Anti-inflammatory Effects of Violaxanthin Isolated from Microalga Chlorella ellipsoidea in RAW 264.7 Macrophages

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Violaxanthin is a major carotenoid of microalgae Chlorella ellipsoidea and is also found in dark-green leafy vegetables, such as spinach. In this study, the anti-inflammatory effect of violaxanthin isolated from C. ellipsoidea was examined using lipopolysaccharide (LPS)-stimulated RAW 264.7 mouse macrophage cells. In addition, the anti-inflammatory activity and mechanism of action of purified violaxanthin was assessed using various assays, such as quantitative real-time polymerase chain reaction (PCR), Western blotting, and electrophoretic-mobility shift assay (EMSA). The results of this combined analysis revealed that violaxanthin significantly inhibited nitric oxide (NO) and the prostaglandin E2 (PGE2). Interestingly, violaxanthin effectively inhibited LPS-mediated nuclear factor-κB (NF-κB) p65 subunit translation into the nucleus, suggesting that the violaxanthin anti-inflammatory activity may be based on inhibition of the NF-κB pathways. In conclusion, violaxanthin of C. ellipsoidea holds promise for use as a potential anti-inflammatory agent for either therapeutic or functional adjuvant purposes.

Key words anti-inflammation; Chlorella ellipsoidea; microalgae; violaxanthin; RAW 264.7 cell; nuclear factor-κB p65

Inflammation is the normal physiological and immune response to tissue injury and occurs when the human body attempts to counteract potentially injurious agents, such as invading bacteria, viruses, and other pathogens. Among immune cells, macrophages play important roles in inflammation by overproducing inflammatory mediators, including nitric oxide (NO) and prostaglandin E2 (PGE2), which are synthesized by inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), respectively. Since inflammatory mediators can cause severe damage, such as sepsis and inflammatory diseases, inhibiting NO and PGE2 production by blocking iNOS and COX-2 at the molecular level may be a useful strategy for the treatment of acute or chronic inflammatory disorders.

Importantly, iNOS is highly expressed in macrophages and can lead to organ destruction in some inflammatory and autoimmune diseases. PGE2 is another important inflammatory mediator and is produced from arachidonic acid metabolites by the catalysis of cyclooxygenase-2 (COX-2). During inflammation, macrophages play a central role in managing many different immunopathological phenomena, including the overproduction of pro-inflammatory mediators, such as NO and PGE2. Experimentally, lipopolysaccharide (LPS) and pro-inflammatory cytokines activate immune cells to up-regulate inflammatory states, and these are therefore useful targets in the development of anti-inflammatory agents for the exploration of molecular anti-inflammatory mechanisms. In cell signal pathways, nuclear factor-κB (NF-κB) is known to stimulate the expression of enzymes, such as inducible iNOS and COX-2, and recent studies have identified that the toll-like receptor 4 (TLR4) is a signal-transducing receptor for LPS. LPS also directly activates the NF-κB pathway via TLR4 that amplifies the inflammatory responses by establishing a positive autoregulatory loop.

A seawater microalga, Chlorella ellipsoidea, which contains a high content of carotenoids, is commonly used as feed for marine fishes. C. ellipsoidea, is photosynthetic organism containing biologically active compounds, such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, and sterols. The carotenoids from microalgae function as accessory pigments in photosynthetic and structural components of light harvesting complexes, as well as photoprotective agents, which play roles in phototaxis. Various microalgae accumulate large quantities of carotenoids, which have been exploited commercially, including β-carotene from Dunaliella, astaxanthin from Haematococcus, and lutein from Chlorophyceae strains. C. ellipsoidea is abundant and commonly used for marine fish and brine shrimp hatcheries in Japan and Korea. There has been only one report that examined the antiproliferative effect of carotenoids from C. ellipsoidea on human colon cancer cells. The main carotenoid from marine C. ellipsoidea was found to be violaxanthin, which also contains minor amounts of two xanthophylls, antheraxanthin and zeaxanthin. The antioxidant potential of carotenoid is primarily due to violaxanthin, which is present in orange colored fruits and green vegetables. Violaxanthin from water spinach (Ipomoea aquatica) was reported to have a more potent scavenging ability than lutein and β-carotene in ABTS radical-scavenging, inhibition of red blood cell hemolysis, and inhibition of lipid peroxidation in liver. However, the potential anti-inflammatory effects of major carotenoids from C. ellipsoidea have not yet been investigated.

