Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Glabridin Downregulates Lipopolysaccharide-Induced Oxidative Stress and Neuroinflammation in BV-2 Microglial Cells via Suppression of Nuclear Factor–κB Signaling Pathway

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Submitted: 01-Jan-2020 Revised: 09-Jan-2020 Accepted: 21-Apr-2020 Published: 20-Oct-2020

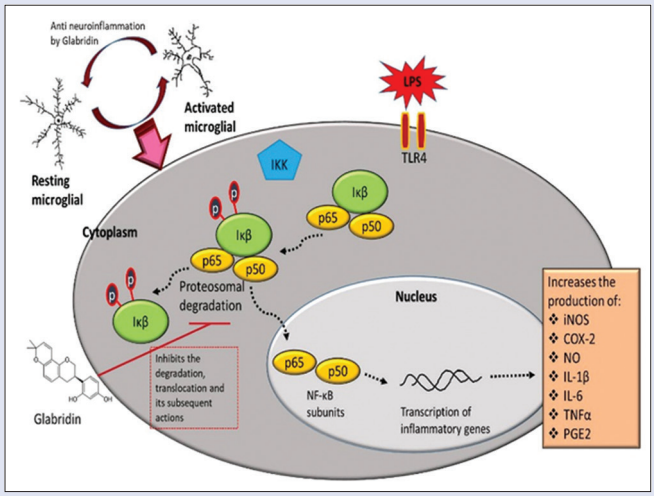
ABSTRACT

Background: Microglia initially undergoes chronic activation in response to the damages caused by stressful stimuli, such as bacterial infection and hypoxia. Inflammatory responses, as well as oxidative stress, perform crucial roles in the process of neuroinflammation, which results in brain damage. Glabridin is a natural organic compound with extensive beneficial properties such as anti-inflammatory and antioxidant properties. To the best of our knowledge, there are no studies conducted on the anti-neuroinflammatory activity of glabridin. Therefore, in this study, we aimed to investigate the potency of glabridin against the expression of lipopolysaccharide (LPS)-stimulated BV-2 cells. **Materials and Methods:** BV-2 cells were preincubated with glabridin followed by the LPS challenge. Subsequently, the cellular status of nitric oxide (NO), reactive oxygen species (ROS), prostaglandin E_2 (PGE_2), and pro-inflammatory modulators (interleukin [IL]-1β and IL-6) were investigated and related signaling pathways were inspected via blotting assay. **Results:** Our results indicate that glabridin appreciably alleviated the LPS-induced accretion of inducible-NO synthase (iNOS), PGE_2, IL-1β, and IL-6. Moreover, it noticeably allayed the NO/iNOS, PGE_2/cyclooxygenase-2 protein statuses, and pro-inflammatory cytokine (tumor necrosis factor-α) on LPS-induced microglia. We also found that LPS severely increased the phosphorylation of Inhibitory kappa B kinases (IKKs), IκBα, p65, and nuclear factor (NF)-κB. Although glabridin supplementation suppressed the phosphorylation of the aforementioned molecules, LPS remarkable caused the nuclear interchange of NF-κBp65. **Conclusion:** Glabridin alleviates LPS-induced neuroinflammation in BV-2 cells by suppressing the accumulation of ROS and cell death and by inhibiting the pro-inflammatory responses via NF-κB-dependent mechanisms. According to our results, glabridin may be beneficial in neuroinflammation-related neurodegenerative disorders.

Key words: BV-2, glabridin, lipopolysaccharide, microglia, neuroinflammation, nuclear factor-κB, reactive oxygen species

SUMMARY

- Glabridin appreciably alleviated the lipopolysaccharide (LPS)-induced neuroinflammatory processes in BV-2 cells by suppressing the nuclear factor-κB (NF-κB) activation
- Glabridin has the potency to suppress neuroinflammation in LPS-induced BV-2 cells, thereby preventing the formation of inflammatory mediators leading to the activation of NF-κB pathway.



Abbreviations used: LPS: Lipopolysaccharide; iNOS: Inducible-nitric oxide synthase; ROS: Reactive oxygen species; NO: Nitric oxide; PGE_2: Prostaglandin-E_2; TNF-α: Tumor necrosis factor-α.

