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Isolation and Characterization of Two Flavonoids, Engeletin and Astilbin, from the Leaves of *Engelhardia roxburghiana* and Their Potential Anti-inflammatory Properties

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ABSTRACT: Engeletin, a flavonoid compound, was isolated from the leaves of *Engelhardia roxburghiana* for the first time, along with astilbin, another flavonoid. The chemical structures of engeletin and astilbin were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and mass spectrometry (MS) spectra, and their anti-inflammatory activities were studied in lipopolysaccharide (LPS)-stimulated mouse J774A.1 macrophage cells. LPS induced the inflammatory state in macrophage cells and increased mRNA expressions of pro-inflammatory cytokines. Engeletin and astilbin exhibited remarkable inhibitory effects on interleukin (IL)-1β and IL-6 mRNA expression. Significant inhibition of LPS-mediated mRNA expressions were also seen in LPS binding toll-like receptor (TLR)-4, pro-inflammatory cytokine tumor necrosis factor (TNF)-α, IL-10, chemoattractant monocyte chemotactic protein (MCP)-1, and cyclooxygenase (COX)-2 genes. The reduced expression of these cytokines may alleviate immune response and reduce inflammatory activation, indicating that engeletin and astilbin may serve as potential anti-inflammatory agents.

KEYWORDS: Anti-inflammatory, engeletin, astilbin, *Engelhardia roxburghiana*

INTRODUCTION

Engelhardia roxburghiana Wall. (Juglandaceae), also known as *Engelhardia chrvsolepis*, is a widely distributed botanical in southeast Asia, including Guangxi, Guangdong, and Fujian provinces in southern China. Leaves of the plant are used in a sweet tea named *huang qi* in China for weight loss and the Kohki tea in Japan. Flavonoids are found as the major and sweet components in the leaves.[1−5] Several biological studies revealed that these flavonoids possess significant activities, such as inhibition of aldose reductase and sorbitol accumulation,[2] lipid-lowering effect,[3] antioxidant activity,[1,3] and enhancing the vanadate-stimulated release of lipoprotein lipase.[4] Many flavonoids have been known as potential anti-inflammatory compounds through inhibiting the expression of pro-inflammatory factors.[6,7] (2*R*,3*R*)-Dihydrokaempferol 3-α-L-rhamnopyranoside (engeletin) and (2*R*,3*R*)-taxifolin 3-*O*-α-L-rhamnopyranoside (astilbin) are two flavonoid compounds first isolated from grapes.[8,9] Both engeletin and astilbin have shown potential in the prevention and treatment of diabetic complications.[10] However, the anti-inflammatory activity of engeletin and astilbin has not previously been reported.

It has been well-recognized that inflammation plays a pivotal role in human immune response. Chronic inflammation can be induced by many factors and may predispose the host to many chronic diseases, such as cardiovascular diseases, cancer, diabetes, arthritis, pulmonary diseases, and Alzheimer's and autoimmune diseases.[6,11−13] Macrophage is a major force in host defense against inflammation. Macrophage can be stimulated and activated by lipopolysaccharide (LPS), which is a component of the cell wall of Gram-negative bacteria, on toll-like receptor 4 (TLR4).[14] During inflammation, the nuclear factor-κB (NF-κB) pathway is activated and macrophage releases a variety of inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, monocyte chemotactic protein-1 (MCP-1), and other cytokines. Once the inflammatory response is triggered, other pathways may also be stimulated and involved, such as the cyclooxygenase (COX) pathway, and lead to the progressive inflammatory state that may result in acute or chronic inflammatory diseases.

In the present study, engeletin and astilbin were isolated from *E. roxburghiana* and confirmed for their chemical structures. Engeletin was isolated from *E. roxburghiana* for the first time. Engeletin and astilbin were also investigated and compared for their potential inhibitory effects on LPS-stimulated expression of TLR4, TNF-α, IL-1β, IL-6, IL-10, MCP-1, and COX-2 on the transcriptional level using mouse J774A.1 macrophage cells. In addition, the antiproliferative effect of the two compounds was also evaluated using LNCaP human prostate cancer cells.

