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Jeju seaweeds suppress lipopolysaccharide-stimulated proinflammatory response in RAW 264.7 murine macrophages

Eun-Jin Yang[1,2], Ji-Young Moon[1,2], Sang Suk Kim[1,3], Kyong-Wol Yang[4], Wook Jae Lee[2], Nam Ho Lee[1], Chang-Gu Hyun[1,5*]

[1]*Cosmetic Science Center, Department of Chemistry, Jeju National University, Jeju 690-756, Korea*

[2]*Jeju Technopark, Ara-1-dong, Jeju-si, Jeju 690-121, Korea*

[3]*Citrus Research Station, National Institute of Horticultural & Herbal Science, RDA, Jeju 699-946, Korea*

[4]*Jeju Love Co., Ltd., 542-5 Haengwon-ri, Gujwa-eup, Jeju 695-975, Korea*

[5]*LINC Agency, Jeju National University, Ara-1-dong, Jeju 690-756, Korea*

PEER REVIEW

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Assistant Prof. Seung-Young KIM, Department of Food Science and Biotechnology, University of Shizuoka, 52-1, Yada, Suruga-ku, Shizuoka, 422-8526, Japan. Tel: +81 54 264-5555, Fax: +81 54 264-5555, E-mail: sykim01@u-shizuoka-ken.ac.jp

Co-reviewers: Rajnish Sahu, Kragujevac, MS, USA. Kil-Nam Kim, Jeju, Korea. Ji-Kwon Park, Jeju, Korea.

Comments

This is a good study in which the authors evaluated the effect of ethylacetate extracts of Jeju seaweed that are effective against LPS-induced inflammation in murine macrophage cells. The results are interesting and suggest that Jeju seaweeds may be used as alternative therapy for anti-inflammatory diseases.

Details on Page 535

ABSTRACT

Objective: To investigate the anti-inflammatory effects of Jeju seaweeds on macrophage RAW 264.7 cells under lipopolysaccharide (LPS) stimulation.

Methods: Ethyl acetate fractions were prepared from five different types of Jeju seaweeds, *Dictyopteris divaricata* (*D. divaricata*), *Dictyopteris prolifera* (*D. prolifera*), *Prionitis cornea* (*P. cornea*), *Grateloupia lanceolata* (*G. lanceolata*), *and Grateloupia filicina* (*G. filicina*). They were screened for inhibitory effects on proinflammatory mediators and cytokines such as nitric oxide (NO), prostaglandin E_2, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).

Results: Our results revealed that *D. divaricata*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* potently inhibited LPS-stimulated NO production (IC_{50} values were 18.0, 38.36, 38.43, 32.81 and 37.14 μg/mL, respectively). Consistent with these findings, *D. divaricata*, *D. prolifera*, *P. cornea*, and *G. filicina* also reduced the LPS-induced and prostaglandin E_2 production in a concentration-dependent manner. Expectedly, they suppressed the expression of inducible NO synthase and cyclooxygenase-2 at the protein level in a dose-dependent manner in the RAW 264.7 cells, as determined by western blotting. In addition, the levels of TNF-α and IL-6, released into the medium, were also reduced by *D. divaricata*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* in a dose-dependent manner (IC_{50} values for TNF-α were 16.11, 28.21, 84.27, 45.52 and 74.75 μg/mL, respectively; IC_{50} values for IL-6 were 37.35, 80.08, 103.28, 62.53 and 84.28 μg/mL, respectively). The total phlorotannin content was measured by the Folin-Ciocalteu method and expressed as phloroglucinol equivalents. The content was 92.0 μg/mg for *D. divaricata*, 151.8 μg/mg for *D. prolifera*, 57.2 μg/mg for *P. cornea*, 53.0 μg/mg for *G. lanceolata*, and 40.2 μg/mg for *G. filicina*.

Conclusions: Thus, these findings suggest that Jeju seaweed extracts have potential therapeutic applications for inflammatory responses.

KEYWORDS

Nitric oxide, Interleukin-6, Prostaglandin E_2, Tumor necrosis factor-α, Seaweeds, Proinflammatory mediators

1. Introduction

The marine ecosystem and organisms supply bioactive and/or secondary metabolites with a number of biological and pharmacological activities. Of marine organisms, seaweeds are an important source of protein, iodine,

*Corresponding author: Chang-Gu Hyun, Cosmetic Science Center and LINC Agency, Jeju National University, Jeju 690-756, Korea.