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DOI: 10.4103/pm.pm_497_19

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INTRODUCTION

Microglia is primarily involved in immune surveillance. In response to neuronal stress, microbial invasion, or inflammation, microglia transform into intrinsic brain macrophages taking care of phagocytosis, producing inflammatory cytokines, and presenting antigen.[1] It is extensively found in the central part of the brain, which includes the Substantia nigra and releases pro-inflammatory cytokines (e.g., tumor necrosis factor-α [TNF-α]) and interleukin [IL]-1β), free radicals, nitric oxide (NO), and reactive oxygen species (ROS). These molecules are responsible for the degenerative progression in brain injury.[2] Previous studies have shown a strong association between the oxidative stress and neuroinflammation. Increased level of intracellular ROS accumulation triggers neuroinflammation.[3] Previous reports have pointed out that ROS accumulation in microglia restricts microglial functions.[4,5]

Lipopolysaccharide (LPS) activates host-defense responses by increasing the oxidative stress and by releasing pro-inflammatory mediators. It induces cytotoxicity in various cells, including glial cells in the central

Cite this article as: Wu Y, Geng J, Lei X, Wu Q, Chen T, Zhong L. Glabridin downregulates lipopolysaccharide-induced oxidative stress and neuroinflammation in BV-2 microglial cells via suppression of nuclear factor-κB signaling pathway. Phcog Mag 2020;16:675-80.

nervous system (CNS) by stimulating apoptosis.[6,7] So far, it has been broadly established that microglial stimulation is responsible for the initiation and development of numerous neurodegenerative ailments. Therefore, it is important to diagnose microglial activation and anti-inflammatory approach might hinder the ailment development before irretrievable injuries and occurring of clinical signs.[8] Thereby, it is important to find therapeutic agents that hinder the activation of microglia and inhibit the production of pro-inflammatory mediators. During the past few years, research on the exploration of anti-inflammatory agents and plant-based compounds has gained greater interest. The inflammatory conditions and excessive oxidative stress in the CNS have been found to be associated with the age-related neurodegenerative disorders. Proper diet might be able to reduce the development of age-related neurodegenerative ailments.[9-11]

Glabridin [(R)-4-(3,4-dihydro-8,8-dimethyl)-2H,8H-benzo[1,2-b: 3,4-b´] dinyran-3yl]-1,3-benzenediol] is a flavonoid compound, and it is a bioactive component present in the licorice extract.[12] It is commonly known as a phytoestrogen and shows antioxidant, anti-inflammatory, neuroprotective, anti-atherogenic, anti-tumor, antinephritic, and antibacterial properties.[13-15] In this study, we used murine BV-2 cells to examine the anti-neuroinflammatory activity of glabridin and investigated the mechanisms of action through nuclear factor-κB (NF-κB) signaling pathway.

MATERIALS AND METHODS

BV-2 cell culture and sample treatments

Immortalized murine BV-2 microglial cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with fetal bovine serum (5%) and 100 units/mL of penicillin/streptomycin (1%) at 37°C in a humidified atmosphere with 5% CO_2. Briefly, the cells were cultured in 75 cm^2 flasks until they reached 80%–90% confluency. Then, the cells were detached via trypsinization and subcultured in 96-well plates and grown overnight. Then, glabridin was added to each well at less than 1% concentration. The concentration of dimethyl sulfoxide (DMSO) was chosen in such a way that it does not cause any cytotoxicity. Next, the cells were preincubated with different concentrations of glabridin for 2 h and subsequently were challenged with LPS (1 μg/mL) except for the control cells.

Cell viability assessment via 3-(4, 5-dimethy lthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2-(4-sulfop henyl)-2H-tetra zolium assay

Cell viability was examined via 3-(4,5-dimeth ylthiazol-2-yl)-5-(3-carboxymeth oxyphenyl)-2-(4-sulfop henyl)-2H-tetr azolium (MTT) method.[16] Briefly, after attaining 80%–90% confluency, the cells in the 96-well plates were supplemented with glabridin (10–100 μg/mL) for 24 h. After this, the medium was replaced with 100 μL of fresh medium and 10 μL of MTT solution (5 mg/mL) and incubated for 4–6 h at 37°C. The formazan crystals formed were solubilized using DMSO, and the absorbance was read at 420–480 nm. All experiments were performed in triplicate.

Determination of nitric oxide production

NO produced in cells was transformed to nitrite in the growth medium that can be examined via a colorimetric test with Griess reagent.[17] Briefly, BV-2 cells (1×10^5 cells/mL) were seeded in 6-well plates in 2 mL culture medium and preincubated for 1 h with indicated doses of glabridin (10, 25, and 50 μg/mL), prior to the incubation of cells in a medium consisting of LPS (1 μg/mL) for 24 h. Culture medium (50 μL) was mixed with an equivalent quantity of Griess reagent `thylethylenediamine and 1% sulfanilamide in 5% H_3PO_4) in 96-well plates and incubated for 10 min at 37°C under dark conditions. The nitrites formed were estimated using sodium nitrite (0–100 μM) as the standard compound. The absorbance was read at 540 nm in a microplate reader (Tecan Trading AG, Switzerland). All tests were performed in triplicate.

