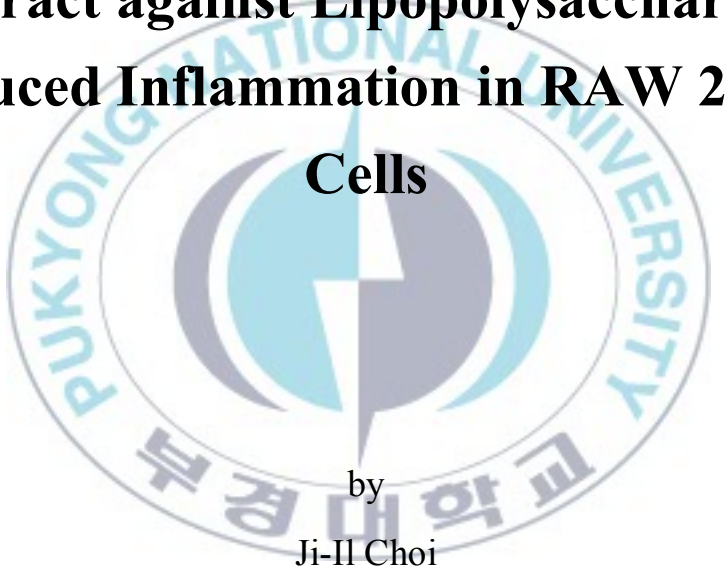
Thesis for the Degree of Master of Science

Effect of Fermented Sea Tangle Extract against Lipopolysaccharide-induced Inflammation in RAW 264.7 Cells



by

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February 2013

다시마 발효 추출물의 lipopolysaccharide 로 유도된 **RAW 264.7** 세포 염증반응에 미치는 영향

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by

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Effect of Fermented Sea Tangle Extract against Lipopolysaccharide-induced inflammation in RAW 264.7 Cells

A dissertation

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February, 2013

다시마 발효 추출물의 lipopolysaccharide 로 유도된 RAW 264.7 세포 염증반응에 미치는 영향

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요약

다시마는 (Laminaria japonica) 전통적으로 한국과 일본에서 식용으로 이용되어 왔고 항산화, 항균, 항당뇨, 항염증에 효과가 있다고 보고되어있다. 최근 다시마 발효 추출물이 항산화 및 간독성을 보호하는 효과가 있다는 보고가 있었다. 그러나, 다시마 추출물의 항 염증에 대한 연구는 많이 이루어져 왔으나 다시마 발효 추출물의 항염증에 대한 연구는 아직 보고된 바 없다. 따라서 본 연구에서는 다시마 발효(FST) 후 외부순환식 감압형 분리막을 이용하여 분자량에 따른 세가지 유형의 FST I (10 kDa 보다 이상), FST II (1-10 kDa)과 FST III (1kDa 이하)를 준비했다. 또한 염증을 유발시키는 Lipopolysaccaride 를 이용하여 RAW 264.7

세포에 염증을 유발시킨 후 FSTs 를 이용하여 염증억제 효능을 검토하였다.

그 결과, 염증반응매개 효소인 iNOS 와 COX-2, 염증성 사이토카인인 IL-6, IL-1β, TNF-α 의 유전자 및 단백질 발현이 억제됨을 확인할 수 있었고 FSTs 중 FST III 이 가장 항염증 효과가 우수함을 확인 할 수 있었다. 또한 FSTs 는 염증 신호전달 경로인 NF-κB 의 활성화를 억제함으로서 염증반응을 제어하는 것을 확인 할 수 있었다.

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List of Abbreviation

BSA	Bovine serum albumin
DCFH-DA	2',7'dichlorofluorescein diacetate
DMEM	Dulbecco's Modified Eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPP	Diphenyl-1-pyrenylphosphine
FBS	Fetal bovine serum
FST	Fermented sea tangle
FST I	Fermented sea tangle with molecular weight over 10 kDa
FST II	Fermented sea tangle with molecular weight between 1and10 kDa
FST III	Fermented sea tangle with molecular weight less than1kDa

IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
kDa	Kilo dalton
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
PCR	Polymerase chane reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
TNF-α	Tumor necrosis factor alpa

Introduction

Inflammation is the result of host response to pathogenic challenges or tissue injuries, and ultimately leads to the restoration of a normal tissue structure and function. Normal inflammatory responses are self-limited by a process which involves the down-regulation of pro-inflammatory proteins and the up-regulation of anti-inflammatory proteins (Lawrence et al., 2002). Thus, acute inflammation is a limited beneficial process, particularly in response to infectious pathogens, whereas chronic inflammation is an undesirable persistent phenomenon that can lead to the developments of inflammatory diseases (Kaplanski et al., 2003). Prolonged inflammation contributes to the pathogenesis of many inflammatory diseases, such as bronchitis, gastritis (Sakagami et al., 1997), inflammatory bowel disease, multiple sclerosis (Klotz et al., 2005), and rheumatoid arthritis (Ponchel et al., 2002). Macrophages play an important role in a variety of disease processes including autoimmune diseases, infections, and inflammatory disorders (Pierce, 1990).

