Supplementary Data

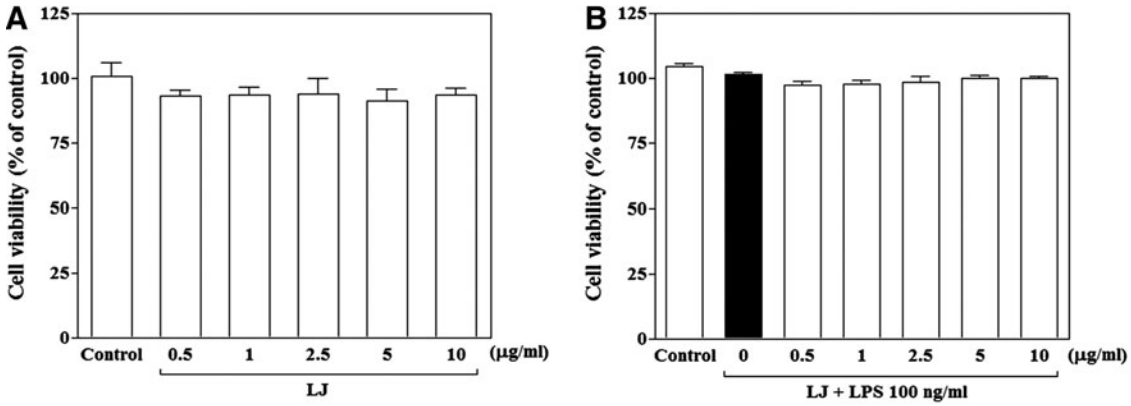
SUPPLEMENTARY MATERIALS AND METHODS

Cell viability assay

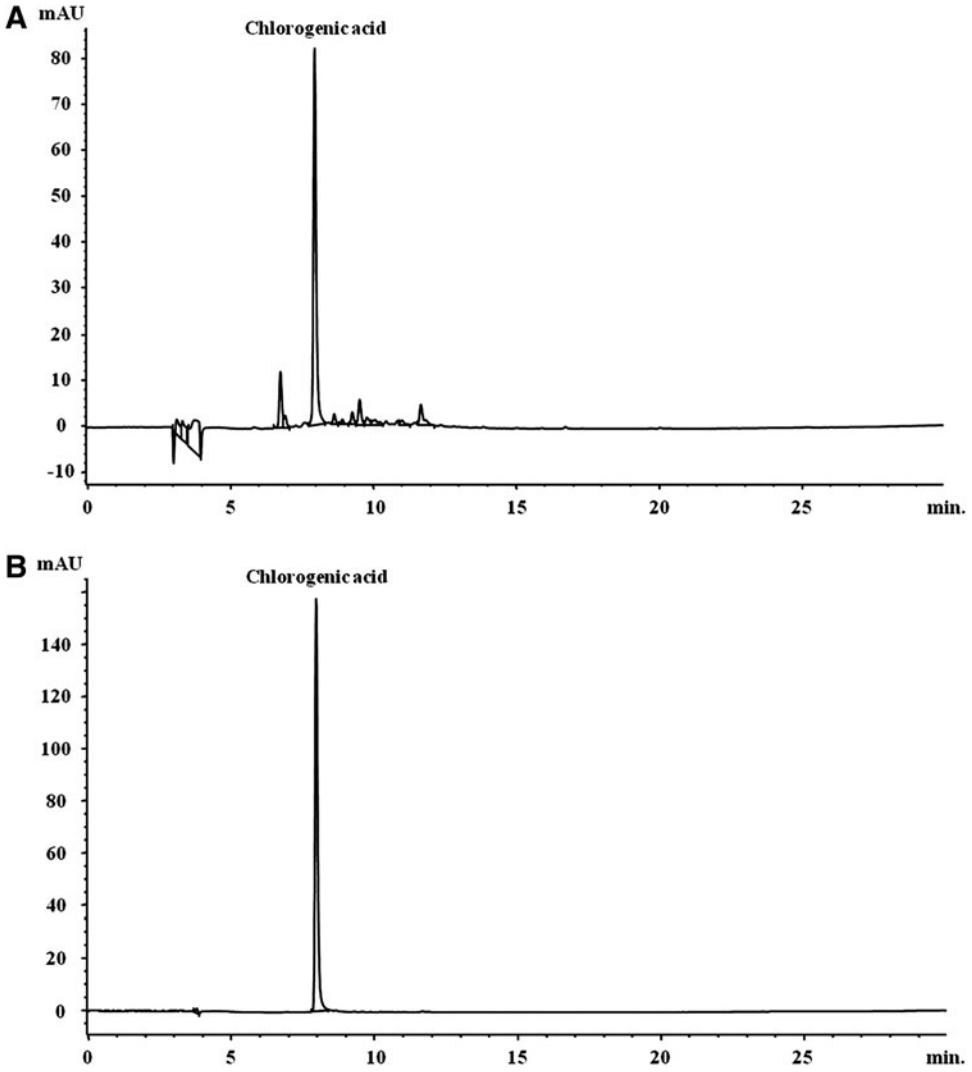
Cell viability was measured based on the formation of blue formazan metabolized from colorless 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial dehydrogenases, which are active only in live cells. BV-2 microglial cells (2.5×10^5 cells/well in 24-well plates) were incubated at 37°C with lipopolysaccharide (LPS) for 24 h with or without *Lonicera japonica* THUNB. (LJ) pretreatment, and then treated with the MTT solution (5 mg/mL) for 2 h. The dark blue formazan crystals formed in intact cells were dissolved in dimethyl sulfoxide (DMSO) and the absorbance at 540 nm was measured with a microplate reader (SpectraMax 250; Molecular Device, Sunnyvale, CA, USA). The results are expressed as the percentage of MTT reduction relative to the absorbance of control cells.

