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Anti-Inflammatory Effect of Freeze-Dried Broccoli Sprout Powder with Antioxidant Activity in RAW264.7 Cells

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Abstract

Sprout products, such as broccoli, alfalfa, and cabbage, have positive health effects. Thus far, sprout foods have attracted attention owing to their good bioavailability. In particular, young broccoli sprouts exhibit anti-inflammatory, antioxidant, and anti-cancer effects. They contain 100 times more chemoprotective substances than adult broccoli. This study examined the anti-inflammatory effects of freeze-dried young sprout broccoli (FD-YB) *in vitro* using RAW264.7 macrophage cells. The FD-YB powder antioxidant ability test showed that the radical-scavenging activity and superoxide dismutase enzyme activity increased in a dose-dependent manner. In addition, FD-YB was not cytotoxic to RAW264.7 cells, and nitric oxide production decreased after the FD-YB treatment of lipopolysaccharide-stimulated RAW264.7 cells in a dose-dependent manner. Furthermore, FD-YB significantly decreased the expression of inflammation-related proteins (Cyclooxygenase-2, Inducible nitric oxide synthase, and Prostaglandin E Synthase 2) and cytokines (Tumor necrosis factor-α and Interleukin-6). In conclusion, FD-YB can be a potential nutraceutical for preventing and regulating excessive immune responses during inflammation.

Key Words : Anti-inflammation, young broccoli sprout, RAW264.7, antioxidant

I. Introduction

The inflammatory response is a defense mechanism that protects the body from invasion by external substances, such as wounds and toxins produced by bacterial and viral infections, or tissue damage. Inflammation transmits a signal that triggers an immune response by secreting inflammatory mediators from immune cells when an influx of foreign substances is detected in the body (Kim et al. 2012). Persistent inflammatory response is a fundamental cause of chronic diseases. The excessive production of inflammatory mediators following a continuous inflammatory response exacerbates inflammatory diseases (Kang et al. 2013; Jin et al. 2014). Therefore, if the inflammatory response is not initially controlled, chronic inflammation may occur due to functional loss and homeostasis imbalance in the body, which may lead to neurodegenerative, autoimmune, and metabolic diseases (De Heradia et al. 2012; Osborn & Olefsky 2012; Cao et al. 2015). Because anti-inflammatory drugs have side effects such as gastrointestinal disorders, nephritis, and heart disease (Markin & Ballinger 2003), the need to develop anti-inflammatory agents from natural products that are more stable, effective, and have no side effects on the human body is emerging, and efforts are being made to use foods that can be consumed in daily life for this purpose (Lee et al. 2011a; Jun et al. 2014).

When reactive oxygen species (ROS) are excessively generated within cells due to smoking, drinking, wrong lifestyle habits, various pollutants and drugs, oxidative stress in the body increases (Ryu et al. 2003). When cells are continuously exposed to oxidative stress, not only cell death but also chronic inflammatory response is induced (Lee et al. 2011b). As modern people's interest in antioxidants that can relieve oxidative stress increases, research on antioxidants and anti-inflammation is actively underway. Synthetic antioxidants are being increasingly used to inhibit inflammatory reactions. However, synthetic antioxidants including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported to have toxicity and side effects such as inducing cancer by interfering with cell metabolism, energy

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production, and respiration (Soory 2009). Therefore, natural antioxidants produced by plants, including polyphenols, flavonoids, and tannins, are preferred because these compounds have little toxicity, are effective in preventing various diseases, and promote health (Salagami et al. 2007). In particular, the consumption and preference for vegetable sprouts has been increasing as a way to prevent and treat chronic inflammation (Lee & Park 2014).

Among natural products, broccoli, a cruciferous vegetable, has almost no fat content, low calories, and is rich in vitamins, minerals, and dietary fiber (Steinmetz & Potter 1996). Broccoli has a detoxifying enzyme and high content of antioxidants known to inhibit chronic inflammation, such as ascorbic acid, β-carotene, rutin, selenium, glutathione, and quercetin (Kwon et al. 2008; Ravikumar 2015). It has a high content of sulforaphane, indole-3-carbinol, and isothiocyanates, which have anti-cancer activities (Fahey et al. 1997; Herr & Buchler 2010), and is known to have anti-obesity and anti-diabetic effects, as well as to reduce high blood pressure and cholesterol (Lee et al. 2009b; Lee et al. 2014).

Sprouts refer to sprouts that develop approximately a week after germination. It refers to the state of young cotyledons with 1-3 leaves before the main leaves appear (Lee et al. 2009a; Kim & Lee 2010). Sprouts contain large amounts of functional and physiologically active substances, such as amino acids, vitamins, minerals, dietary fiber, and enzymes. Plants are known to undergo changes in their constituents as germination progresses and increase the levels of useful physiologically active substances compared to vegetables (Khalil et al. 2007). Sprouts contain 4-100 times more useful bioactive substances than vegetables (Sattar et al. 1995; El-Adawy 2002). In addition, sprouts produce various physiologically active substances to defend themselves against external pathogens such as fungi and bacteria (Badshah et al. 1991). Sprout vegetables do not require pesticides or soil for cultivation, and can be picked and used immediately before consumption. Furthermore, sprouts have thin cell walls; therefore, nutrients can be easily released. Additionally, sprouts contain a large number of enzymes within the vegetable itself and is characterized by good digestion (El-Adawy 2002; Khalil et al. 2007).

Broccoli sprouts contain more sulforaphane and glucosinolates than general broccoli (Almuhayawi et al. 2020), have higher total polyphenol and flavonoid contents, and have superior 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging abilities than general broccoli (Koh et al. 2019). Therefore, broccoli sprouts have excellent preventive and therapeutic effects against organ and tissue damage and immune deterioration caused by reactive oxygen species. In addition, when the nutritional components of general broccoli and its sprouts were compared, the contents of minerals and vitamins and α-amylase activity of sprouts were higher, and similar properties were observed in each freeze-dried powder of sprouts (Kim & Lee 2010).

