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Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages

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ABSTRACT

In this study, potential anti-inflammatory effect of fucoxanthin isolated from brown algae was assessed via inhibitory effect of nitric oxide (NO) production in lipopolysaccharide (LPS) induced RAW 264.7 macrophage cells. The *Myagropsis myagroides* was selected for further experiments due to its profound NO inhibitory effect, and was partitioned with different organic solvents. Highest NO inhibitory effect was detected in the chloroform fraction, and the active compound was identified as fucoxanthin, a kind of carotenoid available in brown algae evidenced high correlation with the inhibitory effect of NO production ($r^2 = 0.9511$). Though, fucoxanthin significantly inhibited the NO production, it slightly reduced the prostaglandin E_2 (PGE_2) production. The inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein expressions were inhibited by fucoxanthin. Further, RT-PCR analysis indicated that the iNOS and COX-2 mRNA expressions were suppressed by fucoxanthin. Moreover, the release of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), and the mRNA expression levels of those cytokines were reduced by the addition of fucoxanthin in a dose-dependent manner. Hence, these results suggest that the use of fucoxanthin may be a useful therapeutic approach for the various inflammatory diseases.

1. Introduction

Inflammation is a complex stereotypical response of the body to cell damage and vascularize tissues. The inflammatory responses are controlled by cytokines, products of the plasma enzyme systems, and lipid mediators including prostaglandins and leukotrienes (Ross and Auger, 2002). However, chronic and uncontrolled inflammations are detrimental to the tissues, which may cause chronic inflammation-derived diseases, such as cardiovascular diseases, rheumatoid arthritis, bronchitis, and cancers (Frostegard et al., 1999; Vernooy et al., 2002; Walsh et al., 2005; Karin et al., 2006).

Macrophages play an important role in inflammatory diseases relating to over production of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, and inflammatory mediators including reactive oxygen species (ROS), nitric oxide (NO), and prostaglandin E_2 (PGE_2), generated by activated inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 (Walsh et al., 2005; Lee et al., 2006). Production of these macrophage mediators has been determined in many inflammatory tissues, along with increased expression of their mRNAs, following exposure to immune stimulants including bacterial endotoxin lipopolysaccharide (LPS). Thus, inhibition of the production of these inflammatory mediators is an important target in the treatment of inflammatory diseases.

Algae are exposing to light and high oxygen concentrations that induces the formation of inflammatory mediators including NO and ROS. Thus, algae are able to generate the necessary compounds to protect themselves from external factors such as pollution, stress and UV radiation (Jimenez-Escrig et al., 2001; Heo and Jeon, 2009). Therefore, algae can be considered as a potential antioxidant and anti-inflammatory sources. Recently, several studies have

Abbreviations: NO, nitric oxide; PGE_2, prostaglandin E_2; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; ROS, reactive oxygen species; LPS, lipopolysaccharide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum.

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done with algae to find their potential bioactivities and some active compounds have already been isolated as chromenes, chlorophylls, phlorotannins, and carotenoids (Salguero et al., 2003; Jang et al., 2005; Ferruzzi and Blakeslee, 2007; Heo et al., 2008a; Le et al., 2009).

The objective of the current study were to isolate fucoxanthin from *Myagropsis myagroides* based on the results of NMR and other analytical data, and to evaluate its anti-inflammatory effect in LPS-stimulated RAW 264.7 macrophages.

2. Materials and methods

2.1. Materials

The marine algae were collected along the coast of Jeju Island, Korea, between October 2007 and March 2008. The samples were washed three times with tap water to remove the salt, epiphytes, and sand attached to the surface, then carefully rinsed with fresh water, and maintained in a medical refrigerator at −20 °C. Then, the frozen samples were lyophilized and homogenized with a grinder prior to extraction.

2.2. Extraction procedure of marine algae

The marine algae samples were pulverized into powder using a grinder. The algal powder (1 g) was extracted with 80% methanol (100 ml) at room temperature for 24 h and filtrated. After filtration, the methanolic extracts were evaporated to dryness under vacuum. The samples were dissolved in dimethylsulfoxide (DMSO) and mixed with Dulbecco's modified Eagle's medium (DMEM; GIBCO Inc., NY, USA) containing cells to adjust final concentration of samples at 200 μg/ml. This extracts were used for biological study.

