The aqueous extract from Toona sinensis leaves inhibits microglia-mediated neuroinflammation

Chao-Chuan Wang, Yee-Jean Tsai, Ya-Ching Hsieh, Rong-Jyh Lin, Chih-Lung Lin

Department of Anatomy, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
Department of Medical Research, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan
Department of Parasitology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
Department of Neurosurgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
Faculty of Medicine, Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Received 5 February 2013; accepted 30 August 2013
Available online 1 November 2013

KEYWORDS
Lipopolysaccharide; Microglia; Neuroinflammation; Toona sinensis; Tumor necrosis factor-α

Abstract The leaves of Toona sinensis, a well-known traditional oriental medicine, have been prescribed for the treatment of enteritis and infection. Recently, aqueous extracts of Toona sinensis leaves (TSL-1) have demonstrated many biological effects both in vitro and in vivo. In the central nervous system, microglial activation and their proinflammatory responses are considered an important therapeutic strategy for neuroinflammatory disorders such as cerebral ischemia, Alzheimer’s disease, and Parkinson’s disease. The present study attempted to validate the effect of TSL-1 on microglia-mediated neuroinflammation stimulated by lipopolysaccharide (LPS). As inflammatory parameters, the production of nitric oxide (NO), inducible NO synthase, and tumor necrosis factor-α were evaluated. Our results demonstrate that TSL-1 suppresses LPS-induced NO production, tumor necrosis factor-α secretion, and inducible NO synthase protein expression in a concentration-dependent manner, without causing cytotoxicity. In addition, the inhibitory effects of TSL-1 in LPS-stimulated BV-2 microglia were extended to post-treatment suggesting the therapeutic potential of TSL-1. Therefore, this work provides the future evaluation of the role of TSL-1 in the treatment of neuroinflammation.

Conflict of Statement: The authors have no conflicts of interest relevant to this article.

Corresponding author. Department of Neurosurgery, Kaohsiung Medical University Hospital, 100 Tz-you 1st Road, Kaohsiung City, Taiwan.
E-mail address: chihlung1@yahoo.com (C.-L. Lin).

1607-551X/$36 Copyright © 2013, Kaohsiung Medical University. Published by Elsevier Taiwan LLC. All rights reserved.
http://dx.doi.org/10.1016/j.kjms.2013.09.012
Introduction

Inflammation is known to play a key role in the progressive damage process in a number of neurodegenerative disorders including Alzheimer’s disease (AD) [1,2], Parkinson’s disease (PD) [3], multiple sclerosis [4,5], and stroke [6,7]. Microglia act as the major immune cells in the central nervous system. In response to brain injury or during neurodegenerative processes, microglia are activated by secreting growth factors, proinflammatory cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and nitric oxide (NO), and reactive oxygen species [3,8–11]. Although microglial activation is necessary and important for host defense, over-activation of microglia is neurotoxic. Studies have shown that microglia activated after ischemic stroke will produce cytokines to trigger neuronal death in response to ischemic injury [6,12]. They also found that the inhibition of inflammation would prevent the progressive brain loss following a stroke. Thus, to develop the agents that reduce microglial activation and their proinflammatory responses is considered an important therapeutic strategy for neuroinflammatory disorders such as cerebral ischemia, AD, and PD.

