

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Delphinidin 3,5-O-diglucoside, a constituent of the maqui berry (*Aristotelia chilensis*) anthocyanin, restores tear secretion in a rat dry eye model

Shigeru Nakamura ^{a,*}, Junji Tanaka ^b, Toshihiro Imada ^a, Hiroshi Shimoda ^b, Kazuo Tsubota ^a

^a Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

^b Research & Development Division, Oryza Oil & Fat Chemical Co., Ltd., Ichinomiya, Aichi, Japan

ARTICLE INFO

Article history:

Received 13 March 2014

Received in revised form 9 June 2014

Accepted 18 June 2014

Available online

Keywords:

Anthocyanin

Ophthalmology

Dry eye

Reactive oxygen species

ABSTRACT

Dry eye disease is a disorder in tear film physiology, which leads to various abnormal states of ocular surface cells. In the present study, we evaluated the potential usefulness of orally applied maqui berry (*Aristotelia chilensis*) extract and the constituent anthocyanin as a preventative intervention in dry eye. Tear secretion capacity was evaluated following the oral administration of maqui berry extract to a rat blink-suppressed dry eye model. This suppressive effect of maqui berry extract and its constituent anthocyanins on reactive oxygen species formation from lacrimal gland was elevated by 2', 7'-dichlorofluorescein diacetate. Maqui berry extract and delphinidin 3,5-O-diglucoside, an anthocyanin specifically contained in the maqui berry, suppressed reactive oxygen species formation from lacrimal gland tissue and preserved tear secretion. The result of the present study demonstrates that maqui berry extract can be used as a preventative intervention for dry eye by managing tear secretion capacity in the lacrimal gland.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Anthocyanins are natural water-soluble phenolic pigments of the flavonoid family that have a wide range of biological effects that have been attributed to their potent antioxidant properties (Tsuda, 2012). Anthocyanins-rich berries and berry extracts have been used as food supplement for the treatment of eye diseases based on the findings of animal and human studies (Ghosh & Konishi, 2007; Matsumoto, Kamm, Stull & Azuma, 2005). The dietary intake of and supplementation with

black currants (*Ribes nigrum* L.), which contain high levels of anthocyanidins, have been shown to be beneficial for visual functions and the subjective symptoms of eye fatigue (Nakaishi et al., 2000; Ohguro et al., 2012; Yagi et al., 2009).

Maqui (*Aristotelia chilensis* Mol. Stuntz) is a plant that belongs to Elaeocarpaceae family, which is grown in the Patagonia region of South America levees. Its fruits (maqui berry) have been used in traditional medicine for inflammation (Misle et al., 2011; Munoz et al., 2011) and are recently used for functional beverages and dietary supplements to offer potential health benefits (Schreckinger et al., 2010). The purple/black edible fruits

* Corresponding author. Tel.: +81-3-5393-3972; fax: +81-3-5363-3974.

E-mail address: vdtwork@gmail.com (S. Nakamura).

Abbreviations: BCE, blackcurrant berry (*R. nigrum* L.) extract; BBE, bilberry (*V. myrtillus* L.) extract; D3G5G, delphinidin 3,5-O-diglucoside; LG, lacrimal gland; MBE, maqui berry extract; ROS, reactive oxygen species; VAMP8, vesicle-associated membrane protein 8 <http://dx.doi.org/10.1016/j.jff.2014.06.027>

1756-4646/© 2014 Elsevier Ltd. All rights reserved.

of this plant contain a variety of phenolic products, anthocyanidins, other flavonoids and phenolic acids, which contribute to the high antioxidant capacity of this fruit (Céspedes et al., 2010). This fruit contains a higher concentration as well as characteristic constitution of anthocyanins than other edible berries (Escribano Bailon et al., 2006; Miranda Rottmann et al., 2002). We previously reported that maqui berry extract (MBE) and its anthocyanidins were able to protect retinal cells against light-induced photoreceptor degeneration in cellular experiments (Tanaka et al., 2013). These findings indicated the possible therapeutic or preventive efficacy of MBE in various ophthalmic disorders.

Tear fluid is secreted from the lacrimal gland (LG), and constantly flows over the ocular surface to provide a properly controlled environment for corneal and conjunctival epithelial cells. Approximately 90% of tear fluid is drained through the lacrimal sac and the rest evaporates from the ocular surface in the intervals between blinking (Mishima, 1965). Dry eye disease is a disorder in tear film physiology that is characterized by misbalance of a tear flow, which leads to various abnormal states of ocular surface cells (Holly & Lemp, 1977). Recent studies have also demonstrated that not only does this disease cause symptoms including ocular discomfort, such as stinging/burning or dryness, but it also affects functional visual acuity (Koh et al., 2008). Dry eye has markedly increased due to the radical expansion in information technology. The incidence of dry eye is particularly high in workers that stare at the screens of technological devices, including desktops, laptops, tablets, and smartphones, which have become ubiquitous in a wide range of age groups (Kawashima et al., 2013; Uchino et al., 2013). We previously reported, in a human epidemiological study and rat blink-suppressed dry eye model, that not only the excess evaporation of tear fluid, but also hypofunction in the LG contribute to the etiology of computer work-associated dry eye (Nakamura et al., 2010). Our findings suggested that the pharmacological modulation of LG dysfunction may be a prospective treatment for this type of dry eye. However, temporal tear replacement therapy, the frequent application of artificial tear eye drops, has long been taken as a basic management for dry eye symptoms. Therefore, an effective intervention that could maintain LG function to reduce the risk of this disease is needed.

In the present study, we assessed the potential usefulness of orally applied MBE and its constituent anthocyanin as a preventative intervention in dry eye associated with the excessive use of digital devices.

