

Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H₂O₂-induced cell damage

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Abstract In this study, the cytoprotective effect of fucoxanthin, which was isolated from *Sargassum siliquastrum*, against oxidative stress induced DNA damage was investigated. Fucoxanthin, a kind of carotenoid, was pretreated to the medium and the protective effect was evaluated via 2',7'-dichlorodihydrofluorescein diacetate, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide, and comet assays. Intracellular reactive oxygen species were over produced by addition of hydrogen peroxide (H₂O₂), but the production was significantly reduced by the treatment with fucoxanthin. The fucoxanthin strongly enhanced cell viability against H₂O₂ induced oxidative damage and the inhibitory effect of cell damage was a dose-dependent manner. Furthermore, a protective effect against oxidative stress-induced cell apoptosis was also demonstrated via nuclear staining with Hoechst dye. These results clearly

indicate that fucoxanthin isolated from *S. siliquastrum* possesses prominent antioxidant activity against H₂O₂-mediated cell damage and which might be a potential therapeutic agent for treating or preventing several diseases implicated with oxidative stress.

Keywords Fucoxanthin · *Sargassum siliquastrum* · Cell damage · Oxidative stress · Reactive oxygen species

Introduction

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radical (O₂⁻), hydroxyl radicals (·OH) and non free-radical species such as H₂O₂ and singlet oxygen (¹O₂), formed during normal metabolic processes, which can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. Free radical scavengers and antioxidants can reduce lipid peroxidation and the generation of ROS. The importance of antioxidants in human health has become increasingly clear due to spectacular advances in understanding the mechanisms of their reaction with oxidants. Furthermore, interest in employing antioxidants from natural sources to increase the shelf-life of foods is considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants [1–3].

Algae, as photosynthetic organisms, are exposed to a combination of light and high oxygen concentrations what induces the formation of free radicals and other oxidative reagents. The absence of structural damage in the algae leads to consider that these organisms are able to generate the necessary compounds to protect themselves against oxidation [4]. In this respect, algae can be considered as an

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important source of antioxidant compounds that could be suitable for protecting our bodies against the ROS formed e.g. by our metabolism or induced by external factors (as pollution, stress, UV radiation, etc.).

Fucoxanthin is found in edible brown algae and, along with β -carotene, is one of the most abundant carotenoids found in nature [5]. There have recently been several reports that fucoxanthin showed biological activities such as radical scavenging, antiproliferative, antiobesity, anti-tumor, and antiangiogenic activities [6–10]. Nonetheless, the protective effects of cell damages related to antioxidant activities of fucoxanthin have not yet been reported. Accordingly, the present study isolated the marine natural carotenoid fucoxanthin from *Sargassum siliquastrum* and evaluated its cytoprotective effects against H₂O₂-induced cell damage.

Materials and methods

Materials

The marine alga *S. siliquastrum* was collected along the coast of Jeju Island, Korea, between October 2005 and March 2006. 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 . Therefore, the frozen samples were lyophilized and homogenized with a grinder prior to extraction.

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. The UV and FT-IR spectra were recorded on a Pharmacia Biotech Ultrospec 3000 UV/Visible spectrometer and a SHIMAZU 8400s FT-IR spectrometer, respectively. NMR spectra were recorded on a Varian INOVA 400 MHz NMR spectrometer. CD₃OD was used as a solvent for the NMR experiments, and the solvent signals were used as an internal reference. ESI and HREI mass spectra acquired using a Finnigan Navigator 30086 and JMS-700 MSTATION high resolution mass spectrometer system, respectively. The HPLC was carried out on a Waters HPLC system equipped with a Waters 996 photodiode array detector and Millennium32 software using C₁₈ column (J'sphere ODS-H80, 150 mm \times 20 mm, 4 μm , YMC Co.).

Extraction and isolation

The powdered *S. siliquastrum* (400 g) was extracted with 80% aqueous MeOH, and was evaporated under vacuo.

Then, the MeOH extract was partitioned with CHCl₃. The chloroform extract (9 g) was fractionated by silica column chromatography using stepwise elution with CHCl₃-MeOH mixture (100:1 \rightarrow 1:1) to afford separated active fractions. A combined active fraction was further subjected to a Sephadex LH-20 column saturated with 100% MeOH, and then finally purified by reversed-phase HPLC to give fucoxanthin (74.9 mg). ¹H and ¹³C NMR data were assigned in Table 1 and the structure was illustrated in Fig. 1.