High-performance liquid chromatography measurement

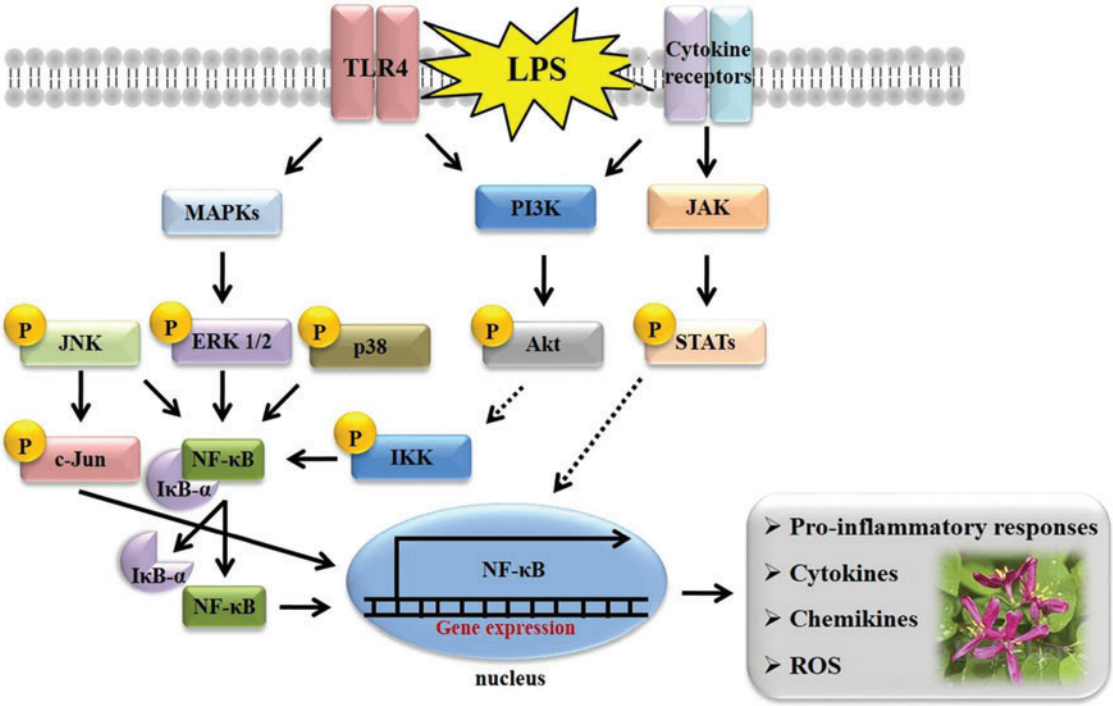
LJ was standardized by the content of chlorogenic acid, an active component of LJ, which some references suggest contains the anti-inflammatory activities observed in some models of neurodegenerative diseases. Chlorogenic acid was measured using reverse-phase high-performance liquid chromatography (HPLC, Agilent 1200 series; Agilent, Santa Clara, CA, USA) with a UV detector, autosampler, column oven, and multichannel pump. Separation was performed using a Phenomenex Luna end-capped C18 column (5 μm, 4.6×250 mm; Phenomenex, Torrance, CA, USA) at 25°C under the following conditions: acetonitrile:water (15:85, v/v) (solvent A) and acetonitrile:methanol (20:80, v/v) (solvent B) as the mobile phase using a linear gradient at a flow rate of 1.0 mL/min. The detector wavelength was set at 327 nm. The LJ contained 0.68% chlorogenic acid (Supplementary Fig. S2).



