

ARTICLE

Open Access



Metabolic profiling and anti-neuroinflammatory effects of *Liriope platyphylla* extract in LPS-stimulated BV2 microglia

Kwan-Woo Kim^{1†}, Woo-Cheol Shin^{2†}, Jin-Kyu Jang², Myoung-Jin Kim¹, Dahye Yoon¹, Young-Seob Lee^{1*} and Dae Young Lee^{2*} 

Abstract

Liriope platyphylla F. T. Wang & T. Tang (*L. platyphylla*) is a perennial member of the Liliaceae family, is predominantly found in East Asian regions such as Korea, Taiwan, and Japan. In this study, we investigated the neuroinflammation-modulating potential of an ethanol-based extract from *L. platyphylla* (LPE) using BV2 microglial cells activated by lipopolysaccharide (LPS). In addition, we examined which bioactive constituents in LPE contribute to its anti-neuroinflammatory effects by isolating and profiling the major compounds. Notably, treating LPE prior to LPS exposure led to a marked decrease in the synthesis of inflammatory mediators, including cyclooxygenase-2 (COX-2), nitric oxide (NO), prostaglandin E₂ (PGE₂), inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines such as IL-6, IL-1 β , and tumor necrosis factor- α (TNF- α) in the microglia. Mechanistic studies revealed that suppressive effects of LPE are linked to the downregulation of LPS-triggered signaling pathways, specifically those involving Toll-like receptor 4 (TLR4), nuclear factor kappa B (NF- κ B), and the p38 and JNK branches of the mitogen-activated protein kinase (MAPK) cascade. Chemical analysis of the extract identified eight distinct compounds, with methylophiopogonone A (**2**) and methylophiopogonanone B (**3**) being primarily responsible for the observed anti-neuroinflammatory activity. Quantitative profiling by ultra-performance liquid chromatography coupled with quadrupole time of flight / mass spectrometry (QTOF/MS) indicated that these two bioactive constituents were present in the ethyl acetate fraction of LPE at concentrations of 0.0007 ± 0.0001 mg/g and 0.0197 ± 0.0009 mg/g, respectively. Collectively, these findings highlight the promise of LPE as a source for developing novel functional ingredients or herbal therapeutics targeting neuroinflammatory conditions.

Keywords Anti-neuroinflammation, BV2 microglial cells, *Liriope platyphylla*, Quantitative analysis

[†]Kwan-Woo Kim and Woo-Cheol Shin contributed equally to this work.

*Correspondence:
Young-Seob Lee
youngseoble@korea.kr

Dae Young Lee
dylee80@knu.ac.kr

¹Department of Herbal Crop Research, Rural Development Administration, National Institute of Horticultural and Herbal Sciences, Eumseong 27709, Republic of Korea

²BK21 FOUR KNU Creative BioResearch Group, School of Life Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

Introduction

Neuroinflammation refers to the immune response that occurs within the central nervous system (CNS), primarily initiated by microglial cells, the resident immune cells of the brain and spinal cord [1]. When exposed to various stimuli, microglia transition from a resting to an activated state, leading to the release of inflammatory mediators and alterations in their phenotype and function [2, 3]. While this activation is essential for CNS defense and tissue repair, persistent or dysregulated microglia activation, especially under chronic stress or exposure to agents like lipopolysaccharide (LPS), inflammatory cytokines, or β -amyloid, can result in excessive production of pro-inflammatory molecules, including prostaglandin E2 (PGE2), nitric oxide (NO), interleukins (IL), and tumor necrosis factor (TNF). Overproduction of pro-inflammatory mediators can disrupt neuronal function, cause cellular damage, and ultimately lead to neuronal loss [4, 5]. Such sustained inflammation is closely linked to the progression of neurodegenerative diseases including Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease [6–8]. Therefore, strategies that suppress microglial activation and limit the release of pro-inflammatory mediators are increasingly recognized as promising therapeutic approaches for preventing or treating neuroinflammation and associated neurodegenerative diseases.

Liriope platyphylla F. T. Wang & T. Tang (*L. platyphylla*) is a perennial herb belonging to the Liliaceae family that is mainly distributed in the mountainous regions of temperate East Asia, including Korea, Taiwan, and Japan. According to the Korean Medical Compendium *Donguibogam*, designated as a UNESCO Memory of the World in 2009, this plant has traditionally been used in both food and medicine for the treatment of treating cough, asthma, lung inflammation, and constipation [9, 10]. In addition to these traditional uses, recent studies have demonstrated new therapeutic properties of *L. platyphylla*, including anti-cancer [11], anti-diabetic [12], anti-obesity [13], and hepatoprotective effects [14]. Furthermore, the Rural Development Administration (RDA) of Korea reported that saponins in *L. platyphylla* extract alleviate dopaminergic dysfunction and repeated nicotine-induced behavioral sensitization [10].

According to the Food Code issued by the Korean Ministry of Food and Drug Safety, *L. platyphylla* is categorized as a “substance permitted for limited use in food,” meaning it can comprise no more than 50% of the raw materials in certain food products. To overcome this limitation, it is essential to develop optimized extraction methods for Korean *L. platyphylla*, to explore its diverse biological activities, and perform safety assessments. Accordingly, we investigated the anti-neuroinflammatory

effects of a 50% ethanol extract of Korean-cultivated *L. platyphylla* on LPS-stimulated BV2 microglial cells.

Materials and methods

Plant materials and the Preparation of ethanolic extract of *L. platyphylla*

The roots of *Liriope platyphylla* were purchased from the Korea Medicine Herbal Association (S. Korea) and authenticated through cultivation in Cheongyang Province. The extraction procedure followed a previously described method [10], in which the roots were coarsely ground and subjected to two rounds of reflux extraction in water at 80 °C for 4 h.

Reagents and instruments

High-resolution mass measurements and quantitative analyses were performed using a Waters Xevo G3 QTOF/MS system (Waters Corp., USA) coupled with an ACQUITY I-Class UPLC (Waters Corp., USA). High-performance liquid chromatography (HPLC)-grade distilled water and acetonitrile (Fisher Scientific Ltd., S. Korea) were used as solvents. Formic acid ($\geq 99.0\%$ purity, Honeywell Fluka™, Fisher Scientific) was added to the mobile phase to ensure compatibility with mass spectrometric detection. All other reagents were of analytical grade or higher. The products and manufacturers used for in vitro experiments are listed in Table S1.

Identification of compounds from *Liriope platyphylla*

Compounds 1–8 were isolated from the roots of *Liriope platyphylla* using successive in-house chromatographic techniques. These compounds were obtained as white powders and identified by electrospray ionization and quadrupole time of flight / mass spectrometry (ESI-QTOF/MS) as methylophiopogonanone A (1; m/z 365.2162 $[M+Na]^+$), methylophiopogonone A (2; m/z 363.2391 $[M+Na]^+$), methylophiopogonanone B (3; m/z 351.2359 $[M+Na]^+$), methylophiopogonone B (4; m/z 349.2187 $[M+Na]^+$), ophiopogonin A (5; m/z 787.2388 $[M+Na]^+$), ophiopogonin D (6; m/z 855.5300 $[M+H]^+$), ophiopogonin D' (7; m/z 855.5300 $[M+Na]^+$), and prosapogenin A (8; m/z 746.4548 $[M+Na]^+$) (Fig. S1, Table 1), which was confirmed by a spectral comparison with previously published NMR data [15–17]. The purity of all compounds was confirmed to be 95–99% by UPLC peak area normalization.