Cell culture

Cells of a monkey kidney fibroblast line (Vero) were maintained at 37 $^{\circ}\text{C}$ in an incubator with humidified atmosphere of 5% CO₂. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 $\mu\text{g}/\text{mL}$), and penicillin (100 U/mL).

Intracellular ROS measurement

For detection of intracellular ROS, Vero cells were seeded in 96-well plates at a concentration of 1×10^5 cells/mL. After 16 h, the cells were treated with various concentrations of fucoxanthin, and incubated at 37 $^{\circ}\text{C}$ under a humidified atmosphere. After 30 min, H₂O₂ was added at a concentration of 1 mM, and then cells were incubated for additional 30 min at 37 $^{\circ}\text{C}$. Finally, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; 5 $\mu\text{g}/\text{mL}$) was introduced to the cells, and 2',7'-dichlorofluorescein (DCF) fluorescence was detected at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, using a Perkin-Elmer LS-5B spectrofluorometer. The percentage of intracellular ROS scavenging activity was calculated in accordance with the following equation:

$$\text{Intracellular ROS scavenging activity(\%)} = [1 - (C_1/C_0)] \times 100$$

where C_1 is the fluorescence intensity of cells treated with H₂O₂ and fucoxanthin, and C_0 is the fluorescence intensity of cells treated with H₂O₂ and distilled water instead of fucoxanthin.

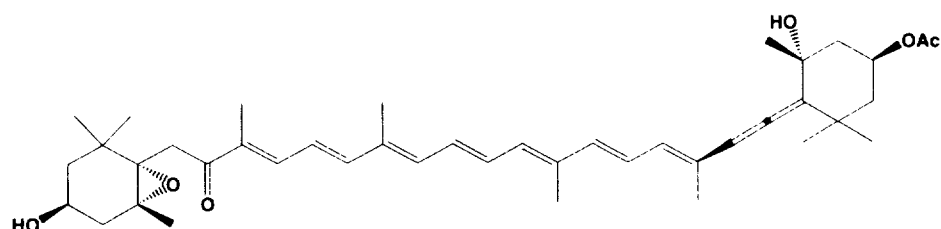
Assessment of cell viability

Cell viability was then estimated via an 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay, which is a test of metabolic competence predicated upon the assessment of mitochondrial performance. It is colorimetric assay, which is dependent on the conversion of yellow tetrazolium bromide to its purple formazan derivative by mitochondrial succinate dehydrogenase in

Table 1 NMR spectroscopic data for fucoxanthin in CDCl₃

| Position | ¹³ C | ¹ H (mult. <i>J</i> = Hz) | Position | ¹³ C | ¹ H (mult. <i>J</i> = Hz) |
|----------|-----------------|--|------------------------|-----------------|---|
| 1 | 35.8 | | 1' | 35.2 | |
| 2 | 47.1 | 1.36 (1H, dd, <i>J</i> = 8.7, 14.2) 1.49 (1H, dd, <i>J</i> = 14.2) | 2' | 45.4 | 1.41 (1H, dd, <i>J</i> = 10.4, 14.9) 2.00 (1H, dd, <i>J</i> = 2.9, 14.9) |
| 3 | 64.3 | 3.80 (1H, m) | 3' | 68.0 | 5.37 (1H, tt, <i>J</i> = 8.8, 12.0) |
| 4 | 41.6 | 1.77 (1H, dd, <i>J</i> = 8.7, 14.2) 2.29 (1H, dd, <i>J</i> = 2.9, 17.8) | 4' | 45.2 | 1.53 (1H, dd, <i>J</i> = 10.4, 14.9) 2.29 (1H, dd, <i>J</i> = 2.9, 17.8) |
| 5 | 66.2 | | 5' | 72.7 | |
| 6 | 67.1 | | 6' | 117.5 | |
| 7 | 40.8 | 2.59, 3.64 (2H, d, <i>J</i> = 20.4) | 7' | 202.4 | |
| 8 | 170.5 | | 8' | 103.4 | 6.04 (1H, s) |
| 9 | 134.5 | | 9' | 132.5 | |
| 10 | 139.1 | 7.14 (1H, d, <i>J</i> = 12.8) | 10' | 128.5 | 6.12 (1H, d, <i>J</i> = 11.6) |
| 11 | 123.4 | 6.58 (1H, m) | 11' | 125.7 | 6.71 (1H, t, <i>J</i> = 12.0) |
| 12 | 145.0 | 6.66 (1H, t, <i>J</i> = 12.8) | 12' | 137.1 | 6.34 (1H, d, <i>J</i> = 11.6) |
| 13 | 135.4 | | 13' | 138.1 | |
| 14 | 136.6 | 6.40 (1H, d, <i>J</i> = 11.6) | 14' | 132.2 | 6.26 (1H, d, <i>J</i> = 11.6) |
| 15 | 129.4 | 6.67 (1H, m) | 15' | 132.5 | 6.71 (1H, t, <i>J</i> = 12.0, 14.2) |
| 16 | 25.0 | 1.37 (3H, s) | 16' | 29.2 | 1.37 (3H, s) |
| 17 | 28.1 | 0.95 (3H, s) | 17' | 32.1 | 1.06 (3H, s) |
| 18 | 21.2 | 1.34 (3H, s) | 18' | 31.3 | 1.34 (3H, s) |
| 19 | 11.8 | 1.80 (3H, s) | 19' | 14.0 | 1.80 (3H, s) |
| 20 | 12.8 | 1.98 (3H, s) | 20' | 12.9 | 1.98 (3H, s) |
| | | | 3'OAc, CH ₃ | 21.4 | 2.03 (3H, s) |
| | | | 3'OAc, C=O | 197.9 | |

