Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Inhibitory effect of Jeju endemic seaweeds on the production of pro-inflammatory mediators in mouse macrophage cell line RAW 264.7*

Eun-Jin YANG[1,2], Ji-Young MOON[1,2], Min-Jin KIM[1,2], Dong Sam KIM[1,2], Chan-Shick KIM[3], Wook Jae LEE[1,2], Nam Ho LEE[2,4], Chang-Gu HYUN[†‡1,2]

([1]*Jeju Biodiversity Research Institute (JBRI), Jeju High-Tech Development Institute (HiDI), Jeju 699-943, Korea*)

([2]*Jeju Seaweed Industry Development Agency, Cheju National University, Jeju 690-756, Korea*)

([3]*Faculty of Biotechnology, Cheju National University, Jeju 690-756, Korea*)

([4]*Department of Chemistry, Cheju National University, Jeju 690-756, Korea*)

[†]E-mail: cghyun@jejuhidi.or.kr

Received Nov. 13, 2009; Revision accepted Feb. 2, 2010; Crosschecked Mar. 30, 2010

Abstract: Seaweed has been used in traditional cosmetics and as a herbal medicine in treatments for cough, boils, goiters, stomach ailments, and urinary diseases, and for reducing the incidence of tumors, ulcers, and headaches. Despite the fact that seaweeds are frequently used in the practice of human health, little is known about the role of seaweed in the context of inflammation. This study aimed to investigate the influence of Jeju endemic seaweed on a mouse macrophage cell line (RAW 264.7) under the stimulation of lipopolysaccharide (LPS). Ethyl acetate extracts obtained from 14 different kinds of Jeju seaweeds were screened for inhibitory effects on pro-inflammatory mediators. Our results revealed that extracts from five seaweeds, *Laurencia okamurae*, *Grateloupia elliptica*, *Sargassum thunbergii*, *Gloiopeltis furcata*, and *Hizikia fusiformis*, were potent inhibitors of the production of pro-inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Based on these results, the anti-inflammatory effects and low cell toxicity of these seaweed extracts suggest potential therapeutic applications in the regulation of the inflammatory response.

Key words: Nitric oxide, Interleukin-6 (IL-6), Prostaglandin E_2 (PGE_2), Tumor necrosis factor-α (TNF-α), Seaweeds, Pro-inflammatory mediators

doi:10.1631/jzus.B0900364 **Document code:** A **CLC number:** Q94

1 Introduction

The inflammatory response serves to protect the host against tissue wounds and microbial infections. An appropriate and proper inflammatory response depends on the careful regulation of a number of mediators such as cytokines, which are secreted by inflammatory cells such as macrophages and neutrophils (Park *et al*., 2006; Zhou *et al*., 2007). Macrophages are known to play a pivotal role in the host's defense against harmful materials and are involved in a variety of diseases including autoimmune diseases, pathogenic infections, and inflammatory disorders (Dokka *et al*., 2001; Kang *et al*., 2008). An inflammatory stimulus such as lipopolysaccharide (LPS) can activate macrophages to produce a variety of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), and other inflammatory mediators including prostaglandins and nitric oxide (NO), which are catalyzed

‡ Corresponding author

* Project partially supported by the Jeju Sea-green Program for the Regional Innovation System and the Regional Technology Innovation Program (No. RTI04-02-07) of the Ministry of Knowledge and Economy, Korea

by cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), respectively.

Overproduction of NO by iNOS has been implicated in the pathology of several inflammatory disorders including septic shock, tissue damage following inflammation, and rheumatoid arthritis. Therefore, NO production induced by LPS through iNOS can reflect the degree of inflammation, and a change in NO level through inhibition of iNOS enzyme activity or iNOS induction provides a means of assessing the effect of agents on the inflammatory process (Zhou *et al*., 2007; Choi *et al*., 2008; Kanwar *et al*., 2009; O'Connor and O'Brien, 2009; Murakami, 2009). Prostaglandins also play a major role as mediators of the inflammatory response. COX is an enzyme that converts arachidonic acid to prostaglandin. It is responsible for the production of large amounts of pro-inflammatory prostaglandins at the inflammatory site, and its uncontrolled activity is thought to play an important role in the pathogenesis of many chronic inflammatory diseases (Zhou *et al*., 2007; Rao and Knaus, 2008; Scher and Pillinger, 2009; Iyer *et al*., 2009). In addition, cytokines like TNF-α and IL-6 have been reported to be pro-inflammatory in vitro and in vivo, and the production of TNF-α is known to be crucial for the induction of NO synthesis in interferon-γ (IFN-γ) and/or LPS-stimulated macrophages. TNF-α elicits a number of physiological effects that include septic shock, inflammation, cachexia, and cell death. Similarly, IL-6 is also considered to be a pivotal pro-inflammatory cytokine; for example, it is regarded as an endogenous mediator of LPS-induced fever (Kim J.Y. *et al*., 2008; de Benedetti, 2009; Fonseca *et al*., 2009; Radovits *et al*., 2009; Esposito and Cuzzocrea, 2009). Therefore, pharmacological interference with the production of NO, PGE_2, and cytokines such as TNF-α and IL-6 is postulated to be useful for reducing many inflammatory disease states that are mediated by excessive and/or prolonged activation of macrophages.