Interest in the functionality of vegetable sprouts has recently increased, and various studies on broccoli sprouts have been conducted. Studies have focused on the antioxidant (Shin et al. 2014; Kim & Kim 2015; Geng et al. 2022) effect, immunomodulatory activity (Koh et al. 2019), and anti-encephalitis effects (Subedi et al. 2019) of broccoli sprouts. Furthermore, studies have been performed on the analysis of nutritional components (Lee et al. 2009a), sprout vegetables manufacturing of processed products (Kim & Lee 2010), anti-diabetic effect of broccoli sprouts (Lee et al. 2014; Sotokawauchi et al. 2018), cholesterol-lowering and obesity-inhibiting effects (Lee et al. 2009b), lipid metabolism improvement effect (Lee et al. 2007a), colitis improvement effect (Zhang et al. 2022), and cognitive improvement effects (Shiina et al. 2015) of sprouts. Also, the anti-inflammatory effects of broccoli sprout ethanol extract have been partially reported (Sim et al. 2023), and this study aims to examine the possibility of a natural product treatment by verifying the antioxidant and atni-inflammatory effects of broccoli sprout water extract. In Korea, the demand for sprout vegetables containing various physiologically active substances is increasing. In particular, to materialize natural foods, such as sprout vegetables, and develop them as new functional foods, systematic physiological activity research should first be conducted.

In this study, we aimed to examine the anti-inflammatory activity of freeze-dried broccoli sprout powder in RAW264.7 cells, which were stimulated with lipopolysaccharide (LPS) to induce an inflammatory response, and investigated the mechanisms associated with anti-inflammation in RAW264.7 cells.

II. Materials and Methods

1. Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from Welgene (Gyeongsan, Korea). Fetal bovine serum (FBS) and antibiotics were obtained from Gibco BRL (Rockville, MD, USA). Dimethyl sulfoxide (DMSO), 2-

amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT), LPS, BHA and ascorbic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies (Inducible nitric oxide synthase (iNOS), Cyclooxygenase-2 (COX-2), Prostaglandin E Synthase 2 (PTGES2), and β-actin) and secondary antibodies were obtained from Cell Signaling Technology Inc. (Beverly, MA, USA) and Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Polyvinylchloride fluoride (PVDF) membrane was obtained from Millipore (Billerica, MA, USA) and Super-signal West Pico Chemiluminescent Substrate was purchased from Pierce Biotechnology (Rockford, IL, USA). Total protein of the cell was extracted using PRO-PREP solution (iNtRON Biotechnology, Seongnam, Korea). Enzyme-linked immunosorbent assay (ELISA) for cytokine detection was purchased from BD Biosciences (San Jose, CA, USA). All other chemicals and solvents were purchased from Sigma-Aldrich unless otherwise stated.

2. Preparation of sample

Young sprout broccoli powder was purchased from Bio Sprout Co. (Yeongcheon, Gyeongsangbuk-do, Korea). Young sprout broccoli powder (15 g) was extracted with 300 mL of boiling purified water for 3 hr long and filtered using a filter paper (Advanced No. 2 Filter paper, Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The filtrate was lyophilized in a freeze drier (Ilshin, Seoul, Korea). The yield of lyophilized young sprout broccoli was 6%. The lyophilized powder was dissolved in purified water and filtered using a 0.22 μM filter (Nalgene, USA) prior to use.

3. Preparation of the standard solutions and samples

Stock solution of Freeze-dried young sprout broccoli (FD-YB) was prepared by dissolving them at a concentration of 20 mg/mL in distilled water. Working solutions were produced by diluting the stock solution with distilled water. The standard stock solutions and working solutions were stored at 4°C.

4. DPPH Radical scavenging activity assay

Electron donating ability was evaluated using DPPH by the method of Blois (Blois 1958). In brief, 180 μL of DPPH solution (0.4 mM in methanol) was added to 20 μL of FD-YB (dissolved in distilled water) at concentrations of 0.25, 0.5, 1 and 2 mg/mL. Ascorbic acid and BHA are used as positive control. The mixture was incubated for 15 min at room temperature. The optical density was measured at 517 nm by a microplate reader (Tecan Group Ltd., Männedorf, Switzerland). The DPPH radical scavenging activity (%) was calculated by the subsequent formula: $[(\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{treatment}})]/[(\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{blank}})]\times 100$. Absorbance $_{\text{control}}$ and absorbance $_{\text{treatment}}$ are the absorbance of the control and the treatment, respectively.

5. Superoxide dismutase (SOD) enzyme activity

Following the manufacturer's instructions, the level of SOD enzyme activity was measured by using of EZ-SOD assay kits (DoGenBio, Seoul, Korea). Absorbance was determined at 450 nm using a microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

6. Cell culture and LPS stimulation

The LAW264.7 cell was obtained from Korean Cell Line Bank (Seoul, South Korea). LAW264.7 cells were cultured in DMEM medium (Welgene, Gyeongsan, Korea) supplemented with 10% heat-inactivated FBS (Welgene, Gyeongsan, Korea), streptomycin (100 μg/mL), and 100 U/mL of penicillin (Gibco-BRL, Grand Island, USA) at 37°C in a humidified incubator containing 5% CO_2. FD-YB dissolved in phenol free DMEM (Corning, New York, USA) as a 20 mg/mL stock solution, and dilutions were made in DMEM. To stimulate cells, the medium was replaced with fresh phenol free DMEM and 1 μg/mL LPS was added after sample treatment in the presence or absence of FD-YB for 24 hr.

7. Cell viability test

Cell viability was detected using the WST (DoGenBio, Seoul, Korea) colorimetric assay. Briefly, LAW264.7 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated at 37°C for 24 hr. FD-YB dissolved in distilled water as a 20 mg/mL stock solution, and diluted solutions were made in DMEM. The LAW264. 7 cells were then treated with FD-YB at various concentrations (0, 7.81, 15.6, 31.2, 62.5, 125, 250 and 500 μg/mL), and then the cells were incubated for another 24 hr at 37°C in an incubator with 5% CO_2. Following incubation, cells were treated with WST solution for 1 hr. Optical density was measured at 540 nm by a microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Cell viability was described relative to the untreated control cells, where viability (% of control) = (optical density of sample treated group)/(optical density of control)×100.

