Effect of the semen extract of Cuscuta chinensis on inflammatory responses in LPS-stimulated BV-2 microglia

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[ABSTRACT]
AIM: To investigate the anti-inflammatory activities of the semen extract of Cuscuta chinensis Lam. (Cuscutae Semen; CS) on the production of inflammatory mediators, nitric oxide (NO), prostaglandin 2 (PGE2), and proinflammatory cytokines in lipopolysaccharide (LPS)-stimulated BV-2 microglia.

METHOD: BV-2 cells were treated with CS extract for 30 min, and then stimulated with LPS or without for 24 h. The levels of NO, PGE2 and proinflammatory cytokines were measured by Griess assay and ELISA. The expression of inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX)-2 mRNA and protein was determined by RT-PCR and Western blot, respectively. The phosphorylation of extracellular signal-regulated kinase 1/2 (ERK 1/2), Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK), and the nuclear expression of nuclear factor (NF)-κB p65 were investigated by Western blot analysis.

RESULTS: CS extract significantly decreased the production of NO and PGE2 by suppressing the expression of iNOS and COX-2 in activated microglia. CS extract decreased the production of TNF-α, IL-1β, and IL-6 by down-regulating their transcription levels. In addition, CS extract suppressed the phosphorylation of ERK1/2, JNK, and p38 MAPK, and the nuclear translocation of NF-κB p65 in activated microglia.

CONCLUSION: These results indicate that CS extract is capable of suppressing the inflammatory response by microglia activation, suggesting that CS extract has potential in the treatment of brain inflammation.

[KEY WORDS] Cuscuta chinensis; Cuscutae Semen; Microglia; Inflammation; NF-κB pathway


Introduction
Inflammation plays an important role in the pathology of neurological disorders, such as traumatic injury, stroke, Alzheimer’s disease, Huntingdon’s disease, Parkinson’s disease, and multiple sclerosis [1]. In the central nervous system (CNS), neuroinflammation is dependent on the synthesis of various inflammatory mediators by local neurons, astrocytes, and microglia [2]. Microglia are the resident immune cells in the brain. They provide the first line of defense when neuronal damage occurs and play a homeostatic role in the CNS [3]. An early pathophysiological insult to the CNS triggers microglia activation with the morphological changes of hypertrophy, the proliferation of resident microglia, and the recruitment of bone marrow-derived precursors into the CNS [4]. Therefore, neuroinflammation is characterized by over-activated microglia causing neuronal death through the rapid accumulation of key inflammatory mediators, such as cytokines, chemokines, prostaglandins, reactive intermediates, proteinases, and complement proteins [3]. The damaged neurons release toxic soluble factors, which, in turn, induce microglia activation termed microgliosis; these toxic factors are thought to contribute to neuronal damage, particularly in neurodegenerative diseases [2]. It is well established that the transcription factor, nuclear fac

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tor-κB (NF-κB), and other signal pathways, such as members of major mitogen-activated protein kinase (MAPK) pathways regulate the expression of pro-inflammatory genes in LPS-induced microglial activation [5]. It is therefore of great interest to control inflammatory responses of the microglia through the NF-κB signaling pathway for the potential therapeutic treatment of inflammation-mediated neurological disorders [6].

Plant-based medicines have long been used in clinical practice to treat various human diseases and to maintain good health. The search for appropriate anti-inflammatory agents has been focused on plants used in traditional medicines because of the leads provided by natural products that may provide better treatment options for chronic inflammatory diseases than currently used drugs [7]. In a search for natural drugs to treat neurological disorders, many plant extracts have been investigated in combination therapy with Western medicines, and effective neuronal protection therapy through anti-neuroinflammation may be an alternative strategy for patients with neurological disorders [8]. The semen of Cuscuta chinensis Lam. (Convolvulaceae, Cuscutae Semen; CS) has traditionally been used as a tonic to improve liver and kidney conditions and sexual function and to prevent senescence as well as abortion [9]. CS has been reported to have various biological activities, such as neuroprotective [10], hepatoprotective, antioxidant [11], osteoblastogenic [12], and genoprotective [13] effects, and to improve renal function in acute renal failure rats [14]. The active constituents of CS include flavonoids, lignan glycosides, quinic acids, and polysaccharides [15], and have been suggested to be responsible for the pharmacological activities, such as immune modulation [16-17], antioxidant [18], neuronal differentiation [19], anti-neuronal apoptosis [10], and phytoestrogenic [20].