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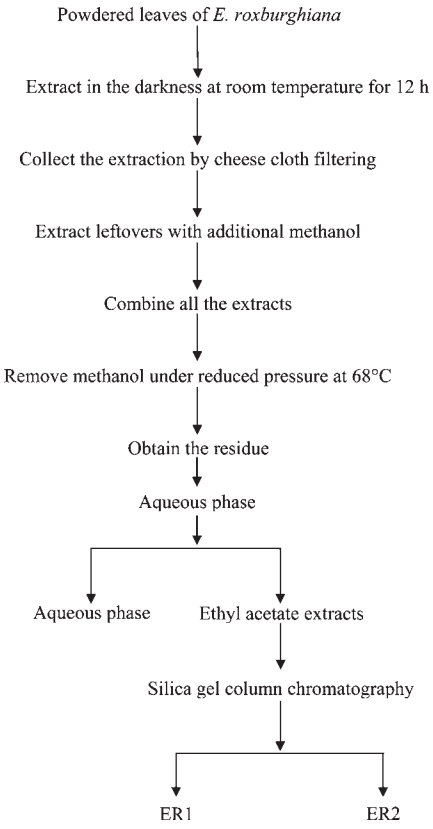

Figure 1. Isolation and purification procedure of engeletin (ER1) and astilbin (ER2).

MATERIALS AND METHODS

Plant Material and Reagents. Leaves of *E. roxburghiana* were collected from the Guangxi Zhuang Autonomous Region, China, in December 2006 and authenticated by Dr. Zhihong Cheng. TRIzol reagent was purchased from Invitrogen (Carlsbad, CA). StrataScript first strand cDNA synthesis kit was obtained from Stratagene (Santa Clara, CA). ABI Prism 7000 sequence detection system and TaqMan universal PCR master mix for real-time polymerase chain reaction (PCR) was developed by Applied Biosystems (Carlsbad, CA). The TaqMan Assay-On-Demand gene expression assays were purchased from Applied Biosystems: Tlr4 (Mm00445273_m1), Tnf (Mm00443258_m1), Il1b (Mm01336189_m1), Il6 (Mm00446190_m1), Il10 (Mm00439614_m1), Ccl2 (Mm00441242_m1), Ptgs2 (Mm01307329_m1), and Tbp (Mm00446973_m1). All other chemicals were of analytical reagent grade and used without any further purification.

General Procedures. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker-AV 400 (Madison, WI) instrument with deuterated methanol (CD_3OD) as the solvent and using tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on silica gel (170−400 mesh, Fisher Scientific, Fair Lawn, NJ) and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden). Silica gel 60 F_{254} TLC plates (Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC) analysis.

Extraction, Isolation, and Separation of Engeletin and Astilbin from *E. roxburghiana*. As shown in Figure 1, leaves of *E. roxburghiana* (5 kg) were air-dried, pulverized, and extracted by cold maceration with 60 L of methanol for 5 times. The extracts were combined, and methanol was removed under the reduced pressure at 68 °C. The obtained residue (1 kg) was resuspended in deionized water and then fractionated by being consecutively partitioned with petroleum ether and ethyl acetate to obtain two fractions. The ethyl acetate fraction was chromatographed on silica gel eluting with a gradient of petroleum ether/ethyl acetate (20:1, 5:1, 1:1, and 0:1) to yield subfractions 1−4. Further purification of subfraction 4 used silica gel chromatography eluting with CH_2Cl_2−CH_3OH (10:1, 5:1, and 2:1; v/v) to obtain three new fractions 4a−4c. Fraction 4a was then purified by Sephadex LH-20 (CH_3OH) with a total solvent volume of about 5 times the column volume to afford ER1 (100 mg) and ER2 (100 mg).

Anti-inflammation Effects in Mouse J774A.1 Macrophage Cells. To determine the anti-inflammatory activity of the two compounds, mouse J774A.1 macrophages were cultured in 6-well plates (Costar, Corning, Inc., Corning, NY) overnight and reached the confluence of 80%. The cells were first treated with the compounds at final concentrations of 10 and 50 μM for 2 h. Then, LPS was added to the media at the initial concentration of 0.5 μg/mL.[6] Cells were incubated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% antibiotic/antimycotic at 37 °C under 5% CO_2. After 24 h, culture media was discarded and cells were collected to perform RNA isolation and real-time PCR.

RNA isolation and real-time PCR were performed according to the previously published protocol.[15] After 24 h of incubation, cells were washed with 1× phosphate-buffered saline (PBS) and TRIzol reagent was added for total RNA isolation. StrataScript first strand cDNA synthesis kit was used to reverse-transcribe cDNA. Real-time PCR was performed on the ABI Prism 7000 sequence detection system using TaqMan universal PCR master mix. The TaqMan Assay-On-Demand Tlr4, Tnf, Il1b, Il6, Il10, Ccl2, and Ptgs2 were used for gene detection. The mRNA amounts were normalized to an internal control, Tbp mRNA. The following amplification parameters were used for PCR: 50 °C for 2 min, 95 °C for 10 min, 46 cycles of amplification at 95 °C for 15 s, and 60 °C for 1 min.

