Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Effect of Simvastatin on Neuroinflammation in Microglial Cells Via Mitogen-activated Protein Kinase and Nuclear Factor κB Pathways

HongYing Ma[1], DingAn Li[2], Thamaraiselvan Rengarajan[3], Kalaivani Manokaran[4]

[1]Department of Neurology, Tangshan GongRen Hospital, Tangshan, Hebei Province, [2]Hanzhong Central Hospital, 22 KangFu Road, Hanzhong, Shaanxi Province, PR China, [3]Scigen Research and Innovation, Periyar Technology Business Incubator, Thanjavur, Tamil Nadu, [4]Department of Medical Laboratory Technology, School of Allied Health Sciences, Manipal, Manipal Academy of Higher Education, Karnataka, India

Submitted: 12-09-2017 Revised: 19-10-2017 Published: 01-03-2018

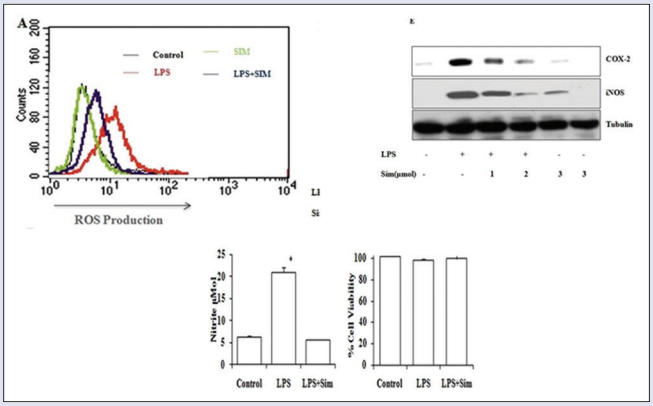
ABSTRACT

Background: Activated microglial cells are found in different sorts of the neurodegenerative process including Parkinson and Alzheimer. Suppressing the activated microglial cells developed as a novel procedure for the treatment of neuroinflammation-based neurodegeneration. **Materials and Methods:** We have investigated the effects of simvastatin on memory impairment and inflammatory cytokines expression induced by transient cerebral ischemia in cultured microglial cells. **Results:** The lipopolysaccharide (LPS)-activated microglial cells treated with simvastatin 3 μmol has decreased the inflammation which was indicated by the reduced levels of the nitric oxide (NO), tumor necrosis factor-α, interleukin-1 β, cyclooxygenase-2, and inducible NO synthase. Simvastatin also delayed the activation of atomic component nuclear factor-κB, p38 mitogen-activated protein kinase, and the reactive oxygen species in LPS-activated microglial cells. Moreover, simvastatin has provoked the outflow of heme oxygenase-1 in BV-2 microglial cells. **Conclusions:** The present study showed that the simvastatin antagonizes neuroinflammation and can be a potential restorative operator for treating neuroinflammatory ailments.

Key words: Lipopolysaccharide, microglial cells, neuroinflammation, simvastatin

SUMMARY

- Simvastatin, applies calming impacts, for the most part by focusing on p38 mitogen-activated protein kinase, atomic component nuclear factor κB, and heme oxygenase-1 signaling pathway
- Simvastatin antagonizes neuroinflammation and can be a potential restorative operator for treating neuroinflammatory ailments.



Abbreviations used: LPS: Lipopolysaccharide, TNF-α: Tumor necrosis factor, NO: Nitric oxide, IL-1β: Interleukin, COX-2: Cyclooxygenase, iNOS: Inducible nitric oxide synthase, MAPK: Mitogen-activated protein kinase, HO-1: Heme oxygenase

Correspondence:

Ms. Kalaivani Manokaran,
Department of Medical Laboratory Technology, School of Allied Health Sciences, Manipal Academy of Higher Education, Madhav Nagar, Near Tiger Circle, Manipal - 576 104, Karnataka, India.
E-mail: kalaivani.m@manipal.edu

DOI: 10.4103/pm.pm_418_17

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Neuroinflammation mediated by glial initiation, recognized as a common characteristic of numerous neurodegenerative diseases.[1] Microglia, the brain-resident macrophages, are considered to assume the essential part in directing neurotoxicity intervened by inflammatory reaction.[2] Microglial can be activated by lipopolysaccharide (LPS), interferon (IFN)-γ or β-amyloid *in vitro*.[3,4]

Activated microglial cells could create some master inflammatory elements, for example, tumor necrosis factor (TNF)-α, prostaglandins, interleukin (IL)-1 β, IL-6, and free radicals such as nitric oxide (NO) and reactive oxygen species (ROS). These neurotoxic variables may prompt neuronal harm, bringing about the advance of neurodegenerative illnesses.[5,6] In parallel, astrocytes activation additionally aggravate the pathogenesis of neurodegenerative diseases.[7,8] Inflammatory initiation of glial cells assumes a vital part in the pathogenesis of neurodegenerative illnesses.[9,10] Microglia and macrophages release TNF-α, IL-1 β, and IL-6 on activation with the bacterial endotoxin LPS *in vitro*. The release of these cytokines is mediated by protein tyrosine kinases, mitogen-activated protein kinases (MAPKs), and transcription factors such as nuclear factor κB (NFκB).[11]

Statins, 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors, have been well-known for their effects on the rate-limiting step in cholesterol synthesis.[12] Simvastatin is currently one of the most common

Cite this article as: Ma H, Li D, Rengarajan T, Manokaran K. Effect of simvastatin on neuroinflammation in microglial cells via mitogen-activated protein kinase and nuclear factor κB pathways. Phcog Mag 2018;14:S237-44.

drugs for old patients with hyperlipidemia, hypercholesterolemia, and atherosclerotic diseases by reducing cholesterol level and antilipid properties. Importantly, simvastatin has also been reported to have anti-inflammatory effect, but the underlying mechanism is largely unknown.[13,14] Few studies[15,16] have demonstrated that simvastatin has immunomodulatory and antagonizes inflammatory impacts. Animal study[17,18] revealed that simvastatin is a great operator that diminishes cytokines levels and leukocyte number in sepsis, and significant for the lipid-bringing down. Simvastatin has vital calming effects on stomach sepsis in rats. Another study[19,20] exhibited that high-dosage of simvastatin may be effective for the treatment of patients with severe systemic inflammation and related vascular hyporeactivity amid endotoxemia. Simvastatin diminishes enrollment and activation of neutrophils, therefore, shielding from LPS-initiated severe lung damage. In the present study, we reported that simvastatin, applies calming impacts, for the most part by focusing on p38 MAPK, atomic component κB (NF-κB), and heme oxygenase (HO)-1 signaling pathway.