2. Materials and methods

2.1. Chemicals

Delphinidin 3-O-sambubioside-5-O-glucoside (D3S5G), delphinidin 3,5-O-diglucoside (D3G5G), and delphinidin 3-O-sambubioside (D3S) were supplied by Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan). Delphinidin 3-O-glucoside (D3G), cyanidin 3-O-glucoside (C3G), petunidin 3-O-glucoside (Pet3G), peonidin 3-O-glucoside (Peo3G), malvidin 3-O-glucoside (M3G), and delphinidin 3-O-rutinoside (D3R) were purchased from Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). MaquiBright™

[maqui berry (*A. chilensis*) extract] (MBE), blackcurrant berry (*R. nigrum* L.) extract (BCE), and bilberry (*Vaccinium myrtillus* L.) extract (BBE) were supplied by Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan). The contents of MBE anthocyanin are D3S5G, D3G5G, cyanidin 3-O-sambubioside-5-O-glucoside, cyanidin 3,5-O-diglucoside, D3S, D3G, cyanidin 3-O-sambubioside, and C3G (Tanaka et al., 2013). Blackcurrant berry extract anthocyanins are D3G, D3R, C3G and cyanidin 3-O-rutinoside (Matsumoto et al., 2006). Bilberry extract anthocyanins are delphinidin, cyanidin, peonidin, petunidin, and malvidin (Matsunaga et al., 2009). The anthocyanin concentrations of these extracts were determined to be 200 mg/g by HPLC.

2.2. Animals

Female 8-week-old Sprague-Dawley rats ($n = 6-8$ in each experiment) were purchased from CREA Japan (Tokyo, Japan). They were quarantined and acclimatized before the experiments for 1 week under standard conditions as follows: room temperature 23 ± 2 °C, relative humidity of $60 \pm 10\%$, alternating 12 hour light–dark cycle (8 AM–8 PM), and water and food available *ad libitum*. All animal experiments were approved by the Animal Care and Use Committee of Keio University, and all procedures were performed in accordance with the Association of Research and Vision in Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research.

2.3. Blink-suppressed dry eye model

The model and methodology used to simulate staring at a computer display have been described previously (Nakamura et al., 2005, 2010). In brief, a series of treatments were performed under dry conditions, with room temperature of 23 ± 2 °C, relative humidity of $25 \pm 5\%$, and a constant air flow of 2–4 m/s produced by an 18-cm diameter electric fan. Sustained suppression of blinking in the rat was achieved by keeping the rat stationary on a swing made of plastic piping (30 mm \times 50 mm in length) suspended 60 cm above the bottom. After 4 hours on the swing, the rats were returned to their cages for 30 minutes for food and water and again placed on a swing for 3.5 hours (Supplementary Fig. S1). They were individually placed in cages with water and food *ad libitum* for the remaining 16 hours. This series of treatments was repeated for 3 days for the screening of berry extracts and 10 days in for the MBE evaluation.

Each material was dissolved in distilled water and was repeatedly administered orally once a day to rats during the swing procedure. Lacrimal function was evaluated on day 11.

2.4. Measurement of tear secretion

We used a modified Schirmer test on the rats' eyes to measure tear fluid secretion under topical anesthesia induced with a 0.4% oxybuprocaine hydrochloride solution (Santen Pharmaceutical, Osaka, Japan). After 3 minutes of anesthesia, a phenol red thread (Zone-Quick; Syowa Yakuin Kako, Ltd, Tokyo, Japan) was placed on the temporal side of the lower eyelid margin for 1 minute. The length of the moistened area from the edge was then measured to within 1 mm.

2.5. Corneal fluorescein staining

The degree of corneal surface damage was determined by the application of a fluorescein solution under a blue-free barrier filter. Corneal staining was graded based on the area of staining according to previously described criteria (Nakamura et al., 2005). The total area of punctate staining was denoted as grade 0 when there was no punctate staining, grade 0.5 when less than one sixteenth was stained, grade 1 when less than one eighth was stained, grade 2 when one fourth was stained, grade 3 when greater than one half was stained, and grade 4 when the entire area was stained.

2.6. Histopathological examination

The fixed LG in 10% formalin was embedded in paraffin and sectioned. Sections were subjected to HE staining or immunostaining. Vesicle-associated membrane protein 8 (VAMP8) was immunostained to evaluate the occupied pattern of secretory vesicles in the acinar cells of specimens. VAMP8 was previously shown to be enriched on the membranes of secretory vesicles (Wang et al., 2004). The primary antibody used for immunostaining was a rabbit monoclonal antibody against VAMP8 (Abcam, Cambridge, UK). Nuclear staining was performed by treating specimens with hematoxylin.

2.7. Reactive oxygen species (ROS) production from the lacrimal gland and cornea

The excised LG or cornea was immersed in cold PBS (25 mg tissue weight/mL) and homogenized using a Mil mixer with a zirconia ball (AS ONE Corporation, Osaka, Japan). To screen the effects of each berry extract and purified anthocyanin (100 nM), tissue homogenates were incubated with each material and 2', 7'-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Inc., Eugene, OR, USA) for 1 hour at 37 °C. DCFH-DA is a nonfluorescent, membrane-permeable compound and ROS in cells cause oxidation of DCFH, yielding the fluorescent product 2', 7'-dichlorofluorescein (DCF). To evaluate MBE on the rat blink-suppressed dry eye model, excised LG homogenates were incubated with DCFH-DA 1 hour at 37 °C. The preparations were washed 3 times with PBS by centrifugation at 800 g for 3 minutes. Washed cells were re-suspended in PBS, followed and read at an excitation of 480 nm and emission of 530 nm. The mean fluorescence intensity was calculated and expressed as percentage of control or vehicle.

2.8. Analysis of LG mitochondrial content

To estimate the LG mitochondrial content, LG homogenates were incubated with 70 μM MitoTracker Green (Molecular Probes, Inc., Eugene, OR, USA) and 20 μM Hoechst 33342 (Dojin Chemical, Kumamoto, Japan) for 30 minutes at 37 °C. After incubation, the preparations were washed 3 times with PBS followed by centrifugation at 800 g for 3 minutes. Washed cells were re-suspended in PBS, and read at an excitation of 490 nm/emission of 516 nm and at an excitation of 361 nm/emission of 486 nm with a Synergy 4 plate reader (Biotek Company, Winooski, VT, USA). The LG mitochondrial content was determined by normalization of MitoTracker signal (Ex. 490 nm, Em.

516 nm) to the cellular DNA content using the Hoechst 33342 signal (Ex. 361 nm, Em. 486 nm) (Modis et al., 2012).