Reactive oxygen species level

The level of ROS in both control and experimental cells was examined quantitatively and qualitatively by employing a fluorescent probe, namely 2´,7´-dichlorodihydrofluorescein diacetate (DCF-DA). Briefly, BV-2 cells were incubated with different concentrations of glabridin (10, 25, and 50 μg/mL) for 24 h. Then, both experimental and control cells were incubated with 20 μM DCF-DA for 45 min at 37°C under dark conditions and then rinsed with phosphate-buffered solution. The changes in the level of ROS were detected fluorometrically on a microplate reader with excitation at 485 nm and 530 nm. The intensity of fluorescence is relatively proportional to the level of ROS. All tests were performed in triplicate.

Measurement of pro-inflammatory cytokines and pro-inflammatory mediators

The accumulation of pro-inflammatory mediators (IL-1β and IL-6) and prostaglandin-E_2 (PGE_2) in BV-2 cells was detected using employing the enzyme-linked immunosorbent assay (ELISA) test kits. Briefly, BV-2 cells (1×10^5 cells/mL) were seeded in 6-well plates in 2 mL medium and were preincubated for 1 h with different concentrations of glabridin (10, 25, and 50 μg/mL), in previous to incubating the medium consisting of LPS (1 μg/mL) for 24 h. Then, the culture medium was analyzed for the amount of pro-inflammatory mediators in accordance with the protocol of the manufacturer. The results are presented as pg/mL of the culture supernatant. All the tests were performed in triplicate.

Gel electrophoresis and western blotting

BV-2 cells (1×10^6 cells) were seeded in a 6-well plate and preincubated with different concentrations of glabridin (10, 25, and 50 μg/mL) in previous to the LPS challenge for 24 h. Then, the cells were cooled suing chilled Radioimmunoprecipitation assay buffer (RIPA buffer) consisting of protease inhibitors. Protein concentration was analyzed using Bradford's method. Then, the proteins were identified via separation on a 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The proteins isolated were transferred into a nitrocellulose membrane. Then, the proteins were blocked with skimmed milk for 1 h and incubated in the presence of respective antibodies overnight at 4°C (TNF-α, TLR4, cyclooxygenase-2 [COX-2], inducible-nitric oxide synthase (iNOS), ikβ, p50, p65, Histone H1, P-IKKα, IKKα, P-IkBα, P-IkBα, and β-actin) (Thermo Scientific). Next, the nitrocellulose membranes were processed with anti-rabbit/mouse immunoglobulin G, horseradish peroxidase-loaded secondary antibodies for 1 h at 37°C. Finally, the membranes were stained with the improved chemiluminescence detection kit and proteins were identified via gel-documentation systems.

Statistical investigation

Data were statistically analyzed by performing a paired Student's *t*-test. The variations in the data were regarded as significant if $P < 0.05$. Data were presented as mean ± standard error of the mean.

RESULTS

Effect of glabridin on the viability of BV-2 cells

The cell viability of BV-2 cells after induction with LPS and after preincubation with glabridin was assessed via MTT assay. According to

our results, glabridin was not found to be toxic to BV-2 cells up to a concentration of 30 μg/mL [Figure 1]. When compared to control cells, 40 μg/mL and 50 μg/mL glabridin showed slight cytotoxicity ($P < 0.05$). The BV-2 cell viability was significantly reduced to almost 70% at 100 μg/mL of glabridin. Therefore, a concentration ranging from 10 μg/mL to 50 μg/mL of glabridin was selected to analyze the anti-neuroinflammatory activity.

Alleviation of nitric oxide accumulation by glabridin in lipopolysaccharide-induced BV-2 cells

The effect of glabridin in alleviating the excessive accumulation of NO after LPS stimulation was studied in BV-2 cells. According to the results, LPS induced the accumulation of NO in BV-2 cells when compared to control cells [Figure 2]. Glabridin decreased the accumulation of NO in a dose-dependent manner ($P < 0.0.5$) when compared with LPS-induced cells. Glabridin at 50 μg/mL concentration decreased the generation NO in BV-2 cells, almost similar to the level of normal cells. The iNOS expression in BV-2 cells was further analyzed through the Western blotting. Our results showed that glabridin downregulated the expression of iNOS in LPS-induced BV-2 cells in a dose-dependent manner [Figure 6].