MATERIALS AND METHODS

Reagents and chemicals

LPS and simvastatin were acquired from Sigma-Aldrich (St. Louis, MO, USA). Recombinant mouse IFN-γ purchased from R and D Systems (Minneapolis, MN, USA).

Cell culture

BV-2 murine microglial cell, HT22 mouse neuroblastoma cell, RAW 264.7 macrophage cell and highly aggressive proliferating immortalized (HAPI) rodent microglial refined in Dulbecco's adjusted Eagle's medium (DMEM) supplemented with 10% fetal ox-like serum (fetal bovine serum), and penicillin (0.1%) streptomycin (0.1%) at 5% CO_2, 37°C. Essential astrocytes and microglial cells were cultured as already portrayed. The infant ICR mice sterilized with 75% liquor for 1–2 days. The skull of mice was stripped, and the entire cerebrum was taken in the dish containing precooling sans serum DMEM. The pia mater and veins deliberately expelled from the cerebral cortex, and the coveted cerebrum tissue was washed 2–3 times with precooling without serum DMEM. The cortex tissue was cut into 1 mm 3 pieces and processed with papain (2 mg/ml) at 37°C for 30 min. The specimen suspended utilizing different pore size of tips and after that centrifuged for 3 min. The cells were seeded on poly-D-lysine-covered 75 mm flagons and refined for 14 days at 37°C, 5% CO_2. The microglial cells acquired from blended glial societies by shaking at 150 rpm for 2 h. The astrocytes cultures isolated by shaking at 280 rpm for 12 h. The refined microglia or astrocytes (>95%) was immune-stained with the counteracting agent of a group of separation 11b (CD11b) or glial fibrillary acidic protein, separately (information not appeared). The present study endorsed by the Institutional Review Board of Soochow University.

Cell viability test

The cell practicality evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) measure as already portrayed. Cells seeded in 96-well plates at the density of 5×10^4 cells/well. The cell culture supernatant disposed after treatment with different operators, and afterward, 30 ml of MTT (0.5 mg/ml) arrangement included into each well. After incubating for 4 h at 37°C, 100 ml of DMSO was added to each well to form its insoluble formazan color, and the microplate reader measured 580 nm of the absorbance solubilized formazan.

Nitrite quantification

The NO in the cell culture supernatants was assessed by Griess reagent as discussed in earlier studies.[20,21] Microplate used at the optical density at 550 nm of absorbance. $NaNO_2$ was utilized as the standard to compute NO_2^- fixations.[22]

Western blot analysis

RIPA buffer with proteinase inhibitors (Roche, Germany) was used for protein extraction from the cell. Protein concentration in the cell extract was determined using Bio-Rad dye reagent (Bio-Rad). An equal volume (40 μg) of the total isolated protein was resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The film was blocked with 5% skim milk and treated with the essential counteracting agent. The membrane was incubated with the primary antibodies for inducible NO synthase (iNOS), cyclooxygenase (COX)-2 (Abcam, Cambridge, MA), IκBα, phospho-Ikka/b and phospho-IκBα (Santa Cruz Biotechnology, Santa Cruz, CA); antagonist to ERK1/2, p38, JNK (Cell Signaling Technology, Beverly, MA, USA); against α-tubulin (Sigma). The membrane was washed with phosphate buffered saline (PBS) with Tween-20 buffer and then incubated with secondary antibodies (Sigma-Aldrich). The intensity of the protein visualized with a ChemiScope 3300 mini (CLINX, Shanghai, China).

Quantitative reverse transcription-polymerase chain reaction

Total RNA extraction from the microglial cells using TRIzol reagent (Takara, Dalian, China). cDNA synthesis was reverse transcribed with 2 μg of total RNA (Takara, Dalian, China). The specifically designed primers and SYBR green Premix II kit (Takara, Dalian, China) utilized for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) examination. The relative edge cycle (CT) estimation of each objective quality was standardized to that of Glyceraldehyde-3-Phosphate dehydrogenase.[23,24]

Enzyme-linked immunosorbent assay

The TNF-α from the cell culture supernatants were measured by TNF-α enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems, Minneapolis, MN, USA) as per manufacturers guideline.[25] ELISA plates were read using microplate reader. Indirect ELISA was performed using specific antibodies. Cell culture medium or the cell lysate precoated onto ELISA plates served as the antigen. O-dianisidine was used as substrate, and the absorbance of the colored horseradish peroxidase product was measured spectrophotometrically at 405 nm by an automated microplate reader (Thermo Multiskan Spectrum).

Measurement of intracellular reactive oxygen species

Intracellular ROS measured using the dichloro-dihydro-fluorescein diacetate (DCFH-DA as described in the previous studies. Cells were harvested in the presence of 10 μmol DCFH-DA in PBS at 37°C for 20 min. The plate was allowed to mix for 3–5 min to help the test to contact cell completely. The cells were washed with sans serum culture medium for three times. Tests were monitored using the flow cytometry at 530 nm (FACS Calibur, Becton Dickinson).

Nuclear factor-κB reporter assay

The BV-2 microglial was conveying NF-κB columnist lentiviral particles seeded to 24-well plate in triplicate. The cells pretreated

with Simvastatin or vehicle for 30 min before LPS treatment. After 16 h of LPS incubation, luciferase movement was measured using the luciferase test pack (Promega) as per the manufacturer's guidelines, and the level of promoter action communicated as individual units.

Establishment of stable BV-2 cell line expressing nuclear factor-kB reporter

BV-2 microglial cells were transduced with NF-kB columnist lentiviral particles. BV-2 cells were seeded on 24-well plate and incubated at 37°C for 12 h. NF-kB journalist lentiviral particles (Cignal™ Lenti Reporters, Qiagen) included in a grouping of 2.5×10^5 transducing units. After incubation of 24 h, the supernatant was removed and replaced with the new medium. Two days later, the medium changed with fresh medium containing 1.2 mg/ml of puromycin. Subsequently, after 5 days, surviving cells were chosen and utilized for further investigations.

