

Research article

Cleistocalyx nervosum var. *paniala* Fruit Extract Attenuates Interleukin-1 β -Induced Inflammation in Human Retinal Pigment Epithelial Cells

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ABSTRACT

Inflammation in retinal pigment epithelial (RPE) cells is a crucial event in the initiation of age-related macular degeneration (AMD). Ripe fruit of *Cleistocalyx nervosum* var. *paniala*, or “Ma-kiang,” contains abundant phytochemicals, especially anthocyanin, which is a plentiful source of antioxidant and anti-inflammatory activities. However, the effects of this ripe fruit on RPE cells have not yet been studied. The present research investigated the inhibitory effect of *C. nervosum* var. *paniala* fruit extract on interleukin (IL)-1 β -mediated inflammation in human retinal pigment epithelial cells (ARPE-19). The ripe fruits of *C. nervosum* var. *paniala* were extracted using ethanol (CEE). Total anthocyanin content of the extract was analyzed using spectrophotometry, which showed high total anthocyanin content (31.54 ± 0.53 mg cyanidin-3-glucoside (C3G) equivalent/100 g DW). For the cell-based investigation, ARPE-19 cells were pretreated with CEE (5-500 μ g/ml) or C3G at 100 μ M for 1 h prior to co-incubation with or without IL-1 β (0.1 ng/ml) for 24 h, and cell viability measured by MTT assay. Thereafter, a culture medium was collected to detect inflammatory mediators, namely, IL-6, IL-8, and monocyte chemoattractant protein-1 using ELISA kit assays. Our results showed that CEE and C3G treatments at all concentrations were not toxic. They also exhibited good potential to significantly inhibit IL-1 β -induced inflammatory cytokines and chemokine in ARPE-19 cells. Consequently, our findings suggest that CEE and its major anthocyanin C3G have good anti-inflammatory potential. This fruit might be utilized as a natural alternative product to prevent inflammation-related AMD.

Key words: *Cleistocalyx nervosum* var. *paniala*, Anti-inflammation, Human retinal pigment epithelial ARPE-19 cells

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บทความวิจัย

สารสกัดผลมะเกี๋ยงลดการอักเสบจากการเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้าในเซลล์จอประสาทตามนุษย์

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บทคัดย่อ

การอักเสบในเซลล์เยื่อจอตาชั้นนอก (จอประสาทตา) เป็นสาเหตุสำคัญเริ่มต้นนำไปสู่ภาวะจอตาเสื่อมหรือจอตารับภาพเสื่อมในผู้สูงอายุ ผลมะเกี๋ยงสุกอุดมไปด้วยสารพฤกษเคมีมากมายโดยเฉพาะสารแอนโทไซยานินซึ่งพบว่ามีฤทธิ์ต้านอนุมูลอิสระและต้านอักเสบได้ดี อย่างไรก็ตาม ยังไม่มีการศึกษาฤทธิ์ของผลมะเกี๋ยงสุกนี้ต่อเซลล์จอประสาทตา วัตถุประสงค์ของงานวิจัยนี้ คือ เพื่อศึกษาผลการยับยั้งของสารสกัดจากผลมะเกี๋ยงต่อการอักเสบที่ถูกเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้า ในเซลล์จอประสาทตามนุษย์ (ARPE-19) ผลมะเกี๋ยงสุกถูกสกัดด้วยเอทานอล (CEE) ปริมาณแอนโทไซยานินรวมของสารสกัดถูกวิเคราะห์โดยเทคนิคสเปกโตรโฟโตเมตรี ผลการศึกษาที่ได้พบว่าสารสกัดผลมะเกี๋ยงสุกมีปริมาณแอนโทไซยานินรวมสูง (31.54 ± 0.53 มิลลิกรัมไซยานิดิน-3-กลูโคไซด์ (C3G) เทียบเท่า/100 กรัมน้ำหนักแห้ง) สำหรับการทดสอบในระดับเซลล์นั้น เซลล์ ARPE-19 ถูกเลี้ยงด้วยอาหารเลี้ยงเซลล์ที่ผสม CEE (5-500 ไมโครกรัม/มิลลิลิตร) หรือ C3G ที่ 100 ไมโครโมลาร์ เป็นเวลา 1 ชั่วโมง ก่อนนำมาเลี้ยงร่วมกับสภาวะที่มีหรือไม่มีอินเตอร์ลิวคิน-1 เบต้า (0.1 นาโนกรัม/มิลลิลิตร) เป็นเวลา 24 ชั่วโมง แล้วตรวจสอบการมีชีวิตอยู่รอดของเซลล์โดยวิธี MTT จากนั้นอาหารเลี้ยงเซลล์ ถูกเก็บเพื่อตรวจสอบหาสารสื่ออักเสบชนิดต่างๆ ได้แก่ อินเตอร์ลิวคิน-6, อินเตอร์ลิวคิน-8 และโมโนไซต์เคโมแอตแทรกแตนท์โปรตีน-1 ทดสอบด้วยวิธี ELISA จากผลการทดสอบ พบว่า CEE และ C3G ในทุกความเข้มข้นไม่มีความเป็นพิษต่อเซลล์และมีศักยภาพที่ดีในการยับยั้งการหลั่งสารสื่ออักเสบต่างๆ ที่เกิดจากการเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้า ในเซลล์ ARPE-19 ได้อย่างมีนัยสำคัญทางสถิติ ดังนั้นข้อมูลที่ได้จากการค้นพบครั้งนี้ ชี้ให้เห็นว่าสารสกัดจากผลมะเกี๋ยงสุกและสารแอนโทไซยานินหลัก C3G มีศักยภาพออกฤทธิ์ต้านการอักเสบได้ดี ผลไม้นี้อาจถูกนำมาใช้เป็นผลิตภัณฑ์ทางเลือกจากธรรมชาติเพื่อป้องกันการเกิดภาวะจอประสาทตาเสื่อมซึ่งสัมพันธ์กับการอักเสบ