In this study, the anti-inflammatory effects of CS extract on inflammatory responses in lipopolysaccharide (LPS)-stimulated BV-2 mouse microglia were investigated. To explore the underlying mechanisms by which the extract inhibited the production/expression of inflammatory mediators in activated microglia, the roles of various signaling molecules, including the mitogen-activated protein kinases (MAPKs) and the nuclear factor (NF)-κB pathways, were determined.

**Materials and Methods**

**Plant material**

The semen of C. chinensis (CS) used in the present study was purchased from an traditional medicine drug store (Om-niherb Co., Yeongcheon, Korea) and authenticated by Prof. YK Park, a medical botanist in the Department of Herbology, College of Korean Medicine, Dongguk University, Republic of Korea. A voucher specimen was deposited in the Herbarium of the Korea Institute of Oriental Medicine (KIOM) under registration number KIOM-211928.

**Preparation of CS extract**

CS extract was prepared by the standard procedure. In brief, the dried semen (300 g) was ground into small pieces and then extracted with 80% aqueous ethanol (3 L × 2) twice, two days each time, at room temperature. The combined ethanol extract was evaporated in vacuo to give a dark brownish residue (27.05 g). The characterization of CS extract is identified the flavonoids contents by HPLC analysis.

**Cell culture**

BV-2 cells, a mouse microglia line, were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL), 100 µg·mL⁻¹ streptomycin, and 100 U·mL⁻¹ penicillin (Gibco BRL). Cells were cultured at 37 °C in a humidified incubator with an atmosphere of 5% CO₂. For experiments, cells were cultured with serum-free DMEM without phenol-red.

**Cell viability assay**

To measure the cytotoxicity of CS extract, the cell viability was determined by a water-soluble tetrazolium salt WST-1 Cell Proliferation Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA). BV-2 cells (5 × 10⁵ cells/well) in 96-well plates were cultured overnight and then treated with different concentrations of CS extract in the presence or absence of LPS (1 µg·mL⁻¹; isotype 0111 : B4, Sigma-Aldrich Co., St. Louis, MO, USA) for 24 h. Next, the reconstituted WST-1 mixture (10 µL) was added to each well and further incubated for 2 h at 37 °C in a CO₂ incubator. The plate was then gently mixed on an orbital shaker for 1 min to ensure homogenous distribution of color. The absorbance of each sample on the plate was measured using an automated microplate reader (SpectraMAX 340; Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 450 nm. Percent viability was calculated by comparison with the control group.

**Nitric oxide assay**

The concentration of nitric oxide (NO) in the cells was determined by measuring the amount of nitrite, a relatively stable oxidation product of nitric oxide. BV-2 cells (1 × 10⁵ cells/well) in 24-well plates were cultured overnight and treated with different concentrations of CS extract in the presence or absence of LPS (1 µg·mL⁻¹) for 24 h. Next, culture medium (100 µL) from each sample was mixed with the same volume of Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% phosphoric acid). Absorbance values were read at 540 nm on an automated SpectraMAX 340 (Molecular Devices, Sunnyvale, CA, USA). Nitrite concentration was calculated with reference to a standard curve of sodium nitrite generated using known concentrations.

**PGE₂ assay**

The cells (1 × 10⁵ cells/well) in 24-well plates were treated with different concentrations of CS extract in the presence or absence of LPS (1 µg·mL⁻¹) for 24 h. Culture media were collected, and the levels of PGE₂ were determined by Enzyme ImmunoAssay (EIA) using commercially available kits (R&D Inc., San Diego, CA, USA) according to the manufacturer’s instructions. The concentration of PGE₂ was calculated according to a standard curve generated using the standard substance in the kit.

**Cytokine assay**

The cells (1 × 10⁵ cells/well) in 24-well plates were treated with different concentrations of CS extract in the
presence or absence of LPS (1 μg·mL⁻¹) for 24 h. Culture media were collected and the levels of TNF-α, IL-1β, and IL-6 were determined by Enzyme-linked Immunosorbent Assay (ELISA) using a commercially available kit (eBioScience Inc., San Diego, CA) according to the manufacturer’s instructions. The concentration of each cytokine was calculated according to the standard curve generated using the recombinant cytokines in the kit.