Marine organisms have proven to be rich sources of structurally novel and biologically active natural compounds. These compounds have served as important chemical prototypes for the discovery of new drugs for use in the treatment of various human diseases (Usami, 2009; Zhang and Kim, 2009; Blunt *et al*., 2009). Jeju Island, the largest island in Korea, is located in the southwest of the Korean Strait, and is well known for its distinctive environment. In particular, the sea levels around this island are known to fluctuate rapidly as a result of global warming. Therefore, in response to this unusual environment, the seaweeds that are present on Jeju Island may possess substantial endogenous protective mechanisms (Kim K.N. *et al*., 2009; Kim M.M. *et al*., 2009).

Some studies on seaweed-derived anti-inflammatory compounds have investigated potential inhibitory effects in in vitro systems, using LPS-stimulated macrophages. Therefore, bacterial LPS has become one of the best characterized stimuli used to induce the up-regulation of pro-inflammatory proteins. However, there is still a lack of methods for evaluating the anti-inflammatory efficacy and mechanisms of action of anti-inflammatory compounds (Dang *et al*., 2008; Kim S.K. *et al*., 2008; Kim K.N. *et al*., 2009; Kim M.M. *et al*., 2009). Hence, in this study we examined the inhibitory effect of Jeju seaweeds on NO, PGE_2, TNF-α, and IL-6 production.

2 Materials and methods

2.1 Plant materials

Most seaweeds were collected between March and July, 2006 from Jeju Island, Korea. The voucher specimens are deposited at the herbarium of the Jeju Biodiversity Research Institute (JBRI), Jeju, Korea. The materials for extraction were cleaned, dried at room temperature for two weeks, and ground into a fine powder. The dried algae (50 g) were extracted with 80% ethanol (EtOH; 2 L) at room temperature for 24 h and then evaporated under vacuum. The evaporated EtOH extract (10 g) was suspended in water (1 L) and partitioned with ethyl acetate (EtOAc; 1 L), and this partition was repeated three times.

2.2 Analysis of total phlorotannin

To determine the total phlorotannin content in the seaweed extracts, the adjusted method with Folin-Ciocalteu reagent (Merck) was used. We added 550 μl of distilled water/Folin-Ciocalteu solution (10:1, v/v) to 50 μl of diluted extract (1 mg/ml of ethanol). After 3 min, 200 μl of 2 mol/L sodium carbonate (Na_2CO_3) and 300 μl of distilled water were added. After 1 h standing at laboratory temperature,

absorbance was measured at 725 nm. The total phlorotannin content was calculated as a phloroglucinol equivalent from the calibration curve of phloroglucinol standard solutions (concentration range, 0–1.0 mg/ml). All measurements were conducted in triplicate.

2.3 Cell culture

Murine RAW 264.7 macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (w/v) fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, all from GIBCO (Grand Island, NY, USA), in an incubator at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.4 Cell viability

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. RAW 264.7 cells were cultured in 96-well plates for 18 h, followed by treatment with various concentrations (3.125, 6.25, 12.5, 25, 50, or 100 μg/ml) of the EtOAc fractions of Jeju seaweeds. After a 24-h incubation, MTT was added to the medium for 4 h. Finally, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulphoxide (DMSO). Absorbance was measured at 540 nm. The percentage of cells showing cytotoxicity was determined relative to the control group.

2.5 Determination of NO concentration

RAW 264.7 cells (5×10^5 cells/well) were incubated in 24-well dishes for 18 h with 1 μg/ml of LPS and various concentrations (3.125, 12.5, 25, 50, or 100 μg/ml) of the EtOAc fractions of Jeju seaweeds. The presence of nitrite, a stable oxidized product of NO, was determined in cell-culture media by a modified Griess method. The culture supernatant (100 μl) was mixed with the same volume of Griess reagent (1% (w/v) sulfanilamide and 0.1% (w/v) *N*-[1-naphthyl]-ethylenediamine dihydrochloride in 5% (w/v) phosphoric acid) for 10 min, and the absorbance was measured at 540 nm.

2.6 Measurement of PGE_2

The RAW 264.7 cells were cultured in 24-well plates for 18 h, followed by treatment with LPS in the presence of various concentrations (3.125, 12.5, 25, 50, or 100 μg/ml) of samples. After a 24-h incubation, PGE_2 in the culture supernatants was measured by an enzyme-linked immunosorbent assay (ELISA) kit.

2.7 Cytokine assays

The amounts of TNF-α and IL-6 in the cell-culture supernatant and in serum were measured using an ELISA kit (R & D, Minneapolis, MI). RAW 264.7 cells were plated in a 24-well cell-culture plate at a density of 2.5×10^5 cells/well and incubated with various concentrations (3.125, 12.5, 25, 50, or 100 μg/ml) of seaweed extracts in 1 μg/ml LPS for 24 h. The culture supernatant was collected and assayed according to the manufacturer's instructions to determine the amounts of TNF-α and IL-6 that had been released from the cells.

