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Protective Effects of Bilberry (*Vaccinium myrtillus* L.) Extract against Endotoxin-Induced Uveitis in Mice

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Endotoxin-induced uveitis (EIU), a useful animal model of ocular inflammation, is induced by injection of lipopolysacharide (LPS). These experiments showed that the nitric oxide (NO) level significantly increased in the whole eye homogenate of BALB/C mice 24 h after footpad injection of LPS at a dosage of 100 mg/mouse. However, the elevated NO level was significantly reduced by oral administration of bilberry extract (containing 42.04% anthocyanins) at dosages of 50, 100, and 200 mg/kg/day for 5 days before the LPS injection. In addition, bilberry extract decreased malondialdehyde (MDA) level and increased oxygen radical absorbance capacity (ORAC) level, glutathione (GSH) level, vitamin C level, and total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Moreover, bilberry extract increased expression of copper/zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and GPx mRNA. Taken together, bilberry extract showed protective effects against EIU, whereas the effects of bilberry extract (100 and 200 mg/kg/day, 5 days) were dose-dependent. In conclusion, these results provide new evidence to elucidate the beneficial effects of bilberry extract on eye health.

KEYWORDS: Bilberry (*Vaccinium myrtillus* L.) extract; anthocyanins; endotoxin; uveitis

INTRODUCTION

Uveitis, an ocular inflammation, is a common and complex eye disease that is likely to outbreak repeatedly and cause blindness. It has been proven that systemic injection of an endotoxin such as lipopolysacharide (LPS) at a sublethal dose can induce uveitis in susceptible animal species including rats and mice (*1*, *2*). The endotoxin-induced uveitis (EIU) serves as a widely applied animal model of acute ocular inflammation in humans. In general, EIU peaks at 24 h after the endotoxin injection in rats and mice. In rats with EIU, acute inflammation develops mainly in the anterior chamber, whereas in mice with EIU, the inflammation develops mainly in the posterior vitreous (*2*−*5*). In addition, EIU is an inflammatory process and induces oxidative stress that leads to increased lipid peroxidation and decreased endogenous antioxidants (*6*−*9*).

Bilberry (*Vaccinium myrtillus* L.) is a low-growing ericaceous dwarf shrub in Europe and North America, which has been found to possess protective effects on various pathophysiological conditions in view of its high content of anthocyanins (*10*−*14*). In our previous study, we have reported that bilberry extract has protective effects on $KBrO_3$-induced kidney damage and restraint stress-induced liver damage (*15*, *16*). Additionally, bilberry extract has the ability to prevent cataracts associated with oxidative stress (*17*) and improve blood vessel conditions in the retina (*18*). Recently, a study has reported that a similar extract containing anthocyanins has anti-inflammatory effects on EIU in rats (*19*).

However, there has been no report on the effects of bilberry extract on the oxidative stress induced by EIU. Therefore, the effects of bilberry extract on the oxidative stress induced by EIU deserved further study. The aim of this study was to evaluate the protective effects of bilberry extract against EIU. For this purpose, we studied modifications of antioxidant capacity, accumulations of lipid peroxidation products, and changes in antioxidant enzyme gene expression in the whole eye of mice.

MATERIALS AND METHODS

Materials and Chemicals. Bilberry extract (containing 42.04% anthocyanins) was purchased from Indena SPA (Milan, Italy) (batch 28870/M). Authentic standards of malvidin-3-*O*-gal, malvidin-3-*O*-glu, cyanidin-3-*O*-gal, and cyanidin-3-*O*-glu were obtained from Extrasynthese S.A. (Genay, France). Vitamin C and LPS from *Salmonella typhimurium* were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Coomassie brilliant blue kit, malondialdehyde (MDA) kit, total superoxide dismutase (SOD) kit, glutathione peroxidase (GPx) kit, and Griess reagent were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Glutathione (GSH) was purchased from Kohjin Co. Ltd. (Tokyo, Japan). Methanol was purchased from Hanbon Science and Technology Co. Ltd. (Jiangsu, China). Sodium 1-octanesulfonate (SOS) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). 2,2-Azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein (FL), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble vitamin E analogue) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Measurement of Anthocyanin Content in Bilberry Extract. Anthocyanins in bilberry extract were qualitatively and quantitatively

