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Note

Protective Effects of Purple Corn (*Zea mays* L.) Byproduct Extract on Blue Light-Induced Retinal Damage in A2E-Accumulated ARPE-19 Cells

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ABSTRACT: This study investigated the antioxidative characteristics of *Zea mays* L. purple corn cob and husk extract (PCHE) and its potential protective effects against blue light (BL)-induced damage in N-retinylidene-N-retinylethanolamine (A2E)-accumulated ARPE-19 retinal pigment epithelial cells. PCHE had a 2,2-diphenyl-1-picrylhydrazyl radical-scavenging capacity and Trolox equivalent antioxidant capacity of 1.28±0.43 mM Trolox equivalents (TE)/g and 2,545.41±34.13 mM TE/g, respectively. Total content of anthocyanins, polyphenols, and flavonoids in the PCHE was 11.13±0.10 mg cyanidin-3-glucoside equivalents/100 g, 227.90±7.38 mg gallic acid equivalents/g, and 117.75±2.46 mg catechin equivalents/g, respectively. PCHE suppressed the accumulation of A2E and the photooxidation caused by BL in a dose-dependent manner. After initial treatment with 25 μM/mL A2E and BL, ARPE-19 cells showed increased cell viability following additional treatment with 15 μg/mL PCHE while the expression of the p62 sequestosome 1 decreased, whereas that of heme oxygenase-1 protein increased compared with that in cells without PCHE treatment. This suggests that PCHE may slow the autophagy induced by BL exposure in A2E-accumulated retinal cells and protect them against oxidative stress.

Keywords: anthocyanins, antioxidants, A2-E (N-retinylidene-N-retinylethanolamine), blue light, zea mays

INTRODUCTION

Exposure to blue light (BL) causes damage and dysfunction in the retinal pigment epithelium (RPE) of the eye (Peng et al., 2022). The increased use of artificial lighting from devices such as mobile phones and computers has led to higher levels of exposure to BL. The BL spectrum within the visible range (400–500 nm) has high levels of energy and can be transmitted through the lens to the retina to damage the RPE and photoreceptor cells (Hunter et al., 2012). Lipofuscin-derived fluorophore N-retinylidene-N-retinylethanolamine (A2E) accumulates in RPE cells and is a major cause of light-induced retinal damage (Brandstetter et al., 2015; Peng et al., 2022). A2E contributes to the formation of reactive oxygen species (ROS) and triggers apoptosis in BL-exposed RPE cells (Jeong et al., 2019). Light exposure increases oxygen consumption, oxidizes polyunsaturated fatty acids, and induces photoreceptor outer segment phagocytosis, generating high levels of free radicals (Upadhyay et al., 2020). Furthermore, RPE cell dysfunction is linked to ocular diseases such as age-related macular degeneration, retinitis pigmentosa, and diabetic retinopathy (Wang et al., 2023). Therefore, inhibiting A2E accumulation and reducing A2E-induced cell death could play crucial roles in preserving RPE function.

Zea mays L. (corn) is a grain used to meet diverse needs, including human food and animal feed requirements. The agricultural waste from corn, mainly corn cobs, is often left in fields, causing pollution (Dhaneswara et al., 2022), and alternative uses for these byproducts are being explored to enhance their reuse. Anthocyanins, including cyanidin-3-glucoside (C-3-G), peonidin-3-glucoside, and pelargonidin-3-glucoside, have been identified in purple corn husk extracts (PCHE), and the relative abundance of C-3-G can be >40% (Li et al., 2008). C-3-G has been reported to protect A2E-accumulated RPE cells from light-induced damage by inhibiting photooxidation and

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photodegradation (Wang et al., 2017). C-3-G also provides a protective effect against hyperglycemia-induced blood-retinal barrier damage (Li et al., 2024), and suppresses endoplasmic reticulum stress in RPE cells (Peng et al., 2022). Autophagy contributes to cell death and regulates proteins involved in antioxidant defense, such as heme oxygenase-1 (HO-1) in the Nrf2 pathway, which interacts with the p62 sequestosome 1 (p62/SQSTM1) that is essential for autophagy (Kaarniranta et al., 2017). Oxidative stress contributes to many ocular diseases, and dysregulated autophagy may exacerbate the condition by impairing cellular stress defense mechanisms. Therefore, this study investigated the protective effects of purple corn byproducts on BL-induced damage in A2E-accumulated RPE cells via changes in autophagy-related protein expression.