8. Detection of nitric oxide (NO)

The LAW264.7 cells (10×10^4 cells/well) incubated for 24 hr in a 24-well plate were pretreated with various concentrations (0, 62.5, 125, 250 and 500 μg/mL) of FD-YB or AMT as positive control (5 μM) for 4 hr and incubated for 20 hr with LPS (1 μg/mL) at 37°C in 5% CO_2 incubator. After incubation, the supernatant was collected and stored at −80°C until use. The NO concentration in the medium was measured using Griess reagent (1% sulfanilamide and 0.1% N-[1-naphtyl]-ethylenediamine dihydrochloride in 5% phosphoric acid, Roche, Switzerland). The Griess reagent to the culture supernatant for 10 min at 25°C in the dark. The absorbance values of mixture were determined using a microplate reader (Tecan Group Ltd., Männedorf, Switzerland) at 540 nm. As NO inhibitor, AMT was used as a positive control.

9. Measurement of cytokines

Following the manufacturer's instructions, the levels of cytokines, tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6) were measured by using of ELISA kits (BD Biosciences, San Jose, CA, USA). Absorbance was determined at 450 nm using a microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

10. Western blot

Sample preparation and western blot analysis were performed. LAW264.7 cells were harvested by centrifugation at 4°C, and then the cells were washed twice with cold phosphate-buffered saline (PBS) and lysed using PRO-PREP solution. The concentration of protein in the supernatants was determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA). After quantification, the protein of the cell was denatured by boiling at 94°C for 5 min, and then the cellular proteins (20 μg/well) were separated using 6-15% gels. After gel electrophoresis, the protein was transferred to PVDF membranes. The PVDF membrane was soaked in 5% skim milk or 3% bovine serum albumin solution to block non-specific responses at room temperature for 1 hr. Next, the membrane was incubated with each specific primary antibody at 4°C overnight incubation. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 1 hr. After another washing step, the protein was visualized with Enhanced Chemiluminescence Detection System (ECL Plus, Thermo Scientific) according to the manufacturer's instructions. Densitometric analysis of the bands was quantitated using ImageJ software.

11. Statistical Analysis

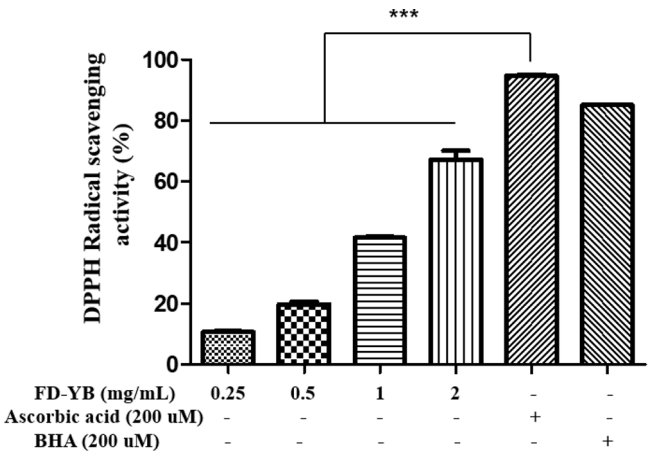
Data were expressed as the means±standard deviations (SD) of at least three independent experiments unless otherwise indicated. Data were compared using one-way ANOVA. Data analysis was carried out using GraphPad Prism version 5 program. P values <0.05, 0.01 and 0.001 were considered significant. All analyses were performed using GraphPad Prism version 5.

III. Results and Discussion

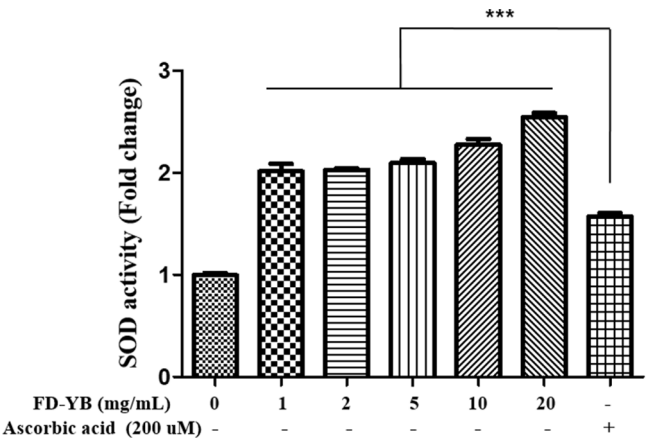
1. Antioxidant ability of FD-YB

When xenobiotics react with the body, antioxidant enzymes, such as SOD, glutathione, and catalase, are produced. Antioxidant enzymes protect biological organs from toxins, chemicals, and reactive oxygen species (Cho et al. 2008). For example, unstable free radicals, including hydroxyl radicals, hydrogen peroxide, and superoxide can damage cell membranes and cause deoxyribo nucleic acid (DNA) mutations (Forman et al. 2014). In addition, excessive production of free radicals causes cell damage and chemically changes the structure and function of biomolecules such as nucleic acids, lipids, and proteins. Natural compounds, including phenolic compounds, flavone derivatives, tocopherols, ascorbic acid, and carotenoids, which are abundant in vegetables and fruits, exert strong antioxidant effects and protect the body from lethal damage induced by oxidative stress. In addition, antioxidant enzymes promote the decomposition of active oxygen, increasing the activity of antioxidant enzymes, such as SOD, catalase, glutathione S-transferase, NADPH quinone oxidoreductase 1, and heme oxygenase 1.

DPPH radical scavenging activity was measured to determine the antioxidant activity of FD-YB. DPPH assay is a simple and economical method for testing the antioxidant activity of substances (Lee et al. 2007b). Treatment with various concentrations of FD-YB (0, 0.25, 0.5, 1, and 2 mg/mL) increased DPPH radical scavenging activity in a dose-dependent manner <Figure 1>. In particular, in a solution treated with 2 mg/mL FD-YB, the observed radical scavenging activity value was 67.17±4.17%. Considering that the activity of BHA, a synthetic antioxidant, is 85.03±0.07%, and that of ascorbic acid, a natural antioxidant, is 94.59±0.60%, the FD-YB 2 mg/mL sample was considered



<Figure 1> DPPH radical scavenging activity of FD-YB.
Ascorbic acid (natural antioxidant, 200 μM) and BHA (synthetic antioxidant, 200 μM) were used as a positive control. FD-YB samples of various concentrations (0, 0.25, 0.5, 1, and 2 mg/mL) were examined. Results shown are representative of 3 independent experiments. Data are presented as the mean±SD. Significance: ***p<0.001 in comparison with ascorbic acid.



