Tropical Journal of Pharmaceutical Research December 2013; 12 (6): 897-903
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v12i6.6

Original Research Article

Salicornia bigelovii Torr Attenuates Neuro-Inflammatory Responses in Lipopolysaccharide-Induced BV-2 Microglia by Regulation of NF-kappa B Signaling

Hyun Kang[1,2], Sushruta Koppula[1] and Tae-Kyu Park[1]*
[1]Department of Biotechnology, Research Institute for Biomedical & Health Science, College of Biomedical and Health Science, Konkuk University, [2]KuGen Healthcare Institute, Konkuk University Business Incubation Center, Chungju, 380-701, Republic of Korea

For correspondence: **Email: taekyupark2@gmail.com; **Tel:** 82-43-840-3870; Fax: 82-43-852-3616*

Received: 8 October 2013 *Revised accepted: 7 November 2013*

Abstract

Purpose: *To investigate the anti-oxidant and anti-neuroinflammatory effects of Salicornia bigelovii extract (SBE) in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells.*
Methods: *Anti-oxidant activity was measured using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. Cell viability was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl-tetrazolium bromide (MTT) assay. BV- microglial cells were stimulated with LPS to study the protein expression and production of inflammatory mediators, determined by Western blot analysis.*
Results: *SBE significantly inhibited the DPPH-generated free radicals showing maximum inhibition at 40 µg/mL ($p < 0.001$). SBE alone did not exhibit any signs of cytotoxicity to BV-2 cells up to 200 µg/mL concentration. The LPS-induced increase in the production of nitric oxide was concentration-dependently suppressed by SBE ($p < 0.05$ for 10 µg/mL, $p < 0.01$ at 20 µg/mL and $p < 0.001$ at 40 µg/mL, respectively). SBE also inhibited the LPS-induced increase in inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions. Further, the production of proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 by LPS-stimulation in BV-2 cells was inhibited by SBE pretreatment. Mechanistic study revealed that SBE acts by regulation of nuclear factor kappa-B signaling pathway in LPS-stimulated BV-2 microglial cells.*
Conclusion: *This study revealed for the first time that SBE possesses anti-oxidant and anti-neuroinflammatory effects and can be developed as a potential therapeutic target in ameliorating microglia-mediated neuroinflammation.*

Keywords: *Salicornia bigelovii, Anti-oxidant, lipopolysaccharide; Neuroinflammation, Microglia, Cyclooxygenase, iNOS, NF-κB.*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Salicornia bigelovii Torr. (*S. bigelovii)* commonly known as "Dwarf saltwort" from the family Amaranthaceae, is a leafless annual salt-marsh herb with green jointed and succulent stems [1]. *S. bigelovii* has an exceptional salt tolerance, adaptation to marginal lands and hot climates, therefore has great potential as a domesticated biomass, oilseed, and forage crop plant [2]. *S. bigelovii* has been successfully cultivated as an oilseed and vegetable crop in the desert coastlines of Mexico, India, the Middle East, Africa and in Southeast China [3]. The seed is rich in oil (30%) and protein (35%) with a high

content of polyunsaturated linoleic (75%) and linolenic (omega-3) fatty acids. In addition to its value in human diet, the oil can be used for the production of biodiesel [4]. Earlier studies suggested that *Salicornia* species has been used as a folk medicine to treat a variety of diseases such as atherosclerosis, hypertension, tumors and claimed as one of the most promising halophytes as immunomodulators [3,5]. However, till date there have been no reports on the antioxidant and anti-neuroinflammatory properties of *S. bigelovii*.

Microglia, the resident immune cells of the central nervous system, become activated thereby inducing significant and highly detrimental neurotoxic effects by excessively producing a large array of cytotoxic and proinflammatory factors [6]. Microglia-mediated neuroinflammation appears to play an essential role in the pathogenesis of various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and multiple sclerosis [7]. Previous reports strongly demonstrated that regulation of microglial activation could reduce neuroinflammation and further neuronal cell damage [8].