คำสำคัญ: มะเกี๋ยง การต้านอักเสบ เซลล์จอประสาทตามนุษย์ชนิด ARPE-19

Introduction

In normal pathology, the inflammatory process is an important mechanism to protect the body from cell or tissue damage, thus affecting the elimination of pathogens and damaged cells¹. However, overwhelming inflammation can destroy cells and cause tissue damage or dysfunction. Consequently, the long-term effects of chronic inflammations can lead to various chronic diseases², including degenerative diseases like age-related macular degeneration (AMD). Presently, it is estimated that 8.7% of the global population suffer from AMD. This number will probably double in the next 20 years with increasing life expectancy³. Globally, AMD is the fourth leading cause of visual impairment and is considered to be an irreversible permanent affliction among older populations⁴. It is characterized by distorted central vision, a dark or gray patch (scotoma) in the central vision, followed by progressive loss of central vision, which lead to difficulties in conducting daily living activities, such as reading fine print or recognizing faces or colors⁵. AMD is classified into two distinct subtypes: dry AMD (geographic atrophy; nonexudative) and wet AMD (neovascular; exudative). Pathological processes include lipofuscin accumulation, drusen formation, retinal pigment epithelial cells (RPE) geographic atrophy, photoreceptor dysfunction and degeneration, plus choroidal neovascularization⁶. These events are associated with chronic inflammation processes via elevated levels of many inflammatory cytokines, such as interleukin (IL)-1, IL-6, etc., and chemokines, such as IL-8 and monocyte chemoattractant protein-1 (MCP-1)⁷. Previous studies have

reported that various cytokines and chemokines are found either in the ocular fluids or tissue or systemically in the serum of AMD patients. Hence, chronic inflammation is involved in facilitating the progression of AMD⁸. Plants and natural substances with antioxidant properties are widely used to prevent or reduce inflammation⁹⁻¹¹. One of the phytochemicals from plants that can play a potential role in reducing inflammation is anthocyanin, which belongs to the sub-group of flavonoids. It is a pigment expressed as blue, red, or purple colors in many fruits and vegetables, especially berry fruits¹²⁻¹³. The health benefits of anthocyanin rest on a variety of biological capabilities, including protection against oxidative damage of DNA, protein, lipid, or other macromolecules due to its antioxidant and anti-inflammation properties¹⁴⁻¹⁶. Interestingly, recent studies have found that foods containing anthocyanin can increase the flow of capillaries in the eyes, relieve tired eyes, suppress the radicals in the eye cells, improve vision in low light conditions, and enhance pupillary response in clinical studies¹⁷⁻¹⁹. *Cleistocalyx nervosum* var. *paniala* (Ma-kiang) is a berry found in Northern Thailand that, when ripe, exhibits a relatively high anthocyanin content²⁰. Previous studies have revealed that *C. nervosum* var. *paniala* possesses numerous biological properties, such as antioxidant, anti-aging, anti-mutagenicity, anti-carcinogenicity, neuroprotection, and immune enhancement²¹⁻²⁴. However, there are no reports on the effect of *C. nervosum* var. *paniala* fruit extract on retinal pigment epithelial cells. Consequently, the present study investigated the effects of *C. nervosum* var. *paniala* fruit extract on IL1 β -



induced inflammation in retinal pigment epithelial cells. The ARPE-19 human retinal pigment epithelial cell line was used as a study model in this experiment.

Materials and methods

Collection and preparation of *C. nervosum* var. *paniala* fruit

C. nervosum var. *paniala* ripe fruits were collected from the Plant Genetic Conservation Project under the Royal initiation of Her Royal Highness Princess Maha Chakri Sirindhorn, in Lampang province, Northern Thailand, during the rainy season from July-August 2018. *C. nervosum* var. *paniala* was identified as the scientific name by Assistant Professor Dr. Thaya Jenjittikul (Department of Plant Science, Faculty of Science, Mahidol University, Thailand). The voucher specimen was No. 9428, which was deposited at Suan Luang Rama IX Herbarium, Bangkok, Thailand. The amounts of ripe fruits ranged from three to five kilograms. These fruits were removed from their stalks, washed with tap water and then deionized water, and then air dried. Thereafter, the flesh was separated from the seed and weighed. Next, the samples were lyophilized and ground into a powder²⁵. Dried samples were packed in aluminum foil under vacuum and stored at -20 °C for further analysis.