Glabridin pretreatment reduced the reactive oxygen species levels in lipopolysaccharide-induced BV-2 cells

ROS is an initial signal inducer of inflammatory reaction in microglia.[5] The level of ROS in LPS-induced BV-2 cells was detected through the fluorescent probe DCF-DA, which gets oxidized by ROS to its fluorescing form DCF.[18] LPS-induced BV-2 cells demonstrated an increased presence of ROS than that of control cells [Figure 3]. Glabridin significantly decreased the levels of ROS in LPS-induced BV-2 cells in a dose-dependent manner. The levels of ROS in LPS-induced BV-2 cells were assuaged equally to a level of control

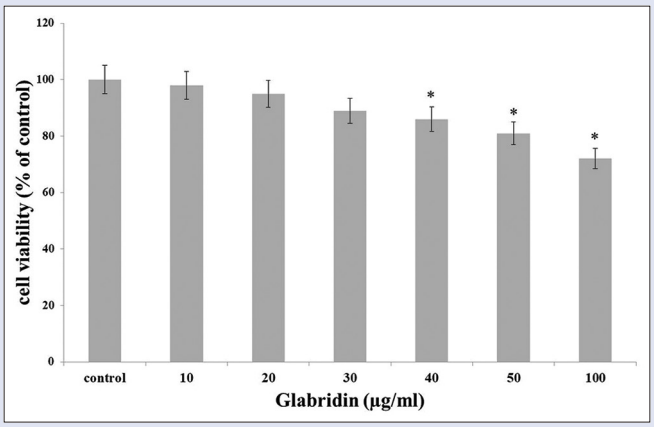

Figure 1: The effect of glabridin on the viability of BV-2 microglial cells. *Significantly different ($P < 0.05$) as compared to the control group. Data are represented as mean ± standard error of mean of three individual experiments

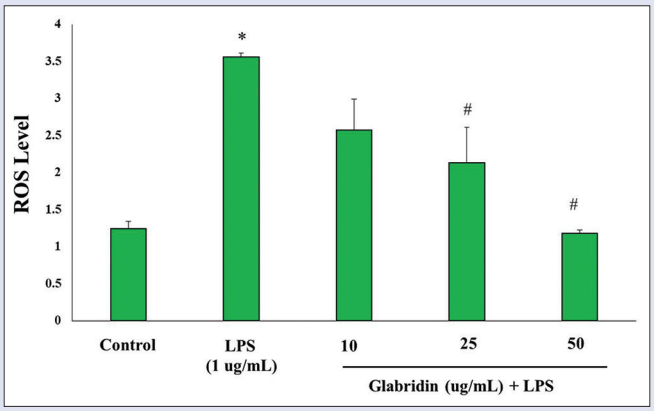

Figure 3: The effect of glabridin on reactive oxygen species levels in lipopolysaccharide-induced BV-2 cells. *Significantly different ($P < 0.05$) as compared to the control group. #Significantly different ($P < 0.05$) as compared to lipopolysaccharide alone-treated group. Data are represented as mean ± standard error of mean of three individual experiments

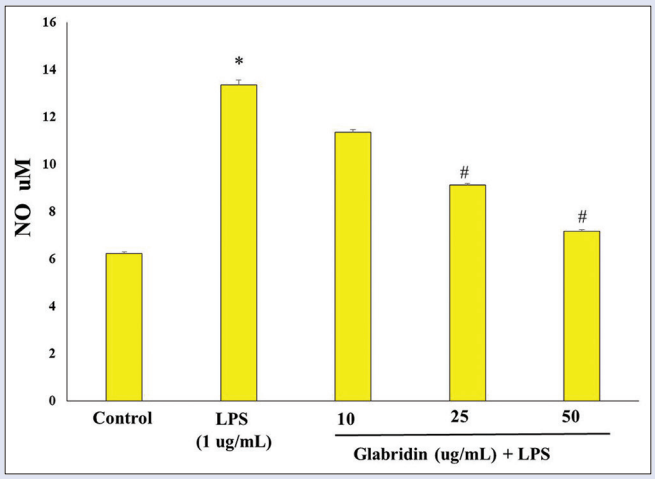

Figure 2: The effect of glabridin on nitric oxide levels in lipopolysaccharide-induced BV-2 cells. *Significantly different ($P < 0.05$) as compared to the control group. #Significantly different ($P < 0.05$) as compared to lipopolysaccharide alone treated group. Data are represented as mean ± standard error of mean of three individual experiments