2.9. Incorporation of anthocyanins into LG

The LG digested by collagenase type 3 (Worthington Biochemical Co., Freehold, NJ, USA) was filtered through a 100 μm nylon mesh (Cell Strainer; BD Biosciences, San Jose, CA, USA) to isolate LG acinar cells. Acinar cells were incubated in saline solution (140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose [pH 7.4]) with anthocyanins for 30 minutes. The anthocyanins, D3G5G, D3G, D3S5G, and D3R, were appropriately prepared in saline solution at a concentration of 200 mM before use.

The anthocyanins were analyzed using UHPLC (Shimadzu Co., Kyoto, Japan) equipped with a C18 column (2.0 mm i.d. × 100 mm, YMC CO., Ltd., Kyoto, Japan). The mobile phase was composed of 0.3% trifluoroacetic acid aqueous solution (v/v, A) and CH₃CN (B). The gradient was as follows: 0 min, 95% (A), 5% (B); 0.60 min, 95% (A), 5% (B); 0.61 min, 87% (A), 13% (B); 3.00 min, 85% (A), 15% (B); 6.00 min, 74% (A), 26% (B); 6.20 min, 10% (A), 90% (B); 7.20 min, 10% (A), 90% (B). The flow rate was set at 0.3 mL/min. The wavelength for detection was 520 nm and the column was kept at 30 °C.

2.10. Statistical analysis

Student's *t*-test was used to compare the two groups and Dunnett's test was used for multiple comparisons. Differences between the measured variables were considered significant if the resultant *P* value was 0.05 or less.

3. Results

3.1. Effects of MBE, BBE, and BCE on ROS formation from ocular tissue

In the LG, MBE and BCE dose-dependently suppressed the concentration of ROS generated between 0.3 and 10 μg/mL. BBE also dose-dependently suppressed the production of ROS, however the effect plateaued at 3 μg/mL (Fig. 1A). The suppression of ROS was significantly stronger with MBE than with BCE at 3 and 10 μg/mL or BBE at 10 μg/mL.

No significant changes were observed in the cornea among each berry extract concentration at 10 μg/mL (Fig. 1B).

3.2. Evaluation of the rat blink-suppressed dry eye model

Figure 1C shows the effects of each berry extract on tear secretion capacity. In the dry eye model, which corresponded to that of our previous study (Nakamura et al., 2010), tear secretion was significantly lower in the vehicle group than the initial value after the three-day treatment. The prevention of a reduction in tear secretion was significant stronger with MBE than with BBC or BCE.

A significant difference was not observed in body weight gain among any dose of MBE and vehicle group (Fig. 2A). Tear

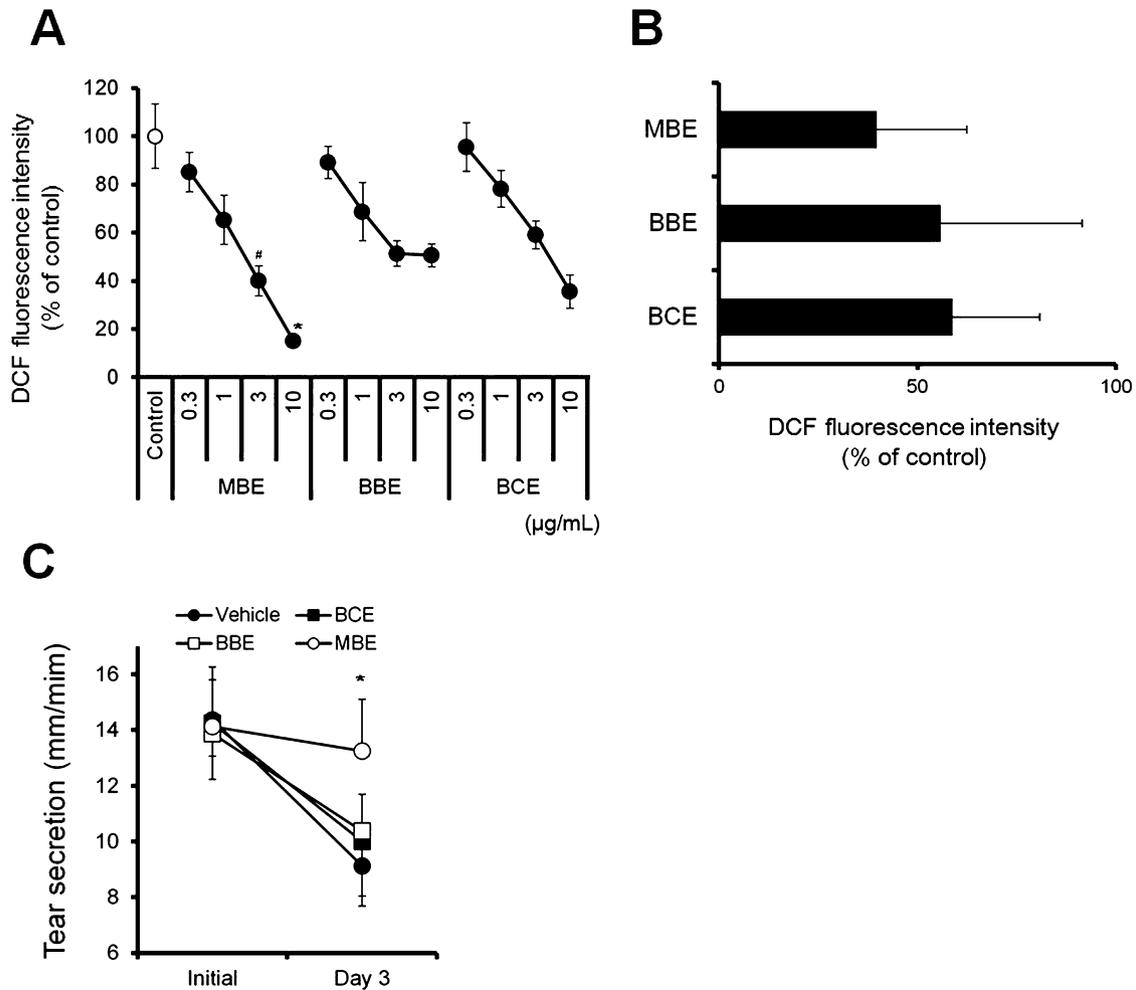


Fig. 1 – Suppressive effect on ROS generation and decrease in tear secretion by three common edible berries. A: ROS generation in the lacrimal gland, B: ROS generation in the cornea ($n = 6–8$ LG or cornea). C: Effects on tear secretion in a rat blink-suppressed dry eye model. MBE, BBE, and BCE were orally administrated at 40 mg/kg, respectively ($n = 5$ rats). All data represent the mean \pm SD. * $P < 0.05$, versus the vehicle.

secretion was significantly less from 5 to 11 days than the initial value in the vehicle group.