Reverse transcription-polymerase chain reaction (RT-PCR)

The expression of iNOS, COX-2, and cytokine mRNAs in the cells was determined by RT-PCR. The cells (5 × 10⁵ cells/well) in 6-well plates were treated with CS extract in the presence or absence of LPS (1 μg·mL⁻¹) for 6 h. Total RNA was prepared from cells using cold Trizol (GibcoBRL) according to the manufacturer’s protocol. For RT-PCR, 1 μg of total RNA from each sample was reverse transcribed for 1 h at 42 °C in a reaction mixture containing 0.5 mmol·L⁻¹ deoxyribonucleotide triphosphate (dNTP), 2 μmol·L⁻¹ oligo-dT primer, 1× reverse transcriptase buffer, 5 units AMV reverse transcriptase, and 5 U RNase inhibitor (Boehringer Mannheim, Indianapolis, IN, USA). PCR was performed using the above-prepared cDNAs as a template, with the following cycle parameters: 94 °C, 2 min; 35 cycles, 94 °C, 30 s, 58–60 °C, 30 s, 72 °C, 1 min; 92 °C, 7 min, 4 °C, ∞. The PCR products were visualized by electrophoresis in 1% agarose gels, followed by staining with ethidium bromide (EtBr; 0.5 μg·mL⁻¹). Verification of specific genes was established by their predicted sizes under UV light. The primer sequences were as follows: 5'-AGT TCC GAA GCA AAC ATC AC-3' (sense), 5'-TAA TGT CCA GGA AGT AGG TG-3' (anti-sense) for iNOS; 5'- CAG CAA ATC CTT GCT GTT CC-3' (sense), 5'-TGG GCA AAG AAT GCA AAC ATC-3' (anti-sense) for COX-2; 5'- ATG AGC ACA GAA AGC ATG ATC-3' (sense), 5'-TAC AGG CTT GTC ACT CGA ATT- 3' (anti-sense) for TNF-α; and 5'- ATG AGG ACA TGA GCC CCT TC-3' (sense), 5'-CAT TGA GGT GGA GAG CCT TC-3' (anti-sense) for IL-6; 5'- AGT TGG CTT CTT GGG ACT GAT-3' (sense), 5'-TCC ACG ATT TCC CAG AGA AC-3' (anti-sense) for IL-1β; and 5'-CTC GTG GAG TCT ACT GGT GT-3' (sense), 5'-GTC ATC ATA CTT GCC AGG TT-3' (anti-sense) for GAPDH as a control for PCR. The band intensity was quantified by densitometric analysis (Digital Image Analysis System).

Western blot analysis

The levels of iNOS, COX-2, ERK1/2, JNK, p38 MAPK, NF-κB p65, and IκB in the cells were determined by Western blot analysis. The cells (5 × 10⁵ cells/well) in 6-well plates were treated with CS extract in the presence or absence of LPS (1 μg·mL⁻¹) for 15 min (for MAPKs), 30 min (for NF-κB and IκB), or 24 h (for iNOS and COX-2). After LPS (1 μg·mL⁻¹) stimulation, cells were washed twice with ice-cold PBS, scraped off with a rubber policeman, and centrifuged at 5 000 r·min⁻¹ for 5 min at 4 °C. Cell pellets were resuspended in an appropriate volume of lysis buffer (1% Triton X-100, 1% deoxycholate, and 0.1% NaCl) and incubated for 30 min on ice. Lysates were then centrifuged at 12 000 r·min⁻¹ for 10 min at 4 °C and collected for further analysis. For nuclear extraction, cells were washed twice with cold PBS and lysed with NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL, USA). After centrifugation at 12 000 r·min⁻¹ for 10 min, the supernatant was stored at –80 °C until use. Protein concentrations of samples were determined by Bradford assay (Bio-Rad, Hemel, Hempstead, UK) using samples equilibrated to 2 mg·mL⁻¹ with lysis buffer. Twenty μg·mL⁻¹ of cytoplasmic (for iNOS, MAPK) or nuclear protein (for NF-κB) were separated on 10% SDS-polyacrylamide gels and then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). Each membrane was incubated for 1 hr with 5% skim milk in TBS-T buffer (0.1 mol·L⁻¹ Tris-HCl, pH 7.4, 0.9 % NaCl, 0.1% Tween-20) to block non-specific binding and then incubated with primary antibodies that recognized iNOS (Santa Cruz Biotech, Santa Cruz, CA, USA), β-actin (Sigma-Aldrich Corp.), and the phospho- or total forms of NF-κB p65 (Cell Signaling Technology). The membranes were subsequently incubated with peroxidase-conjugated affinity goat anti-rabbit IgG (Santa Cruz Biotech). Each protein was detected by using a chemiluminescence detection system according to the manufacturer’s instructions (ECL, Amersham, Berkshire, UK). The band intensity was quantified by densitometric analysis (Digital Image Analysis System).