Antiproliferative Activity in LNCaP Cells. The antiproliferation study was performed according to a published laboratory protocol.[16] LNCaP cells (2.5×10^4 cells/well) were plated in 6-well plates (Costar, Corning, Inc., Corning, NY). Cells were incubated in a humidified atmosphere at 37 °C and 5% carbon dioxide in a culture media of RPMI media supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic for 24 h, and then cells were treated with the compounds. Dimethyl sulfoxide (DMSO) was used as a vehicle. The initial treatment concentrations were 10, 50, and 100 μM, and fresh medium with treatment was replaced every 24 h. Cell growth was analyzed using a previously reported sulforhodamine B (SRB) assay.[17]

Statistical Analysis. Tests were conducted in triplicate with data reported as the mean ± standard deviation (SD). The significance level of differences in means was detected using one-way analysis of variation (ANOVA) and Tukey's test. Statistics were analyzed using SPSS for Windows (version release 10.0.1, 1999, SPSS, Inc., Somers, NY). Statistical significance was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Structure Identification of Engeletin and Astilbin from *E. roxburghiana*. ER1 was obtained as colorless needles from methanol. Its ^{1}H and ^{13}C NMR data, listed in Table 1, agreed well with the data reported for engeletin.[11,18,19] Thus, ER1 was identified as engeletin, also known as (2*R*,3*R*)-dihydrokaempferol 3-α-L-rhamnopyranoside. This is the first report on the isolation of this compound from the leaves of *E. roxburghiana*.

ER2 was obtained as colorless needles from methanol. Its ^{1}H and ^{13}C NMR data, listed in Table 1, agreed well with the data reported for (2*R*,3*R*)-taxifolin 3-*O*-α-L-rhamnopyranoside.[5] Thus, ER2 was identified as (2*R*,3*R*)-taxifolin 3-*O*-α-L-rhamnopyranoside, also named as astilbin.

Table 1. NMR Data for ER1 and ER2 (CD_3OD)[a]

	^{1}H NMR		^{13}C NMR	
C number	ER1	ER2	ER1	ER2
2	5.13 (1H, d, J = 10.8 Hz)	5.08 (1H, d, J = 10.8 Hz)	83.83	83.96
3	4.62 (1H, d, J = 10.8 Hz)	4.58 (1H, d, J = 10.4 Hz)	78.66	78.56
4			196.05	196.00
5			165.47	165.52
6	5.92 (1H, d, J = 1.6 Hz)	5.89 (1H, d, J = 2.0 Hz)	97.39	97.37
7			168.53	168.62
8	5.89 (1H, d, J = 1.6 Hz)	5.91 (1H, d, J = 2.0 Hz)	96.25	96.25
9			164.10	164.11
10			102.20	102.48
1′			128.58	129.18
2′	7.36 (1H, d, J = 8.4 Hz)	6.95 (1H, d, J = 1.6 Hz)	130.05	116.30
3′	6.85 (1H, d, J = 8.4 Hz)		116.41	146.54
4′			159.42	147.38
5′	6.85 (1H, d, J = 8.4 Hz)	6.85 (1H, m)	116.41	115.46
6′	7.36 (1H, d, J = 8.4 Hz)	6.85 (1H, m)	130.05	120.48
1″	3.99 (1H, s)	4.04 (1H, d, J = 1.6 Hz)	102.48	102.14
2″	4.30 (1H, m)	4.28 (1H, m)	71.74	71.77
3″	3.67 (1H, dd, J = 9.2, 2.8 Hz)	3.67 (1H, dd, J = 9.6, 3.2 Hz)	72.12	72.15
4″	3.50 (1H, m)	3.53 (1H, dd, J = 3.2, 1.6 Hz)	73.76	73.79
5″	3.34 (1H, m)	3.32 (overlap, m)	70.50	70.50
6″	1.19 (3H, d, J = 6.0 Hz)	1.18 (3H, d, J = 6.0 Hz)	17.85	17.85

[a] ER1 stands for engeletin, and ER2 stands for astilbin.