MATERIALS AND METHODS

Purple corn extract

The PCHE used in this study was prepared according to the method patented under KR 10-2016-0081814 and obtained from Gangwondo Agricultural Research and Extension Services (Chuncheon, Korea).

Total anthocyanin content

The total anthocyanin content (TAC) of the PCHE was determined as described by Moazami Goodarzi et al. (2020). PCHE (1 mg) was mixed with potassium chloride (pH 1.0) and sodium acetate (pH 4.5) buffers, and the absorbance was measured at 510 and 700 nm with an EPOCH2 microplate reader (Biotek Instruments). The TAC was calculated using the following equations and expressed in mg of C-3-G equivalents (C3GE) per 100 g of extract.

$$A=(Abs510-Abs700)\ pH\ 1.0 - (Abs510-Abs700)\ pH\ 4.5 \quad (1)$$

$$TAC\ (mg/100\ g) = (A\times MW\times DF\times V/W\times 100)/(\varepsilon\times L) \quad (2)$$

where MW=449.2 g/mol, DF is the dilution factor, V is the final volume, W is the extract weight, ε=26,900 L/mol/cm, and L=1 cm.

Total phenolic content

The total phenolic content (TPC) of the PCHE was measured using the Folin-Ciocalteu method (Gujral et al., 2011; Muhialdin et al., 2020). PCHE (20 μL/mL) was mixed with 10% Folin-Ciocalteu reagent (500 μL) and 20% sodium carbonate (1.5 mL), incubated for 2 h in darkness, and the absorbance was measured at 765 nm using an EPOCH2 microplate reader. The TPC was quantified using gallic acid as a standard and expressed as mg of gallic acid equivalents (GAE) per g of extract.

Total flavonoid content

The total flavonoid content (TFC) of the PCHE was determined as described by Kim et al. (2018). The PCHE (100 μL) was mixed with 1.25 mL of distilled water and 75 μL of 5% sodium nitrate for 6 min. Then, 150 μL of 10% aluminum chloride was added, and the mixture was incubated for a further 5 min before 500 μL of 1 M sodium hydroxide was added. The absorbance was measured at 510 nm using an EPOCH2 microplate reader. Results were calculated using catechin as the standard and expressed as mg of catechin equivalents (CE) per g of extract.

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging capacity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity of PCHE was measured as previously described (Aung et al., 2022). The PCHE (50 μL) was mixed with 0.1 mM DPPH in 100% methanol (1,950 μL). After 30 min of incubation in darkness, the absorbance was measured at 515 nm using an EPOCH2 microplate. Results were expressed as μM of Trolox equivalents (TE) per g of extract.

Trolox equivalent antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC) was determined as previously described (Lee et al., 2022). An $ABTS^+$ stock solution was prepared by combining 7.4 mM 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 2.6 mM potassium persulfate and allowing this to react overnight in darkness. The $ABTS^+$ stock solution was diluted with 100% methanol to an absorbance of 0.7 at 734 nm. Then, the $ABTS^+$ stock solution (2,960 μL) and PCHE (40 μL) were mixed for 7 min, and the absorbance was measured at 734 nm using an EPOCH2 microplate reader (Biotek Instruments).

N-retinylidene-N-retinylethanolamine (A2E) synthesis and purification

A2E was synthesized as described by Furso et al. (2020) and Parish et al. (1998). Briefly, 50 mg of all-trans-retinal, 4.75 μL of ethanolamine, and 4.65 μL of acetic acid were combined in 1.5 mL of 100% ethanol and stirred at 250 rpm for 48 h in darkness. The ethanol was then evaporated, and the residue was dissolved in 10 mL of acetonitrile. A2E was purified by washing several times with hexane and 1 M sodium acetate (1:1), collecting the middle layer each time, and followed by a final wash with distilled water and drying with nitrogen (Guan et al., 2020).

