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Protective Role of Alginic Acid and Fucoidan from Nitric Oxide-Induced Oxidative Stress

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Abstract

The protective activities of alginic acid and fucoidan from oxidative stress (OS) were investigated under cellular system using LLC-PK1 renal epithelial cells that are widely used for study cellular OS. The cellular OS in LLC-PK1 cells was induced by the free radical generators. The treatment of pyrogallol, sodium nitroprusside or 3-morpholinosydnonimine led to the production of superoxide anion (O_2^-), nitric oxide (NO) and peroxynitrite ($ONOO^-$), respectively. The free radical generators decreased cell viability and elevated lipid peroxidation compared OSwith non-treated cells. However, the treatment of alginic acid and fucoidan attenuated OS significantly induced by NO, O_2^- and $ONOO^-$, resulting in the significant increase of cell viability and inhibition of lipid peroxidation in concentration-dependent manner. In particular, fucoidan showed the stronger protective effect from oxidative stress than alginic acid. These results indicated that alginic acid and fucoidan played the protective role from NO-induced OS. The present study suggests the promising antioxidative agents of alginc acid and fucoidan with protective activity from free radical-induced OS.

Keywords: alginic acid, fucoidan, nitric oxide, peroxide, oxidative stress, antioxidant therapy, protective effect

1. Introduction

The oxidative stress (OS) caused by nitric oxide (NO) has attracted much attention because the metabolic products, such as peroxynitrite ($ONOO^-$) and the hydroxyl radical (·OH), as well as NO itself have been suggested to be important pathogenic causative agents of cellular damage, alterations of biomolecules and organ dysfunction. The toxicity and damage caused by NO in tissues and cells are multiplied enormously if NO reacts with the superoxide anion (O_2^-) to yield $ONOO^-$, an extremely reactive radical (Akaike, Suga, & Maeda, 1998; Radi, Beckman, Bush, & Freeman, 1991). The reactions between $ONOO^-$ and biological molecules mediate toxic oxidative and nitrosative reactivity, leading to impaired function, toxicity and alterations in signaling

pathways that are implicated in diverse forms of free radical-induced tissue injury. The pathophysiological importance of NO suggests that regulation of NO formation may be an efficient intervention strategy to improve or alleviate these pathological conditions. Therefore, antioxidant therapy to reduce the toxicity of $ONOO^-$ as well as that of NO is considered to be a new avenue of therapeutic intervention and may have beneficial effects by ameliorating the damage and lesions involved in various pathological conditions. Unfortunately, only very few studies on the effects of antioxidants against NO-induced oxidative damage have been carried out. Although several synthetic antioxidants have been suggested for the prevention and treatment of diseases, their side effects and toxicities have become issues during or in post-treatment period. Therefore, dietary natural antioxidants have attracted much attention as preventive and therapeutic agents attenuating oxidative damage because of their safe role and effectiveness. Edible seaweeds have been studied for their various biological effects. Although the active components from brown seaweeds have not been clearly elucidated, they contain abundant alginate and fucoidan which are soluble dietary polysaccharide fibers (Ruperez, Ahrazem, & Leal, 2002). Alginic acid consists of alternating units of mannuronic and guluronic acids. Several reports have shown biological functions of alginic acid such as hypocholesterolemic, antidiabetic, antimutagenic, anticancer and antioxidative activities (Torsdottir, Alpsten, Holm, Sandberg, & Tolli, 1991; Kimura, Watanabe, & Okuda, 1996; Cho, Rhee, & Park, 1998; Xue, Yu, Hirata, Terao, & Lin, 1998; Lee, Choi, & Seo, 2004; So *et al*., 2007). Fucoidan contains a number of fucose and sulfate ester groups and it has been reported to exhibit various biological activities, such as antioxidant (Ruperez *et al*., 2002), antitumor (Yamamoto, Takahashi, Suzuki, Seino, & Mori, 1984), anticoagulant (Nishino, Kiyohara, Yamada, & Nagumo, 1991) and antiviral (Feldman, Reynaldi, Stortz, Cerezo, & Damonte, 1999) activities. However, the protective activities of alginic acid and fucoidan from NO-induced OS have not been clearly elucidated. In particular, under the oxidative cellular system using LLC-PK1 renal epithelial cells that are widely used for the study on the protective effect from OS, the antioxidative activity of fucodian and alginic acid has not been investigated. In this study, we investigated the protective role of fucoidan and alginic acid under cellular OS model using LLC-PK1 renal epithelial cells.