Lipopolysaccharide (LPS), an endotoxin derived from Gram-negative bacterial outer membrane, can directly activate macrophages to produce a variety of pro-inflammatory cytokines, such as tumor necrosis factor-α

(TNF-α) and interleukins (ILs), and the formation of other inflammatory mediators, including prostaglandins (PGs) and nitric oxide (NO). NO is endogenously produced from L-arginine and molecular oxygen by the action of NO synthases (NOSs). In mammals, there are three isoforms of NOS; neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). nNOS and eNOS are constitutively expressed in neuronal and endothelial, respectively. In contrast, iNOS is inducible and its expression is increased in cells that are exposed to LPS or cytokines (Vane et al., 1994). Accordingly, high expression and activity of iNOS are observed in chronic diseases, such as inflammation and cancer (Maeda et al., 1998., Liu et al., 1998).

Prostaglandins (PGs) are synthesized by the action of cyclooxygenase (COX) in arachidonic acid metabolism (Vane et al., 1994). COX has two isoforms; COX-1 and COX-2. COX-1 is constitutively expressed in most cells and the COX-1-produced prostaglandins which involves in normal physiological functions. COX-2 is inducible in many types of cells including macrophages after the exposure of LPS, growth factors, and tumor promoters (Prescott et al., 2000, Hinz et al., 2002). In view of the importance of iNOS and COX-2 as plausible targets for the treatment of inflammatory disorders, I was interested in the effect of fermented sea

tangle (FST) on the suppression of iNOS and COX-2 expressions in LPS-induced RAW 264.7 cells.

Marine algae have been identified as rich sources of structurally diverse bioactive compounds with great pharmaceutical potential (Blunt et al., 2010, Abad et al., 2008). Variety of biological compounds including phlorotannins and fucoxanthin were isolated from Laminariacceae and characterized on their biological activities (Okuzumi et al., 1993, Kim et al., 2005, Woo et al.,2009). Sea tangle (*Laminaria japonica*) is representative marine brown alga that is commonly used as seasonings, condiments and health food in Korea, Japan and China. Variety *in vitro* and *in vivo* studies have focused on the antioxidant (Huang et al., 2004., Yuan et al., 2006., Park et al., 2009) and chemopreventive (Zhang et al., 2008) activities of the extracts from *L. japonica.*

As part of my ongoing is the investigation for anti-inflammatory activity of fermented sea tangle extract (FST). To the best of my knowledge, there are no reports about the anti-inflammatory activity of γ-aminobutyric acid (GABA)-enriched sea tangle extract (FST) and prompted me to investigate signaling mechanism of FST to inflammatory proteins in the LPS-induced RAW 264.7 cells. In the present study, anti-inflammatory activity of FST

and its possible mechanisms in LPS-induced RAW 264.7 cells was investigated.

Materials and Methods

1. Preparation of fermented sea tangle extracts (FSTs)

Sea tangle (*Laminaria japonica*) was purchased at Gi-jang market, Busan, Korea in March 2007. The Sea tangle was washed with fresh water to eliminate foreign materials such as sand, shells, and others. Then, the sample was added to water at a ratio of 1:15 (w/v) and 2% (w/w of dry sea tangle) rice flour was added to aid fermentation. After autoclaving at 121°C for 30 min, *Lactobacillus. brevis* BJ20 (Accession no. KCTC 11377BP) culture was added to the sea tangle extract at a concentration of 2% (v/v), followed by thorough mixing and incubation at 37°C. The fermented product was obtained by filtration and was freeze-dried. γ-Amino Butyric Acid (GABA)-enrich fermented sea tangle extract (FST) were further fractionated into three types, FST I (over than 10 kDa), FST II (1-10 kDa) and FST III (less than 1kDa) according to the molecular weight using a ultrafiltration .

2. Cell culture and cell viability assay

RAW 264.7 cells were grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) at 37ºC in a humidified atmosphere of 5% CO_2. Cytotoxicity of various molecular weight of FSTs was evaluated by MTT assay, a method based on the reduction of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Weislow et al., 1989). The medium containing RAW 264.7 cells were cultured into a 96-well plate at a density of 1 x 10^5 cells per ml. The plate was incubated overnight and treated with 100 μl of DMEM medium containing different concentrations of FSTs. After 24 h of incubation, MTT solution (1 mg/ml) was added to each well and the plate was incubated for another 4 h at 37ºC. The blue formazan salt was dissolved in DMSO. Optical density was measured at 540 nm with a GENios microplate reader (Tecan, Austria GmbH, Austria). The optical density of formazan formed by untreated cells was taken as 100% of viability.

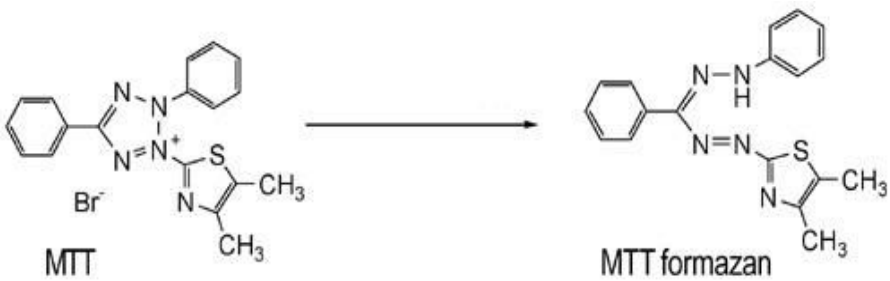


Fig. 1. Molecular structure of MTT and its corresponding reaction product

3. Determination of intracellular ROS formation

Intracellular formation of reactive oxygen species (ROS) was assessed as described previously using 2',7'dichlorofluorescein diacetate (DCFH-DA) as the substrate (Rajapakse et al., 2007). RAW 264.7 cells growing in fluorescence 96-well plates were loaded with 20 μM DCFH-DA in HBSS and incubated for 20 min in the dark. Non-fluorescent DCFH-DA dye is freely penetrated into cells, gets hydrolyzed by intracellular esterases to 2',7'dichlorodihydrofluororescein (DCFH), and traps inside the cells. Cells were then treated with different concentrations of FSTs and incubated for 1 h. After washing the cells with PBS three times, 500 μM H_2O_2 dissolved in HBSS were added to the cells. The formation of 2',7'dichlorofluorescein (DCF) due to oxidation of DCFH in the presence of various ROS was read