The purity of the A2E was confirmed using Waters e2695 high-performance liquid chromatography (HPLC;

Waters Corp.) with a Capcell Pak C18 column (5 μm, 250×4.6 mm, Osaka Soda Corp.) and a photodiode array (PDA) detector. The mobile phase comprised 0.1% trifluoroacetic acid (TFA) (Solvent A) and acetonitrile with 0.1% TFA (Solvent B). The flow rate was 1 mL/min with a 10 μL injection. The gradient elution conditions started with 85% B, increased to 85%–96% B at 10 min, then to 96% for 5 min, 96%–100% for 17 min, and finally at 100% for 25 min. The PDA detector was set at 340 and 430 nm (Kim et al., 2020).

Cell-free A2E photooxidation assay

A2E (100 μM) was added at 180 μL per well to a 96-well plate and mixed with 20 μL of either PCHE diluted in phosphate-buffered saline (PBS) or PBS only as a control. The plate was then exposed to BL for 20 min, and then the absorbance was measured at 440 nm using an EPOCH2 microplate reader (Wang et al., 2017). The difference in absorbance before and after BL exposure was used to determine the A2E oxidation, with an external A2E standard for reference.

Cell culture and cell viability assay

Human RPE cells, ARPE-19 (ATCC CRL-2302), were cultured in Dulbecco's modified Eagle medium/nutrient mixture F-12 (WelGENE Inc.) with 10% fetal bovine serum (WelGENE Inc.), 100 units/mL penicillin, and 100 μg/mL streptomycin (WelGENE Inc.) in a humidified incubator with 5% CO_2 at 37°C. To determine the cytotoxicity of A2E and PCHE and the inhibitory effects of PCHE on BL-induced damage, cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Kaczara et al., 2010). Briefly, ARPE-19 cells were seeded at 2×10^4 cells per well in a 96-well plate and incubated at 37°C for 24 h. A2E and PCHE in fresh media were added to cells, which were incubated for another 24 h. Subsequently, 100 μL of the MTT stock solution (5 mg/mL) was added per well and incubated for 4 h. The medium was then removed, and 100 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. The absorbance was measured at 595 nm using an EPOCH2 microplate reader. Cytotoxicity results were expressed as the percentage of absorbance compared to the control group (without A2E or PCHE).

Western blotting

ARPE-19 cells were treated with 25 μM of A2E and 5, 10, and 15 μg/mL of PCHE in phenol red-free media for 24 h. Cells were then washed with PBS and exposed to BL for 20 min. After a further 24-h incubation, cells were lysed with radioimmunoprecipitation assay buffer, and the total protein content was quantified using a Bradford assay; 25 μg of protein was then separated using sodium-dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Protein detection was conducted using primary (HO-1 (E3F4S) Rabbit mAb #43966; LC3A/B (D3U4C) XP® Rabbit mAb #12741; SQSTM1/p62 (D1Q5S) Rabbit mAb #39749) and secondary (Anti-rabbit IgG, HRP-linked Antibody #7074) antibodies (Cell Signaling Technology). Protein bands were detected using an iBright CL1500 imaging system (Thermo Fisher), and quantified using ImageJ software (Huang et al., 2023); β-actin was used as a loading control.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 software (GraphPad Software). Data are expressed as the mean±standard deviation of triplicate measurements. Significant differences between groups were determined using a one-way analysis of variance (ANOVA) with Tukey's post hoc test.

RESULTS AND DISCUSSION

Antioxidant properties of purple corn cob and husk extracts (PCHE)

As shown in Table 1, the TAC of PCHE was 11.13±0.10 mg C3GE/100 g while the TPC and TFC were 227.90±7.38 mg GAE/g and 117.75±2.46 mg CE/g, respectively. The antioxidant capacity of PCHE determined using DPPH-scavenging and TEAC assays was 1.28±0.43 and 2,545.41±34.13 mM TE/g, respectively. Li et al. (2008) showed that extracts prepared from different parts of the corn plant (husk, cob, and leaf) predominantly contained C-3-G and cyanidin derivatives, which are known to exhibit DPPH radical-scavenging activity. Furthermore, Yang and Zhai (2010) used linear regression analysis to reveal a strong positive correlation ($r>0.828$) between