2.8 Statistical analysis

All results were expressed as mean±standard error (SE). Each experiment was repeated at least three times. Statistical significances were compared between each treated group and analyzed by the Student's *t*-test. Data with $P<0.05$ were considered statistically significant.

3 Results and discussion

Since the overproduction of NO is harmful and results in various inflammatory and autoimmune diseases, pharmacological interference with the NO production cascade offers promising strategies for therapeutic intervention in inflammatory disorders. Therefore, we first investigated the effect of Jeju seaweed extracts on NO synthesis in activated macrophages. Murine macrophage RAW 264.7 cells can induce iNOS transcription and protein synthesis, and subsequent NO production in response to LPS stimulation alone. This cell system is an excellent model for evaluating topical agents and for screening potential inhibitors of the pathways that induce iNOS and NO production. In our search for natural products that possess anti-inflammatory activity, we prepared 80% ethanol crude extracts from 14 native seaweeds from Jeju Island, Korea. The evaporated EtOH extract was partitioned with ethyl acetate. All ethyl acetate seaweed fractions were dissolved in 80% ethanol and diluted with sterile water to normalise the concentration

of the test sample. The Griess reaction, a spectrophotometric determination for nitrite, was carried out to quantify the nitrite levels in the conditioned medium of RAW 264.7 cells treated with LPS. The final concentration of ethanol in the culture media was 0.1%, and this concentration of ethanol did not show any effect on the assay systems.

Table 1 shows the inhibitory effect of seaweed extracts on NO production by LPS-activated macrophages. Of the 14 extracts, 13 extracts showed greater than 50% inhibition of NO production at a concentration of 100 μg/ml in the culture medium. Among these 12 extracts, 5 (*Laurencia okamurae*, *Grateloupia elliptica*, *Sargassum thunbergii*, *Gloiopeltis furcata*, and *Hizikia fusiformis*) showed the most potent inhibition in a concentration-dependent manner (Fig. 1). Their half maximal inhibitory concentration (IC_{50}) values were 12.6, 28.5, 20.2, 21.2, and 26.8 μg/ml, respectively. The numbers of viable activated macrophages were not significantly altered by the 14 extracts as determined by MTT assays (Table 1 and Fig. 1), thereby indicating that the inhibition of NO synthesis by the seaweed extracts was not simply the result of cytotoxic effects (Yang *et al*., 2009).

COX is a key enzyme catalyzing the rate-limiting step in the biosynthesis of prostaglandins from arachidonic acid. Growing evidence indicates that COX-2 plays a key role in several biological processes such as inflammation and tumorigenesis. This is because the targeted inhibition of COX-2 is a promising approach to inhibiting inflammation and carcinogenesis. Therefore, we examined the effects of Jeju seaweed extracts on PGE_2 production in LPS-stimulated RAW 264.7 macrophages.

When the macrophages were stimulated with LPS (1 μg/ml) for 24 h, the levels of PGE_2 increased in the culture medium. Except for *Ishige okamurae* and *Bonnemaisonia hamifera*, 100 μg/ml of all seaweed samples suppressed the LPS-induced PGE_2 production (Table 1). Among the seaweed species, extracts from *L. okamurae*, *G. elliptica*, *S. thunbergii*, *G. furcata*, and *H. fusiformis* produced the most active preparations, giving IC_{50} values of 11.7, 15.0, 14.9, 9.7, and 15.0 μg/ml, respectively. They also inhibited LPS-induced PGE_2 production in a dose-dependent manner (Fig. 2).

Inflammation is activated in response to appropriate extracellular stimulation, most often by stress

Table 1 Inhibitory effect of Jeju seaweeds on the production of pro-inflammatory mediators

