Maqui berry (*Aristotelia chilensis*) and the constituent delphinidin glycoside inhibit photoreceptor cell death induced by visible light

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ABSTRACT

The protective effects of maqui berry (*Aristotelia chilensis*) extract (MBE) and its major anthocyanins [delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G)] against light-induced murine photoreceptor cells (661W) death were evaluated. Viability of 661W after light treatment for 24 h, assessed by the tetrazolium salt (WST-8) assay and Hoechst 33342 nuclear staining, was improved by addition of MBE, D3G5G, and D3S5G. Intracellular radical activation in 661W, evaluated using the reactive oxygen species (ROS)-sensitive probe 5-(and-6)-chloromethyl-2, 7-dichlorodihydro fluorescein diacetate acetyl ester (CM-H$_2$DCFDA), was reduced by MBE and its anthocyanins. The anti-apoptosis mechanism of MBE was evaluated by light-induced phosphorylation of p38. MBE significantly suppressed the light-induced phosphorylation of p38. These findings indicate that MBE and its anthocyanidins suppress the light-induced photoreceptor cell death by inhibiting ROS production, suggesting that the inhibition of phosphorylated-p38 may be involved in the underlying mechanism.

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1. Introduction

Everyday people are exposed to various visible light. It is generated by the sun as well as by a wide variety of artificial illumination sources such as fluorescent lights, light-emitting diodes (LED), and the monitors of computers, mobile phones, and televisions. However, excessive exposure to light can be a source of damage to the eye, as evidenced by photoreceptor degeneration in rats and mice following prolonged light exposure (LaVail, Gorrin, Repaci, Thomas, & Ginsberg, 1987; Noell, Walker, Kang, & Berman, 1996). In addition, sunlight exposure has a role in the progression of age-related macular degeneration (AMD) (Hirakawa et al., 2008) and can also contribute to retinitis pigmentosa (RP) as indicated in a human population-based study (Tomany, Cruickshanks, Klein, Klein, & Knudtson, 2004). In the USA, AMD and RP are the most frequent causes of blindness in adults (Dewan et al., 2006). Photoreceptor cell death is an irreversible injury and can cause night blindness and constriction of the visual field, leading to the loss of central vision. Light-induced photoreceptor cell death can be caused by a variety of cellular mechanisms that involve oxidative stress, reactive oxygen species (ROS), activation of caspase-1, and depletion of NF-κB (Krishnamoorthy et al., 1999). Light exposure also causes enhancement of the phosphorylation of p38 (stress activated protein kinase-2) of photoreceptor cells by ROS (Yang, Zhu, & Tso, 2007).

The human retina, in particular, requires large amounts of oxygen; thus, it readily generates ROS, such as superoxide anion radical ($\cdot O_2^-$) and hydrogen peroxide. Although the oxidising capability of these radicals is weak, they react with metals in living tissues and are immediately changed to hydroxyl radical ($\cdot OH$) if exposed to ultraviolet light. This radical has great capacity to injure DNA and the cell membrane. The eye therefore depends on the presence of antioxidants such as ascorbic acid to protect the retina from light-induced free radical damage (Li, Tso, Wang, & Organisciak, 1985; Organisciak, Wang, Li, & Tso, 1985). However, oxidative stress conditions can overwhelm this internal antioxidant system, resulting in the progression of many diseases including retinal diseases.
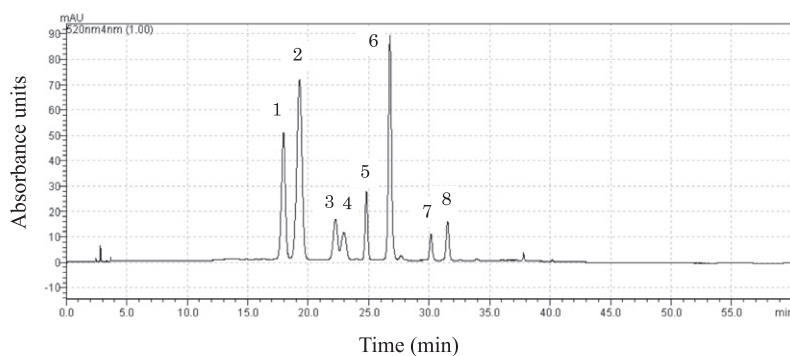
Maqui berry [*Aristotelia chilensis* (Molina) Stuntz] is a plant of the Elaeocarpaceae family and cultivated in central and southern Chile. Maqui berry has a particularly high concentration of anthocyanins, and 8 anthocyanins found are glycosylated forms of delphinidin and cyanidin, such as delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G) (Fig. 1)

A

B

Fraction No.	Retention Time (min)	Anthocyanins	R_1	R_2	R_3
1	18.0	Delphinidin 3-*O*-sambubioside-5-*O*-glucoside	OH	Sam	Glu
2	19.3	Delphinidin 3,5-*O*-diglucoside	OH	Glu	Glu
3	22.3	Cyanidin 3-*O*-sambubioside-5-*O*-glucoside	H	Sam	Glu
4	23.0	Cyanidin 3,5-*O*-diglucoside	H	Glu	Glu
5	24.8	Delphinidin 3-*O*-sambubioside	OH	Sam	H
6	26.8	Delphinidin 3-*O*-glucoside	OH	Glu	H
7	30.2	Cyanidin 3-*O*-sambubioside	H	Sam	H
8	31.5	Cyanidin 3-*O*-glucoside	H	Glu	H

Fig. 1. HPLC separation of MBE. (A) Use of 520 nm as a selective wavelength allowed identification of 8 anthocyanins. The various peak numbers correspond to the 'Fraction No.' in (B). (B) Chemical structure of MBE. Glu, glucose; Sam, sambubiose.