MBE dose-dependently suppressed the reduction in tear secretion. This suppression was significantly stronger with 40 mg/kg MBE than with the vehicle at all time points throughout the experiment. Figure 2D (upper) shows representative photographs of tear secretion patterns as measured using the modified Schirmer test. The wetted length on the tread showed by a red discolored area was shorter in vehicle eyes than in MBE eyes.

We performed corneal epithelial fluorescein staining on 40 mg/kg MBE to investigate whether the effects of MBE on tear secretion altered corneal epithelial damage under dry eye conditions.

The grade of corneal epithelial fluorescein staining was significantly higher in the vehicle than the initial value at all time points throughout the experiment. The intake of 40 mg/kg MBE maintained the graded score at the same level as the initial value, and the graded score was significantly less than that of the vehicle. Representative corneal staining patterns re-

stored by MBE are shown in Fig. 2D (lower). Punctate staining appeared in the whole area of the cornea surface with the vehicle treatment. In contrast, staining was cleared following intake of MBE.

3.3. Effects of MBE on the morphology of the LG and ROS formation under dry eye conditions

Figure 2A shows a representative microphotograph of the LG stained by HE (upper) and secretory vesicle-occupied pattern by VAMP8 immunostaining (lower) under dry eye condition. The cytoplasm of acinar cells was occupied by more enlarged acini with expanded cytoplasm in the vehicle group than that in the normal group. Acinar cell density was significantly lower in the vehicle than the normal value under dry eye conditions. MBE maintained the acinar cell density at the same level as the normal value, and this value was significantly higher than that of the vehicle (Fig. 2B).

VAMP8 was primarily localized near the lumen in the normal state, and was distributed diffusely in the entire cytoplasm of

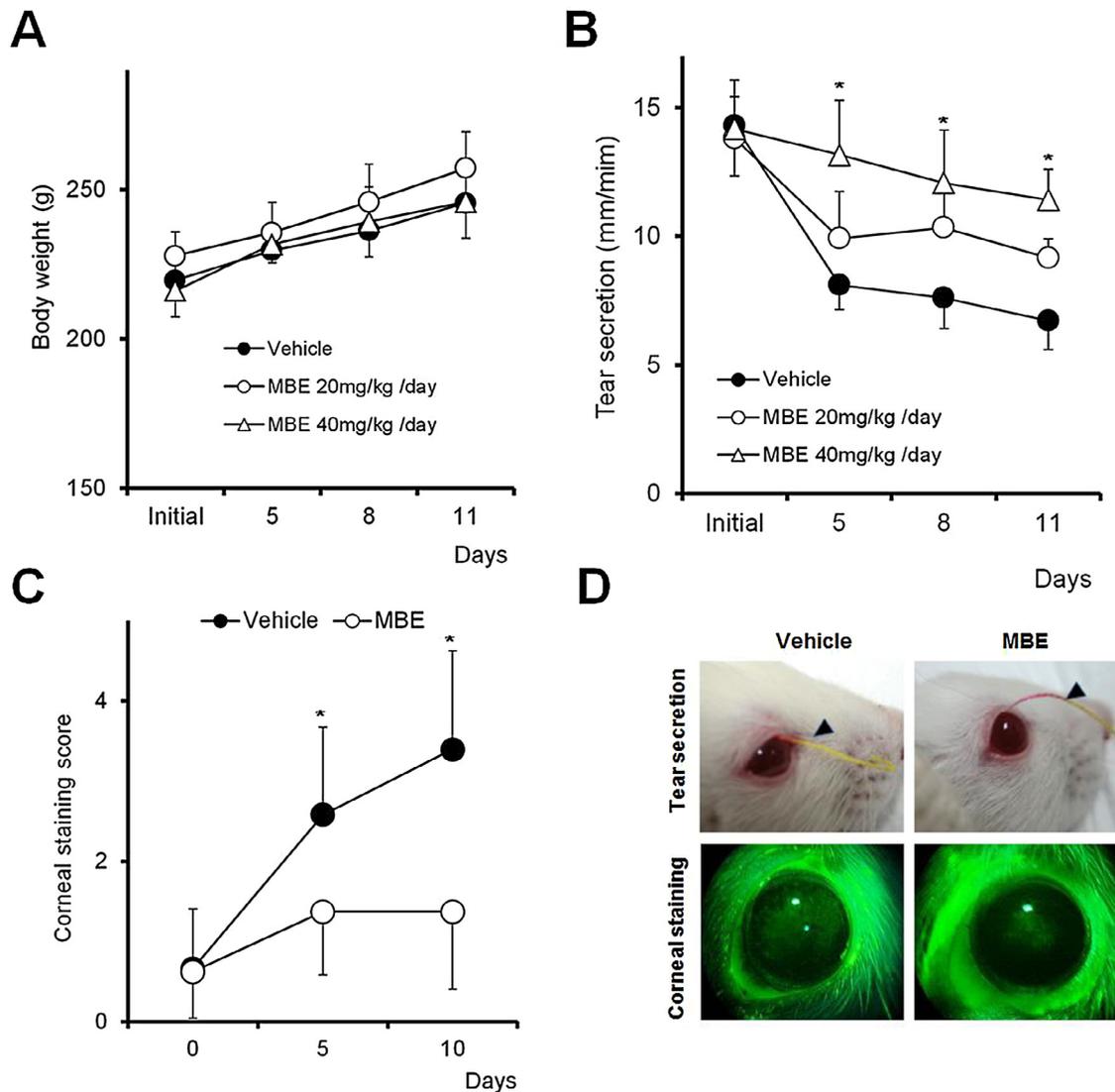


Fig. 2 – MBE restored tear secretion and corneal staining in a rat blink-suppressed dry eye model. **A:** Changes in body weight. **B:** Changes in tear secretion ($n = 6$ rats). **C:** Effects of MBE on corneal surface damage. Changes in the grading score ($n = 6$ rats). All data represent the mean \pm SD. * $P < 0.05$ versus the vehicle. **D:** Typical tear secretion patterns measured by a cotton thread (upper) and corneal staining (lower). Punctate staining appeared in the whole corneal surface with the vehicle treatment. The arrow shows the wetted length by tear secretion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

acinar cells in the vehicle group. VAMP8 was localized to the proximity of the lumen in the MBE group and its distribution was identical to that found in the normal state. These results suggested that acinar cells were enlarged by the accumulation of secretory vesicles and returned to the normal state with the intake of MBE in this dry eye model.