Specimen number	Scientific name	Inhibition rate (%)					Phlorotannin content (μg/ml)
		NO	PGE_2	IL-6	TNF-α	Cytotoxicity	
JBR243	*Sargassum nipponicum*	72.1±2.7**	86.2±0.8**	74.9±7.2**	90.0±2.6**	132.6±5.2	58.0
JBR256	*Myelophycus simplex*	81.9±0.3**	65.0±2.2**	74.5±4.5**	51.8±1.2**	103.5±1.6	33.0
JBR270	*Chondrus ocellatus*	78.2±0.3**	90.7±1.0**	83.1±2.4**	71.0±1.9**	106.1±12.4	37.2
JBR271	*Ishige okamurae*	30.8±0.9*	NE	47.1±0.7*	3.1±0.8*	118.6±3.8	332.5
JBR274	*Bonnemaisonia hamifera*	81.8±0.1**	NE	18.0±0.6**	73.8±0.0**	99.5±1.0	72.2
JBR345	*Scytosiphon lomentaria*	70.4±0.2**	89.9±1.5**	90.4±0.2**	55.2±0.3**	93.6±6.9	65.0
JBR354	*Callophyllis crispata*	83.6±0.0**	84.7±3.1**	90.4±0.5**	75.7±0.9**	79.7±5.9	40.0
JBR368	*Padina arborescens*	59.7±0.4**	80.5±0.9**	71.3±3.7**	80.9±0.7**	87.8±8.7	124.0
JBR375[a]	*Laurencia okamurae*	82.4±0.1**	82.1±4.2**	76.9±0.7**	52.5±0.0**	91.4±2.4	37.3
JBR376	*Chondria crassicaulis*	75.7±0.0**	88.4±1.6**	84.8±0.6**	67.1±0.3**	87.5±5.3	54.7
JBR510[b]	*Grateloupia elliptica*	78.2±0.4**	81.0±4.8**	85.7±0.8**	63.8±0.8**	89.4±6.9	47.7
JBR511[b]	*Sargassum thunbergii*	77.3±0.2**	73.8±4.0**	46.9±4.4**	63.1±1.2**	98.0±3.7	44.8
JBR518[b]	*Gloiopeltis furcata*	77.6±0.5**	85.6±0.6**	82.1±2.2**	70.9±1.3**	108.3±4.8	53.3
JBR538[b]	*Hizikia fusiformis*	77.2±0.1**	81.0±4.8**	69.9±6.7**	61.5±3.9**	105.8±3.5	43.3

The presence of nitrite, as a stable oxidized product of NO, was determined in cell-culture media by a modified Griess method. The amounts of PGE_2, TNF-α, and IL-6 in the cell-culture supernatant and serum were measured using an ELISA kit. RAW 264.7 cells were incubated with various concentrations of seaweed extracts (25[a], 50[b], or 100 μg/ml). Cell viability was determined by MTT assay. The data represent the mean±SD of triplicate experiments. * $P<0.05$, ** $P<0.01$ vs. LPS alone. NE: no effects

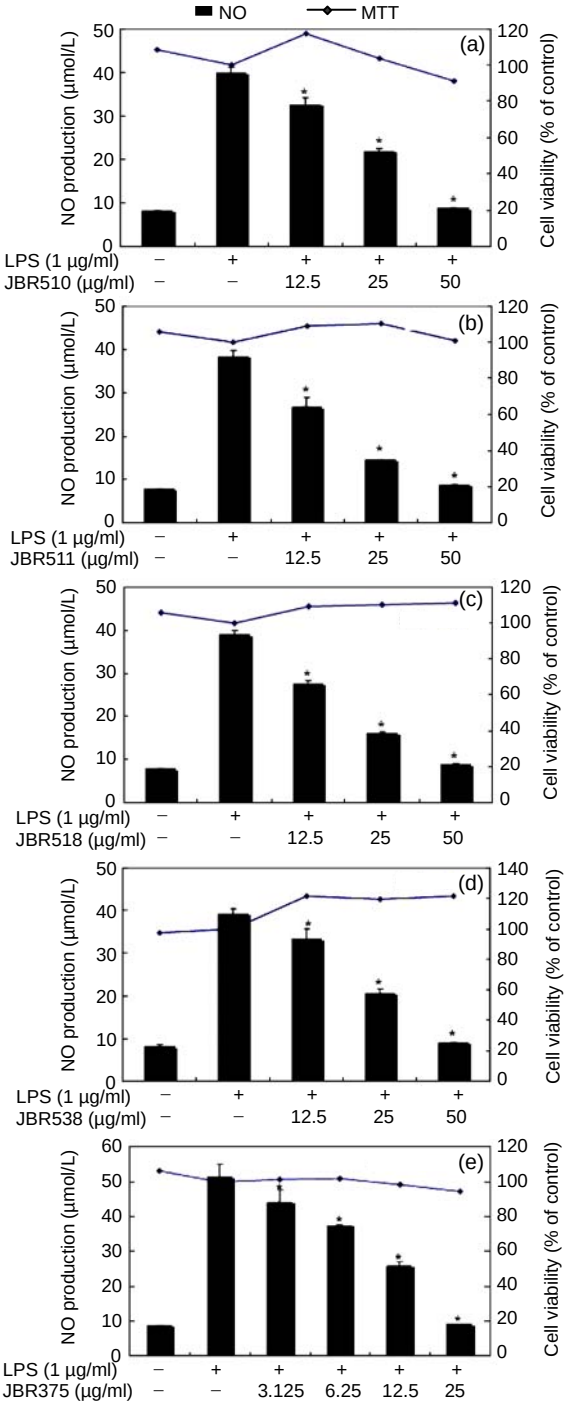

Fig. 1 Effect of Jeju seaweed extracts on nitric oxide production in LPS-stimulated RAW 264.7 cells

The cells were stimulated with 1 μg/ml of LPS only or with a combination of LPS and various concentrations (3.125, 12.5, 25, or 50 μg/ml) of seaweed varieties JBR510 (a), JBR511 (b), JBR518 (c), JBR538 (d), and JBR375 (e) for 24 h. Nitric oxide production was determined by the Griess reagent method. Cell viability was determined using the 24-h culture of cells stimulated with LPS (1 μg/ml) in the presence of each sample. The data represent the mean±SD of triplicate experiments. * P<0.05, ** P<0.01 vs. LPS alone