(Maria, Cristina, Orlando, Julian, & Celestino, 2006). D3G5G and D3S5G are not contained in bilberry (*Vaccinium myrtillus* L.) or blackcurrant berry (*Ribes nigrum* L.) (Matsumoto, Nakamura, Iida, Ito, & Ohguro, 2006; Matsunaga et al., 2009). Many studies conducted on the biological activities of maqui berry extract (MBE) have reported, such as antioxidant (Miranda-Rottmann et al., 2002; Ruiz et al., 2010), antimicrobial (Mølgaard et al., 2011), cardioprotective (Cespedes, El-Hafidi, Pavon, & Alarcon, 2008), antidiabetic (Rojo et al., 2012), anti-inflammatory (Schreckinger, Wang, Yousef, Lila, & de Mejia, 2010), and α-glucosidase/α-amylase inhibitory (Rubilar et al., 2011) effects. Moreover, it has been previously shown that the protective effect of purple rice (*Oryza sativa* L.) bran extract and cyanidin of the main constituents against light induced retinal damage (Tanaka et al., 2011). However, the effects of MBE, D3G5G, and D3S5G on light-induced photoreceptor cell death have not yet been extensively examined.

The purpose of the present study was to examine the potential protective effects of MBE and its constituents (D3G5G and D3S5G) against murine photoreceptor cells (661W) death induced by light exposure. Furthermore, MBE was compared with data obtained for

other berry fruits of bilberry and blackcurrant berry. The mechanisms underlying the effects of MBE on ROS production and induction of phosphorylation of p38 by light were also investigated.

2. Materials and methods

2.1. Materials

Maqui berry (*A. chilensis*) extract (MBE), blackcurrant berry (*R. nigrum* L.) extract (BCE), and bilberry (*V. myrtillus* L.) extract (BBE) were supplied by Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan), which were extracted with aqueous ethanol. Delphinidin 3,5-*O*-diglucoside and delphinidin 3-*O*-sambubioside-5-*O*-glucoside were purchased from Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). Delphinidin, cyanidin, malvidin, and peonidin were purchased from Extrasynthese (Genay Cedex, France). A Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Hoechst 33342 and propidium iodide (PI)

were purchased from Molecular Probes (Eugene, OR, USA). 5-(and-6)-Chloromethyl-2,7-dichlorodihydro fluorescein diacetate acetyl ester (CM-H_2DCFDA) was purchased from Invitrogen Co. (Eugene, OR, USA). Radio-immunoprecipitation assay (RIPA) buffer, protease inhibitor cocktail, phosphatase inhibitor cocktail 1, and phosphatase inhibitor cocktail 2 were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). BCA Protein Assay Kit, SuperSignal® West Femto Maximum Sensitivity Substrate, goat anti-rabbit HRP-conjugated, and goat anti-mouse HRP-conjugated were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). Phosphorylated-p38 rabbit monoclonal antibody was purchased from Promega Co. (Madison, WI, USA). p38 Mouse monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). 661W were a kind gift from Dr. Muayyad R. Al-Ubaidi (University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA).

2.2. HPLC analysis

The anthocyanins in MBE were analysed by HPLC (Shimadzu, Kyoto, Japan) equipped with a C18 column (SunFire C18, 4.6 mm *i.d.* × 150 mm, Waters Corp., USA). The mobile phase was composed of 0.3% trifluoroacetic acid aqueous solution v/v (A) and CH_3CN (B). The gradient was as follows: 0 min, 95% (A), 5% (B); 4 min, 95% (A), 5% (B); 4.5 min, 90% (A), 10% (B); 27 min, 85% (A), 15% (B); 47 min, 45% (A), 55% (B); 48 min, 10% (A), 90% (B); 50 min, 10% (A), 90% (B); 51 min, 95% (A), 5% (B); 60 min, 95% (A), 5% (B). The flow rate was set at 0.7 ml/min. The wavelength for detection was 520 nm and the column was kept at 30 °C (Maria et al., 2006).

2.3. Cell culture

Murine photoreceptor cells (661W) were maintained in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin under a humidified atmosphere of 5% CO_2 at 37 °C. 661W were passaged by trypsinization every 2–3 days (Tanaka et al., 2011).

2.4. Cell survival following visible light irradiation

661W (3×10^4 cells/ml) were seeded into a 96-well plate and cultured at 37 °C for 24 h. The medium was replaced with 1% FBS-DMEM containing each samples and cultured at 37 °C for 1 h. After preincubation, the cells were exposed from underneath to 2500 lux of white fluorescent light (Nikon, Tokyo, Japan) for 24 h at 37 °C. The viable cell numbers were measured using a water-soluble tetrazolium salt, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H* tetrazolium monosodium salt (WST-8). Briefly, 10 µl of CCK-8 solution was added to each well, incubated at 37 °C for 3 h, and then the absorbance was measured at 492 nm (reference wave length, 660 nm) using a spectrophotometer (Varioskan; Thermo Electron Corporation, Vantaa, Finland) (Tanaka et al., 2011).

2.5. Hoechst 33342 staining

At the end of the light exposure treatment, Hoechst 33342 (excitation: 360 nm, emission: 490 nm) and PI were added to the culture medium (final concentrations of 8.1 and 1.5 µM, respectively) and incubated for 15 min. Microscopic images through fluorescence filters for Hoechst 33342 (U-MWU; Olympus Co., Tokyo, Japan) and PI staining (U-MWIG; Olympus) were captured by a CCD camera (DP30BW; Olympus).

2.6. Measurement of ROS production in 661W

Intracellular radical activation within 661W was determined using CM-H_2DCFDA. CM-H_2DCFDA being taken into the cell is converted to dichlorodihydrofluorescin (DCFH) by an intracellular esterase. Then, the ROS oxidises DCFH (non-fluorescent) to dichlorodihydrofluorescein (fluorescent). At the end of the light exposure period, CM-H_2DCFDA was added to the culture medium and incubated at 37 °C for 1 h at a final concentration of 10 µM. The 96-well plate was loaded into a plate-holder in a fluorescence spectrophotometer. The reaction was carried out at 37 °C, and fluorescence was measured at 488 nm for excitation and 525 nm for emission. The number of cells was determined by Hoechst 33342 staining and used to calculate ROS production per cell (Tanaka et al., 2011).