*Toona sinensis* (TS), a well-known Chinese herb, is widely available in Asia. All parts of TS, including its root, bark, petioles, leaves, fruits, and seeds, have been used for medicinal purposes [13–15]. The leaves of TS are a popular vegetable amongst vegetarians in Taiwan. It also serves as an ingredient in some Chinese and Malaysian recipes. In the field of traditional Chinese medicine, the leaves of TS have been used for treating enteritis, dysentery, diabetes, infection, and itch, with no irreversible side effects observed after treatment [16]. Recent studies have also revealed that the aqueous extracts of TS leaves (TSL-1) have a variety of biological functions, including: (1) antioxidant activities [17,18]; (2) the lowering of blood sugar levels via mediating adipose glucose transporter [19,20]; (3) the alleviation of liver fibrosis via reducing tumor growth factor-\(\beta\)1 and collagen [21]; (4) the inhibition of coronavirus replication in severe acute respiratory syndrome [22]; (5) the decrease of steroidogenesis in mouse Leydig cells [23]; and (6) the inhibition of vascular endothelial growth factor (VEGF)-induced angiogenesis in vascular endothelial cells [24]. In addition, TSL-1 has anti-proliferative properties in human lung cancer cells [25–28], oral squamous carcinoma cells [29], and human pre-myelocytic leukemia cells [30] *in vitro*. Furthermore, there was no acute lethal effect even at a maximal oral tested dose of 5000 mg/kg of body weight in mice [31,32]. Interestingly, one of these findings demonstrated that daily dietary supplement of TSL-1 in senescence-accelerated mice (an AD model) improved brain degeneration caused by the incidence of \(\beta\) amyloid plaques [17]. This points to the possibility that TSL-1 may pass through the blood—brain barrier to affect the central nervous system. Recent studies have also found that the appearance of amyloid plaques in the brain coincides with a dramatic phenotypic activation of the surrounding microglia, which release proinflammatory cytokines and neurotoxic substances, for disease progression [33,34]. Liao et al.’s findings [17] indicated that TSL-1 supplement in aged mice had a potential effect on neuroinflammation due to the recruitment of activated microglia in amyloid plaques. Therefore, this study aimed to examine whether TSL-1 would modulated neuroinflammation-associated diseases such as AD through microglia. At the same time, because the systemic administration of lipopolysaccharide (LPS, a heat-stable bacterial cell wall component) in mouse brain, causing amyloid protein accumulation and neuroinflammation, was being used to study the underlying mechanisms of AD [35–37], we tested the potential anti-inflammation effect of TSL-1 in the *in vitro* model of LPS-induced microglial activation system.

Materials and methods

Plant materials and preparation of TSL-1

TS leaves were obtained in Tuku (Yunlin County, Taiwan). The leaves were picked and washed with water as described by Chang et al. in 2002 [25]. Reverse osmosis water was added to TS leaves at a proportion of 4 L of reverse osmosis water to 1 kg of leaves. The mixture was boiled for 30 minutes, after which the leaves were removed and the remaining liquid concentrated over low heat and filtered with a sieve (70 meshes). The filtered concentrate was lyophilized with a Virtis apparatus (Gardiner, NY, USA) to obtain a crude extract. The crude extracts were centrifuged at 1400 g for 12 minutes, and the supernatant-labeled TSL-1 (an advanced bioactive fraction of TS) was used for this study. The extracts were then concentrated in a vacuum, freeze-dried to form a powder, and stored at \(-20^\circ\)C for subsequent analysis. Various doses of TSL-1, from 5 \(\mu\)g/mL to 50 \(\mu\)g/mL, were used in this study.

**BV-2 microglial cell culture***

A murine cell line (BV-2) was generated by infecting primary microglial cell cultures with a v-raf/v-myec oncogene carrying retrovirus (J2), with most of the morphological, phenotypical, and functional properties described for freshly isolated microglial cells retained [38]. In the present study, BV-2 microglial cell line was a gift from Professor Hong, Jau-Shyong (Research Triangle Park, NIEH; NIH, Bethesda, MA, USA). Cells (\(1 \times 10^5\) cells/mL) were cultured

neurodegenerative diseases by inhibition of inflammatory mediator production in activated microglia.

Copyright © 2013, Kaohsiung Medical University. Published by Elsevier Taiwan LLC. All rights reserved.
in Dulbecco’s modification of Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, ThermoFisher Scientific, New Zealand), 100 U/mL penicillin-streptomycin (Gibco), and 4 mM L-glutamine (Gibco) and were maintained in a 5% carbon dioxide incubator. In all experiments except the post-treatment scheme, cells were treated with TSL-1 (5 µg/mL, 10 µg/mL, or 50 µg/mL) 30 minutes prior to the addition of lipopolysaccharide (LPS, 1 µg/mL, *Escherichia coli*, Serotype 055:B5; Sigma-Aldrich, St Louis, MO, USA) in DMEM with 2% FBS. Passages 3–8 of the BV-2 cell lines were used in this study.