2. Materials and Methods

2.1 Materials

Alginic acid, fucoidan, 3-morpholinosydnonimine (SIN-1), pyrogallol, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H* tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) and sodium nitroprusside (SNP) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The LLC-PK1 porcine renal epithelial cells were obtained from ATCC (Manassas, VA, USA). Dulbecco's modified eagle medium/nutrient mixture F-12 (DMEM/F-12) and fetal bovine serum (FBS) were purchased from Invitrogen Co. (Grand Island, NY, USA).

2.2 Cell Culture

Commercially available LLC-PK1 cells were maintained at 37℃ in a humidified atmosphere of 5% CO_2 in culture plates with 5% FBS-supplemented DMEM/F-12 medium. The cells were sub-cultured weekly with 0.05% trypsin-EDTA in calcium- and magnesium-free phosphate buffer.

2.3 Treatment of Radical Generator

After confluence of LLC-PK1 cells had been reached, the cells were seeded into 96-well culture plates at 10^4 cells/mL. Two hours later, 1.2 mM of pyrogallol, 1.2 mM of SNP or 1.0 mM of SIN-1 were treated for 24 hr, and then alginic acid or fucoidan was added for 24 hr in the test wells at various concentrations. (Yokozawa, Cho, Hara, & Kitani, 2000; Yokozawa, Satoh, Cho, Kashiwada, & Ikeshiro, 2005; So *et al*., 2007).

2.4 MTT Assay

Cell viability was determined using a MTT colorimetric assay (Mosmann, 1983). Fifty microliters of MTT (1 mg/mL) solution was added to each well. After incubation for 4 hr at 37℃, MTT solution was removed from the medium. The resultant formazan crystals in the renal cells were solubilized with 100 μL of dimethylsulfoxide. The absorbance of each well was then read at 540 nm using a microplate reader (model SPECTRAmax 340PC, Molecular Devices, Sunnyvale, CA, USA).

2.5 Thiobarbituric Acid Reactive Substances (TBARS)

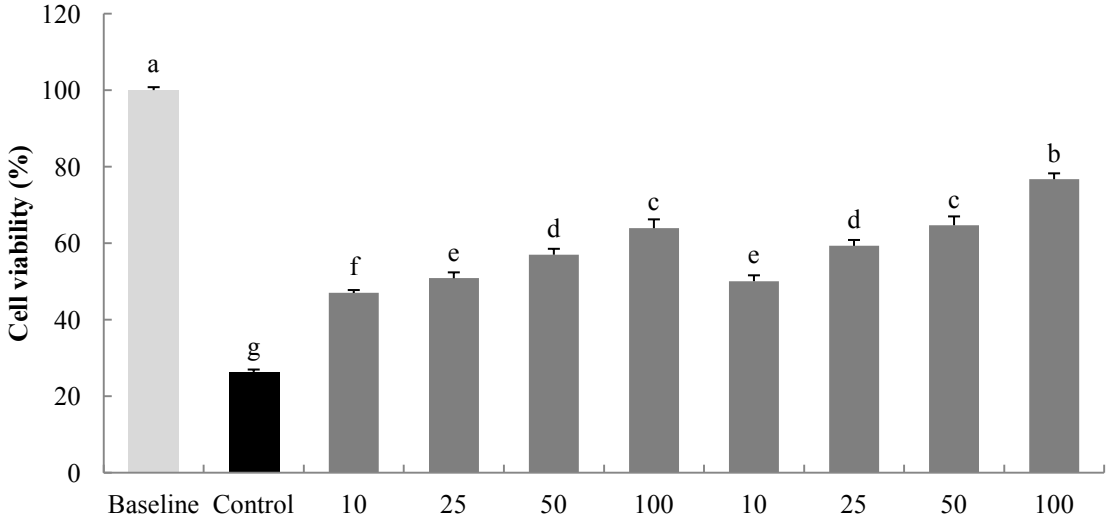
The level of lipid peroxidant released from the cultured cells was estimated as TBARS according to the methods of Yagi (1976) and Yokode *et al.* (1988) with a slight modification. One aliquot of medium was mixed with 1.5 mL of 0.67% TBA aqueous solution and 1.5 mL of 20% trichloroacetic acid, and boiled at 95 ~ 100℃ for 45 min. The mixture was cooled and shaken vigorously with 3.0 mL of *n*-butanol. After centrifuging at 4,000 ×g for 10 min, the fluorescence of the n-BuOH layer was measured at an excitation wavelength of 515 nm and an emission wavelength of 553 nm using a fluorescence spectrophotometer.