Cell culture

BV2 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μ g/mL streptomycin, and 100 units/mL penicillin. The culture was maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

Table 1 Relative percentages (%) of eight secondary metabolites in the LPE-EtOAc fraction

No.	Component name	Formula	Molecular Weight (MW)	Observed m/z	Observed RT (min)	Adducts	Relative percentages (Response,%) LPE-EtOAc fr.
1	methylophiopogonanone A	C ₁₉ H ₁₈ O ₆	342.22	365.2162	11.88	+Na	6283.8(3.3%)
2	methylophiopogonone A	C ₁₉ H ₁₆ O ₆	340.14	363.2391	10.70	+Na	2619.0(1.4%)
3	methylophiopogonanone B	C ₁₉ H ₂₀ O ₅	328.13	351.2359	12.48	+Na	13916.4(7.4%)
4	methylophiopogonanone B	C ₁₉ H ₂₀ O ₅	326.11	349.2187	13.49	+Na	14571.4(7.8%)
5	ophiopogonin A	C ₄₁ H ₆₄ O ₁₃	764.43	787.2388	13.89	+Na	2036.7(1.1%)
6	ophiopogonin D	C ₄₄ H ₇₀ O ₁₆	854.46	855.5300	14.22	+H	79218.8(42.2%)
7	ophiopogonin D'	C ₄₄ H ₇₀ O ₁₆	854.46	877.5126	14.80	+Na	11784.8(6.3%)
8	prosapogenin A	C ₃₉ H ₆₂ O ₁₂	722.42	746.4548	15.00	+H, +Na	57315.8(30.5%)

Cell viability assay

The MTT assay was employed to evaluate *L. platyphylla* ethanolic extract (LPE) cytotoxicity in BV2 microglial cells. Cells were seeded in 48-well plates at 2×10^4 cells/well and allowed to adhere for 24 h before exposure to varying concentrations of LPE (dissolved in dimethyl sulfoxide [DMSO]) for an additional 24 h. The final DMSO concentration in all treatments was maintained at $\leq 0.1\%$ relative to the total culture medium. Following treatment, cells were incubated with 50 μ L of MTT solution (0.5 mg/mL) at 37 °C for 1 h to allow formazan crystal formation. The insoluble formazan product was subsequently dissolved using 150 μ L DMSO, and optical density measurements were conducted at 540 nm using a microplate reader.

Measurement of nitric oxide (NO) levels

BV2 microglial cells seeded in 24-well plates and co-treated with LPS (1 μ g/mL) and varying concentrations of LPE for 24 h. To quantify NO production, 100 μ L aliquots of conditioned media were combined with an equal volume of Griess reagent (Sigma-Aldrich) and incubated at room temperature for 15 min. Absorbance was measured at 540 nm using a microplate reader, and the NO concentration was calculated using a sodium nitrite standard curve.

PGE₂ assay

BV2 cells were plated in 24-well plates and incubated for 24 h to allow cell attachment. After this period, the cells were exposed to LPE for 1 h before being challenged with LPS for an additional 24 h. Upon completion of the treatments, the amount of PGE₂ released into the culture medium was measured using an ELISA kit, following the instructions provided by the manufacturer.

Quantitative real-time reverse transcription polymerase chain reaction PCR (qRT-PCR)

Total RNA was extracted from BV2 microglial cells using TRIzol reagent (Invitrogen), following the supplier's protocol. The RNA concentration was determined by spectrophotometric measurements at 260 nm. For cDNA

synthesis, 4 μ g of purified total RNA served as the template for reverse transcription using ReverTra Ace™ qPCR RT Master Mix (TOYOBO). Relative gene expression was quantified using qTOWER3 G Real-Time PCR Thermal Cycle (Analytik Jena, Jena, Germany) with THUNDERBIRD™ Next SYBR® qPCR Mix (TOYOBO). The oligonucleotide primers sequences used in this investigation were designed and purchased from Cosmo Genetech (Seoul, Republic of Korea), and are listed in Table S2. Gene expression levels were adjusted to the endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Cyclic threshold (Ct) values of target genes were analyzed with the comparative $\Delta\Delta C_t$ method ($2^{-\Delta\Delta C_t}$) using qPCR analysis software (version 4.0, Analytik Jena, Jena, Germany).

Western blot analysis

BV2 microglial cells were pre-treated with LPE at concentrations of 250, 500, and 1,000 μ g/mL, followed by stimulation with LPS to induce inflammation. The detailed procedures for the Western blot analysis of iNOS, COX-2, NF- κ B-related proteins, and MAPK-associated proteins were conducted by referring to previously published literature [18].

Preparation of the cytosolic and nuclear fractions

After collecting BV2 microglial cells, the cells were centrifuged at $16,000 \times g$ for 5 min to form pellets. The separation of cytoplasmic and nuclear fractions was then performed using NE-PER nuclear and cytoplasmic extraction reagents, following the manufacturer's recommended protocol.

Quantitative multiple reaction monitoring (MRM) analysis and compound profiling using LC–ESI–QTOF/MS

For profiling and quantitative analysis, standard solutions of methylophiopogonone A (2) and methylophiopogonanone B (3) were prepared by dissolving each compound in methanol. Calibration curves were generated using five to seven concentration points in the range of 0.06–2 ppm. Dried *Liriope platyphylla* root powder was extracted twice by refluxing in 50% fermented ethanol at

60 °C for 4 h per cycle, followed by filtration using filter paper (ADVANTEC, Japan). The pooled aqueous layers were evaporated under reduced pressure and freeze-dried to obtain the crude extract. A portion of the dried extract (2 g, yield: 62.61%) was reconstituted in 40 mL of water and subjected to liquid-liquid extraction with ethyl acetate (2 × 40 mL) using a separatory funnel. The ethyl acetate (EtOAc) fraction was concentrated, after which 5 mg (0.23%) was reconstituted in 50% ethanol, passed through a 0.22 µm polyvinylidene fluoride (PVDF) syringe filter, and subjected to compound profiling and quantitative analysis.

The analysis was performed on a UPLC system coupled to a ESI-Q-TOF/MS operated in positive ion mode. Separation was achieved using an ACQUITY BEH C18 column (2.1 mm × 100 mm, 1.7 µm) maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) with the following gradient: 0–1 min, 25% B; 1–5 min, 25–40% B; 5–19 min, 40–50% B; 19–20 min, 50–85% B; 20–22 min, 85% B; 22–23 min, 85–25% B; 23–25 min, 25% B. A total of 2 µL of sample was injected, and the mobile phase was delivered at a flow rate of 0.4 mL/min.

Mass spectrometric parameters were set as follows: desolvation temperature, 550 °C; source temperature, 120 °C; cone gas flow, 30 L/h; capillary voltage, 3.0 kV; desolvation gas flow, 800 L/h; cone voltage, 40 V; mass scan range, *m/z* 100–2,000; low collision energy, 6 V; high collision energy ramp, 20–45 V. Data acquisition was performed using MassLynx software and data processing was performed with UNIFI software (version 3.8.0.23, Waters Corp., USA).

Statistical analysis

Data are expressed as mean ± SD from a minimum of three biological replicates. Statistical comparisons were performed using one-way analysis of variance (ANOVA)

followed by Tukey's post-hoc test in GraphPad Prism (version 4.0; GraphPad Software, San Diego, CA, USA). The significance thresholds were defined as follows: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 compared to the LPS-treated group.