Microglial/neuron co-culture

Microglial/neuron coculture was performed with transwell embed. The microglial cells were seeded at the density of 5×10^4 on the transwell embed (pore measure 0.4 mm; Corning, CA, USA) in 24-well plates and incubated overnight. Cells were preincubated with Simvastatin for 30 min preceding LPS treatment. After 6 h of LPS treatment, the supplements containing microglial cells exchanged to HT-22 cells. After corefined for 36 h, the additions expelled, and MTT controlled the cytotoxicity of HT-22 cells examine or AnnexinV/propidium iodide apoptosis measure.

Data analysis

All experiments were concluded at least three times, and data were expressed as mean ± stdardard deviation (SD). Statistical analyses were completed using GraphPad Prism 4 (GraphPad Software, La Jolla, California, USA). The results shown are the means ± SD of three independent experiments. Statistical significance was determined by one-way analysis of variance followed by the Student Newman Keuls *post hoc* comparison tests and $P < 0.05$ was considered statistically significant.

RESULTS

Simvastatin inhibited lipopolysaccharide induced nitric oxide and tumor necrosis factor-α production in microglial cells

The effect of the Simvastatin on LPS-activated NO generation in BV-2 microglial cells [Figure 1a and b] and primary microglial cultures [Figure 1c and d] was evaluated. Simvastatin-treated groups showed a strong decrease in LPS-induced NO generation in both the BV-2 microglial and primary microglial cultures [Figure 1a and c]. The potency of suppression of NO production was ranked based on 94% inhibitory concentration 50 as Simvastatin (5.32 ± 0.05 mM). Cell viability was measured to know the possibility that the decrease of NO generation was due to the cytotoxicity of the Simvastatin. As shown in the Figure 1b and d, the Simvastatin did not display obvious cytotoxicity on microglial cells. Based on its powerful NO inhibitory effects in LPS activated microglial cells, the anti-inflammatory effects of Simvastatin further studied. The inhibitory effect of Simvastatin on NO generation further confirmed in HAPI rat microglial cells, RAW 264.7 murine macrophage cells, and primary astrocytes [Figure 2a-c]. Besides, we have also tested the effect of Simvastatin on TNF-α level in cell culture supernatant. LPS-induced TNF-α production in BV-2 microglial or primary microglia was significantly decreased by Simvastatin [Figure 3a and b].

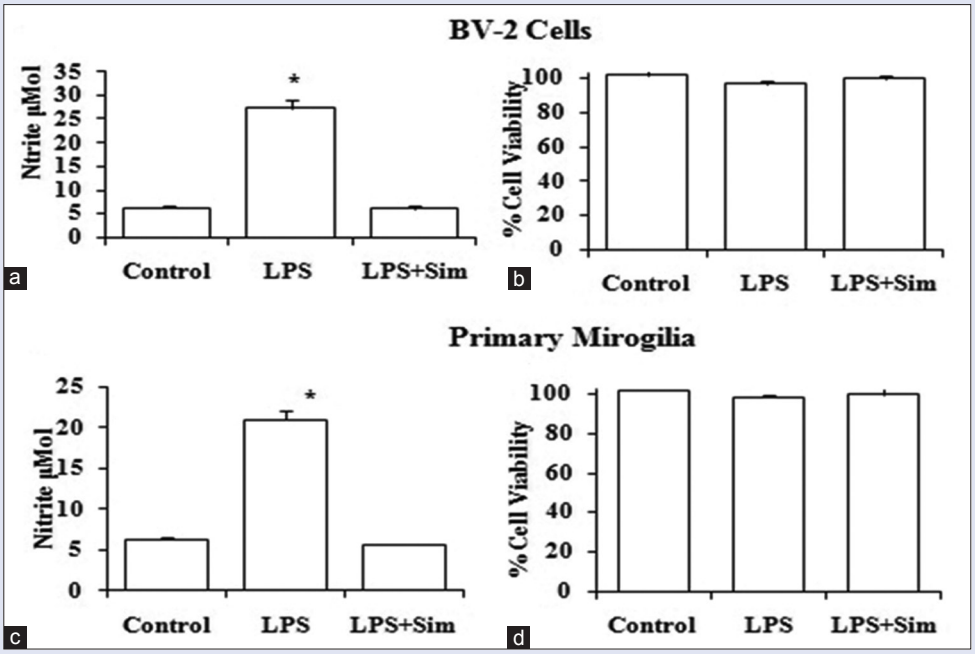


Figure 1: Effect of simvastatin (Sim) on lipopolysaccharide-induced nitric oxide generation in microglial cells. Microglial cells pretreated with compounds for 30 min, and then 0.1 mg/ml of lipopolysaccharide was added and cultured for 24 h. Nitrite generation in culture media in cells (a) BV-2 cell; (c) primary microglial was determined using Griess reaction. The cytotoxicity of compounds determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in cells (b) BV-2 cell; (d) primary microglial. Data represent mean ± standard deviation of at least three independent experiments. Lipopolysaccharide-treated cells compared to simvastatin-treated cells. Symbol mark indicates significant differences from lipopolysaccharide alone treatment group (*$P < 0.05$)