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analyzed according to the method described by Dugo (*20*) and Baj (*21*). The anthocyanins in bilberry were analyzed by ESI-MS/RP-HPLC at 535 nm. The analysis was performed with a Waters RP-18 column (4.6 mm × 250 mm) with the following mobile phases: (A) water/formic acid (90:10, v/v) and (B) methanol/acetonitrile/water/formic acid (22.5:22.5:40:10, v/v/v/v). The solvent gradient was held at 9% B in the initial 45 min and then increased from 9 to 35% B in the following 45 min. ESI-MS was in positive ion mode.

Animals and Treatments. Seven-week-old male BALB/C mice were purchased from Guangdong Medical Laboratory Animal Center, Guangzhou, China. All mice were kept in a specific pathogen-free animal room under controlled condition. A 12 h light−dark cycle was maintained, with lights on from 6:00 a.m. to 6:00 p.m.; temperature was 23 ± 1 °C. The mice were provided with standard laboratory diet and tap water and allowed to acclimatize to the environment for 1 week before the experiment. The mice were randomly divided into five groups with 18 animals in each group. Experimental groups received oral administration of bilberry extract dissolved in drinking water at final concentrations of 5, 10, and 20 mg/mL, respectively, whereas the control group and LPS group received drinking water only. The intake of bilberry extract solution was 0.1 mL/10 g of weight for 5 days. On the fifth day, EIU in the experimental groups was induced via injection of 100 mg of LPS diluted in 0.1 mL of saline into the footpad immediately after the administration of bilberry extract, whereas EIU in the LPS group was induced in the same way after administration of drinking water. All mice were killed 24 h after the injection of LPS in a 100% ether atmosphere, and the eyes were collected immediately. The care and treatment of the animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (NIH Publication 85-23, revised 1985), and the experiment was in accordance with animal ethics standards.

Collection of Eyes and Measurement of Protein Level in Eyes. Eyes were enucleated and collected in test tubes and were homogenized in chilled saline using an Ultra-Turrax T8 homogenizer (Ika Co., Germany) and centrifuged at 12000 rpm for 15 min at 4 °C by refrigerated centrifuge (Sigma Co., Germany). A 1% eye homogenate was used to determine the protein concentration using a Coomassie brilliant blue kit.

Measurement of Nitric Oxide (NO) Level in Eyes. NO level of 40% eye homogenate was determined according to the Griess method (*22*). A 40 μL sample was transferred into 96-well microplates, and 160 μL of Griess reagent was added at room temperature. After 20 min, the purple-azo-dye product was detected at 540 nm with an MK3 microplate reader (Labsystems Co., Finland).

Measurement of MDA Level in Eyes. MDA level was measured with 40% eye homogenate by a commercial MDA kit. In acidic medium, MDA reacted with thiobarbituric acid (TBA) upon boiling, and the resultant MDA−TBA adducts were pink in color and measured by an MK3 microplate reader (Labsystems Co.) at 532 nm.

Measurement of ORAC Level in Eyes. Four percent eye homogenate was deproteinized by adding 3% perchloric acid (PCA) (1:1) and centrifuging at 12000 rpm for 15 min at 4 °C. The supernatant was assayed. Automated ORAC assay was carried out on a Labsystems Fluoroskan Ascent plate reader (Labsystems Co.) with fluorescent filters (Infinite F200, excitation wavelength, 485 nm; emission wavelength, 527 nm) as previously described (*23*). AAPH was used as a radical generator, and the reaction was initiated with fluorescein; Trolox was used as a standard. Final results were calculated on the basis of the difference in the area under the fluorescein decay curve between the AAPH control and each sample.