SUPPLEMENTARY FIG. S1. Effects of LJ on cell viability in BV-2 microglial cells **(A)**. Cells were treated with the indicated concentrations of LJ for 24 h. Effects of LJ on LPS-induced cell viability in BV-2 microglial cells **(B)**. Cell viability was assessed by the MTT assay and expressed as percentages of the corresponding values for the control group. Cells were pretreated with the indicated concentrations of LJ for 30 min, then exposed to 100 ng/mL of LPS for 24 h. LJ, *Lonicera japonica* THUNB.



SUPPLEMENTARY FIG. S2. Representative HPLC chromatogram of LJ **(A)** and chlorogenic acid standard **(B)**. Chlorogenic acid was detected at about 8 min in this system. LJ also produced a chlorogenic acid peak at this time, which corresponded to a chlorogenic acid level of 0.68%. HPLC, high-performance liquid chromatography.



SUPPLEMENTARY FIG. S3. Proposed schematic of the molecular mechanisms for the anti-inflammatory effects of LJ on LPS-induced proinflammatory responses in BV-2 cells. LPS induces intracellular ROS accumulation, triggering phosphorylation of MAPKs, PI3K/Akt, and JAK1, as well as activation of STAT1/3. In addition, phosphorylation and activation of these proteins may induce neuroinflammation leading to proinflammatory responses, release and/or expression of cytokines and chemokines, and subsequent accumulation of ROS, which initiates activation of NF-κB localization from the cytosol to the nucleus, thereby suppressing the proinflammatory signaling. The signaling components, on which the effects of LJ were determined in this study, are indicated by shaded rectangles. Dotted lines represent incompletely defined pathways. IKK, inhibitor of kappaB kinase; IκBα, nuclear factor-κB inhibitor α; JAK, Janus kinase; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; STATs, signal transducer and activator of transcriptions; TLR4, Toll-like receptor 4.

Supplementary Table S1. Primers Used in Reverse Transcription–Polymerase Chain Reaction Experiments

Target	*Forward primer (5′ → 3′)*	*Reverse primer (5′ → 3′)*	*Size (bp)*
iNOS	CTGCAGCACTTGGATCAG GAACCTG	GGGAGTAGCCTG TGTGCACCTGGAA	311
COX-2	TTGAAGACCAGGAGTACAGC	GGTACAGTTCCATGACATCG	324
TNF-α	CGTCAGCCGATTTGCTATCT	CGGACTCCGCAAAGTCTAAG	206
IL-1β	GCCCATCCTCTGTGACTCAT	AGGCCACAGGTATTTTGTCG	230
MCP-1	CTTCTGGGCCTGC TGTTCA	CCAGCCTACTCATTGGGATCA	127
MMP-9	GAGCTGTGCGTCTTCCCCTTC	GGAATGATCTAAGCCCAGTGC	204
β-actin	AGCCATGTACGTAGCCATCC	GCTGTGGTG GTGAAGCTGTA	222