2.7. Immunoblotting

661W (3×10^3 cells/ml) were seeded into a 12-well plate and cultured at 37 °C for 24 h in a humidified atmosphere of 5% CO_2. The medium was replaced with 1% FBS-DMEM containing MBE at 10 µg/ml, which is the most effective concentration without cell toxicity, and cultured at 37 °C for 1 h. After preincubation, the cells were exposed from underneath to 2500 lux of white fluorescent light (Nikon, Tokyo, Japan) for 1 h at 37 °C. The 661W were washed twice with PBS and then lysed in RIPA buffer supplemented with protease inhibitor cocktail, phosphatase inhibitor cocktail 1 and phosphatase inhibitor cocktail 2. The cell lysate was stocked at −80 °C. The protein concentration was measured using a BCA Protein Assay Kit with bovine serum albumin as a standard. A mixture of equal parts of an aliquot of protein and sample buffer with 10% 2-mercaptoethanol was subjected to 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The separated proteins were then transferred to a polyvinylidene difluoride membrane. For immunoblotting, the following primary antibodies were used: phosphorylated-p38 rabbit monoclonal antibody (1:1000) and p38 mouse monoclonal antibody (1:1000). The secondary antibody used was either goat anti-rabbit HRP-conjugated (1:2000) or goat anti-mouse HRP-conjugated (1:2000). The immunoreactive bands were visualised using SuperSignal® West Femto Maximum Sensitivity Substrate. The band intensity was measured using a Lumino Imaging Analyzer (LAS-4000, Fujifilm, Tokyo, Japan).

2.8. Statistical analysis

Data are presented as means ± SEM. Statistical comparisons were made using a one-way analysis of variance (ANOVA) followed by a Student's *t*-test or Dunnett's multiple-comparison test. A value of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Determination of anthocyanins in MBE

Eight anthocyanin components of MBE were confirmed by HPLC analysis (Fig. 1A), and these are listed in Fig. 1B. Our HPLC data were almost the same as previous studies (Maria et al., 2006). The contents of delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G), delphinidin 3,5-*O*-diglucoside (D3G5G), cyanidin 3-*O*-sambubioside-5-*O*-glucoside, cyanidin 3,5-*O*-diglucoside, delphinidin 3-*O*-sambubioside, delphinidin 3-*O*-glucoside, cyanidin 3-*O*-sambubioside, and cyanidin 3-*O*-glucoside in the MBE preparation were 7.1%, 14.3%, 2.7%, 2.1%, 1.6%, 6.1%, 0.6%, and 0.9%, respectively. The total anthocyanin content in the MBE was 35.4%.
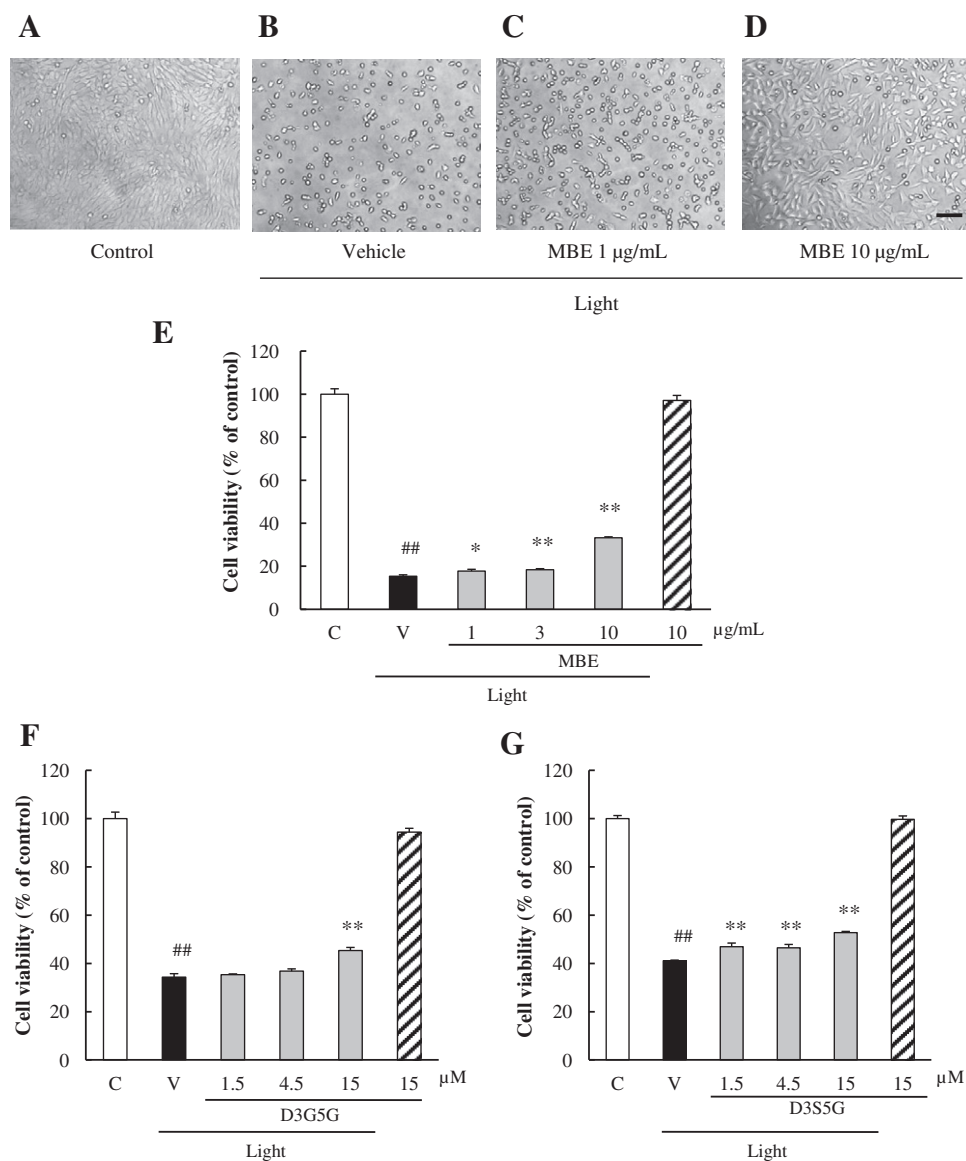
Fig. 2. Effects of MBE and its anthocyanin constituents on visible light-induced 661W cell damage, as determined by WST-8 assay. (A–D) Representative microscopy at 24 h after light exposure. (A) Nontreated cells showed normal nuclear morphology. (B) Light-induced cell death, with cells showing nuclear condensation. (C and D) Pretreatment with MBE at 1–10 μg/ml reduced nuclear condensation, respectively. (E–G) Effects of MBE, D3G5G, and D3S5G on light-induced 661W cell death, as determined by WST-8 assay. The last bars in each figure show data obtained following the application of each drug alone but without light exposure. Data are shown as mean ± SEM ($n = 6$). C, control; V, vehicle. $^{##}p < 0.01$ versus control, and $^*p < 0.05$, $^{**}p < 0.01$ versus vehicle. The scale bar (D) represents 250 μm.