<Figure 2> SOD enzyme activity of FD-YB.
Ascorbic acid (200 μM) was used as a positive control. FD-YB samples of various concentrations (0, 1, 2, 5, 10, and 20 mg/mL) were examined. Results shown are representative of 3 independent experiments. Data are presented as the mean±SD. Significance: ***p<0.001 in comparison with ascorbic acid.

to have some degree of antioxidant ability.

SOD catalyzes the conversion of superoxide, which is harmful to cells, to hydrogen peroxide. It is known to protect living organisms by suppressing the reactivity of superoxide and is present in large amounts in most plants, including phytochemicals (Jin 2011). Treatment with various concentrations of FD-YB (0, 1, 2, 5, 10, and 20 mg/mL) increased the SOD activity in a dose-dependent manner <Figure 2>. When treated with 1 mg/mL FD-YB, the SOD activity was higher than that of ascorbic acid, a natural antioxidant.

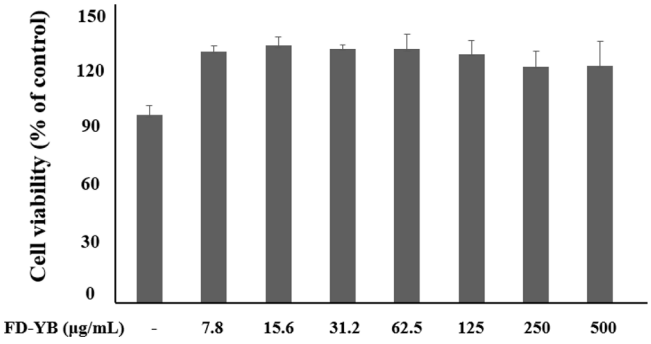
Chronic inflammation is associated with many diseases, including various cancers, inflammatory bowel diseases, and persistent pain (Sherwood & Toliver-Kinsky 2004). Among cruciferous vegetables, broccoli is rich in nutrients that confer antioxidant and anti-inflammatory properties (Lafarga et al. 2018). Broccoli sprouts are known to have positive inflammatory and antioxidant effects. More specifically, glucosinolates, which are abundant in cruciferous vegetables, can be metabolized into various constituents. Glucosinolates can be metabolized into isothiocyanates, a group of bioactive metabolites with anti-inflammatory effects (Holman et al. 2023). Recently, sulforaphane has become one of the most well-studied isothiocyanates. Sulforaphane is more abundant in broccoli sprouts than that in other plant sprouts (Mahn et al. 2022). Sulforaphane increases antioxidant defense system by regulating nuclear factor erythroid 2-related factor 2 (Nrf2)/kelch-like ECH-associated protein 1 and inhibiting the NF-κB pathway (Kubo et al. 2017). According to Kim Hyo-In's newspaper article report in 2024, it has been reported that broccoli sprouts contain 7 times more polysulfide as an antioxidant than mature broccoli. In addition, glucoraphanin that is glucosinolates found in broccoli sprouts was confirmed to prevent carcinogenesis in rodent models (Mahn et al. 2022). In addition to glucosinolates, it also contains phenolic acids and anthocyanins to prevent inflammation (Garcia-Ibañez et al. 2023). Therefore, it can be concluded that the nutritional components of broccoli sprouts regulate the antioxidant system in the body, which is involved in anti-inflammation.

2. Effects of FD-YB on cell viability

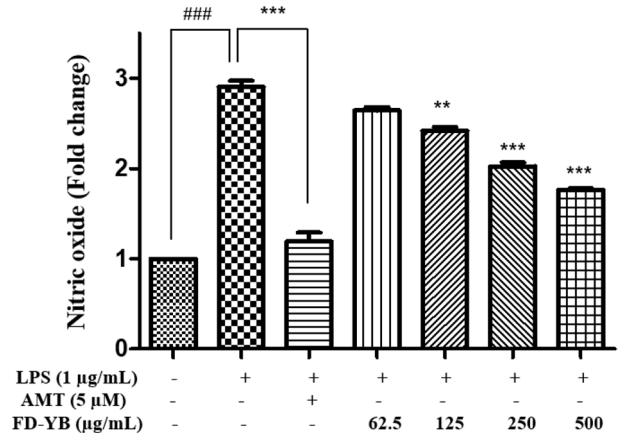
To determine the effects of FD-YB on viability of RAW264.7 cells, WST assay was performed by treating cells with various concentrations of FD-YB (0, 7.8, 15.6, 31.2, 62.5, 125, 250 and 500 μg/mL) for 24 hr. As shown in <Figure 3>, no cytotoxicity was observed in RAW264.7 cells treated with FD-YB at concentrations up to 500 μg/mL. Rather, it was confirmed that the proliferation of RAW264.7 cells is increased when treated with FD-YB comparing to the control group. Therefore, further experiments were conducted at the maximum concentration of 500 μg/mL.

3. Effects of FD-YB on NO and pro-inflammatory proteins in RAW264.7 cells

NO is a highly reactive radical known to be a biologically important substance in the human body and is involved in



<Figure 3> Effect of FD-YB on cell viability in RAW264.7 cells. Cytotoxicity of FD-YB on LAW264.7 cells by WST assay with different concentrations (0, 7.8, 15.6, 31.2, 62.5, 125, 250, and 500 μg/mL) of FD-YB for 24 hr incubation. Results shown are representative of 3 independent experiments. Data are the mean±SD.



<Figure 4> Effect of FD-YB on production of NO in RAW264.7 cells. The level of NO was detected with different concentrations (0, 62.5, 125, 250, and 500 μg/mL) of FD-YB in RAW264.7 cells. It was measured using Griess reagent after 24 hr incubation. LPS (1 μg/mL) used as inducer of inflammation. AMT (5 μM) was used as positive control, NO inhibitor. Results shown are representative of 3 independent experiments. Data are the mean±SD. Significance: ###p<0.001, significant difference from control group. **p<0.01, ***p<0.001, significant difference from LPS-treated group.

nerve transmission, blood coagulation, platelet inhibition, and vasodilation (Bae et al. 2016). However, when excessive NO is produced due to stimuli such as LPS, interleukin, and TNF, tissue and nervous system damage and bronchitis are induced by inflammatory responses. It causes pathological reactions, such as arthritis, and has harmful effects on the body (Knowles & Moncada 1992; Nagayama et al. 2002). Furthermore, NO is produced by iNOS and reacts with superoxide to form peroxynitrite, which causes cytotoxicity (Lee & Kang 2018), damage to surrounding tissues, and chronic inflammation (Coleman 2001). Therefore, the inhibition of NO production is an indicator of the suppression of inflammatory diseases (Yi et al. 2017).