after every 20 min at the excitation wavelength (Ex) of 485 nm and the emission wavelength (Em) of 528 nm using a fluorescence microplate reader (Tecan Austria GmbH, Salzburg, Austria). Dose and time-dependant effects of the sample treated groups were plotted and compared with fluorescence intensity of the control and blank groups.

4. Membrane lipid peroxidation

Intracellular lipid hydroperoxide levels were determined by the fluorescence probe, diphenyl-1-pyrenylphosphine (DPPP) as described previously (Rajapakse et al., 2007). RAW 264.7 cells growing in culture dishes were washed three times with PBS and labeled with 13 mM DPPP (dissolved in DMSO) for 30 min at 37ºC in the dark. Cells were washed three times with PBS and seeded into fluorescence microtiter 96-well plates at a density of 1 x 10^8 cells per ml using serum free media. Following complete attachment, cells were treated with various concentrations of sample and incubated for 1 h. After incubation, 3 mM AAPH in PBS was added and DPPP-oxide fluorescence intensity was measured after 9 and 18 h at the excitation wavelength (Ex) of 361 nm and the emission wavelength

(Em) of 380 nm using GENios fluorescence microplate reader (Tecan Austria GmbH,Austria).

5. Measurement of NO

RAW 264.7 cells (1 x 10^6) were plated and incubated with 0-100 μg/mL FSTs in the absence or presence of LPS (1 μg/mL) for 24 hr. After treatment of LPS and FSTs, RAW 264.7 cells culture medium was saved for measured as an of nitrite. The nitrite concentration in the culture medium was measured as an indicator of NO production, according to the Griess reaction (Kim et al., 1995). One hundred microliters of culture supernatant was mixed with the same volume of Griess reagent (0.1% naphtylethylenediamine dihydrochloride and 1% sulfanilamide in 5% H_3PO_4). The absorbance of the mixture was measured with a microplate reader (Infinite F200 pro, TECAN) at 540 nm. The concentration of nitrite was calculated with sodium nitrite as a standard.

6. RNA extraction and reverse transcription-polymerase chain reaction

Total RNA was isolated using a Trizol reagent (Invitrogen Co., CA, USA) following the manufacture's recommendations. Total RNA was digested with RNase-free DNase (Roche, IN, USA) for 15 min at 37ºC and repurified by the RNeasy kit according to the manufacture's protocol (Quiagen, CA, USA). cDNA was synthesized from 2 μg total RNA. By incubation at 37ºC for 1 h with MLV reverse transcriptase (Promega) with random hexanucleotide according to the manufacture's instruction. Primers to specifically amplify the genes interested were showed in Table 1. Amplification was performed in a master-cycler (Eppendorf, Hamburg, Germany) with cycles of denaturation at 95ºC 30 sec, annealing at 60ºC 45 sec, and extension at 72ºC for 1 min, respectively. The amplified PCR products were run in 1.0% agarose gels and visualized by ethidium bromide (EtBr).

7. Western blot analysis

Western blotting was performed according to standard procedures. Briefly, cells were lysed in RIPA buffer containing 50 mM Tris–HCl (pH

8.0), 0.4% Nonidet P-40, 120 mM NaCl, 1.5 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, leupeptin (80 μg/ml), 3 mM NaF and 1 mM DTT at 4°C for 30 min. Cell lysates (50 μg) were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech., England, UK), blocked with 5% skim milk, and hybridized with primary antibodies (diluted 1:1000). After incubation with horseradish-peroxidase-conjugated secondary antibody at room temperature, immunoreactive proteins were detected using a chemiluminescent ECL assay kit (Amersham Pharmacia Biosciences, England, UK) according to the manufacturer's instructions. Western blot bands were visualized using a LAS3000® Luminescent image analyzer (Fujifilm Life Science, Tokyo, Japan).

Table 1. Chemicals used for reverse transcription PCR

PCR Chemical	Amount used	Stock
MMLV reverse ranscriptase	0.5 µl	200 U/ µl
dNTP mixture	0.5 µl	10 mM
DTT	1 µl	100 mM
5X reaction buffer	5 µl	
RNase inhibitor	0.5 µl	80 U/ µl

Table 2. RT-PCR conditions

Temperature	Time
37ºC	1h
70ºC	5 min
4ºC	∞

Table 3. Chemicals used for PCR reaction

PCR Chemical	Amount used	Stock
Taq polymerase	0.125 µl	5 U/ µl
dNTP	0.5 µl	2.5 mM
Reverse Primer	0.5 µl	25 pmole/ µl
Forward Primer	0.5 µl	25 pmole/ µl
5X reaction buffer	5 µl	
DW	17.375 µl	

Table 4. PCR conditions

Temperature	Time	Cycle
95ºC	2 min	1
95ºC	30 sec	
60ºC	45 sec	28
72ºC	1 min	
72ºC	5 min	1
4ºC	∞	