Tel: +82 64 754 4419

Fax: +82 64 751 3127

E-mail: cghyun@jejunu.ac.kr

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vitamins, and minerals, and secondary metabolites might be promising anti-inflammatory agents[1]. Indeed, seaweed extracts and their natural products are known to possess a wide range of biological functions with diverse health benefits[2-5]. Recently, many studies have confirmed the anti-inflammatory activity of seaweeds. Yang and others reported that an anti-inflammatory effect of *Petalonia binghamiae*, brown algae was mediated by the suppression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)[6], while Yoon and others reported that *Sargassum muticum*, also brown algae, inhibited the expression of proinflammatory cytokines, iNOS, and COX-2 in the RAW 264.7 cells[7]. Hwang *et al.* and Kazłowska *et al.* reported the inhibition of lipopolysaccharide (LPS)-induced inflammatory responses of marine sulfated polysaccharide and phenolic compounds[8,9]. Also, several anti-inflammatory natural products such as apo-9′-fucoxanthinone, sargaquinoic acid, and sargachromenol have been discovered and described[10,11]. These compounds are abundant in *Sargassum* sp.; they show anti-inflammatory activity and have various other functions[12].

Inflammation is an essential host response to external stimuli (*e.g.*, microbial infection, endotoxin exposure, or tissue injury). It ultimately releases the complex array of inflammatory mediators, leading to the restoration of normal cell structure and function. However, inflammation is in a way a double-edged sword in that the prolonged inflammation contributes to the development of a number of chronic diseases such as atherosclerosis, rheumatoid arthritis, Alzheimer's disease, asthma, and allergies[13-17]. In inflammatory processes, macrophages, activated immunocytes, play a crucial role in providing an immediate defense against microbial invasion and tissue injury. On activation with an LPS, a well-studied component from the outer membrane of Gram-negative bacteria, macrophages produces proinflammatory mediators and cytokines, including nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6[18-20]. Hence, a down-regulation of these mediators and cytokines in macrophages is essential in suppressing chronic inflammatory process and provides a rationale for the development of therapeutic agents that might be used in various inflammatory diseases[21,22].

As part of our continuous effort to develop value-added utilization of seaweeds, the present work aimed to evaluate the anti-inflammatory properties of five kinds of Jeju seaweeds for human benefit. In this study, we investigated whether ethyl acetate (EtOAc) fractions of *Dictyopteris divaricate* (*D. divaricate*), *Dictyopteris prolifera* (*D. prolifera*), *Prionitis cornea* (*P. cornea*), *Grateloupia lanceolata* (*G. lanceolata*), and *Grateloupia filicina* (*G. filicina*) inhibit NO and PGE_2 production as well as iNOS and COX-2 protein expression in the LPS-induced murine macrophage system. Our study may provide a molecular basis for the therapeutic use of Jeju seaweeds in various inflammatory diseases.

2. Materials and methods

2.1. Algal material

All seaweeds in this study were collected from the Jeju Island, Jeju Province, South Korea, and identified by Dr. Wook Jae Lee, Jeju Technopark, South Korea. The voucher specimen was deposited in the herbarium of the Jeju Biodiversity Research Institute, Jeju, Korea. The fresh seaweeds were washed in freshwater to remove sediment, epifauna, and epiphytes, and then dried in the air for 2 weeks, ground into fine powder, and stored in plastic bags at 4 °C until further experiment. The dried seaweeds (1 kg) were extracted with 80% ethanol (5 L) at room temperature for 24 h and then evaporated under vacuum. The evaporated seaweed extracts (10 g each) were suspended in water (1 L) and partitioned with EtOAc (1 L; repeated 3 times).

2.2. Cell culture and viability

Murine RAW 264.7 macrophages were obtained from the Korean Cell Line Bank (Seoul, South Korea). Cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin sulfate (all from GIBCO, Grand Island, NY, USA), in an incubator at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. For this study the cells were mechanically passaged by dissociation every other day; they underwent fewer than 25 passages. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was undertaken as described previously[10]. Cells were counted with a haemocytometer, and the number of viable cells was assessed by trypan blue dye exclusion method. The RAW 264.7 macrophages were seeded in 96-well plates for 18 h and then stimulated with various concentrations (12.5, 25, 50, or 100 μg/mL) of the Jeju seaweeds (*D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina*) or LPS (1 μg/mL) for the indicated time. On the day of collection, cells were incubated with MTT solution for 4 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The MTT-containing supernatant was removed and the formazan crystals were solubilized in dimethylsulfoxide. Absorbance of each well at 540 nm was measured using an automatic microplate reader (PowerWave X340, Bio-tech Instruments, Inc., Winooski, VT, USA).

2.3. Determination of NO concentration

The NO concentration in *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* extracts was determined based on the method of Yang *et al*[10]. RAW 264.7 cells (5×10^5 cells/well) were cultured on 24-well dishes with 1 μg/mL of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina*. After 18 h, culture supernatants

were collected and nitrite, a stable oxidized product of NO, was measured by a modified Griess method. Equal volumes (100 μL) of the Griess reagent [1% sulfanilamide and 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 5% phosphoric acid] and *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, or *G. filicina* were incubated together at room temperature for 10 min. Absorbance of each well at 540 nm was measured using an automatic microplate reader (PowerWave X340, Bio-tech Instruments).