2.6 Statistical Analysis

The results for each group were expressed as mean±SD values (n=3). Data were analyzed by one way ANOVA between control and sample treated groups using SAS software (SAS Institute Inc., Cary, NC, USA). Significant differences were determined among groups at $p<0.05$.

3. Results

3.1 Protective Activity against SNP-Induced OS

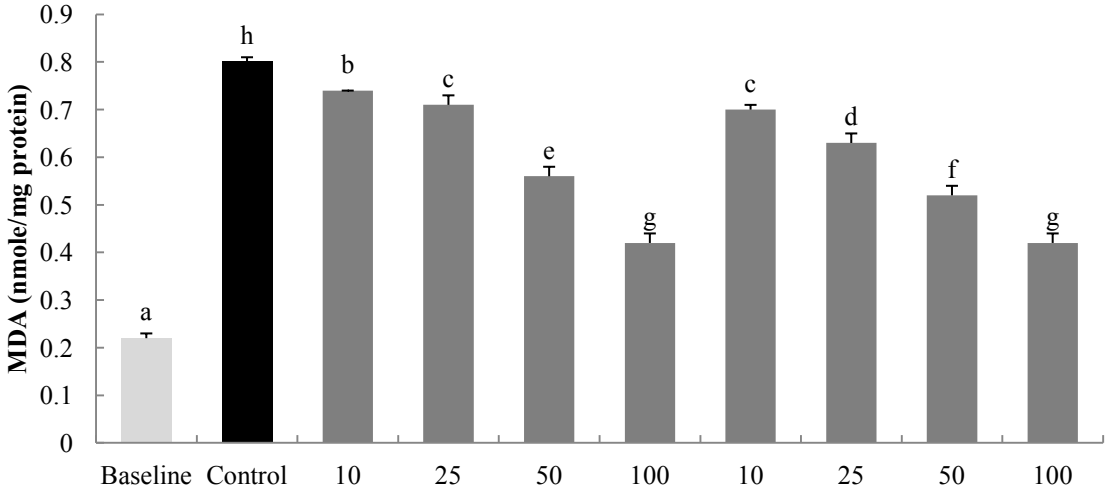


Data are presented mean±SD (n=3)
[a~g]Mean values with different superscripts are statistically different from each other at $p<0.05$

Figure 1. Protective effect of alginic acid and fucoidan on cell viability of LLC-PK_1 cells treated with SNP