**Cell viability assay**

BV-2 microglial cells (1 × 10⁵ cells/mL, 24-well plate) were allowed to adhere and grow overnight. Cells were then incubated in 2% FBS-containing medium with different concentrations of TSL-1 for 6 hours and 24 hours. After incubation, 2 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) reagent replaced the medium and was incubated in a 5% carbon dioxide incubator at 37°C for an additional 4 hours. The BV-2 cells were then harvested in 50 µL dimethyl sulfoxide and transferred to 96-well plates. The absorbance was measured at 540 nm using a microplate reader (Beckman Coulter Inc., Brea, CA, USA).

**Measurement of TNF-α production by enzyme-linked immunosorbent assay**

BV-2 microglial cells were pretreated with medium or TSL-1 (50 µg/mL) for 30 minutes prior to incubation with LPS (1 µg/mL) in 24-well plate. At different time points (6 hours and 24 hours after treatment with LPS), the supernatants were collected for TNF-α measurements by enzyme-linked immunosorbent assay kits (Endogen mouse/rat TNF-α ELISA kit; Thermo Fisher Scientific, Rockford, IL, USA). Each sample was tested in duplicate.

**NO analysis**

NO was evaluated by measuring the amount of nitrite in the cell culture supernatant, using Griess reagent (0.1% naphthylethylenediamine, 1% sulfanilamide in 2.5% H₃PO₄). BV-2 microglial cells were grown on 24-well plates and pretreated with medium or TSL-1 (5 µg/mL, 10 µg/mL, or 50 µg/mL) for 30 minutes prior to incubation with LPS (1 µg/mL). For the post-treatment study, TSL-1 (50 µg/mL) was added during, or 1 hour, 2 hours, 4 hours, or 6 hours after, LPS (1 µg/mL) treatment in BV-2 microglial cell cultures. The supernatant were collected 24 hours after LPS treatment. The production of NO was determined basing on the Griess reaction. In short, 50 µL of culture supernatant was allowed to react with an equal volume of Griess reagent in 96-well plates for 10 minutes at room temperature in the dark. The absorbance at 550 nm was determined using a microplate reader (MRX ELISA reader; Dynex, Chantilly, VA, USA). A standard nitrite curve was generated in the same fashion using NaNO₂.

**Western blot analysis**

BV-2 microglial cells were grown on 6-well plates and pre-treated with medium or TSL-1 (5 µg/mL, 10 µg/mL, or 50 µg/mL) for 30 minutes prior to incubation with LPS (1 µg/mL). For the post-treatment study, TSL-1 (50 µg/mL) was added during, or 1 hour, 2 hours, 4 hours, or 6 hours after, LPS (1 µg/mL) treatment in BV-2 microglial cell cultures. After treatment, cell lysates were washed twice with phosphate-buffered saline and harvested in Laemmli sodium dodecyl sulfate sample buffer. The protein concentration in the supernatant was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). Equal amounts of whole cell lysates were separated in 10% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membranes were first incubated with 5% nonfat milk in PBS for 1 hour at room temperature to reduce nonspecific binding. The membranes were washed with PBS containing 0.1% Tween-20, and then incubated for 1 hour at room temperature with the indicated antibodies including inducible NO synthase (iNOS; 1:1000; BD Biosciences, Franklin Lakes, NJ, USA), HO-1 (1:10,000; Santa Cruz Biotechnologies, Santa Cruz, CA, USA), and β-actin (1:20,000, Sigma-Aldrich). This was followed by the addition of horseradish peroxidase-conjugated secondary antibody. After the final wash, membranes were probed using enhanced chemiluminescence (Amersham Pharmacia Biotech) and autoradiographed. The optical density of the bands (integrated area, arbitrary units) was measured by an Imaging Densitometer (Bio-1D V.97; Vilber Lourmat, Torcy, France).

**Statistical analysis**

Data are expressed as mean ± standard deviation for the separate experiments. The differences among treatment groups were determined by analysis of variance (ANOVA) with post hoc compared Dunnett’s test with p < 0.05 as the criterion of significance. The statistical analysis was computed by SAS 9.20 (SAS Institute, Inc., Chicago, IL, USA).