Statistical analysis

GraphPad Prism (GraphPad Software, Inc., San Diego) was used for statistical analysis. Data were expressed as Mean ± SEM (standard error of mean) of three separate experiments and were analyzed for statistical significance using analysis of variance (ANOVA), followed by Tukey’s test for multiple comparison. *P < 0.05 was considered significant.

Results

Effect of CS extract on cell viability

The cytotoxic effect of CS extract was evaluated by measuring the cell viability in BV-2 cells stimulated with LPS (1 μg·mL⁻¹) in the presence of CS extract at different concentrations for 24 h using the WST-1 assay. As shown in Fig. 1, CS extract had a dose-dependent effect on the viability of BV-2 cells. The cell viability was significantly reduced in a dose-dependent manner, with a significant difference observed between the control group and the LPS group (Fig. 1).

Fig. 1 Effect of CS extract on cell viability in LPS-stimulated BV-2 cells. Cells were treated with CS extract (0.1, 0.2, 0.5, 1, and 2 mg·mL⁻¹) in the presence of LPS (1 μg·mL⁻¹) for 24 h. Mean ± SEM, n = 3. **P < 0.01 vs LPS alone.
extract concentrations of 0.1, 0.2, 0.5, and 1 mg·mL⁻¹ did not decrease cell viability. Therefore, CS extract ranging from 0.1 to 1 mg·mL⁻¹ was used to investigate the anti-inflammatory activities and its mechanism of action in BV-2 cells. 

**Effect of CS extract on NO production and iNOS expression**

Microglial activation results in overproduction of free radicals and NO, which induce neuronal loss, axonal damage, and oligodendroglial death in the development of brain injury [21]. Therefore, the effect of CS extracts on the production of NO and the expression of iNOS in LPS-stimulated BV-2 cells was investigated by RT-PCR and Western blot. CS extract significantly inhibited NO production in LPS-stimulated BV-2 cells in a dose-dependent manner (Fig. 2A). CS extract also significantly attenuated the expression of iNOS mRNA (Fig. 2B) and protein (Fig. 2C) in the cells. CS extract alone did not affect the NO production and iNOS expression in the cells.

![Fig 2](image)

**Effect of CS extract on PGE₂ production and COX-2 expression**

In the brain, COX-2 expression has been associated with inflammatory and neurodegenerative processes of neurological disorders [22]. Therefore, the effect of CS extract on the production of PGE₂ and the expression of COX-2 in LPS-stimulated BV-2 cells was investigated by enzyme immunoassay, RT-PCR, and Western blot analysis. CS extract significantly inhibited the production of PGE₂ in LPS-stimulated BV-2 cells in a dose-dependent manner (Fig. 3A). CS extract also significantly suppressed the expression of COX-2 mRNA (Fig. 3B) and protein (Fig. 3C) in the cells. CS extract alone did not affect the release of PGE₂ and the expression of COX-2 in the cells.

**Effect of CS extract on pro-inflammatory cytokine production**

In pathological conditions, over-activated microglia contribute to early events in the progression of pathophysiology that is causally linked to synaptic dysfunction, behavioral deficits, and neuronal death through the release of pro-inflammatory cytokines [23]. Therefore, the effects of CS extract on the production of inflammatory cytokines, TNF-α, IL-1β, and IL-6, induced by LPS stimulation in BV-2 cells were investigated by ELISA and RT-PCR. The CS extract significantly inhibited the production of TNF-α, IL-1β, and IL-6 (Fig. 4A), and their mRNA expression (Fig. 4B) in LPS-stimulated BV-2 cells. CS extract alone did not affect the production of these cytokines, nor did it affect gene expression in the cells.