2.4. Measurement of PGE_2 and cytokine assays

The inhibitory effect of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* on the production of PGE_2 and pro-inflammatory cytokines from the LPS-stimulated RAW 264.7 cells was determined using a previously described method with slight modifications[10]. Macrophage RAW 264.7 cells (1.8×10^5 cells/mL) were cultured in 24-well plates for 18 h, and then treated with LPS (1 μg/mL) in the presence of various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina*. After 24-hour incubation, PGE_2 levels in the culture medium were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MI, USA). The release of PGE_2 was measured relative to that of the control value. For cytokine immunoassays, the RAW 264.7 cells (1.8×10^5 cells/mL) were cultured in 12-well plates, incubated with various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* in 1 μg/mL LPS for 24 h. Cell supernatants were then collected, and the levels of TNF-α and IL-6 were measured using the ELISA kit (R&D Systems) according to the manufacturer's instructions.

2.5. Western blot analysis

The western blot analysis was performed to evaluate the effect of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* on iNOS and COX-2 expression as described previously[10]. The RAW 264.7 cells (2.5×10^6 cells/mL) were cultivated in a 6-well plate for 18 h and then treated with the various concentrations of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* extracts in the absence or presence of LPS. After treatment for 24 h with LPS, the RAW 264.7 cells were harvested and the total protein was extracted with RIPA lysis buffer (Santa Cruz, CA, USA) supplemented with protease inhibitors, and kept on ice for 30 min. β-actin was used as control. The protein was denatured and separated on sodium dodecyl sulfonate (SDS)-polyacrylamide gels and then transferred onto a polyvinylidene fluoride membrane (Bio-Rad, HC, USA). The membranes were blocked and incubated with iNOS or COX-2 antibodies, followed by incubation with the horseradish peroxidase-linked secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). The signals were detected using an enhanced chemiluminescence reagent (Amersham Life Sciences, Arlington Heights, IL, USA).

2.6. Analysis of total phlorotannin

The total phlorotannin content of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* was determined by the Folin-Ciocalteu colorimetric method according to the method of Huang *et al*[23], as modified by Yang *et al*[24]. Sample absorbances were read at 725 nm. Phloroglucinol was used as the standard for a calibration curve; the total phlorotannin content of the seaweed extracts was expressed as phloroglucinol equivalents. The analysis of each seaweed extract was conducted in quadruplicate.

2.7. Statistical analysis

All results were expressed as mean±SE. Each experiment was repeated at least 3 times. Statistical significances were compared between each treated group and analyzed by the Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of Jeju seaweed fractions on the viability of the RAW 264.7 cells

Examination of cytotoxicity on the RAW 264.7 cells by an MTT assay indicated that none of the Jeju seaweed extracts at a concentration of up to 100 μg/mL affected the viability of the cells after 48-hour incubation (data not shown). Similarly, none of the Jeju seaweed extracts had a significant effect on the viability of the RAW 264.7 cells at various concentrations (up to 100 μg/mL) after 24-hour incubation (Figure 1).

3.2. Inhibition of LPS-induced NO production by Jeju seaweed fractions

In this study, to identify whether Jeju seaweeds have anti-inflammatory activity, we prepared 80% ethanol extracts from *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* from the Jeju Island, South Korea. All five types of evaporated 80% ethanol extracts were partitioned with EtOAc. All EtOAc fractions of the seaweeds were dissolved in 80% ethanol and diluted with sterile water to normalize the concentration of the experimental samples. After treatment with LPS (1 μg/mL) for 24 h, nitrite concentrations in the medium increased remarkably by about six-fold. When the RAW 264.7 cells were treated with 1 μg/mL of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of the EtOAc fractions of the Jeju seaweeds for 18 h, a significant ($P<0.05$) concentration-dependent inhibition of nitrite production was detected in the medium. All EtOAc fractions of the Jeju seaweeds showed more than 50% inhibition of NO production at a concentration of 40 μg/mL in the culture medium. IC_{50} values of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* were 18.0, 38.36, 38.43, 32.81, and 37.14 μg/mL, respectively.