The formation of ROS in the LG was significantly higher in the vehicle group than in the normal group. The intake of MBE significantly suppressed the increase in ROS formation in the vehicle group (Fig. 3B). Mitochondrial content was significantly lower in the vehicle group than in the normal group. Mitochondrial content was restored significantly more in the MBE group than in the vehicle group (Fig. 3C).

3.4. Delphinidin 3,5-O-diglucoside suppressed ROS formation in the LG due to its high incorporation rate

Figure 4A shows the effects of anthocyanin aglicones and their glycosides on ROS formation in the LG. Delphinidin 3-O-sambubioside-5-O-glucoside and D3G5G are major anthocyanins in MBE, but are not contained in either BBE or BCE (Escrignano Bailon et al., 2006; Tanaka et al., 2013). Delphinidin 3-O-glucoside is the main anthocyanin in BBE, while D3R is specific to BCE. Other anthocyanins are contained in MBE, BBE, or/and BCE. All anthocyanins suppressed the formation of ROS more in the LG than the control. Delphinidin 3,5-O-diglucoside significantly inhibited the formation of ROS in the LG more than all other anthocyanins.

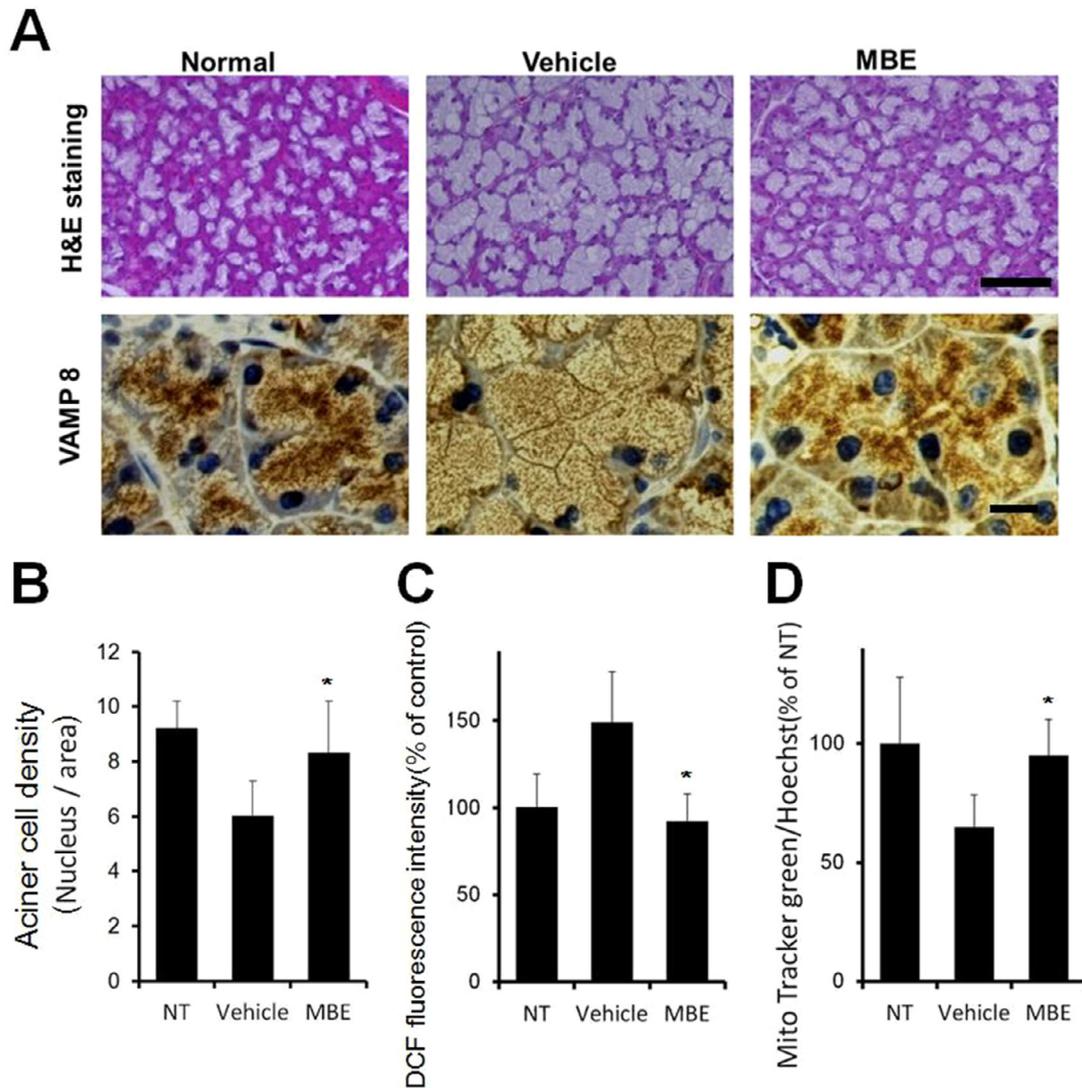


Fig. 3 – Effects of MBE on lacrimal gland function in a rat blink-suppressed dry eye model. A: Histopathological changes in the LG. HE staining (upper). VAMP8 immunostaining (lower). Scale bar, 100 μm (HE) or 10 μm (VAMP8). **B:** Acinar cell size ($n = 6$ LG). **C:** ROS formation in the LG, **D:** Mitochondria content. All data represent the mean \pm SD. * $P < 0.05$ versus the vehicle.

The incorporation rate of D3G5G into the LG was 10-fold higher than that of D3S5G, D3G, and D3R (Fig. 4B).