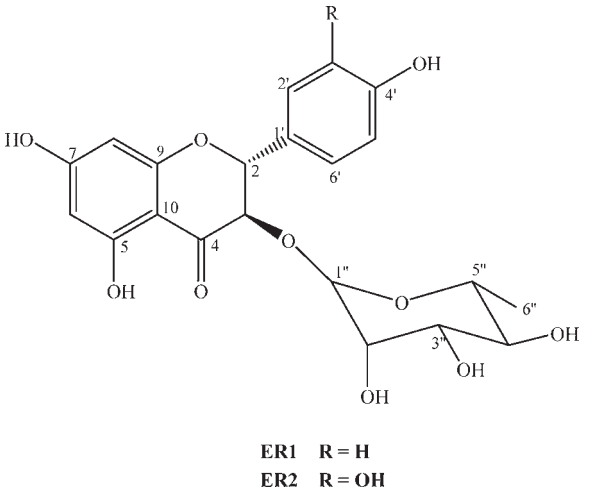

Figure 2. Chemical structures of engeletin (ER1) and astilbin (ER2).

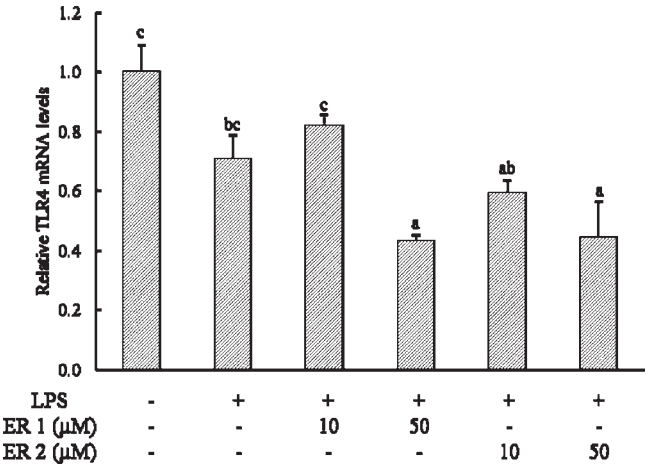

Figure 3. Effects of engeletin and astilbin on mRNA levels of TLR4 in mouse J774A.1 macrophage cells. ER1 stands for engeletin, and ER2 stands for astilbin. Cells (1.5×10^5/mL) were incubated with vehicle, LPS (0.5 μg/mL), or LPS plus 10 or 50 μM of ER1 and ER2. The mRNA levels of TLR4 were determined as described in the Materials and Methods. Each column represents the mean ± SD (n = 3). Columns marked by different letters are significantly different from each other ($p \leq 0.05$).

The structures of engeletin (ER1) and astilbin (ER2) are shown in Figure 2.

Anti-inflammatory Effect of ER1 and ER2 on the mRNA Expression of Inflammatory Cytokines. Engeletin and astilbin both exhibited a significant anti-inflammatory effect on the transcriptional level. Mouse J774A.1 macrophage cells were employed to simulate the innate immune system. The macrophage cells were first preincubated with samples for 2 h and then stimulated with LPS at the final concentration of 0.5 μg/mL to induce inflammation in the cells. LPS, the major component of the outer membrane of Gram-negative bacteria, is also known as endotoxin, which elicits strong immune responses in the macrophage. After 24 h of incubation, mRNA was isolated to perform real-time PCR.

TLRs recognize pathogen-associated molecular patterns indicating bacteria, fungi, and viruses to protect the host from foreign invasion.[20] A recent study showed that TLRs can