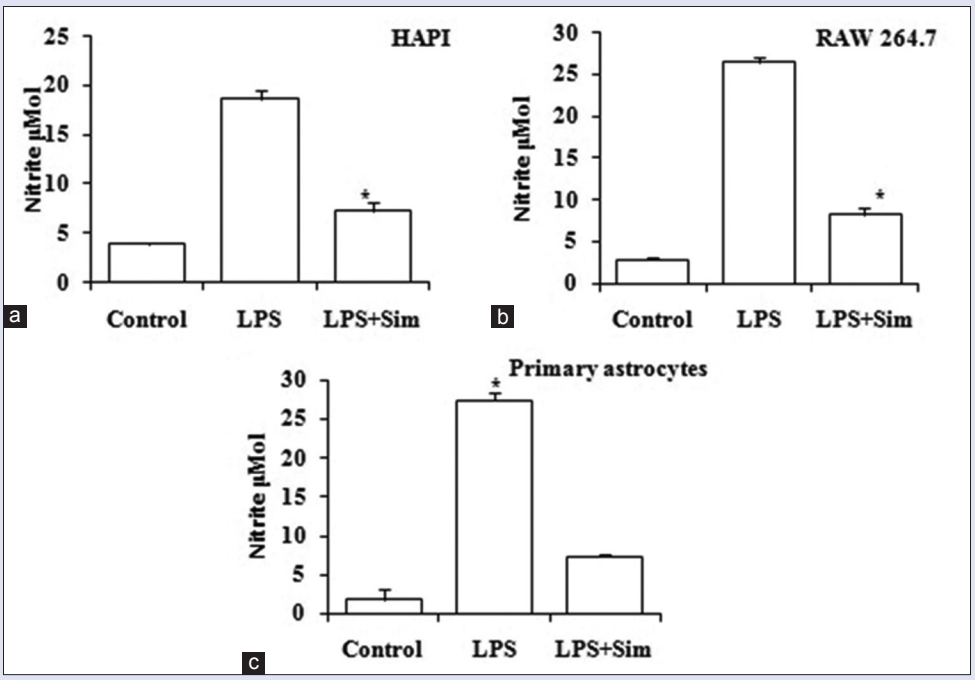


Figure 2: Effect of simvastatin (Sim) on nitric oxide generation in activated highly aggressive proliferating immortalized rat microglial, macrophage cells, and primary astrocytes cells. Microglial cells pretreated with simvastatin for 30 min, and then lipopolysaccharide or lipopolysaccharide/interferon-γ was added and cultured for 24 h. Nitrite generation in culture media (a) highly aggressive proliferating immortalized cell; (b) RAW 264.7 murine macrophage; (c) primary astrocytes was determined using Griess assay. Data represent mean ± standard deviation of at least three independent experiments. Lipopolysaccharide-treated cells compared to simvastatin-treated cells. Symbol mark indicates significant differences from lipopolysaccharide alone treatment group (*$P < 0.05$)

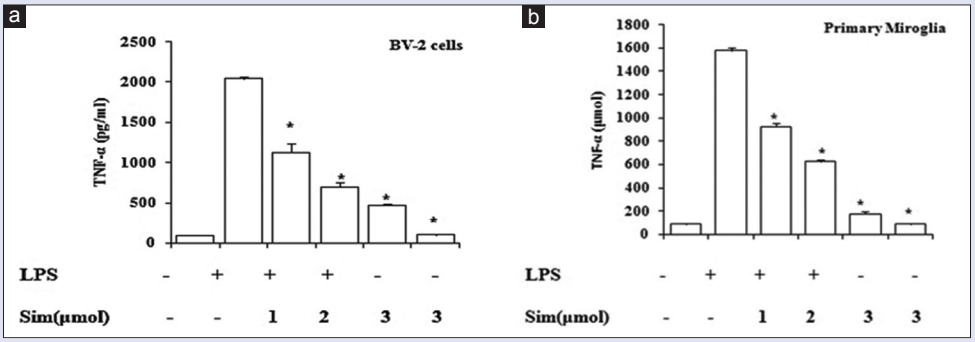


Figure 3: Effect of simvastatin (Sim) on lipopolysaccharide-induced tumor necrosis factor-α generation in BV-2 microglial cells (a) or primary microglial cells. (b) Microglial cells pretreated with simvastatin for 30 min, and then, lipopolysaccharide was added and cultured for 24 hours. tumor necrosis factor-α generation in culture media was determined using enzyme-linked immunosorbent assay kit. Data represent mean ± standard deviation of at least three independent experiments. Lipopolysaccharide-treated cells compared to simvastatin-treated cells. Symbol mark indicates significant differences from lipopolysaccharide alone treatment group (*$P < 0.05$)

Simvastatin inhibited expression of interleukin-1 β, inducible nitric oxide synthase, cyclooxygenase-2, and tumor necrosis factor-α in lipopolysaccharide-activated microglial cells

The impacts of Simvastatin inspected on the LPS-activated microglial cells, the mRNA levels of iNOS, TNF-α, IL-1 β, and COX-2 measured by qRT-PCR. Simvastatin decreased the mRNA expression of iNOS, TNF-α, IL-1 β, and COX-2 significantly [Figure 4a-d]. The inhibitory impacts of simvastatin on protein level of iNOS and COX-2 in LPS-initiated BV-2 microglial cells were likewise acknowledged by Western blotting [Figure 4e].

Simvastatin the lipopolysaccharide-induced activation of nuclear factor-kB in microglial cells

NF-kB is a key transcription complex that controls the cytokines production in microglial reactions. With this regards, we explored whether NF-kB pathway included in calming impacts of Simvastatin. LPS starts a flagging course prompting kinase IκB kinase (Ikk) phosphorylation, taken after by IκB phosphorylation, which activates IκB debasement and translocation of p65 subunit into atomic. LPS-activated phosphorylation of Ikkα/β, IκB, and corruption of IκB was notably repressed by simvastatin [Figure 5a]. The inhibitory impact of simvastatin on LPS-activated atomic increased p65 and NF-kB luciferase action in BV-2 microglial cells was additionally