Lipopolysaccharide (LPS) is a common toxin used to investigate the impact of inflammation on neuronal death. LPS can directly activate microglia triggering the production of inflammatory mediators, such as nitric oxide (NO), cyclooxygenase (COX)-2, pro-inflammatory cytokines and leukotrienes [9]. Therefore, LPS-induced inflammatory mediators *in vitro* can be considered as one of the important tools to evaluate new and existing agents for their anti-neuroinflammatory actions. Recent studies have shown a convincing link between reactive oxygen species (ROS) and neuroinflammation. Inhibition of ROS by several anti-oxidants may suppress microglial activation and thus protect neuronal cell death [10,11]. In this study we evaluated the antioxidant potential and anti-neuroinflammatory properties of *S. bigelovii* extract in LPS-stimulated BV-2 microglial cells.

EXPERIMENTAL

Preparation of *S. bigelovii* extract

The dried whole plant of *S. bigelovii* (5 kg) collected during May 2012, were purchased from a local market in South Korea and authenticated by Prof Jong-Bo Kim, a taxonomist at Konkuk University, South Korea. A voucher specimen (SB-KU2012) has been kept in our laboratory herbarium, Konkuk University, South Korea, for future reference. To obtain the *S. bigelovii* extract, the dried plant material was ground in a blender and defatted three times with three volumes of ethanol. The residue was extracted with absolute ethanol at 1:10 ratio (w/v) for 2 h in a heating mantle at 70 - 80 °C, and the supernatant was filtered and concentrated in a vacuum evaporator system at 50°C. For further fractionation, the extract (1 kg) was partitioned into hexane, chloroform and ethyl acetate fractions to yield 220 mg, 50 mg and 456 mg, respectively. The active ethyl acetate fraction of *S. bigelovii* extract (SBE, 45.6%) was lyophilized and stored in a refrigerator (-20 °C) until use. SBE extract was re-dissolved in distilled water and filtered on 0.22 µm filters to evaluate its anti-oxidant and anti-neuroinflammatory activities.

DPPH radical scavenging activity

The anti-oxidant activity of the SBE was determined using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA). The radical scavenging capacity was evaluated by employing a reaction mixture constituted by aliquots of the SBE extract and a DPPH methanolic solution as described previously [12]. Briefly, a sample solution of 60 µl of each OFP-EA extract, was added to 60 µl of DPPH (60 µM) in methanol. After mixing vigorously for 10 s, the mixture was then transferred into a 100 µl Teflon capillary tube and the scavenging activity of each sample on DPPH radical was measured using a JES-FA ESR spectrometer (Jeol Ltd., Tokyo, Japan). A spin adduct was measured on an ESR spectrometer exactly after 2 min. Experimental conditions were as follows: central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5, and temperature, 298°K.

Cell cultures and viability

BV-2 microglia cells were cultured at 37 °C in 5 % CO2 in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 5 % foetal bovine serum (FBS, Hyclone, Logan, UT, USA) and 50 µg/ml penicillin-streptomycin (Invitrogen). In all experiments, cells were pre-treated with the indicated concentrations of SBE for 1 h before the addition of LPS (1 µg/ml, Sigma-Aldrich, St Louis, MO, USA) in serum free DMEM. An equal volume of sterile water was added to all control treatments. Cell viability was determined by 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [13]. Cells were incubated with various concentrations (0.1, 1.0, 10, 40, 60, 80, 100 and 200 µg/ml) of SBE for 24 h followed by MTT for 4

h, and then 100 µl of isopropanol (in 0.04N hydrochloric acid) was added to dissolve the formazan crystals. The absorbance was read at 570 nm using the Anthos 2010 spectrophotometer (Salzburg, Austria). Cell viability was calculated as relative absorbance compared to control.

Nitric oxide assay

The amount of stable nitrite, the end product of NO generation, by activated microglia was determined by a colorimetric assay as previously described [14]. Briefly,BV-2 cells (2 x 10^5 cells/ml) were seeded in 6-well plates in 500 µl complete culture medium and treated with the SBE extract at indicated concentrations (10, 20 and 40 µg/ml) for 1 h prior stimulation with LPS (1 µg/ml) for 2 h. 50 µl of culture supernatant was mixed with an equal volume of Griess reagent and incubated at room temperature for 10 min. The absorbance at 540 nm was read using a PowerWavex Microplate Scanning spectrophotometer (Bio-Tek Instrument, Winooski, VT, USA). Nitrite concentration was determined by extrapolation from a sodium nitrite standard curve.