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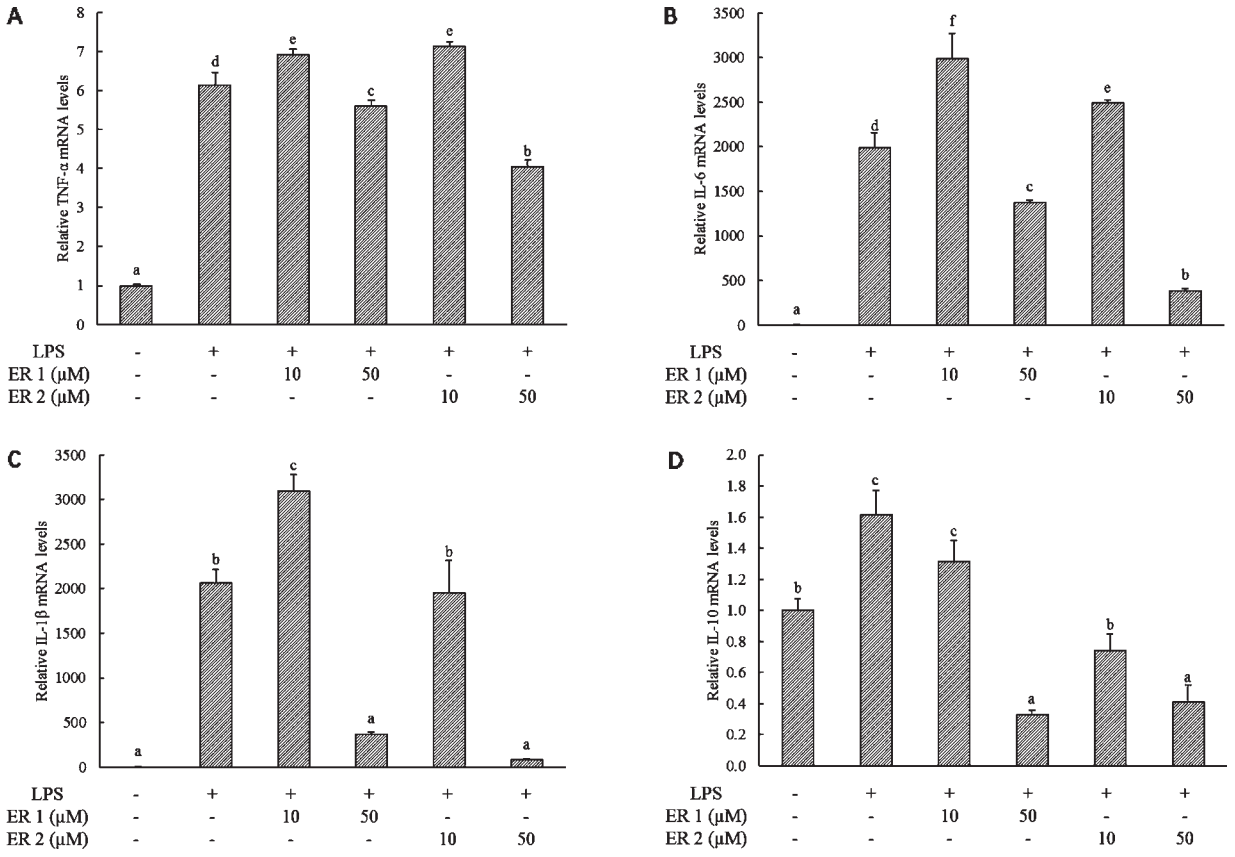

Figure 4. Effects of engeletin and astilbin on mRNA levels of (A) TNF-α, (B) IL-6, (C) IL-1β, and (D) IL-10 in mouse J774A.1 macrophage cells. ER1 stands for engeletin, and ER2 stands for astilbin. Cells (1.5×10^5/mL) were incubated with vehicle, LPS (0.5 μg/mL), or LPS plus 10 or 50 μM ER1 and ER2. The mRNA levels of TNF-α, IL-6, IL-β and IL-10 were determined as described in the Materials and Methods. Each column represents the mean ± SD (n = 3). Columns marked by different letters are significantly different from each other ($p \leq 0.05$).

recognize a wide variety of exogenous and endogenous molecular ligands involving self-recognition and host damage and repair.[21] TLR4 is the specific receptor of LPS, and binding of LPS will lead to the activation of the inflammatory cascade NF-κB pathway.[22] The expression of TLR4 is in response to the presence of LPS, as shown in Figure 3. The present study showed that LPS treatment significantly attenuated TLR4 mRNA expression in macrophage cells, which may be due to endotoxin tolerance.[23] Engeletin and astilbin treatments showed inhibition of TLR4 gene expression, and both engeletin and astilbin exhibited about 40% inhibition on TLR4 gene expression at the higher treatment concentration of 50 μM. A reduced expression of TLR4 will put the inflammatory response under control and attenuate the activation of downstream inflammatory cascades, including the NF-κB pathway, which is associated with the expression of a number of other inflammatory cytokines.