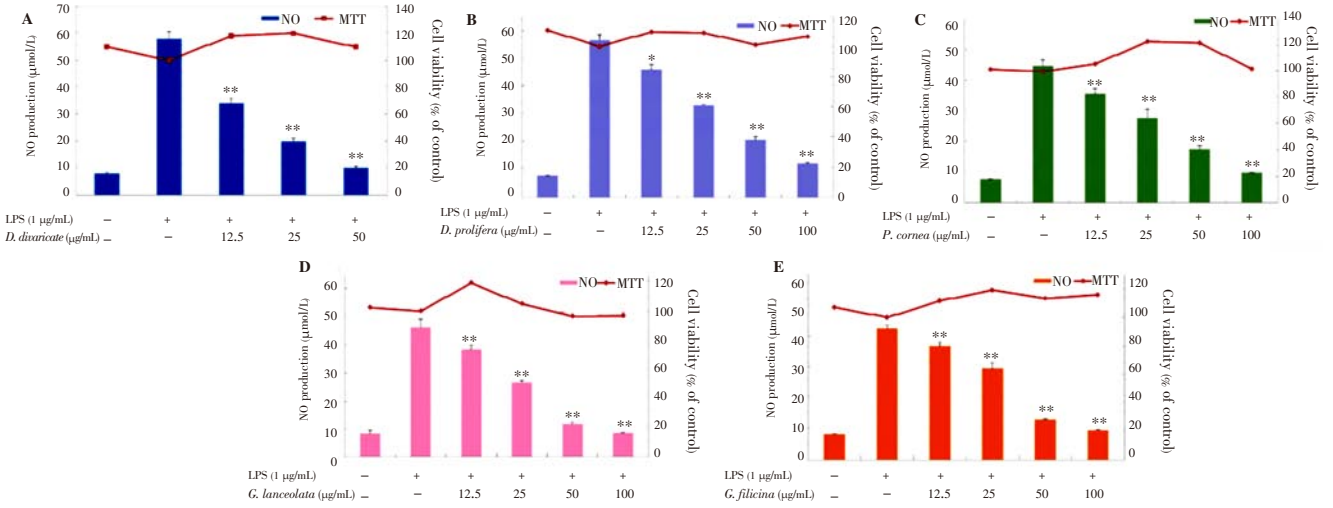

Figure 1. Effect of Jeju seaweed extracts on NO production in LPS-stimulated RAW 264.7 cells.

The cells were stimulated with 1 μg/mL of LPS alone or with a combination of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of seaweed extracts [*D. divaricate* (A), *D. prolifera* (B), *P. cornea* (C), *G. lanceolata* (D), and *G. filicina* (E)] for 24 h. NO production was determined by the Griess reagent method. Cell viability was determined using the 24-hour 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.

3.3. Inhibition of LPS-induced PGE_2 produced by Jeju seaweed fractions

A large body of data indicated that COX-2 played a key role in several biological processes such as chronic inflammation; therefore, we next examined the effects of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* 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. As shown in Figure 2, except for *G. lanceolata*, other samples (*D. divaricate*, *D. prolifera*, *P. cornea*, *G. filicina*) suppressed the LPS-induced PGE_2 production in a dose-dependent manner. Among these 4 extracts, 3 extracts—*i.e.*, those from *D. divaricate*, *D. prolifera*, and *P. cornea*—showed the most potent inhibition with IC_{50} values below of 12.5 μg/mL.

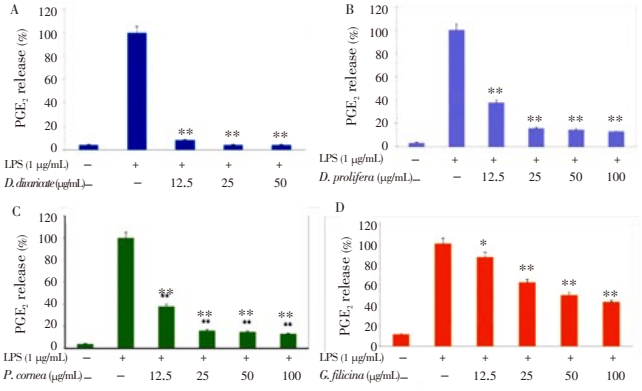

Figure 2. Effect of Jeju seaweed extracts on PGE_2 production in LPS-stimulated RAW 264.7 cells.

The cells were stimulated with 1 μg/mL of LPS alone or with a combination of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate* (A), *D. prolifera* (B), *P. cornea* (C), and *G. filicina* (D) 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.

3.4. Inhibitory effects of Jeju seaweed extracts on LPS-induced expression of COX-2 and iNOS

The western blot analysis was performed to determine whether the inhibitory effects of the Jeju seaweed extracts on the proinflammatory mediators, NO and PGE_2, were related to the modulation of iNOS and COX-2 expression. As shown in Figures 3 and 4, we did not detect iNOS and COX-2 proteins in non-stimulated RAW 264.7 cells. On the contrary, a significant increase in the levels of COX-2 and iNOS proteins was observed in response to LPS. In addition, treatment with EtOAc fractions of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* (12.5, 25, 50, or 100 μg/mL) resulted in a dose-dependent inhibition of the levels of LPS-stimulated COX-2 and iNOS proteins. These results were consistent with the inhibitory effects of Jeju seaweeds on PGE_2 and NO production in LPS-stimulated RAW 264.7 cells.

