Regular article

Protective effect of BlingLife®-berry extract on blue light-induced eye damage and its mechanism

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Abstract

This study aims to investigate the protective effects of BlingLife®-berry extract on the eyes. BlingLife®-berry extract is a mixture of high-quality natural berries, including blackcurrant, aromia, bilberry and maquiberry. The main active ingredient responsible for theeye-protective effects is anthocyanins. Anthocyanins are natural water-soluble pigments belonging to the flavonoid class, and theyhave multiple benefits, including improving vision, anti-inflammatory and antibacterial properties, and antioxidant effects. Dueto the dual benefits of anthocyanins in protecting vision and eliminating free radicals, this study explores the comprehensive eye protective effects of BlingLife®-berry extract in terms of combating blue light-induced eye damage and oxidative stress-inducedvisual fatigue. The results provide robust evidence for the *in vivo* effects and further technological applications of BlingLife®-berry extract.

Keywords: BlingLife®-berry extract combination; anthocyanins; antioxidant; protecting vision; eliminating radicals

1 Introduction

Natural products with the function of relieving visual fatigue can be divided into 5 categories, namely carotenoids, polyunsaturated fatty acids, vitamins, minerals and secondary metabolites. Carotenoids are naturally occurring pigments found in most fruits, vegetables, plants, algae, and photosynthetic bacteria. Omega-3 polyunsaturated fatty acids are not only the basic structure of cell membranes, but also the precursors of the synthesis of a variety of bioactive substances, with anti-inflammatory, antioxidant, anti-proliferative and anti-angiogenic effects. These polyunsaturated fatty acids mainly include EPA, DHA, ALA and DPA [1]. Deficiency of omega-3 polyunsaturated fatty acids predisposes to a variety of eye diseases, such as dry eye, retinitis pigmentosa, glaucoma, etc., which can be alleviated and prevented after supplementation [2,3]. Vitamin A is the main vitamin commonly used to relieve eye strain [2]. Vitamin A, also known as

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retinol, is involved in visual phototransduction and is a key part of photopigments, the first molecules to convert photons into electrical signals. The deficiency of vitamin A in the retina will affect the impaired function of rod cells, leading to night blindness and it can affect cone cell dysfunction and impaired vision during the day, inducing eye fatigue. Therefore, vitamin A is an essential micronutrient for the maintenance of normal visual function. Some secondary metabolites also play a key role in alleviating eye fatigue, such as bilberry, blueberry and grape seed extract. These berry extracts are rich in anthocyanins, proanthocyanidins, anthocyanins, and polyphenols which are also effective in alleviating eye fatigue [2].

Riva et al. have reported that the bilberry extract is a natural and bioavailable delivery form of anthocyanins, which has strong antioxidant potential and can alleviate dry eye symptoms [4]. The dietary supplement, a combination of two phenolic extracts from bilberry, was shown to lower elevated intraocular pressure [5]. In a randomized, double-blind, placebo-controlled clinical trial, the protective effect of blackcurrant extract on adults with eye fatigue was evaluated [6].

Eye fatigue, also known as visual fatigue, refers to a group of symptoms that occur when the eyes are subjected to visual tasks beyond their functional capacity [7]. This may lead to visual impairments, eye discomfort, and even systemic symptoms that hinder normal visual work. Subjective symptoms of eye fatigue usually include decreased vision, pain, soreness, dryness, and light sensitivity. It can also lead to systemic symptoms such as fatigue, dizziness, headaches, and decreased memory.

Electronic devices have become ubiquitous, especially in recent years with the advent of online classes and remote learning, making smartphones, tablets, and other devices indispensable tools for students and even children. These electronic screens emit blue light, increasing everyone's exposure to blue light. Blue light has a short wavelength and high energy, which can penetrate the eye's lens and directly reach the retina. This will lead to atrophy or even death of the retinal pigment epithelial cells, resulting in vision loss, myopia progression, or even complete blindness.