2.3. Isolation and structural identification of active compounds

The algal powder was extracted three times with 80% aqueous methanol, and was evaporated under vacuum at 40 °C. The methanol extract was dissolved in distilled water and partitioned with chloroform. Then, the chloroform extract was fractionated by silica column chromatography with stepwise elution of chloroform–methanol mixture (100:1 → 1:1) to separate active fractions in chloroform extract. A combined active fraction was further subjected to a Sephadex LH-20 column saturated with 100% methanol, and then purified by reversed-phase high performance liquid chromatography (HPLC) using a Waters HPLC system (Alliance 2690, NY, USA) equipped with a Waters 996 photodiode array detector and C18 column (J'sphere ODS-H80, 150 × 20 mm, 4 μm, YMC Co., Kyoto, Japan) by stepwise elution with methanol–water gradient (UV range: 440 nm, flow rate: 0.8 ml/min). Finally, the purified compound was identified by comparing its ^{1}H and ^{13}C NMR data with literature (Heo et al., 2008b).

2.4. Cell culture

The murine macrophage cell line RAW 264.7 was purchased from the Korean Cell Line Bank (KCLB; Seoul, KOREA). RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO Inc., NY, USA) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin and 10% fetal bovine serum (FBS; GIBCO Inc., NY, USA). The cells were incubated in an atmosphere of 5% CO_2 at 37 °C and were sub-cultured every 3 days.

2.5. Determination of nitric oxide (NO) production

After pre-incubation of RAW 264.7 cells (1.5×10^5 cells/ml) with LPS (1 μg/ml) plus algae extracts or fucoxanthin at 37 °C for 24 h, the quantity of nitrite accumulated in the culture medium was measured as an indicator of NO production (Lee et al., 2007). Briefly, a 100 μl of cell culture medium was mixed with 100 μl of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid), the mixture was incubated at room temperature for 10 min, and the absorbance at 540 nm was measured in a microplate reader (ThermoMax, CA, USA). Fresh culture medium was used as a blank in every experiment.

2.6. LDH cytotoxicity assay

RAW 264.7 cells (1.5×10^5 cells/ml) plated in 96 well plates were pre-incubated and then treated with LPS (1 μg/ml) plus aliquots of fucoxanthin at 37 °C for 24 h. The medium was carefully removed from each well, and the LDH activity in the medium was determined using an LDH cytotoxicity detection kit (Promega, Madison, WI, USA). Briefly, a 100 μl of reaction mixture was added to each well, and the reaction was incubated for 30 min at room temperature in the dark. The absorbance of each well was measured at 490 nm using a microplate reader (ThermoMax).

2.7. Determination of PGE_2 production

Fucoxanthin was diluted with DMEM before treatment. Cells were treated with LPS (1 μg/ml) to allow cytokine production for 24 h. The PGE_2 concentration in the culture medium was quantified using a competitive enzyme immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The production of PGE_2 was measured relative to that of control value.

2.8. Measurement of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) production

Fucoxanthin solubilized with DMSO was diluted with DMEM before treatment. The inhibitory effect of fucoxanthin on the pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) production from LPS (1 μg/ml) treated RAW 264.7 cells was determined as described by Cho et al. (2000). Supernatants were used for pro-inflammatory cytokines assay using mouse ELISA kit (R&D Systems Inc., MN, USA).

2.9. RNA isolation and RT-PCR analysis

Total RNA from LPS (1 μg/ml)-treated RAW 264.7 cells was prepared with Tri-Reagent (MRC, Cincinnati, OH, USA), according to the manufacturers protocol. RNA was stored at −70 °C until used. The reverse transcription of 1 μg RNA was carried out with M-MuLV reverse transcriptase (Promega, WI, USA), oligo dT-18 primer, deoxyribonucleotide triphosphates (dNTP, 0.5 μM) and 1 U RNase inhibitor. After this reaction cocktail was incubated at 70 °C for 5 min, 25 °C for 5 min, and 37 °C for 60 min in series, M-MuLV reverse transcriptase was inactivated by heating at 70 °C for 10 min. Polymerase chain reaction (PCR) was performed in reaction buffer (cDNA, 1.25 U Taq DNA polymerase (Promega, WI, USA), 3′- and 5′-primer (50 μM each) and 200 mM dNTP in 200 mM Tris–HCl buffer (pH 8.4) containing 500 mM KCl and 1–4 mM $MgCl_2$). The PCR was performed in a DNA gene cycler (BIO-RAD, HC, USA) with amplification by 30 cycles of 94 °C for 45 s (denaturing), 60–65 °C for 45 s (annealing) and 72 °C for 1 min (primer extension). The primers (Bioneer, Seoul, Korea) used in this experiment were indicated in Table 1. The PCR products were electrophoresed in 1.2% agarose gels and stained with ethidium bromide.