Table 5. Gene-specific primers used for the RT-PCR

Gene	Direction	Sequence
iNOS	Forward	5'- CAC CTT GGA GTT CAC CCA GT -3'
	Reverse	5'- ACC ACT CGT ACT TGG GAT GC -3'
COX-2	Forward	5'- TGA AAC CCA CTC CAA ACA CA -3'
	Reverse	5'- GAG AAG GCT TCC CAG CTT TT -3'
TNF-α	Forward	5'-AGG CCT TGT GTT GTG TTT CCA-3'
	Reverse	5'-TGG GGG ACA GCT TCC TTC TT-3'
IL-1β	Forward	5'- CTG TCC TGC GTG TTG AAA GA -3'
	Reverse	5'- TTC TGC TTG AGA GGT GCT GA -3’
IL-6	Forward	5'- AGG AGA CTT GCC TGG TGA AA -3'
	Reverse	5'- CAG GGG TGG TTA TTG CAT CT -3'
β-actin	Forward	5'- CCA CAG CTG AGA GGG AAA TC-3'
	Reverse	5'-AAG GAA GGC TGG AAA AGA GC-3'

Results and Discussion

1. Effect of FSTs on RAW 264.7 cells viability

The Effect of FSTs on the viability of LPS-activated RAW 264.7 cells was determined by MTT assay. LPS-activated RAW 264.7 cells were treated with or without FSTs at concentrations of 25, 50 and 100 μg per ml. As shown in (Fig. 2), FSTs did not show any significant cytotoxicity. These results revealed that FSTs are safe materials for *in-vitro* cell culture experiments up to a concentration of 100 μg per ml.

2. Effect of FSTs on NO production in RAW 264.7 cells

Macrophage-derived intercellular NO is a free radical with a short lifespan that plays an important role in the physiological and pathophysiological mechanisms in immunological systems (Asamitsu et al., 2003). NO is synthesized from the amino acid arginine by nitric oxide synthase (NOS). Under pathological conditions, NO production is increased by the inducible NOS (iNOS) and, subsequently, brings about cytotoxicity and tissue damage (Kim et al., 1998). Compounds able to reduce NO

production by iNOS may be attractive as anti-inflammatory agents, and for this reason, the effects of polyphenoles on iNOS activity have been intensively studied to develop anti-inflammatory drugs (Santangelo et al., 2007). LPS can induce the formation of iNOS and NO in macrophage cells (Mendis et al., 2008). Therefore, it was investigated whether FSTs could inhibit NO production in LPS- induced RAW 264.7 cells. As an indicator of NO production, nitrite ($NO2^-$) accumulation in the cultured media was determined by the Griess method. FSTs inhibited LPS-induced NO production in RAW 264.7 macrophages compared to control group (Fig. 3). To my knowledge, this is the first report regarding the inhibitory effects of FSTs on NO production in RAW 264.7 cells. Hence, these findings suggest that the inhibition of NO production by FSTs might be due to the suppression of LPS-induced iNOS transcription.

3. Cellular radical scavenging effect

Intracellular ROS scavenging was examined using fluorescence sensitive dye. The DCFH-DA fluorescent intensity was significantly decreased by FSTs extract in a time and concentration dependent manners on RAW 264.7 cells. As shown in (Fig. 4), the progressive increments in DCF fluorescence

intensity due to the hydrogen peroxide generation were observed with the incubation time up to 100 min. The FSTs significantly reduced DCF fluorescence intensity, resulting in the increased scavenging activity against intracellular ROS in a concentration-dependent manner. The presence of FSTs extract at the concentrations of 50 and 100 μg per ml led to a remarkable reduction in fluorescent intensity. These results confirmed that FSTs could exert a substantial effect against intracellular ROS formation.