3.2. Effects of MBE, D3G5G, and D3S5G on light-induced cell death in 661W

The effects of MBE, D3G5G, and D3S5G on light-induced damage in 661W were investigated. Typical images of microscopy are shown in Fig. 2A–D. Untreated control cells displayed normal nuclear morphology (Fig. 2A), while the cells irradiated with light for 24 h revealed shrunken and condensed nuclei (Fig. 2B). After light exposure, nuclei in the cells treated with MBE at 10 μg/ml were morphologically similar to those of the control cells (Fig. 2D). The WST-8 assay indicated that light induced a death rate of approximately 60–80% of the cells. MBE significantly inhibited this cell death at 1–10 μg/ml (Fig. 2E). D3G5G at 15 μM and D3S5G at 1.5–15 μM also significantly inhibited the light-induced cell death (Fig. 2F and G). At these concentrations, MBE, D3G5G, or D3S5G alone had no effects on the viability of the cells (Fig. 2E–G). Typical images of Hoechst 33342 staining are shown in Fig. 3A–C. With this dye, condensed nuclei of dam-

aged cells stained more intensely than those of normal cells did. Untreated control cells displayed normal nuclear morphology (Fig. 3A), while the cells irradiated with light for 24 h revealed shrunken and condensed nuclei (Fig. 3B). After light exposure, nuclei in the cells treated with MBE at 10 μg/ml were morphologically similar to those of the control cells (Fig. 3C). As shown in Fig. 3D, MBE at 3 and 10 μg/ml significantly reduced the number of apoptotic cells which was detected by PI labelling. D3G5G and D3S5G at 1.5–15 μM also significantly inhibited cell apoptosis (Fig. 3E and F). At these concentrations, MBE, D3G5G, or D3S5G alone had no effects on the viability of untreated control 661W (Fig. 3D–F).