**Results**

**Effect of TSL-1 on the cell viability of BV-2 microglial cells**

To test whether TSL-1 treatment would affect the cell viability of BV-2 microglial cells, cells were incubated in different concentrations of TSL-1 for 6 hours and 24 hours. In the normal condition without TSL-1 treatment, there was an increase in BV-2 microglial cell viability at 24 hours’ incubation when compared to 6 hours of incubation (Fig. 1). Within our tested concentration range of TSL-1 (5–50 µg/mL), the cell viability was comparable to those corresponding controls at 6 hours’ and 24 hours’ incubation (Fig. 1). At the same time, there was no cell death found (data not shown). The TSL-1 treatment alone did not change the cell viability of BV-2 microglial cells.
Effect of TSL-1 on BV-2 microglial cell morphology after LPS stimulation

Traditionally, the microglial cells were classified into two primary phenotypic states in vivo: “quiescent” or “activated”. The transformation of “quiescent” microglia with ramified morphology to the “activated” phenotype with round or amoeboid shape was associated with inflammation and disease [39]. LPS, as a potent activator of microglia, will stimulate microglia to become activated and to undergo a series of morphologic and phenotypic changes [40].

In this study, BV-2 microglial cell morphology was observed by phase-contrast microscope. In the treatment of LPS alone for 24 hours, BV-2 cell morphology transformed from a predominantly rod cell morphology to a round or oval shape (Fig. 2). Clustering of BV-2 cells was usually observed in the LPS-alone plates. There were no compatible morphologic changes between the control and TSL-1 alone plates. In the group of pretreatment with TSL-1 (50 μg/mL) for 30 minutes then incubated with LPS for an additional 24 hours, although some cell debris was found, the BV-2 cell morphology showed the same shapes as the controls and TSL-1 alone ones.

TSL-1 inhibited LPS-induced TNF-α production in BV-2 microglial cells

As demonstrated in Fig. 3, treatment of BV-2 cells with LPS (1 μg/mL) caused a substantial increase in the production of TNF-α, dependent on time. Pretreatment with TSL-1 (50 μg/mL) prior to incubation with LPS resulted in a significant inhibition of the LPS-induced TNF-α production in both the 6 hours and 24 hours treatment groups (p < 0.001

Figure 1. Effect of aqueous extract of Toona sinensis leaves (TSL-1) on cell viability of BV-2 microglial cells. BV-2 microglial cells were incubated with various concentrations of TSL-1 for 6 hours and 24 hours. MTT assay was performed to detect viability of the cells and the results were expressed as the absorbance at 570 nm. Six independent experiments were performed at each time and dose points. Data are expressed as mean ± standard deviation.

Figure 2. Effect of aqueous extract of Toona sinensis leaves (TSL-1) on BV-2 microglial cell morphology. BV-2 microglial cells were pretreated with medium or TSL-1 (50 μg/mL) for 30 minutes prior to stimulation with lipopolysaccharide (LPS; 1 μg/mL) for an additional 24 hours. The photomicrographs were taken directly from culture plates by converted light microscopy with phase contrast. Some cell fragments (arrows) were found in the group pretreated with TSL-1 for 30 minutes then incubated with LPS for an additional 24 hours (TSL-1 + LPS). Control group: cell incubated in the 2% FBS medium for 24 hours. TSL-1 group: cells incubated in 2% FBS medium including 50 μg/mL of TSL-1 for 24 hours. Three independent experiments were performed. Scale bar = 20 μm.
for both). There was no effect on the TNF-α production in BV-2 cells treated with TSL-1 alone for 6 hours and 24 hours.

**Pretreatment with TSL-1 reduced the NO production and iNOS protein expression in LPS-stimulated BV-2 microglial cells**

Previous study has demonstrated that NO would be released from microglia following exposure to LPS [41]. In this series of experiments (Fig. 4A), treatment of BV-2 microglial cells with LPS (1 μg/mL) for 24 hours caused a robust increment of NO level (11.8 ± 2.44 μM). Pretreatment with TSL-1 prior to incubation with LPS resulted in a concentration-dependent inhibition of the LPS-induced NO production in BV-2 cells (TSL-1 5 μg/mL + LPS: 7.7 ± 2.33, TSL-1 10 μg/mL + LPS: 7.5 ± 1.96, TSL-1 50 μg/mL + LPS: 3.8 ± 1.5, n = 5). Moreover, the LPS treatment markedly increased the protein level of iNOS in BV-2 microglial cell culture as with as little as 6 hours’ incubation. This induction was drastically inhibited by pretreatment with TSL-1 in a time- and concentration-dependent manner (Fig. 4B,C).