Blue light not only directly damages the photoreceptor cells and accelerates the oxidation of ester compounds on cell membranes, but also inhibits the body's antioxidant capacity and reduce the efficiency of scavenging free radicals. Therefore, both cellular damage and oxidative stress should be considered in evaluating the eye-protective effects on blue light [8].

Nowadays, children are increasingly using electronis, and their eye health problems are becoming more serious, especially the damage caused by blue light to developing eyes. Considering the effectiveness of eye health care and the acceptance of nutritional supplements by the population, in this study, we investigated the eye protection effects of BlingLife®-berry extract, aiming to explore the protective effect of these natural ingredients on blue light damage and the possibility of further developing them into dietary supplements acceptable to more children.

2 Materials and methods

2.1 Extraction of BlingLife®

BlingLife®-berry extract is purchased from Mom-Garden GmbH, Germany. BlingLife®-berry extract combination is a mixture of high-quality natural berries and the anthocyanin content is more than 30% by HPLC.

2.2 Construction of the blue light-induced eye damage model in mice

After one week of adaptive feeding, mice were randomly divided into different groups (*n* = 6), namely normal control group, light irradiation control group, solvent control group, anthocyanin control group, low-dose BlingLife®-berry extract group (10 mg/kg), and high-dose BlingLife® extract group (50 mg/kg). The BlingLife®-berry extract group received the corresponding concentration of BlingLife®-berry extract (positive control), the solvent control group received an equal volume of sunflower seed oil instead of treatment, and the mice were orally gavaged for 45 d. Then, except the normal control group, the mice were placed in a dark room for 24 h for dark adaptation. Then, the mice in the other groups were exposed to blue light using a light damage device (LED blue light strip with a wavelength of 452 nm, light intensity of 1400-1500 lux, and light exposure time of 1.5 h) [9]. After light exposure, the mice were returned to the dark room for continued feeding. After 24 h, the mice were euthanized, and their eyeballs were collected for subsequent tests.

2.3 Retinal structure and cellular morphological changes

After the extraction of mouse eyeballs, paraffin sections were prepared and stained with hematoxylin and eosin (HE) for observation. The retinal structure and cellular morphology of each group of mice were compared. The thickness of the outer nuclear layer (ONL) of the retina after HE staining was measured under a 40×10 optical microscope. The measurement was made at the deepest point of retinal damage [10,11].

2.4 Measurement of BlingLife® antioxidant indices

Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were selected as representative oxidative stress enzymes related to blue light-induced damage and the rescue of visual fatigue [12-14]. The contents of MDA, SOD, and GSH-Px in mouse serum were measured according to the instructions of the respective kits to evaluate the preventive and protective effects of BlingLife®-berry extract on oxidative stress damage.

2.5 Mechanism study of BlingLife®-berry extract antioxidant activity

The expression levels of key marker proteins, such as HO-1, NQO-1, p62, and LC3, in the retinal tissues were monitored [15]. These proteins are involved in key signaling pathways related to retinal physiological functions, such as oxidative stress and autophagy. The study aimed to reveal how BlingLife®-berry extract participates in and achieves its antioxidant effects at the protein level.

2.6 Statistics and reproducibility

Error bars in all the plots indicate mean ± S.D. *P* value < 0.05 was considered statistically significant. $^{**}p$ value < 0.001 and $^{*}p$ value < 0.05 were determined by two tailed Student's *t* tests. All experiments were conducted at least three times unless otherwise indicated. Statistics was calculated with GraphPad Prism 8.

3 Results and discussion

3.1 BlingLife®-berry extract combination analysis

High-quality natural berries such as blackcurrant, wild cherry and cranberry were mixed according to a specific optimized process to make BlingLife®-berry extract combination. The anthocyanin content is more than 30% by HPLC. The information of chromatographic system was shown in Table 1 and mobile phase was shown in Table 2. Anthocyanosides in the mixture included more than 30% of delphinidin-3-*O*-rutinoside and six other analytes, namely delphinidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside and cyanidin-3-*O*-rutinoside (Fig. 1). Therefore, according to the source of BlingLife®-berry extract combination and the analysis results, the extract has certain antioxidant damage function.