Results and discussion

Impact of LPE on NO and PGE₂ production in BV2 microglial cells exposed to LPS

To determine the cell survival rate and cytotoxicity of the extract, we investigated the cytotoxicity of LPE using the MTT assay. Treatment of BV2 microglial cells with LPS at 125–1,000 µg/mL concentration had no effects of the cell viability, while 2,000 µg/mL of LPE reduced cell viability (Fig. 1A). Thus, in subsequent experiments, a concentration of 1,000 µg/mL was set as the highest concentration for measuring the anti-neuroinflammatory effects of LPE. NO acts as a pleiotropic signaling molecule and plays dual roles in physiological processes (vasodilation and neurotransmission) and pathological conditions, such as neuroinflammation and neurodegenerative disorders. Its overproduction in the activated microglia contributes to neuronal oxidative stress and synaptic dysfunction, thereby driving disease progression [19]. In addition, excessive production of PGE₂ is closely linked to various inflammatory diseases as it promotes the upregulation of growth factors, cytokines, and other pro-inflammatory molecules that drive and amplify pathological inflammation [8, 19]. Therefore, we investigated the inhibitory effect of LPS on NO and PGE₂ production. The increased levels of NO and PGE₂ caused by due to LPS exposure was decreased by pre-treatment with LPE (Fig. 1B, C). Based on these results, LPE has a remarkable suppressive effect on the production of inflammatory mediators.

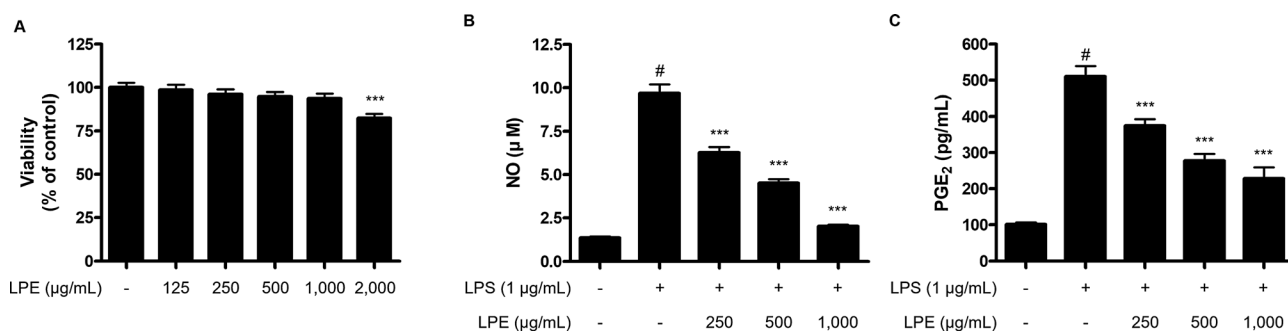


Fig. 1 Inhibitory effects of LPE on LPS-stimulated NO and PGE₂ production in BV2 microglial cells. **(A)** Cells viability was evaluated using the MTT assay after treatment with LPE (125–2,000 µg/mL) for 24 h. Data are presented as mean ± SD (*n* = 3). ****p* < 0.001 versus the control group. **(B, C)** BV2 microglial cells were pre-treated with LPE (250–1,000 µg/mL) for 1 h, followed by stimulation with LPS (1 µg/mL) for 24 h. NO levels **(B)** in the culture medium were determined using the Griess assay, and PGE₂ levels **(C)** were quantified by ELISA under the same experimental conditions. Data represent the mean values of three independent experiments ± SD. #*p* < 0.05 versus the control group; ****p* < 0.001 versus the LPS-treated group. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; NO, nitric oxide; PGE₂, prostaglandin E₂

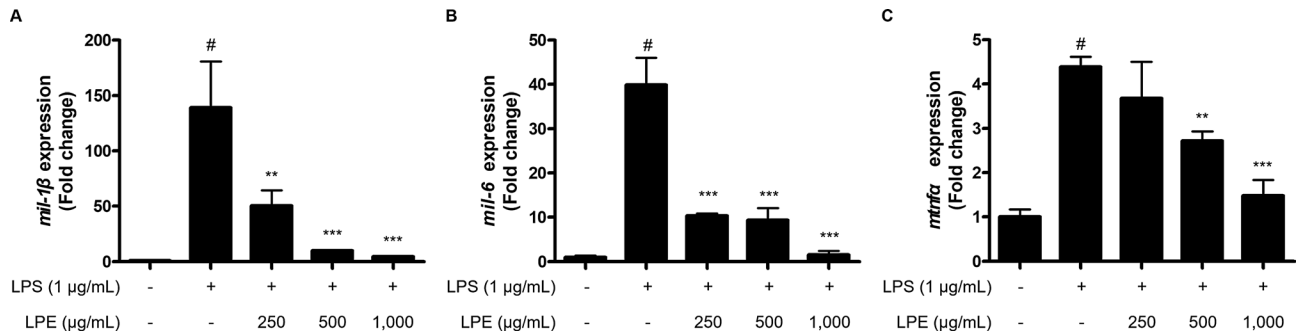


Fig. 2 LPE suppresses the mRNA expression of inflammatory genes in LPS-stimulated BV2 microglial cells. Cells were pre-treated with LPE (125–1,000 μg/mL) for 1 h and then stimulated with LPS (1 μg/mL) for 6 h. The mRNA expression levels of IL-1β, IL-6, and TNF-α were analyzed by qRT-PCR. Significant differences are indicated by # $p < 0.001$ when compared to control and ** $p < 0.01$, and *** $p < 0.001$ when compared to the LPS-treated group. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor alpha

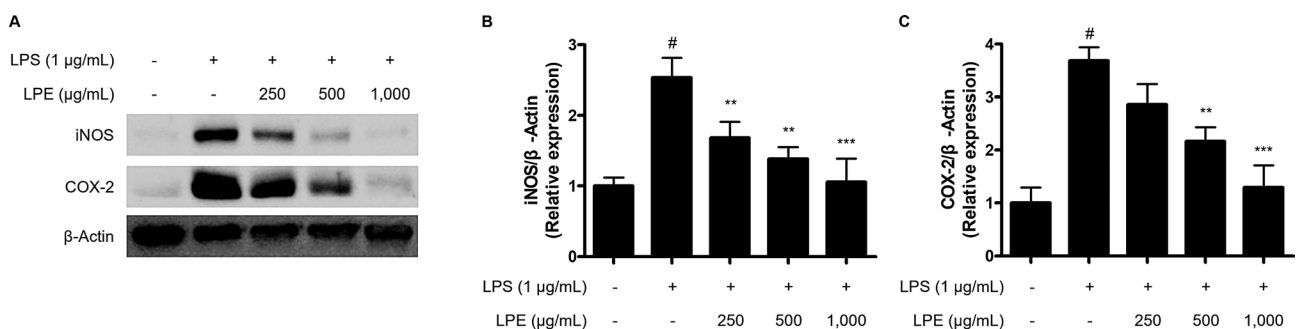


Fig. 3 LPE reduces LPS-stimulated protein expression of iNOS and COX-2 in BV2 microglial cells. Cells were pre-treated with LPE (125–1,000 μg/mL) for 1 h, and then stimulated with LPS (1 μg/mL) for 24 h. The alteration of protein levels of iNOS and COX-2 was determined by western blot analysis. β-Actin was used as a loading control. (A) Representative blots from three independent experiments are shown. (B, C) Bar graphs show densitometric quantification. Compared to the control group, # p value are less than 0.001; compared to the LPS-treated group, significance is shown as ** $p < 0.01$, and *** $p < 0.001$. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2

Modulation of cytokine mRNA levels by LPE in BV2 microglial cells treated with LPS

Pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), are signaling molecules released by immune cells such as macrophages, T lymphocytes, and dendritic cells to mediate inflammatory responses. Elevated levels of these proteins amplify neuroinflammatory responses by activating microglia and astrocytes, which, in turn, release additional inflammatory mediators. This creates a self-sustaining cycle that disrupts neuronal function and promotes neurodegeneration [20, 21]. To explore how LPS suppresses the expression of pro-inflammatory cytokines at the molecular level, qRT-PCR was employed to measure the mRNA levels of cytokines including IL-6, IL-1β, and TNF-α in the treated cells. As shown in Fig. 2, the mRNA expression of IL-6, IL-1β, and TNF-α which was markedly upregulated by LPS stimulation, inhibited by pre-treatment with LPE. These results suggested that LPE regulates the expression of pro-inflammatory cytokines at the transcriptional level.