Table 1. TAC, TPC, TFC, DPPH, and TEAC values of PCHE

	TAC (mg C3GE/100 g)	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (mM TE/g)	TEAC (mM TE/g)
PCHE	11.13±0.10	227.90±7.38	117.75±2.46	1.28±0.43	2,545.41±34.13

Values are expressed as the mean of triplicate measurements±standard deviation.
TAC, total anthocyanin content; C3GE, cyanidin-3-glucoside equivalents; TPC, total phenolic content; GAE, gallic acid equivalent; TFC, total flavonoid content; CE, catechin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; TE, Trolox equivalent; TEAC, trolox equivalent antioxidant capacity; PCHE, purple corn cob and husk extract.

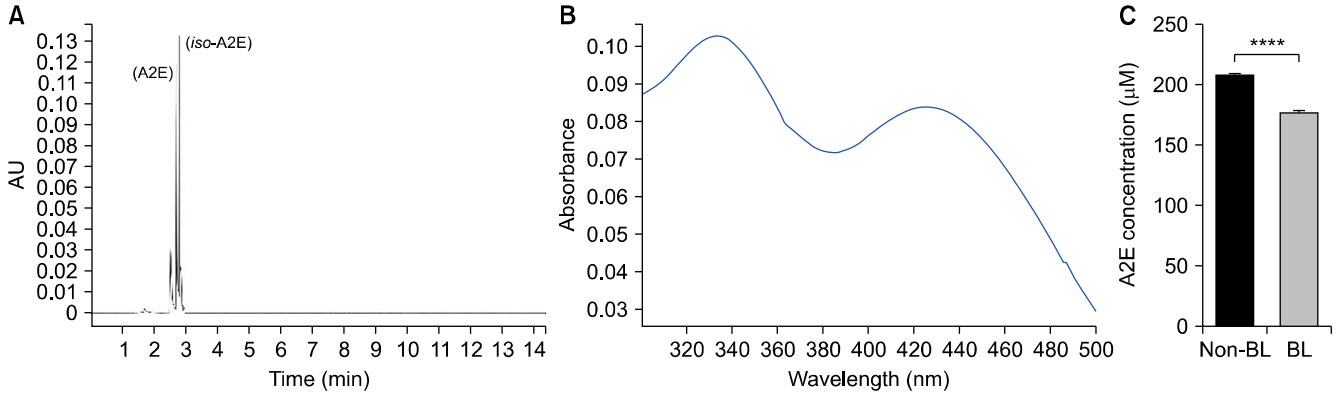

Fig. 1. Total ion chromatograms from high-performance liquid chromatography of synthesized A2E. Chromatograms of A2E and *iso*-A2E (A), ultraviolet spectra of synthesized *iso*-A2E (λmax=333.8 and 425.1 nm) (B), and photooxidation of A2E by blue light (BL) irradiation (C). Values are expressed as the mean of triplicate measurements. ****P<0.0001. A2E, N-retinylidene-N-retinylethanolamine.

the antioxidant activity, assessed by both DPPH-scavenging and TEAC assays, and the TAC of the studied extracts. Similarly, Orak et al. (2012) found a close association between TFC, TPC, DPPH-scavenging activity, and reducing power in pomegranate (*Punica granatum* L.) peel, which is rich in phenolic and flavonoid compounds, whereas PCHE contains significantly higher levels of anthocyanins. Therefore, based on the TAC and TPC of the PCHE, purple corn byproducts could be potential sources of antioxidants.

Chromatogram of high-performance liquid chromatography (HPLC)-purified A2E and blue light (BL)-induced A2E oxidation

The synthesized A2E was purified by liquid partitioning and its purity was confirmed via HPLC. The A2E and iso-A2E were detected at 44.87% and 41.55% of the total HPLC peak area, respectively (Fig. 1A). The ultraviolet spectra of *iso*-A2E exhibited λmax values of 333.8 and 425.1 nm, which is consistent with previously reported values of 335 and 426 nm (Parish et al., 1998), (Fig. 1B). The decrease in A2E concentration after BL exposure was measured at 440 nm (Fig. 1C). The initial A2E concentration of 208.69±0.54 μM decreased to 177.74±0.92 μM after BL exposure, indicating that A2E may be photooxidized upon BL exposure.