2.10. Immunoblotting

RAW 264.7 cells (1.0×10^6 cells/ml) were treated with LPS (1 μg/ml) plus fucoxanthin for 24 h, and cellular proteins were extracted from the cells. Protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad, CA, USA) with bovine serum albumin (BSA) as a standard. Cell lysates (30–50 μg) were electrophoresed in SDS–polyacrylamide gels (8–12%), and the separated proteins were transferred to PVDF membranes (Bio-Rad) for 2 h. The membranes were pre- incubated with blocking solution (5% skim milk in Tris buffered saline containing Tween-20) at room temperature for 2 h and then incubated with anti-mouse iNOS (1:1000; Calbiochem, La Jolla, CA, USA) and anti-mouse COX-2 (1:1000; BD Biosciences Pharmingen, San Jose, CA, USA) for 2 h at room temperature. After washing, the blots were incubated with horseradish peroxidase conjugated goat anti-mouse IgG secondary antibody (1:5000; Amersham Pharmacia Biotech, Little Chalfont, UK) for 30 min. The bands were visualized on X-ray film using ECL detection reagent (Amersham Biosciences, Piscataway, NJ, USA).

2.11. Statistical analysis

The Student's *t*-test and one-way ANOVA were used to determine the statistical significance of differences between the values for the various experimental and control groups. Data are expressed as means ± standard errors (SE) and the results are taken from at least three independent experiments performed in triplicate. *p*-Values of 0.05 or less were considered statistically significant.

3. Results

3.1. Inhibitory effect of NO production of brown algae extracts

To evaluate whether the brown algae extracts possess potential anti-inflammatory effect in LPS-stimulated RAW 264.7 macrophages, we investigated inhibitory effect of nine species of brown algae on NO production (Fig. 1). Among those extracts obtained with 80% methanol, *M. myagroides* extract showed the highest level of inhibitory effect (91.08%) on NO production, whereas the other extracts evidenced less than 60% inhibitory activities. Thus, *M. myagroides* was selected for further experiments and partitioned with chloroform and ethyl acetate to detect bioactive compounds, such as carotenoid or polyphenolic compounds. It was noted that the chloroform fraction (70.31%) evidenced higher levels of NO

Table 1
Sequence of primers and fragment sizes of the investigated genes in a RT-PCR analysis.

Gene	Primer	Sequence	Fragment size (bp)
iNOS	Sense Antisense	5′-CCCTTCCGAAGTTTCTGGCAGCAGC-3′ 5′-GGCTGTCAGAGCCTCGTGGCTTTGG-3′	496
COX-2	Sense Antisense	5′-CACTACATCCTGACCCACTT-3′ 5′-ATGCTCCTGCTTGAGTATGT-3′	696
IL-1p	Sense Antisense	5′-CAGGATGAGGACATGAGCACC-3′ 5′-CTCTGCAGACTCAAACTCCAC-3′	447
IL-6	Sense Antisense	5′-GTACTCCAGAAGACCAGAGG-3′ 5′-TGCTGGTGACAACCACGGCC-3′	308
TNF-a	Sense Antisense	5′-TTGACCTCAGCGCTGAGTTG-3′ 5′-CCTGTAGCCCACGTCGTAGC-3′	364
β-Actin	Sense Antisense	5′-GTGGGCCGCCCTAGGCACCAG-3′ 5′-GGAGGAAGAGGATGCGGCAGT-3′	603

