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ORIGINAL ARTICLE

Oroxylum indicum Kurz (L.) Seed Extract Exerts Antioxidant and Anti-Inflammatory Effects on Lipopolysaccharide-Stimulated BV2 Microglial Cells

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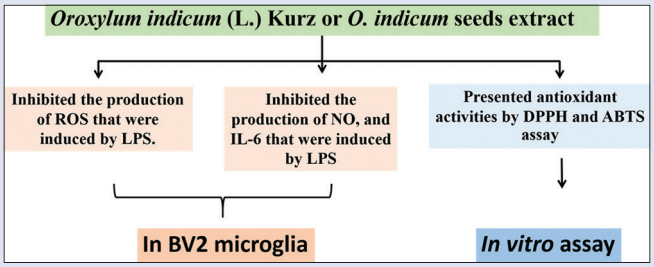
ABSTRACT

Background: *Oroxylum indicum* (L.) Kurz is a plant that has been extensively used as the traditional medicine in several Asian countries. However, the role of *Oroxylum indicum* seeds (OISs) in the antioxidant and anti-inflammatory functions of activated microglia have not yet been identified. **Objectives:** The present study aimed to investigate the anti-inflammatory and antioxidant role of OIS extract in a neuroinflammatory model of lipopolysaccharide (LPS)-stimulated microglia cells. **Materials and Methods:** BV2 microglial cells were treated with OIS extract in the presence or absence of LPS for 24 h. Subsequently, the levels of interleukin (IL)-6, nitric oxide (NO), and reactive oxygen species (ROS) were detected through enzyme-linked immunosorbent assay, Griess reagent, and the 2′,7′-dichlorofluorescein diacetate fluorescent probe, respectively. *In vitro* antioxidant capacity was assessed through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The total flavonoid and phenolic contents were also investigated. **Results:** OIS extract increased the levels of antioxidants detected by the DPPH and ABTS assays. The total flavonoid and phenolic content of OIS extract was 325.64 ± 4.95 and 50.47 ± 1.53 mg/g of dried extract, respectively. In addition, the levels of IL-6, NO, and ROS significantly decreased in LPS-induced BV2 microglia cells following treatment with OIS compared with the control. **Conclusion:** Taken together, the results of the present study demonstrated the antioxidant and anti-inflammatory properties of OIS in activated-BV2 cells. Thus, OIS extract may be used as a potential source of nutraceuticals for the development of health food supplements or as a novel anti-inflammatory herbal medicine.

Key words: Anti-inflammatory activity, antioxidant activity, BV2 microglia, neuroinflammation, *Oroxylum indicum* seeds

SUMMARY

- This study aimed to explore the antioxidant and anti-inflammatory activities of *Oroxylum indicum* seed (OIS) extracts in lipopolysaccharide (LPS)-stimulated BV2 microglia cells
- OIS extract dosedependently inhibited the production of reactive oxygen species that were induced by LPS
- OIS extract dose-dependently inhibited the production of nitric oxide and interleukin-6 that were induced by LPS
- OIS presented antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay.



Abbreviations used: ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); AD: Alzheimer's disease; ALS: Amyotrophic lateral sclerosis; DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's modified Eagle's medium; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FBS: Fetal bovine serum; LPS: Lipopolysaccharide; MS: Multiple sclerosis; MSU: Mahasarakham University; NFkB: Nuclear factor kappa B; NO: Nitric oxide; PD: Parkinson's disease; ROS: Reactive oxygen species; SD: Standard deviation.

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INTRODUCTION

Oroxylum indicum Vent. (*O. indicum*) is a plant commonly found in the tropical and subtropical countries, including Thailand, India, China, Japan, Malaysia, and Sri Lanka.[1] Major compounds that are present in the several parts of *O. indicum* include baicalein-7-O-diglucoside, baicalein-7-O-glucoside, apegenin, chrysin, oroxindin, prunetin, ellagic acid, sitosterol, biochanin-A, baicalein and its 6- and 7-glucuronides, tetuin, scutellarein, aloe-emodin, and anthraquinone.[2-5] Several parts of this plant, including the stem, seeds, fruits, roots, leaves, and bark, have been used to treat fever, diarrhea, cancer, jaundice, and ulcer in the Ayurveda and folk medicine.[1] Previous studies have demonstrated the anti-inflammatory, antioxidant, anticancer, antiarthritic, antiulcer, hepatoprotective, photocytotoxic, anti-proliferative, antimicrobial, antimutagenic, and immunostimulant properties of *O. indicum*, both *in vivo* and *in vitro*.[6-16] A previous study also reported that the extract from *O. indicum* fruit can decrease the apoptosis of neuronal SH-SY5Y cells following treatment with Aβ25-35.[17] Although *O. indicum* has been

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extensively applied as the herbal medicine in several countries, the effect of *O. indicum* seeds (OIS) on oxidative stress and neuroinflammation has not yet been investigated.