Nuclear protein extraction and Western blot analysis

Cells were washed in cold PBS three times and lysed in a buffer containing 50 mM Tris-HCl, pH 7.4, 1 % (v/v) Tergitol- type *NP-40* (NP-40), 0.25 % sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 25 mM NaF, 2 mM Sodium orthovanadate (Na3VO4) and protease inhibitor cocktail (Complete MiniTM, Roche, Mannheim, Germany) at 4 °C. The lysate was clarified by centrifugation at 10,000 g for 20 min at 4 °C to remove insoluble components. Cell lysates were normalized for protein content using bicinchoninic acid (BCA) reagent (Pierce, Rockford, IL, USA). Equal amounts of protein were loaded onto 10 % poly acryl amide gel electrophoresis (PAGE) and separated by standard sodium dodecyl sulphate (SDS)-PAGE procedure. Proteins were transferred to an NC membrane (S&S, Dassel, Germany) and blocked with 5% non-fat dry milk in TBS. To detect protein expression, the blots were probed with specific antibodies for iNOS (1:1000), COX-1 (1:1000), COX-2 (1:1000), NF-*κ*B, I*κ*B-*α* (1:1000), anti-p-I*κ*B-*α and β*-actin (1:2000) followed by a 1 h incubation with horseradish peroxidase–conjugated secondary antibodies (1:1000 – 2000)(Bio-Rad, Herculus, CA, USA) with β-actin as internal control. The immunoreactive proteins on the membrane were detected by chemiluminescence using the West-Save substrate (Lab-Frontier, Seoul, Korea) on X-ray film. The antibodies against inducible nitric oxide synthase (iNOS), cyclooxygenase (COX) - 1, COX-2, nuclear factor kappa-B (NF-κB), I kappa B-alpha (IκB-α) and β-actin were purchased from Cell Signaling Technology Inc (Beverly, MA, USA).

IL-6 assay

BV-2 microglia cells (1 x 10^5 cells/well) were cultured on 96-well plates and treated with SBE at indicated concentrations with or without LPS (1µg/ml). At 4 h of post LPS treatment, the cells were collected and the supernatants were subjected to assay of IL-6 contents using a murine IL-6 ELISA kit from BD Biosciences (San Jose, CA, USA) according to the manufacturer's instruction.

TNF-α assay

BV-2 microglia cells (1 x 10^5 cells/well) were cultured on 96-well plates and treated with the SBE at indicated concentrations for 1 h and stimulated with LPS (1 µg/ml). At 4 h post-LPS treatment, the cells were collected and the supernatants were evaluated for TNF-α level using a murine TNF-α ELISA kit from BD Biosciences (San Jose, CA, USA) according to the manufacturer's instructions.

Statistical analysis

All data are represented as the mean ± SEM of at least three independent experiments. Statistical analyses were performed using SAS statistical software (SAS Institute, Cray, NC, USA) using one-way analysis of variance, followed by Dunnett's multiple range tests. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of SBE extract on DPPH radical scavenging activity

As shown in Fig 1A, SBE exhibited significant DPPH radical scavenging activity in a concentration-dependent manner showing a maximum effect at 40 µg/ml of concentration ($p < 0.001$). The ESR spectroscopy data is represented in Fig 1B.

Effect of SBE on BV-2 microglial cell viability

As shown in Fig. 2, SBE treatment for 24 hr at various concentrations ranging from 0.1 µg/ml to 200 µg/ml did not exhibit any significant cytotoxicity on BV-2 microglial cells.