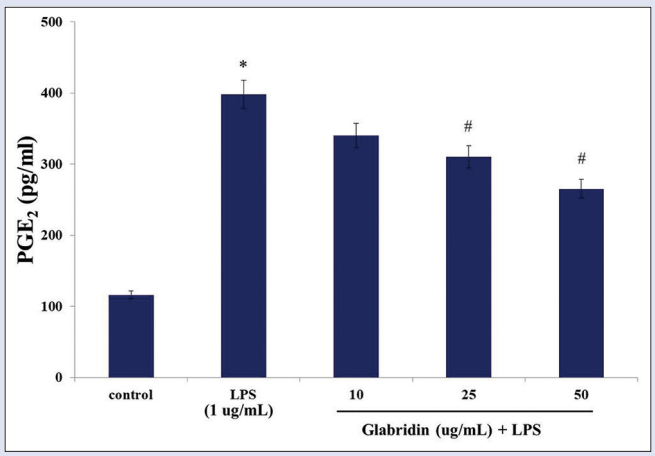

Figure 4: The effect of glabridin on prostaglandin-E_2 levels in lipopolysaccharide-induced BV-2 cells. *Significantly different ($P < 0.05$) as compared to the control group. #Significantly different ($P < 0.05$) as compared to lipopolysaccharide alone-treated group. Data are represented as mean ± standard error of mean of three individual experiments

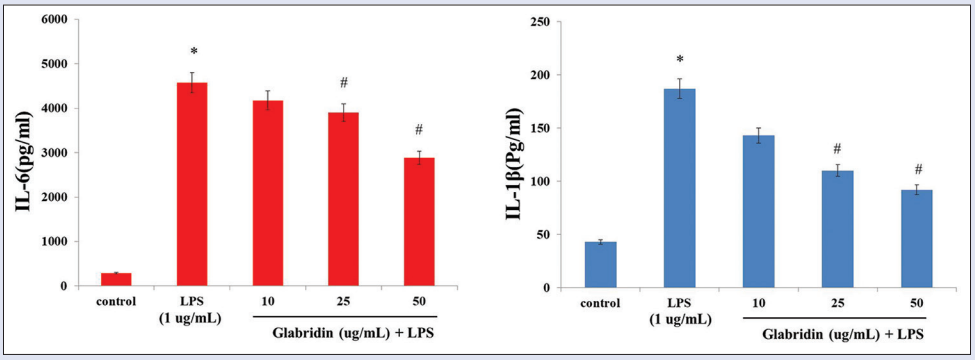

Figure 5: The effect of glabridin on interleukin-6 and interleukin-1β levels in lipopolysaccharide-induced BV-2 cells. *Significantly different ($P < 0.05$) as compared to control group. #Significantly different ($P < 0.05$) as compared to lipopolysaccharide alone-treated group. Data are represented as mean ± standard error of mean of three individual experiments

cells with 50 μg/mL of glabridin treatment. This shows that glabridin inhibited the formation of ROS in LPS-induced BV-2 cells.

Suppression of prostaglandin-E_2, interleukin-1β, and interleukin-6 by glabridin in lipopolysaccharide-induced BV-2 cells

Pro-inflammatory mediators such as IL-6, IL-1β, and PGE_2 are important indicators of cellular inflammatory processes. The expression levels of PGE_2, IL-6, and IL-1β in LPS-induced BV-2 cells were examined by ELISA. LPS significantly upregulated the expression levels ($P < 0.05$) of PGE_2 [Figure 4], IL-6 [Figure 5], and IL-1β [Figure 5b] in BV-2 cells when compared to control cells. This effect was reversed the pretreatment of the cells with glabridin ($P < 0.05$). COX-2 synthesizes PGE_2 during an inflammatory reaction.[19] Hence, we evaluated the expression of COX-2 via the Western blot technique. According to our results, COX-2 expression was significantly unregulated in LPS-induced BV-2 cells when compared with normal cells. Glabridin dose dependently decreased the expression of COX-2 [Figure 6]. This shows the efficacy of glabridin in preventing neuroinflammation by suppressing the production of pro-inflammatory mediators (PGE_2, IL-6, and IL-1β) and related enzymes (COX-2).