Data were obtained using 400 MHz for ¹H and 100 MHz for ¹³C

Fig. 1 Chemical structure of fucoxanthin

viable cells [11]. The cells were seeded in 96-well plate at a concentration of 1×10^5 cells/mL. After 16 h, the cells were treated with fucoxanthin at difference concentrations. Then, 10 μ L of H₂O₂ (1 mM) was added to the cell culture medium, and incubated for 24 h at 37 °C. MTT stock solution (50 μ L; 2 mg/mL) was then applied to the wells, to a total reaction volume of 200 μ L. After 4 h of incubation, the plates were centrifuged for 5 min at 800 \times g, and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μ L of dimethylsulfoxide (DMSO), and the absorbance was measured via ELISA at a wavelength of 540 nm. Relative cell viability was evaluated in accordance with the quantity of MTT converted to the insoluble formazan salt. The optical density of the

formazan generated in the control cells was considered to represent 100% viability. The data are expressed as mean percentages of the viable cells versus the respective control.

Determination of DNA damage by comet assay

Comet assay was performed to determine the oxidative DNA damage [12]. The cell suspension was mixed with 75 μ L of 0.5% low melting agarose (LMA), and added to the slides precoated with 1.0% normal melting agarose. After solidification of the agarose, slides were covered with another 75 μ L of 0.5% LMA and then immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and

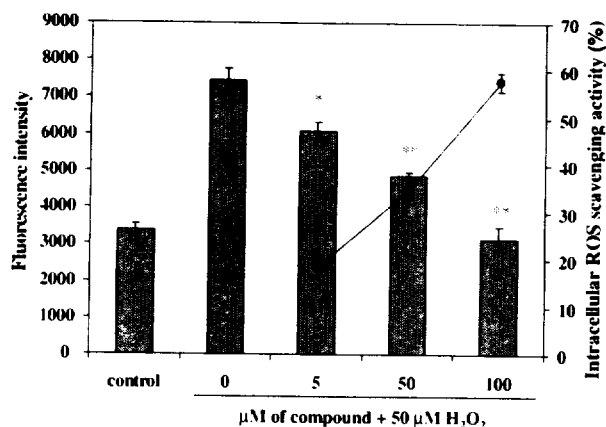


Fig. 2 Effect of fucoxanthin isolated from *S. siliquastrum* on scavenging ROS. The intracellular ROS generated was detected by DCFH-DA assay. **Dark filled square** indicates fluorescence intensity; **Dark filled circle** indicates intracellular ROS scavenging activity. Statistical evaluation was performed to compare the experimental groups and corresponding control groups. * $p < 0.05$, ** $p < 0.01$

1% sodium lauryl sarcosine; 1% Triton X-100 and 10% DMSO) for 1 h at 4 °C. The slides were next placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na₂EDTA (pH 13.0) for 40 min for DNA unwinding. For electrophoresis of the DNA, an electric current of 25 V/300 mA was applied for 20 min at 4 °C. The slides were washed three times with a neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4 °C, and then treated with ethanol for another 5 min before staining with 50 μL of ethidium bromide (20 μg/mL). Measurements were made by image analysis (Kinetic Imaging, Komet 5.0, UK) and fluorescence microscope (LEICA DMLB, Germany), determining the percentage of fluorescence in the tail (tail intensity; 50 cells from each of two replicate slides).