Due to their scarcity, it is difficult to evaluate the effects of purified anthocyanins on tear secretion in the rat model. Therefore, we used mouse stress induced dry eye model to confirm the relationship between the incorporation rate and the effect on tear secretion capacity of D3G5G. In this mouse model, tear secretion was significantly lower in the vehicle group than the initial value after the 1 day treatment. Orally applied D3G5G preserved tear secretion to the same value as the initial value. The prevention of a reduction in tear secretion was significantly stronger with D3G5G than with D3S5G or/and D3G (Supplementary Fig. S2).

4. Discussion

We previously reported a positive relationship between the duration of working on computer and the decrease in tear secretion in an epidemiological study on office workers (Nakamura et al., 2010). A mode of a computer worker is characterized by a lack of blinking, low humidity occupational environment, and sustained static postures during repetitive tasks (Smith, 1997). The rat blink-suppressed dry eye model used in this study was created to simulate a mode with excess computer users by repeatedly exposing rats to stressful conditions; persistent strain by swing board settlement in combination with exposure to an evaporative environment. Consistent with the findings of the epidemiological study on office workers, this rat model

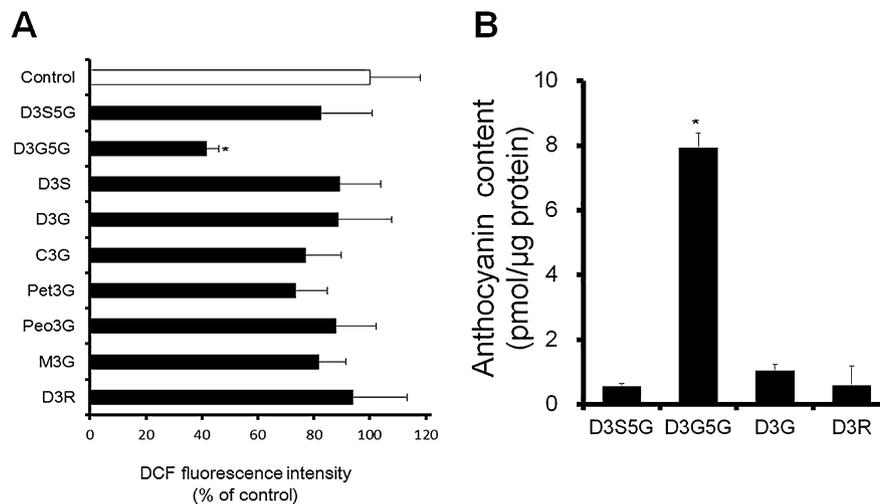


Fig. 4 – Delphinidin 3,5-O-diglucoside suppressed lacrimal gland ROS formation in the LG due to its high incorporation rate. A: Effects of anthocyanins and their glycosides on ROS formation in the LG (n = 8 LG), B: Incorporation rate of MBE anthocyanins into the LG (n = 6 LG). All data represent the mean ± SD. *P < 0.05 versus the control and other anthocyanins.

showed persistent decreases in tear secretion that depended on strain duration. The usefulness of this model has already been demonstrated by us to be a good model for studying effective treatments to avoid the progression of decreases in tear secretion due to lacrimal hypofunction (Nakamura et al., 2010). The present study showed that MBE had a more potent restorative effect on tear secretion capacity than the common edible berries in which ophthalmic activity has been documented. In addition, consistent with the effects of MBE on tear secretion capacity, the corneal staining score, a hallmark of ocular surface damage in dry eye, was apparently reduced by the application of MBE. This effect on the cornea was considered to be a consequence of providing a proper environment for corneal cells due to the restoration of an abundant tear fluid status by the intake of MBE. Thus, these findings suggest that the daily intake of MBE has the potential to be an effective intervention to preventing dry eye.

Many studies have demonstrated that exposure to stressful stimuli can induce a disruption in homeostasis and an unbalanced antioxidant status in several organs (Atif, Yousef, & Agrawal, 2008; Fontella et al., 2005; Lee et al., 2006). In this dry eye model, a decrease in tear secretion was accompanied by an increase in the formation of ROS in the LG of immobilized rats on a swing board continuously exposed to a desiccated air blower. Several studies have shown that the accumulation of oxidative damage causes the functional decline of the tear secretion capacity of the lacrimal gland. Deficiency of Cu,Zn-superoxide dismutase-1, a well-known enzyme in the antioxidant defense systems, in mice leads to the decreased production of tears, and a decline in total protein secretion from the LG (Kojima et al., 2012). Mice with a mitochondrial respiratory chain dysfunction that induced the overproduction of ROS exhibit a corneal epithelial disorder accompanied by decrease in tear secretory function (Uchino et al., 2012). The formation of tear fluid by the LG is achieved by a process of secretory vesicle-mediated exocytosis in acinar cells, and increased intracellular ROS is known to inhibit this process

(Keating, 2008). We also previously showed that the characteristic feature of morphological changes in the LG in this model was the enlargement of acinar cells accompanied by filling with secretory vesicles that indicate functional disorder in the secretory system of the LG (Nakamura et al., 2010). Based on the finding of these studies, a decrease in tear secretion in our dry eye model can be explained by an impairment in the LG secretory system due to the excessive formation of ROS during stressful treatment that simulated the condition of a computer worker.

We found that the treatment with MBE, which restored tear secretion, was accompanied by the suppressed accumulation of secretory vesicles and formation of ROS in the LG. Furthermore, consistent with changes in tear secretion capacity, our in vitro results revealed that the suppressive effects of MBE on ROS production in the LG, not in the cornea, were more potent than BBE or BCE. Taken together, these results suggest that suppression of ROS production by MBE in the LG under stressful condition was involved in the tear secretion capacity of LG.