**Effect of CS extract on the MAPK/NF-κB pathways**

The MAPK pathway is a key signal transduction pathway involved in the production of pro-inflammatory cytokines [24]. Therefore, to determine whether CS extract influences the MAPK pathway, the phosphorylation of three MAPK molecules, ERK1/2, JNK, and p38 MAPK were
investigated in activated microglia. As shown in Fig. 5, LPS stimulation induced the phosphorylation of ERK1/2, JNK and p38 MAPK in BV-2 cells. However, CS extract treatment in LPS-stimulated BV-2 cells significantly inhibited phosphorylation of the three MAPK molecules, particularly ERK1/2, while their non-phosphorylated forms remained the same.
Fig. 5 Effect of CS extract on the phosphorylation of MAPK molecules in LPS-stimulated BV-2 cells. The cellular proteins from the cells were used for the detection of phosphorylated or total forms of ERK1/2, JNK, and p38 MAPK. Mean ± SEM, n = 3. *P < 0.05, **P < 0.01 vs control (a) or LPS alone (b)

Next, to determine whether CS extract blocked the NF-κB pathway, which is implicated in the transcriptional regulation of inflammatory mediators in activated microglia, the expression of NF-κB p65 and I-κB were analyzed in the cytosol or the nucleus by Western blot. As shown in Figure 6, the expression of NF-κB p65 and I-κB was abundantly observed in the cytosol of unstimulated cells (Fig. 6A) but not in the nucleus. Marked translocation of NF-κB p65 from the cytosol to the nucleus was observed in LPS-stimulated cells (Fig. 6B). Treatment with CS extract significantly inhibited the nuclear expression of NF-κB p65 in BV-2 cells (Fig. 6B). In addition, in the NF-κB p65 and DAPI immunofluorescence staining, marked enhancement of the nuclear expression in LPS-stimulated cells was attenuated by CS extract treatment (Fig. 6C).

Discussion

Inflammation in the CNS is a common feature in the progression of stroke and neurodegenerative diseases, although the mechanisms through which this occurs are still unclear [25]. The inflammatory response during most chronic neurodegenerative diseases is dominated by the microglia, and the mechanisms by which microglia contributes to neuronal damage and degeneration are the subject of intense study [26]. Microglia are immune cells in the brain, and are primarily involved in immune surveillance but, when activated in response to pathological conditions, have macrophage-like capabilities, including phagocytosis, inflammatory mediator production, and antigen presentation in the CNS [27-29]. Acute neurodegenerative disorders, such as stroke, hypoxia, and trauma, compromise neurological survival, and indirectly trigger neuroinflammation; microglia become activated in response to the insult itself, developing a phagocytic phenotype and secreting inflammatory mediators, mainly cytokines and chemokines. This acute neuroinflammatory response is more beneficial to the CNS, since it tends to minimize further neuronal damage and it contributes to the repair of damaged tissues [30]. In contrast, chronic neurodegenerative diseases such as AD, PD, Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS) are associated with chronic neuroinflammation characterized by long-standing activation of microglia and subsequent maintained release of inflammatory mediators leading to increased oxidative stress [31]. This, in turn, establishes a permanent inflammatory cycle, activating additional microglia through proliferation and resulting in further release of inflammatory substances. Therefore, anti-inflammatory therapies involving intervention in the microglia activation process may be a promising therapy for the treatment of numerous neurodegenerative conditions [32].
Fig. 6  Effect of CS extract on the expression of NF-κB p65 in LPS-stimulated BV-2 cells. The expression of NF-κB p65 was determined in cytosol (A) and nuclear extract (B) of the cells by Western blotting. TBP was used as an internal control in nuclear extract. Mean ± SEM, n = 3. *P < 0.05, **P < 0.01 vs control (a) or LPS alone (b). (C) The cells were immunostained with FITC-labeled anti-NF-κB p65 antibody (green) and DAPI (blue).

Recently, it should be considered that anti-inflammatory drugs have the multiple targets, thus leading to the hypothesis that they can operate through other pathways, including inflammatory ones. Therefore, due to the complex pathophysiology, including a cascade of neuroinflammatory or neurotoxic events that results in neuronal death, eventual dementia and etiology of neurological disorders, the development and use of multifunctional pharmaceuticals from natural sources may offer an innovative approach for neuroprotection [33].

Plant-based medicines have been widely used in clinical practice to treat brain disorders for thousands of years, and many studies have recently shown their efficacy with fewer side effects in treating neurodegeneration [34]. CS is a traditional herbal medicine commonly used as a tonic for the liver and the kidney, to improve sexual function prevent senescence, and to regulate the immune system [11, 14, 16]. In this study, the anti-inflammatory effect of CS as a natural source of neuroprotection from neurodegeneration in the over-activation of microglia was examined.