Normally, TNF-α is expressed at the baseline level. After LPS stimulation, a 4−6-fold increase in TNF-α expression was observed (Figure 4), which may lead to enhanced inflammatory response. Astilbin showed a stronger inhibitory effect on TNF-α expression at 50 μM concentration, although both engeletin and astilbin had significantly suppressed the LPS-stimulated TNF-α expression. At 10 μM concentration, neither engeletin nor astilbin could suppress TNF-α expression under the experimental conditions. With interleukins, the effects of engeletin and astilbin on IL-6 and IL-1β were studied. Both of them increased thousands of folds, induced by LPS treatment, suggesting that interleukins are the major target of LPS stimulation in inflammatory responses. Engeletin and astilbin exhibited remarkable inhibitory effects on both IL-6 and IL-1β at 50 μM concentration. More than 60 and 80% of induced IL-6 mRNA expression was suppressed by engeletin and astilbin, respectively (Figure 4), although they may further induce IL-6 mRNA expression at 10 μM concentration. Furthermore, engeletin and astilbin were able to inhibit about 80 and 95% IL-1β mRNA expression at a final treatment concentration of 50 μM (Figure 4). Interestingly, engeletin at 10 μM concentration was able to significantly further induce the expression of IL-1β mRNA, whereas astilbin showed no effect on IL-1β mRNA expression at 10 μM. These data suggested that the potential anti-inflammatory effect of astilbin and engeletin is highly dose-dependent.

TNF-α and IL-1β are known to be key activators of the NF-κB pathway.[24,25] TNF-α has been proven to play an important role in inflammation in mediating the proliferation and differentiation of immune cells and development of immune response.[26] TNF-α is one of the major inflammatory mediators secreted by activated macrophage and involved in many crucial events for the initiation of both acute and chronic inflammation, such as regulating the production of several cytokines, upregulation of adhesion molecule expression, and activation of leukocyte-specific chemotactic cytokines.[6,27] IL-1β can initiate signal transduction by activation of NF-κB and the mitogen-activated

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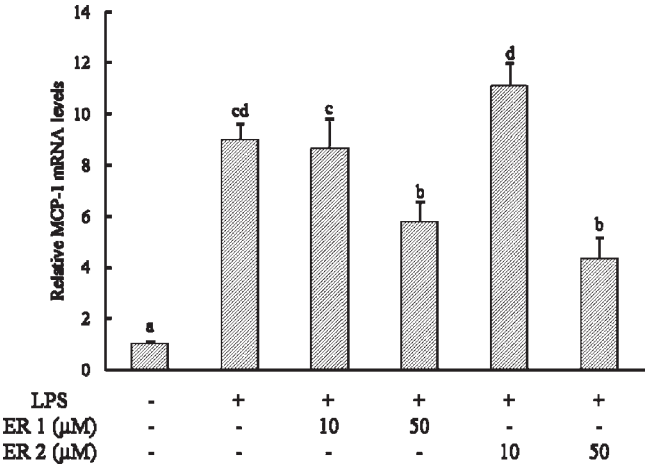

Figure 5. Effects of engeletin and astilbin on mRNA levels of MCP-1 in mouse J774A.1 macrophage cells. ER1 stands for engeletin, and ER2 stands for astilbin. Cells (1.5×10^5/mL) were incubated with vehicle, LPS (0.5 μg/mL), or LPS plus 10 or 50 μM of ER1 and ER2. The mRNA levels of MCP-1 were determined as described in the Materials and Methods. Each column represents the mean ± SD ($n = 3$). Columns marked by different letters are significantly different from each other ($p \leq 0.05$).

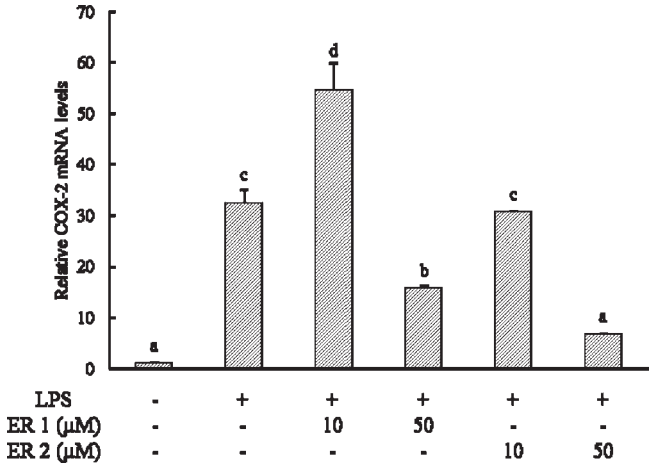

Figure 6. Effects of engeletin and astilbin on mRNA levels of COX-2 in mouse J774A.1 macrophage cells. ER1 stands for engeletin, and ER2 stands for astilbin. Cells (1.5×10^5/mL) were incubated with vehicle, LPS (0.5 μg/mL), or LPS plus 10 or 50 μM of ER1 and ER2. The mRNA levels of COX-2 were determined as described in the Materials and Methods. Each column represents the mean ± SD ($n = 3$). Columns marked by different letters are significantly different from each other ($p \leq 0.05$).