**Post-treatment effect of TSL-1 on NO production and iNOS protein expression in LPS-stimulated BV-2 microglial cells**

In addition to pretreatment, we also evaluated the effects of post-treatment with TSL-1 on the LPS-induced microglial activation. TSL-1 (50 μg/mL) was added during, or 1 hour, 2 hours, 4 hours, or 6 hours after LPS (1 μg/mL) treatment in BV-2 cells. Supernatant and cell lysates were collected after 24 hours of LPS incubation for the detection of NO production. As shown in Fig. 5A, LPS treatment significantly increased NO release from BV-2 microglial cells. Post-treatment with TSL-1 up to 6 hours after LPS treatment attenuated LPS-induced release of NO in BV-2 microglial cells by 50% to 80%. A similar pattern was observed in the group of post-treatment with TSL-1 on the LPS-induced iNOS (Fig. 5B) production. When the iNOS production at 24 hours after LPS treatment was determined, the addition of TSL-1 at 0 hours and 1 hour after LPS treatment still exhibited an inhibitory effect on LPS-induced iNOS production (0 hours: 47.5%; 1 hour: 50.18% of LPS alone). However, the addition of TSL-1 at 2 hours after LPS treatment showed no obvious inhibitory effect on iNOS production.

**Discussion**

This is the first report to demonstrate that TSL-1 markedly inhibited LPS-induced inflammatory responses in the murine microglial BV-2 cell line. NO production and iNOS expression were significantly inhibited by TSL-1 in a concentration-dependent manner in the microglial BV-2 cell line. This anti-inflammatory effect of TS was also evidenced by inhibiting TNF-α release. Moreover, the cell viability assay showed that treatment with TSL-1 alone did not have cytotoxic effects at concentrations of 5–50 μg/mL, whereas TSL-1 significantly inhibited those inflammatory factors stimulated by LPS. In this connection, we suggest that TSL-1 might have a potent antineuroinflammatory activity via the inhibition of LPS-stimulated production of TNF-α, NO, as well as iNOS protein in microglia.

In the present study, we also used microglia BV-2 cell to evaluate the potential therapeutic effect of TSL-1 after LPS treatment. The results indicated that even post-treatment with TSL-1 (50 μg/mL), later than LPS application, is effective in the reduction of NO release and iNOS protein level. Accumulating evidence indicates that iNOS is the most important contributor to NO production in the brain after inflammatory assault, compared with other isoforms of NOS, namely eNOS and nNOS [42,43]. Furthermore, a novel approach with NO-donating nonsteroidal anti-inflammatory drugs develops a safe profile that strongly reduces their untoward side effects without altering the anti-inflammatory effectiveness [44–47]. These findings
correlate the important role of NO in the function of the central nervous system. In the central nervous system, activated microglia are the major cellular source of iNOS. Therefore, the inhibitory effect that TSL-1 exerts on iNOS might be beneficial not only in the protection of neurons but also as therapy through microglia.

The neuroprotective potential of TSL-1 might be due to the biological activities of compounds in the leaf extract. More than eight compounds, including gallic acid, methyl gallate, ethyl gallate, kaempferol, kaempferol-3-O-b-D-glucoside, quercetin, quercitrin, quercetin-3-O-b-D-glucoside, and rutin, have been isolated from TSL-1, as previously described [30,48]. Of these compounds, gallic acid isolated directly from TSL-1 has been demonstrated to possess effective antioxidant activity against various oxidative stress such as leukemia, atherogenesis, prostate cancer, oral carcinoma, and angiogenesis in the liver, kidney, and testis [18,24,29,30,49,50]. Quercetin purified from TSL-1 specifically elevates the activities of antioxidant...
enzymes only in the testis [50]. In the central nervous system, gallic acid and quercetin have also been found to reduce neuronal damage by inhibiting microglia-mediated NO release, TNF-α production, and oxidative stress [51–55]. Therefore, it is reasonable to infer the potential therapeutic effect of TSL-1 or its compounds, such as gallic acid or quercetin, on the suppression of inflammatory-related neuronal injury and oxidative stress in neurodegenerative diseases.