Nuclear staining with Hoechst 33342

The nuclear morphology of the cells was evaluated using the cell-permeable DNA dye, Hoechst 33342. Cells with homogeneously stained nuclei were considered viable, whereas the presence of chromatin condensation and/or fragmentation was indicative of apoptosis [13, 14]. The cells were placed in 24-well plates at a concentration of 1×10^5 cells/mL. Sixteen hours after plating, the cells were treated with various concentration of fucoxanthin, and further incubated for 1 h prior to expose to H₂O₂ (1 mM). After 24 h, 1.5 μL of Hoechst 33342 (stock 10 mg/mL), a DNA-specific fluorescent dye, were added to each well, followed by 10 min of incubation at 37 °C. The stained cells were then observed under a fluorescence microscope equipped with a CoolSNAP-Pro color digital camera, in order to examine the degree of nuclear condensation.

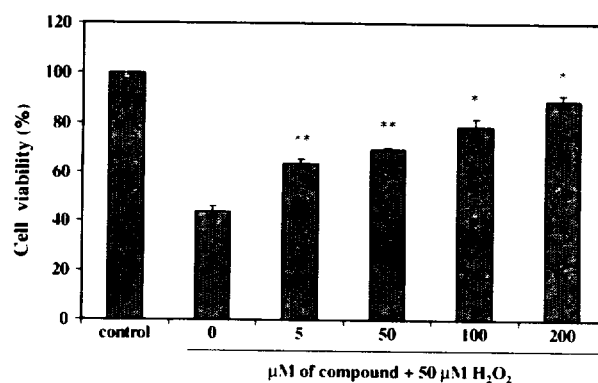


Fig. 3 Protective effect of fucoxanthin isolated from *S. siliquastrum* on H₂O₂ induced oxidative damage of vero cells. The viability of cells on H₂O₂ treatment was determined by MTT assay. Statistical evaluation was performed to compare the experimental groups and corresponding control groups. * $p < 0.05$, ** $p < 0.01$

Statistical analysis

The data are expressed as the mean \pm standard error. A statistical comparison was performed via the SPSS package for Windows (Version 10). p -Values of <0.05 were considered to be significant.

Results and discussion

Cells are protected from ROS-induced damage by a variety of endogenous ROS-scavenging enzymes, chemical compounds, and natural products. Recently, interest has been increased in the therapeutic potential of natural products to be used as natural antioxidants in the reduction of such free