Berry fruits, including MBE, are a rich source of dietary anthocyanins (Seeram, 2008). Most natural anthocyanins are present in the pulp or coat as derivatives of pelargonidin, cyanidin, delphinidin, and their glycosides, and each of these exhibits potent antioxidant properties (Galvano et al., 2007; Kahkonen & Heinonen, 2003). In this study, we showed that D3G5G, an anthocyanin specifically contained in MBE (Escrignano Bailon et al., 2006; Tanaka et al., 2013), suppressed the formation of ROS from LG tissue and restoration of tear secretion was more potent than other anthocyanins naturally contained in MBE, BBE, or BCE. In spite of the large number of studies conducted, the correlation between antioxidant activity and chemical structure is not yet fully understood and D3G5G has not yet been examined in detail (Galvano et al., 2007; Kahkonen & Heinonen, 2003). We previously reported that D3G5G and D3S5G, the main anthocyanins in MBE, protected against light-induced retinal photoreceptor death by modulating ROS production; however, the reason why these

delphinidin glycosides, but not other anthocyanin aglycons potentially protected against this form of cell death has not been identified (Tanaka et al., 2013). Most intra-cellular ROS are generated due to the electron leakage from mitochondria (Starkov, 2008). Therefore, cellular uptake level of anthocyanins may be a critical determinant for the potential antioxidant effects of anthocyanins. As expected, the uptake of D3G5G into the LG was markedly higher than that of the other major maqui berry anthocyanins. Orally administered berries' anthocyanins have been shown to be absorbed through the intestinal tract and rapidly appear in the plasma and the ocular tissue (Kalt et al., 2008; Matsumoto et al., 2001; McGhie et al., 2003); high transport rate of D3G5G by the LG via plasma is the mechanism by which MBE restores tear secretion capacity. Further investigations on the transcellular transport pathways and molecular antioxidant mechanisms of D3G5G are needed to clearly elucidate the availability of MBE.

In conclusion, this study is the first to demonstrate that orally administered MBE restored tear secretion capacity in dry eye. This effect was associated with the modulation of the LG secretory system stimulated by MBE containing the anthocyanin D3G5G. Since the health benefits of maqui berries, apart from the ophthalmic uses, have been well established, further clinical studies on the prevention or treatment of dry eye by MBE and, with a focus on information technology-associated dry eye, will contribute to the establishment of new therapeutic interventions for this disease.

Contributions

S.N., J.T. and K.T. conceived the project. S.H. and K.T. supervised all research. S.N. and J.T. wrote the manuscript. S.N. and J.T. prepared the figures. S.N., J.T. and T.I. designed the experiments. S.N., J.T. and T.I. performed the experiments. S.N. and T.I. performed analyzed data and statistical analysis. All authors reviewed the manuscript.

Conflict of interest

Shigeru Nakamura, Toshihiro Imada and Kazuo Tsubota declare no conflicts of interest. Junji Tanaka and Hiroshi Shimoda are employees of Oryza Oil & Fat Chemical Co., Ltd.

Acknowledgments

This research was supported by a grant from Oryza Oil & Fat Chemical Co., Ltd. The authors thank Takashi Kadekaru, Sachiko Shimoda, and Kohei Yoshimoto for their assistance.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2014.06.027.

REFERENCES

- Atif, F., Yousuf, S., & Agrawal, S. K. (2008). Restraint stress-induced oxidative damage and its amelioration with selenium. *European Journal of Pharmacology*, 600, 59–63.
- Céspedes, C. L., Valdez-Morales, M., Avila, J. G., El-Hafidi, M., Alarcón, J., & Paredes-López, O. (2010). Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae). *Food Chemistry*, 119, 886–895.
- Escribano Bailon, M. T., Alcalde Eon, C., Munoz, O., Rivas Gonzalo, J. C., & Santos Buelga, C. (2006). Anthocyanins in berries of maqui (*Aristotelia chilensis* (Mol.) Stuntz). *Phytochemical Analysis*, 17, 8–14.
- Fontella, F. U., Siqueira, I. R., Vasconcellos, A. P., Tabajara, A. S., Netto, C. A., & Dalmaz, C. (2005). Repeated restraint stress induces oxidative damage in rat hippocampus. *Neurochemical Research*, 30, 105–111.
- Galvano, F., La Fauci, L., Vitaglione, P., Fogliano, V., Vanella, L., & Felgines, C. (2007). Bioavailability, antioxidant and biological properties of the natural free-radical scavengers cyanidin and related glycosides. *Annali Dell'istituto Superiore Di Sanita*, 43, 382–393.
- Ghosh, D., & Konishi, T. (2007). Anthocyanins and anthocyanin-rich extracts: Role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition*, 16, 200–208.
- Holly, F. J., & Lemp, M. A. (1977). Tear physiology and dry eyes. *Survey of Ophthalmology*, 22, 69–87.
- Kahkonen, M. P., & Heinonen, M. (2003). Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry*, 51, 628–633.
- Kalt, W., Blumberg, J. B., McDonald, J. E., Vinqvist Tymchuk, M. R., Fillmore, S. A., Graf, B. A., O'Leary, J. M., & Milbury, P. E. (2008). Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. *Journal of Agricultural and Food Chemistry*, 56, 705–712.
- Kawashima, M., Uchino, M., Kawazoe, T., Kamiyashiki, M., Sano, K., & Tsubota, K. (2013). A field test of Web-based screening for dry eye disease to enhance awareness of eye problems among general Internet users: A latent strategy to promote health. *Journal of Medical Internet Research*, 15, e209.
- Keating, D. J. (2008). Mitochondrial dysfunction, oxidative stress, regulation of exocytosis and their relevance to neurodegenerative diseases. *Journal of Neurochemistry*, 104, 298–305.
- Koh, S., Maeda, N., Hirohara, Y., Mihashi, T., Bessho, K., Hori, Y., Inoue, T., Watanabe, H., Fujikado, T., & Tano, Y. (2008). Serial measurements of higher-order aberrations after blinking in patients with dry eye. *Investigative Ophthalmology and Visual Science*, 49, 133–138.
- Kojima, T., Wakamatsu, T. H., Dogru, M., Ogawa, Y., Igarashi, A., Ibrahim, O. M., Inaba, T., Shimizu, T., Noda, S., Obata, H., Nakamura, S., Wakamatsu, A., Shirasawa, T., Shimazaki, J., Negishi, K., & Tsubota, K. (2012). Age-related dysfunction of the lacrimal gland and oxidative stress: Evidence from the Cu,Zn-superoxide dismutase-1 (Sod1) knockout mice. *American Journal of Pathology*, 180, 1879–1896.
- Lee, Y. J., Choi, B., Lee, E. H., Choi, K. S., & Sohn, S. (2006). Immobilization stress induces cell death through production of reactive oxygen species in the mouse cerebral cortex. *Neuroscience Letters*, 392, 27–31.
- Matsumoto, H., Inaba, H., Kishi, M., Tominaga, S., Hirayama, M., & Tsuda, T. (2001). Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. *Journal of Agricultural and Food Chemistry*, 49, 1546–1551.