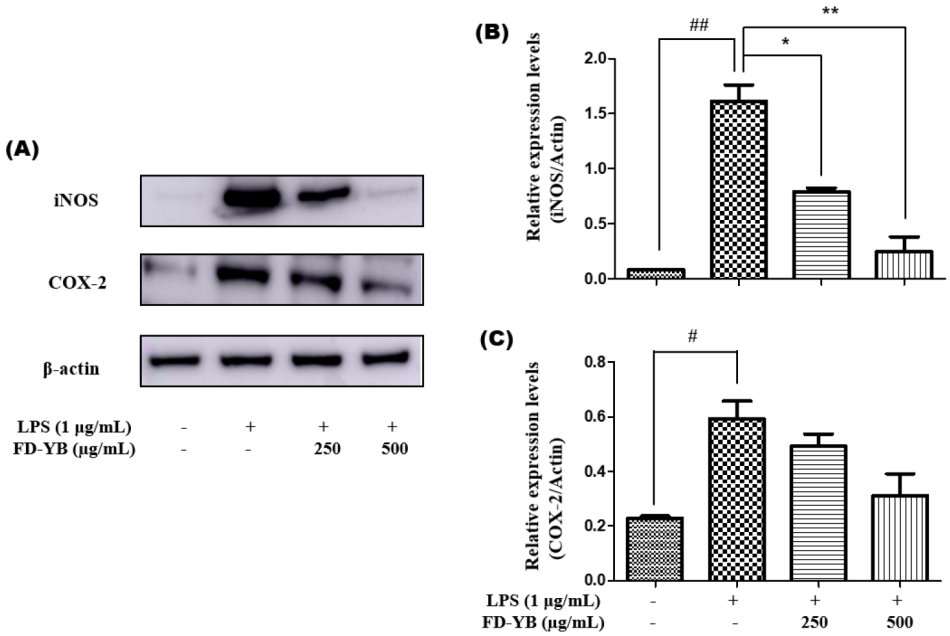
Further analyses were performed to determine whether FD-YB reduced NO production in LPS-stimulated RAW264.7 cells. NO production was detected by measuring the amount of nitrite in the cell culture media using the Griess reagent. Cells treated with LPS alone showed a dramatic increase in NO levels compared to the control. AMT, which was used as a positive control, is an NO inhibitor, and a significant reduction in NO was confirmed in this study. Although it was lower than NO reduction rate of AMT, the NO reduction rate of FD-YB was attenuated at various concentrations (0, 62.5, 125, 250, and 500 μg/mL) in a dose-dependent manner <Figure 4>.

COX-2 and iNOS are involved in many inflammatory processes and play important roles in the induction of various cancers (Kim 2018; Kim 2019). During an inflammatory reaction occurs, iNOS and COX-2 secrete NO and PGE_2, which are inflammatory mediators (Lim & Shin 2010). This promotes inflammation, which leads to edema, as well as tissue and nerve damage. Specifically, COX-2 is involved in the production of inflammatory mediators, the suppression of tumor apoptosis, and the induction of angiogenesis (Duerksen-Hughes et al. 1992).

Therefore, the expression of inflammatory proteins was confirmed in LPS-stimulated RAW264.7 cells. When the cells were treated with different concentrations FD-YB (250, 500 μg/mL), the expression of proteins (COX-2, iNOS) due to LPS stimulation decreased in a dose-dependent manner <Figure 5>. When the cells were treated with 500 μg/mL FD-YB, iNOS expression was found to be slightly higher than that of the control group. These results suggest that FD-YB contains ingredients with anti-inflammatory effects, as it has been shown to be effective in suppressing the production of NO and the expression of COX-2 and iNOS.

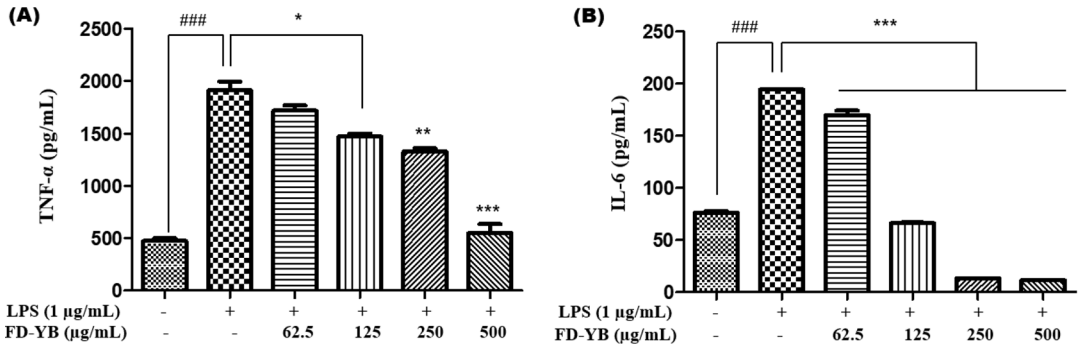
4. Effects of FD-YB on pro-inflammatory cytokines and mediators

Macrophages, which play an important role in the inflammatory response, produce pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α and inflammatory mediators including NO, iNOS, and PGE_2 as inflammation progresses (Stuehr et al. 1991). In response to LPS stimulation, activated immune cells produce various cytokines and mediators (Cavaillon 2018). IL-6 results in toll-like receptor (TLR) activation leading to pro-inflammatory signaling, cancer cell survival, and evasion of immune cell detection by. Sulforaphane, which is present in broccoli or broccoli sprouts, can alleviate



<Figure 5> Effects of FD-YB on the expression of iNOS and COX-2 in RAW264.7 cells.