3.5. Inhibitory effects of Jeju seaweed extracts on LPS-induced TNF-α and IL-6 production

Considering that all extracts potently inhibited the proinflammatory mediators (PGE_2 and NO), we continued to investigate whether they suppressed the production of proinflammatory cytokines such as TNF-α and IL-6 in LPS-stimulated RAW 264.7 cells. After 24-hour incubation with both LPS (1 μg/mL) and EtOAc fractions of the seaweeds (12.5, 25, 50, or 100 μg/mL), a remarkable inhibition of TNF-α and IL-6 release was noted. As shown in Figures 5 and 6, *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* significantly inhibited the production of TNF-α and IL-6 in a dose-dependent manner with IC_{50} values of 37.35, 80.08, 103.28, 62.53, and 84.28 μg/mL for TNF-α and 16.11, 28.21, 84.27, 45.52, and 74.75 for IL-6, respectively.

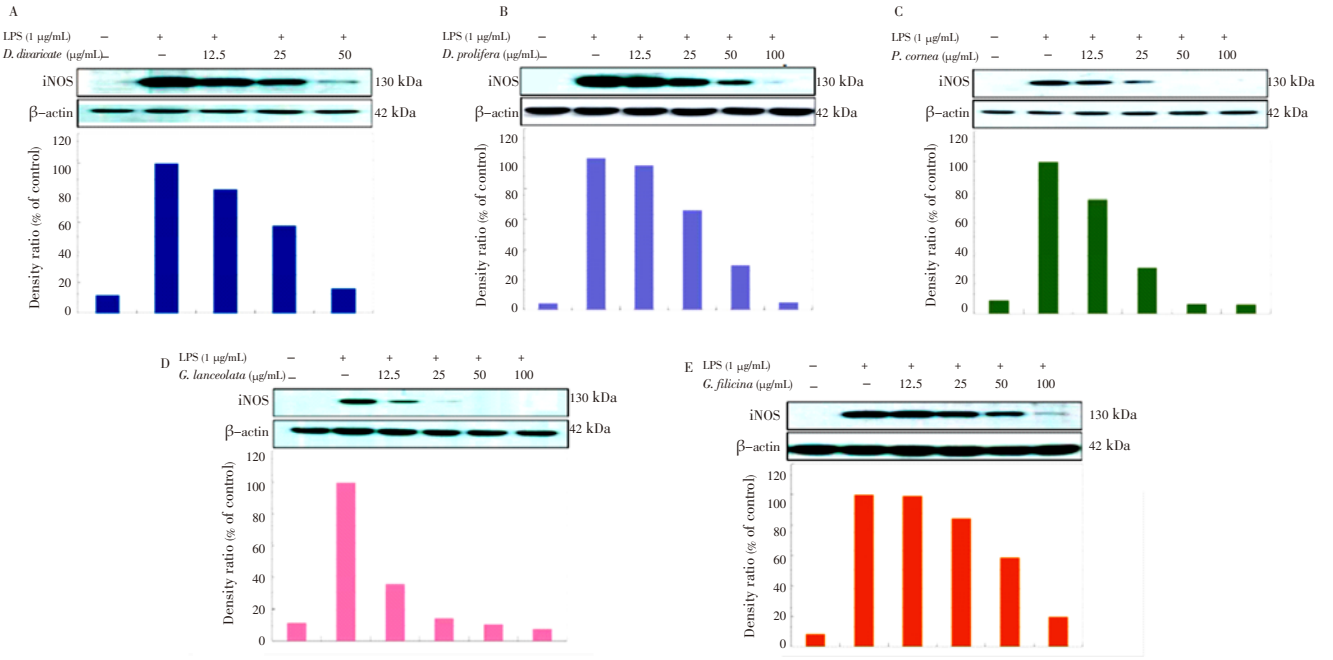

Figure 3. Effect of Jeju seaweed extracts on the activation of iNOS in LPS-stimulated RAW 264.7 cells.

The RAW 264.7 cells (2.5×10^6 cells/mL) were stimulated with LPS (1 μg/mL) in Jeju seaweed extracts (12.5, 25, 50, and 100 μg/mL) for 24 h. Whole-cell lysates (25 μg) were prepared, and the protein lysates were subjected to 10% SDS-PAGE; the expressions of iNOS and β-actin were determined by western blotting. Quantification of band intensities from three independent results was determined by densitometric analysis. The figure is representative of 3 similar experiments. The Jeju seaweed extract fractions were prepared as described in the materials and methods section.