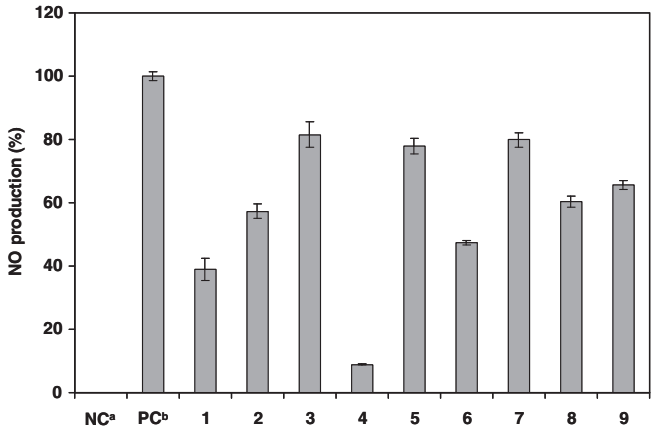

Fig. 1. Inhibitory effect of brown algae extracts on LPS-induced NO production in RAW 264.7 macrophages. The production of nitric oxide was assayed in the culture medium of macrophages stimulated with LPS (1 μg/ml) for 24 h in the presence of brown algae extracts. Each value indicates that the mean ± SE from three independent experiments. (1) *Dictyota coriacea*, (2) *Ecklonia cava*, (3) *Hizikia fusiformis*, (4) *Myagropsis myagroides*, (5) *Sargassum coreanum*, (6) *Sargassum hemiphyllum*, (7) *Sargassum horneri*, (8) *Sargassum muticum*, (9) *Sargassum patens*. NC[a]; negative control, PC[b]; positive control.

inhibition activity than that of ethyl acetate fraction (30.83%, data not shown).

3.2. Isolation and identification of the active compound

The chloroform fraction was subjected to silica gel and Sephadex LH-20 column chromatography, due to its prominent NO inhibitory effect. Finally, the active compound of this fraction was isolated via HPLC and was identified as fucoxanthin (Fig. 2) on the basis of a comparison of NMR spectroscopic data (data not shown) with previous literature (Heo et al., 2008b). The molecular formula of fucoxanthin was deduced as $C_{42}H_{58}O_6$ on the basis of NMR and HREI-MS data (M+, *m*/*z*: 658.0898 Calcd. For $C_{42}H_{58}O_6$ *m*/*z*: 658.0901).

3.3. Correlation of fucoxanthin contents and NO production

Fucoxanthin was isolated from all the tested brown algae and the contents were exhibited in Fig. 3A. *M. myagroides* possess the highest contents (9.01 mg/g) of fucoxanthin in the tested brown algae and *Dictyota coriacea* also evidenced high contents (6.42 mg/g) of fucoxanthin. Moreover, it was observed that the brown algae having high contents of fucoxanthin evidenced high correlation ($r^2 = 0.9511$) with NO production (Fig. 3B).

3.4. Effects of fucoxanthin on LPS-induced NO production and cytotoxicity

To evaluate the effect of fucoxanthin on NO production, RAW 264.7 macrophages were stimulated with LPS (1 μg/ml) for 24 h to evoke NO, and the accumulation of its metabolite, nitrite, in the culture medium was measured. NO was produced by the treatment of LPS, which was inhibited around 80% by the addition of fucoxanthin at 60 μM and LPS-induced NO production was decreased in a fucoxanthin dose-dependently (Fig. 4). To exclude the possibility that the inhibition of NO production was due to cytotoxicity caused by fucoxanthin treatment, LDH assays were investigated in RAW 264.7 macrophages treated with fucoxanthin. As shown in Fig. 4, fucoxanthin did not affect cell viability in macrophages. Thus, the inhibitory effect of fucoxanthin on LPS-induced NO production was not due to any cytotoxic action on RAW 264.7 macrophages.

3.5. Effects of fucoxanthin on LPS-induced PGE_2 production

The inhibitory effect of fucoxanthin on PGE_2 production in LPS-induced RAW 264.7 macrophages were similar pattern to its effect on NO inhibition, in that fucoxanthin inhibited LPS-induced PGE_2 production by 32% at 60 μM (Fig. 5). Although fucoxanthin had

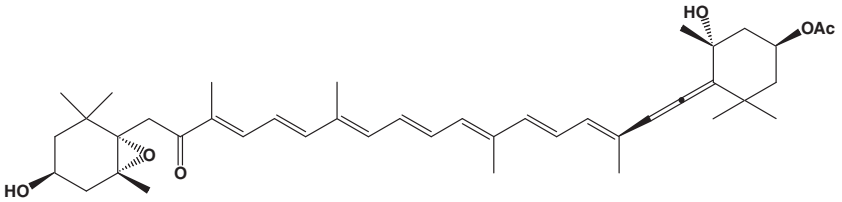

Fig. 2. Chemical structure of fucoxanthin.