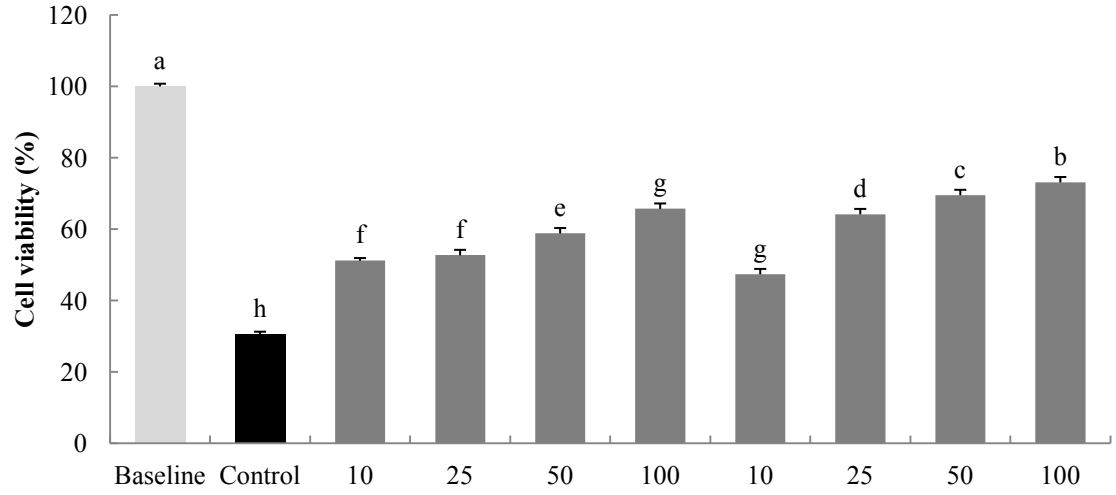
Figure 1 shows the effects of alginic acid and fucoidan under SNP-induced OS in LLC-PK1 cells. Treatment of SNP led to decrease in cell viability from 100% to 26.18%. However, the treatment of alginic acid and fucoidan exerted protective activity against SNP-induced cellular damage. The cell

viability was elevated by their treatments concentration-dependently, showing the cell viability of 63.90% and 76.70% at the concentrations of 100 μg/mL of alginic acid and fucoidan, respectively. In addition, the treatment of SNP elevated MDA production markedly, whereas alginic acid and fucoidan led to the reduction the levels as concentration-dependent manner (Figure 2). At the treatment of concentration of 25, 50 and 100 μg/mL of alginic acid, lipid peroxidation was decreased from 0.80 nmole/mg protein to 0.71, 0.56 and 0.42 nmole/mg protein, respectively. In addition, fucoidan at 25, 50 and 100 μg/mL lowered the MDA level to 0.63, 0.52 and 0.42 nmole/mg protein, respectively.



Data are presented mean±SD (n=3).
[a~h]Mean values with different superscripts are statistically different from each other at $p<0.05$

Figure 2. Protective effect of alginic acid and fucoidan on lipid peroxidation in LLC-PK_1 cells treated with SNP

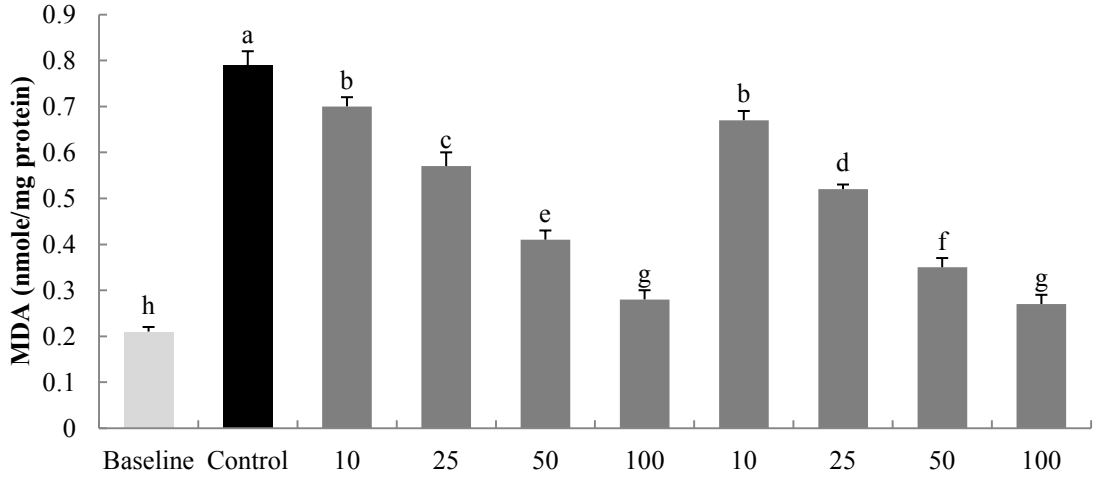


Data are presented mean±SD (n=3)
[a~h]Mean values with different superscripts are statistically different from each other at $p<0.05$