The aim of this study was to investigate the effect of the major carotenoid isolated from C. ellipsoidea on anti-inflammatory potencies by evaluating the inhibition of NO, PGE2 production, and the expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in LPS-stimulated RAW 264.7 cells by determining the molecular mechanism of violaxanthin action in LPS-induced NF-κB signaling pathway.

The authors declare no conflict of interest.

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MATERIALS AND METHODS

Materials Dimethyl sulfoxide (DMSO), Griess reagent, Celecoxib, and N°-Methyl-l-arginine acetate salt (l-NMMA) were purchased from Sigma-Aldrich (MO, U.S.A.). Polymeric chain reaction (PCR) primers were purchased from Bi-one (Deajeon, South Korea). All other chemicals used were of analytical grade.

Preparation of the Microalgaes Extract Chlorella ellipsoidea was obtained from the Daesang company (Seoul, South Korea). Chlorella cells were harvested from culture solution by centrifugation at 7000 rpm for 10 min at 4°C and freeze-dried in a vacuum freezer-dryer (Samwon Freezing Engineering, Seoul, South Korea). The dried sample was finely ground in a mortar and stored at −80°C before extraction.

The Chlorella powder (4 g) was extracted with 200 mL of 90% ethanol for 30 min by ultrasound-assisted extraction at a frequency of 35 kHz (Berlin, Germany) and room temperature, which was repeated three times. The combined extracts were filtered through Whatman No. 2 filter paper and saponified by adding 6% KOH (w/v) (24 g), and then heated at 50°C for 30 min with shaking at 100 rpm. After cooling to room temperature, the mixture was evaporated under vacuum at 40°C. The ethanol extract was dissolved in distilled water (300 mL), and then partitioned with ethyl acetate (300 mL) three times. The concentrated ethyl acetate fraction (300 mL) was washed by centrifugation at 7000 × g for 10 min at 4°C and freeze-dried in a vaccum freezer-dryer (Samwon Freezing Engineering, Deajeon, South Korea). All other chemicals used were of analytical grade.

Preparation of the Microalgaes Extract Chlorella ellipsoidea was obtained from the Daesang company (Seoul, South Korea). Chlorella cells were harvested from culture solution by centrifugation at 7000 rpm for 10 min at 4°C and freeze-dried in a vacuum freezer-dryer (Samwon Freezing Engineering, Seoul, South Korea). The dried sample was finely ground in a mortar and stored at −80°C before extraction.

The Chlorella powder (4 g) was extracted with 200 mL of 90% ethanol for 30 min by ultrasound-assisted extraction at a frequency of 35 kHz (Berlin, Germany) and room temperature, which was repeated three times. The combined extracts were filtered through Whatman No. 2 filter paper and saponified by adding 6% KOH (w/v) (24 g), and then heated at 50°C for 30 min with shaking at 100 rpm. After cooling to room temperature, the mixture was evaporated under vacuum at 40°C. The ethanol extract was dissolved in distilled water (300 mL), and then partitioned with ethyl acetate (300 mL) three times. The concentrated ethyl acetate fraction (300 mL) was washed several times with distilled water until the water phase was colorless. After separation, the ethyl acetate fraction was evaporated on a rotary evaporator at 40°C.

Isolation and Structural Identification of Violaxanthin

The ethyl acetate fraction was subjected to a silica gel column (2.0 × 60 cm), which was eluted with hexane (150 mL), followed by hexane–acetone (7:3, v/v) (400 mL). The target compound fractions were collected based on the spectral characteristics of violaxanthin, which were examined over a range of 350–550 nm using a spectrophotometer, by comparing the wavelengths of maximum absorption and spectral fine structural values (%III/II). In the epoxide test, 10 mL of 0.1 M HCl was added to a 1 mL ethanolic carotenoid solution and the absorption maxima were determined using a spectrophotometer. The purified compound was identified by liquid chromatography/mass spectrometry (LC/MS) (HP-1100 MSD, Agilent Technologies, CA, U.S.A.) equipped with an electrospray ionization (ESI). Mass spectra were acquired over the m/z 400–700 scan range using a 0.1 unit step size. Ten μL of isolated compound in methanol was injected into the LC, Eclipse XDB-C18 column (5 μm, 150 × 46 mm i.d., Waters, MA, U.S.A.) and eluted using isocratic solvent system, acetonitrile–methyl–dichloromethane (71:22:7, v/v/v), at a flow rate of 0.5 mL/min. Finally, the structure of the isolated compound was determined by spectroscopic methods, including 1H-NMR and 13C-NMR. 1H and 13C-NMR were recorded in chloroform-d6 (CDCl6) on a Bruker DRX-600 spectrometer (Kaisruhe, Germany).