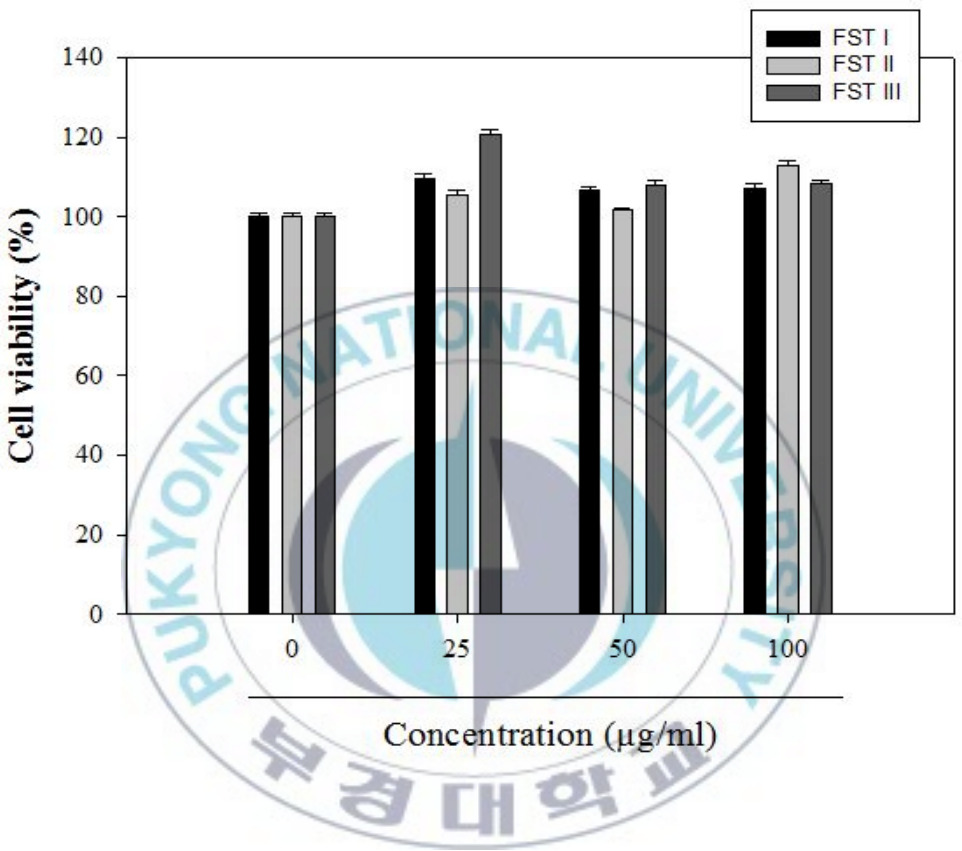


Fig. 2. Effect of fermented sea tangle extracts (FSTs) on cell cytotoxicity in RAW 264.7 cells.

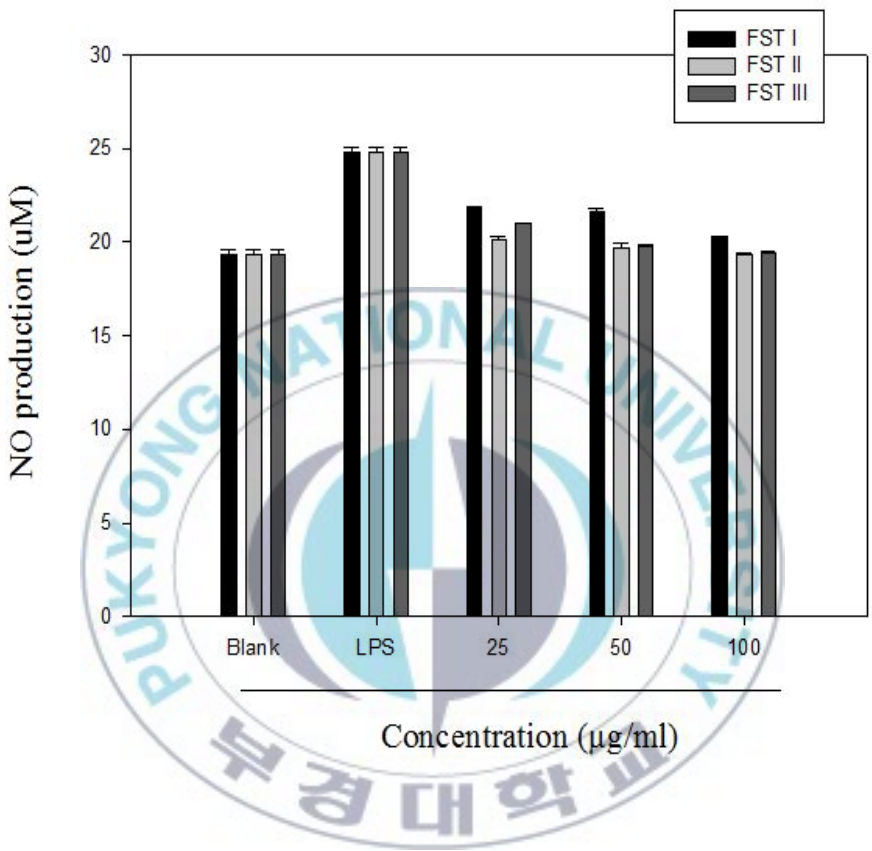


Fig. 3. Effect of fermented sea tangle extracts (FSTs) on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 cells. FSTs were treated with in the presence of LPS (1 μg/mL). Blank: -LPS; LPS: LPS (1 μg/mL); FST I (>10 kDa), FST II (1-10 kDa) and FST III (<1kDa).

3. Cellular radical scavenging effect

Intracellular ROS scavenging was examined using fluorescence sensitive dye. The DCFH-DA fluorescent intensity was significantly decreased by FSTs extract in a time and concentration dependent manners on RAW 264.7 cells. As shown in (Fig. 4), the progressive increments in DCF fluorescence intensity due to the hydrogen peroxide generation were observed with the incubation time up to 100 min. The FSTs significantly reduced DCF fluorescence intensity, resulting in the increased scavenging activity against intracellular ROS in a concentration-dependent manner. The presence of FSTs extract at the concentrations of 50 and 100 μg per ml led to a remarkable reduction in fluorescent intensity. These results confirmed that FSTs could exert a substantial effect against intracellular ROS formation.