3.3. Effects of MBE, D3G5G, and D3S5G on light-induced ROS production in 661W

CM-H$_2$DCFDA, a cell-permeant indicator of reactive oxygen species (ROS), is nonfluorescent until removal of its acetate groups by
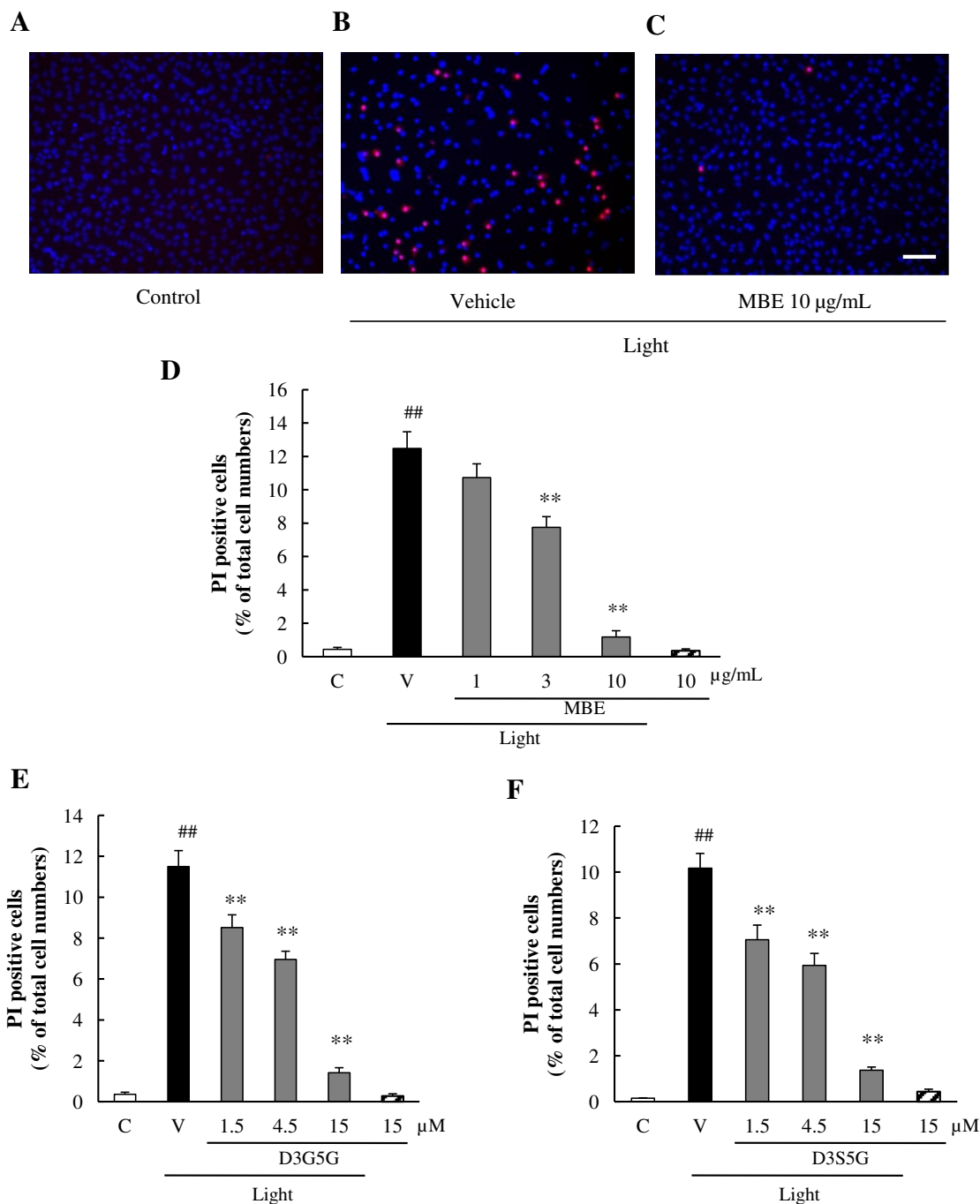
Fig. 3. Effects of MBE and its anthocyanin constituents on light-induced photoreceptor cell death in 661W cells. (A–C) Representative fluorescence microscopy of Hoechst 33342 staining at 24 h after light exposure. (A) Nontreated cells showed normal nuclear morphology. (B) Light-induced cell death, with cells showing nuclear condensation. (C) Pretreatment with MBE at 10 μg/ml reduced nuclear condensation. (D–F) The number of cells exhibiting PI fluorescence was counted, and positive cells were expressed as the percentage of PI-positive to Hoechst 33342-positive cells. Effects of MBE, D3G5G, and D3S5G on light-induced 661W cell death. The last bars in each figure show data obtained following application of each drug alone but without light exposure. Data are shown as mean ± SEM ($n = 6$). C, control; V, vehicle. $^{##}p < 0.01$ versus control, and $^{**}p < 0.01$ versus vehicle. The scale bar (C) represents 250 μm.

intracellular esterase. Within the cell, esterases cleave CM-H$_2$DCFDA to release CM-H$_2$DCFDH, which is converted to a fluorescent product (CM-H$_2$DCF) when exposed to ROS. MBE at 1–10 μg/ml, D3G5G at 1.5–15 μM, and D3S5G at 1.5–15 μM significantly inhibited the light-induced radical activity in 661W (Fig. 4A–C), indicating the inhibition of ROS production in 661W.

3.4. Effects of MBE on light-induced phosphorylation of p38

The effect of MBE on the activation of signalling pathways known to be induced by light was analysed. Yang et al. indicated that SB203580, a p38 inhibitor, inhibits the light-induced 661W cell death (Yang et al., 2007). Exposure to light for 1 h increased the phosphorylation of p38 (p-p38) to approximately 3-fold
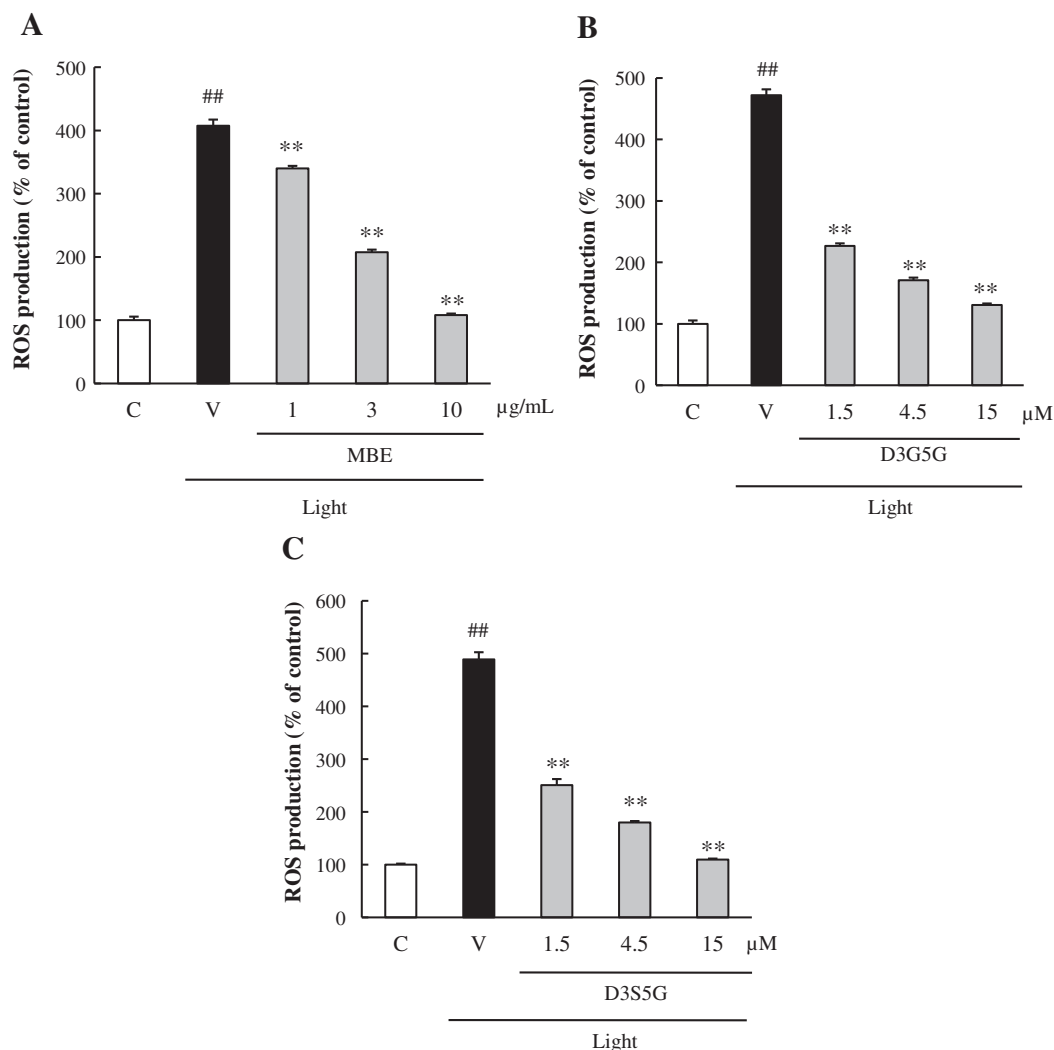
A