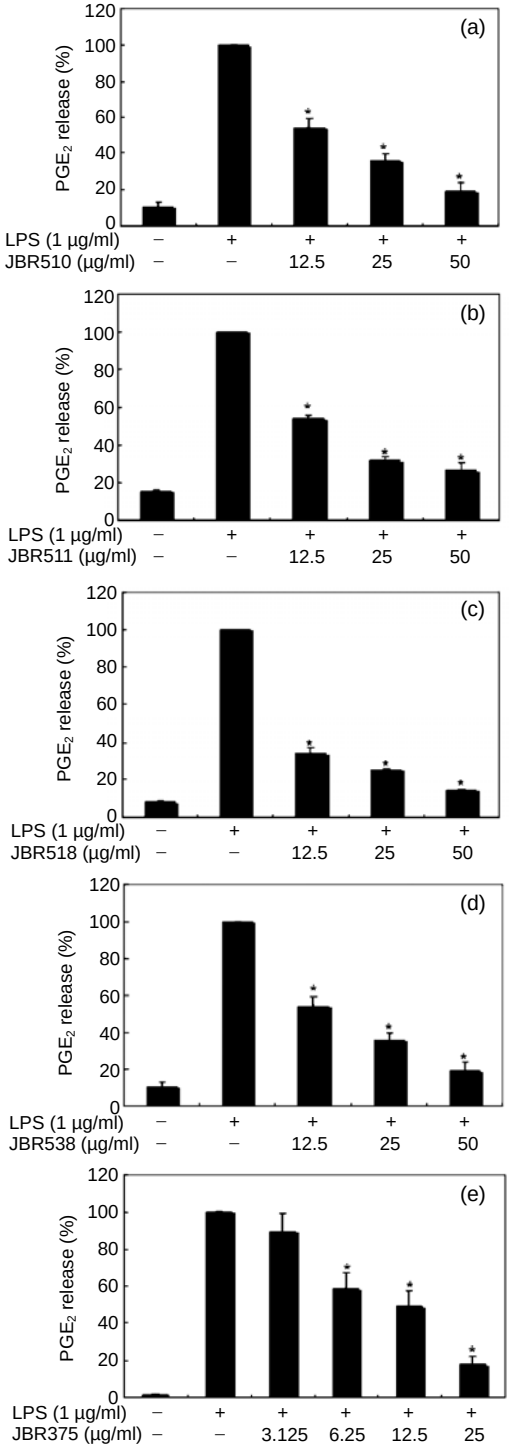

Fig. 2 Effect of Jeju seaweed extracts on PGE_2 production in LPS-stimulated RAW 264.7 cells

The cells were stimulated with 1 μg/ml of LPS only or with a combination of LPS and various concentrations (3.125, 12.5, 25, or 50 μg/ml) of JBR510 (a), JBR511 (b), JBR518 (c), JBR538 (d), and JBR375 (e) for 24 h. PGE_2 produced and released into the culture medium was assayed by the ELISA method. The data represent the mean±SD of triplicate experiments. * P<0.05, ** P<0.01 vs. LPS alone