<table>
<thead>
<tr>
<th>Retention time</th>
<th>λmax (nm)</th>
<th>Hypochromic shift (nm)</th>
<th>Epoxide test</th>
<th>% III/II</th>
<th>ESI-MS (positive, m/z)</th>
<th>Tentative identification</th>
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</thead>
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<td>4.084</td>
<td>417, 441, 470</td>
<td>380, 400, 426</td>
<td>Diepoxide</td>
<td>95</td>
<td>601.4</td>
<td>Violaxanthin</td>
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</table>

(a) The wavelength of maximum absorption. A mobile phase of acetonitrile–methanol–dichloromethane (71:22:7, v/v/v) was used. (b) Ratio of the height of the longest wavelength absorption peak, designated III, and that of the middle absorption peak, designated II, taking the minimum between two peaks as baseline, multiplied by 100, in mobile phase.
1-NMMA was compared as a positive control. The concentration of nitrite (µM) was calculated from a standard curve that was established using known concentrations of sodium nitrite dissolved in RPMI-1640 medium.

**Prostaglandin E$_2$ (PGE$_2$) Assay** The cells (10$^6$ cells/mL) were incubated with LPS (1 µg/mL) in the presence of violaxanthin at different concentrations (10 to 60 µM) for 24 h in 24 well-plates. Celecoxib (3 µM) was used as a positive control. The level of PGE$_2$ in the supernatants from macrophage cultures was determined using a competitive enzyme immunoassay kit (R&D System, MN, U.S.A.) according to the manufacturer’s protocol.

**Quantitative Real-Time Polymerase Chain Reaction (PCR) Assay** The RAW 264.7 cells were seeded at a density of 5×10$^5$ cells/mL in a 6 well plate and pre-incubated for 12 h. The cells were then treated with LPS (1 µg/mL) in the absence or presence of violaxanthin at different concentrations (10, 30, 60 µM) for 24 h. Total RNAs were prepared from cultured cells using the Trizol method (Invitrogen). Complementary DNA (cDNA) was synthesized from ribonucleic acid (RNA) by the reverse transcription of 1 µg of total RNA using the Improm-II reverse transcription system and oligo dT primers in a total volume of 20 µL (Promega, WI, U.S.A.). PCR amplification was performed using the primers described in Table 2 (Bioneer, Deajeon, South Korea). Quantitative real-time PCR (qPCR) reactions were run on a Rotor-Gene 6000 (Corbett Research, Sydney, Australia) using SYBR Green PCR Master Mix (Qiagen, CA, U.S.A.) in 20 µL reaction mixtures. Each real-time-PCR master mix contained 10 µL of 2X enzyme mastermix, 7.0 µL of RNase free water, 1 µL of each primer (10 pmol each) and 1 µL of diluted template. PCR was performed with an initial pre-incubation step for 10 min at 95°C, followed by 45 cycles of 95°C for 15 s, annealing at 52°C for 15 s and extension at 72°C for 10 s. Melting curve analysis was used to confirm formation of the expected PCR product, and products from all assays were subjected to 1.2% agarose gel electrophoresis to confirm that the products had the correct lengths. An inter-run calibrator was used, and a standard curve was created for each gene to determine PCR efficiencies. Relative sample expression levels were calculated using Rotor-Gene 6000 Series Software 1.7 (Corbett Research, Sydney, Australia), and expressed relative to β-actin and corrected for between run variability. Data for the experimental samples were expressed as the percentage of the internal control gene. Relative sample expression levels were calculated using the ΔΔCT method, followed by statistical analysis using one-way ANOVA with post-hoc Dunnett’s test, which was performed using SPSS software (v.13) (IL, U.S.A.). Statistical significance was set a priori at p<0.05.