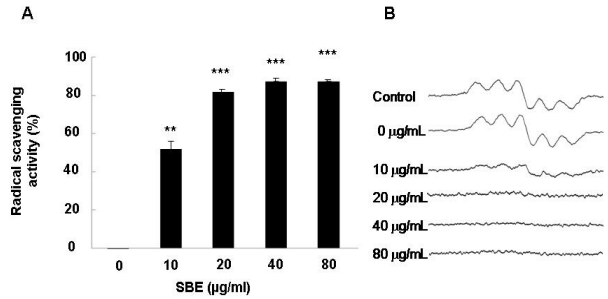

Fig 1: Effect of SBE on DPPH radical scavenging activity. The capacity to scavenge DPPH free radical by different concentrations of SBE (A) and ESR spectra (B) was measured. BV-2 cells were treated with or without SBE at the various concentrations (10, 20, 40 and 80 μg/ml). The scavenging activity of each sample on DPPH radical was measured using a JES-FA ESR spectrometer. A spin adduct was measured on an ESR spectrometer exactly 2 min later. Data are presented as the mean ± SEM (n = 3); $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with control group by one-way analysis of variance, followed by Dunnett's multiple range tests. SBE = *Salicornia bigelovii* extract.

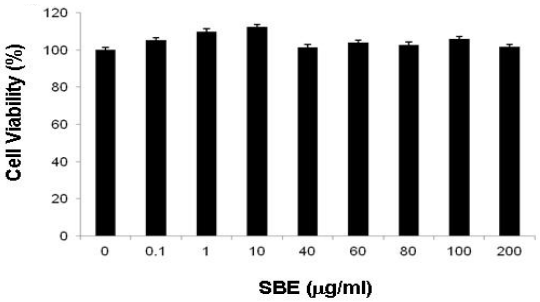

Fig. 2: Effect of SBE on the viability of BV- 2 microglial cells. Viability in-SBE treated cells was determined using MTT assay. The results are depicted as percentage of control samples. Data are presented as the mean ± SEM (n = 3). SBE = *Salicornia bigelovii* extract.

Effect of SBE on LPS-induced NO production in BV-2 microglial cells

As shown in Fig 3, cells treated with LPS alone significantly increased the NO levels ($p < 0.001$). Pretreatment with SBE (10, 20, 40 and 80 μg/ml) significantly suppressed the LPS-stimulated increased NO release in BV-2 cells in a dose-dependent manner compared to LPS only treated cells. The maximum effect was observed at a concentration of 100 μg/ml ($p < 0.001$).

Effect of SBE on LPS-induced expression of iNOS and COX levels in BV-2 microglial cells

SBE exhibited a broad spectrum of inhibitory effect on the expression of pro-inflammatory mediators and reduced the LPS-stimulated increase of protein expression such as iNOS and inducible COX-2 in a concentration-dependent manner. However, constitutive COX-1 protein expressional levels were uninterrupted (Fig 4).

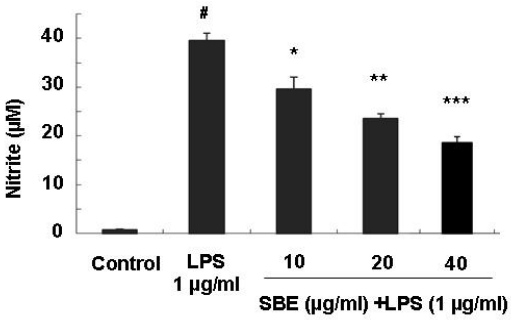

Fig 3: Effect of SBE on NO Production in LPS-stimulated BV-2 microglial cells. BV-2 cells were treated with SBE at indicated concentrations (10, 20 and 40 μg/ml) with or without LPS (1 μg/ml) for 4 h. The nitrite in the culture supernatant was evaluated using Griess reagent. Data are presented as the mean ± S.E.M. (n = 3); $^{\#}p < 0.001$, when compared with control group. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, when compared with LPS alone treated group by one-way analysis of variance, followed by Dunnett's multiple range tests. SBE = *Salicornia bigelovii* extract.