- Matsumoto, H., Kamm, K. E., Stull, J. T., & Azuma, H. (2005). Delphinidin-3-rutinoside relaxes the bovine ciliary smooth muscle through activation of ETB receptor and NO/cGMP pathway. *Experimental Eye Research*, 80, 313–322.
- Matsumoto, H., Nakamura, Y., Iida, H., Ito, K., & Ohguro, H. (2006). Comparative assessment of distribution of blackcurrant anthocyanins in rabbit and rat ocular tissues. *Experimental Eye Research*, 83, 348–356.
- Matsunaga, N., Imai, S., Inokuchi, Y., Shimazawa, M., Yokota, S., Araki, Y., & Hara, H. (2009). Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage in vitro and in vivo. *Molecular Nutrition and Food Research*, 53, 869–877.
- McGhie, T. K., Ainge, G. D., Barnett, L. E., Cooney, J. M., & Jensen, D. J. (2003). Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. *Journal of Agricultural and Food Chemistry*, 51, 4539–4548.
- Miranda, R. S., Aspillaga, A. A., Perez, D. D., Vasquez, L., Martinez, A. L., & Leighton, F. (2002). Juice and phenolic fractions of the berry *Aristotelia chilensis* inhibit LDL oxidation in vitro and protect human endothelial cells against oxidative stress. *Journal of Agricultural and Food Chemistry*, 50, 7542–7547.
- Mishima, S. (1965). Some physiological aspects of the precorneal tear film. *Archives of Ophthalmology*, 73, 233–241.
- Misle, E., Garrido, E., Contardo, H., & González, W. (2011). Maqui [*Aristotelia chilensis* (Mol.) Stuntz]-the amazing Chilean tree: A review. *Journal of Agricultural Science and Technology*, B1, 473–482.
- Modis, K., Gero, D., Erdelyi, K., Szoleczky, P., DeWitt, D., & Szabo, C. (2012). Cellular bioenergetics is regulated by PARP1 under resting conditions and during oxidative stress. *Biochemical Pharmacology*, 83, 633–643.
- Munoz, O., Christen, P., Cretton, S., Backhouse, N., Torres, V., Correa, O., Costa, E., Miranda, H., & Delporte, C. (2011). Chemical study and anti-inflammatory, analgesic and antioxidant activities of the leaves of *Aristotelia chilensis* (Mol.) Stuntz, Elaeocarpaceae. *The Journal of Pharmacy and Pharmacology*, 63, 849–859.
- Nakaishi, H., Matsumoto, H., Tominaga, S., & Hirayama, M. (2000). Effects of black current anthocyanoside intake on dark adaptation and VDT work-induced transient refractive alteration in healthy humans. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 5, 553–562.
- Nakamura, S., Kinoshita, S., Yokoi, N., Ogawa, Y., Shibuya, M., Nakashima, H., Hisamura, R., Imada, T., Imagawa, T., Uehara, M., Shibuya, I., Dogru, M., Ward, S., & Tsubota, K. (2010). Lacrimal hypofunction as a new mechanism of dry eye in visual display terminal users. *PLoS ONE*, 5, e11119.
- Nakamura, S., Shibuya, M., Nakashima, H., Imagawa, T., Uehara, M., & Tsubota, K. (2005). D-beta-hydroxybutyrate protects against corneal epithelial disorders in a rat dry eye model with jogging board. *Investigative Ophthalmology and Visual Science*, 46, 2379–2387.
- Ohguro, H., Ohguro, I., Katai, M., & Tanaka, S. (2012). Two-year randomized, placebo-controlled study of black currant anthocyanins on visual field in glaucoma. *Ophthalmologica. Journal International D'ophtalmologie. International Journal of Ophthalmology. Zeitschrift fur Augenheilkunde*, 228, 26–35.
- Schreckinger, M. E., Lotton, J., Lila, M. A., & de Mejia, E. G. (2010). Berries from South America: A comprehensive review on chemistry, health potential, and commercialization. *Journal of Medicinal Food*, 13, 233–246.
- Seeram, N. P. (2008). Berry fruits: Compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *Journal of Agricultural and Food Chemistry*, 56, 627–629.
- Smith, M. J. (1997). Psychosocial aspects of working with video display terminals (VDTs) and employee physical and mental health. *Ergonomics*, 40, 1002–1015.
- Starkov, A. A. (2008). The role of mitochondria in reactive oxygen species metabolism and signaling. *Annals of the New York Academy of Sciences*, 1147, 37–52.
- Tanaka, J., Kadekaru, T., Ogawa, K., Hitoe, S., Shimoda, H., & Hara, H. (2013). Maqui berry (*Aristotelia chilensis*) and the constituent delphinidin glycoside inhibit photoreceptor cell death induced by visible light. *Food Chemistry*, 139, 129–137.
- Tsuda, T. (2012). Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Molecular Nutrition and Food Research*, 56, 159–170.
- Uchino, M., Yokoi, N., Uchino, Y., Dogru, M., Kawashima, M., Komuro, A., Sonomura, Y., Kato, H., Kinoshita, S., Schaumberg, D. A., & Tsubota, K. (2013). Prevalence of dry eye disease and its risk factors in visual display terminal users: The Osaka study. *American Journal of Ophthalmology*, 156, 759–766.
- Uchino, Y., Kawakita, T., Miyazawa, M., Ishii, T., Onouchi, H., Yasuda, K., Ogawa, Y., Shimmura, S., Ishii, N., & Tsubota, K. (2012). Oxidative stress induced inflammation initiates functional decline of tear production. *PLoS ONE*, 7, e45805.
- Wang, C. C., Ng, C. P., Lu, L., Atlashkin, V., Zhang, W., Seet, L. F., & Hong, W. (2004). A role of VAMP8/endobrevin in regulated exocytosis of pancreatic acinar cells. *Developmental Cell*, 7, 359–371.
- Yagi, A., Fujimoto, K., Michihiro, K., Goh, B., Tsi, D., & Nagai, H. (2009). The effect of lutein supplementation on visual fatigue: A psychophysiological analysis. *Applied Ergonomics*, 40, 1047–1054.