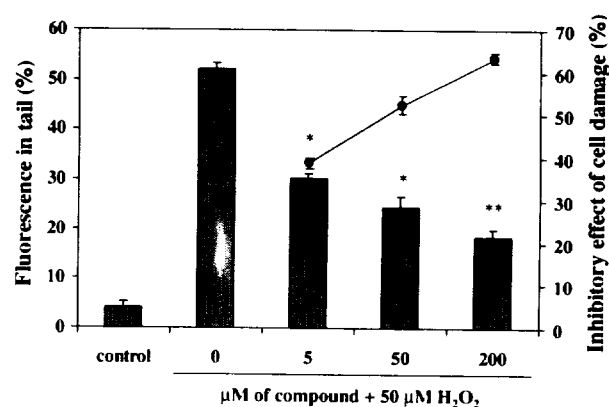
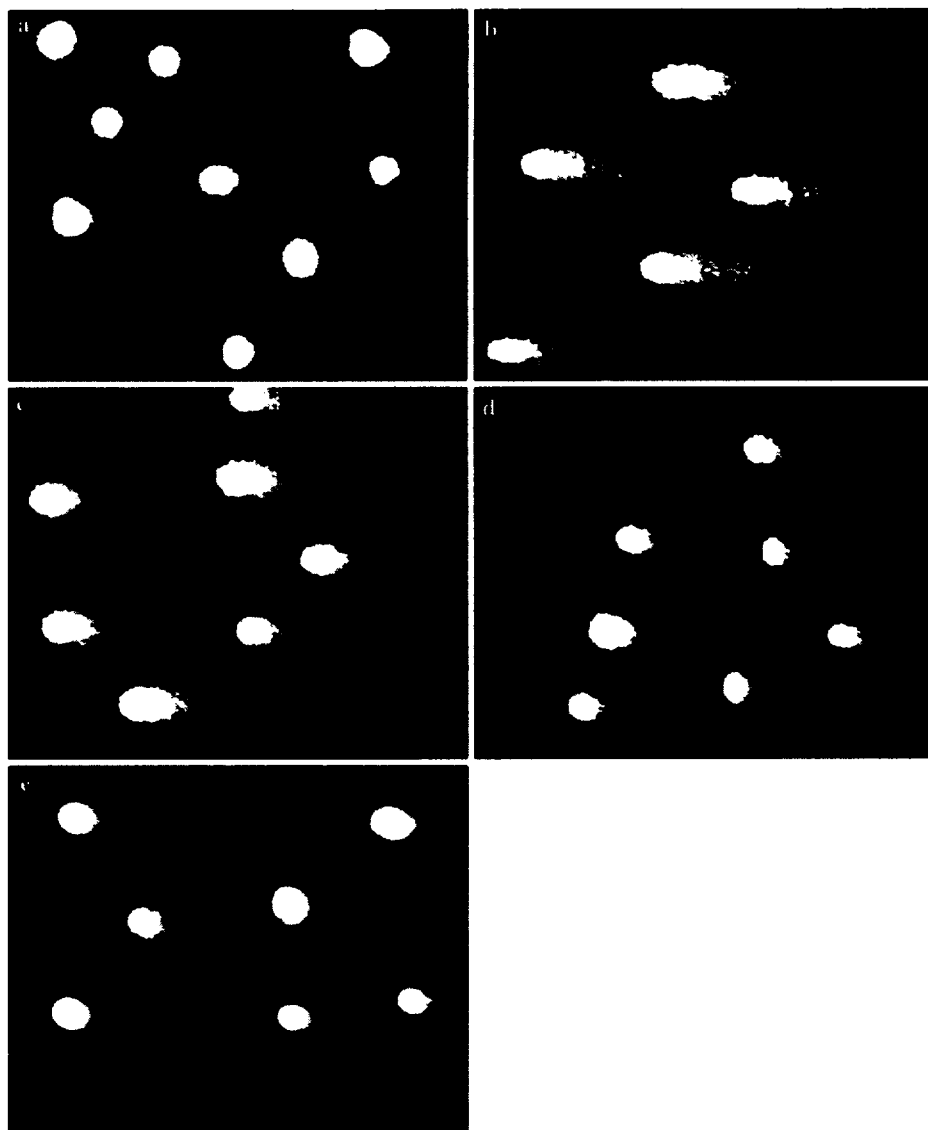


Fig. 4 Inhibitory effect of different concentration of fucoxanthin isolated from *S. siliquastrum* on H₂O₂ induced DNA damage. The damaged cells on H₂O₂ treatment was determined by comet assay. **Dark filled square** indicates percentage of fluorescence in tail; **Dark filled circle** indicates inhibitory effect of cell damage. Statistical evaluation was performed to compare the experimental groups and corresponding control groups. * $p < 0.05$, ** $p < 0.01$

Fig. 5 Photomicrographs of DNA damage and migration observed under fucoxanthin isolated from *S. siliquastrum* where the tail moments were decreased. **a** control, **b** cells treated with 50 μM H_2O_2 , **c** cells treated with 5 μM compound + 50 μM H_2O_2 , **d** cells treated with 50 μM compound + 50 μM H_2O_2 , **e** cells treated with 200 μM compound + 50 μM H_2O_2