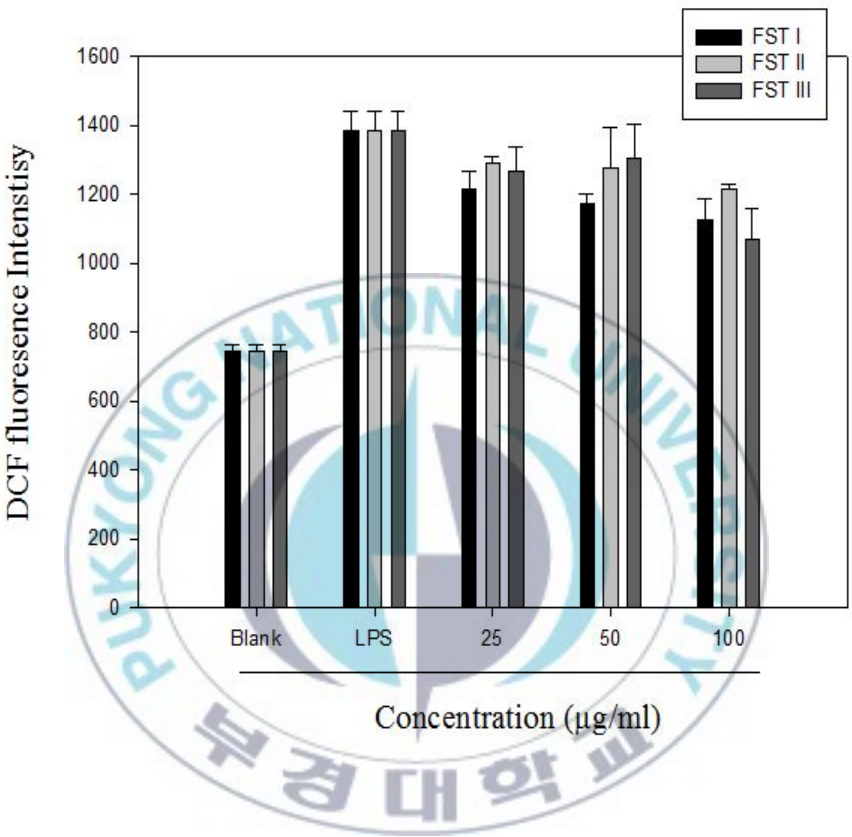


Fig. 4. Scavenging effect of fermented sea tangle extracts (FSTs) on intracellular generation of ROS in Lipopolysaccharide (LPS)-induced Raw 264.7 cells. FSTs were treated with in the presence of LPS (1 μg/mL). Blank: -LPS; LPS: +LPS (1 μg/mL); FST I (>10 kDa), FST II (1-10 kDa) and FST III (<1kDa).

4. Inhibition of membrane lipid peroxidation

The levels of lipid hydroperoxides in the presence or absence of FSTs were examined by use of specific fluorescence probe, DPPP (Fig. 5). DPPP has been used as a sensitive method for the measuring lipid hydroperoxides of cell membrane, since it successfully incorporate into membranes and oxidize with hydroperoxides to emit DPPP-oxide fluorescence (Rajapakse et al., 2007). When the DPPP-labeled RAW 264.7 cells were treated with LPS, the fluorescent intensity derived from DPPP oxide steadily increased about 1.5- to 1.8-fold due to peroxyl radical mediated membrane lipid peroxidation. Treatment with FSTs led to concentration-dependent reduction in the fluorescent intensity. The reduction in fluorescent intensity was compared to the blank (LPS non-stimulated), confirming FSTs could exert a substantial effect against oxidation of membrane lipids. At high concentration more than 100 μg per ml, FSTs has exhibited a similar fluorescent intensity with blank group. In comparison analysis, FST III showed highest activity on the inhibition of NO and radical formation than FST I and FST II in LPS-induced RAW 264.7 cells. Accordingly, FST III was selected to evaluate further anti-inflammatory effect.

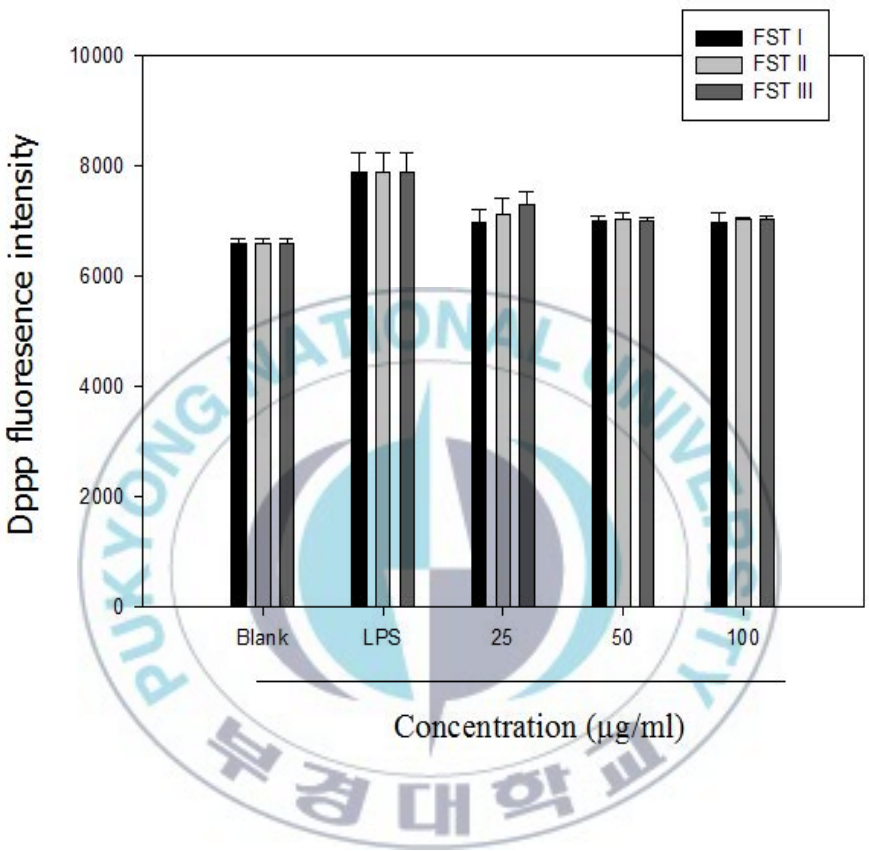


Fig. 5. Inhibition of fermented sea tangle extracts (FSTs) on membrane lipid peroxidation in lipopolysaccharide (LPS)-induced Raw 264.7 cells. FSTs were treated with in the presence of LPS (1 μg/mL). Blank: -LPS; LPS: +LPS (1 μg/mL); FST I (>10 kDa), FST II (1-10 kDa) and FST III (<1kDa).