Oxidative stress and neuroinflammation are considered major pathological factors in brain aging and neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis.[18-21] Microglia are innate immune cells located in the central nervous system, which play an essential role in these processes. Chronic microglial activation participates in the inflammatory response by producing reactive oxygen species (ROS), releasing cytokines, such as tumor necrosis factor α (TNF-α), interleukin (IL)-1 β, and IL-6, as well as inflammatory mediators, such as nitric oxide (NO).[22-24] Overproduction of these agents endangers neuronal population. Thus, controlling microglial activation may be a therapeutic approach to the prevention and treatment of neuroinflammation and neurodegenerative diseases.

The present study aimed to investigate the beneficial effects of an ethanolic extract of OIS on the antioxidant and anti-inflammatory activities in lipopolysaccharide (LPS)-activated BV2 microglial cells.

MATERIALS AND METHODS

OIS extract

OIS extract were used as previously described.[17]

Cell culture and treatments

Murine BV2 microglial cells were kindly gifted by Dr James R Connor from the Department of Neurosurgery, The Pennsylvania State University, College of Medicine (Hershey, PA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin (all purchased from HyClone; Cytiva), at 37°C in a humidified incubator with 95% O_2 and 5% CO_2.

BV-2 cells were seeded into 96-well plates to determine cell viability. Once the cells reached 80% confluence, the growth medium was replaced with serum-free DMEM containing different concentrations of OIS extract (0–50 μg/ml) in the presence or absence of LPS.

To determine the levels of NO, IL-6, and ROS, the growth medium was replaced with medium containing LPS, in the presence or absence of specifiable concentrations of OIS extract. Cells in serum-free DMEM served as the untreated control.

Cells viability assay

Cells viability assay was detected by using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Following treatment with different concentrations of OIS extract (0-50 μg/ml) in the presence or absence of LPS for 24 h, the medium was replaced with 0.4 mg/ml MTT reagent (Sigma-Aldrich; Merck KGaA) in serum-free DMEM. Following incubation with MTT for 2 h at 37°C in a humidified incubator with 95% O_2 and 5% CO_2, the MTT reagent was removed and the purple formazan crystals were dissolved using dimethyl sulfoxide and cell viability was subsequently analyzed at a wavelength of 570 nm, using a plate reader (Spectramax 340 PC).

Nitric oxide assay

To determine nitrite accumulation, NO levels in the cell culture supernatants were detected using Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine dinydrochloride/2.5% phosphoric acid) (Invitrogen; Thermo Fisher Scientific, Inc.). Briefly, 150 μl samples were seeded into a 96-well plate, to which 20 μl Griess reagent and 130 μl deionized water were added. Following incubation for 30 min at the room temperature, absorbance values were detected at a wavelength of 540 nm, using a microplate reader.

Measurement of reactive oxygen species generation

The 2',7'-dichlorofluorescein diacetate (DCFH-DA) fluorescent probe is commonly used to detect intracellular ROS production. Briefly, the samples were cultured with 10 μM DCFH-DA for 20 min at 37°C with 5% CO_2. Subsequently, cells were treated with medium containing LPS, in the presence or absence of specifiable concentrations of OIS extract for 24 h. The fluorescence intensity was measured at 495 nm excitation and 525 nm emission filter.

Enzyme-linked immunosorbent assay

The levels of IL-6 in the culture supernatant were detected using enzyme-linked immunosorbent assay (ELISA) kits from R&D System (Minneapolis, MN) according to manufacturer's instruction. The supernatant was quantified at a wavelength of 450 nm, using a microplate reader. The level of IL-6 in each sample was calculated with reference to the standard curves.