Figure 3. Protective effect of alginic acid and fucoidan on cell viability of LLC-PK_1 cells treated with pyrogallol

3.2 Protective Activity against Pyrogallol-Induced OS

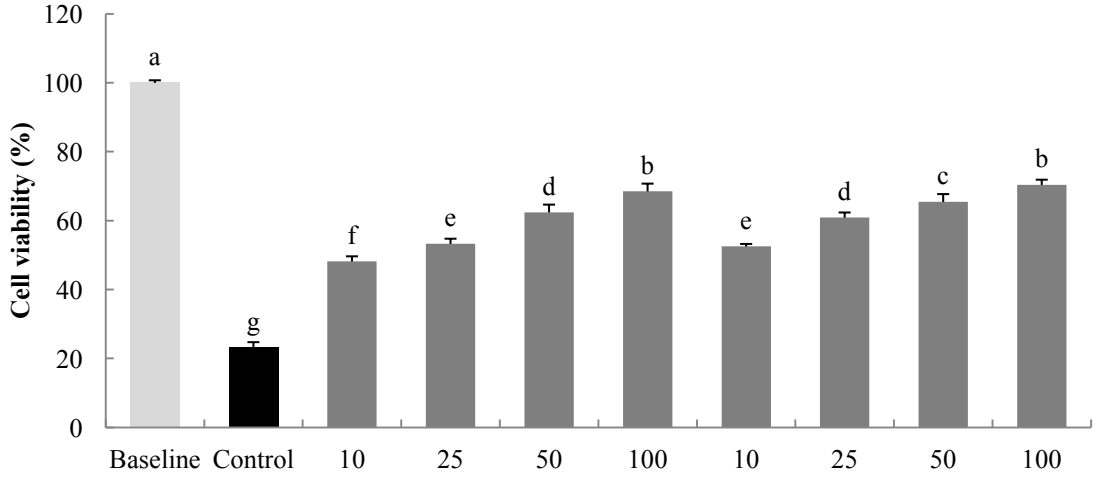
Figure 3 represents the result on the cell viability under OS induced by pyrogallol. By the treatment of alginic acid and fucoidan at the concentration of 100 μg/mL, cell viability was increased from 30.56% to 65.70% and 73.10%, respectively. Moreover, MDA generation was significantly elevated by pyrogallol, but the treatment of alginic acid and fucoidan showed the significant and dose-dependent declines of lipid peroxidation (Figure 4). At 100 μg/mL of alginic acid and fucoidan treatment, MDA level was decreased from 0.79 nmole/mg protein to 0.28 nmole/mg protein and 0.27 nmole/mg protein.



Data are presented mean±SD (n=3)
[a~h]Mean values with different superscripts are statistically different from each other at $p<0.05$