Influence of LPE on iNOS and COX-2 protein levels in BV2 microglial cells treated with LPS

The iNOS enzyme is implicated in elevating oxidative stress within neurons and glial cells by facilitating the conversion of L-arginine to NO and L-citrulline, with N-hydroxy-L-arginine serving as an intermediate in this reaction [22, 23]. COX-2 also plays a pivotal role in neuroinflammatory processes, being responsible for the biosynthesis of prostaglandins from arachidonic acid [24, 25]. In this study, we assessed how LPE modulates the expression of these pro-inflammatory proteins in BV2 microglial cells following LPS stimulation. As illustrated in Fig. 3, treatment with LPE led to a substantial decrease in the protein levels of both iNOS and COX-2 in LPS-challenged BV2 microglial cells. These findings indicate that LPE suppresses the production of pro-inflammatory mediators and cytokines in microglia by downregulating iNOS and COX-2 expression.

Regulation of NF-κB signaling by LPE in BV2 microglial cells following LPS exposure

Nuclear factor kappa B (NF-κB) pathways serve as central regulators of innate and adaptive immune responses

and their dysregulation has been implicated in chronic inflammatory diseases and autoimmune disorders [26]. Under basal conditions, NF- κ B transcription factors (primarily p65/p50 heterodimers) remain sequestered in the cytoplasmic compartment through binding to the inhibitory protein I κ B- α . Upon inflammatory stimulation, the canonical NF- κ B pathway becomes activated, leading to the phosphorylation of I κ B- α by the I κ B kinase (IKK) complex. This phosphorylation event targets I κ B- α for ubiquitination and subsequent degradation by the proteasome, thereby releasing NF- κ B to translocate into the nucleus and regulate gene expression including cytokines, chemokines, and adhesion molecules [27]. Thus, to clarify how LPE suppresses LPS-induced upregulation of pro-inflammatory enzymes and mediators, we assessed its impact on the phosphorylation and degradation of cytoplasmic I κ B- α , as well as the nuclear translocation of the NF- κ B subunits p65 and p50. Pre-treatment with LPE efficiently inhibited LPS-stimulated phosphorylation and subsequent degradation of I κ B- α in the cytoplasm (Fig. 4A–C). As a result, the nuclear accumulation of p65 and p50 was reduced, as demonstrated by Western blot analysis (Fig. 4D–F). Taken together, these findings indicate that LPE exerts anti-neuroinflammatory effects by interfering with the canonical NF- κ B activation pathway, highlighting its potential as a therapeutic agent for neuroinflammatory conditions.

LPE-mediated modulation of MAPK phosphorylation in BV2 microglial cells following LPS challenge

MAPKs are a group of serine/threonine-specific protein kinases that play vital roles in regulating numerous cellular activities, such as cell proliferation, differentiation, survival and inflammatory responses [28]. Phosphorylation of the three major MAPK subunits, p38, ERK, and JNK, correlates with the neuroinflammatory response upon stimulation [2]. Once these signaling cascades are activated, they promote the transcription of pro-inflammatory mediators by influencing downstream transcription factors, such as activator protein-1 (AP-1) and NF- κ B. This modulation results in elevated synthesis of cytokines, chemokines, and inflammatory enzymes including COX-2 [29]. Therefore, we assessed whether LPE affected MAPK phosphorylation. To investigate the effects of LPE on MAPK signaling in neuroinflammation, the phosphorylation status of p38, ERK, and JNK in LPS-stimulated BV2 microglial cells was assessed by western blotting. LPE pre-treatment resulted in a notable of LPS-induced phosphorylation of both p38 and JNK in BV2 microglial cells (Fig. 5A, C), whereas ERK activation remained unaffected (Fig. 5B). These data demonstrate that LPE selectively inhibits p38/JNK-dependent MAPK signaling and synergistically attenuates NF- κ B activation, collectively reducing downstream inflammatory mediator synthesis.

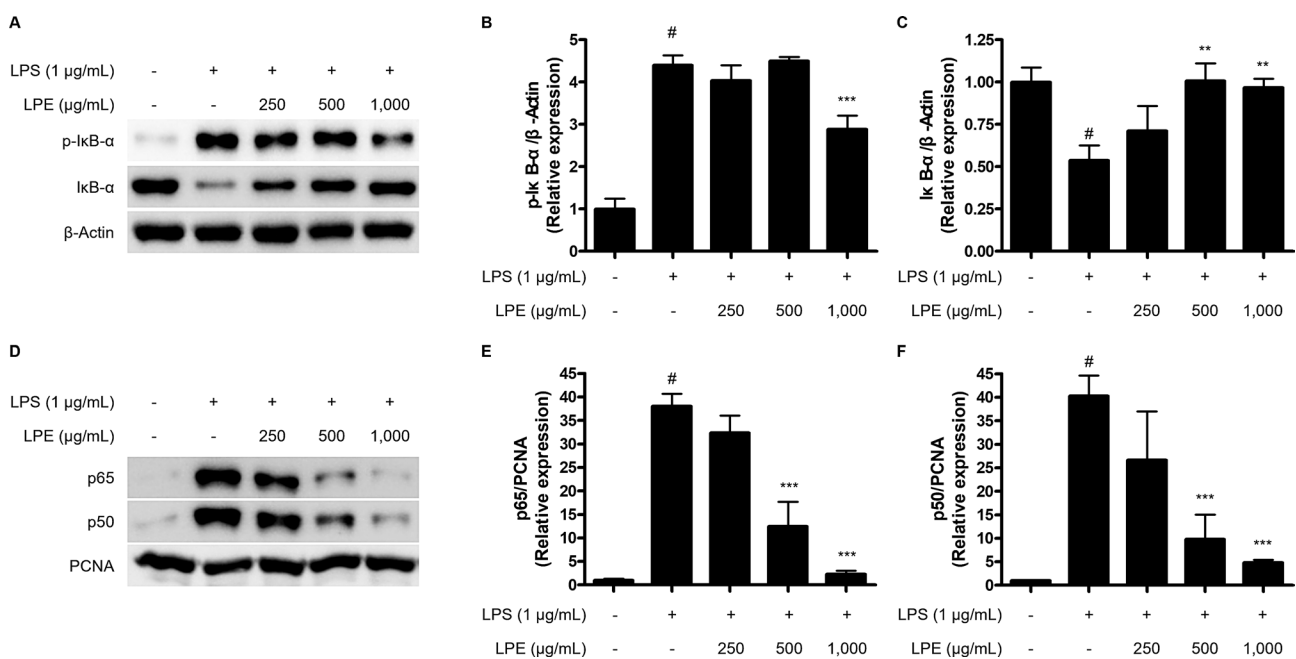


Fig. 4 LPE inhibits LPS-stimulated activation of NF- κ B signaling pathway in BV2 microglial cells. After pre-treatment with LPE (250–1,000 μ g/mL) for 1 h, cells were stimulated with LPS (1 μ g/mL) for 1 h. Cytoplasmic protein were analyzed for p-I κ B- α and I κ B- α and nuclear protein for p65 and p50 via Western blotting. β -Actin and PCNA were used as a loading control for cytoplasm and nuclear, respectively. (A, D) Representative blots of p-I κ B- α and I κ B- α in the cytosolic fraction (A) and p65 and p50 in the nuclear fraction (D) from three independent experiments are shown. (B, C, E, F) Relative expression of p-I κ B- α (B) I κ B- α (C), p65 (E), and p50 (F). Symbol # denotes $p < 0.001$ relative to control; symbol ** and *** represent $p < 0.01$ and $p < 0.001$, respectively, relative to LPS stimulation. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; NF- κ B, nuclear factor kappa B; PCNA, proliferating cell nuclear antigen