Measurement of GSH Level in Eyes. The measurement of the GSH level was performed according to the method described by Haramaki (*24*). The GSH level in eyes was determined by high-performance liquid chromatography (HPLC): Cosmosil 5C18 column (4.6 mm × 150 mm); mobile phase, 99% phosphate buffer (pH 2.5)−1% methanol containing 100 mg/L SOS and 5 mg/L EDTA; flow rate, 0.5 mL/min; electrochemical detection system, ECD-100 (Eicom Co., Japan) operated at room temperature. Forty percent eye homogenate was deproteinized by adding 3% PCA (1:1) and then centrifuged at 12000 rpm for 15 min at 4 °C. The supernatant was injected after being filtered through a 0.45 μm filter disk.

Measurement of Vitamin C Level in Eyes. The measurement of the vitamin C level was performed according to the method described by Wu (*25*). The following chromatographic conditions were applied: Cosmosil RP-C18 column (4.6 mm × 150 mm) at room temperature, 99 mM potassium phosphate buffer (pH 3.0) consisting of 1% methanol at a flow rate of 1 mL/min, and UV detection at 245 nm. Forty percent eye homogenate was deproteinized by adding 3% PCA (1:1) and then centrifuged at 12000 rpm for 15 min at 4 °C. The supernatant was injected after being filtered through a 0.45 μm filter disk.

Measurement of Total SOD Activity in Eyes. Total SOD activity was measured with 40% eye homogenate by a commercial SOD kit. SOD in samples can inhibit $O_2^{\bullet-}$ and reduce the level of nitrite. When it reacted with color-developing reagent, nitrite was purple-red in color and measured by an MK3 microplate reader (Labsystems Co.) at 550 nm.

Measurement of GPx Activity in Eyes. GPx activity was measured with 40% eye homogenate by a commercial GPx kit. The activity of GPx was calculated by determining the optical density of the enzyme tube and the nonenzyme tube measured by an MK3 microplate reader (Labsystems Co.) at 412 nm after GSH had reacted with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB).

Measurement of Expression of CuZnSOD, MnSOD, and GPx mRNA Levels in Eyes. Antioxidant enzyme gene expression was semiquantitatively assessed utilizing reverse transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from samples of eyes by using Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). A 3 μg amount of total RNA was reverse-transcribed into cDNA at 42 °C for 1 h in 20 μL of reaction mixture containing mouse moloney leukemia virus reverse transcriptase (Tiangen, Beijing, China) with oligo (dT)15 primer (Tiangen) followed by PCR amplification. PCR was carried out with 1 μL of cDNA, 2.5 μL of 10× Taq reaction buffer (Tiangen), 2 μL of dNTP mixture,1 μM forward primer, 1 μM reverse primer, and 1 μL of Taq polymerase (Tiangen) in a total volume of 25 μL. The cDNA was amplified using specific primers with 30 cycles at 94 °C for 30 s, an annealing temperature of 58 °C for 40 s, and then 72 °C for 50 s, with a final incubation at 72 °C for 7 min. The PCR primers for mouse CuZnSOD (GenBank accession no. NM_011434) mRNA were forward, 5′-ATGGCGATGAAAGCGGTGTG-3′, and reverse, 5′-TTACTGCGCAATCCCAATCAC-3′, and the product size was 456 bp. The PCR primers for mouse MnSOD (GenBank accession no. NM_013671) mRNA were forward, 5′-AAGCACAGCCTCCCAGACCT-3′, and reverse, 5′-TCACTTCTTGCAAGCTGTGTATCTT-3′, and the product size was 597 bp. The PCR primers for mouse GPx (GenBank accession no. NM_008160) mRNA were forward, 5′-GAAGTGCGAAGTGAATGG-3′, and reverse, 5′-TGGGACAGCAGGGTTT-3′, and the product size was 255 bp. The primers for the mouse housekeeping gene β-actin (GenBank accession no. NM_007393) mRNA were forward, 5′-GAGGGAAATCGTGCGTGAC-3′, and reverse, 5′-GCTGGAAGGTGGACAGTGAG-3′, and the product size was 446 bp. The PCR products were fractionated on a 1% agarose gel and visualized by ethidium bromide staining. The band intensity of ethidium bromide fluorescence was measured by using an image analysis system (Bio-Rad, Hercules, CA), then quantified with Quantity One analysis software (Bio-Rad), and expressed as the ratios to β-actin.