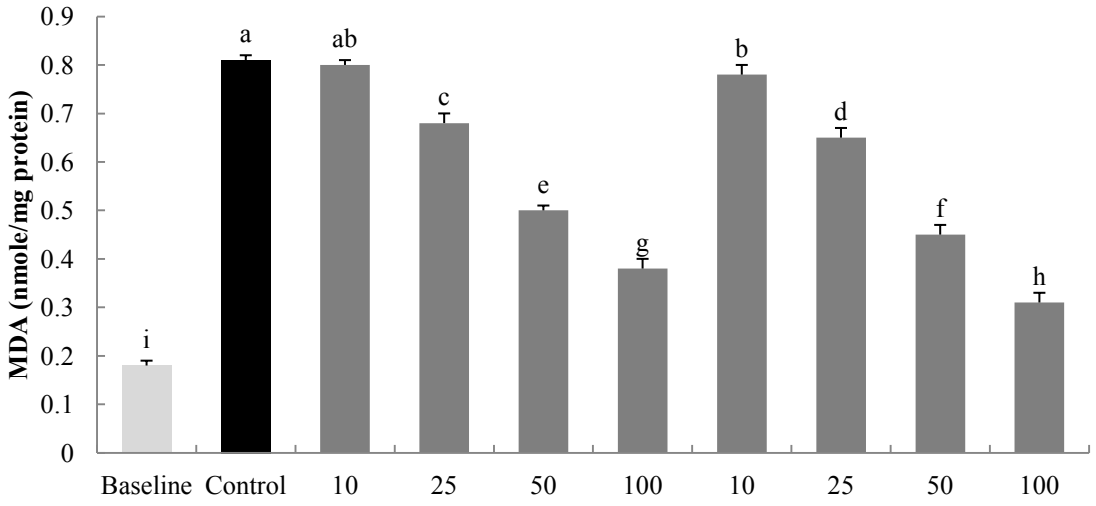
Figure 4. Protective effect of alginic acid and fucoidan on lipid peroxidation in LLC-PK_1 cells treated with pyrogallol

3.3 Protective Activity against SIN-1-Induced OS



Data are presented mean±SD (n=3)
[a~g]Mean values with different superscripts are statistically different from each other at $p<0.05$

Figure 5. Protective effect of alginic acid and fucoidan on cell viability of LLC-PK_1 cells treated with SIN-1



Data are presented mean±SD (n=3).
[a~i]Mean values with different superscripts are statistically different from each other at $p<0.05$.

Figure 6. Protective effect of alginic acid and fucoidan on lipid peroxidation in LLC-PK_1 cells treated with SIN-1

Figure 5 and 6 show the protective activities of alginic acid and fucoidan from $ONOO^-$. The treatment of SIN-1 declined cell viability to 23.14%, whereas the treatment of alginic acid and fucoidan at 100 μg/mL led to the increase in cell viability to 68.50% and 70.40% (Figure 5). In addition, the elevated lipid peroxidation by SIN-1 was significantly decreased by the two antioxidants (Figure 6). The treatment of alginic acid at 25, 50 and 100 μg/mL inhibited lipid peroxidation from 0.81 nmole/mg protein to 0. 68, 0.50 and 0.38 nmole/mg protein, respectively. In addition, fucoidan also exerted the protective effect from lipid peroxidation by SIN-1, indicating a decline trend in MDA level of 0.65, 0.45 and 0.31 nmole/mg protein at the concentrations of 25, 50 and 100 μg/mL. Fucoidan had stronger protective effect than alginic acid from $ONOO^-$.

4. Discussion

The over production of NO results in oxidative damage and contributes to disorder conditions (Halliwell, 1987; Halliwell, Zhao, & Whiteman, 1999). In addition, reaction of O_2^- with NO leads to the formation of $ONOO^-$ which is far more reactive and toxic than its precursors (Beckman, Beckman, Chen, Marshall, & Freeman, 1990; Patel *et al*., 1999). This reaction is extremely rapid, and it can generate the most toxic and reactive radical, ·OH, that can react with unsaturated fatty acids of membrane phospholipids to generate free radicals, which in turn react quickly with oxygen to form peroxides (Blough, & Zafiriou, 1985).