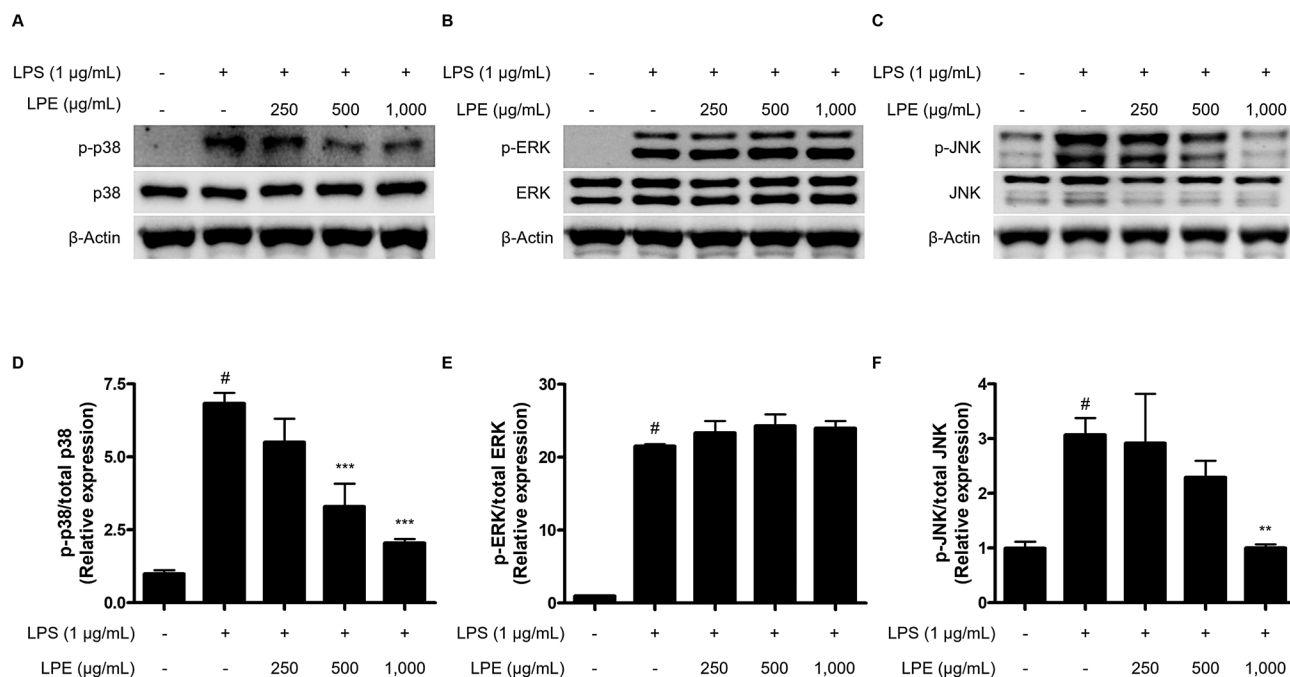


Fig. 5 Effects of LPE on the activation of MAPK signaling pathway in LPS-stimulated BV2 microglial cells. Cells were pre-treated with LPE (250–1,000 µg/mL) for 1 h, and then exposed to LPS (1 µg/mL) for 30 min. The alteration of protein levels of p-p38/p38, p-ERK/ERK, and p-JNK/JNK was determined by western blot analysis. β-Actin and was used as a loading control. (A, B, C) Representative blots from three independent experiments are shown. (D, E, F) Quantitative data are presented as mean ± SD ($n=3$). # $p<0.001$ versus the control group; ** $p<0.01$ and * $p<0.001$ versus the LPS-treated group. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase

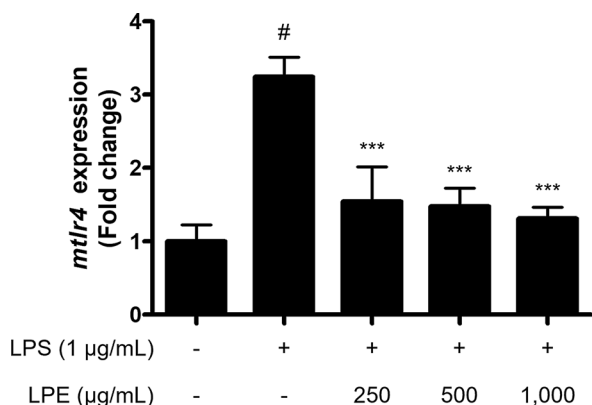


Fig. 6 LPE downregulates TLR4 mRNA expression in LPS-stimulated BV2 microglial cells. Cells were treated with LPE (250–1,000 µg/mL) for 1 h and then stimulated with LPS (1 µg/mL) for 6 h. The expression of level of TLR4 mRNA was quantified by qRT-PCR. Data are expressed as mean ± SD ($n=3$). Significant differences are indicated by # $p<0.001$ when compared to control; *** $p<0.001$ when compared to the LPS-treated group. LPE, *L. platyphylla* ethanolic extract; LPS, lipopolysaccharide; TLR4, Toll-like receptor 4

Modulatory effect of LPE on TLR4 mRNA levels in BV2 microglial cells stimulated with LPS

Upon priming with to LPS, microglial cells recognize and bind to LPS via TLR4, triggering robust immune and inflammatory responses [30]. Engagement of TLR4 by LPS initiates downstream signaling cascades, notably both NF-κB and MAPK pathways, which, in turn, drive the production of various pro-inflammatory mediators

[31]. To investigate the regulatory effects of LPE on TLR4 signaling, TLR4 mRNA expression was quantified by qRT-PCR. LPS stimulation significantly upregulated TLR4 transcription, whereas LPE pre-treatment inhibited this increase in a dose-dependent manner (Fig. 6), demonstrating its ability to suppress TLR4-mediated inflammatory priming at the transcriptional level.

LC-ESI-QTOF/MS profiling and MRM quantitative analysis

LC-ESI-QTOF/MS profiling of eight isolated compounds from *L. platyphylla* revealed the following retention times: methylphiopogonone A (2) at 10.58 min, methylphiopogonanone A (1) at 11.88 min, methylphiopogonanone B (3) at 12.47 min, methylphiopogonanone B (4) at 13.49 min, ophiopogonin A (5) at 13.89 min, ophiopogonin D (6) at 14.22 min, ophiopogonin D' (7) at 14.80 min, and prosapogenin A (8) at 15.00 min. A comparative analysis of the relative contents of the eight compounds revealed that ophiopogonin D (6) and prosapogenin A (8) were detected at relatively high levels (Table 1). Quantitative analysis of compounds 2 and 3, which exhibited the highest bioactivity, was performed using UPLC-ESI-QTOF/MS in the QTOF-MRM mode. The precursor ions were set at m/z 341.1020 for methylphiopogonone A (2) and m/z 329.1479 for methylphiopogonanone B (3) (Fig. 7). Fragmentation was induced using low and high collision energies and the