Inhibition of A2E photooxidation by PCHE in a cell-free system

A cell-free system was then used to evaluate the direct effects of PCHE on A2E photooxidation. According to Lee et al. (2023), when A2E is exposed to light at its peak absorption of 440 nm, singlet oxygen is produced, which oxidizes A2E. BL irradiation catalyzes the conversion of A2E into an oxidized form. The concentration of oxidized A2E induced by BL was 62.83±4.58 μM but decreased to 46.43±6.09, 36.52±1.93, and 27.86±4.91 μM with 25, 50, and 100 μg/mL PCHE, respectively (Fig. 2), indicating that PCHE inhibits BL-induced A2E oxidation. Similarly, Lee et al. (2016) demonstrated that bog bilberry (*Vaccinium uliginosum* L.) extract, particularly the polyphenol fraction without polar components, effectively suppressed BL-induced A2E photooxidation, with flavonoids and anthocyanins showing the strongest inhibitory effects.

Cytotoxicity effects of PCHE and A2E in ARPE-19 cells

ARPE-19 cells were exposed to various concentrations of PCHE (5–50 μg/mL) for 24 h, yielding a cell viability of 80.69%±0.84% after treatment with 20 μg/mL of PCHE (Fig. 3A). To determine the suitable A2E concentration for further experiments, the cytotoxicity of A2E in ARPE-19 cells with BL exposure was measured at 10–100 μM of A2E, which showed that 10 μM of A2E with BL did not induce cytotoxicity. However, the cell viability decreased to 60.72% and 34.67% with 25 and 30 μM A2E, respectively, under BL exposure (Fig. 3B and 3C). Therefore, 25 μM of A2E was chosen for fur-

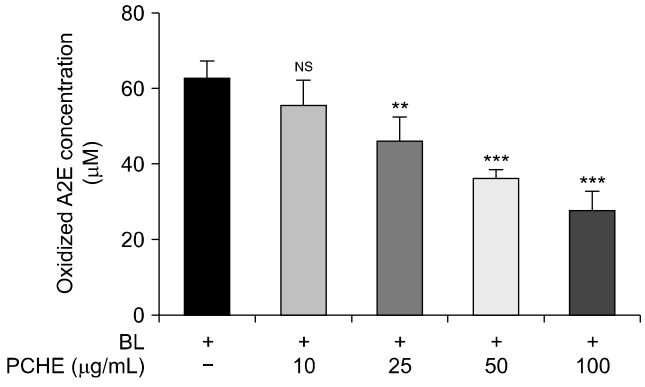

Fig. 2. Effect of purple corn cob and husk extract (PCHE) on A2E photooxidation in a cell-free system. Values are expressed as the mean of triplicate measurements±standard deviation. **P<0.01 and ***P<0.001 compared to the blue light (BL)-irradiated group (BL+, PCHE−). A2E, N-retinylidene-N-retinylethanolamine; NS, not significant.