Table 1 Chromatographic system

Column	ZORBAX Eclipse Plus C_{18} 4.6 mm × 250 mm, 5 μm
Column temperature	30 ℃
Detector	uv-vis 535 nm
Flow rate	0.8 mL/min
Injection size	10 μL
Solution A	Formic acid and water (8.5:91.5)
Solution B	Acetonitrile, methanol, Formic acid and water (22.5:22.5:8.5:41.5)

Table 2 Mobile phase

Time/min	Solution A/%	Solution B/%
0	93	7
40	75	25
60	35	65
61	0	100
66	0	100
67	93	7
75	93	7

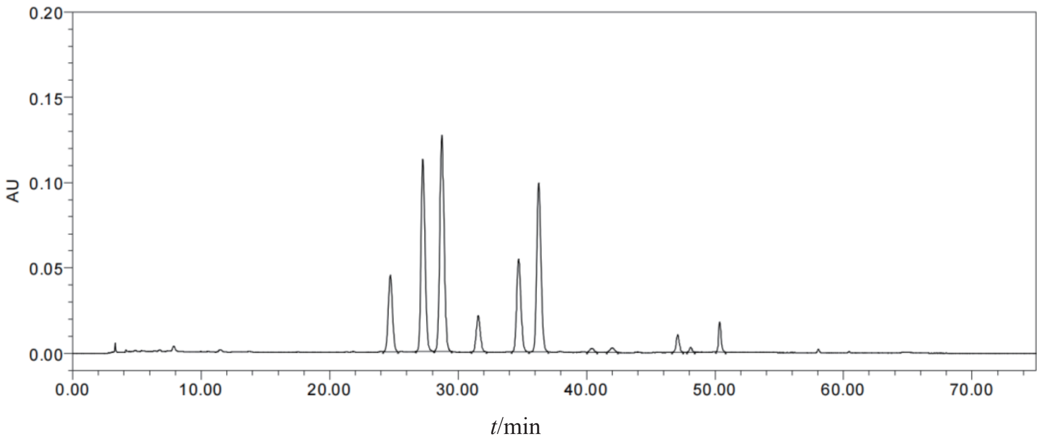

Fig. 1 BlingLife®-berry extract combination analysis

3.2 Changes in retinal structure and cellular morphology

The retina is the innermost layer of the eye responsible for receiving and transmitting light signals to the brain. In this study, we observed the changes in retinal structure and cellular morphology in each group of mice using HE staining. In the normal control group, the retina exhibited a normal structure and orderly cellular morphology. Different layers of the retina were clearly visible, and the cells were arranged in an organized manner. In the light irradiation control group, significant retinal damage was observed due to light exposure. The retinal structure was disrupted, and the cellular layers became blurred and disorganized. In the solvent control group, there were no significant changes in retinal structure and cellular morphology compared with the normal control group, indicating that the solvent did not affect the retina. In the BlingLife®-berry extract group, a significant protective effect was observed. The retinal structure was similar to that of the normal control group, and the cellular morphology was well-organized, indicating that the BlingLife®-berry extract could alleviate retinal damage caused by light exposure (Fig. 2).