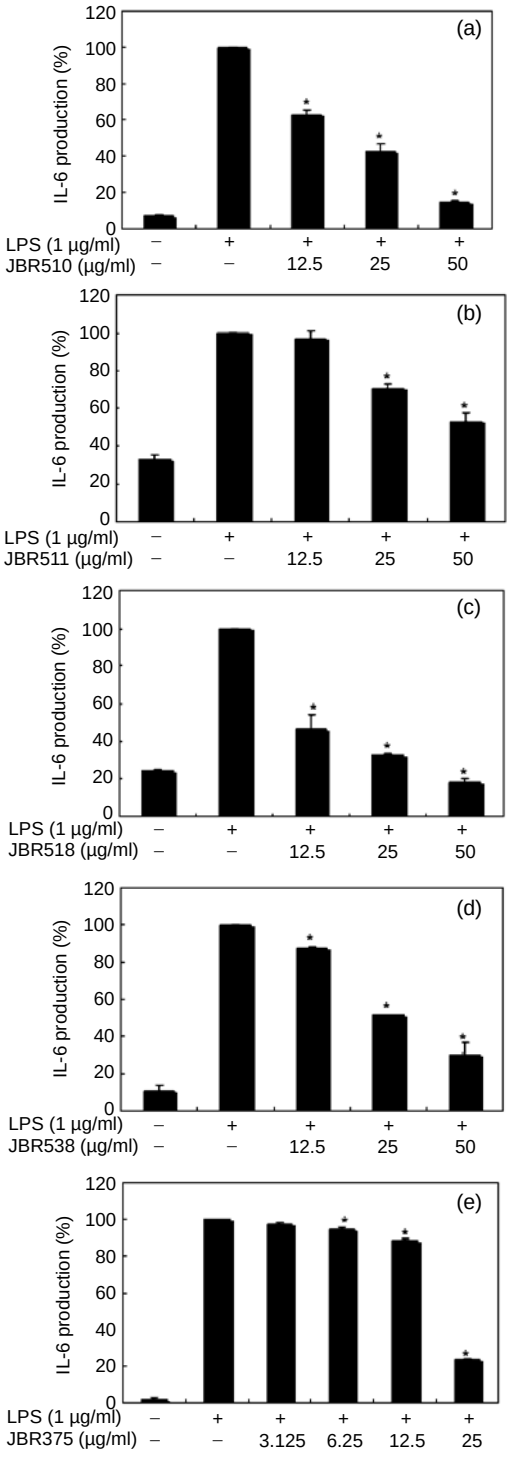

Fig. 3 Effect of Jeju seaweed extracts on IL-6 production in LPS-stimulated RAW 264.7 cells

The cells were stimulated with 1 μg/ml of LPS only or with a combination of LPS and various concentrations (3.125, 12.5, 25, or 50 μg/ml) of JBR510 (a), JBR511 (b), JBR518 (c), JBR538 (d), and JBR375 (e) for 24 h. IL-6 produced and released into the culture medium was assayed by the ELISA method. The data represent the mean±SD of triplicate experiments. * P<0.05, ** P<0.01 vs. LPS alone

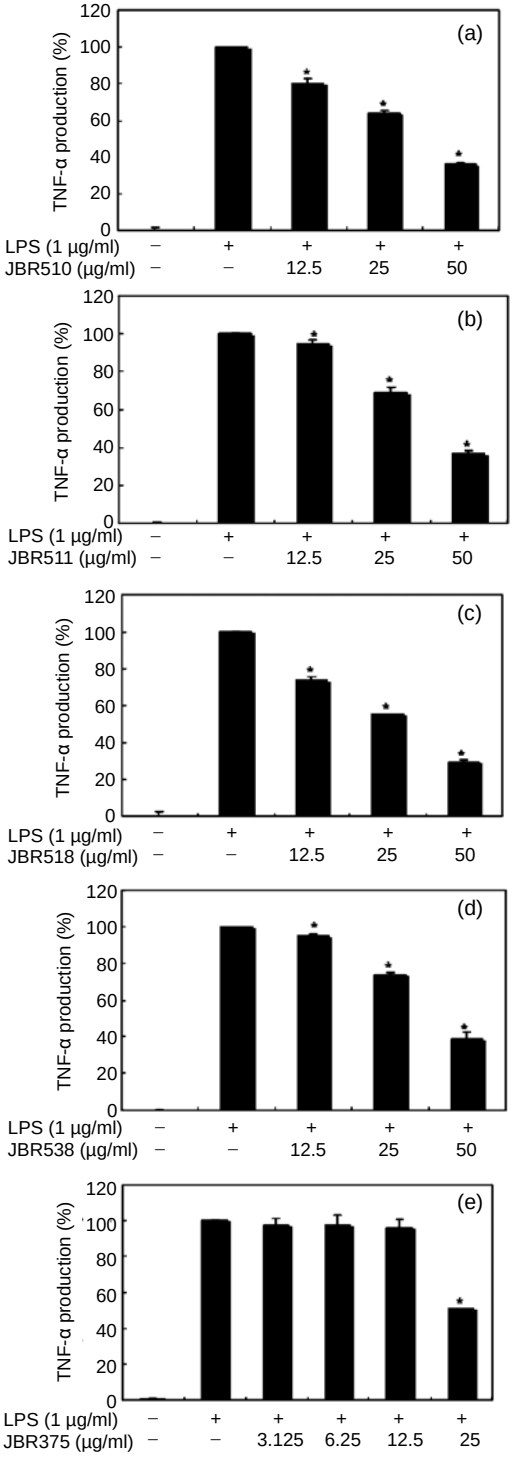

Fig. 4 Effect of Jeju seaweed extracts on TNF-α production in LPS-stimulated RAW 264.7 cells

The cells were stimulated with 1 μg/ml of LPS only or with a combination of LPS and various concentrations (3.125, 12.5, 25, or 50 μg/ml) of JBR510 (a), JBR511 (b), JBR518 (c), JBR538 (d), and JBR375 (e) for 24 h. TNF-α produced and released into the culture medium was assayed by the ELISA method. The data represent the mean±SD of triplicate experiments. * P<0.05, ** P<0.01 vs. LPS alone

or pro-inflammatory cytokines including TNF-α and IL-6, and by bacterial components including LPS through the Toll-like receptors. As Jeju seaweed extracts potently inhibited the pro-inflammatory mediators, we further investigated their effects on LPS-induced IL-6 and TNF-α release by enzyme immunoassay. After 24-h incubation with both LPS (1 μg/ml) and solvent fractions of seaweed (12.5, 25, or 50 μg/ml), there was remarkable inhibition of TNF-α and IL-6. Extracts from *L. okamurae*, *G. elliptica*, *S. thunbergii*, *G. furcata*, *H. fusiformis* and other seaweeds inhibited the production of IL-6 and TNF-α in a dose-dependent manner (Figs. 3 and 4). Furthermore, the *L. okamurae* fractions (25 μg/ml) significantly decreased the levels of IL-6 and TNF-α production by up to 76.9% and 52.5%, respectively.