RAW264.7 cells were treated with different concentrations (250 and 500 μg/mL) of FD-YB for 24 hr, collected and then lysed. Lysates from the cells were subjected to western blot assay for iNOS, COX-2, and β-actin. β-actin was used as a loading control. LPS was used as inducer of inflammation. (A) The level of iNOS, COX-2 was detected by western blot analysis. (B) Specific band intensities were measured sing a densitometer and reduction of iNOS, COX-2 was calculated as iNOS, COX-2/β-actin ratio. Results shown are representative of 3 independent experiments. Data are the mean±SD. Significance: $^{\#}p<0.05$, significant difference from control group. *p<0.05, **p<0.01, significant difference from LPS-treated group.

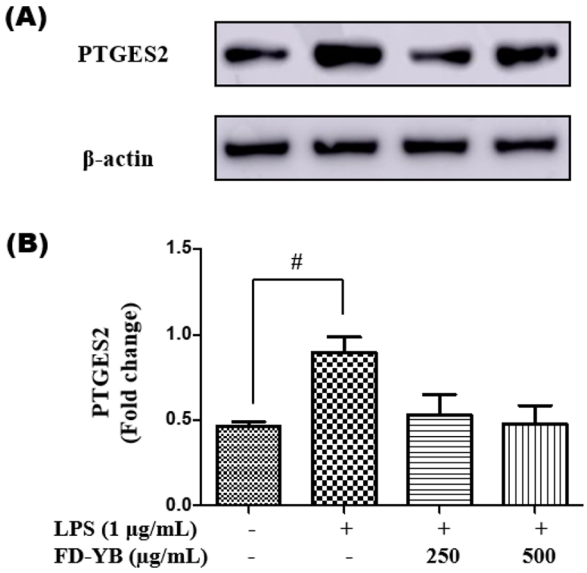


<Figure 6> Effects of FD-YB on production of pro-inflammatory cytokines in RAW264.7 cells.

The level of cytokines (TNF-α, IL-6) in FD-YB-treated RAW264.7 cells were evaluated by ELISA. RAW264.7 cells were treated with various concentrations (62.5, 125, 250, and 500 μg/mL) of FD-YB. The amount of cytokines in the supernatants of RAW264.7 cells was measured. LPS was used as inducer of inflammation. Results shown are representative of 3 independent experiments. Data are the mean±SD. Significance: $^{\#\#\#}p<0.001$, significant difference from control group. *p<0.05, **p<0.01, and ***p<0.001, significant difference from LPS-treated group.

inflammatory responses (Holman et al. 2023). Similarly, broccoli sprouts decrease the LPS-stimulated acute inflammatory response in mouse livers (Sim et al. 2023). Normally, high consumption of cruciferous vegetables has been associated with decreased serum levels of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α (Burnett et al. 2017; Liu et al. 2017).

We measured the production of pro-inflammatory cytokines such as TNF-α and IL-6 in LPS-stimulated RAW264.7 cells. The level of both TNF-α and IL-6 were dramatically increased in the LPS-treated group, whereas treatment with FD-YB (250 and 500 μg/mL) suppressed the production of both TNF-α and IL-6 in a dose-dependent manner <Figure 6>. Based on the results of inflammatory cytokine and mediator production, PTGES2 expression was determined via western blot analysis. The protein levels of PTGES2 increased after LPS treatment, whereas PTGES2 expression



<Figure 7> Effects of FD-YB on inflammatory related protein in RAW264.7 cells.

RAW264.7 cells were treated with different concentrations (250 and 500 μg/mL) of FD-YB for 24 hr, collected and then lysed. Lysates from the cells were subjected to western blot assay for PTGES2, β-actin. β-actin was used as a loading control. LPS was used as inducer of inflammation. (A) The level PTGES2 was detected by western blot analysis. (B) Specific band intensities were measured sing a densitometer and PTGES2 reduction was calculated as PTGES2/β-actin ratio. Results shown are representative of 3 independent experiments. Data are the mean±SD. Significance: $^{\#}p<0.05$, significant difference from control group.

was suppressed in the 250 and 500 μg/mL FD-YB-treated groups after LPS treatment <Figure 7>.

IV. Summary and Conclusion

This study demonstrated that the antioxidant effects of FD-YB increased DPPH radical scavenging activity and SOD activity. FD-YB suppressed inflammatory mediators including NO, COX-2, iNOS, and PTGES2 and pro-inflammatory cytokines including TNF-α and IL-6 in LPS-stimulated RAW264.7 cells. Therefore, we suggest the use of FD-YB obtained from natural sources as an anti-inflammatory supplement.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

References

Almuhayawi MS, AbdElgawad H, Al Jaouni SK, Selim S, Hassan AHA, Khamis G. 2020. Elevated CO_2 improves glucosinolate metabolism and stimulates anticancer and anti-inflammatory properties of broccoli sprouts. Food. Chem., 328:127102

Badshah A, Zeb A, Sattar A. 1991. Effect of soaking, germination and autoclaving on selected nutrients of rapeseed. Pakistan. J. Indus. Res., 34(11):446-448

Bae NY, Kim MJ, Kim KBWR, Park JH, Park SH, Sung NY, Byun EH, Ahn DH. 2016. Anti-inflammatory effect of *Chondrus ocellatus* holmes ethanol extract on lipopoly-saccharide-induced inflammatory responses in RAW264.7 cells. Micr*obiol. Biotechnol. Lett., 44(3):268-277.