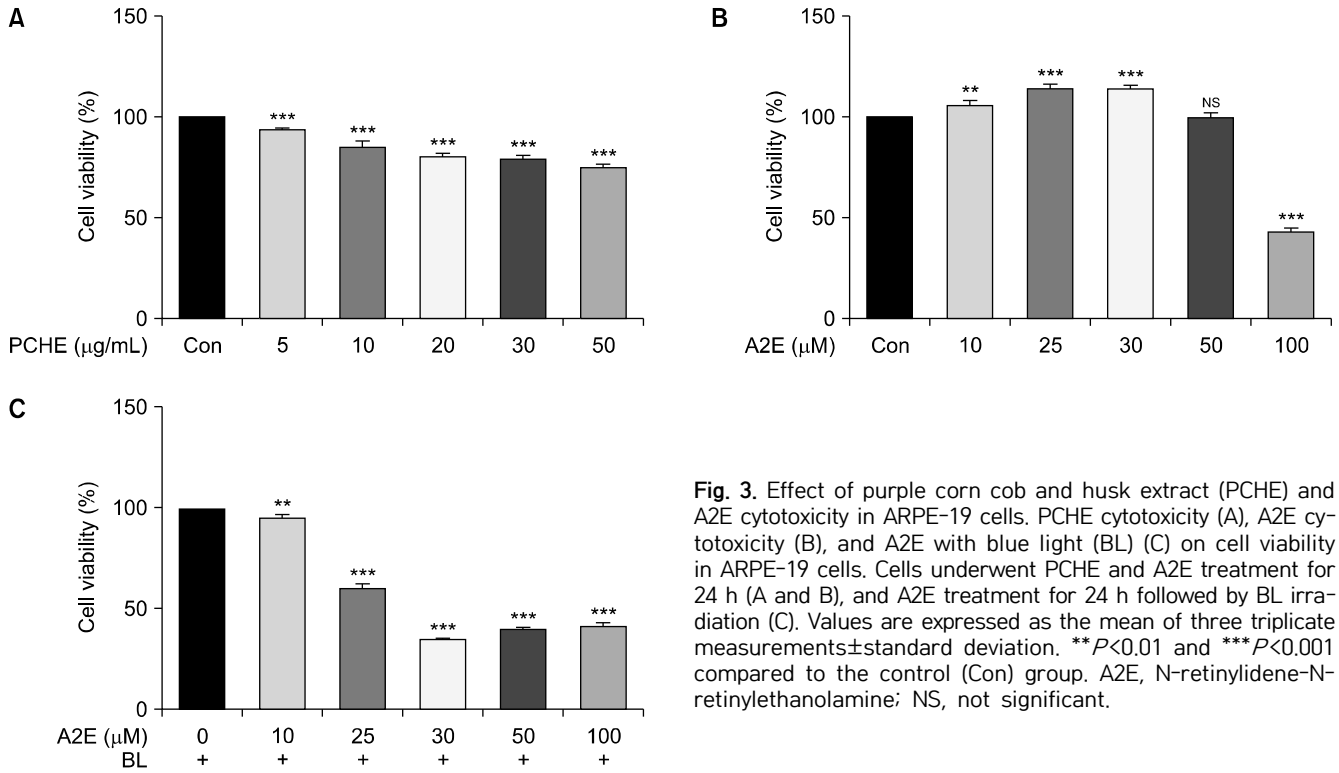

Fig. 3. Effect of purple corn cob and husk extract (PCHE) and A2E cytotoxicity in ARPE-19 cells. PCHE cytotoxicity (A), A2E cytotoxicity (B), and A2E with blue light (BL) (C) on cell viability in ARPE-19 cells. Cells underwent PCHE and A2E treatment for 24 h (A and B), and A2E treatment for 24 h followed by BL irradiation (C). Values are expressed as the mean of three triplicate measurements±standard deviation. $^{**}P<0.01$ and $^{***}P<0.001$ compared to the control (Con) group. A2E, N-retinylidene-N-retinylethanolamine; NS, not significant.

ther experiments.

Inhibitory effect of PCHE on BL-induced damage in A2E-accumulated ARPE-19 cells

To investigate how PCHE influences BL-induced cell death in A2E-accumulated ARPE-19 cells, cells were incubated with 25 μM of A2E and different concentrations (5–15 μg/mL) of PCHE, and then irradiated under BL. The cell viability of the A2E-treated group (without PCHE) decreased by 35.23%±0.79% ($P<0.001$), whereas the addition of 15 μg/mL of PCHE slightly increased the cell viability to 44.48%±0.11% ($P<0.001$). Thus, the BL-induced cell death in A2E-accumulated ARPE-19 cells could be ameliorated by PCHE treatment (Fig. 4). Drusen impairs the barrier function of the Bruch's membrane and hinders waste transport by RPE cells (Fields et al., 2020). Light exposure causes lipofuscin, a type of drusen, to produce ROS, causing severe disruption of the Bruch's membrane (Różanowska and Różanowski, 2022) and resulting in the atrophy and loss of RPE and photoreceptor cells (van Lookeren Campagne et al., 2014). Therefore, suppressing oxidative stress holds significant potential for preventing cell damage. The mechanisms of light-induced cell death primarily involve apoptosis and ROS production (Tanaka et al., 2011). As indicated by the high TAC, TPC, and TFC, PCHE can effectively scavenge DPPH and ABTS radicals, confirming its ability to neutralize ROS. These antioxidant properties could effectively prevent BL-induced cell death in A2E-accumulated ARPE-19 cells. These findings are consistent with results from studies on bog bilberries (Lee et al., 2016), blueberries (Liu et al., 2012), astaxanthin (Otsuka et al., 2013), purple rice extract (Tanaka et al., 2011), and *Arctium lappa* L. leaves (Kim et al., 2020) where apoptosis was inhibited by antioxidants. Previous studies have also demonstrated that flavonoids and anthocyanins (Lee et al., 2016), especially C-3-G (Wang et al., 2017), effectively protect against A2E photooxidation damage. Thus, flavonoids and anthocyanins are likely the active compounds in PCHE.