B

C

Fig. 4. Effects of MBE and its anthocyanin constituents on light-induced ROS production. Intracellular ROS levels were determined by measuring the fluorescence of CM-H$_2$DCFDA (at excitation 488 nm/emission 525 nm) after light exposure at 24 h in 661W. (A–C) Effects of MBE, D3G5G, and D3S5G on light-induced intracellular radical activation. Data are shown as mean ± SEM (n = 6). C, control; V, vehicle. [##]$p < 0.01$ versus control, and [**]$p < 0.01$ versus vehicle.

(Fig. 5). MBE at 10 µg/ml significantly inhibited light-induced increase in p-p38 (Fig. 5).

3.5. Comparison of the effects of berry extracts or anthocyanidins on light-induced cell death in 661W

A comparison was made of the effects of MBE, BCE, and BBE on light-induced damage in 661W. The total anthocyanin content in the MBE, BCE, and BBE were all 20%. The WST-8 assay indicated that light irradiation induced the death of approximately 80% of the cells. MBE significantly inhibited this cell death at 10 µg/ml, which was even stronger than those of BCE and BBE (Fig. 6A). Secondly, we also made a comparison among delphinidin, cyanidin, malvidin, and peonidin on light-induced 661W cell damage. Delphinidin, a main anthocyanidin in MBE, significantly inhibited this cell death at 30 µM, which was even stronger than those of malvidin and peonidin (Fig. 6B).

4. Discussion

MBE anthocyanins were analysed by HPLC, in which eight kinds of anthocyanins were determined (Fig. 1). Actually, MBE contains two kinds of anthocyanidins (delphinidin and cyanidin), and each

of these can be linked to different sugars, which may be 3-glucosides, 3,5-diglucosides, 3-sambubiosides, and 3-sambubioside-5-glucosides (Maria et al., 2006). The total anthocyanin content in the MBE was 35.4%, of which the anthocyanin proposition is 82.3% of delphinidin, and cyanidin being the rest. Further, anthocyanins containing delphinidin exhibit higher absorption rations than the other anthocyanins (Talavéra et al., 2003). Main anthocyanins in MBE are D3G5G and D3S5G, whose contents are 14.3% and 7.1% respectively. On the basis of these findings, D3G5G and D3S5G were used in the *in vitro* studies.

In the present study, *in vitro* supply of MBE and its constituents (D3G5G and D3S5G) protected cultured 661W against visible light-induced cell death. The 661W served as a useful *in vitro* model for photoreceptor cell responses to visible light, as the cells in culture also showed light-induced cell death pathways that are similar to those observed in photoreceptor cells *in vivo* (Krishnamoorthy et al., 1999). Irradiation with white fluorescent light (2500 lux for 24 h) in the present study was sufficient to induce cell damage, such as necrosis and apoptosis, as shown in Figs. 2 and 3. MBE, D3G5G, and D3S5G treatments were able to inhibit the light-induced 661W cell death. Delphinidin glycosides (glucoside, galactoside, and arabinoside) have been previously reported to protect against A2E oxidation induced by blue light, while delphin-
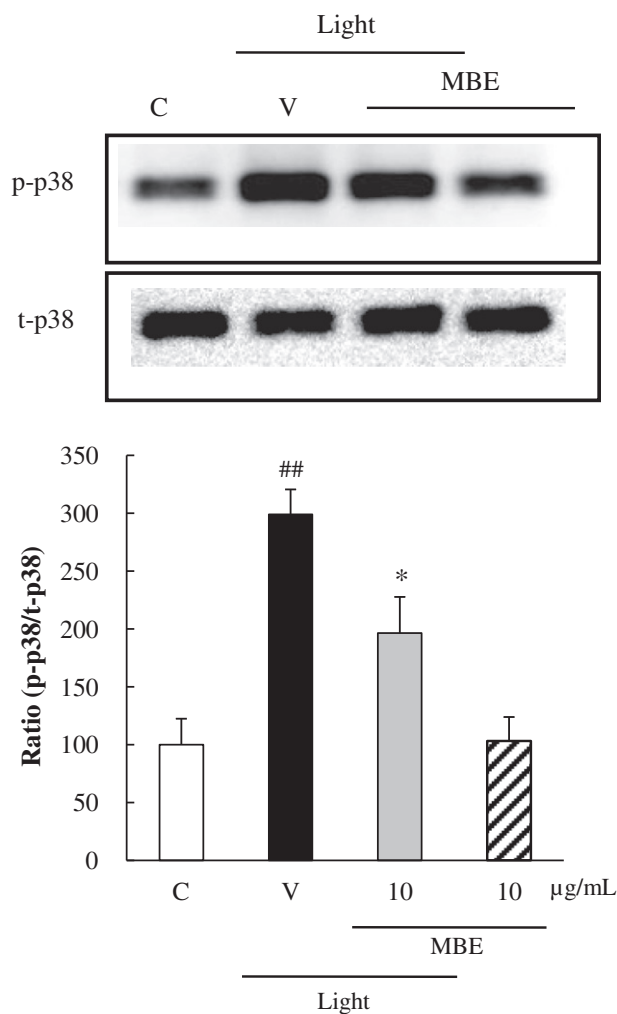
Fig. 5. Effects of MBE on phosphorylation of p38 induced by light. Phosphorylation of p38 was determined by immunoblotting assays. Blots were scanned and quantified by densitometric analysis, with the phosphorylated-p38 blots (p-p38) expressed relative to the total-p38 (t-p38). Data are shown as means ± SEM ($n = 6$). C, control; V, vehicle. [##]$p < 0.01$ versus control, and [*]$p < 0.05$ versus vehicle.