Statistical Analysis. The data were presented as mean ± SE. Statistical analysis of the data was performed using the SPSS 13.0 statistical package. One-way analysis of variance (ANOVA) was applied to analyze differences in data of biochemical parameters among the different groups, followed by Dunnett's significant post hoc test for pairwise multiple comparisons.

RESULTS

Concentrations of Anthocyanins in Bilberry Extract. Structure and concentrations of the 15 anthocyanins in bilberry extract are shown in **Figure 1** and **Table 1**, respectively (*16*).

Effects of Bilberry Extract on NO and MDA Levels in Eyes. Observations of retinal edema and hemorrhage confirmed uveitis in the LPS group compared with the control group (**Figure 2**). **Table 2** shows that the NO level markedly increased in the LPS group (38.60 ± 2.53 μmol/mL) compared with the control group (25.53 ± 2.18 μmol/mL) ($P < 0.001$). However, the elevated NO level was obviously decreased by the daily treatment of bilberry extract (50, 100, and 200 mg/kg/day, for 5 days).

MDA level in eyes of the LPS group was significantly higher than that of the control group (from 1.18 ± 0.02 to 0.97 ± 0.09 nmol/mg of protein) ($P < 0.001$). Bilberry extract (50, 100, and 200 mg/kg/day, for 5 days) remarkably reduced the level of MDA in eyes compared with the LPS group (**Table 2**).

Effects of Bilberry Extract on ORAC Level in Eyes. ORAC level in the LPS group decreased to 80% of control group. Bilberry extract (100 mg/kg/day, for 5 days) elevated the ORAC level to 721.04 ± 38.41 U/mL ($P < 0.05$), and bilberry extract (200 mg/kg/day, for 5 days) elevated the ORAC level to 758.96 ± 31.66 U/mL ($P < 0.01$) (**Table 3**).

Effects of Bilberry Extract on GSH and Vitamin C Levels in Eyes. GSH level in eyes of the LPS group significantly decreased to 133.76 ± 10.12 μg/g of tissue compared with that of the control group, 156.92 ± 12.45 μg/g of tissue ($P < 0.01$). Bilberry extract (50, 100, and 200 mg/kg/day, for 5 days) significantly increased the GSH level in eyes (**Table 4**).

EIU also lowered the vitamin C level in eyes (256.62 ± 21.71 μg/g of tissue) compared with the control group (309.43 ± 26.57 μg/g of tissue) ($P < 0.01$). By contrast, bilberry extract (100 and

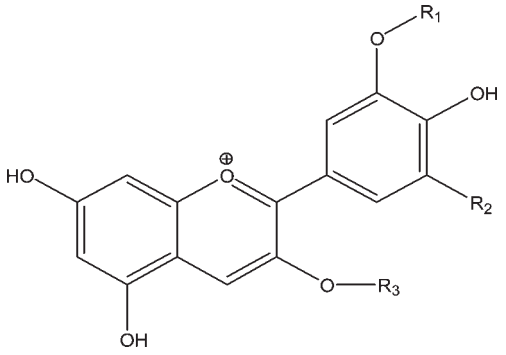

Figure 1. Structure of the 15 anthocyanins in bilberry extract.