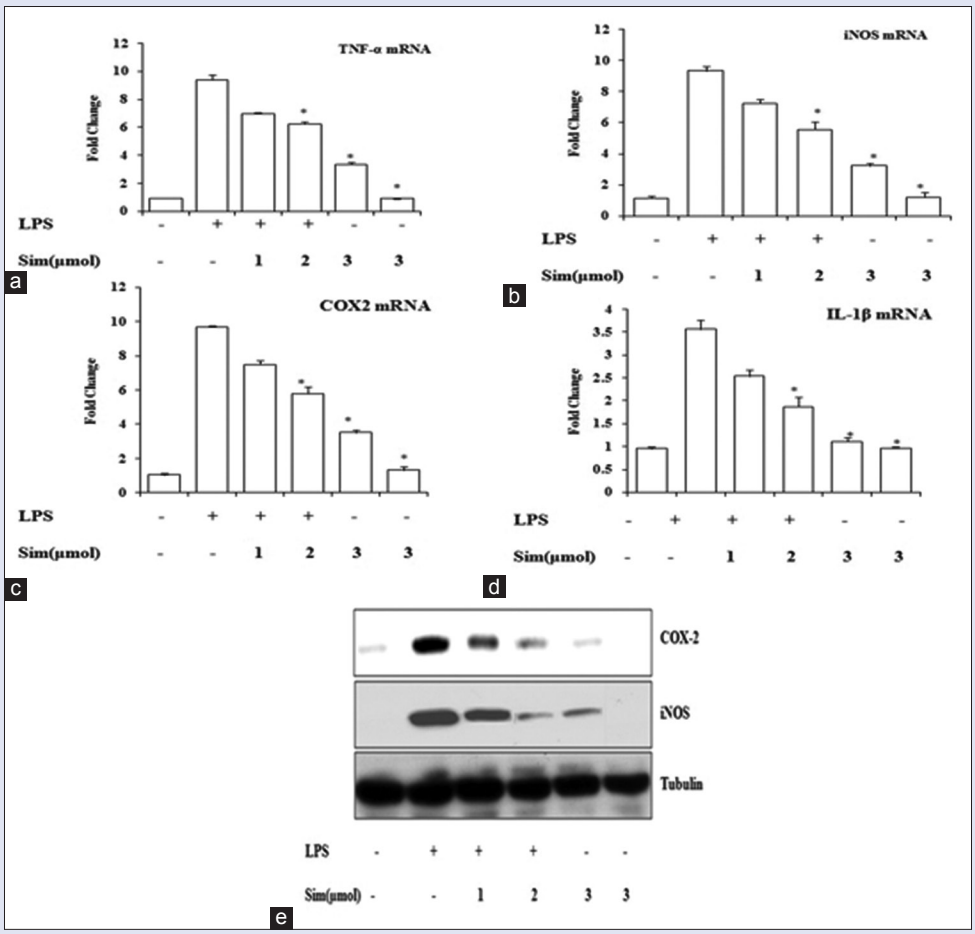


Figure 4: Effect of simvastatin (Sim) on lipopolysaccharide-induced pro-inflammatory genes expression in microglial cells, BV-2 cells pretreated with simvastatin for 30 min, and then lipopolysaccharide was added and cultured for 24 hours. The mRNA levels of (a) tumor necrosis factor-α, (b) inducible nitric oxide synthase, (c) cyclooxygenase-2 and interleukin-1β (d) assessed by quantitative reverse transcription-polymerase chain reaction. The protein levels of inducible nitric oxide synthase and cyclooxygenase-2 evaluated by Western blot (e). The tubulin used as an internal control. Data represent mean ± standard deviation of three independent experiments. Symbol mark indicates significant differences from lipopolysaccharide alone treatment group (*$P < 0.05$)

confirmed [Figure 5b]. Consequently, the impact of simvastatin on the MAPKs (ERK, JNK, and p38) pathways was analyzed. After activation of BV-2 microglial cells with LPS for 20 min, the initiation of MAPKs was identified by Western blot utilizing immune responses particular for individual phospho-MAPKs. The level of p38 phosphorylation was restricted by simvastatin, while phosphorylation of ERK and JNK was not attuned [Figure 5c]. Thus seem to show that the p38 MAPK and NF-kB pathways might be related to the mitigating systems of simvastatin in microglial cells.

Simvastatin suppressed the lipopolysaccharide-induced intracellular reactive oxygen species generation

LPS-activated microglial cells creates ROS that is related with the neuroaggravation and neurodegeneration.[26] To test the impacts of simvastatin on ROS, the intracellular ROS was measured by FACS examination. As appeared in Figure 6a, simvastatin diminished ROS in LPS-activated BV-2 microglial cells. Since NADPH oxidase primarily catalyzed ROS in microglial cells,[27] the impacts of simvastatin on the articulation of NADPH oxidase were inspected utilizing qRT-PCR. The outcomes demonstrated that simvastatin uniquely decreased the articulation of gp91phox [Figure 6b] and p47phox [Figure 6c] of NADPH oxidase yet not significantly the other subunit (p22phox) [Figure 6d]. We next analyzed the impacts of simvastatin on the articulation of HO-1, which is an inducible chemical that catalyzes the corruption of heme and applies against oxidant and mitigating impacts under different conditions.[28]

DISCUSSION

Neuroinflammation brought on by activated glial cells is extremely vital to the improvement of neurodegenerative sicknesses.[20] Activated microglial could deliver different cytokines furthermore, neurotoxic elements that add to neuronal cell degeneration. In the present study, the impacts of Simvastatin have the strongest inhibitor on LPS-activated microglial cells and NO generation. The high liposolubility acetyl gathering characteristic of simvastatin made the compound less demanding to penetrate into the cells, accordingly displaying the calming properties of activated microglia. Known that NO and PGE_2 generation were mostly intervened by iNOS and COX.[21] TNF-α and IL-1 β are similarly important pro-inflammatory elements, which are effectively produced during the neuroinflammatory illnesses.[22] We found that simvastatin controlled TNF-α, IL-1 β, and COX-2 gene

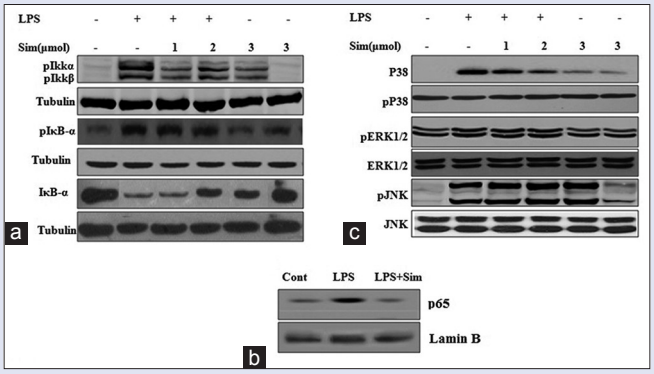


Figure 5: Impact of simvastatin (Sim) on nuclear factor-kB initiation in activated microglial: BV-2 cells pretreated with simvastatin for 30 min, then lipopolysaccharide was added and cultured for indicated times. The levels of phospho-IκB kinase -α/β (5 min), phospho-IκB-α (10 min) and IκB-α (20 min) assessed by Western blot . LPS-activated phosphorylation of Ikkα/β, IκB, and corruption of IκB was notably repressed by simvastatin. The α-tubulin used as an internal control (a). The expression of p65 (1 h) in nuclear assessed by Western blot and the LPS induced P65 is decreased upon simvastatin treatment. The Lamin β used as an internal control (b). After activation of BV-2 microglial cells with LPS for 20 min, MAPKs expression was observed, utilizing immune responses particular for individual phospho-MAPKs. The level of p38 phosphorylation was decreased upon simvastatin treatment, while phosphorylation of ERK and JNK was not changed significantly (c)

expression and protein levels in LPS-activated microglial cells. The outcomes projected that simvastatin might be a potential prospect for therapeutic applications for neuroinflammatory illnesses.