The application of traditional Chinese herbs for medicinal use has attracted attention in recent years. Increasing evidence has suggested that Chinese herbs have therapeutic effects on neurodegenerative diseases such as PD and AD through their anti-inflammatory features [56–58]. These traditional Chinese medicines, such as the extracts of Tripterygium wilfordii Hook. f. and Anemarrhena asphodeloides Bunge, or the more recently studied grape seed extract, have been shown to promote neuronal survival and neurite growth, to facilitate functional recovery after brain injury, and to act as inhibitors of neuroinflammatory toxicity of activated-microglia. However, it has been proposed that an exacerbated inflammatory response was responsible for causing the impairment in the phagocytosis of amyloid protein deposits by microglia in the AD brain. In this connection, the discovery of agents that are capable of increasing amyloid protein uptake by phagocytic cells is of potential therapeutic interest for AD. In fact, with amyloid phagocytosis in microglia while inhibiting intracellular ion channel 1 will stimulate amyloid β phagocytosis in microglia while inhibiting iNOS induction and TNF-α production [59,60]. In the present study, pretreatment with TSL-1 has proved protective against LPS-induced microglial activation. Treatment with TSL-1 at 0 hours and 1 hour post-LPS treatment also exhibited similar degrees of antineuroinflammatory effect comparable to that observed with pretreatment. Therefore, it is likely that the attenuation of LPS-stimulated NO release and NO production are at least partially responsible for neuroinflammation of TSL-1. However, the potential effect of TSL-1 in modifying microglial activation with enhancing amyloid β clearance needs to be addressed.

In conclusion, our results indicate that TSL-1 possesses effective anti-inflammatory features, including the suppression of LPS-induced NO production, as well as the synthesis of TNF-α and iNOS protein in BV-2 microglial cells. Because therapeutic agents from herbal sources are usually perceived as being natural and devoid of side effects. It is reasonable to consider TSL-1 as another potential therapeutic agent that works by inhibiting the inflammatory response of microglia in neurodegenerative diseases. To confirm its effect, further studies in in vivo animal models are necessary.

Acknowledgments

This study was supported by a grant (NSC 93-2320-B-037-031) from the National Science Council, Taiwan. The authors wish to thank Dr. Hung-Pin Tu (Department of Public Health and Environmental Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan) for participating in the data analysis.