Free radical-induced OS has been implicated in a variety of degenerative diseases as well as in the aging process (Bokov, Chaudhuri, & Richardson, 2004; Scott, & King, 2004; Gibson, & Huang, 2005). Therefore, antioxidants that scavenge free radicals and prevent free radical induced damage have attracted much attention, and there have been a great deal of effort to identify safe and effective therapeutic agents for OS-related diseases. Edible seaweeds are rich sources of dietary fibers, minerals, vitamins, proteins and antioxidants (Cahyana, Shuto, & Kinoshita, 1992; Yan, Chuda, Suzuki, & Nagata, 1999). In particular, fucoidan and alginic acid have been known as the main active compounds with various health beneficial effects including hypocholesterolemic, antidiabetic, anticancer, antiviral and antioxidative activities (Torsdottir *et al*., 1991; Kimura *et al*,

1996; Cho, Rhee, & Park, 1998; Xue *et al*., 1998; Lee *et al*., 2004). In the present study, the protective role of alginic acid and fucoidan from free radical-induced OS using LLC-PK_1 cells, which are vulnerable to OS induced by free radicals.

The cellular model of OS using LLC-PK_1 cells is well established and it is useful for searching for agents that can provide effective protection from free radicals (Yokozawa *et al*., 2000; Yokozawa *et al*., 2005; Piao *et al*., 2005). Free radical generators such as SNP, pyrogallol, and SIN-1, were treated to induce cellular OS in LLC-PK_1 cells. NO and O_2^- generated by SNP and pyrogallol, respectively, led to significant decline in cell viability and elevation of lipid peroxidation compared to non-treated cells. It indicated that the cellular OS was induced by the generators of NO and O_2^-. However, the treatment of alginic acid and fucoidan showed the significant protective effects from cellular loss and lipid peroxidation induced by NO and O_2^-. Peroxynitrite derived from NO and O_2^- is a strong oxidant and nitrating agent and can lead for the production of the most toxic ·OH radical (Beckman *et al*., 1990). SIN-1 simultaneously generates both NO and O_2^-, which then combine rapidly to form $ONOO^-$. The treatment of SIN-1 led to the significant decline of cell viability and elevation of lipid peroxidation compared with non-treated cells. However, alginic acid and fucoidan attenuated the OS by increase of cell viability and decrease of lipid peroxidation. These results supported that alginic acid and fucoidan played the protective role from NO-induced OS.

References

[1] Akaike, T., Suga, M., & Maeda, H. (1998). Free radicals in viral pathogenesis: molecular mechanisms involving superoxide and NO. *Experimental Biology and Medicine, 217*(1)**,** 64-73.

[2] Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., & Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences of the United States of America (PNAS), 87*(4), 1620-1624.

[3] Blough, N. V., & Zafiriou, O. C. (1985). Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution. *Inorganic Chemistry, 24*(22)**,** 3502-3504.

[4] Bokov, A., Chaudhuri, A., & Richardson, A. (2004). The role of oxidative damage and stress in aging. *Mechanisms of Ageing and Development, 125*(10-11), 811-826.

[5] Cahyana, A. H., Shuto, Y., & Kinoshita, Y. (1992). Pyropheophytin a as an antioxidative substance from the marine alga, Arame (*Eisenia bicyclis*). *Bioscience, Biotechnology, and Biochemistry, 56*(10), 1533-1535.

[6] Cho, E. J., Rhee, S. H., & Park, K. Y. (1998). Antimutagenic and anticarcinogenic effects of alginic acid extracted from sporophyll of sea mustard. *Journal of Food Science and Nutrition, 3*(2)*,* 169-174.

[7] Feldman, S. C., Reynaldi, S., Stortz, C. A., Cerezo, A. S., & Damonte, E. B. (1999). Antiviral properties of fucoidan fractions from *Leathesia difformis*. *Phytomedicine, 6*(5)*,* 335-340.

[8] Gibson, G. E., & Huang, H. M. (2005). Oxidative stress in Alzheimer's disease. *Neurobiology of Aging, 26*(5)*,* 575-578.

[9] Halliwell, B, (1987). Oxidants and human disease: some new concepts. *The FASEB Journal, 1*(5)*,* 358-364.

[10] Halliwell, B., Zhao, K., & Whiteman, M. (1999). Nitric oxide and peroxynitrite. The ugly, the uglier and the not so good. *Free Radical Research, 31*(6), 651-669.