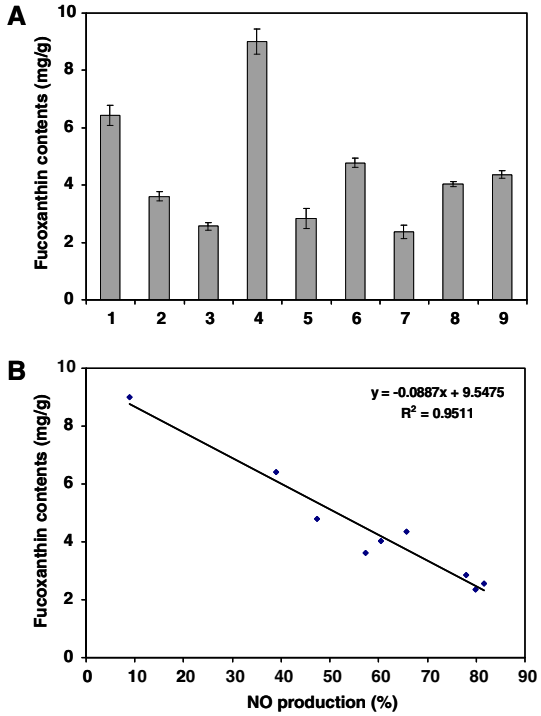

Fig. 3. Fucoxanthin contents isolated from 9 species of brown algae (A), and correlation between fucoxanthin contents and LPS-induced NO production in RAW 264.7 macrophages (B). The production of nitric oxide was assayed in the culture medium of macrophages stimulated with LPS (1 μg/ml) for 24 h in the presence of brown algae extracts. Each value indicates that the mean ± SE from three independent experiments. (1) *Dictyota coriacea*, (2) *Ecklonia cava*, (3) *Hizikia fusiformis*, (4) *Myagropsis myagroides*, (5) *Sargassum coreanum*, (6) *S. hemiphyllum*, (7) *S. horneri*, (8) *S. muticum*, (9) *S. patens*.

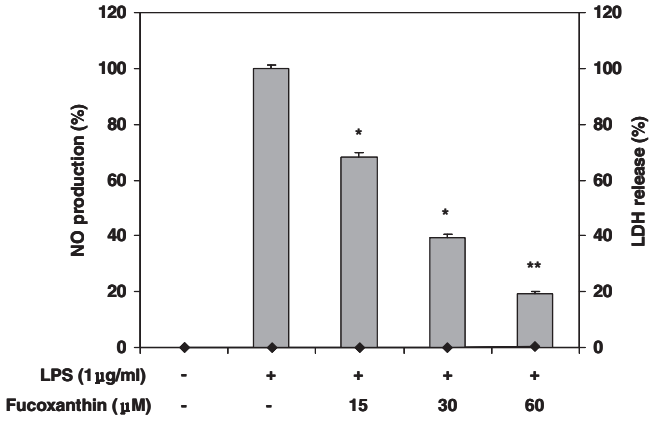

Fig. 4. Inhibitory effect of fucoxanthin on LPS-induced NO production in RAW 264.7 macrophages (■) and cytotoxicity (–♦–). The production of NO was assayed in the culture medium of macrophages stimulated with LPS (1 μg/ml) for 24 h in the presence of fucoxanthin (15, 30, and 60 μM). Each value indicates that the mean ± SE from three independent experiments. Cytotoxicity was determined using the LDH method. $^{*}p < 0.05$, $^{**}p < 0.01$.

an inhibitory effect on PGE_2 production, the effect was not as strong as that exhibited in the inhibition of NO production.

3.6. Effects of fucoxanthin on LPS-induced iNOS and COX-2 protein and mRNA expressions

To determine the mechanism by which fucoxanthin reduces LPS-induced NO and PGE_2 production, we investigated the ability

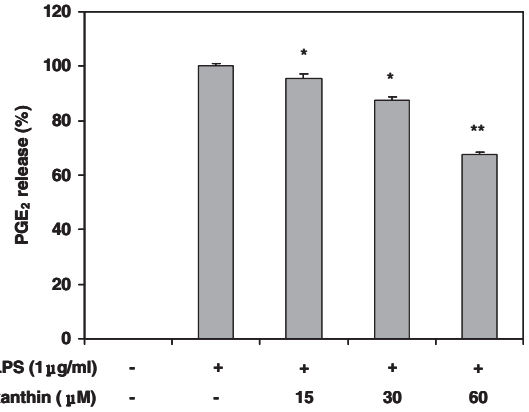

Fig. 5. Inhibitory effect of fucoxanthin on LPS-induced PGE_2 production in RAW 264.7 macrophages. RAW 264.7 macrophages were stimulated with LPS (1 μg/ml) for 24 h in the presence of fucoxanthin (15, 30, and 60 μM). PGE_2 concentration was measured in culture media using an ELISA kit. Each value indicates that the mean ± SE from three independent experiments.