Fig. 6. Effects of berry extracts and anthocyanidins on visible light-induced 661W cell damage. Cells were cultured in 96-well plates for 24 h, and the cells were exposed to 2500 lux of white fluorescent light for 24 h. Cell viability was assessed by immersing cells in CCK-8 for 3 h at 37 °C, with photometric data being recorded at 492/660 nm. (A) Effects of MBE, CSE, and BBE on light-induced 661W cell death, as determined by WST-8 assay. (B) Effects of delphinidin, cyanidin, malvidin, and peonidin on light-induced 661W cell death, as determined by WST-8 assay. Data are shown as mean ± SEM ($n = 6$). C, control; V, vehicle. [##]$p < 0.01$ versus control, [**]$p < 0.01$ versus vehicle, [§§]$p < 0.01$ versus MBE, and [¶]$p < 0.05$, [¶¶]$p < 0.01$ versus delphinidin.

idin galactoside protected A2E-laden retinal pigment epithelial cells against blue light–induced damage (Jang, Zhou, Nakanishi, & Sparrow, 2005). Consequently, these data indicate that D3G5G and D3S5G are active anthocyanin components of MBE.

Previous reports demonstrated that light exposure triggered apoptosis in the photoreceptor cells (Abler, Chang, Ful, Tso, & Lam, 1996), and that apoptosis was prevented by antioxidants such as ascorbic acid (Li et al., 1985; Organisciak et al., 1985), dimethylthiourea (Organisciak, Darrow, Jiang, Marak, & Blanks, 1992), and thioredoxin (Tanito, Masutani, Nakamura, Ohira, & Yodoi, 2002). The main effects of light-induced cell death have been reported to depend on the apoptotic pathway and also on ROS production (Krishnamoorthy et al., 1999). ROS such as H_2O_2, ($\cdot O_2^-$), and $\cdot OH$, have been implicated in the regulation of many important cellular events, including transcription-factor activation (Schreck, Rieber, & Baeuerle, 1991), gene expression (Lo & Cruz, 1995), and cell proliferation (Murrell, Francis, & Bromley, 1990). However, excessive production of ROS gives rise to events that lead to the death of several types of cells (Wolfe, Ross, & Cohen, 1994). The ROS level of 661W increased at 24 h after light exposure in the vehicle group, while MBE, D3G5G, and D3S5G treatment prevented this increase (Fig. 4). Hence, D3G5G and D3S5G may be two of the active components in MBE that cause inhibition of ROS production
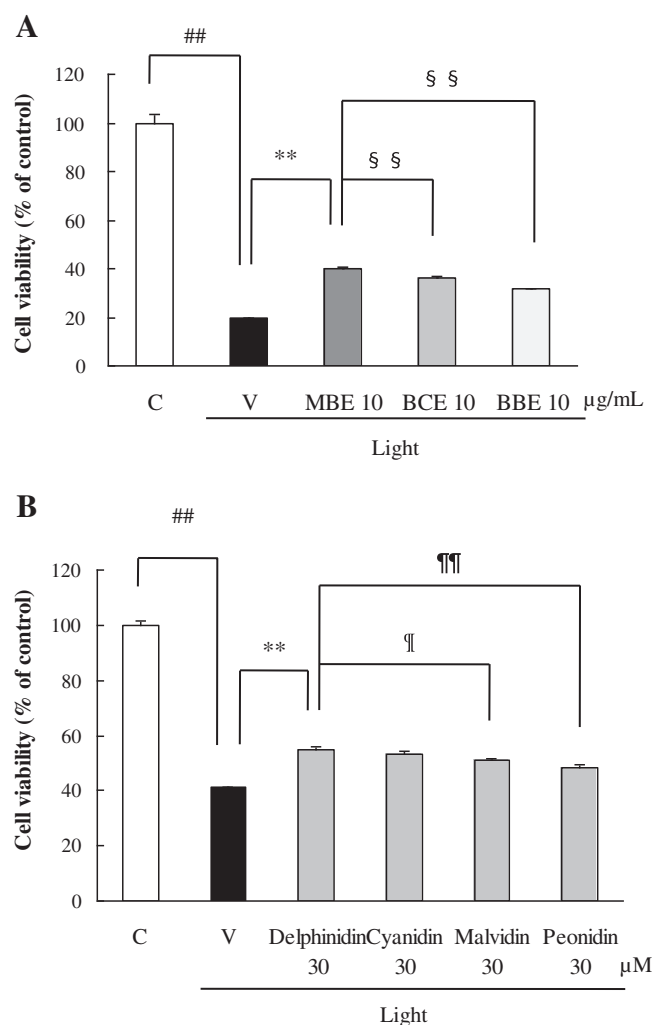
in 661W cells following irradiation by visible light. Excessive light exposure induces large amount of ROS, including free radicals, and their production can be overcome by a retinal defensive mechanism, such as superoxide dismutase (SOD) (Dong et al., 2006). Maqui berry juice has previously been reported to protect human endothelial cells against H_2O_2 induced oxidative stress (Ruiz et al., 2010). Delphinidin and delphinidin glycosides have also been reported to show potent $\cdot O_2^-$ and $\cdot OH$ scavenging activities (Noda, Kaneyuki, Mori, & Packer, 2002). Taken together, these findings indicate that MBE has strong antioxidant activity and can scavenge light-induced free radicals, including ($\cdot O_2^-$) and $\cdot OH$, resulting in a protective effect against photoreceptor cell death. Overall, the findings of the present study indicate that MBE is effective at attenuating the dysfunction of photoreceptor and inner retinal cells induced by light exposure, and thus, MBE can ameliorate visual function.