LPS provokes the inflammatory elements by the official of Toll-like receptor 4 (TLR4). After the response of TLR4 by LPS, various intracellular components counting Ikk complex and MAPKs gets activated with cytoplasmic particles and thus activating the downstream transcriptional elements including NF-kB and activator protein-1.[25] It is notable that a few events control NF-kB activation, for example, Ikk phosphorylation, IκB phosphorylation, IκB degradation, and nuclear translocation of NF-kB. In nuclear, the p65 subunit of the NF-kB complex ties to a kB site in the DNA area of target qualities to direct quality interpretation.[23] In the present study, we observed that simvastatin suppressed Ikk phosphorylation, IκB phosphorylation, IκB degradation and NF-kB promoter movement in LPS-activated microglial cells which were predictable with past perception that Gamma-linolenic acid restricted the activation of NF-kB.[24] Nevertheless, NF-kB and p38 are another essential modulator of the statement of star inflammatory cytokines and compounds, for example, iNOS, COX-2, IL-6, and ROS in glial cells.[21]

Simvastatin suppressed LPS-activated phosphorylation of p38. The crosstalk between NF-kB and p38 pathways is most certainly not predictable. In the past review, it was accounted that, p38 particular inhibitor SB 203580 substantially blocked NF-kB initiation while NF-kB inhibitor pyrrolidinedithiocarbamate did not piece p38 initiation, recommending that NF-kB be a downstream flag atom of p38 in macrophage cells.[29] Furthermore, various reviews proposed that NF-kB and p38 be two independent flag pathways in macrophages reaction to inflammatory boosts. Different mitigating specialist's decreased NF-kB initiation in LPS-activated microglial cells, though p38 activation was not influenced.[30] In this manner, crosstalk among p38 and NF-kB in the control of the inflammatory reaction is likely subject to cell sort and nature of traumas.

Despite the fact that the immediate target particle of simvastatin is unclear, this perception showed that hindrance of NF-kB and p38 MAPK signaling pathway might include in molecular components of the suppressive impacts of simvastatin on mRNA expression of iNOS, COX-2, TNF-α, and IL-1 β. ROS are reliably created by activated microglial cells and assume a fundamental part in the aggravation intervened neurodegeneration.[31] Intracellular ROS, as an optional representative for inflammatory responses, passes different downstream flagging atoms including NF-kB, protein kinase C (PKC), and MAPK,[32,33] in this way managing the statement of fiery master qualities in glial cells reaction to inflammatory boosts. The ROS controlled by NADPH oxidase which is a noteworthy hotspot for cell ROS in LPS-activated microglial. There are developing confirming support that LPS causes upregulation of NADPH oxidase in microglia cells, and hindrance of NADPH oxidase stifles inflammatory initiation of microglial cells.[33,34]

NADPH oxidase was made out of two-layer parts (gp91phox and p22phox) and three cytosolic parts (p47phox, p67phox, and p40phox). In addition, microglial cells disengaged from gp91phox or p47phox lacking mice indicated less generation of proinflammatory between and ROS in cells reaction to LPS.[35] The present study reveals that LPS-activated ROS furthermore, articulates gp91phox and p47phox were altogether diminished by simvastatin in BV-2 microglial cells. Contradictorily, it was accounted for that simvastatin, essentially elevated ROS in human HL-60 leukemia cells.[26] These outcomes recommended that the impact of glaucocalyxins on the ROS may rely on upon cell sort and centralization of combinations. Since NF-kB and p38 are downstream proteins of ROS,[27,28] hindrance of ROS/NF-kB, and ROS/p38 pathways included in the calming systems of Simvastatin.

HO-1 is an inducible compound that catalyzes the oxidative corruption of heme into carbon monoxide, biliverdin, and free press.[36] Few studies have exhibited that acceptance of HO-1 expression in glia cells showed calming, antagonistic to oxidant furthermore, cytoprotective properties under different conditions.[37,38] The mitigating activity of HO-1 in microglial cells interceded by restraint of NF-kB and MAPK flagging pathway.[39] We also found that simvastatin initiated HO-1 expression in microglial cells, proposing that enlistment of HO-1 may be included in the mitigating properties of simvastatin. The atomic instruments of HO-1 expression are additionally questionable. Studies have detailed that MAPKs, ROS, PKC, and NF-kB were flagging which is a fundamental part in the control of HO-1 expression to oxidative anxiety.[40] Our work have observed that simvastatin alone did not activate MAPK (p38, JNK, ERK) and NF-kB, demonstrating that these flagging pathways not included in the Simvastatin activated HO-1 expression in microglial cells.

Few studies additionally showed that both NF-kB inhibitor and MAPK inhibitor did not activate HO-1 expression in microglial cells, recommending that these two pathways not be upstream of HO-1 at slightest in our exploratory conditions.[41] In any event, two pathways are conceivably involved in the calming mechanisms of simvastatin: NADPH oxidase intervened concealment of ROS/NF-kB and ROS/p38 activation; HO-1 acceptance intervened hindrance of ROS/NF-kB, and ROS/p38 activation. Microglial cells have both beneficial and adverse capacity on neuronal homeostasis.[42,43] Unreasonable microglial activation gives neurotoxic microenvironment encompassing neuron through the generation of star inflammatory cytokines and free radicals, adds to neurodegenerative procedures.[32,42] In this manner, hindrance of glial activation can anticipate neuronal wounds in neurodegenerative sicknesses conditions.[44] Numerous calming operators displayed neuroprotective properties by restraining microglial initiation and resulting generation of pro-inflammatory middle people under focal sensory system (central nervous system) diseases conditions.[45]