References

[1] von Bernhardi R, Ramirez G. Microglia-astrocyte interaction in Alzheimer’s disease: friends or foes for the nervous system? Biol Res 2001;34:123–8.
[2] von Bernhardi R. Glial cell dysregulation: a new perspective on Alzheimer disease. Neurotox Res 2007;12:215–32.
[3] Lee JK, Tran T, Tansey MG. Neuroinflammation in Parkinson’s disease. J Neuroimmune Pharmacol 2009;4:419–29.
[4] Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med (Berl) 1997;75:165–73.
[5] Gao Z, Tsirka SE. Animal models of MS reveal multiple roles of microglia in disease pathogenesis. Neurol Res Intern 2011;2011:383087.
[6] Wang Q, Tang XY, Yenari MA. The inflammatory response in stroke. J Neuroimmunol 2007;184:53–68.
[7] Park JS, Shin JA, Jung JS, Hyun JW, Le TK, Kim DH, et al. Anti-inflammatory mechanism of compound K in activated microglia and its neuroprotective effect on experimental stroke in mice. J Pharmacol Exp Ther 2012;341:59–67.
[8] Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 2005;76:77–98.
[9] González-Scarano F, Baltuch G. Microglia as mediators of inflammatory and degenerative diseases. Annu Rev Neurosci 1999;22:219–40.
[10] Muñoz-Fernández MA, Fresno M. The role of tumour necrosis factor, interleukin 6, interferon-gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. Prog Neurobiol 1998;56:307–40.
[11] Stolp HB, Dziegielewsk KM. Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. Neuropathol Appl Neurobiol 2009;35:132–46.
[12] Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 2007;27:2596–605.
[13] Cho EJ, Yokozawa T, Ryu DY, Kim HY, Shibahara N, Park JC. The inhibitory effects of 12 medicinal plants and their component compounds on lipid peroxidation. Am J Chin Med 2003;31:907–17.
[14] Cho EJ, Yokozawa T, Ryu DY, Kim SC, Shibahara N, Park JC. Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical. Phytomedicine 2003;10:544–51.
[15] Luo XD, Wu SH, Ma YB, Wu DG. Limonoids and phytol derivatives from Cedrela sinensis. Fitoterapia 2000;71:492–6.
[16] Edmonds JM, Staniforth M. Toona sinensis (Meliaceae). Curtis’ Bot Mag 1998;15:186–93.
[17] Liu JW, Hsu CK, Wang MF, Hsu WM, Chan YC. Beneficial effect of Toona sinensis Roemor on improving cognitive performance and brain degeneration in senescence-accelerated mice. Br J Nutr 2006;96:400–7.
[18] Hseu YC, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, et al. Antioxidant activities of Toona sinensis leaves extracts using different antioxidant models. Food Chem Toxicol 2008;46:105–14.
[19] Wang PH, Tsai MJ, Hsu CY, Wang CY, Hsu HK, Weng CF. Toona sinensis Roem (Meliaceae) leaf extract alleviates hyperglycemia via altering adipose glucose transporter 4. Food Chem Toxicol 2008;46:2554–60.
[20] Zhang JF, Yang JY, Wen J, Wang DY, Yang M, Liu QQ. Experimental studies on hypoglycemic effects of total flavonoid from Toona sinensis. Zhong Yao Cai 2008;31:1712–4 [Article in Chinese].
[21] Fan S, Chen HN, Wang CJ, Tseng WC, Hsu HK, Weng CF. Toona sinensis Roem (Meliaceae) leaf extract alleviates liver fibrosis via reducing TGFbeta1 and collagen. Food Chem Toxicol 2007;45:2228–36.

[22] Chen CJ, Michaelis M, Hsu HK, Tsai CC, Yang KD, Wu YC, et al. Toona sinensis Roem tender leaf extract inhibits SARS coronavirus replication. J Ethnopharmacol 2008;120:108–11.

[23] Poon SL, Leu SF, Hsu HK, Liu MY, Huang BM. Regulatory mechanism of Toona sinensis on mouse Leydig cell steroidogenesis. Life Sci 2005;76:1473–87.

[24] Hseu YC, Chen SC, Lin WH, Hung DZ, Lin MK, Kuo YH, et al. Toona sinensis (leaf extracts) inhibit vascular endothelial growth factor (VEGF)-induced angiogenesis in vascular endothelial cells. J Ethnopharmacol 2011;134:111–21.

[25] Chang HC, Hung WC, Huang MS, Hsu HK. Extract from the leaves of Toona sinensis roemor exerts potent anti-proliferative effect on human lung cancer cells. Am J Chin Med 2002;30:307–14.

[26] Wang CY, Lin KH, Yang CJ, Tsai JR, Hung JY, Wang PH, et al. Toona sinensis extracts induces cell cycle arrest and apoptosis in the human lung large cell carcinoma. Kaohsiung J Med Sci 2010;26:68–75.

[27] Yang CJ, Huang YJ, Wang CY, Wang CS, Wang PH, Hung JY, et al. Antiproliferative and antitumorigenic activity of Toona sinensis leaf extracts in lung adenocarcinoma. J Med Food 2010;13:54–61.

[28] Yang CJ, Huang YJ, Wang CY, Wang PH, Hsu HK, Tsai MJ, et al. Antiproliferative effect of Toona sinensis leaf extract on non-small-cell lung cancer. Transl Res 2010;155:305–14.

[29] Chia YC, Rajbanshi R, Calhoun C, Chiu RH. Anti-neoplastic effects of gallic acid, a major component of Toona sinensis leaf extract, on oral squamous carcinoma cells. Molecules 2010;15:8377–89.

[30] Yang HL, Chang WH, Chia YC, Huang CJ, Lu FJ, Hsu HK, et al. Toona sinensis extracts induces apoptosis via reactive oxygen species in human premyelocytic leukemia cells. Food Chem Toxicol 2006;44:1978–88.