In microglia, NO is generated by the inducible isoform of NO synthase (iNOS or NOS-2) and has been described as a neurotoxic substance in the processes of CNS inflammation [35]. Thus, NO, and the pathways triggering its release, have emerged as an important research focus in the search for strategies to prevent, halt, or treat neurodegenerative disorders [36]. In this study, CS extract strongly inhibited NO production by suppressing iNOS expression in activated microglia. This result indicates that CS extract acts principally by regulating NO generation at the post-transcriptional level and could be beneficial for preventing the progression of neuroinflammation by microglial activation.

Prostanoids, another type of neuroinflammatory mediator, are arachidonic acid metabolites generated by the COX pathway [6]. Prostaglandins (PGs), which are critical mediators of physiologic processes and inflammation, are largely produced by activated microglia and reactive astrocytes during brain inflammation. PGE2, the most abundant prostaglandin in the CNS, is a critical mediator of physiologic processes and inflammation, and is largely produced by activated microglia during brain inflammation [37]. Microglia can express both COX-1 and COX-2, and much evidence has shown that COX-2, but not COX-1, plays a crucial role in neuroinflammation [38]. Therefore, treatment with selective COX-2 inhibitors has been demonstrated to reduce neuroinflammation and increase neuronal survival in chronic neurodegeneration [8]. In this study, CS extract significantly inhibited PGE2 release by suppressing COX-2 expression in activated microglia.

Both in vitro and in vivo, microglia activated by various
stimulants such as LPS, amyloid-β, ganglioside, metallocproteinase-3, and thrombin, are shifted towards a strongly pro-inflammatory phenotype and abundantly produce the pro-inflammatory cytokines, TNF-α, IL-1β, and IL-6, as well as potentially neurotoxic substances, such as NO, oxygen radicals, and proteolytic enzymes. Therefore, the over-production of pro-inflammatory cytokines by activated microglia is involved in the disruption of the blood-brain barrier (BBB), and is a possible etiological factor of neurological disorders \[39\]. In this study, CS extract significantly inhibited the release of these pro-inflammatory cytokines in activated microglia by suppressing their transcription. This result implies that CS extract can modulate the activity of pro-inflammatory cytokines at the transcriptional or mRNA stabilization level in activated microglia.

The MAPK pathway is a key signal transduction pathway involved in the production of inflammatory mediators and may be a viable target for modulating inflammatory responses in neurological disorders \[24\]. Inhibitors of MAPK, especially p38 MAPK, have been demonstrated to reduce LPS-induced metabolic activity and to up-regulate pro-inflammatory cytokines, such as TNF-α and IL-1β, from neurotoxicity \[40\]. In this study, the activation of the MAPK pathway appeared to be involved in LPS-induced ERK1/2, JNK, and p38 MAPK phosphorylation, and the CS extract inhibited the MAPK pathway. On the other hand, NF-κB is an inflammatory transcription factor involved in mediating inflammatory responses in microglia. The activation of NF-κB through the degradation of inhibitor protein, IκB, leads to the up-regulation of inflammatory mediators \[41\]. Therefore, NF-κB is the target of some anti-inflammatory drugs in neurodegenerative disorders. In this study, CS extract inhibited the nuclear translocation of NF-κB p65 subunit in activated microglia, suggesting that this extract exerts its anti-inflammatory activity by suppressing the MAPK/NF-κB pathway.

Many scientific reports have supported the long-held belief of anti-inflammatory natural sources from plant medicines that control the activation of microglia by suppressing the over-production of inflammatory mediators with multifunction and multi-target characteristics in neurological disorders \[39\]. However, the main constituents of CS extract have been reported to include flavonoids, lignin glycosides, quinic acids and polysaccharides \[35\]. Further studies are required to evaluate the neuroprotective and anti-inflammatory properties of CS extract and its main compounds in vivo in neurological disorders to understand the capacity to cross the blood-brain barrier and achieve effective concentrations in the brain.

In summary, these data demonstrate that the CS extract has anti-inflammatory properties in LPS-induced microglial activation through the down-regulation of inflammation-related gene expression, including iNOS, COX-2, and proinflammatory cytokines, by blocking the MAPK/NF-κB pathway. These data imply that CS extract inhibits the over-activation of microglia, suggesting that this plant-based medicine may play a therapeutic role in preventing and/or delaying the onset and progression of neurodegenerative disorders.

**References**


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