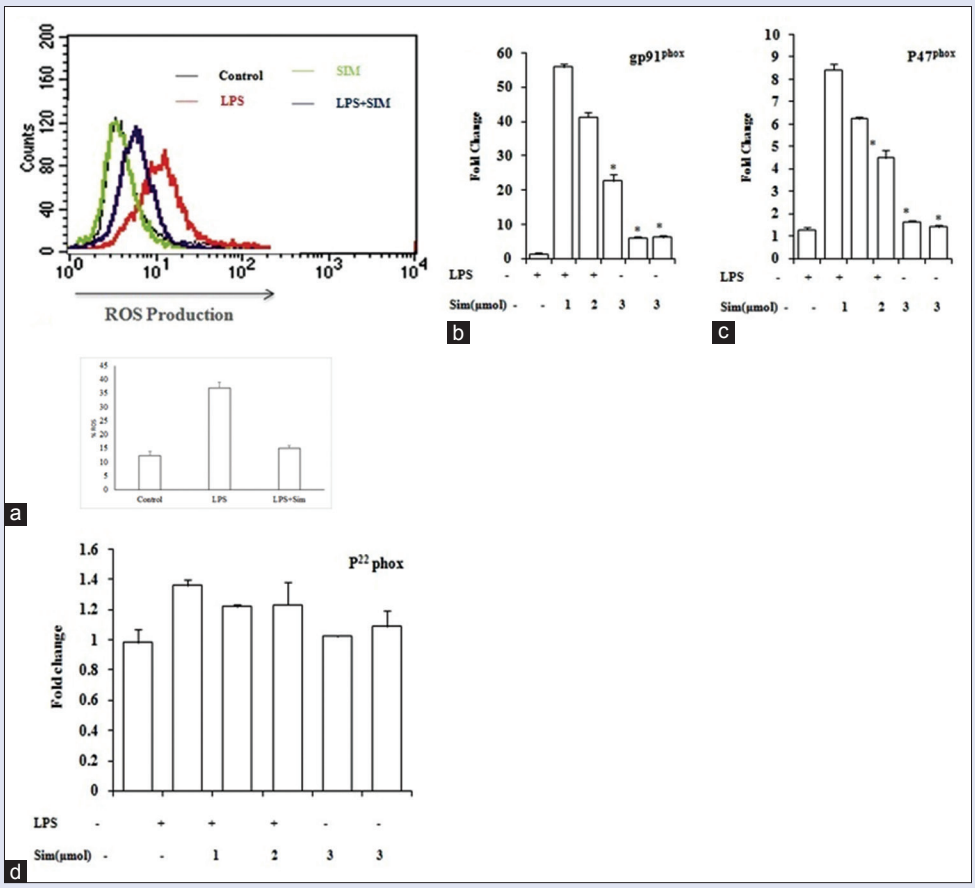


Figure 6: Impacts of simvastatin (Sim) on intracellular reactive oxygen species and articulation of NADPH oxidase and heme oxygenase-1 in microglial cells: BV-2 cells pretreated with simvastatin for 30 min, and then, lipopolysaccharide was added and cultured for four hours. After incubation with 10 mM of dichloro-dihydro-fluorescein diacetate, the generation of intracellular reactive oxygen species measured by flow cytometry (a) The mRNA expression of gp91phox (b), p47phox (c), and p22phox (d) was determined by quantitative reverse transcription-polymerase chain reaction. The relative expression value of control group set to 1. Data represent mean ± standard deviation of three independent experiments. Symbol mark indicates significantly differences from lipopolysaccharide alone treatment group (*$P < 0.05$, **$P < 0.01$)

Future reviews are required to recognize the specific molecular focus of simvastatin and to assess the neuroprotective impacts utilizing appropriate neuroinflammatory sickness creature models. The present study concludes that simvastatin has the neuroprotective properties by hindering microglial activation and might have a significant potential against neuroinflammatory diseases.

CONCLUSION

Our study showed that the simvastatin mitigates the neuroinflammation and can be a potential restorative operator for treating neuroinflammatory ailments.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Miller DW, Cookson MR, Dickson DW. Glial cell inclusions and the pathogenesis of neurodegenerative diseases. Neuron Glia Biol 2004;1:13-21.
2. Brown A. Understanding the MIND phenotype: Macrophage/microglia inflammation in neurocognitive disorders related to human immunodeficiency virus infection. Clin Transl Med 2015;4:7.
3. Bi XL, Yang JY, Dong YX, Wang JM, Cui YH, Ikeshima T, *et al.* Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. Int Immunopharmacol 2005;5:185-93.
4. Pyo H, Jou I, Jung S, Hong S, Joe EH. Mitogen-activated protein kinases activated by lipopolysaccharide and beta-amyloid in cultured rat microglia. Neuroreport 1998;9:871-4.
5. Nakamura Y, Si QS, Kataoka K. Lipopolysaccharide-induced microglial activation in culture: Temporal profiles of morphological change and release of cytokines and nitric oxide. Neurosci Res 1999;35:95-100.
6. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, *et al.* Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem 2006;281:21362-8.
7. Ye J, Jiang R, Cui M, Zhu B, Sun L, Wang Y, *et al.* Etanercept reduces neuroinflammation and lethality in mouse model of Japanese encephalitis. J Infect Dis 2014;210:875-89.
8. Laroni A, Novi G, Kerlero de Rosbo N, Uccelli A. Towards clinical application of mesenchymal stem cells for treatment of neurological diseases of the central nervous system. J Neuroimmune Pharmacol 2013;8:1062-76.
9. Heppner FL, Ransohoff RM, Becher B. Immune attack: The role of inflammation in Alzheimer disease. Nat Rev Neurosci 2015;16:358-72.
10. Liu B, Hong JS. Role of microglia in inflammation-mediated neurodegenerative diseases: Mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther 2003;304:1-7.
11. Bozic I, Savic D, Laketa D, Bjelobaba I, Milenkovic I, Pekovic S, *et al.* Benfotiamine attenuates inflammatory response in LPS stimulated BV-2 microglia. PLoS One 2015;10:e0118372.

