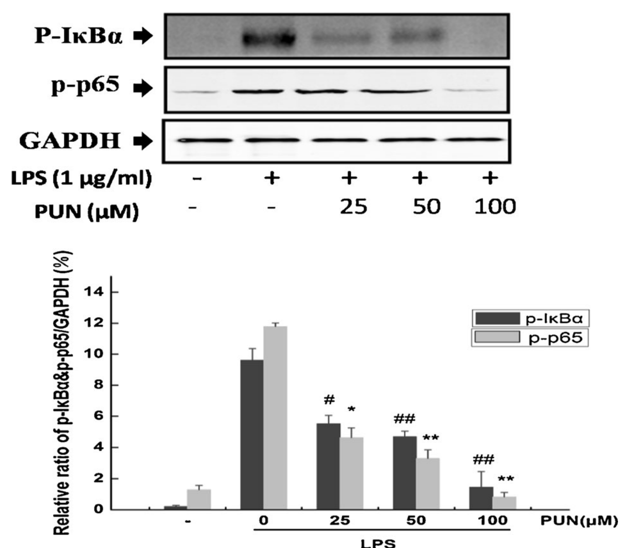

Fig. 5. Effect of PUN on LPS-induced NF-κB activation in RAW264.7 cells. Effect of PUN in different concentrations on LPS-induced NF-κB activation in RAW264.7 cells. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 30 min, and protein samples were analyzed by Western blot. Phosphorylation of IκBα and p65 were analyzed using phospho-specific anti-pIκBα, phospho-specific anti-pp65 antibodies. Data represent the mean ± S.E.M of three independent experiments, and differences between mean values were assessed by one-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$ indicate significant differences of pIκBα compared with the LPS-treated group. $^{\#}p<0.05$, $^{\#\#}p<0.01$ indicate significant differences of pp65 compared with the LPS-treated group.

phosphorylated subunit p65 plays an important role in triggering transcription of certain genes. Results in our study

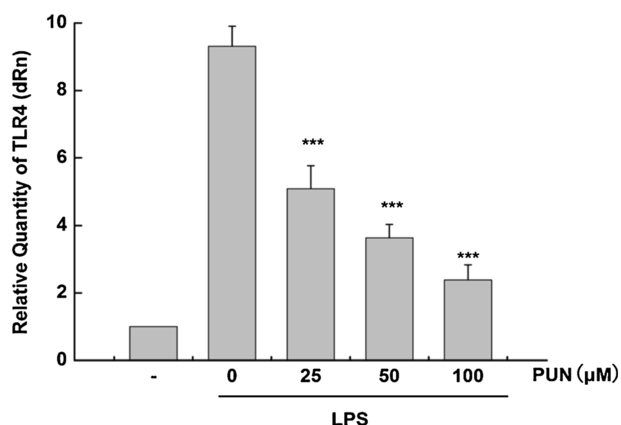

Fig. 6. Effect of PUN on LPS-induced TLR4 mRNA expression. Effect of PUN in different concentration on TLR4 mRNA expression in RAW264.7 cells. Cells were pretreated with PUN (25, 50, and 100 μM) and exposed to 1 μg/ml LPS for 12 h, mRNA expression were detected using RT-PCR. Data represent the mean ± S.E.M of three independent experiments, and differences between mean values were assessed by one-way ANOVA. $^{***}p<0.001$ indicate significant differences compared with the LPS-treated group.

showed that PUN strongly inhibited LPS-induced NF-κB activation by suppressing the phosphorylation and degeneration of IκBα and thus decreased the phosphorylation of p65. Therefore, we inferred that PUN inhibited transcription of target genes including iNOS, COX-2, IL-1β, IL-6, and TNF-α mRNA by suppressing the activity of NF-κB.

Previous studies indicated that MAPKs, the upstream regulators of NF-κB, acted as a key role in LPS-induced inflammatory factors release in macrophages [34, 35]. Regulation of p38, JNK, and ERK, three important MAPK family proteins, are major targets for understanding the mechanism of NF-κB signaling with treatments [36, 37]. However, which target or targets could be affected by PUN and play important roles in NF-κB signaling remained unclear before this research. So we tested whether PUN downmodulates LPS-induced p38, JNK, and ERK MAPK activation. Results showed that PUN attenuated LPS-induced phosphorylation of all the three MAPKs, suggesting that PUN suppressed NF-κB signaling pathway by inhibiting the activation of p38, JNK, and ERK.

TLR4, which is expressed on various of proinflammation cells, is a critical key factor in regulating innate immune response. Previous studies indicate that TLR4 is the major signaling receptor in LPS-induced inflammatory responses [38] by regulating activity of downstream mediators including MAPKs and NF-κB [39, 40]. In the present study, data indicated that PUN had a strong inhibitory effect on TLR4 mRNA overexpression in RAW264.7 cells when exposed to LPS, suggesting that PUN inhibited activation of MAPKs and NF-κB by preventing TLR4-signaling pathway.

In summary, our investigation indicated that PUN has potential inhibitory effect on LPS-induced release of proinflammatory factors and cytokines including NO, PGE_2, IL-1β, IL-6, and TNF-α in RAW264.7 cells by suppressing the activation of TLR4-mediated MAPKs and NF-κB signaling pathways. These observations therefore suggest that PUN, an ellagitannin from pomegranate polyphenols, has potentials to prevent a variety of inflammation diseases in macrophage.

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Conflict of Interest. The authors declare that they do not have any conflict of interest.

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