**RESULTS**

- **Isolation and Structural Identification of Violaxanthin**

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**Table 2. Primer Sequences Used for Real-Time RT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Amino acid sequences</th>
<th>Product size (bp)</th>
<th>Accession No.</th>
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<td>iNOS</td>
<td>5’ Primer</td>
<td>5’- TGCCCTTGGGAGTTTCTCTT-3’</td>
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<td>M87039.1</td>
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<td></td>
<td>3’ Primer</td>
<td>5’- ACTGGCCCAGTTTGCACG-3’</td>
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<tr>
<td>COX-2</td>
<td>5’ Primer</td>
<td>5’- CCCAGGCTCTCTTTCTTAAC-3’</td>
<td>241</td>
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<tr>
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<td>3’ Primer</td>
<td>5’- AATTGGCCATTTTCTTCCC-3’</td>
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<td></td>
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<tr>
<td>β-Actin</td>
<td>5’ Primer</td>
<td>5’- TACCAGCTTACCCACACAGGC-3’</td>
<td>291</td>
<td>NM_007393</td>
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<tr>
<td></td>
<td>3’ Primer</td>
<td>5’- AAGGAAGGCTGGAAGAGAC-3’</td>
<td></td>
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</table>
The ethyl acetate fraction of *C. ellipsoidea* was further purified to isolate violaxanthin using silica gel and the target compound fraction was collected based on the absorption spectra. The visible spectrum characteristics (418, 442, 472 nm) and spectral fine structural values (% III/II), are presented in Fig. 1A and Table 1, respectively, which were in agreement with previous reports. In the epoxide test, a hypsochromic shift of 40 nm was observed after the addition of HCl, which confirmed the presence of two epoxides. The peak purity of violaxanthin was determined to be 86% based on the chromatographic purity (Fig. 1B). The mass spectrum corresponding to the molecular weight of violaxanthin was 600 Da according to \( m/z \) 601 [M+H\(^+\)] and fragments at \( m/z \) 583 [M+H−H\(_2\)O\(^+\)] represented the elimination of one H\(_2\)O (Fig. 1C). This compound was identified as 5,6:5'-6'-Diepoxy-5,5'-6,6'-tetrahydro-\( \beta \)-carotene-3,3'-diol or (all-E)-violaxanthin (Fig. 2) by comparing its \(^1\)H-,\(^13\)C-NMR with those found in the literature.

**Effect of Violaxanthin on RAW 264.7 Cell Viability and NO Production**  The cytotoxic effects of different concentrations of violaxanthin (10–60 \( \mu \)M) on RAW 264.7 cells were determined after incubation for 24h. In these experiments, violaxanthin did not affect the viability of RAW 264.7 at concentrations <100 \( \mu \)M. Hence, the inhibitory effect of violaxanthin on the LPS-induced RAW 264.7 macrophages was deemed not to be attributable to cytotoxicity. The effect of violaxanthin on the cytotoxicity of RAW 264.7 cells was then investigated over a broader range of concentrations (1 to 250 \( \mu \)M). Incubation of RAW 264.7 cells with violaxanthin produced a dose- and time-dependent increase in cell cytotoxicity as measured by LDH release. Figure 3A shows that violaxanthin concentrations under 100 \( \mu \)M did not significantly increase LDH release after exposure for up to 24h, but a higher concentration (250 \( \mu \)M) induced a significant increase in LDH release when incubated for 24h. The greatest cytotoxic effect (>90%) was
observed with 250 \mu M violaxanthin. Because the toxicity with 250 \mu M violaxanthin was so profound, higher concentrations were not tested. Based on this result, a violaxanthin concentration below 100 \mu M was used for further studies.

Effect of Violaxanthin on the LPS-Induced iNOS and COX-2 Expression

To determine if violaxanthin inhibits pro-inflammatory repertoires (iNOS and COX-2) at the gene level, RAW 264.7 cells were stimulated by LPS for 24h. L-NMMA or Celecoxib were used as a positive control. iNOS (A) and COX-2 (B) mRNA and protein levels were analyzed using Rotor-Gene 6000 Series Software 1.7 and 10% SDS-PAGE, respectively, as described in the Materials and Methods. For Western blot analysis, 60 \mu M violaxanthin was treated. The experiments were performed in triplicate and the results are expressed as the mean±S.D. ***p<0.001 vs. LPS-treated control group.