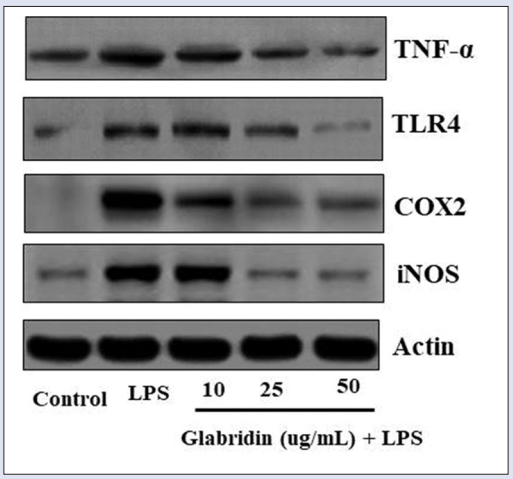

Figure 6: Western blot analysis on the effect of glabridin on the expressions of inflammatory mediator proteins in lipopolysaccharide-induced BV-2 cells. β-actin was used as internal control. Representation of three individual experiments is shown

Glabridin prevented the tumor necrosis factor-α, TLR4, and nuclear factor-κB stimulation in lipopolysaccharide-induced BV-2 cells

The expression patterns of pro-inflammatory mediator TNF-α, inflammatory regulator TLR4, and NF-κB activation were investigated through the Western blot analysis. The expression of TNF-α and TLR4 was significantly upregulated after LPS induction in BV-2 cells when compared to the control cells; however, the expression was downregulated by glabridin in a dose-dependent manner [Figure 6]. NF-κB activation is a pro-inflammatory response which is initiated by the phosphorylation of IKKα and IκBα by external factors such as LPS.[5] The outcome of NF-κB activation in LPS-induced BV-2 cells was positive as IKKα and IκBα were phosphorylated by LPS [Figure 7]. The NF-κB stimulation in LPS-induced BV-2 cells was further evidenced by the translocation of p65 and p50 heterodimers from the cytosol into the nucleus. The expression of NF-κB, p65, and p50 was upregulated by LPS [Figure 7]. The phosphorylation of IKKα and IκBα and the expression of p65 and p50 were reversed by glabridin in LPS-induced BV-2 cells. This shows that glabridin suppressed the effects of LPS-stimulation in BV-2 cells by alleviating pro-inflammatory

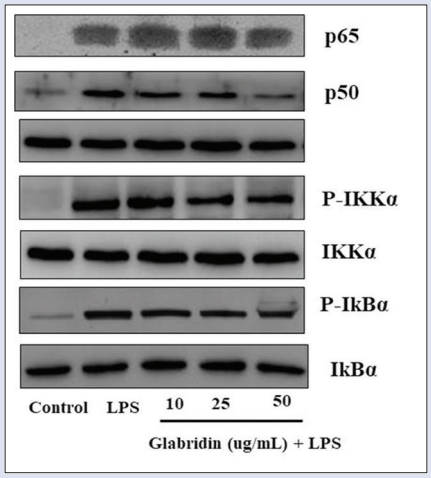

Figure 7: Western blot analysis on the effect of glabridin on nuclear factor-κB protein expressions in lipopolysaccharide-induced BV-2 cells. β-actin was used as internal control. Representation of three individual experiments is shown

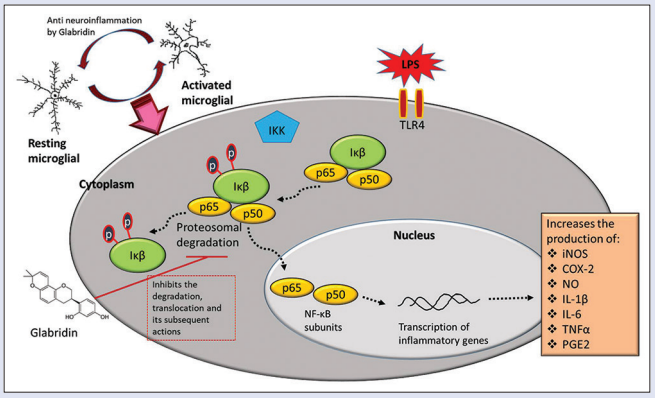

Figure 8: Representation of anti-neuroinflammatory mechanism of glabridin in lipopolysaccharide-induced microglia through the prevention of nuclear factor-κB activation. Glabridin prevented the proteosomal degradation of IκB complex thereby inhibited the translocation of nuclear factor-κB subunits into nucleus thus suppressed the activation of gene expression encoded for pro-inflammatory proteins

mediator (TNF-α), inflammatory regulator (TLR4), and the NF-κB activation.

DISCUSSION

Neuroinflammation is a common manifestation in neurodegenerative and neurological disorders which is mediated through the activation of inflammatory cytokines and pathways.[7] Oxidative stress is the prime inducer of neuroinflammation via the formation of ROS in microglial cells.[20,21] The stimulation of microglia stimulates the assembly of a number of neuroinflammatory cytokines and mediators; therefore, it is important to prevent the activation of microglia. This study was performed to examine the anti-neuroinflammatory potency of glabridin in LPS-induced neuroinflammation in BV-2 microglial cells. Glabridin demonstrates very potent antioxidant effects, but so far, there are no scientific findings on the anti-neuroinflammatory activity of glabridin. The formation of ROS formation in LPS-induced BV-2 cells was dose dependently reversed by glabridin, which prevented the activation of the microglia.