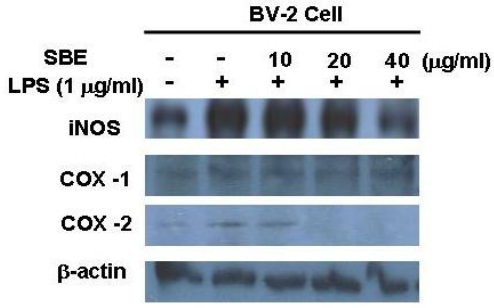

Fig 4: Effect of SBE on iNOS, COX-1 and COX-2 protein expressional levels in LPS-stimulated BV-2 microglial cells. The expression levels of iNOS, COX-1 and COX-2 production in the LPS-stimulated BV-2 cells by various concentrations (10, 20 and 40 μg/ml) of the SBE was monitored by immunoblot analyses with the specific antibodies against iNOS, COX-1 and COX-2. The internal control used was β-actin. SBE = *Salicornia bigelovii* extract.

Effect of SBE on TNF-α and IL-6 production in LPS-stimulated BV-2 microglial cells

As shown in Fig 5, TNF-α level increased significantly after LPS treatment when compared to those in untreated cells ($p<0.001$). However, SBE significantly inhibited TNF-α production in a concentration dependent manner ($p < 0.01$ at 10 μg/ml and $p < 0.01$ at 20 and $p < 0.001$ at 40 μg/ml, respectively). LPS stimulation increased the IL-6 expression in BV- cells. However, pretreatment with SBE at various concentrations significantly and dose dependently decreased ($p < 0.05$ at 10 μg/ml, $p < 0.01$ at 20 μg/ml and p <

0.001 at 40 µg/ml) the LPS-induced IL-6 levels in BV-2 microglia (Fig 6).

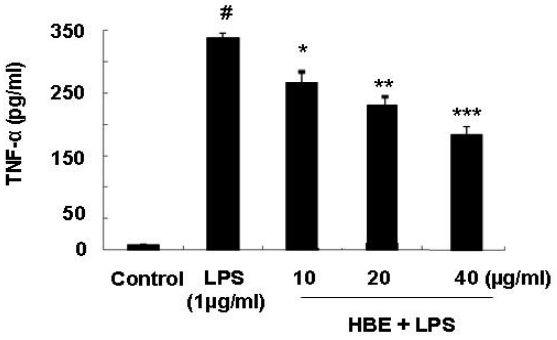

Fig 5: Effect of SBE on pro-inflammatory cytokine TNF-α level in LPS-stimulated BV-2 cells. BV-2 cells were treated with SBE at indicated concentrations (10, 20 and 40 µg/ml) with or without LPS (1 µg/ml) for 4 h. The TNF-α level in the culture supernatant was evaluated using a specific ELISA kit from BD Sciences according to the manufacturer's instruction. Data are presented as the mean ± S.E.M. (n = 3). $^{\#}p < 0.001$, compared with control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with LPS alone treated group by one-way analysis of variance, followed by Dunnett's multiple range tests. SBE = *Salicornia bigelovii* extract.

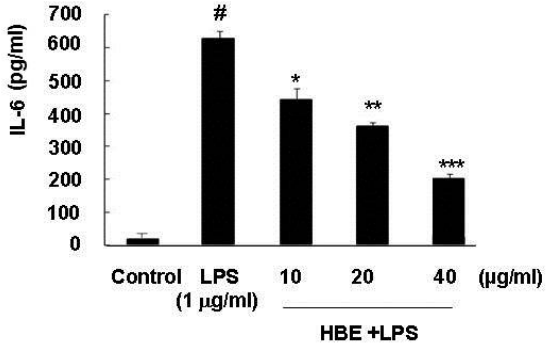

Fig 6: Effect of SBE on pro-inflammatory cytokine IL-6 expression in LPS-stimulated BV-2 cells. BV-2 cells were treated with SBE at indicated concentrations (10, 20 and 40 µg/ml) with or without LPS (1 µg/ml) for 4 hr. The IL-6 in the culture supernatant was evaluated using a murine IL-6 ELISA kit from BD Sciences according to the manufacturer's instruction. Data are presented as the mean ± SEM (n = 3). $^{\#}p<0.001$, when compared with control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with LPS alone treated group by one-way analysis of variance, followed by Dunnett's multiple range tests. SBE = *Salicornia bigelovii* extract.

Effect of SBE on NF-κB in LPS-stimulated BV-2 microglial cells

SBE inhibited the LPS-induced phosphorylation and degradation of IκB-α, and nuclear translocation of p65 NF-κB in a concentration dependent manner (Fig 7).