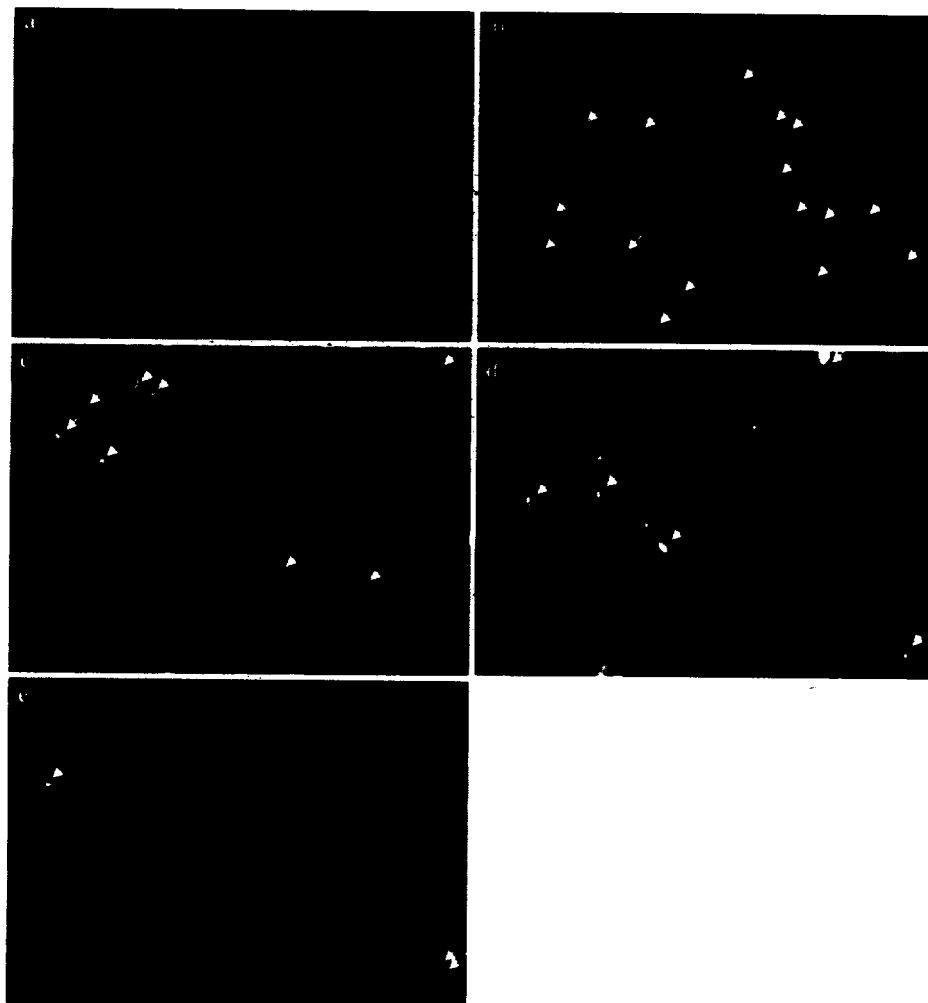
radical-induced tissue injuries, thereby suggesting that many natural products may prove to possess therapeutically useful antioxidant compounds [15–17].

H_2O_2 has been extensively used as an inducer of oxidative stress in in vitro models. It readily crosses the cellular membranes giving rise to the highly reactive hydroxyl radical, which has the ability to react with macromolecules, including DNA, proteins, and lipids, and ultimately damage to whole cell [18, 19]. Several studies have shown that oxidative stress is a major cause of cellular injuries in a variety of human diseases including cancer, neurodegenerative, and cardiovascular disorders [20–22]. Recently, many researchers have made considerable efforts to search natural antioxidants. Many studies have revealed that seaweeds have potential to be used as a candidate for natural antioxidant. Potent antioxidative compounds have

already been isolated from seaweeds and identified as phylophoeophytin in *Eisenia bicyclis* (arame), fucoxanthin in *Hijikia fusiformis* (hijiki), phlorotannin in *Ecklonia stolonifera*, bromophenols in *Polysiphonia urceolata*, and sulfated polysaccharides [6, 23–27]. Among these antioxidants, fucoxanthine, a kind of carotenoid, showed radical scavenging activity. In this study, we investigated the antioxidant effects of natural carotenoid fucoxanthin, after the administration of H_2O_2 in cell lines.