Phlorotannins are aromatic secondary plant metabolites, widespread in the seaweeds that are associated with the nutritional, anti-inflammatory and antioxidant properties of various foods. Therefore, the total phlorotannin content of each of the 14 seaweeds was assessed. Four extracts, from *Ishige okamurae*, *Padina arborescens*, *Bonnemaisonia hamifera*, and *Scytosiphon lomentaria*, showed high phlorotannin content (>60 μg/mg) (Table 1).

Considering these results, we suggest that the Jeju seaweed extracts in this study, especially those from *L. okamurae*, *G. elliptica*, *S. thunbergii*, *G. furcata*, and *H. fusiformis*, may be considered as possible candidates for anti-inflammatory agents. Further investigations will focus on the in vivo assessment of the biological activity of seaweed extracts and on the chemical identification of the major active components responsible for anti-inflammatory activity in the efficacious seaweed extracts. The efficacies of *I. okamurae* (Kim K.N. *et al*., 2009; Kim M.M. *et al*., 2009), *S. thunbergii* (Samee *et al*., 2009) and *G. furcata* (Bae and Choi, 2007) extracts have already been reported. To the best of our knowledge, this is the first report demonstrating the in vitro anti-inflammatory activities of *S. nipponicum*, *M. simplex*, *C. ocellatus*, *B. hamifera*, *S. lomentaria*, *C. crispata*, *P. arborescens*, *L. okamurae*, *C. crassicaulis*, *G. elliptica*, and *H. fusiformis* extracts, and providing a scientific basis for their application in human health.

References

Bae, S.J., Choi, Y.H., 2007. Methanol extract of the seaweed *Gloiopeltis furcata* induces G2/M arrest and inhibits cyclooxygenase-2 activity in human hepatocarcinoma HepG2 cells. *Phytotherapy Research*, **21**(1):52-57. [doi:10.1002/ptr.2020]

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H., Northcote, P.T., Prinsep, M.R., 2009. Marine natural products. *Natural Product Reports*, **26**(2):170-244. [doi:10.1039/b805113p]

Choi, H.J., Eun, J.S., Park, Y.R., Kim, D.K., Li, R., Moon, W.S., Park, J.M., Kim, H.S., Cho, N.P., Cho, S.D., *et al*., 2008. Ikarisoside A inhibits inducible nitric oxide synthase in lipopolysaccharide-stimulated RAW 264.7 cells via p38 kinase and nuclear factor-kappaB signaling pathways. *European Journal of Pharmacology*, **601**(1-3):171-188. [doi:10.1016/j.ejphar.2008.09.032]

Dang, H.T., Lee, H.J., Yoo, E.S., Shinde, P.B., Lee, Y.M., Hong, J., Kim, D.K., Jung, J.H., 2008. Anti-inflammatory constituents of the red alga *Gracilaria verrucosa* and their synthetic analogues. *Journal of Natural Products*, **71**(2):232-240. [doi:10.1021/np070452q]

de Benedetti, F., 2009. Targeting interleukin-6 in pediatric rheumatic diseases. *Current Opinion in Rheumatology*, **21**(5):533-537. [doi:10.1097/BOR.0b013e32832f1445]

Dokka, S., Shi, X., Leonard, S., Wang, L., Castranova, V., Rojanasakul, Y., 2001. Interleukin-10-mediated inhibition of free radical generation in macrophages. *The American Journal of Physiology-Lung Cellular and Molecular Physiology*, **280**(6):L1196-L1202.

Esposito, E., Cuzzocrea, S., 2009. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia-reperfusion injury and trauma. *Current Medicinal Chemistry*, **16**(24):3152-3167. [doi:10.2174/092986709788803024]

Fonseca, J.E., Santos, M.J., Canhão, H., Choy, E., 2009. Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmunity Reviews*, **8**(7):538-542. [doi:10.1016/j.autrev.2009.01.012]

Iyer, J.P., Srivastava, P.K., Dev, R., Dastidar, S.G., Ray, A., 2009. Prostaglandin E(2) synthase inhibition as a therapeutic target. *Expert Opinion on Therapeutic Targets*, **13**(7):849-865. [doi:10.1517/14728220903018932]

Kang, O.H., Chae, H.S., Choi, J.G., Oh, Y.C., Lee, Y.S., Kim, J.H., Seung, M.J., Jang, H.J., Bae, K.H., Lee, J.H., *et al*., 2008. Ent-pimara-8(14), 15-dien-19-oic acid isolated from the roots of *Aralia cordata* inhibits induction of inflammatory mediators by blocking NF-kappaB activation and mitogen-activated protein kinase pathways. *European Journal of Pharmacology*, **601**(1-3):179-185. [doi:10.1016/j.ejphar.2008.10.012]