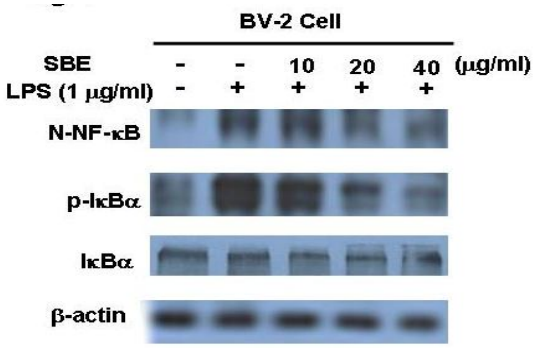

Fig 7: Effect of SBE on NF-κB activity in LPS-stimulated BV2 microglia. The expression levels of IκB-α, and nuclear translocation of p65 NF-κB in the LPS-stimulated BV-2 cells by indicated concentrations (10, 20 and 40 µg/ml) of the SBE was monitored by immunoblot analyses with the specific antibodies. The internal control used was β-actin. Data are presented as the mean ± SEM (n = 3) for three independent experiments. SBE = *Salicornia bigelovii* extract.

DISCUSSION

In the present study, proinflammatory stimulus by LPS to BV-2 cells resulted in excessive production of NO. Earlier studies revealed that prolonged activation of microglial cells leads to increased release of NO by iNOS in the brain. NO, an important regulatory mediator involved in cell survival and death exerts a number of pro-inflammatory effects during several physiological and pathological processes leading to increased inflammatory reaction. It was well known that COX-1 is constitutively expressed in many cell types and COX-2 is normally not present in most cells, but its expression is induced in response to inflammatory cytokines linked to pathological events [15]. COX-2 is upregulated in response to various inflammatory stimuli including LPS in BV-2 microglia. Therefore, agents that inhibit the release of NO and attenuate iNOS and COX-2 expression could be beneficial for preventing and delaying the progression of neuroinflammatory disease. [16]. Data from our study clearly showed that SBE attenuated LPS-induced iNOS and COX-2 expression and downstream NO production. However, SBE has no influence on the constitutive COX-1 expression.

Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 play central roles in microglia-mediated inflammation [17]. In particular, increased levels of brain TNF-α and IL-6 has been associated with severe cognitive impairments, neuronal damage and neuroinflammation [17]. Therefore, the effects of SBE on proinflammatory cytokine TNF- α and IL-6 production in LPS-stimulated BV-2 microglial cells were evaluated. LPS-stimulation increased the levels of TNF- α and IL-6 in BV-2 cells.

However, pretreatment with SBE suppressed the increased TNF- α and IL-6 production indicating that SBE may convincingly be an effective anti-neuroinflammatory agent.

NF-κB, a mammalian transcription factor, activated by LPS, is known to control the expression of cell survival genes as well as pro-inflammatory enzymes and cytokines [18]. Our result showed that SBE inhibited the LPS-induced phosphorylation/degradation of IκB-α and translocation of NF-κB/p65 sub unit in a concentration-dependent manner. Considering the above data, we can conclude that NF-κB is a major target of SBE. However, the exact molecular target of SBE on NF-κB activation remains to be elucidated.

The mechanism of neuro-inflammation is partly attributed, to release of toxic free radicals and ROS from activated microglia which may participate in the neurodegenerative process. DPPH radical assay is one of the widely used methods for screening the free radical scavenging activities of several agents in a relatively short period of time. In the present study, SBE significantly scavenged the DPPH free radicals. Reports from earlier studies indicated that *S. bigelovii* posses active constituents such as triterpenoids, flavones, glycosides, saponins, vitamins and minerals [3,19]. *Salicornia* species were also reported to posses' strong anti-oxidant properties [20,21]. In light of such reports, the strong anti-oxidant activity exhibited by SBE supports the notion that SBE might play a promising role in exhibiting anti-neuroinflammatory properties in LPS-stimulated BV-2 cells.