2,2-diphenyl-1-picrylhydrazyl and 2,2-azinobis (3-ethylbenzothiazoline-6 -sulfonic acid) radical scavenging activity assays

Total free radical scavenging capacity of OIS was determined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, as previously described.[25,26]

Determination of total flavonoid and total phenol contents

Total flavonoid and total phenol contents of OIS were determined, as previously described.[17]

Statistical analysis

Data are presented as the mean ± the standard error of the mean. One-way ANOVA and Bonferroni *post hoc* test were used to compare the differences between multiple groups. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Effects of *Oroxylum indicum* seed extract on cell viability

The MTT assay was performed to assess the cytotoxicity of OIS extract on BV2 cells. The results demonstrated that OIS extract at 50 μg/ml was not toxic against BV2 cells [Figure 1]. Thus, 12.5, 25.0, and 50.0 μg/ml of OIS were selected for subsequent experimentation.

Oroxylum indicum seed extract decreases nitric oxide production in lipopolysaccharide-treated BV-2 microglia

To determine the effect of OIS on the production of NO, the accumulation of nitrite was measured in the culture medium of BV2 cells

through the Griess reagent assay. As presented in Figure 2, treatment with LPS significantly increased NO production in BV2 cells (17.22 fold) compared with the control group. However, LPS microglial activation significantly decreased NO production in a concentration-dependent manner [Figure 2].

Oroxylum indicum seed extract decreases reactive oxygen species production in lipopolysaccharide-treated BV-2 microglia

Intracellular ROS production was assessed to determine the antioxidant effect of OIS extract on microglia activation. As presented in Figure 3, treatment with LPS significantly increased ROS accumulation (2.22 fold) in BV-2 cells compared with untreated cells. Following treatment with OIS extract, ROS accumulation significantly decreased in LPS-activated BV-2 cells in a concentration-dependent manner [Figure 3].

Oroxylum indicum seed extract decreases IL-6 expression in lipopolysaccharide-treated BV-2 microglia

ELISA was performed to detect IL-6 levels to determine the anti-inflammatory properties of OIS extract in LPS-activated BV2 microglial cells. As presented in Figure 4, IL-6 expression significantly increased in LPS-induced BV2 cells (132.47 fold) compared with the control group. However, IL-6 level significantly decreased in cells treated with LPS and OIS extract in a concentration-dependent manner [Figure 4].

In vitro antioxidant activities of *Oroxylum indicum* seed extract

The antioxidative potential of OIS was assessed *in vitro* through the DPPH and ABTS assays [Table 1].

Total phenolic and flavonoid content of *Oroxylum indicum* seed extract

The values of phenolic content were 50.47 ± 1.53. In addition, the flavonoid content values were 325.64 ± 4.95 mg/g of dried extract [Table 2].

DISCUSSION

Oxidative stress and neuroinflammation mediated by microglial activation play key roles in neurodegenerative diseases.[27-29] Oxidative stress usually occurs due to an imbalance between ROS production

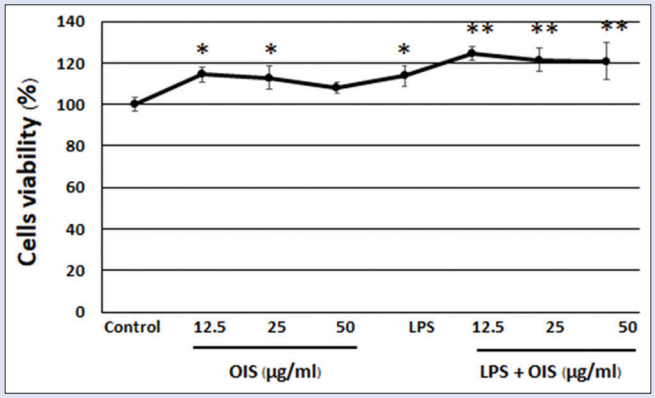

Figure 1: Effect of OIS on the viability of BV2 cells. BV2 cells were incubated with OIS extract for 24 h, in the presence or absence of 1 µg/ml LPS, and cell viability was determined via the MTT assay. Data are presented as the mean ± the standard error of the mean ($n = 3$). *$P < 0.05$, **$P < 0.01$ versus control group. OIS: *Oroxylum indicum* seed; LPS: Lipopolysaccharide