Table 1. Concentrations of 15 Anthocyanins in Bilberry Extract

ingredient	positive MS	concentration (mg/g)
delphinidin-3-*O*-gal	303, 465	49.07
delphinidin-3-*O*-glu	303, 465	54.21
cyanidin-3-*O*-gal	287, 449	40.03
delphinidin-3-*O*-ara	303, 435	49.06
cyanidin-3-*O*-glu	287, 449	41.81
cyanidin-3-*O*-ara	287, 419	32.18
petunidin-3-*O*-gal	317, 479	17.92
petunidin-3-*O*-glu	317, 479	38.49
peonidin-3-*O*-gal	301, 463	4.34
petunidin-3-*O*-ara	317, 449	13.44
peonidin-3-*O*-glu	301, 463	15.90
malvidin-3-*O*-gal	331, 493	13.23
peonidin-3-*O*-ara	ND	1.75
malvidin-3-*O*-glu	331, 493	40.15
malvidin-3-*O*-ara	331, 463	8.75
total anthocyanins		420.33

Table 2. Effects of Bilberry Extract (BE) on NO and MDA Levels in Eyes of Mice Treated with LPS[a]

treatment	NO (μmol/mL)	MDA (nmol/mg of protein)
control	25.53 ± 2.18	0.97 ± 0.09
LPS	38.60 ± 2.53 c	1.18 ± 0.02 c
LPS + BE (50 mg/kg/day)	34.35 ± 2.61 d	1.14 ± 0.03 d
LPS + BE (100 mg/kg/day)	33.49 ± 3.09 d	1.09 ± 0.05 e
LPS + BE (200 mg/kg/day)	32.18 ± 2.19 f	1.03 ± 0.06 f

[a] Seven-week-old male BALB/C mice were injected with LPS in the footpad before measurement of biomakers in eyes. The results are presented as mean ± SE obtained from 18 animals in each group. c, significantly different from control group at $P < 0.001$. d, significantly different from LPS group at $P < 0.05$. e, significantly different from LPS group at $P < 0.01$. f, significantly different from LPS group at $P < 0.001$ (one-way ANOVA followed by Dunnett's test).

Table 3. Effects of Bilberry Extract (BE) on ORAC Level in Eyes of Mice Treated with LPS[a]

treatment	ORAC (U/mL)
control	800.46 ± 47.93
LPS	638.98 ± 62.00 c
LPS + BE (50 mg/kg/day)	668.53 ± 45.75
LPS + BE (100 mg/kg/day)	721.04 ± 38.41 d
LPS + BE (200 mg/kg/day)	758.96 ± 31.66 e

[a] Seven-week-old male BALB/C mice were injected with LPS in the footpad before measurement of biomakers in eyes. The results are presented as mean ± SE obtained from 18 animals in each group. c, significantly different from control group at $P < 0.001$. d, significantly different from LPS group at $P < 0.05$. e, significantly different from LPS group at $P < 0.01$ (one-way ANOVA followed by Dunnett's test).

Table 4. Effects of Bilberry Extract (BE) on GSH and Vitamin C Levels in Eyes of Mice Treated with LPS[a]

treatment	GSH (μg/g of tissue)	vitamin C (μg/g of tissue)
control	156.92 ± 12.45	309.43 ± 26.57
LPS	133.76 ± 10.12 b	256.62 ± 21.71 b
LPS + BE (50 mg/kg/day)	145.27 ± 7.29 d	263.88 ± 19.44
LPS + BE (100 mg/kg/day)	152.01 ± 8.21 e	283.72 ± 13.45 d
LPS + BE (200 mg/kg/day)	154.27 ± 7.16 e	290.25 ± 15.26 d

[a] Seven-week-old male BALB/C mice were injected with LPS in the footpad before measurement of biomakers in eyes. The results are presented as mean ± SE obtained from 18 animals in each group. b, significantly different from control group at $P < 0.01$. d, significantly different from LPS group at $P < 0.05$. e, significantly different from LPS group at $P < 0.01$ (one-way ANOVA followed by Dunnett's test).