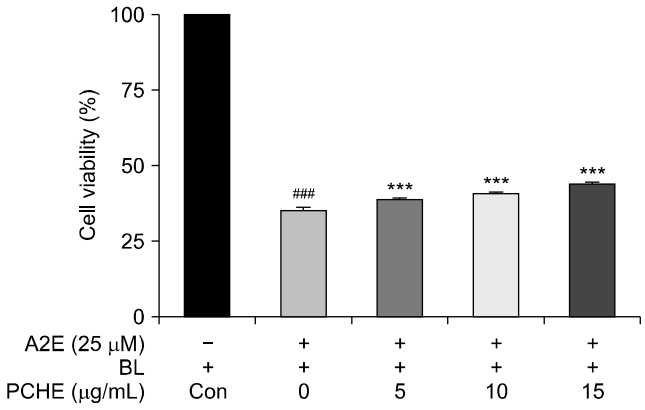

Fig. 4. Inhibitory effect of purple corn cob and husk extract (PCHE) on blue light (BL)-induced damage in A2E-accumulated ARPE-19 cells. Values are expressed as the mean of triplicate measurements±standard deviation. $^{###}P<0.001$ compared to the control (Con) group (A2E–, BL+, PCHE–). $^{***}P<0.001$ compared to the A2E BL group (A2E+, BL+, PCHE0). A2E, N-retinylidene-N-retinylethanolamine.

Protective effects of PCHE on heme oxygenase-1 (HO-1) expression and autophagy in BL-irradiated, A2E-accumulated ARPE-19 cells

A2E and BL are known to increase the intracellular microtubule-associated protein light chain 3-II (LC3-II) levels in RPE cells (Chen et al., 2016). In addition, BL-exposed A2E-accumulated ARPE-19 cells have been shown to exhibit a substantial increase in LC3-II levels (Jeong et al., 2019). Changes in LC3-II expression are known to correspond to autophagosome formation (Zhang et al., 2020), and the transition from soluble LC3-I to autophagy vesicle-associated LC3-II has been shown to indicate the autophagy induction (Schaaf et al., 2016). Herein, the photooxidation of A2E triggered by BL, rather than A2E alone, was shown to trigger the significant upregulation of LC3-II expression in RPE cells. Exposure to BL increased the LC3-II/LC3-I ratio, inducing the formation of autophagosomes, which was suppressed by treatment with PCHE. p62/SQSTM1 functions as an autophagy receptor and serves as an indicator of autophagy (Jeong et al., 2019). Compared with that in the control and A2E-only treatment groups, PCHE treatment induced a decrease in p62 expression in the A2E and BL treatment group (Fig. 5). Removing ROS with antioxidants reactivates lysosomes, enhances autophagic pathways, and prevents cell death, implying that photooxidized A2E disrupts autophagy by inhibiting the ROS-mediated lysosomal function (Jeong et al., 2019). When ARPE-19 cells accumulate A2E, BL irradiation of this A2E significantly increases ROS levels (Cho et al., 2022), indicating that A2E produces photooxidation products and singlet oxygen upon BL irradiation. In the current study, BL exposure increased HO-1 expression in A2E-accumulated ARPE-19 cells, which was further enhanced by PCHE treatment. These results suggest that cells undergoing photooxidation strive to rebalance the cellular oxidation-reduction equilibrium by activating the expression of antioxidant-response genes (Lu et al., 2016). In several studies, an increase in HO-1 levels has been demonstrated as a crucial mechanism in protecting RPE cells from oxidative stress (Wang et al., 2016).