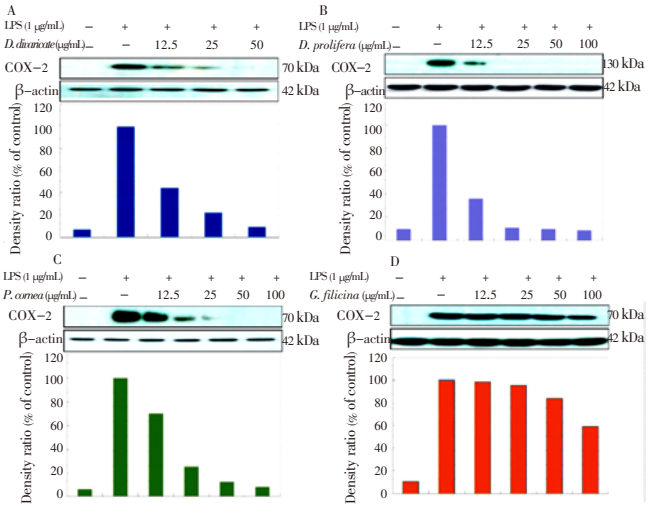

Figure 4. Effect of Jeju seaweed extracts on the activation of COX-2 in LPS-stimulated RAW 264.7 cells.

The RAW 264.7 cells (2.5×10^6 cells/mL) were stimulated with LPS (1 μg/mL) in Jeju seaweed extracts (12.5, 25, 50, and 100 μg/mL) for 24 h. Whole-cell lysates (25 μg) were prepared, and the protein lysates were subjected to 10% SDS-PAGE; the expressions of iNOS and β-actin were determined by western blotting. Quantification of band intensities from three independent results was determined by densitometric analysis. The figure is representative of 3 similar experiments. The Jeju seaweed extract fractions were prepared as described in the materials and methods section.

3.6. Phlorotannin contents of the Jeju seaweed extracts

Phlorotannins are aromatic secondary plant metabolites, which are widespread in brown seaweeds and associated with anti-inflammatory activity[25,26], hepatoprotective effects[27,28], anti-diabetic effects[29], radioprotective properties[30], and angiotensin-converting-enzyme inhibitory activity[31]. Therefore, the total phlorotannin content in all extracts was assessed, and the results were as follows: 92.0 μg/mg for *D. divaricate*, 151.8 μg/mg for *D. prolifera*, 57.2 μg/mg for *P. cornea*, 53.0 μg/mg for *G. lanceolata*, and 40.2 μg/mg for *G. filicina*. *D. prolifera*, brown algae, showed the highest phlorotannin content (>150 μg/mg).

4. Discussion

Marine algae are popular sea vegetables, which are consumed as health food by many people. Since ancient times, they have also been used in alternative medicine in such countries as Korea, China, and Japan[32]. Marine algae are a source of numerous natural products because they contain a wide variety of chemical structures and exhibit a broad range of biological and pharmacological activities (*e.g.*, anti-inflammatory[10-11], hepatoprotective[27,28], antioxidant[33,34], anti-proliferative[35,36], antiviral[37,38], and anti-diabetic[29,39]). Recent studies have also shown that marine algae contain a wide range of highly bioactive secondary metabolites with therapeutic potential that might represent useful leads in the development of functional ingredients for neutraceuticals, cosmeceuticals, and pharmaceuticals[32,40,41]. However, the possibility of developing these functional ingredients is limited because

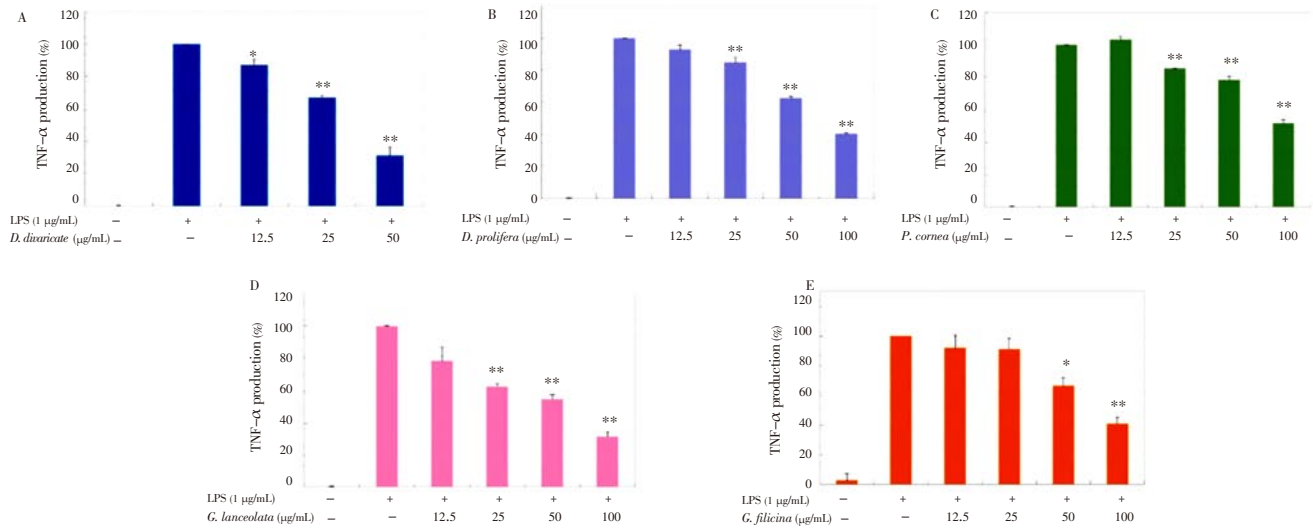

Figure 5. Effect of Jeju seaweed extracts on TNF-α production in LPS-stimulated RAW 264.7 cells.