of fucoxanthin (15, 30, and 60 μM) to influence the LPS-induced production of iNOS and COX-2. Fig. 6A shows the effect of fucoxanthin on iNOS and COX-2 protein expression in RAW 264.7 macrophages by western blot analysis. The iNOS and COX-2 protein expression was significantly increased when the macrophages treat with only LPS (1 μg/ml) compared to the control without LPS and fucoxanthin. However, the inhibitory effect of fucoxanthin on iNOS protein expression was significantly suppressed in a concentration-dependent manner with the addition of fucoxanthin to the macrophages mixed with LPS. Moreover, the effect of fucoxanthin on the expression of iNOS and COX-2 mRNA level was determined by RT-PCR analysis. As shown in Fig. 6B, the expression of iNOS and COX-2 mRNA correlated with their protein levels. Especially, fucoxanthin at 60 μM completely suppressed iNOS protein and mRNA expression, while the fucoxanthin had less effect on the expression of COX-2 protein and mRNA.

3.7. Effects of fucoxnathin on LPS-induced IL-1β, IL-6, and TNF-α

To determine the effects of fucoxanthin on the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, the RAW 264.7 macrophages were incubated with fucoxanthin (0, 15, 30, and 60 μM) in the presence or absence of LPS (1 μg/ml) for 24 h, and the cytokine levels were measured by ELISA. It was found that pretreatment of macrophages with fucoxnathin considerably reduced the production of IL-1β, IL-6, and TNF-α (Fig. 7A–C). Especially, fucoxanthin was significantly inhibited the TNF-α production in LPS-induced macrophages, and the production rate was recorded as 68.4%, 59.7%, 35.3% at 15, 30, and 60 μM, respectively. Consistently, RT-PCR was performed to determine whether fucoxanthin reduce the expression of those cytokines at the mRNA levels. All the mRNA levels were increased by treatment of LPS, and these increases were significantly decreased in a concentration-dependent manner by treatment with fucoxanthin (Fig. 7D).

4. Discussion

In the present study, we screened inhibitory effect of NO production from 9 species of brown algae, and we confirmed the NO production was correlated with fucoxanthin contents. Thereafter, *M. myagroides* was selected as a potential source to act as an effective source on the production of inflammatory mediators in RAW 264.7 macrophages due to its profound NO inhibition affect and high content of fucoxanthin.

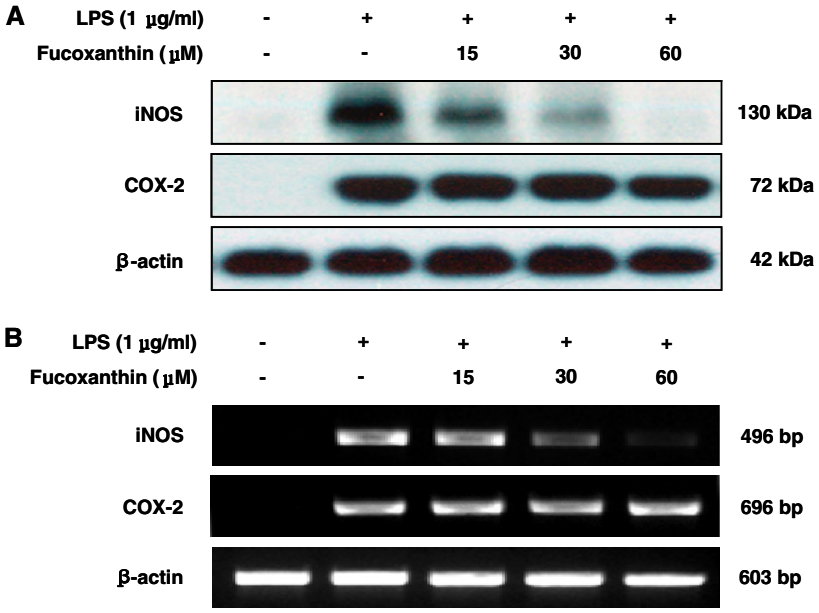

Fig. 6. Inhibitory effect of LPS-induced iNOS and COX-2 protein (A) and mRNA (B) expression by fucoxanthin in RAW 264.7 macrophages. (A) RAW 264.7 macrophages (1.0×10^6 cells/ml) were pre-incubated for 18 h, and the macrophages were stimulated with LPS (1 μg/ml) for 24 h in the presence of fucoxanthin (15, 30, and 60 μM). Cell lysates were electrophoresed, and the expression levels of iNOS and COX-2 were detected with specific antibodies. (B) After LPS treatment, total RNA was prepared from RAW 264.7 macrophages and RT-PCR was preformed for the iNOS and COX-2 genes. β-actin was used as internal control for western blot analysis and RT-PCR assays. This experiment was preformed in triplicate and similar results were obtained.