Overall, this study showed that CHE protected ARPE-19 cells from A2E photooxidation-induced cell death *in vitro*, impacting autophagy and oxidative stress response. BL irradiation increased LC3-II levels, indicating enhanced autophagosome formation, whereas PCHE treatment decreased p62/SQSTM1 levels and increased HO-1 expression, suggesting autophagy inhibition and the activation of antioxidant pathways. These results underscore the potential of PCHE to alleviate oxidative stress and enhance cellular protection mechanisms, suggesting this is a promising dietary intervention for BL-induced cell damage.

Further study should be conducted to evaluate the effects of PCHE *in vivo* as gastrointestinal processes in clinical delivery may alter the results. Moreover, only the short-term effects were studied, leaving long-term impacts uncertain. Thus, *in vivo* studies and research on prolonged exposure are necessary to confirm the protective effects of PCHE and understand the precise molecular mechanisms involved. The current study suggests the potential for validating the therapeutic potential of PCHE for BL-induced damage in A2E-accumulated RPE cells.

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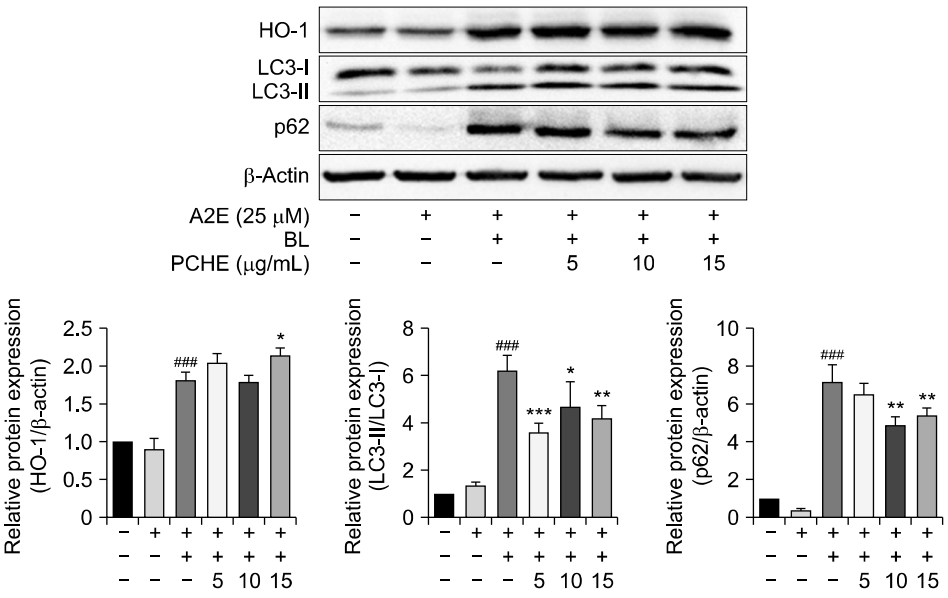

Fig. 5. Western blot of HO-1, LC3-II/I, p62/SQSTM1, and β-actin expression levels in ARPE-19 cells. Values are expressed as the mean of triplicate measurements±standard deviation. β-Actin was used as a loading control. $^{###}P<0.001$ compared to the control group (A2E–, BL–, PCHE–). $^{*}P<0.1$, $^{**}P<0.01$, and $^{***}P<0.001$ compared to the A2E BL group (A2E+, BL+, PCHE–). BL, blue light; PCHE, purple corn cob and husk extract; HO-1, heme oxygenase-1; LC3, microtubule-associated protein light chain 3; p62/SQSTM1, p62 sequestosome 1; A2E, N-retinylidene-N-retinylethanolamine.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: MJK. Analysis and interpretation: TYK, YRS, GIK, SWP. Data collection: SWP, HJL, JK, SC, MJK. Obtained funding: TYK, YRS, GIK. Writing the article: SWP, HJL. Critical revision of the article and final approval of the article: MJK.

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