protein kinases (MAPKs) during the inflammatory process.[25] The activation of the NF-κB pathway will in turn induce further expression of a series of cytokines, including IL-1β, TNF-α, and IL-6, and promote the inflammatory process. IL-6 is a pleiotropic cytokine that is involved in initiation and development of inflammation, cardiovascular disease, type-II diabetes, and tumor growth and regulates cell proliferation and apoptosis.[28,29]

As shown in Figure 4, engeletin and astilbin showed inhibitory effects on IL-1β, IL-6, and TNF-α at the treatment concentration of 50 μM, which attenuated the inflammatory response by downregulating expression of cytokines and signal pathways. The decreased level of these cytokines will also reduce the further activation of the NF-κB pathway and, thus, alleviate immune response. Therefore, treatment with engeletin and astilbin at a required concentration not only modulates the inflammatory response through reducing cytokine expression, but also, more importantly, downregulates downstream signal pathways and attenuates further inflammatory responses.

In this study, it was observed that IL-10 mRNA expression was induced by LPS and inhibited by engeletin and astilbin treatments (Figure 4). LPS slightly induced IL-10 mRNA expression, while engeletin and astilbin were able to suppress the induced IL-10 mRNA expression. At a treatment concentration of 50 μM, IL-10 mRNA expression was reduced under the baseline level. IL-10 is expressed by various cells in the immune system, including macrophage.[30] IL-10 is a key immune-modulatory cytokine, which has shown both immunosuppressive and immunostimulatory effects.[31] It has been established as an immunosuppressive molecule, which modulates the function of several adaptive immunity-related cells by reducing the production of cytokines, such as TNF-α, IL-1β, and IL-6.[30,32] However, immunostimulatory properties of IL-10 in leukocyte recruitment, endothelial cell adhesion, molecule expression, and natural killer (NK) cell cytotoxicity have been reported in previous studies.[33−35] The inhibition of IL-10 mRNA expression is similar to adding a scabbard on the double-edged sword. The reduced expression of IL-10 will lead to increased production of cytokines in macrophage. However, these cytokines can be suppressed by engeletin and astilbin. Furthermore, the reduced expression of IL-10 mRNA also attenuates its immunostimulatory effects.

MCP-1 is a potent chemoattractant for monocytes during local immune response and plays an important role in the initiation of atherosclerosis.[36,37] It has been demonstrated that MCP-1 expression is regulated through the NF-κB pathway.[38] As shown in Figure 5, strong induction of MCP-1 mRNA expression by LPS was observed in this study, and this protein can also be stimulated by TNF-α, IL-1, IL-6, and IL-10.[36] After engeletin and astilbin treatments, reduced MCP-1 mRNA expression was detected in the cells, which may be due to their direct inhibitory effects on MCP-1 mRNA or indirect anti-inflammatory effects on related pathways. For instance, upstream attenuation of the NF-κB pathway may lead to reduced expression of MCP-1 mRNA, which may alleviate local immune response because of the fact that fewer immune cells will be attracted to the site.

COXs are a family of key enzymes that convert arachidonic acid to prostaglandins (PGs). PGs regulate various pathophysiological processes, including inflammatory reactions.[39] COX-2 is an isoform of the COX family and has been proven to be in response to many proinflammatory stimuli, growth factors, tumor promoters, and oncogenes and carcinogens.[40,41] Extensive evidence supports that COX-2 plays an important role in the development of inflammation. During inflammation, a 10−80-fold increase in the level of COX-2 mRNA expression can be observed in monocytes, macrophages, and endothelial cells.[42] As shown in Figure 6, COX-2 was significantly induced by LPS and engeletin and astilbin at the final treatment concentration of 50 μM could reduce the COX-2 mRNA expression by 50 and 80%, respectively, in LPS-stimulated cells. In contrast, engeletin at 10 μM significantly further induced COX-2 mRNA expression, while astilbin had no effect on COX-2 mRNA expression under the experimental conditions (Figure 6). In addition, this study showed that engeletin and astilbin could inhibit mRNA