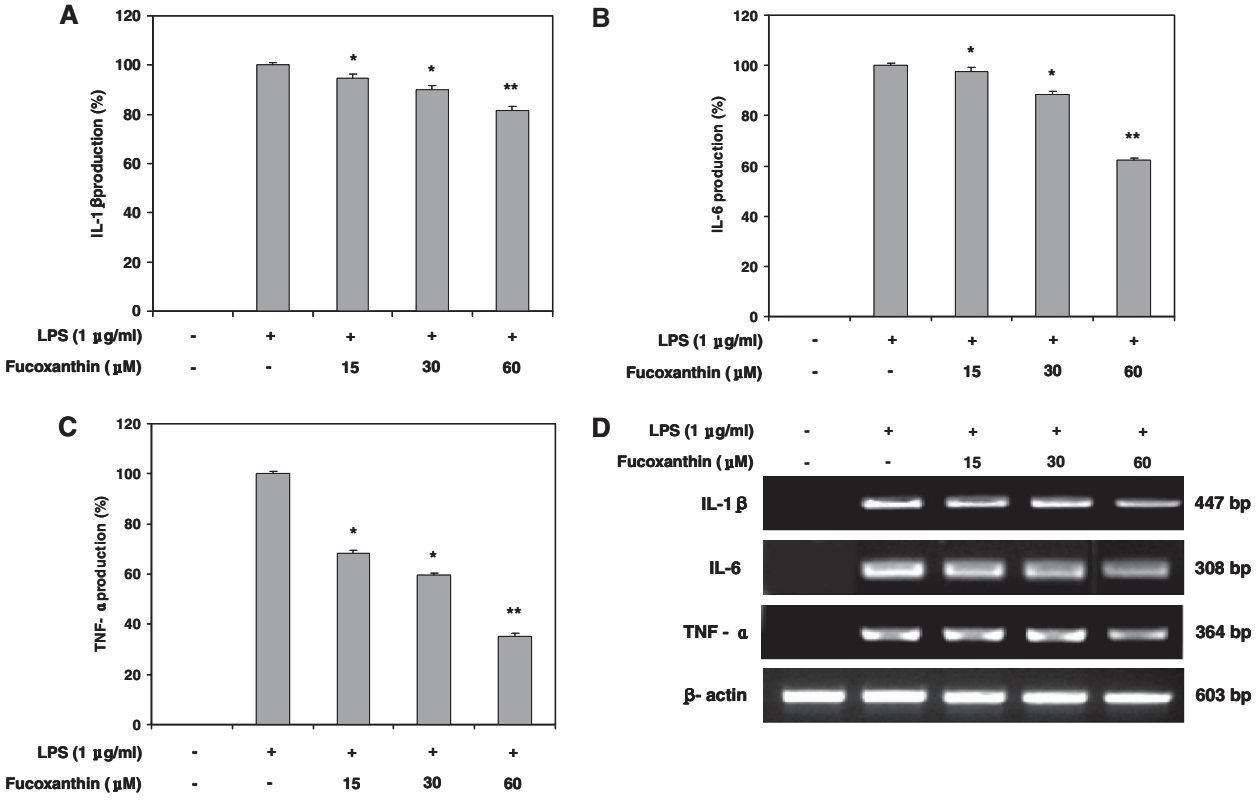

Fig. 7. Inhibitory effect of fucoxanthin on LPS-induced IL-1β, IL-6, and TNF-α production in RAW 264.7 macrophages. RAW 264.7 macrophages (1.0×10^6 cells/ml) were pre-incubated for 18 h, and the macrophages were stimulated with LPS (1 μg/ml) for 24 h in the presence of fucoxanthin (15, 30, and 60 μM). Extracellular levels of IL-1β (A), IL-6 (B), and TNF-α (C) were measured in culture media using an ELISA kits. The levels of IL-1β, IL-6, and TNF-α mRNA were determined by RT-PCR (D). Each value indicates that the mean ± SE from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$.