The potential anti-inflammatory properties of violaxanthin in RAW 264.7 cells after 24h of treatment with a mixture of LPS (1 \mu g/mL) and violaxanthin (10–60 \mu M) or LPS (1 \mu g/mL) alone (positive control) was examined by adding the Griess reagent to determine the concentration of nitrates (\mu M) in cell supernatants. Violaxanthin markedly inhibited the NO production in LPS (1 \mu g/mL)-treated RAW 264.7 cells in a dose-dependent manner (Fig. 3B) and this effect was maximal at 60 \mu M (p<0.001).

Effect of Violaxanthin on LPS-Induced PGE2 Production

The effect of violaxanthin on PGE2 production was examined in RAW 264.7 macrophages. When the macrophages were stimulated with LPS (1 \mu g/mL) for 24h, the levels of PGE2 production increased in the culture medium. Treatment with violaxanthin at different concentrations significantly inhibited PGE2 production (Fig. 4). A dose-dependent effect of violaxanthin was observed at 30 \mu M and maximized at 60 \mu M (p<0.01 and p<0.001, respectively), which closely corresponded to the positive control, celecoxib.

Effect of Violaxanthin on the LPS-Induced iNOS and COX-2 Expression

To determine if violaxanthin inhibits pro-inflammatory repertoires (iNOS and COX-2) at the gene level, RAW 264.7 cells were stimulated by LPS for 24h and the expression of iNOS and COX-2 mRNAs was examined in the presence of varying concentrations of violaxanthin (10, 30, 60 \mu M). L-NMMA, an inhibitor of NO generation

Fig. 3. Effect of Violaxanthin on LPS-Induced NO Production and Cytotoxicity in RAW 264.7 Cells

(A) RAW 264.7 cells were exposed to violaxanthin (1, 10, 50, 100 and 250 \mu M) for the indicated times. The concentration dependent and time dependent cytotoxicity, measured as %LDH released into culture supernatant, was compared with cells treated with 1% Triton X-100. (B) The NO production was assayed from the culture medium of cells stimulated with LPS (1 \mu g/mL) in the presence of violaxanthin for 24h. The experiments were performed in triplicate and the results are expressed as the mean±S.D. **p<0.01, ***p<0.001 vs. LPS-treated control group.

Fig. 4. Inhibitory Effect of Violaxanthin on LPS-Induced PGE2 Production in RAW 264.7 Cells

PGE2 production was assayed from the culture medium of cells stimulated with LPS (1 \mu g/mL) in the presence of violaxanthin for 24h. Each value indicates the mean±S.D. The experiments were performed in triplicate and the results are expressed as the mean±S.D. **p<0.01 and ***p<0.001 vs. LPS-treated control group.

Fig. 5. Quantitative mRNA Analysis and Western Blot Profiles of iNOS and COX-2

RAW 264.7 cells were stimulated with LPS (1 \mu g/mL) in the absence or presence of violaxanthin for 24h. L-NMMA or Celecoxib were used as a positive control. iNOS (A) and COX-2 (B) mRNA and protein levels were analyzed using Rotor-Gene 6000 Series Software 1.7 and 10% SDS-PAGE, respectively, as described in the Materials and Methods. For Western blot analysis, 60 \mu M violaxanthin was treated. The experiments were performed in triplicate and the results are expressed as the mean±S.D. *** indicates a significant difference from the LPS-treated control group (p<0.001).
inhibits the transcriptional activity of NF-κB, and ERK1/2, which is increased phosphorylation via LPS stimulation, were remarkably attenuated in the presence of violaxanthin at 60 μM (Fig. 6B). Interestingly, these results suggest that violaxanthin might have an effective anti-inflammatory property in macrophages that was comparable to the l-NMMA and celecoxib controls.