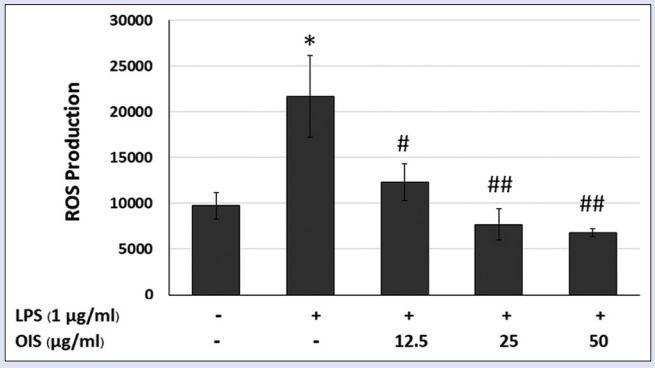

Figure 3: Effect of OIS on ROS production. BV2 cells were treated with 1 µg/ml LPS, in the presence or absence of OIS. Data are presented as the mean ± the standard error of the mean ($n = 3$). **$P < 0.01$ versus control group; ##$P < 0.01$ versus LPS-treated group. OIS: *Oroxylum indicum* seed; ROS: Reactive oxygen species; LPS: Lipopolysaccharide

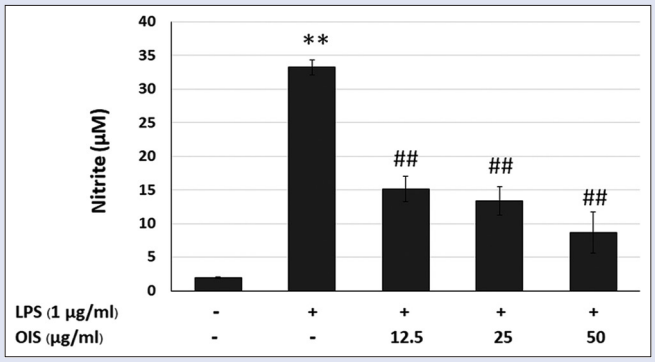

Figure 2: Effect of OIS on NO production. BV2 cells were incubated with OIS extract for 24 h, in the presence or absence of 1 µg/ml LPS, and NO production was assessed through the Griess reaction assay. Data are presented as the mean ± the standard error of the mean ($n = 3$). *$P < 0.05$ versus control group; #$P < 0.05$, ##$P < 0.01$ versus LPS-treated group. OIS: *Oroxylum indicum* seed; NO: Nitric oxide; LPS: Lipopolysaccharide

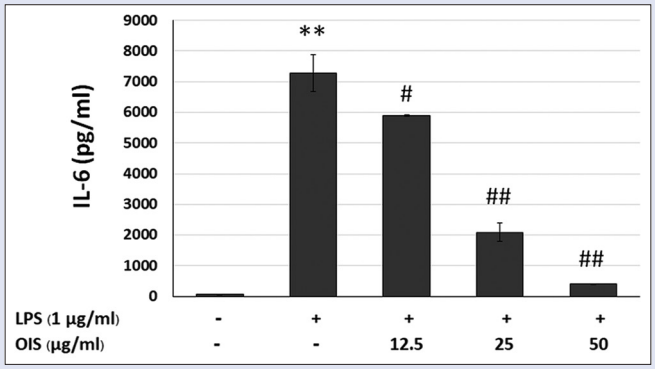

Figure 4: Effect of OIS on IL-6 production. BV2 cells were treated with 1 µg/l LPS for 24 h, in the presence or absence of OIS, and IL-6 production was assessed using enzyme-linked immunosorbent assay kits. Data are presented as the mean ± the standard error of the mean ($n = 3$). **$P < 0.01$ versus control group; #$P < 0.05$, ##$P < 0.01$ versus LPS-treated group. OIS: *Oroxylum indicum* seed; IL: Interleukin; LPS: Lipopolysaccharide

Table 1: *In vitro* antioxidant activities of *oroxylum indicum* seeds extract

Assay	Results
DPPH assay (mg Trolox equivalent/g extract)	174.526±0.48
ABTS assay (mg Trolox equivalent/g extract)	103.805±0.57

DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

Table 2: Total phenolic and flavonoid content of *oroxylum indicum* seeds *extract*

Assay	Results
Total phenolic content[a]	50.47±1.53
Total flavonoid content[b]	325.64±4.95