The cells were stimulated with 1 μg/mL of LPS alone or with a combination of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate* (A), *D. prolifera* (B), *P. cornea* (C), *G. lanceolata* (D), and *G. filicina* (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.

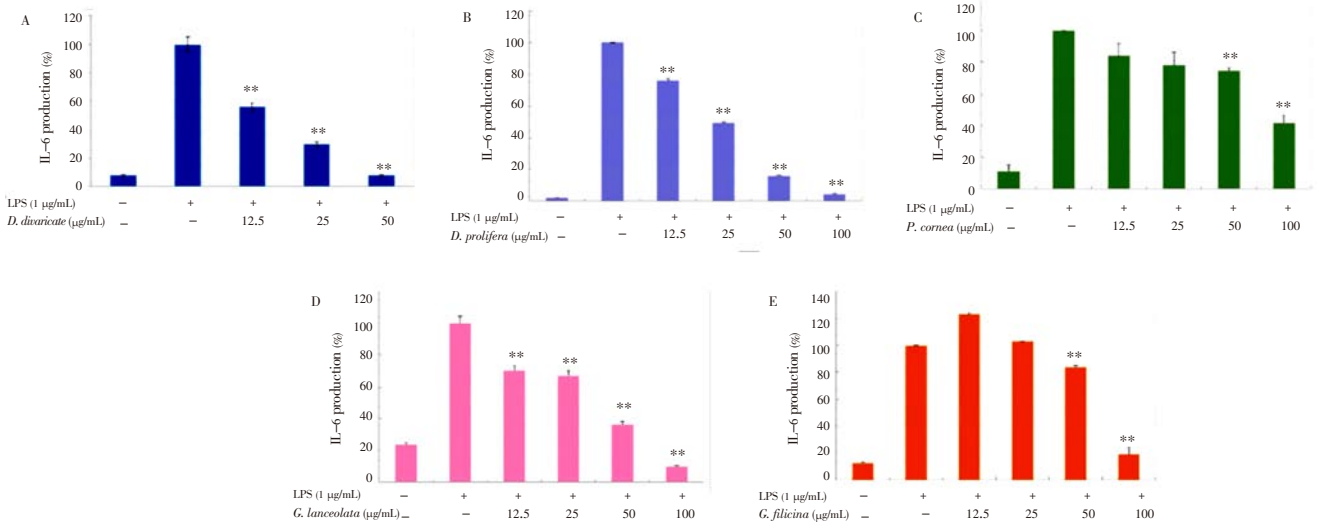

Figure 6. Effect of Jeju seaweed extracts on IL-6 production in LPS-stimulated RAW 264.7 cells.

The cells were stimulated with 1 μg/mL of LPS alone or with a combination of LPS and various concentrations (12.5, 25, 50, or 100 μg/mL) of *D. divaricate* (A), *D. prolifera* (B), *P. cornea* (C), *G. lanceolata* (D), and *G. filicina* (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.

the primary activity of algal ethanol extracts could be easily missed owing to the salts of the seaweeds on the surface. For this reason, the present study investigated the effect of Jeju seaweeds on inflammation by using their EtOAc fractions, which completely eliminates the salts of the seaweeds on the surface. Indeed, the activity of the ethanol extracts might be significantly different from that of the EtOAc fractions.

The proinflammatory mediators, PGE_2 and NO, which are produced by activated macrophages, play an important role in the pathogenesis of various chronic inflammatory diseases such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer. Hence, the blocking effects of these proinflammatory mediators or iNOS and COX-2 expression in inflammatory cells provide an important therapeutic strategy for the treatment of chronic inflammation. Indeed, a number of natural products that inhibit the production of these inflammatory mediators have been previously considered as potential anti-inflammatory candidates[42-45].

Murine macrophage RAW 264.7 cells are an example of inflammatory cells that play a crucial role in specific and nonspecific immune responses during the inflammatory process. After macrophages are activated by stimuli, large amounts of the proinflammatory mediators (PGE_2 and NO) and cytokines (TNF-α and IL-6) are released. Interestingly, the production of these inflammatory molecules by macrophages can be easily induced in response to LPS stimulation[46-49]. Therefore, LPS-stimulated macrophages have been typically used for assessing the anti-inflammatory potential of natural products.