[31] Liao JW, Chung YC, Yeh YJ, Lin YC, Lin YG, Wu SM, et al. Safety evaluation of water extracts of Toona sinensis Roemor leaf. Food Chem Toxicol 2007;45:1393–9.

[32] Liao JW, Yeh YJ, Lin YC, Wei MM, Chung YC. Mutagenicity and safety evaluation of water extract of fermented Toona sinensis Roemor leaves. J Food Sci 2009;74:T7–13.

[33] Lee CY, Landreth GE. The role of microglia in amyloid clearance from the rachis of Cedrela sinensis. Korea J Pharmacognosy 1996;27:219–23.

[34] Chen HM, Wu YC, Chia YC, Chang FR, Hsu HK, Hsieh YC, et al. Gallic acid, a major component of Toona sinensis leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. Cancer Lett 2009;286:161–71.

[35] Yu WJ, Chang CC, Kuo TF, Tsai TC, Chang SJ. Toona sinensis Roem leaf extracts improve antioxidant activity in the liver of rats under oxidative stress. Food Chem Toxicol 2012;50:1860–5.

[36] Chen JC, Ho FM, Pei-Dawn Lee C, Chen CP, Jeng KC, Hsu HB, et al. Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IkappaB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia. Eur J Pharmacol 2005;521:9–20.

[37] Kao TK, Ou YC, Raung SL, Lai CY, Liao SL, Chen CJ. Inhibition of nitric oxide production by quercetin in endotoxin/cytokine-stimulated microglia. Life Sci 2010;86:315–21.

[38] Lee MK, Jeon HY, Lee KY, Kim SH, Ma CJ, Sung SH, et al. Inhibitory constituents of Eucaphis japonica on lipopolysaccharide-induced nitric oxide production in BV2 microglia. Planta Med 2007;73:782–6.

[39] Lu J, Wu DM, Zheng YL, Hu B, Zhang ZF, Shan Q, et al. Quercetin activates AMP-activated protein kinase by reducing PP2C expression protecting old mouse brain against high cholesterol-induced neurotoxicity. J Pathol 2010;222:199–212.

[40] Kim MJ, Seong AR, Yoo JY, Jin CH, Lee YH, Kim YJ, et al. Gallic acid, a histone acetyltransferase inhibitor, suppresses microglial cell line BV-2. Neurochem Res 2001;26:1209–16.

[41] Chen PS, Wang CC, Bortner CD, Peng GS, Wu X, Pang H, et al. Valproic acid and other histone deacetylase inhibitors induce microglial apoptosis and attenuate lipopolysaccharide-induced dopaminergic neurotoxicity. Neuroscience 2007;149:203–12.

[42] Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, et al. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med 1999;5:1403–9.

[43] Iravani MM, Kashefi K, Mander P, Rose S, Jenner P. Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 2002;110:49–58.

[44] L’Episcopo F, Tirolo C, Caniglia S, Testa N, Serra PA, Impagliafatto F, et al. Combining nitric oxide release with anti-inflammatory activity preserves nigrostriatal dopaminergic innervation and prevents motor impairment in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson’s disease. J Neuroinflamm 2010;7:83–96.
beta-amyloid neurotoxicity by inhibiting microglial-mediated neuroinflammation. Mol Nutr Food Res 2011;55:1798–808.

[56] Pan XD, Chen XC. Advances in the study of immunopharmacological effects and mechanisms of extracts of *Tripterygium wilfordii* Hook. f. in neuroimmunologic disorders. Yao Xue Xue Bao 2008;43:1179–85 [Article in Chinese].

[57] Lee B, Jung K, Kim DH. Timosaponin AIII, a saponin isolated from *Anemarrhena asphodeloides*, ameliorates learning and memory deficits in mice. Pharmacol Biochem Behav 2009;93:121–7.

[58] Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, Pollard A, et al. Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer’s disease mouse. Neurotox Res 2009;15:3–14.

[59] Kopec KK, Carroll RT. Phagocytosis is regulated by nitric oxide in murine microglia. Nitric Oxide 2000;4:103–11.

[60] Paradisi S, Matteucci A, Fabrizi C, Denti MA, Abeti R, Breit SN, et al. Blockade of chloride intracellular ion channel 1 stimulates Abeta phagocytosis. J Neurosci Res 2008;86:2488–98.