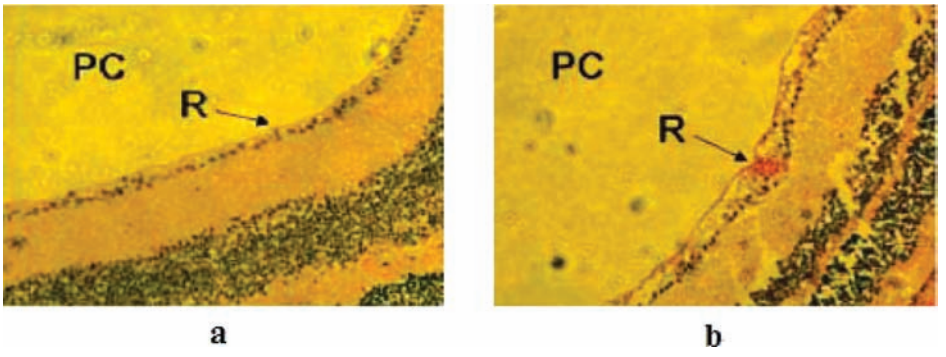

Figure 2. Photomicrographs of hematoxylin−eosin-stained sections of eyes from (**a**) control group and (**b**) LPS group 24 h after the LPS injection. Represented are views of the posterior chamber (PC) and retina (R). Magnification, ×400.

Table 5. Effects of Bilberry Extract (BE) on Total SOD and GPx Activities in Eyes of Mice Treated with LPS[a]

treatment	SOD (U/mg of protein)	GPx (U/mg of protein)
control	0.28 ± 0.08	49.03 ± 3.42
LPS	0.13 ± 0.05 b	43.72 ± 2.24 b
LPS + BE (50 mg/kg/day)	0.22 ± 0.05 e	46.81 ± 1.61 d
LPS + BE (100 mg/kg/day)	0.25 ± 0.04 e	47.02 ± 2.85 d
LPS + BE (200 mg/kg/day)	0.27 ± 0.06 e	48.49 ± 2.47 e

[a] Seven-week-old male BALB/C mice were injected with LPS in the footpad before measurement of biomakers in eyes. The results are presented as mean ± SE obtained from 18 animals in each group. b, significantly different from control group at $P < 0.01$. d, significantly different from LPS group at $P < 0.05$. e, significantly different from LPS group at $P < 0.01$ (one-way ANOVA followed by Dunnett's test).

200 mg/kg/day, for 5 days) significantly increased the vitamin C levels to levels similar to the control group (283.72 ± 13.45 and 290.25 ± 15.26 vs 309.43 ± 26.57 μg/g of tissue, respectively) (**Table 4**).

Effects of Bilberry Extract on Total SOD and GPx Activities in Eyes. Total SOD activity significantly decreased in eyes of the LPS group compared with the control group (0.13 ± 0.05 vs 0.28 ± 0.08 U/mg of protein) ($P < 0.01$). Bilberry extract (50, 100, and 200 mg/kg/day, for 5 days) increased the total SOD activity in the LPS-treated group ($P < 0.01$). Especially, bilberry extract (200 mg/kg/day, for 5 days) increased total SOD activity to 0.27 ± 0.06 U/mg of protein, which is almost recovered to the level of the control group (**Table 5**).

The GPx activity in eyes of the control group was 49.03 ± 3.42 U/mg of protein, whereas the GPx activity of the LPS group was 43.72 ± 2.24 U/mg of protein ($P < 0.01$). Compared with the LPS group, bilberry extract (50, 100, and 200 mg/kg/day, for 5 days) elevated the GPx activity to 46.81 ± 1.61, 47.02 ± 2.85, and 48.49 ± 2.47 U/mg of protein, respectively (**Table 5**).

Effects of Bilberry Extract on Expression of CuZnSOD, MnSOD, and GPx mRNA Levels in Eyes. The expression of CuZnSOD, MnSOD, and GPx mRNA levels in the LPS group all decreased compared with the control group. Bilberry extract (100 and 200 mg/kg/day, for 5 days) obviously enhanced the mRNA levels of CuZnSOD, MnSOD, and GPx (**Figure 3**).