[a]Mg gallic acid equivalent/g dry weight; [b]Mg rutin equivalent/g dry weight. Values are presented as the mean of three biological replicates

and availability of antioxidants. Excessive ROS production causes oxidative damage to biological molecules, such as lipids, proteins, and DNA. ROS accumulation also plays a pivotal role in the progression of neuroinflammation by regulating transcriptional factors, including nuclear factor kappa B, which increases the expression of pro-inflammatory genes. The results of the present study demonstrated that OIS extract exhibited antioxidant activity *in vitro* through the DPPH and ABPS assays. Taken together, these results suggest that OIS extract suppressed ROS production in activated BV2 microglia, which is consistent with a previous report.[30]

In the central nervous system, NO is an inflammatory mediator derived by activated microglia. High concentrations of NO in the brain are associated with the onset of neurodegenerative diseases, such as multiple sclerosis, AD, and PD.[31] In addition, NO overproduction rapidly reacts with superoxide anion to form the highly toxic peroxynitrite anion, which can directly react with several biological molecules.[32] The results of the present study demonstrated that LPS significantly increased NO production in BV2 cells, the effects of which were reversed following treatment with OIS. These results are consistent with previous findings, which suggest that OIS suppresses NO production in LPS-activated RAW264.7 macrophages.[30]

To confirm the anti-inflammatory effect of OIS extract in LPS-activated BV-2 microglia, the effect of OIS on IL-6 production was investigated, as high levels of this pro-inflammatory cytokine is considered a hallmark of neuroinflammatory diseases.[33] The induction of LPS caused inflammation in BV-2 microglia by releasing IL-6; however, treatment with OIS significantly decreased IL-6 expression in LPS-activated cells. These results are consistent with previous findings, which suggest that treatment with OIS decreases IL-6 expression in LPS-activated RAW264.7 macrophages.[30] Taken together, the results of the present study suggest that OIS exerts anti-inflammatory effects by inhibiting NO and IL-6 production of microglia cells.

It has been reported that OIS comprises various flavonoid compounds, such as apigenin, baicalein, chrysin, luteolin, and oroxylin A, which exert anti-inflammatory effects by modulating ROS generation and suppressing the release of proinflammatory cytokines, including IL-6, TNF-α, and IL-1 β.[34] Consistent with previous findings, the results of the present study demonstrated that total flavonoid and phenolic contents of OIS were 325.64 ± 4.95 and 50.47 ± 1.53 mg/g of dried extract, respectively.[34] These results suggest that flavonoid-enriched OIS may have potent antioxidant and anti-inflammatory properties.

CONCLUSION

The results of the present study demonstrated that OIS exerted antioxidative and anti-inflammatory effects on LPS-stimulated BV2 microglial cells by decreasing intracellular ROS, NO, and IL-6 levels. Thus, OIS extract may be used to develop food supplements to allay neuroinflammation.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Harminder VS, Chaudhary AK. A review on the taxonomy, ethnobotany, chemistry and pharmacology of *Oroxylum indicum* vent. Indian J Pharm Sci 2011;73:483-90.
2. Chen LJ, Games DE, Jones J. Isolation and identification of four flavonoid constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography. J Chromatogr A 2003;988:95-105.
3. Chen LJ, Song H, Lan XQ, Games DE, Sutherland IA. Comparison of high-speed counter-current chromatography instruments for the separation of the extracts of the seeds of *Oroxylum indicum*. J Chromatogr A 2005;1063:241-5.
4. Tomimori T, Imoto Y, Ishida M, Kizu H, Namba T. Studies on the Nepalese crude drug. VIII. On the flavonoid constituents of the seed of *Oroxylum indicum*. Shoyakugaku Zasshi 1988;42:98-101.
5. Bhattacharje AK, Das AK. Phytochemical screening of some Indian plants. QJ Crude Drug Res 1969;9:1408.
6. Kandaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT, *et al.* The antitumor activities of flavonoids. *In Vivo* 2005;19:895-909.
7. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Med Res Rev 2003;23:519-34.
8. Kao YC, Zhou C, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site-directed mutagenesis study. Environ Health Perspect 1998;106:85-92.
9. Hecker M, Preiss C, Klemm P, Busse R. Inhibition by antioxidants of nitric oxide synthase expression in murine macrophages: Role of nuclear factor kappa B and interferon regulatory factor 1. Br J Pharmacol 1996;118:2178-84.
10. Fishkin RJ, Winslow JT. Endotoxin-induced reduction of social investigation by mice: Interaction with amphetamine and anti-inflammatory drugs. Psychopharmacology (Berl) 1997;132:335-41.
11. Upaganlawar AB, Tende CR, Yeole PG. Antiinflammatory activity of aqueous extract of *Oroxylum indicum* vent. leaves extract-preliminary study. Pharmacology online 2009;1:22-6.
12. Roy MK, Nakahara K, Na TV, Trakoontivakorn G, Takenaka M, Isobe S, *et al.* Baicalein, a flavonoid extracted from a methanolic extract of *Oroxylum indicum* inhibits proliferation of a cancer cell line *in vitro* via induction of apoptosis. Pharmazie 2007;62:149-53.
13. Zaveri M, Gohil P, Jain S. Immunostimulant activity of n-butanol fraction of root bark of *Oroxylum indicum*, vent. J Immunotoxicol 2006;3:83-99.
14. Nakahara K, Onishi-Kameyama M, Ono H, Yoshida M, Trakoontivakorn G. Antimutagenic activity against trp-P-1 of the edible Thai plant, *Oroxylum indicum* vent. Biosci Biotechnol Biochem 2001;65:2358-60.
15. Laupattarakasem P, Houghton PJ, Hoult JR, Itharat A. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. J Ethnopharmacol 2003;85:207-15.
16. Ong CY, Ling SK, Ali RM, Chee CF, Samah ZA, Ho AS, *et al.* Systematic analysis of *in vitro* photo-cytotoxic activity in extracts from terrestrial plants in Peninsula Malaysia for