Blois M. 1958. Antioxidant determinations by the use of a stable free radical. Nature., 181(4617):1199-1200

Burnett JP, Lim G, Li Y, Shah RB, Lim R, Paholak HJ, MaDermott SP, Sun L, Tsume Y, Bai S, Wicha MS, Sun D, Zhang T. 2017. Sulforaphane enhances the anticancer activity of taxanes against triple negative breast cancer by killing cancer stem cells. Cancer. Lett., 394:52-64

Cao Y, Zhao D, Xu AT, Shen J, Ran ZH. 2015. Effects of immunosuppressants on immune response to vaccine in inflammatory bowel disease. Chin. Med. J (Engl). 128(6):835-838

Cavaillon JM. 2018. Exotoxins and endotoxins: inducers of inflammatory cytokines. Toxicon., 149:45-53

Cho HY, Noh KH, Cho MK, Jang JH, Lee MO, Kim SH, Song YS. 2008. Anti-oxidative and anti-inflammatory effects of genistein in BALB/c mice injected with LPS. J. Korean. Soc. Food. Sci. Nutr., 37(9):1126-1135

Coleman JW. 2001. Nitric oxide in immunity and inflammation. Int. Immnopharmacol. 1(8):1397-1406

De Heredia FP, Gomez-Martinez S, Marcos A. 2012. Obesity, inflammation and the immune system. Proc. Nutr. Soc., 71(2):332-338

Duerksen-Hughes PJ, Day DB, Laster SM, Zachariades NA, Aquinol, Gooding LR. 1992. Both tumor necrosis factor and nitric oxide participate in lysis of simian virus 40-transformed cells by activated macrophages. J. Immunol., 149(6):2114-2122

El-Adawy TA. 2002. Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum L.*) undergoing different cooking methods and germination. Plant. Foods. Hum. Nutr., 57(1):83-97

Fahey JW, Zhang Y, Talalay P. 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci., 94(19):10367-10372

Forman HJ, Davies KJ, Ursini F. 2014. How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging *in vivo*. Free. Radic. Biol. Med., 66:24-35

Garcia-Ibañez P, Núñez-Sánchez MA, Oliva-Bolarín A, Martínez-Sánchez MA, Molina Ramos B, Ruiz-Alcaraz AJ, Moreno DA. 2023. Anti-inflammatory potential of digested Brassica sprout extracts in human macrophage-like HL-60 cells. Food. Funct., 14(1):112-121

Geng J, Li J, Zhu F, Chen X, Du B, Tian H, Li J. 2022. Plant sprout foods: biological activities, health benefits, and bioavailability. J. Food. Biochem., 46(3):e13777

Herr I, Buchler MW. 2010. Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. Cancer. Treat. Rev., 36(5):377-383

Holman J, Hurd M, Moses PL, Mawe GM, Zhang T, Ishaq SL, Li Y. 2023. Interplay of broccoli/broccoli sprout bioactives with gut microbiota in reducing inflammation in inflammatory bowel diseases. J. Nutr. Biochem., 113:109238

Jin KS, Lee JY, Kwon HJ, Kim BW. 2014. Antioxidative and anti-inflammatory activities of *Ardisia arborescens* ethanol extract. Korean. J. Life. Sci., 24(7):713-720

Jin SY. 2011. Study on antioxidant activities of extracts from different parts of Korean and Iranian pomegranates. J. Korean. So. Food. Sci. Nutr., 40(8):1063-1072

Jun HI, Kim YA, Kim YS. 2014. Antioxidant activities of Rubus coreanus Miquel and Morus alba L. fruits. J. Korean. Soc. Food. Sci. Nutr., 43(3):381-388

Kang CH, Han SH, So JS. 2013. Anti-inflammatory effect of chloroform extract from *Potentilla chinensis*. Korean. Soc. Biotechnol. Bioeng. J., 28(1): 13-17

Khalil AW, Zeb A, Mahmood F, Tariq S, Khattak AB, Shah H. 2007. Comparison of sprout quality characteristics of desi and kabuli type chickpea cultivars (*Cicer arietinum* L.). LWT-Food. Sci. Technol., 40(6):937-945

Kim DS, Lee KB. 2010. Physiological characteristics and manufacturing of the processing products of sprout vegetables. Korean. J. Food. Cookery Sci., 26(3):238-245

Kim EJ, Kim MH. 2015. Antioxidant activity of solvent fraction from Broccoli sprouts cultivated at the plant factory system. Korean. J. Food. Nutr., 28(1):1-8

Kim HK. 2018. The functional effects of antimicrobaial activity and anti-inflammatory seaweed polysaccharide extracts. J. Converg. Inf. Technol., 4(2):155-163

Kim HK. 2019. The anti-inflammatory effects of heat-treated broccoli extract. J. Convergence. Cult. Technol., 5(2):397-404

Kim YS, Lee SJ, Hwang JW, Kim EH, Park PJ, Jeong JH. 2012. Anti-inflammatory effects of extracts from *Ligustrum ovalifolium* H. leaves on RAW 264.7 macrophages. J. Korean. Soc. Food. Sci. Nutr., 41(9):1205-1210

Knowles RG, Moncada S. 1992. Nitric oxide as a signal in blood vessels. Trends. Biochem., 17(10);399-402

Koh JH, Kim H, Hwang JH, Yu KW. 2019. Anti-oxidative and immunomodulating activities of solvent extracts from broccoli (*Brassica oleracea*) sprouts. Korean. J. Food. Nutr., 32(1):1-10

Kubo E, Chhunchha B, Singh P, Sasaki H, Singh DP. 2017. Sulforaphane reactivates cellular antioxidant defense by inducing Nrf2/ARE/Prdx6 activity during aging and oxidative stress. Sci. Rep., 7:14130

Kwon YD, Ko ES, Hong SJ, Park SW. 2008. Comparison of sulforaphane and antioxidant contents according to different parts and maturity of broccoli. Korean. J. Hort. Sci. Technol., 26(3):344-349

Lafarga T, Bobo G, Vinas I, Collazo C, Aguiló-Aguayo I. 2018. Effects of thermal and non-thermal processing of cruciferous vegetables on glucosinolates and its derived forms. J. Food. Sci. Technol., 55(6):1973-1981

Lee HS, Kang HJ, Jeon IH, Youm JH, Jang SI. 2014. Anti-diabetic effects of sprouts in high-fat diet and streptozotocin-induced Type II diabetes mellitus mice. J. Korean. Soc. Food. Sci. Nutr., 43(11):1658-1664

Lee JJ, Lee YM, Kim AR, Lee MR. 2009a. Physicochemical Composition of Broccoli Sprouts. J. Life. Sci., 19(2):192-197

Lee JJ, Lee YM, Shin HD, Jeong YS, Lee MY. 2007a. Effects of vegetable sprout power mixture on lipid metabolism in rats fed high fat diet. J. Korean. Soc. Food. Sci. Nutr., 36(8):965-974