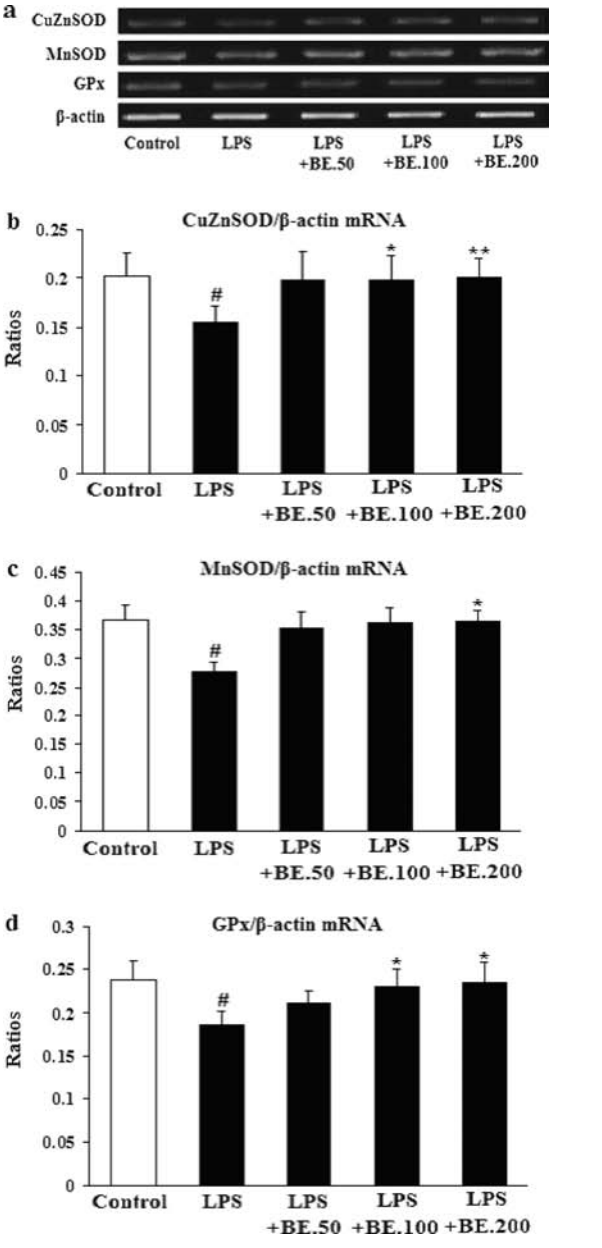

Figure 3. Effects of bilberry extract on expression of CuZnSOD, MnSOD, and GPx mRNA levels in eyes of mice treated with LPS: (**a**) agarose gel electrophoresis of RT-PCR amplication of CuZnSOD mRNA, MnSOD mRNA, GPx mRNA, and β-actin mRNA in the eyes of five groups; (**b**) ratios of CuZnSOD/β-actin mRNA expression; (**c**) ratios of MnSOD/β-actin mRNA expression; (**d**) ratios of GPx/β-actin mRNA expression in five groups. #, significantly different from the control group at $P < 0.05$. ∗, significantly different from the LPS group at $P < 0.05$. ∗∗, significantly different from LPS group at $P < 0.01$ (one-way ANOVA followed by Dunnett's test).

DISCUSSION

NO is a highly reactive free radical. Excess NO reacts with oxygen free radicals and produces the cytotoxic radical $ONOO^-$, which can damage cellular functions. Our results showed that NO was produced in large amounts in eyes of rats with EIU, which was consistent with previous reports (*26*) indicating that NO is an important parameter associated with EIU regardless of animal species. MDA, the final metabolite of lipid peroxidation, which is utilized as an available parameter of oxidative stress, not only translates reactive oxygen species (ROS) into active chemicals but magnifies the function of ROS through chain reaction, inducing cellular metabolism and functional impairment (*27*). Enhanced lipid peroxidation of eyes was also observed in the LPS group, reflected by the increased level of MDA, which was in good agreement with the previous results (*9*, *28*). Moreover, bilberry extract significantly inhibited the production of NO and the increase in MDA level compared with the LPS group, suggesting that it may attenuate EIU via an antioxidative pathway.