NO and PGE_2 are important mediators in the pathogenesis of various pathological and physiological inflammatory conditions.[21-23] The synthesis of NO and PGE_2 is catalyzed by L-arginine via endogenous iNOS and arachidonic acid via COX-2, respectively. Neuroprotection or inhibition of neuroinflammation can be achieved by preventing the formation of NO and PGE_2.[22,23] Glabridin has the potential to prevent the LPS-induced increase in the level of NO and PGE_2 in BV-2 cells in a dose-dependent manner. These results show that glabridin alleviates the expression of iNOS and COX-2.

Pro-inflammatory mediators directly contribute to the processes of neuroinflammation induced by LPS.[20,24] Hence, the expression patterns of pro-inflammatory mediators such as TNF-α, IL-6, and IL-1β were evaluated in LPS-induced BV-2 cells. From these findings, we can say that LPS substantially upregulates the expression of TNF-α, IL-6, and IL-1 β, and glabridin suppressed the expression of pro-inflammatory cytokine in a dose-dependent manner in LPS-induced BV-2 cells. This finding suggests that glabridin alleviates LPS-induced neuroinflammation via downregulating the expression of pro-inflammatory mediators in BV-2 cells.

LPS is known to be recognized by TLR4 which is located in an exterior of microglial cells which eventually initiates the activation of microglia toward inflammatory reactions.[25] It has been reported previously that the expression of TLR4 is upregulated during neuroinflammatory conditions, resulting in the stimulation of major transcription factors of inflammatory response such as the NF-κB pathway.[5,25] Our results show that LPS stimulation upregulated the expression of TLR4 and activators of NF-κB pathway such as IKKα, IκBα, NF-κB, p65, and p50 in BV-2 cells. The expression of TLR4 was downregulated by glabridin in LPS-induced BV-2 cells in a dose-dependent manner. Glabridin also alleviated the LPS-induced neuroinflammation in BV-2 cells by suppressing the activation of NF-κB. Rearrangement of NF-κB, p65, and p50 from the cytosol into the nuclei of microglia was prevented by the inhibition of IκBα phosphorylation by glabridin; therefore, the transcription of neuroinflammatory mediators was not initiated. These results show that glabridin suppresses LPS-induced neuroinflammation in BV-2 cells by alleviating the NF-κB signaling pathway, which is a consequence of preventing the formation of inflammatory mediators.

CONCLUSION

The results of this study have shown that glabridin has the potential to prevent ROS formation and downregulate the expression of TLR4 and pro-inflammatory mediators (NO, TNF-α, PGE_2, IL-6, and IL-1 β). It can also inhibit the activation of NF-κB, thereby ameliorating the neuroinflammation processes due to LPS induction in BV-2 cells. The results of this study demonstrate the efficiency of glabridin as a potent neuroprotective agent. In summary, glabridin protected BV-2 cells against LPS-induced neuroinflammation. Therefore, glabridin can be utilized as an efficient neuroprotective agent. We recommend further research on the *in vivo* efficiency and other pharmacological evaluations of glabridin [Figure 8].

Acknowledgements

The authors would like to thank the Department of Neurology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan-650032, China, for instrumentation facilities support.

Financial support and sponsorship

Yunnan Applied Basic Research Projects (2018FE001(-145)) and also National Natural Science Foundation of China,81760226.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science 2005;308:1314-8.
2. Garden GA, Möller T. Microglia biology in health and disease. J Neuroimmune Pharmacol 2006;1:127-37.
3. Qin L, Crews FT. NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration. J Neuroinflammation 2012;9:5.
4. Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, Hong JS. Role of nitric oxide in inflammation-mediated neurodegeneration. Ann N Y Acad Sci 2002;962:318-31.
5. Jeong YH, Park JS, Kim DH, Kang JL, Kim HS. Anti-inflammatory mechanism of lonchocarpine in LPS- or poly (I: C)-induced neuroinflammation. Pharmacol Res 2017;119:431-42.
6. Kim WG, Mohney RP, Wilson B, Jeohn GH, Liu B, Hong JS. Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: Role of microglia. J Neurosci 2000;20:6309-16.
7. Lee CM, Lee DS, Jung WK, Yoo JS, Yim MJ, Choi YH, *et al.* Benzyl isothiocyanate inhibits inflammasome activation in *E. coli* LPS-stimulated BV2 cells. Int J Mol Med 2016;38:912-8.
8. Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, *et al.* Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor

4-dependent pathway. Proc Natl Acad Sci U S A 2003;100:8514-9.