2',7'-Dichlorodihydrofluorescein diacetate was used as a probe for ROS measurement. DCFH-DA crosses cell membranes and is hydrolyzed enzymatically by intracellular esterase to nonfluorescent DCFH. In the presence of ROS, DCFH is oxidized to highly fluorescent DCF. It is well known that H_2O_2 is the principal ROS responsible for the oxidation of DCFH to DCF [28]. In the present study,

Fig. 6 Protective effect of fucoxanthin isolated from *S. siliquastrum* against H_2O_2 -induced apoptosis in vero cells. Cellular morphological changes were observed under a fluorescence microscope after Hoechst 33342 staining. **a** untreated, **b** 500 μM H_2O_2 , **c** 5 μM compound + 500 μM H_2O_2 , **d** 50 μM compound + 500 μM H_2O_2 , **e** 200 μM compound + 500 μM H_2O_2 . Apoptotic bodies are indicated by arrows



we investigated scavenging activity of fucoxanthin on intracellular ROS and the results are illustrated in Fig. 2. Fluorescence intensity of control cells was recorded as 3398 while H_2O_2 treated cells showed 7452. However, the addition of fucoxanthin to the cells mixed with H_2O_2 reduced intracellular ROS accumulation to 6079, 4867, and 3149 at 5, 50, and 100 μM , respectively. Moreover, the scavenging activity of fucoxanthin on intracellular ROS was dose-dependently increased as 18.4, 34.7, and 57.7% at 5, 50, and 100 μM , respectively. Hence these results indicate that fucoxanthin has efficient antioxidant properties which can be developed into a potential bio-molecular candidate to inhibit ROS formation of cellular.

To evaluate whether fucoxanthin protect from cellular damage induced by H_2O_2 , cells were pretreated with fucoxanthin for 24 h in the absence or presence of oxidative stress. After then, cell viability was measured via MTT assay. As shown in Fig. 3, H_2O_2 treatment without fucoxanthin decreased cell viability to 43.7%, while fucoxanthin prevented cells from H_2O_2 -induced damage, restoring cell

survival to 63.6, 69.4, 78.5, and 89.2% at the concentrations of 5, 50, 100, and 200 μM , respectively. Active mitochondria of living cells can cleave MTT to produce formazan, the amount of which is directly correlated to the living cell number [29]. The results of MTT assay showed that formazan content was reduced due to H_2O_2 treatment, however, significantly increased with addition of fucoxanthin. These results suggest that fucoxanthin has ability to protect vero cells from oxidative stress-related cellular injuries.

The protective effect of fucoxanthin on DNA damages was also confirmed by comet assay and was presented as tail DNA percent. This assay can reflect different types of DNA damage, such as DNA single strand breakage, or incomplete DNA repairing and shows high sensitivity in detecting oxidative stress [30]. As shown in Fig. 4, H_2O_2 treatment increased the tail moments in the cells versus the control cells. However, the increased tail moments caused by H_2O_2 induced oxidative DNA damage were decreased in the cells treated with fucoxanthin. The inhibition effect

of fucoxanthin on DNA damage was 38.8, 52.5, and 63.4% at the concentrations of 5, 50, and 200 μM , respectively. The DNA migration profiles of tested cells when treated with different concentrations of fucoxanthin are presented in Fig. 5. The DNA was completely damaged and the amount of tail DNA was significantly increased in the group treated with only H_2O_2 (Fig. 5b), compared to the control (Fig. 5a). However, the amount of tail DNA was increasingly decreased with increased concentrations of fucoxanthin (Fig. 5c–e). In a previous study, Kang et al. [23] reported that some natural compounds from brown seaweeds have an ability to increase catalase located at peroxisome in cell that converts H_2O_2 into molecular oxygen and water. In this study, we showed that fucoxanthin has prominent effect on the H_2O_2 -induced DNA damage. The inhibition activities on the DNA damage and the decrease of the amounts of tail DNA might be related to the ability of fucoxanthin to increase catalase.

The protective effect of fucoxanthin on apoptosis induced by H_2O_2 was investigated by nuclear staining with Hoechst 33342. Hoechst 33342 dye specifically stains DNA, and is widely used to detect the nuclear shrinkage such as chromatin condensation, nuclear fragmentation, and the appearance of apoptotic bodies, which are all indications of apoptosis [31]. The microscopic photograph in Fig. 6, the negative control, which contained no compound or H_2O_2 , possessed intact nuclei (Fig. 6a), and the positive control (H_2O_2 treated cells) exhibited significant nuclear fragmentation, indicating apoptosis (Fig. 6b). However, the addition of fucoxanthin with H_2O_2 reduced the apoptotic bodies (Fig. 6c–e), which suggested the ability of fucoxanthin to protect against nuclear fragmentation amidst oxidative stress.