The ORAC level reflects the antioxidative capacity of water-soluble low molecular antioxidants such as GSH and vitamin C (*29*, *30*). The LPS group showed an approximately 20% decrease of ORAC level compared with the control group. We also observed that bilberry extract (100 and 200 mg/kg/day, for 5 days) significantly ameliorated the decreased ORAC level compared with the LPS group. GSH is a very important antioxidant in tissues. It is beneficial for cells in eyes to increase its GSH level, and thus they are able to quench ROS. The existence of oxidative challenge has been found in eyes of EIU rats in view of a significant decrease in GSH level (*9*). Eyes of mice generally have high levels of vitamin C. However, we found the vitamin C level in eyes of mice was obviously reduced by EIU. Our results demonstrated that bilberry administration could inhibit the reduced levels of GSH and vitamin C in the eyes treated with LPS. These results indicated that bilberry extract could attenuate inflammation-induced oxidative stress in EIU by increasing levels of antioxidants.

Furthermore, two important antioxidant enzymes, SOD and GPx, are major scavengers of ROS in the body. There are two major forms of SOD, including cytoplasmic SOD, which is a copper/zinc-containing enzyme (CuZnSOD), and mitochondrial manganese superoxide dismutase (MnSOD). SOD can catalyze the dismutation of superoxide anions to oxygen and hydrogen peroxide that can be cleared by GPx by converting it into water. In this study, we reported for the first time that the total SOD activity in the whole eye of the LPS group significantly decreased. As for GPx, we observed a decreased activity in the LPS group, which was in line with the results of Bosch-Morell (*9*), and thus it could be further supported by its susceptibility to oxidative modifications (*31*) acting as a sensory molecule for the detection of oxidative stress (*32*). In the LPS group, we found that both the mRNA expression and the activities of two antioxidant enzymes decreased, which indicates that activities of enzymes are controlled at the level of their genes. Thus, the decreased total SOD activity may result from the changes of CuZnSOD and MnSOD mRNA expression. Similarly, decreased expression of GPx mRNA may lead to decreased GPx activity. Contrary to Pittman (*33*), who observed an elevated mRNA expression of MnSOD in the ciliary body of rats with EIU, we observed the decreased mRNA expression in the whole eye of mice with EIU. Oxidative stress has been reported to cause changes in the balance between oxidation and reduction in a cell that affects the translocation of redox-sensitive transcription factors into the nucleus (*34*). It has been also suggested that these changes in mRNA expression of antioxidant enzymes in EIU may be due to the effect of some transcriptional factors responsible for the initiation of transcription process of antioxidant enzymes, which would be further investigated in our next study. For instance, nuclear factor-κB (NF-κB), a redox-sensitive transcriptional factor, has been found to play an important role in the activation of antioxidant enzymes in uveitis (*35*). In addition, the mRNA expression and the activities of total SOD and GPx were all increased by bilberry administration compared with the LPS group, suggesting that bilberry extract can improve the activities of antioxidant enzymes from gene level in EIU.

Bilberry extract is well-known as an antioxidant; however, less attention has been given to its effects on oxidative stress in EIU. In the present study, our results demonstrated that LPS elevated the level of NO and the content of MDA and reduced the levels of ORAC, GSH, and vitamin C, as well as decreased activities of total SOD and GPx in eyes of mice. Additionally, mainly due to the effects of anthocyanins on antioxidative stress, bilberry extract (containing 42.04% anthocyanins) bears remarkable activities for protecting eyes against EIU reflected by the reduced levels of NO and MDA and the elevated levels of ORAC, GSH, and vitamin C, as well as the increased activities of SOD and GPx. Furthermore, the protective effects of bilberry extract (100 and 200 mg/kg/day, 5 days) were dose-dependent. Our results further demonstrated that bilberry extract could reduce oxidative stress in EIU by improving the status of antioxidant and antioxidant enzyme in eyes of mice treated with LPS, which indicates the protective effects of bilberry extract on uveitis and its benefits to eye health. These inspiring findings may encourage us to further investigate the effects of other plant extracts with antioxidant activity on EIU and eye health.

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