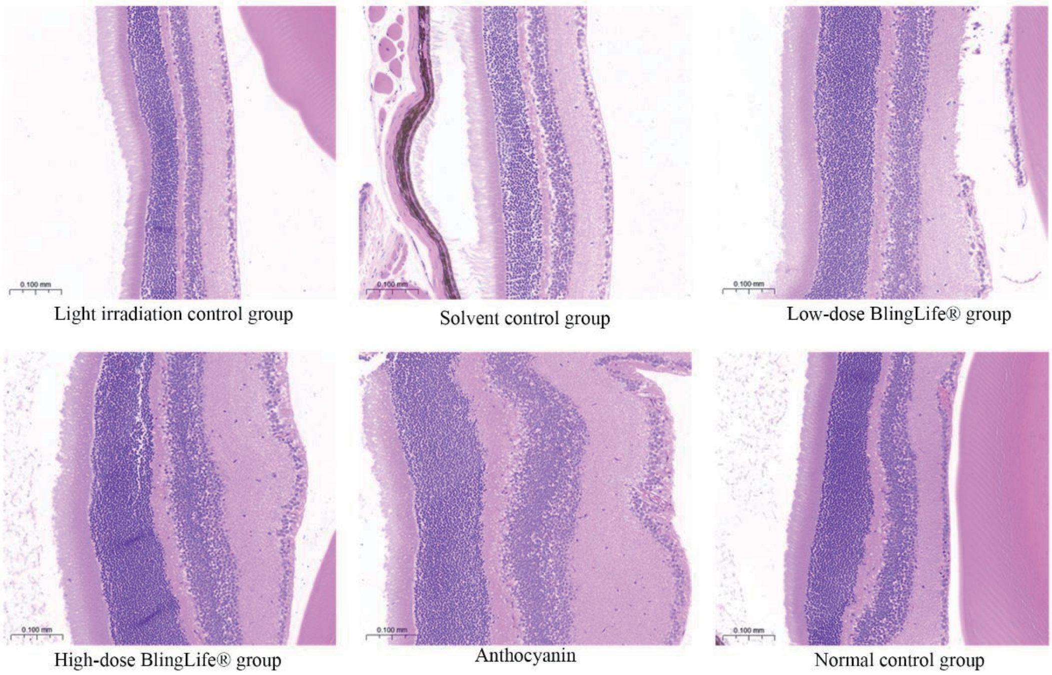

Fig. 2 HE staining of the 6 groups of treated mice ($n = 6$)

By measuring the thickness of the outer nuclear layer (ONL) of the retina after HE staining, we quantitatively evaluated the extent of retinal damage. In the light irradiation control group, the thickness of the ONL was significantly reduced, while in the BlingLife®-extract group, the thickness of the ONL was similar to that of the normal control group (Fig. 3). Data are represented as mean ± SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

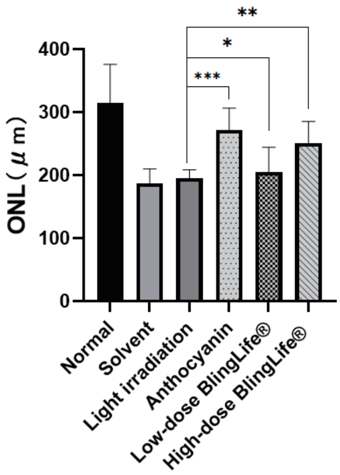

Fig. 3 Thickness of the outer nuclear layer (ONL) in micrometers (n = 6)

In conclusion, the BlingLife®-berry extract can protect the retina from damage caused by light exposure and maintain the normal structure and cellular morphology of the retina. This provides strong evidence for further research and development of BlingLife®-berry extract.

3.3 Content and activity of key enzymes involved in oxidative stress in mouse ocular tissue homogenate

MDA, also known as malondialdehyde, is a metabolic byproduct found in the body. Under oxidative stress conditions, such as blue light stimulation, lipids within cells will be oxidized, leading to the production of high-level MDA and the increase of cellular oxidative stress. Therefore, MDA is used as an indicator of oxidative stress by measuring its content within cells. The higher the level of MDA, the stronger the oxidative stress on the tissue cells. As shown in Table 1, the MDA content in the mouse ocular tissue of the blue light control group and solvent control group significantly increased under blue light stimulation, reaching approximately three times that of the blank control group. However, the MDA content in the positive control group, while slightly higher than the blank control, demonstrated a protective effect on cellular damage caused by blue light oxidative stress. The table shows the obvious dose-response relationship in the groups treated with different doses of BlingLife®-berry extract. Although their MDA content was still higher than the positive control, significant differences were observed compared with the blue light control group and solvent control group. Therefore, it can be concluded that oral administration of higher dose of BlingLife®-berry extract can reduce the damage to eye cells caused by factors such as blue light.