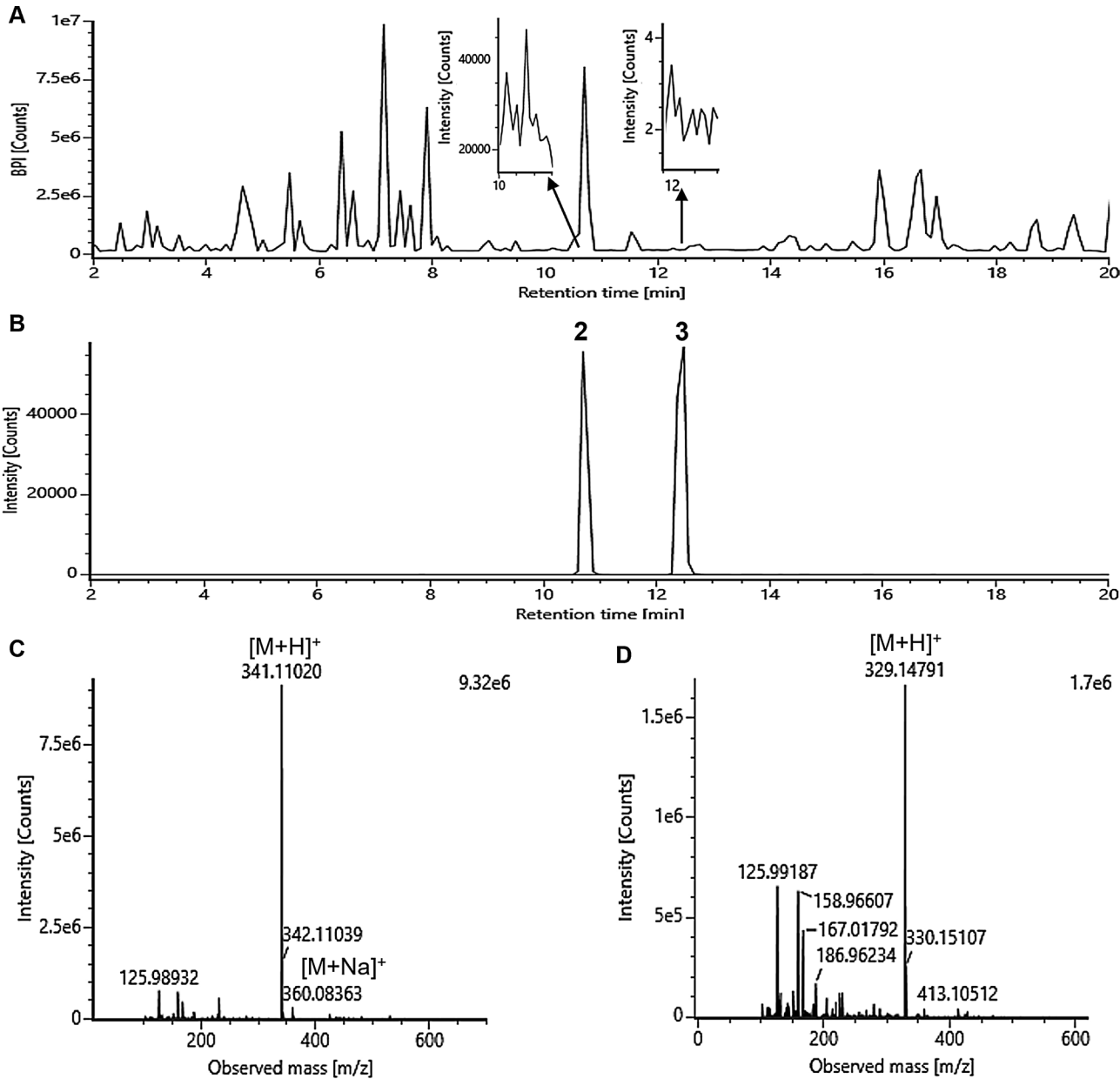


Fig. 7 LC-ESI-QTOF/MS in positive-ion mode was performed (A) BPI chromatogram of LPE-EtOAc fr (B) overlaid extracted ion chromatograms (EIC) of two standard compounds, (C) mass spectrum of methylophiopogonone A (2), and (D) mass spectrum of methylophiopogonone B (3). LC-ESI-QTOF/MS, ultra-performance liquid chromatography combined with electrospray ionization and quadrupole time-of-flight mass spectrometry; LPE-EtOAc fr, *L. platyphylla* ethyl acetate fraction; EIC, extracted ion chromatogram; BPI, base peak intensity

Table 2 MRM analysis of compounds 2 and 3 using UPLC-QTOF/MS

Compounds	Time range (min)	Molecular Formula	MW	Measured Value ^a	TOF-MRM Fragmentation ^a (low and high energy)
2	9–15	C ₁₉ H ₁₈ O ₆	340.33	341.1102[M+H] ⁺	341[M+H] ⁺ ; 121[M-C ₁₂ H ₁₁ O ₄ +H] ⁺ ;
3	7–13	C ₁₉ H ₂₀ O ₅	328.36	329.1479[M+H] ⁺	329[M+H] ⁺ ; 219[M-C ₁₂ H ₁₃ O ₄ +H] ⁺ ;

^a Mass accuracy < 10.0 ppm

most sensitive product ions were selected for quantification. The major product ions were *m/z* 269 for compound 2 and *m/z* 301.1077 for compound 3 (Table 2, Fig. S2). Calibration curves were constructed using by linear

regression analysis over five to seven concentration levels of the standard. The correlation coefficients (*R*²) were 0.999 and 0.998 for compounds 2 and 3, respectively, indicating excellent linearity. The contents of active

Table 3 The quantification analysis of compounds **2** and **3** using UPLC-QTOF/MS

Com- pounds	RT (min)	Calibration Curve	R ²	Linear Range (μg/mL)	Amount (mg/g)
2	10.70	y = 32705x + 9144.8	0.999	0.06 ~ 1	0.0007 ± 0.0001
3	12.48	y = 67542x + 2635.6	0.998	0.06 ~ 2	0.0197 ± 0.0009

Table 4 Inhibitory effects of eight compounds from LPE on LPS-induced pro-inflammatory mediators in BV2 microglial cells

Compound	NO (μM)	IL-6 (μM)	TNF-α (μM)
1	18.89 ± 2.3	49.1% at 40 μM	37.19 ± 3.3
2	9.55 ± 0.7	32.77 ± 2.7	23.85 ± 2.86
3	3.79 ± 0.2	11.26 ± 0.6	10.91 ± 0.85
4	38.29 ± 3.28	7.4% at 80 μM	25.1% at 80 μM
5	15.0% at 20 μM	n.d.	n.d.
6	20.53 ± 4.4	n.d.	n.d.
7	3.15 ± 1.1	n.d.	n.d.
8	31.4% at 40 μM	n.d.	n.d.

compounds (**2** and **3**) in the EtOAc fraction of *L. platyphylla* were determined to be 0.0007 ± 0.0001 mg/g and 0.0197 ± 0.0009 mg/g, respectively (Table 3).

The inhibitory effect of active components from LPE on the production of pro-inflammatory mediators in LPS-induced BV2 microglial cells

To determine which substances are responsible for the anti-neuroinflammatory activity of LPE, we compared the effects of eight compounds derived from LPE on the production of NO, IL-6, and TNF-α induced by LPS. First, we evaluated the cytotoxicity of the eight compounds through an MTT assay. Cell viability remained above 70% at the following concentration ranges: **1** to 40 μM, **2** to 40 μM, **3** to 20 μM, **4** to 80 μM, **5** to 20 μM, **6** to μM, **7** to 5 μM, and **8** to 40 μM (Fig. S3). Based on these results, the effects of the eight compounds isolated from LPE on LPS-induced NO, IL-6, and TNF-α production were assessed at concentrations where cell viability was maintained at 70% or higher. Regarding NO production, six compounds, excluding **5**, and **8**, demonstrated concentration conditions that inhibited LPS-induced NO production by more than 50% (Fig. S4, Table 4). For IL-6, **2** and **3** showed inhibition rates of more than 50% (Fig. S5), while for TNF-α, **1**, **2**, and **3** demonstrated inhibition rates exceeding 50% (Fig. S6, Table 4). Notably, compounds **5**, **6**, **7**, and **8** either failed to inhibit the LPS-induced production of IL-6 or TNF-α, or even increased their production compared to the LPS group (Figure S5, S6, Table 4). Considering these results collectively, it can be inferred that the anti-neuroinflammatory activity of *L. platyphylla* extract is primarily attributed to compounds **2** and **3**. Through future research, it is necessary to verify

whether **2** and **3** exhibit effects in line with the anti-neuroinflammatory activity demonstrated by LPE.