Kanwar, J.R., Kanwar, R.K., Burrow, H., Baratchi, S., 2009. Recent advances on the roles of NO in cancer and chronic inflammatory disorders. *Current Medicinal Chemistry*, **16**(19):2373-2394. [doi:10.2174/092986709788682155]

Kim, J.Y., Park, S.J., Yun, K.J., Cho, Y.W., Park, H.J., Lee, K.T., 2008. Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. *European Journal of Pharmacology*, **584**(1):175-184. [doi:10.1016/j.ejphar.2008.01.032]

Kim, K.N., Ham, Y.M., Moon, J.Y., Kim, M.J., Kim, D.S., Lee, W.J., Lee, N.H., Hyun, C.G., 2009. In vitro cytotoxic activity of *Sargassum thunbergii* and *Dictyopteris divaricata* (Jeju seaweeds) on the HL-60 tumour cell line. *International Journal of Pharmacology*, **5**(5):298-306. [doi:10.3923/ijp.2009.298.306]

Kim, M.M., Rajapakse, N., Kim, S.K., 2009. Anti-inflammatory effect of *Ishige okamurae* ethanolic extract via inhibition of NF-kappaB transcription factor in RAW 264.7 cells. *Phytotherapy Research*, **23**(5):628-634. [doi:10.1002/ptr.2674]

Kim, S.K., Lee, D.Y., Jung, W.K., Kim, J.H., Choi, I., Park, S.G., Seo, S.K., Lee, S.W., Lee, C.M., Yea, S.S., *et al*., 2008. Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: role of suppressor of cytokine signaling. *Biomedicine and Pharmacotherapy*, **62**(5):289-296. [doi:10.1016/j.biopha.2007.07.009]

Murakami, A., 2009. Chemoprevention with Phytochemicals Targeting Inducible Nitric Oxide Synthase. *In*: Yoshikawa, T. (Ed.), Food Factors for Health Promotion. Forum of Nutrition, Basel, Karger, Vol. 61, p.193-203. [doi:10.1159/000212751]

O'Connor, D.M., O'Brien, T., 2009. Nitric oxide synthase gene therapy: progress and prospects. *Expert Opinion on Biological Therapy*, **9**(7):867-878. [doi:10.1517/14712590903002047]

Park, J.H., Oh, S.M., Lim, S.S., Lee, Y.S., Shin, H.K., Oh, Y.S., Choe, N.H., Park, J.H., Kim, J.K., 2006. Induction of heme oxygenase-1 mediates the anti-inflammatory effects of the ethanol extract of *Rubus coreanus* in murine macrophages. *Biochemical and Biophysical Research Communications*, **351**(1):146-152. [doi:10.1016/j.bbrc.2006.10.008]

Radovits, B.J., Kievit, W., Laan, R.F., 2009. Tumour necrosis factor-alpha antagonists in the management of rheumatoid arthritis in the elderly: a review of their efficacy and safety. *Drugs and Aging*, **26**(8):647-664. [doi:10.2165/11316460-000000000-00000]

Rao, P., Knaus, E.E., 2008. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *Journal of Pharmacy and Pharmaceutical Sciences*, **11**(2):81s-110s.

Samee, H., Li, Z.X., Lin, H., Khalid, J., Guo, Y.C., 2009. Anti-allergic effects of ethanol extracts from brown seaweeds. *Journal of Zhejiang University-SCIENCE B*, **10**(2):147-153. [doi:10.1631/jzus.B0820185]

Scher, J.U., Pillinger, M.H., 2009. The anti-inflammatory effects of prostaglandins. *Journal of Investigative Medicine*, **57**(6):703-708.

Usami, Y., 2009. Recent synthetic studies leading to structural revisions of marine natural products. *Marine Drugs*, **7**(3):314-330. [doi:10.3390/md7030314]

Yang, E.J., Yim, E.Y., Song, G., Kim, G.O., Hyun, C.G., 2009. Inhibition of nitric oxide production in lipopolysaccharide-activated RAW 264.7 macrophages by Jeju plant extracts. *Interdisciplinary Toxicology*, **2**(4):245-249. [doi:10.2478/v10102-009-0022-2]

Zhang, C., Kim, S.K., 2009. Matrix metalloproteinase inhibitors (MMPIs) from marine natural products: the current situation and future prospects. *Marine Drugs*, **7**(2):71-84. [doi:10.3390/md7020071]

Zhou, H.Y., Shin, E.M., Guo, L.Y., Zou, L.B., Xu, G.H., Lee, S.H., Ze, K.R., Kim, E.K., Kang, S.S., Kim, Y.S., 2007. Anti-inflammatory activity of 21(alpha,beta)-methylmelianodiols, novel compounds from *Poncirus trifoliata* Rafinesque. *European Journal of Pharmacology*, **572**(2-3):239-248. [doi:10.1016/j.ejphar.2007.07.005]