Because excessive NO production has been shown to cause acute and chronic inflammation, pharmacological

interference of NO production has been speculated to offer promising strategies for alleviating inflammatory disorders. To validate the use of Jeju seaweed extracts (*D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, or *G. filicina*) as anti-inflammatory ingredients, we first investigated the effect of the extracts on NO synthesis in LPS-stimulated macrophages RAW 264.7 cells, and the results indicated that all extracts were effective inhibitors of LPS-induced NO production in these cells. The study showed that those inhibitory effects were accompanied by a decrease in the levels of iNOS expression as evidenced by dose-dependent reductions, mediated by *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, or *G. filicina*. These results suggest that Jeju seaweeds have potent inhibitory activity on proinflammatory mediator production.

PGE_2 is also an important mediator of the inflammatory response produced by COX-2 expression, which is induced by cytokines, LPS, growth factors, mitogens, cytokines, and tumor promoters during inflammation, resulting in the production of large amounts of PGE_2 at the site of inflammation[50-52]. In this study, we showed that in LPS-stimulated macrophages, *D. divaricate*, *D. prolifera*, *P. cornea*, or *G. filicina* inhibited PGE_2 production in a dose-dependent manner. COX-2 was also downregulated by *D. divaricate*, *D. prolifera*, *P. cornea*, and *G. filicina*, suggesting that this was the mechanism underlying the observed suppression of PGE_2 production.

It has been reported that proinflammatory cytokines such as TNF-α and IL-6 are involved in a variety of immune reactions and interactions with a variety of target cells both *in vitro* and *in vivo*[53-55]. They are considered to be important initiators of the inflammatory response and mediators of the development of various inflammatory diseases. Therefore, we chose these parameters to investigate additional anti-inflammatory effects of the Jeju seaweed extracts. Their inhibitory effects on LPS-stimulated TNF-α and IL-6 were assessed using the EIA kit, and the data showed that treatment with the extracts significantly inhibited the secretion of LPS-induced TNF-α and IL-6 in a concentration-dependent manner. These results suggest that *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* exert their anti-inflammatory effects via inhibition of TNF-α and IL-6 production, which has important implications for the development of therapeutic strategies in chronic inflammation.

In summary, as part of our research on the anti-inflammatory potential of the Jeju seaweeds, we previously reported their numerous inhibitory effects on the release of various inflammatory mediators induced by LPS. The results of the present study show that the anti-inflammatory effects of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* are achieved via the modulation of macrophage-mediated inflammatory markers such as NO, PGE_2, IL-1β, and IL-6, as well as protein levels of iNOS and COX-2. Thus, considering their potent effects, these extracts—whether used separately or in combination—may become extremely useful in the prevention and treatment of various inflammatory diseases. Our study provides a better understanding of the health-promoting effects of a phytochemical that is widely consumed worldwide. In future studies, we will investigate the molecular mechanisms underlying the anti-inflammatory effect of *D. divaricate*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina*, such as NF-κB and/or MAPKs signaling pathways, and the ability of the seaweed extracts to stimulate the immune system in an *in vivo* model.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Many attempts have been made to derive new anti-inflammatory agents from natural sources of phytochemicals that have been considered safe, less toxic, and readily available, though their modes of action mostly remain unclear. Thus, elucidating the molecular mechanisms underlying the anti-inflammatory actions of naturally occurring phytochemicals might be a good strategy for identifying new therapeutic agents.

Research frontiers

The present study depicts the anti-inflammatory effects of *D. divaricata, D. prolifera, P. cornea, G. lanceolata, and G. filicina* on LPS-induced inflammation in murine RAW 264.7 macrophage cells, and the underlying mechanisms.

Related reports

A series of studies have been done for centuries to discover the anti-inflammatory potential of certain seaweeds. The extracts of *D. divaricata*, *D. prolifera*, *P. cornea*, *G. lanceolata*, and *G. filicina* have not been tested for any anti-inflammatory assay and this study is considered novel in their specific field.

Innovations and breakthroughs

The study have showed the anti-inflammatory potentials

of Jeju seaweed extract (*D. divaricata, D. prolifera, P.cornea, G. lanceolata, and G. filicina*). The study is highly significant in the field of pharmacology in which the developing novel drugs pose anti-inflammatory agents in near future.

Applications

This study provides basis for the traditional use of Jeju seaweeds in the treatment of inflammatory pathologies. Other pharmacological effects such as anti-aging and anti-melanogenesis could be explored.

Peer review

This is a good study in which the authors evaluated the effect of ethylacetate extracts of Jeju seaweed that are effective against LPS-induced inflammation in murine macrophage cells. The results are interesting and suggest that Jeju seaweeds may be used as alternative therapy for anti-inflammatory diseases.

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