5. Effect of FSTs on the regulation of inflammatory response genes and proteins

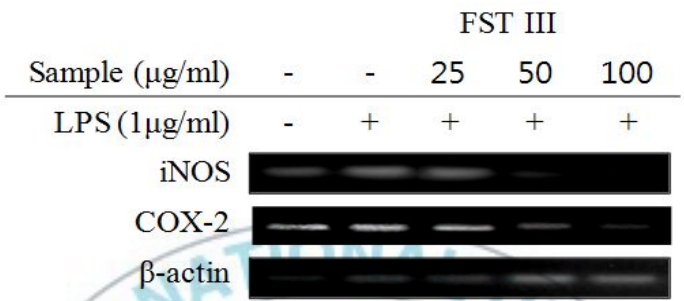
Inflammatory processes are mediated by multiple molecular mechanisms. iNOS and COX-2 play a pivotal role in immunity against infectious agents by producing an excess amount of NO and PGE2, respectively; these enzymes have attracted attention for their detrimental roles in inflammation related disease (Yun et al., 1996, Kim et al., 2009)

TNF-a, IL-1β and IL-6 are primary inflammatory cytokines which play an essential role during the inflammatory process (Trikha et al., 2003). The pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β are small secreted proteins, which mediate and regulate immunity and inflammation (Huang et al., 2006). TNF-α production is crucially required for the synergistic induction of NO synthesis in LPS-induced macrophages. TNF-α elicits a number of physiological effects such as septic shock, inflammation, cachexia, and cytotoxicity. IL-1β is found in thecirculation following Gram-negative sepsis, which is a mediator of the gost inflammatory response in innate immunity (Roshak st al., 1996). Additionally, the production of IL-6 is induced by several factors, TNF-α, IL-1β as well as the bacterial endotoxin, LPS. IL-6, a pro-inflammatory cytokine, acts as an endogenous

pyrogen in addition to its multiple effects on the immune system and in particular on hematopotesis (Van Snick., 1990).

It was evaluated whether FST III affects the inflammatory response cytokine mRNA and protein expression in LPS-induced RAW 264.7 cells, using RT-PCR and Western blot analysis, respectively (Fig. 6). The mRNA transcription and protein levels of iNOS, COX-2, IL-6, IL-1β and TNF-α were reduced by FST III treatment, which was consistent with the results obtained from NO production. Therefore, FST III prevented the production of NO and inflammatory cytokines by suppressing their mRNA transcription and protein expression in LPS-induced RAW 264.7 cells (Fig. 7). As a result, FST III was shown to have anti-inflammatory activities by reducing mRNA and protein expression levels of inflammatory cytokines.

(A)



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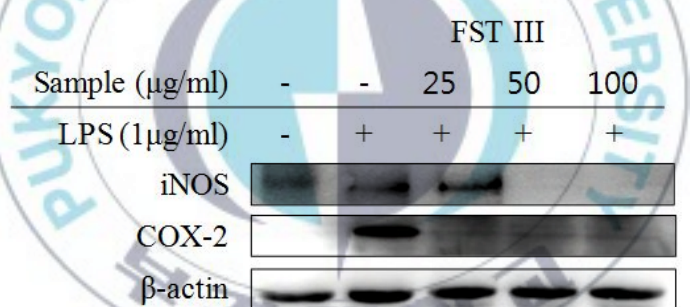
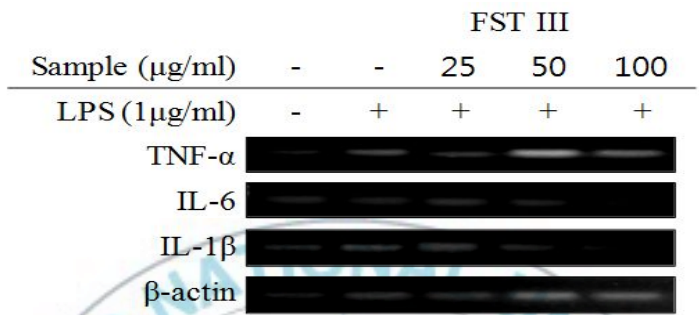


Fig. 6. Effect of fermented sea tangle extract sub-fraction III (FST III) on the regulation of iNOS and COX-2 expression in LPS- induced in RAW 264.7 cells. RAW264.7 cells were co-cultured with various concentrations of FST III for 1 h and then stimulated with lipopolysaccharide (LPS; 1 μg/ml) for 24 h. Gene (a) and protein (b) expression levels were determined by RT-PCR and Western blot analysis, respectively.