[11] Kimura, Y., Watanabe, K., & Okuda, H. (1996). Effects of soluble sodium alginate on cholesterol excretion and glucose tolerance in rats. *Journal of Ethnopharmacology, 54*(1)*,* 47-54.

[12] Lee, K. S., Choi, Y. S., & Seo, J. S. (2004). Sea tangle supplementation lowers blood glucose and supports antioxidant systems in streptozotocin-induced diabetic rats. *Journal of Medicinal Food, 7*(2)*,* 130-135.

[13] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1-2)*,* 55-63.

[14] Nishino, T., Kiyohara, H., Yamada, H., & Nagumo, T. (1991). An anticoagulant fucoidan from the brown seaweed *Ecklonia kurome*. *Phytochemistry, 30*(2)*,* 535-539.

[15] Patel, R. P., McAndrew, J., Sellak, H., White, C. R., Jo, H., Freeman, B. A., & Darley-Usmar, V. M. (1999). Biological aspects of reactive nitrogen species. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1411*(2-3)*,* 385-400.

[16] Piao, X. L., Kim, H. Y., Yokozawa, T., Lee, Y. A., Piao, X. S., & Cho, E. J. (2005). Protective effects of broccoli (*Brassica oleracea*) and its active components against radical-induced oxidative damage. *Journal of Nutritional Science and Vitaminology, 51*(3)*,* 142-147.

[17] Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics*, *288*(2), 481-487.

[18] Ruperez, P., Ahrazem, O., & Leal, J. A. (2002). Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry, 50*(4)*,* 840-845.

[19] Scott, J. A., & King, G. L. (2004). Oxidative stress and antioxidant treatment in diabetes. *Annals of the New York Academy of Sciences, 1031,* 204-213.

[20] So, M. J., Kim, B. K., Choi, M. J., Park, K. Y., Rhee, S. H., & Cho, E. J. (2007). Protective activity of fucoidan and alginic acid against free radical-induced oxidative stress under in vitro and cellular system. *Journal of Food Science and Nutrition, 12*(4), 191-196.

[21] Torsdottir, I., Alpsten, M., Holm, G., Sandberg, A. S., & Tolli, J. (1991). A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *Journal of Nutrition, 121*(6)*,* 795-799.

[22] Xue, C., Yu, G., Hirata, T., Terao, J., & Lin, H. (1998). Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents. *Bioscience, Biotechnology, and Biochemistry,* 62(2), 206-209.

[23] Yamamoto, I., Takahashi, M., Suzuki, T., Seino, H., & Mori, H. (1984). Enhancement of antitumor activity by sulfation of a crude fucoidan fraction from *Sargassum kjellmanianum*. *Japanese Journal of Experimental Medicine, 54*(4), 143-151.

[24] Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical Medicine, 15*(2), 212-216.

[25] Yokode, M., Kita, T., Kikawa, Y., Ogorochi, T., Narumiya, S., & Kawai, C. (1988). Stimulated arachidonate metabolism during foam cell transformation of mouse peritoneal macrophages with oxidized low-density lipoprotein. *The Journal of Clinical Investigation, 81*(3)*,* 720-729.

[26] Yokozawa, T., Satoh, A., Cho, E. J., Kashiwada, Y., & Ikeshiro, Y. (2005). Protective role of Coptidis Rhizoma alkaloids against peroxynitrite-induced damage to renal tubular epithelial cells. *Journal of Pharmacy and Pharmacology, 57*(3)*,* 367-374.

[27] Yokozawa, T., Cho, E. J., Hara, Y., & Kitani, K. (2000). Antioxidative activity of green tea treated with radical initiator 2, 2'-azobis(2-amidinopropane) dihydrochloride. *Journal of Agricultural and Food Chemistry, 48*(10)*,* 5068-5073.

[28] Yan, X. J., Chuda, Y., Suzuki, M., &, Nagata, T. (1999). Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Bioscience, Biotechnology, and Biochemistry, 63*(3), 605-607.

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