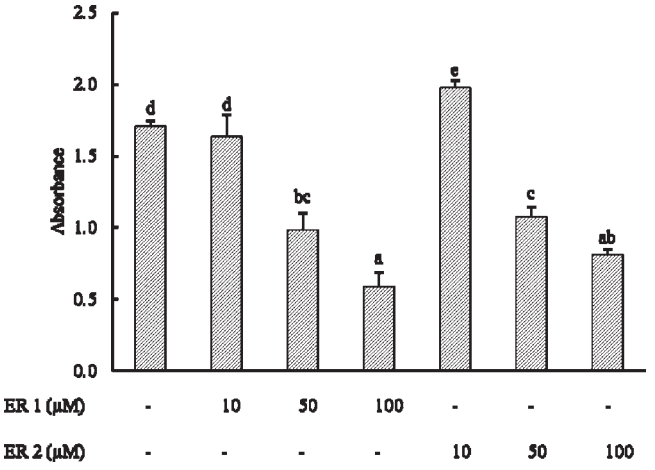

Figure 7. Effects of engeletin and astilbin on human LNCaP prostate cancer cell growth. ER1 stands for engeletin, and ER2 stands for astilbin. Cells (2.5×10^4/mL) were incubated with vehicle or 10, 50, or 100 μM of ER1 and ER2. The 96 h end-point cell numbers were determined as described in the Materials and Methods. Each column represents the mean ± SD ($n = 3$). Columns marked by different letters are significantly different from each other ($p \leq 0.05$).

expression of several inflammatory cytokines, which may lead to reduced activation of COX-2 and further reduce synthesis of PGs and inflammatory reactions.[40,42]

NF-κB, a ubiquitous transcription factor, plays an important role in the regulation of various inflammatory, apoptotic, and oncogenic genes.[43] Engeletin and astilbin showed suppressive effects on all cytokines and chemokines, and the mechanism involved may include the downregulation of the NF-κB pathway, suggesting that engeletin and astilbin may be considered possible candidates for anti-inflammatory compounds.

It was noticed that LPS treatment significantly induced the expression of inflammatory cytokines in macrophage cells, such as IL-6, IL-1β, and MCP-1, which indicated a progressive inflammatory state. Certain extents of inhibition on the expression of mRNA were observed in all of the high concentration treatments. Astilbin exhibited a relatively more potent inhibitory effect on mRNA expression of these inflammatory factors than engeletin under the experimental conditions. At lower concentration, the mRNA expression of TLR4, TNF-α, IL-6, IL-1β, and COX-2 was slightly increased. Other inflammatory factors and responses may be involved, and further investigation is required to obtain more evidence to explain this trend.

Antiproliferative Effect of Engeletin and Astilbin in LNCaP Cells. Growing evidence has gradually built up the link between inflammation and cancer.[44,45] Chronic inflammation has been shown to induce tumorigenesis. TNF-α, IL-1, IL-6, COX-2, and NF-κB are involved in various steps of carcinogenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis.[43,46] It is well-accepted that the inhibition of pro-inflammatory cytokines and chemokines may shed light on cancer prevention and therapy.

In this study, engeletin and astilbin have shown inhibitory effects on TNF-α, IL-1β, IL-6, and COX-2 in LPS-stimulated mouse macrophage cells. The potential antiproliferative effect was also tested in LNCaP human prostate cancer cells. As recently reported, prostate cancer has become the most common type of cancer in men and the second leading cause of cancer deaths in the United States.[47] Engeletin and astilbin exhibited a dose-dependent antiproliferative effect on human prostate cancer LNCaP cells in the concentration range of 10−100 μM (Figure 7). With a final concentration of 50 μM, engeletin and astilbin showed approximately 50% inhibition on cell growth; with 100 μM treatment, the cell proliferation was reduced by 65 and 60%, respectively, compared to the control group. Treatment with the concentration under 10 μM did not show significant inhibition of the cell growth.

Previous studies have revealed antitumor activity of flavonoids on human prostate cancer cells, which is further confirmed by this study.[48,49] However, other studies only observed suppressed proliferation on PC-3 cells, an androgen-independent cell line; in the present study, an antiproliferative effect was achieved in LNCaP cells, an androgen-responsive cell line. The inhibition of tumorigenesis may be at the transcriptional level, but the exact mechanism of the antiproliferative effect exhibited by engeletin and astilbin may be a combination of effects on several signal pathways, including the inflammatory NF-κB pathway and some proliferation pathways. The link between anti-inflammatory effects and antiproliferative effects may not be direct; however, the results from the present research warrant additional *in vitro* and *in vivo* studies to further investigate the anti-inflammatory effects of engeletin and astilbin and their potential effect in the prevention and treatment of prostate cancer.

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