12. Ridker PM, Pradhan A, MacFadyen JG, Libby P, Glynn RJ. Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: An analysis from the JUPITER trial. Lancet 2012;380:565-71.
13. Parihar SP, Hartley MA, Hurdayal R, Guler R, Brombacher F. Topical simvastatin as host-directed therapy against severity of cutaneous leishmaniasis in mice. Sci Rep 2016;6:33458.
14. Wang G, Cao R, Wang Y, Qian G, Dan HC, Jiang W, *et al.* Simvastatin induces cell cycle arrest and inhibits proliferation of bladder cancer cells via PPARγ signalling pathway. Sci Rep 2016;6:35783.
15. Maneechotesuwan K, Wongkajornsilp A, Adcock IM, Barnes PJ. Simvastatin suppresses airway IL-17 and upregulates IL-10 in patients with stable COPD. Chest 2015;148:1164-76.
16. Wu BQ, Luo JM, Wang YH, Shi YF, Liu H, Ba JH, *et al.* Inhibitory effects of simvastatin on *Staphylococcus aureus* lipoteichoic acid-induced inflammation in human alveolar macrophages. Clin Exp Med 2014;14:151-60.
17. Rezaie-Majd A, Maca T, Bucek RA, Valent P, Müller MR, Husslein P, *et al.* Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194-9.
18. Yu L, Da XW, Wu XL, He AD, Long D. Simvastatin prevents lipopolysaccharide-induced septic shock in rats. J Huazhong Univ Sci Technolog Med Sci 2017;37:226-30.
19. Boyd AR, Hinojosa CA, Rodriguez PJ, Orihuela CJ. Impact of oral simvastatin therapy on acute lung injury in mice during pneumococcal pneumonia. BMC Microbiol 2012;12:73.
20. Balez R, Steiner N, Engel M, Muñoz SS, Lum JS, Wu Y, *et al.* Neuroprotective effects of apigenin against inflammation, neuronal excitability and apoptosis in an induced pluripotent stem cell model of Alzheimer's disease. Sci Rep 2016;6:31450.
21. McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG, *et al.* Microglia in degenerative neurological disease. Glia 1993;7:84-92.
22. Kim BW, Koppula S, Kim IS, Lim HW, Hong SM, Han SD, *et al.* Anti-neuroinflammatory activity of Kamebakaurin from *Isodon japonicus* via inhibition of c-jun NH_2-terminal kinase and p38 mitogen-activated protein kinase pathway in activated microglial cells. J Pharmacol Sci 2011;116:296-308.
23. Ghosh S, May MJ, Kopp EB. NF-kappa B and rel proteins: Evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998;16:225-60.
24. Kim BW, Koppula S, Hong SS, Jeon SB, Kwon JH, Hwang BY, *et al.* Regulation of microglia activity by glaucocalyxin-A: Attenuation of lipopolysaccharide-stimulated neuroinflammation through NF-κB and p38 MAPK signaling pathways. PLoS One 2013;8:e55792.
25. Guha M, Mackman N. LPS induction of gene expression in human monocytes. Cell Signal 2001;13:85-94.
26. Clutterbuck RD, Millar BC, Powles RL, Newman A, Catovsky D, Jarman M, *et al.* Inhibitory effect of simvastatin on the proliferation of human myeloid leukaemia cells in severe combined immunodeficient (SCID) mice. Br J Haematol 1998;102:522-7.
27. Su YW, Chiou WF, Chao SH, Lee MH, Chen CC, Tsai YC, *et al.* Ligustilide prevents LPS-induced iNOS expression in RAW 264.7 macrophages by preventing ROS production and down-regulating the MAPK, NF-κB and AP-1 signaling pathways. Int Immunopharmacol 2011;11:1166-72.
28. Cindrova-Davies T. Gabor than award lecture 2008: Pre-eclampsia – From placental oxidative stress to maternal endothelial dysfunction. Placenta 2009;30 Suppl A:S55-65.
29. Kwon CH, Moon HJ, Park HJ, Choi JH, Park DY. S100A8 and S100A9 promotes invasion and migration through p38 mitogen-activated protein kinase-dependent NF-κB activation in gastric cancer cells. Mol Cells 2013;35:226-34.
30. Lim JY, Hwang BY, Hwang KW, Park SY. Methylalpinumisoflavone inhibits lipopolysaccharide-induced inflammation in microglial cells by the NF-kappaB and MAPK signaling pathway. Phytother Res 2012;26:1948-56.
31. Gao HM, Liu B, Hong JS. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J Neurosci 2003;23:6181-7.
32. Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol 2011;11:775-87.
33. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol 2002;2:725-34.
34. Kang G, Kong PJ, Yuh YJ, Lim SY, Yim SV, Chun W, *et al.* Curcumin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression by inhibiting activator protein 1 and nuclear factor kappab bindings in BV2 microglial cells. J Pharmacol Sci 2004;94:325-8.
35. Choi SH, Aid S, Kim HW, Jackson SH, Bosetti F. Inhibition of NADPH oxidase promotes alternative and anti-inflammatory microglial activation during neuroinflammation. J Neurochem 2012;120:292-301.
36. Koistinaho M, Koistinaho J. Role of p38 and p44/42 mitogen-activated protein kinases in microglia. Glia 2002;40:175-83.
37. Jeon NR, Koppula S, Kim BW, Park SH, Lee HW, Choi DK, *et al.* MMHD [(S, E)-2-methyl-1-(2-methylthiazol-4-yl) hexa-1,5-dien-ol], a novel synthetic compound derived from epothilone, suppresses nuclear factor-kappaB-mediated cytokine expression in lipopolysaccharide-stimulated BV-2 microglia. J Pharmacol Sci 2010;112:158-66.
38. Bhat NR, Zhang P, Lee JC, Hogan EL. Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. J Neurosci 1998;18:1633-41.
39. Lee IS, Lim J, Gal J, Kang JC, Kim HJ, Kang BY, *et al.* Anti-inflammatory activity of xanthohumol involves heme oxygenase-1 induction via NRF2-ARE signaling in microglial BV2 cells. Neurochem Int 2011;58:153-60.
40. Nguyen CN, Kim HE, Lee SG. Caffeoylserotonin protects human keratinocyte HaCaT cells against H2 O2 -induced oxidative stress and apoptosis through upregulation of HO-1 expression via activation of the PI3K/Akt/Nrf2 pathway. Phytother Res 2013;27:1810-8.
41. Zhou X, Gan P, Hao L, Tao L, Jia J, Gao B, *et al.* Antiinflammatory effects of orientin-2″-O-galactopyranoside on lipopolysaccharide-stimulated microglia. Biol Pharm Bull 2014;37:1282-94.
42. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. Nat Rev Neurol 2014;10:217-24.
43. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. Nat Rev Neurosci 2007;8:57-69.
44. Guyton KZ, Gorospe M, Kensler TW, Holbrook NJ. Mitogen-activated protein kinase (MAPK) activation by butylated hydroxytoluene hydroperoxide: Implications for cellular survival and tumor promotion. Cancer Res 1996;56:3480-5.
45. Zheng LT, Ock J, Kwon BM, Suk K. Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. Int Immunopharmacol 2008;8:484-94.