In conclusion, the results obtained in the present study show that fucoxanthin isolated from *S. siliquastrum* can effectively inhibit intracellular ROS formation, DNA damage, and apoptosis induced by H_2O_2 . Moreover, fucoxanthin showed evidence in cell survival rate. These results revealed that fucoxanthin can be used not only as the easily accessible source of natural antioxidants but also as an ingredient of the functional food and cosmetic agents related to the prevention and control oxidative stress.

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References

- Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Huynh-Ba T, Lambelet P, McPhail D, Skibsted LH, Tijburg L (2001) Eur Food Res Technol 212:319–328
- Farag RS, El-Baroty GS, Basuny AM (2003) Int J Food Sci Technol 38:81–87
- Heo SJ, Park EJ, Lee KW, Jeon YJ (2005) Bioresour Technol 96:1613–1623
- Jimenez-Escrig A, Jimenez-Jimenez I, Pulido R, Saura-Calixto F (2001) J Sci Food Agric 81:530–534
- Berhard K, Moss GP, Toth GY, Weedon BLC (1976) Tetrahedron Lett 17:115–118
- Yan X, Chuda Y, Suzuki M, Nagata T (1999) Biosci Biotechnol Biochem 63:605–607
- Hosokawa M, Kudo M, Maeda H, Kohno H, Tanaka T, Miyashita K (2004) Biochim Biophys Acta 1675:113–119
- Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K (2005) Biochem Biophys Res Commun 332:392–397
- Kotake-Nara E, Asai A, Nagao A (2005) Cancer Lett 220:75–84
- Sugawara T, Matsubara K, Akagi R, Mori M, Hirata T (2006) J Agric Food Chem 54:9805–9810
- Mosmann T (1983) J Immunol Methods 65:55–63
- Singh NP (2000) Mutat Res 455:111–127
- Gschwind M, Huber G (1995) J Neurochem 65:292–300
- Lizard G, Fournel S, Genestier L, Dhedin N, Chaput C, Flacher M, Mutin M, Panaye G, Revillard JP (1995) Cytometry 21:275–283
- Lee SE, Ju EM, Kim JH (2002) Exp Mol Med 34:100–106
- Jung WK, Rajapakse N, Kim SK (2005) Eur Food Res Technol 220:535–539
- Ahn GN, Kim KN, Cha SH, Song CB, Lee J, Heo MS, Yeo IK, Lee NH, Jee YH, Kim JS, Heo MS, Jeon YJ (2007) Eur Food Res Technol 226:71–79
- Satoh T, Sakai N, Enokido Y, Uchiyama Y, Hatanaka H (1996) J Biochem 120:540–546
- Halliwell B, Clement MV, Long LH (2000) FEBS Lett 486:10–13
- Al-Enezi KS, Alkhalaf M, Benov LT (2006) Free Radic Biol Med 40:1144–1151
- Zhang L, Yu H, Sun Y, Lin X, Chen B, Tan C, Cao G, Wang Z (2007) Eur J Pharmacol 564:18–25
- Strazzullo P, Puig JG (2007) Nutr Metab Cardiovasc Dis 17:409–414
- Kang HS, Chung HY, Jung HA, Son BW, Choi JS (2003) Chem Pharm Bull 51:1012–1014
- Cahyana AH, Shuto Y, Kinoshita Y (1992) Biosci Biotechnol Biochem 56:1533–1535
- Li K, Li XM, Ji NY, Wang BG (2007) Bioorg Med Chem 15:6627–6631
- Zhang Q, Li N, Liu W, Zhao Z, Li Z, Xu Z (2004) Carbohydr Res 339:105–111
- Kim SH, Choi DS, Athucorala Y, Jeon YJ, Senevirathne M, Rha CK (2007) Int J Food Sci Nutr 12:65–73
- LeBel CP, Ischiopoulos H, Bondy SC (1992) Chem Res Toxicol 5:227–231
- Hu Z, Guan W, Wang W, Huang L, Xing H, Zhu Z (2007) Cell Biol Int 31:798–804
- Senthilmohan ST, Zhang J, Stanley RA (2003) Nutr Res 23:1199–1210
- Kerr JF, Gobe GC, Winterford CM, Harmon BV (1995) Methods Cell Biol 46:1–27