CONCLUSION

This study revealed for the first time that SBE inhibits neuro-inflammatory responses via NF-κB signaling in LPS-stimulated BV-2 microglial cells. Further, the antioxidant potential of SBE might partly be involved for the observed effects.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists regarding this work.

ACKNOWLEDGEMENT

This work was supported by Konkuk University in the year (2013). The author's Kang H and Koppula S contributed equallyto this work.

REFERENCES

1. *Edward PG, Oleary JW, Watson MC, Thompson T L, Kuehl RO. An oil seed halophyte for seawater irrigation. Science 1991; 251 (4997), 1065-1067.*
2. *Masters DG, Benes SE, Norman HC. Biosaline agriculture for forage and livestock production. Agr Ecosyst Environ 2007; 119: 234–248.*
3. *Lu D, Zhang M, Wang S, Cai J, Zhou X, Zhu C. Nutritional characterization and changes in quality of Salicornia bigelovii Torr. during storage. LWT - Food Sci Tech 2010; 43: 519–524.*
4. *Anwar F, Bhanger M I, Nasir MKA, Ismail S. Analytical characterization of Salicornia bigelovii seed oil cultivated in Pakistan. J Agri Food Chem 2002; 50 (15): 4210–4214.*
5. *Im SA, Kim KJ, Lee CK. Immunomodulatory activity of polysaccharides isolated from Salicornia herbacea. Int Immunopharmacol 2006; 6(9): 1451–1458.*
6. *Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol, 2005; 76: 77–98.*
7. *Perry VH, Gordon S. Macrophages and microglia in the nervous system. Trends Neurosci 1988; 11: 273-277.*
8. *Liu B, Hong J.S. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention.* J Pharmacol Exp Ther *2003; 304: 1-7.*
9. *Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase J Biol Chem 1994; 269 (7): 4705–4708.*
10. *Agullo G, Gamet-Payrastre L, Manenti S, Viala C, Rémésy C, Chap H, et al. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. Biochem Pharmacol 1997; 53:2087–2094.*
11. *Araki E, Forster C, Dubinsky JM, Ross ME, Iadecola C. Cyclooxygenase-2 inhibitor NS-398 protects neuronal cultures from lipopolysaccharide-induced neurotoxicity. Stroke 2001; 32: 2370–2375.*
12. *Sanches-Moreno C, Plaza L, Ancos B, Cano MP. Nutritional characterization of commercial trnaditional pasteurized tomato juices: carotenoids, vitamin and radical-scavenging activity. Food Chem 2006; 98: 749-756.*
13. *Reis K, Halldin J, Fernaeus S, Pettersson C, and Land T. NADPH oxidase inhibitor diphenyliodonium abolishes lipopolysaccharide induced down regulation of transferrin receptor expressions in N2a and BV-2 cell. J Neurosci Res 2006; 84:1047-1052.*
14. *Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [^{15}N] nitrite in biological fluids. Anal Biochem 1982; 126: 131-138.*
15. *Teismann P, Vila M, Choi DK, Tieu K, Wu DC, Jackson-Lewis V, Przedborski S. COX-2 and neurodegeneration in Parkinson's disease. Ann. N.Y. Acad. Sci 2003; 991: 272 - 277.*
16. *Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cell Mol Life Sci 1999; 55:1015–1028.*
17. *Hunot S, Hartmann A, Hirsh EC. The inflammatory response in the Parkinson brain. Clin Neurosci Res 2001; 1: 434-443.*
18. *Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 1996; 14: 649–683.*
19. *Xia GP, Feng X, Chen Y, Wang, M, Dong YF. Chemical constituents of Salicornia bigelovii Torr. Natural*

Product Research and Development , 2010; 22(6): 1012-1014.

20. *Daffodil ED, Rajalakshmi K, Mohan VR. Antioxidant avtivity, total phenolics and flavonoids of Salicornia brachiata Rox. Leaf extracts (Chenopodiaceae). World J Pharm Pharm Sci 2013; 2(1): 352-366.*
21. *Kim YA, Kong CS, Um YR, Lim SY, Yea SS, Seo Y . Evaluation of Salicornia herbacea as a potential antioxidant and anti-inflammatory agent .J. Med. Food. 2009; 12:3; 661-668.*