photodynamic therapy. J Photochem Photobiol B 2009;96:216-22.

17. Mairuae N, Connor JR, Buranrat B, Lee SY. *Oroxylum indicum* (L.) extract protects human neuroblastoma SHSY5Y cells against βamyloidinduced cell injury. Mol Med Rep 2019;20:1933-42.
18. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, *et al.* Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383-421.
19. Hailer NP. Immunosuppression after traumatic or ischemic CNS damage: It is neuroprotective and illuminates the role of microglial cells. Prog Neurobiol 2008;84:211-33.
20. Kim YS, Joh TH. Microglia, major player in the brain inflammation: Their roles in the pathogenesis of Parkinson's disease. Exp Mol Med 2006;38:333-47.
21. 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.
22. Boje KM, Arora PK. Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain Res 1992;587:250-6.
23. Chao CC, Hu S, Peterson PK. Glia, cytokines and neurotoxicity. Crit Rev Neurobiol 1995;9:189-205.
24. Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A. Neuroinflammation in Alzheimer's disease and Parkinson's disease: Are microglia pathogenic in either disorder? Int Rev Neurobiol 2007;82:235-46.
25. Yang H, Dong Y, Du H, Shi H, Peng Y, Li X. Antioxidant compounds from propolis collected in Anhui, China. Molecules 2011;16:3444-55.
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
27. Rubio-Perez JM, Morillas-Ruiz JM. A review: Inflammatory process in Alzheimer's disease, role of cytokines. ScientificWorldJournal 2012;2012:1-15.
28. Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of chronic oxidative stress on neuroinflammatory response mediated by CD4(+) T Cells in neurodegenerative diseases. Front Cell Neurosci 2018;12:114.
29. Gao HM, Zhou H, Hong JS. Oxidative stress, neuroinflammation and neurodegeneration neuroinflammation and neurodegeneration. Springer 2014;81:104.
30. Benjawan D, Talabnin C, Murphy M, Kanjana T, Patcharawan S, Tanaporn H, *et al.* Intracellular ROS scavenging and anti-inflammatory activities of *Oroxylum indicum* Kurz (L.) Extract in LPS plus IFN-γ-Activated RAW264.7 Macrophages. Evid Based Complement Alternat Med 2020;20:1-5.
31. Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, *et al.* Role of microglia in central nervous system infections. Clin Microbiol Rev 2004;17:942-64.
32. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 2007;15:252-9.
33. Wu YY, Hsu JL, Wang HC, Wu SJ, Hong CJ, Cheng IH. Alterations of the neuroinflammatory markers IL-6 and TRAIL in Alzheimer's disease. Dement Geriatr Cogn Dis Extra 2015;5:424-34.
34. Ribeiro D, Freitas M, Tomé SM, Silva AM, Porto G, Fernandes E. Modulation of human neutrophils' oxidative burst by flavonoids. Eur J Med Chem 2013;67:280-92.