This research evaluated the anti-neuroinflammatory properties of an ethanolic extract from *L. platyphylla* (LPE) in BV2 microglial cells activated by LPS, employing both qualitative and quantitative methodologies. LPE administrated prior to LPS exposure led to a significant reduction in the production of major inflammatory mediators, such as NO, PGE₂, IL-1β, IL-6, and TNF-α. Immunoblot analysis further demonstrated that LPE treatment resulted in decreased expression of iNOS and COX-2 proteins. On a mechanistic level, LPE was found to mitigate neuroinflammatory signaling by interfering with TLR4-dependent activation of the NF-κB and MAPK signaling pathways, as shown by lower phosphorylation levels of IκB-α, p38, and JNK (Fig. 8). Through the analysis of the components contained in LPE, we identified that LPE concludes eight metabolites, including methylophiopogonanone A (**1**), methylophiopogonone A (**2**), methylophiopogonanone B (**3**), methylophiopogonone B (**4**), ophiopogonin A (**5**), ophiopogonin D (**6**), ophiopogonin D' (**7**), and prosapogenin A (**8**), and concluded that among these, methylophiopogonone A (**2**) and methylophiopogonanone B (**3**) are predominantly involved in the anti-neuroinflammatory activity of LPE. LC-ESI-QTOF/MS analysis of the LPE-EtOAc fraction revealed relatively high levels of the isolated compounds (**1**–**8**) in the roots of *Liriope platyphylla*. Subsequent quantitative analysis of the two bioactive constituents (**2** and **3**) using TOF-MRM demonstrated that both compounds were present at trace concentrations in the LPE-EtOAc fraction. In this study, TOF-MRM mode on a LC-ESI-QTOF/MS platform was utilized for the quantitative analysis of the EtOAc fraction from *Liriope platyphylla*, taking advantage of its high sensitivity and analytical precision. The TOF-MRM mode selectively monitors specific compound transitions and minimizes matrix interference, thereby enabling the precise detection of trace-level compounds in complex natural extracts. An analysis of these trace constituents provides essential baseline data for accurately identifying minor bioactive compounds in *L. platyphylla* and facilitates further functional evaluation. In conclusion, our findings indicate that LPE shows promise as an anti-neuroinflammatory agent, warranting further research on its possible application in the treatment of neurodegenerative disorders.

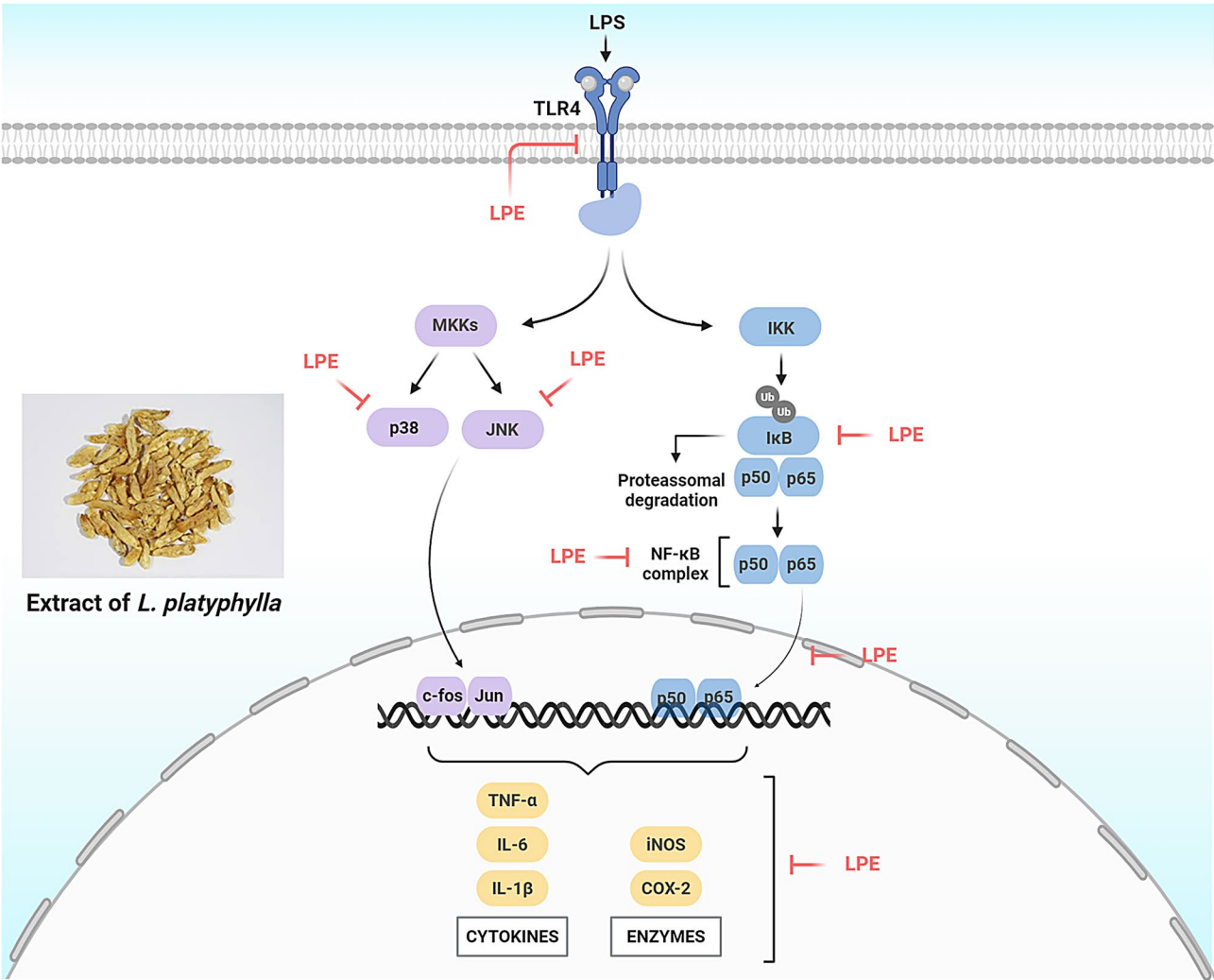


Fig. 8 The schematic diagram of the molecular mechanisms of the anti-neuroinflammatory effects of LPE. Figure is drawn using Biorender. LPE, *L. platyphylla* ethanolic extract

Abbreviations

CNS	Central nervous system
COX-2	Cyclooxygenase-2
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-jun NH ₂ -terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MTT	3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide sodium
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PGE2	Prostaglandin E2
RIPA	Radioimmunoprecipitation assay
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TLR4	Toll-like receptor 4

TNF	Tumor necrosis factor
UNESCO	United Nations Educational, Scientific and Cultural Organization
LPE	Fermented ethanol extract of <i>Liriope platyphylla</i>
EtOAc fr	Ethyl acetate fraction
UPLC-QTOF/MS	UPLC combined with electrospray ionization and quadrupole time-of-flight mass spectrometry

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-025-01015-8>.

Supplementary Material 1

Acknowledgements

This investigation was funded by the “Cooperative Research Program for Agricultural Science & Technology Development” (PJ01717002) of the Rural Development Administration, Republic of Korea. This study was supported by the 2025 RDA Fellowship Program of the National Institute of Horticultural and Herbal Science, Rural Development Administration, Republic of Korea.