NO is an important inflammatory mediator which is synthesized from arginine by nitric oxide synthase (NOS). Generally, NO plays an important role as a vasodilator, neurotransmitter and in the immunological system as a defense against tumor cells, parasites, and bacteria (Nakagawa and Yokozawa, 2002). However, under pathological condition, NO production is increased by the

inducible NOS (iNOS), subsequently, brings about cytotoxicity, and tissue damage (Kim et al., 1999). Therefore, NO inhibitors are essential for the prevention of inflammatory diseases. PGE_2 is produced at inflammatory site by COX-2 and it also has been implicated as important mediator in the processes of inflammation (Ahmad et al., 2002). Chang et al. (2006) reported that the induction of COX-2 activity and subsequent generation of PGE_2 are closely related to the NO production. Thus, reducing the levels of PGE_2 and COX-2 may be an effective strategy for inhibiting the inflammation. Our results demonstrated that fucoxanthin inhibited LPS-induced NO and PGE_2 production in a concentration-dependent manner in RAW 264.7 macrophages which were attributed to its ability to down regulate the protein and mRNA expression of iNOS and COX-2. Especially, 60 μM of fucoxanthin completely suppressed NO production and iNOS expression. Moreover, the inhibitory effects of fucoxanthin on the LPS-induced expressions of iNOS and COX-2 in RAW 264.7 macrophages were not due to the cytotoxicity of fucoxanthin, as assessed by LDH assay. Thus, fucoxanthin can be considered as an effective therapeutic agent for preventing inflammatory diseases.

It has been reported that an abnormality in the production or function of cytokines, such as TNF-α, IL-6, and IL-1β, play roles in many inflammatory lesions (De Nardin, 2001). TNF-α is a potent activator of macrophages and can stimulate the production or expression of IL-6, IL-1β, PGE_2, collagenase, and adhesion molecules. It elicits a number of physiological effects, including septic shock, inflammation, and cytotoxicity (Aggarwal and Natarajan, 1996). Interleukin 6 is well known pro-inflammatory cytokine and regarded as an endogenous mediator of LPS-induced fever (Kim et al., 2008). Interleukin 1β is also considered to be a pivotal pro-inflammatory cytokine, primarily released by macrophages, and it is believed to play an important role in the pathophysiology of rheumatoid arthritis (Jung et al., 2008). Inflammatory stimuli, such as LPS, induce cytokines in the process of macrophage activation, which mediates tissue response in different phases of inflammation (Laskin and Pendino, 1995; Hseu et al., 2005). Thus, the inhibition of cytokine production or function is a key mechanism in the control of inflammation. Our results exhibited that fucoxanthin significantly inhibited the production of pro-inflammatory cytokine TNF-α in RAW 264.7 macrophages stimulated by LPS but showed less effect on IL-6 and IL-1β formation, suggesting that the inhibition of iNOS/NO pathway by fucoxanthin may be associated with the attenuation of TNF-α formation and less mediated by enhancing IL-6 and IL-1β release.

It has been shown that several natural antioxidant compounds directly inhibit the expression of cytokines, iNOS and COX-2, and thus reduce inflammation (Hehner et al., 1998; Ma et al., 2003). The suppressive effects of these antioxidant compounds on the production of the associated inflammatory mediators are associated with their antioxidant activities. Several antioxidant compounds have already been isolated from algae and identified as chromenes, phlorotannins, pyropheophytin, bromophenols, fucoidans, and carotenoids (Jang et al., 2005; Heo et al., 2008a; Cahyana et al., 1992; Ruperez et al., 2002; Li et al., 2007; Kamath et al., 2008). Recently, Ohgami et al. (2003) and Shiratori et al. (2005) reported that carotenoids astaxanthin and fucoxanthin prevents the inflammation of endotoxin-induced uveitis. Fucoxanthin, found in edible brown algae is one of the most abundant carotenoids found in nature (Bernard et al., 1976). In a previous study, we isolated fucoxanthin from *S. siliquastrum* and evaluated its potential antioxidant activities in cell lines (Heo et al., 2008b). Accordingly, it is possible that the ability of fucoxanthin to reduce ROS formation may be involved in the inhibition of iNOS, COX-2, and pro-inflammatory cytokines expression, and thus reduced inflammation.

In conclusion, we provided a mechanism to explain the anti-inflammatory activity of fucoxanthin by suppressing NO production and iNOS expression, which may be associated with the attenuation of TNF-α formation in LPS-stimulated RAW 264.7 macrophages. Hence, these results suggest that the fucoxanthin possesses potential anti-inflammatory activity and might have a beneficial effect on the treatment for inflammatory diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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