SOD, also known as superoxide dismutase, is an enzyme with strong antioxidant activity in the body. It can inhibit the synthesis and release of inflammatory factors such as histamine, 5-hydroxytryptamine, prostaglandins, and leukotrienes, and has beneficial effects of anti-aging, anti-inflammatory, anti-radiation, and anti-fatigue. The higher the SOD level, the stronger the anti-fatigue ability. As shown in Table 1, the SOD content in the mouse ocular tissue of the blue light control group and solvent control group significantly decreased under blue light stimulation, while the SOD content in the positive control group was almost equal to or even slightly higher than that of the blank control group. This indicates that the cells' own antioxidant capacity is greatly affected by blue light irradiation. The table shows the dose-response relationship in the groups treated with different doses of BlingLife®-berry extract. Although the SOD content in each group was still lower than that in the positive control and blank control groups, significant differences were observed compared with the blue light control group and solvent control group. Therefore, it can be concluded that oral administration of higher dose of BlingLife®-berry extract can enhance the anti-fatigue ability of eye cells.

GSH-Px, also known as glutathione peroxidase, is one of the main enzymes in the glutathione redox cycle that catalyze the oxidation of reduced glutathione (GSH). GSH-Px can not only specifically catalyze the reaction of reduced glutathione with reactive oxygen species (ROS) to produce oxidized glutathione (GSSG), protecting biological membranes from ROS damage and maintaining normal cellular function, but also protect the liver, enhance immune function, antagonize the harmful effects of metal ions on the body, and increase the body's resistance to radiation. The higher the level of GSH-Px, the stronger the radiation resistance on the surface of biological membranes. As shown in Table 1, the GSH-Px content in the mouse ocular tissue of the blue light control group and solvent control group significantly decreased under blue light stimulation, reaching only 30% of the blank control group. However, the GSH-Px content in the positive control group was almost equal to that of the blank control group. This indicates that the activity of GSH-Px, which catalyzes the oxidation of reduced glutathione, is greatly affected by blue light stimulation. The table shows the significant dose-response relationship in the groups treated with different doses of BlingLife®-berry extract. Although the GSH-Px content in each group was still lower than that in the positive control and blank control groups, significant differences were observed compared with the blue light control group and solvent control group. Therefore, it can be concluded that oral administration of higher dose of BlingLife®-berry extract can enhance the ability of eye cells to resist damage.

The measurement results of MDA, SOD, and GSH-Px in the mouse ocular tissue of each group are shown in Table 3.

Table 3 MDA, SOD, and GSH-Px content measurement results (n = 3)

Group	MDA/(nmol/mgprot)	SOD/(U/mgprot)	GSH-Px/(U/mgprot)
Normal control	0.0360	39.1352	9.9892
Light irradiation	0.1195	25.3274	2.9910
Solvent control	0.1082	25.3513	2.9593
Anthocyanin	0.0368	40.0935	9.5775
Low-dose BlingLife®	0.0803	34.2850	5.1996
High dose BlingLife®	0.0485	38.5961	8.9010

3.4 Mechanism of antioxidant and anti-fatigue effects of BlingLife®-berry extract

The main active components of BlingLife®-berry extract are anthocyanins. Numerous studies have reported that anthocyanins can exert their effects by regulating oxidative stress responses and participating in cellular autophagy [16-18]. Thus, in this study, mice were treated with BlingLife®-berry extract for 45 d and then exposed to blue light-induced eye damage, after which their eyeballs were collected for further analysis. Retina and choroid tissues were separated from the eyeballs of mice in the blank control group, blue light irradiation model group, and high-dose BlingLife®-berry extract [19,20] (Fig. 4). Data are represented as mean ± SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