Author contributions

K-WK and DYL conceived and designed the experiments. K-WK and W-CS performed the experiments and writing-original draft preparation. J-KJ and DY performed data curation and formal analysis. Y-SL managed the research project and funding acquisition. DYL writing-reviewing, editing, and supervision. All authors read and approved the final manuscript.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval

Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, have complied with relevant institutional, national, and international guidelines and legislation.

Conflict of interest

The authors declare that they have no conflict of interest.

Received: 16 May 2025 / Accepted: 6 July 2025

Published online: 06 August 2025

References

- Shabab T, Khanabdali R, Moghadamtousi SZ, Kadir HA, Mohan G (2017) Neuroinflammation pathways: a general review. *Int J Neurosci* 127(7):624–633
- Poonasri M, Mankhong S, Chiranthan N, Srisook K (2023) 4-methoxycinnamyl p-coumarate reduces neuroinflammation by blocking NF- κ B, MAPK, and Akt/GSK-3 β pathways and enhancing Nrf2/HO-1 signaling cascade in microglial cells. *Biomed Pharmacother* 168:115808
- Liu B, Hong J-S (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther* 304(1):1–7
- Fu R, Shen Q, Xu P, Luo JJ, Tang Y (2014) Phagocytosis of microglia in the central nervous system diseases. *Mol Neurobiol* 49(3):1422–1434
- Subramanyam CS, Wang C, Hu Q, Dheen ST (2019) Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin Cell Dev Biol* 94:112–120
- Perry VH, Teeling J (2013) Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol* 35(5):601–612
- Yang I, Han SJ, Kaur G, Crane C, Parsa AT (2010) The role of microglia in central nervous system immunity and glioma immunology. *J Clin Neurosci* 17(1):6–10
- Li B, Wang M, Chen S, Li M, Zeng J, Wu S, Tu Y, Li Y, Zhang R, Huang F, Tong X (2022) Baicalin mitigates the neuroinflammation through the TLR4/MyD88/NF- κ B and MAPK pathways in LPS-Stimulated BV-2 microglia. *Biomed Res Int* 2022:3263446
- Truong V-L, Bae YJ, Rarison RHG, Bang J-H, Park S-Y, Jeong W-S (2023) Anti-Inflammatory and antioxidant activities of lipophilic fraction from *Liriope platyphylla* seeds using network pharmacology, molecular docking, and in vitro experiments. *Int J Mol Sci* 24(19):14958
- Yoon D, Ryu IS, Shin WC, Ka M, Kim H-G, Jang EY, Kim O-H, Lee Y-S, Seo J-W, Lee DY (2020) Mitigating effects of *Liriope platyphylla* on Nicotine-Induced behavioral sensitization and quality control of compounds. *Brain Sci* 10(9):654
- Wang H-C, Wu C-C, Cheng T-S, Kuo C-Y, Tsai Y-C, Chiang S-Y, Wong T-S, Wu Y-C, Chang F-R (2013) Active constituents from *Liriope platyphylla* root against Cancer growth in vitro. *Evid Based Complement Alternat Med* 2013:857929
- Choi SB, Wha JD, Park S (2004) The insulin sensitizing effect of homoisoflavone-enriched fraction in *Liriope platyphylla* Wang et Tang via PI3-kinase pathway. *Life Sci* 75:2653–2664
- Jeong S, Chae K, Jung YS, Rho YH, Lee J, Ha J, Yoon KH, Kim GC, Oh KS, Shin SS, Yoon M (2008) The Korean traditional medicine Gyeongshingangjeehwan inhibits obesity through the regulation of leptin and PPAR α action in OLETF rats. *J Ethnopharmacol* 119:245–251
- Wu F, Cao J, Jiang J, Yu B, Xu Q (2001) Ruscogenin glycoside (Lm-3) isolated from *Liriope muscari* improves liver injury by dysfunctioning liver-infiltrating lymphocytes. *J Pharm Pharmacol* 53:681–688
- Tada A, Kasai R, Saitoh T, Shoji J (1980) Studies on the constituents of ophiopogonis tuber. V. Isolation of a novel class of homoisoflavonoids and determination of their structures (1). *Chem Pharm Bull* 28(5):1477–1484
- Watanabe Y, Sanada S, Tada A, SHOJI J (1977) Studies on the constituents of ophiopogonis tuber. IV. On the structures of ophiopogonin A, B', C', and D'. *Chem Pharm Bull* 25(11):3049–3055
- Sautour M, Mitaine-Offer AC, Miyamoto T, Dongmo A, Lacaille-Dubois MA (2004) A new steroidal saponin from *Dioscorea cayenensis*. *Chem Pharm Bull* 52(11):1353–1355
- Kim KW, Lee YS, Yoon D, Lee KGS (2022) The ethanolic extract of *Curcuma longa* grown in Korea exhibits anti-neuroinflammatory effects by activating of nuclear transcription factor erythroid-2-related factor 2/heme oxygenase-1 signaling pathway. *BMC Complement Med Ther* 22(1):343
- Dilshara MG, Lee K-T, Jayasooriya RGPT, Kang C-H, Park SR, Choi YH, Choi I-W, Hyun J-W, Chang W-Y, Kim Y-S, Lee H-J, Kim G-Y (2014) Downregulation of NO and PGE $_2$ in LPS-stimulated BV2 microglial cells by trans-isoferulic acid via suppression of PI3K/Akt-dependent NF- κ B and activation of Nrf2-mediated HO-1. *Int Immunopharmacol* 18(1):203–211
- Galic MA, Riaz K, Pittman QJ (2012) Cytokines and brain excitability. *Front Neuroendocrinol* 33(1):116–125
- Zhang W, Xiao D, Mao Q, Xia H (2023) Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther* 8(1):267
- Förstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. *Eur Heart J* 33(7):829–837
- Behl T, Rana T, Sehgal A, Makeen HA, Albratty M, Alhazmi HA, Meraya AM, Bhatia S, Sachdeva M (2023) Phytochemicals targeting nitric oxide signaling in neurodegenerative diseases. *Nitric Oxide* 130:1–11
- Ricciotti E, FitzGerald GA (2011) Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol* 31(5):986–1000
- Nango H, Tsuruta K, Miyagishi H, Aono Y, Saigusa T, Kosuge Y (2023) Update on the pathological roles of prostaglandin E2 in neurodegeneration in amyotrophic lateral sclerosis. *Transl Neurodegener* 12(1):32
- Liu T, Zhang L, Joo D, Sun S-C (2017) NF- κ B signaling in inflammation. *Signal Transduct Target Ther* 2:17023
- Zhang T, Ma C, Zhang Z, Zhang H, Hu H (2021) NF- κ B signaling in inflammation and cancer. *MedComm* (2020) 2(4):618–653
- Cargnello M, Roux PP (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Rev* 75(1):50–83
- Lai J-L, Liu Y-H, Liu C, Qi M-P, Liu R-N, Zhu X-F, Zhou Q-G, Chen Y-Y, Guo A-Z, Hu C-M (2017) Indirubin inhibits LPS-Induced inflammation via TLR4 abrogation mediated by the NF- κ B and MAPK signaling pathways. *Inflammation* 40(1):1–12
- Rong S, Yang C, Wang F, Wu Y, Sun K, Sun T, Wu Z (2022) Amentoflavone exerts Anti-Neuroinflammatory effects by inhibiting TLR4/MyD88/NF- κ B and activating Nrf2/HO-1 pathway in Lipopolysaccharide-Induced BV2 microglia. *Mediators Inflamm* 2022:5184721
- Wang W, Liang D, Song X, Wang T, Cao Y, Yang Z, Zhang N (2015) Magnolol inhibits the inflammatory response in mouse mammary epithelial cells and a mouse mastitis model. *Inflammation* 38(1):16–26

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.