(A)



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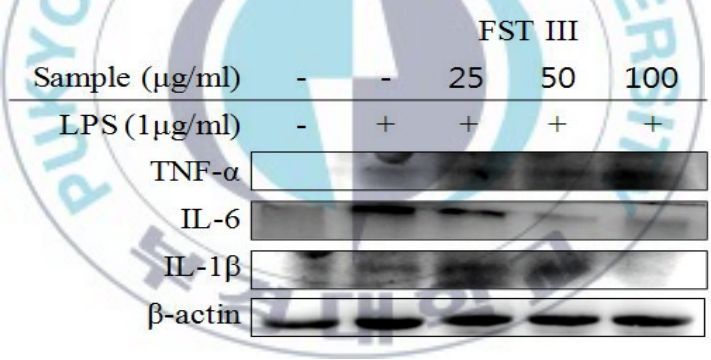

Fig. 7. Effect of fermented sea tangle extract sub-fraction III (FST III) on the regulation of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β in RAW 264.7 cells. RAW 264.7 cells were co-cultured with various concentrations of FSTs for 1 h and then stimulated with lipopolysaccharide (LPS; 1 μg/ml) for 24 h. Gene (a) and protein (b) expression levels were determined by RT-PCR and Western blot analysis, respectively

6. Effect of FSTs on NF-κB signaling pathway

Nuclear factor kappa B (NF-κB) is known to be involved in the inducible expression of various genes that regulate inflammatory actions. (Aggarwal et al., 2006). In unstimulated cells, NF-κB is constitutively localized in the cytosol as a heterodimer by physical association with an inhibitory protein called inhibitor κB (IκB). Various stimuli, such as LPS, cytokines, activators of protein kinase C, oxidants and viruses, activate several signal transduction pathways that all lead to phosphorylation and degradation of IκB and subsequent activation of NF-κB (Hawiger, 2001). Following activation, the NF-κB heterodimer is rapidly translocated to nucleus, where it activates the transcription of target genes, including genes encoding for pro-inflammatory cytokines, adhesion molecules, chemokines and inducible enzymes such as iNOS and COX-2 (Hayden et al., 2006; Ghosh et al., 2008).

The role of the NF-κB signaling pathway in the anti-inflammatory responses in RAW 264.7 cells treated with FST III was confirmed using Western blot analysis (Fig. 8). Inflammatory gene expression can be regulated by NF-κB, which is important for mediating cytokine production in LPS- induced macrophages. As shown in (Fig. 8), NF-κB p65 and p50

were suppressed depending on the concentrations of FSTs treatment. These results indicated that signal transduction of NF-κB might be suppressed by FST III.

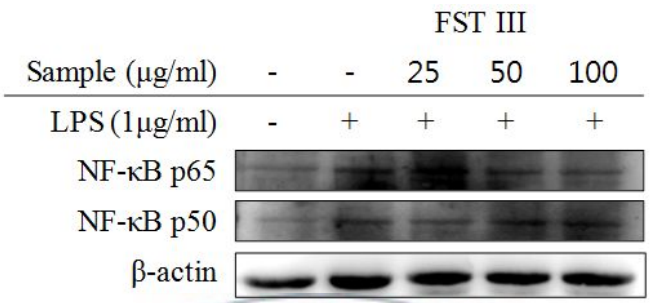

Fig. 8. Effect of fermented sea tangle extract sub-fraction III (FST III) on the transcriptional activation of nuclear factor kappa B (NF-κB) in lipopolysaccharide (LPS)-induced RAW 264.7 cells. RAW 264.7 cells were co-cultured with various concentrations of FST III for 1 h and then stimulated with LPS (1 μg/ml) for 24 h.

Conclusion

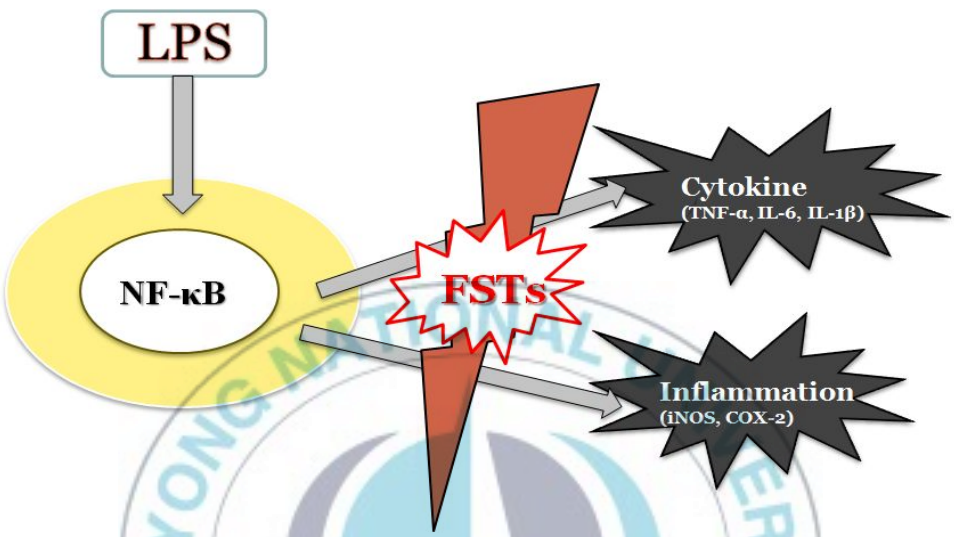

Fig. 9. Effect of fermented sea tangle extracts (FSTs) against lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 cells

This study demonstrated that FST effectively inhibited excessive production in inflammatory mediators such as NO, TNF-α, IL-1β and IL-6. These results suggest that FSTs inhibited NO production, biosynthesis of cytokines and expression of inflammatory-related genes in LPS-activated RAW 264.7 cells. Moreover, these anti-inflammatory profiles of FSTs were

mediated through the inhibition of NF-κB transcriptional inductions. Accordingly, these results underscore the nutraceutical value of FSTs as a potential anti-inflammatory agent via attenuation of inflammatory responses or processes.

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