Mitogen-activated protein kinases (MAPKs) are stress-related kinases, and members of the MAPKs subfamily (JNK, p38, and ERK1/2) have been implicated in neuronal injury and diseases.

Activation of p38, which are stimulated by various stresses, including ischaemia, UV exposure, and oxidative stress, are involved in cell differentiation and apoptosis. It has been reported that light-induced photoreceptor apoptosis was related to MAPKs activation *in vitro* and *in vivo* (Imai et al., 2010). Furthermore, Yang et al. indicated that SB203580, a p38 inhibitor, inhibits the light-induced 661W cell death (Yang et al., 2007). The phosphorylated-p38 level of 661W increased after light exposure in the vehicle group, while MBE treatment at 10 μg/ml prevented this increase (Fig. 5). In addition, activator protein-1 (AP-1) is an important factor for light-induced retinal degeneration (Grimm, Wenzel, Hafezi, & Remé, 2000). Light exposure leads to activation of AP-1 in mouse retina, and their complexes are mainly composed of c-Fos and c-Jun proteins. Furthermore, AP-1 is activated through phosphorylations of c-Jun and c-Fos, induced by p38 in retinal pigment epithelium cells (Roduit & Schorderet, 2008). These reports suggest that AP-1 activation through p38 is essential for light-induced photoreceptor apoptosis in mouse retina. Delphinidin and cyanidin have been reported to have inhibitory effects against activation of p38 and AP-1 induced by UV in epidermal cells (Ding et al., 2006; Kwon et al., 2009). Therefore, there is a possibility that MBE containing delphinidin glycosides and cyanidin glycosides may inhibit AP-1 activation by inhibiting p38 activation.

The effects of MBE, BCE, and BBE on light-induced damage in 661W were compared at the contents of that the total anthocyanins in the MBE, BCE, and BBE were all 20%. MBE suppressed the light-induced 661W cell death, which was even stronger than those of BCE and BBE (Fig. 6A). The composition MBE anthocyanin is 82.3% of delphinidin, and the rest of cyanidin. The composition BCE anthocyanin is 54.7% of delphinidin, and the rest of other anthocyanins, and the composition BBE anthocyanin is 41.4% of delphinidin, and the rest of other anthocyanins, such as malvidin and peonidin (Kähkőnen, Heinämäki, Ollilainen, & Heinonen, 2003). In view of their composition features in the contents of anthocyanins, a comparison was also made among delphinidin, cyanidin, malvidin, and peonidin on light-induced 661W cell damage. Delphinidin inhibited this cell death, which was even stronger than those of malvidin and peonidin (Fig. 6B). The availability of the hydroxyl group on the B ring of anthocyanidin (delphinidin) is fundamental to the antioxidant activity of these compounds because these hydroxyl moieties can donate hydrogen to scavenge radicals (Torel, Cillard, & Cillard, 1986). Indeed, the donated hydrogen serves to scavenge oxygen radicals such as ($\cdot O_2^-$) and $\cdot$OH. Rahman et al. and Noda et al. indicated delphinidin glycoside show the strongest ($\cdot O_2^-$), $\cdot$OH, and peroxynitrite radical scavenging activity when compared to other anthocyanins (Noda et al., 2002; Rahman, Ichiyanagi, Komiyama, Hatano, & Konishi, 2006). Taken together, these findings indicate that MBE possesses antioxidant effects and suppress the light-induced 661W cell death, which is stronger than other berry extracts, due to the higher proportion of delphinidin glycosides content in MBE than in other berry extracts.

Maqui berry anthocyanins, such as D3G5G and D3S5G, have been reported to be detectable in the model of the human gastrointestinal tract (TIM-1) (Lila et al., 2012). Kalt et al. have reported that bilberry anthocyanins had been identified in the eyes, liver, and brain in pig by oral administration (Kalt et al., 2008), suggesting the possible protective effect of oral MBE for retinal diseases. Although there are no pharmacokinetic studies of MBE, previous pharmacokinetic study has demonstrated that the intraperitoneal administration of blackcurrant anthocyanin at 108 mg/kg showed the maximum concentrations of blackcurrant anthocyanin were 6.72 μg/ml in aqueous fluid and 6.89 μg/g in retina (Matsumoto et al., 2006). These results suggest the possibility for MBE anthocyanins to distribute in aqueous fluid and retina at about the same contents when the dose is reasonably administrated. The *in vitro*

study showed the MBE at 3–10 μg/ml, which corresponds to 1.06–3.54 μg/ml MBE anthocyanins, exhibited protective effects of light-induced photoreceptor cell death. These effective doses were lower than the anthocyanins distributed in the aqueous fluid and retina, therefore, it is possible for MBE to exert a protective effect on the retinal diseases *in vivo*. However, further experiments would be necessary.

In conclusion, MBE, in part through the activity of its constituent anthocyanidins (D3G5G and D3S5G), was able to protect retinal cells against light-induced photoreceptor degeneration in *in vitro* models. As the light-induced retinal damage model is one of the best models of dry-atrophic AMD (Marc et al., 2008), MBE may prove useful as prophylactic health food for the prevention of retinal diseases.

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