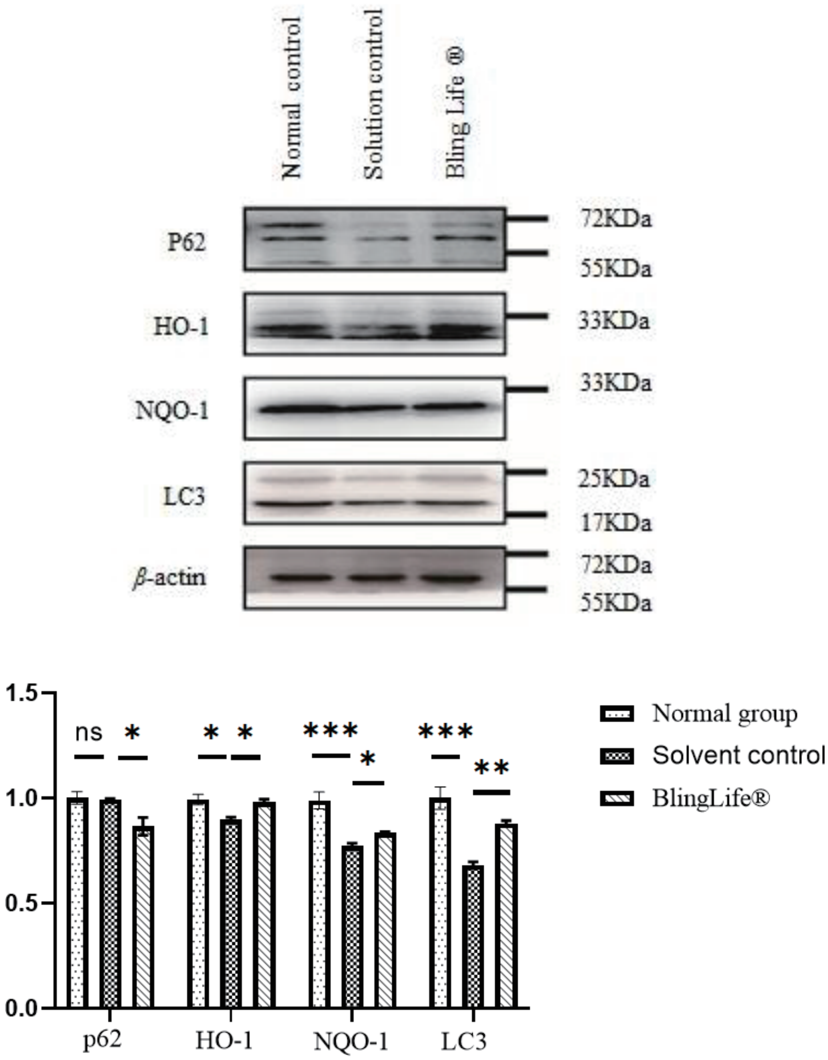

Fig. 4 Changes in the expression levels of HO-1, NQO-1, p62, and LC3 were analyzed using western blot and quantitative analyzation of the western blotting ($n = 3$)

4 Conclusion

In this study, the thickness of outer nuclear layer of retina (ONL) was used to characterize the degree of oxidative stress, antioxidant capacity and self-protection ability of the body. The content of MDA, SOD and GSH-Px in serum of each group of mice after modeling was determined. It was concluded that BlingLife®-berry extract composition had a protective effect on the prevention of ocular oxidative stress injury, providing strong evidence for the effect of BL extract *in vivo* and further technical transformation application. The discovery is the development of a plant extract with anthocyanins as the main effective component, which can protect the eyes. This study provides a theoretical basis for the subsequent development of food nutritional supplements.

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