Lee JJ, Shin HD, Lee YM, Kim AR, Lee MY. 2009b. Effect of broccoli sprouts on cholesterol-lowering and anti-obesity effects in rats fed high fat diet. J. Korean. Soc. Food. Sci. Nutr, 38(3):309-318

Lee JM, Chung H, Chang PS, Lee HJ. 2007b. Development of a method predicting the oxidative stability of edible oils using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Food. Chem., 103(2):662-669

Lee JW, Kang YJ. 2018. Anti-inflammatory effects of *Abeliophyllum distichum* flower extract and associated MAPKs and NF-κB pathway in RAW 264.7 cells. Korean. J. Plant. Res., 31(3):202-210

Lee KS, Park GS. 2014. Studies in the consumption and preference for sprout vegetable. J. East. Asian. Soc. Dietary. Life., 24(6):896-9-5

Lee SE, Lee JH, Kim JK, Kim GS, Kim YO, Soe JS, Choi JH, Lee ES, Noh HJ, Kim SY. 2011a. Anti-inflammatory activity of medicinal plant extracts. Korean. J. Med. Crop. Sci., 19(4):217-226

Lee SG, Jeong HJ, Lee BJ, Kim JB, Choi SW. 2011b. Antioxidant and anti-inflammatory activities of ethanol extracts from medicinal herb mixture. Kor. J. Food. Sci. Technol., 43(2):200-205

Lim HR, Shin SW. 2010. Effects of the essential oil components from *Ligusticum chuanxiong* on proinflammatory mediators of RAW264.7 macrophage cells. Korean. Sci. Pharm., 16(4):259-264

Liu XL, Zhang XT, Meng J, Zhang HF, Zhao Y, Li C, Sun Y, Mei QB, Zhang F, Zhang T. 2017. ING5 knock down enhances migration and invasion of lung cancer cells by inducing EMT via EGFR/PI3K/Akt and IL-6/STAT3 signaling pathways. Oncotarget., 8(33):54265-54276

Mahn A, Pérez CE, Zambrano V, Barrientos H. 2022. Maximization of sulforaphane content in broccoli sprouts by blanching. Foods., 11(13):1906

Makins R, Ballinger A. 2003. Gastrointestinal side effects of drugs. Expert. Opin. Drug. Saf., 2(4):421-429

Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T. 2002. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. J. Antimicrob. Chemother., 50(6);889-893

Osborn O, Olefsky JM. 2012. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med., 18(3):363-374

Ravikumar C. 2015. Therapeutic potential of Brassica oleracea (Broccoli) - A review. Int. J. Drug. Dev. Res., 7(2):9-10

Ryu JH, Ahn H, Kim JY, Kim YK. 2003. Inhibitory activity of plant extracts on nitric oxide synthesis in LPS-activated macrophage. Phytother. Res., 17(5):485-489

Salagami H, Kobayashi M, Chien CH, Kanegae H, Kavase M. 2007. Selective toxicity and type of cell death induced by various natural and synthetic compounds in oral squamous cell carcinoma. In. Vivo., 21(2):311-320

Sattar A, Badshah A, Aurangzeb. 1995. Biosynthesis of ascorbic acid in germinating rapeseed cultivars. Plant. Foods. Hum. Nutr., 47(1):63-70

Sherwood ER, Toliver-Kinsky. 2004. The mechanisms of the inflammatory response. Best. Pract. Res. Clin. Anaesthesiol., 18(3):385-405

Shiina A, Kanahara N, Sasaki T, Oda Y, Hashimoto T, Hasegawa T, Yoshida T, Lyo M, Hashimoto K. 2015. An open study of sulforaphane-rich broccoli sprout extract in patients with schizophrenia. Clin. Psychopharmacol. Neurosci., 13(1):62-67

Shin GH, Lee YJ, Kim JH, Kim YH, Kim DB, Lee JS, Lim JH, Lee OH. 2014. Antioxidant activities of commonly used *Brassica* spp. Sprout vegetables in Korea. Korean. J. Food. Preserv., 21(4):587-592

Sim HW, Lee WY, Lee R, Yang SY, Ham YK, Lim SD, Park HJ. 2023. The anti-inflammatory effects of Broccoli (*Brassica oleracea* L. var. italica) sprout extract in RAW264.7 macrophages and a lipopolysaccharide-induced liver injury model. Curr. Issues. Mol. Biol., 45(11):9117-9131

Soory M. 2009. Relevance of nutritional antioxidant in metabolic syndrome, ageing and cancer: potential for therapeutic targeting. Infect. Disord. Drug. Targets., 9(4):400-414

Sotokawauchi A, Ishibashi Y, Matsui T, Yamagishi SI. 2018. Aqueous extract of glucoraphanin-rich broccoli sprouts inhibits formation of advanced glycation end products and attenuates inflammatory reactions in endothelial cells. Evid. Based. Complement. Alternat. Med., 8:2018:9823141

Steinmetz KA, Potter JD. 1996. Vegetables, fruit, and cancer prevention: a review. J. Am. Diet. Assoc. 96(10):1027-1039

Stuehr DJ, Cho HJ, Kwon NS, Weise MF, Nathan DF. 1991. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. Proc. Natl. Acad., 88(17):7773-7777

Subedi L, Cho K, Park YU, Choi HJ, Kim SY. 2019. Sulforaphane-enriched broccoli sprouts pretreated by pulsed electric fields reduces neuroinflammation and ameliorates scopolamine-induced amnesia in mouse brain through its antioxidant ability via Nrf2-HO-1 activation. Oxid. Med. Cell. Longev., 27:2019:3549274

Yi MR, Kang CH, Bu HJ. 2017. Anti-inflammatory and tyrosinase inhibition effects of amaranth (*Amaranthus spp* L.) seed extract. Korean. J. Plant. Res., 30(2):144-151

Zhang T, Holman J, McKinstry D, Trindade BC, Eaton KA, Mendoza-Castrejon J, Ho S, Wells E, Yuan H, Wen B, Sun D, Chen GY, Li Y. 2022. A steamed broccoli sprout diet preparation that reduces colitis via the gut microbiota. J. Nutr. Biochem., 112:109215

Kim Hyo-In. Antioxidants in broccoli sprouts, 7 times more than adult broccoli. Available from: http://www.chosun.com, [accessed 2024. 03. 29]

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