**DISCUSSION**

In this study, violaxanthin was purified from *C. ellipsoidea* and the anti-inflammatory effect of violaxanthin on LPS-induced NO and PGE₂ was examined *in vitro*. The mechanism of this anti-inflammatory effect was also assessed by examining the expression of iNOS and COX-2 in LPS-stimulated RAW 264.7 macrophages using qPCR and Western blot analysis. In murine macrophage RAW 264.7 cells, which naturally express TLR4 complexes, LPS induces iNOS transcription and transduction with subsequent NO production. Furthermore, LPS stimulation is known to induce 1αB proteolysis and NF-κB nuclear translocation. Therefore, RAW 264.7 cells provide an excellent model for drug screening and subsequent evaluation of potential inhibitors of the pathway leading to iNOS induction and NO production. The reactive free radical NO, which is synthesized by iNOS, is a major macrophage-derived inflammatory mediator and has also been reported to be involved in the development of inflammatory diseases. Moreover, a large body of evidence suggests that prostaglandins are involved in various pathophysiological processes, including inflammation and carcinogenesis, and the inducible cyclooxygenase isoform, COX-2, is mainly responsible for the production of large amounts of these mediators. Based on this information, we explored the anti-inflammatory activities of *S. micracanthum* in LPS-induced NO and PGE₂ production in RAW 264.7 cells as well as NF-κB nuclear translocation. In the present study, *S. micracanthum* downregulated the expression of iNOS and COX-2 proteins and RNA, indicating that effects of *S. micracanthum* occur at the transcriptional level.

**NO and PGE₂** are produced by NOS and COX-2, respectively, which are pro-inflammatory mediators involved in the development of inflammatory human diseases. Commonly, NO plays an important role as an immune regulator and neurotransmitter in a variety of tissues at physiological concentrations. Overproduction of NO derived from iNOS plays an important role in the pathogenesis of inflammation. On the other hand, PGE₂ is a pleiotropic mediator that causes pain, swelling, and stiffness. Hence, inhibitors of NO and PGE₂ production could be used as potential anti-inflammatory agents. The results of this study demonstrated that violaxanthin inhibited the production of NO and PGE₂ in a dose-dependent manner in RAW 264.7 cells. The effect of violaxanthin on NO and PGE₂ production was consistent with the carotenoids, where β-carotene, lutein, and fucoxanthin were shown to suppress NO production. Violaxanthin concentrations of 30 and 60 μM significantly inhibited LPS-induced NO production by RAW 264.7 cells. At 60 μM, violaxanthin significantly inhibited NO production by as much as l-NMMA. Coincidently, iNOS mRNA and protein expression were also significantly down-regulated. Moreover, violaxanthin reduced PGE₂ production and expression of COX-2 at the mRNA and protein level.
TLRs are essential for innate host defense as well as for controlling adaptive immune responses. Of these, TLR4 plays an important role in activating NF-κB of LPS-stimulated macrophages, followed by up-regulation of iNOS and COX-2 expression. At the molecular level, we investigated if violaxanthin appreciably suppressed the synthesis of iNOS and COX-2 by inhibiting NF-κB nuclear translocation. NF-κB activation can induce the overexpression of pro-inflammatory genes in the nucleus, thereby initiating the inflammatory processes. Interestingly, violaxanthin was shown to inhibit activation of NF-κB in LPS-activated RAW 264.7 cells in the gel shift assay (EMSA). Coincidently, violaxanthin well inhibited phosphorylation of ERK and IκBα, which induce the activation of LPS-dependent NF-κB and mitogen-activated protein kinase (MAPK). These experimental findings suggest that violaxanthin may exert its inhibitory effects on inflammation by inhibiting the activation of NF-κB, followed by a reduction in iNOS and COX-2 expression.

Several previous studies have reported that antioxidants, such as β-carotene and astaxanthin, can inhibit NF-κB activity and suppress the expression of pro-inflammatory genes as well as production of NO and PGE₂, which might inhibit NF-κB activity. These observations indicate that reactive oxygen species are related to inflammatory gene expression though the NF-κB signaling pathway. Recently, violaxanthin was reported to have strong antioxidant properties in oxidative stress and function as a chain breaking antioxidant in lipid peroxidation. Therefore, the antioxidant ability of violaxanthin may be originated from inhibition of iNOS and COX-2 expression.

In summary, we suggested that LPS stimulated RAW 267.4 via the TLR4-ERK/NF-κB in the pro-inflammatory signaling pathway, and violaxanthin effectively inhibited these pathways. Taken together, our results revealed that violaxanthin isolated from Chlorella ellipsoidea can be a favorable candidate for the treatment of inflammatory disorders, and the anti-inflammatory activity of violaxanthin may be based on its ability to inhibit the expression of iNOS and COX-2 via inactivation with NF-κB. Therefore, violaxanthin, a nontoxic natural product, may serve as a safe and effective anti-inflammatory agent, which could be used for therapeutic purposes.

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