9. Shukla SM, Sharma SK. Sinomenine inhibits microglial activation by Aβ and confers neuroprotection. J Neuroinflammation 2011;8:117.
10. Sung SC, Dong HL, Sang HL. Blueberry protects LPS stimulated BV-2 microglia through inhibiting activities of p38 MAPK and ERK1/2. Food Sci Biotechnol 2012;21:1195-201.
11. Wu WY, Wu YY, Huang H, He C, Li WZ, Wang HL, *et al.* Biochanin A attenuates LPS-induced pro-inflammatory responses and inhibits the activation of the MAPK pathway in BV2 microglial cells. Int J Mol Med 2015;35:391-8.
12. Hsieh MJ, Lin CW, Yang SF, Chen MK, Chiou HL. Glabridin inhibits migration and invasion by transcriptional inhibition of matrix metalloproteinase 9 through modulation of NF-KB and AP-1 activity in human liver cancer cells. Br J Pharmacol 2014;171:3037-50.
13. Kang MR, Park KH, Oh SJ, Yun J, Lee CW, Lee MY, *et al.* Cardiovascular protective effect of glabridin: Implications in LDL oxidation and inflammation. Int Immunopharmacol 2015;29:914-8.
14. Yu XQ, Xue CC, Zhou ZW, Li CG, Du YM, Liang J, *et al. In vitro* and *in vivo* neuroprotective effect and mechanisms of glabridin, a major active isoflavan from glycyrrhiza glabra (licorice). Life Sci 2008;82:68-78.
15. Simmler C, Pauli GF, Chen SN. Phytochemistry and biological properties of glabridin. Fitoterapia 2013;90:160-84.
16. Chattopadhyay M, Kodela R, Olson KR, Kashfi K. NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid is a potent inhibitor of colon cancer cell growth *in vitro* and in a xenograft mouse model. Biochem Biophys Res Commun 2012;419:523-8.
17. Stockert JC, Blázquez-Castro A, Cañete M, Horobin RW, Villanueva A. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. Acta Histochem 2012;114:785-96.
18. Mühleisen L, Alev M, Unterweger H, Subatzus D, Pöttler M, Friedrich RP, *et al.* Analysis of hypericin-mediated effects and implications for targeted photodynamic therapy. Int J Mol Sci 2017;18. pii: E1388.
19. Weng L, Zhang H, Li X, Zhan H, Chen F, Han L, *et al.* Ampelopsin attenuates lipopolysaccharide induced inflammatory response through the inhibition of the NF-κB and JAK2/STAT3 signaling pathways in microglia. Int Immunopharmacol 2017;44:1-8.
20. Amor S, Puentes F, Baker D, van der Valk P. Inflammation in neuro degenerative diseases. Immunology 2010;129:154-69.
21. Kim DC, Quang TH, Yoon CS, Ngan NTT, Lim SI, Lee SY, *et al.* Anti-neuroinflammatory activities of indole alkaloids from Kanjang (Korean fermented soy source) in lipopolysaccharide-induced BV2 microglial cells. Food Chem 2016;213:69-75.
22. Dilshara MG, Lee KT, Jayasooriya RG, Kang CH, Park SR, Choi YH, *et al.* Down regulation of NO and PGE_2 in LPS stimulated BV2 microglial cells by trans-isoferulic acid via suppression of PI3K/Akt dependent NF kappa B and activation of Nrf2 mediated HO-1. Int Immunopharmacol 2014;18:203-11.
23. Guo C, Yang L, Wan CX, Xia YZ, Zhang C, Chen MH, *et al.* Anti-neuroinflammatory effect of Sophoraflavanone G from Sophora alopecuroides in LPS-activated BV2 microglia by MAPK, JAK/STAT and Nrf2/HO-1 signaling pathways. Phytomedicine 2016;23:1629-37.
24. Kwon YW, Cheon SY, Park SY, Song J, Lee JH. Tryptanthrin suppresses the activation of the LPS-treated BV2 microglial cell line via Nrf2/HO-1 antioxidant signaling. Front Cell Neurosci 2017;11:18.
25. Luo Q, Yan X, Bobrovskaya L, Ji M, Yuan H, Lou H, *et al.* Anti-neuroinflammatory effects of grossamide from hemp seed via suppression of TLR-4 mediated NF-κB signaling pathways in lipopolysaccharide stimulated BV2 microglia cells. Mol Cell Biochem 2017;428:129-37.