Sample extraction

The lyophilized sample was extracted with 95% ethanol at the ratio 1:15 (weight of sample: volume of solvent extract) for 3 times, according to the slightly modified technique of Nantacharoen et al. (2022)²⁵. Briefly, the samples were dissolved in 95% ethanol, mixed by a vortex

mixer for 1 min, then sonicated in ultrasonic bath for 10 mins, and then centrifuged at 4600 rpm for 10 mins (Hettich® Instruments, Rotina 38R, UK). The supernatants of extract were collected and evaporated using a vacuum rotary evaporator. Next, the extract was solubilized with 2 ml of solvent extract and transferred to an amber vial prior to blowing with nitrogen gas until dry. The dried sample was kept at -20 °C until use.

Total anthocyanin content analysis

The total anthocyanin analysis was determined according to the procedure of Sukprasansap et al. (2017)²⁴. Briefly, the sample was extracted with acidified methanol (ratio 1:10) for 30 min on a magnetic stirrer, then centrifuged to collect the supernatants, and kept in darkness at 4 °C. This process was conducted over three cycles, and the supernatants were combined and mixed well for analysis. The extract was measured at 525 nm by spectrophotometer. Total anthocyanin content was calculated using a calibration curve of cyanidin-3-glucoside (C3G) as a reference, and data were presented as mg C3G equivalent/100 g dry weight (DW)^{24, 26}.

Cell culture

The human retinal pigment epithelial cell line (ARPE-19) was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's Medium/Nutrient Mixture F-12 Ham 1:1 (DMEM/F-12) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) and 1% (v/v) 100 µm/ml of penicillin and streptomycin. Cells were maintained at 37°C in an incubator in a humidified atmosphere of 5% CO₂.

Cell viability assay

The cytotoxic effect of the ethanolic extract of *C. nervosum* var. *paniala* (CEE), C3G or IL-1 β on ARPE-19 cells was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide tetrazolium (MTT) assay. ARPE-19 cells were cultured in 48-well plates at a density of 1×10^5 cells/well, and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 24 h. The cells were washed with serum-free medium, and then added to serum-free medium containing various concentrations of CEE (50-1000 μ g/ml), C3G (6.25-100 μ M), or IL-1 β (0.1-10 ng/ml) for 24 h to identify the appropriate concentration for subsequent experiments. After incubation, the cells were treated with 0.5 mg/ml MTT solution at 37°C for 4 h. Next, MTT solution was removed and the formazan crystals were dissolved with dimethyl sulfoxide (DMSO). Likewise, the pretreatment cells containing different concentrations of CEE or C3G for 1 h, followed by IL-1 β in a complete medium at 37°C for 24 h, were also examined for cell viability. This procedure was conducted using a similar process as mentioned above. Absorbance was measured by microplate reader (BioTek® Instruments, Vermont, USA) at 540 nm. The percentages of cell viability were calculated and compared with the control group. The acceptable cell viability value in each treatment group should be more than 80%.

Determination of the production of inflammatory mediators

After IL-1 β treatment of the cells, the culture media were collected to measure the inflammatory cytokines and chemokines, namely, IL-6 and IL-8, and MCP-1 by a quantitative

“sandwich” enzyme-linked immunosorbent assay (ELISA) (Biolegend Inc., San Diego, CA, USA). In brief, high-binding plates (NUNC, Roskilde, Denmark) were coated with capture antibody for mouse IL-6, IL-8 and MCP-1 overnight at 4 °C. Excessive antibodies were washed and blocked unbinding sites with 1% bovine serum albumin (BSA) in PBS for 1 h at 25 °C. Thereafter, culture medium or recombinant human (IL-6, IL-8 or MCP-1) standards were added to each well for binding with capture antibodies for 2 h at 25 °C. After incubation and washing with PBS, the immune complex was detected using a streptavidin HRP-tetramethylbenzidine detection system incubated for 30 min at 25 °C. The reactions in each well were stopped with sulfuric acid (H₂SO₄). Absorbance was determined at 450 nm by a microplate reader (BioTek® Instruments, Vermont, USA). The concentrations of IL-6, IL-8 or MCP-1 in samples were calculated by comparing absorbance with their standard curves.

Statistical analysis

All data are presented as mean $\pm$ SD from at least three independent experiments. Statistical significance was analyzed by one-way analysis of variance (ANOVA) with the post-hoc Duncan for multiple comparisons to identify mean differences among treatment groups. Statistical significance was set at $p < 0.05$. SPSS (version 19.0, SPSS Inc., Chicago, IL) was used to analyze the data.

Results

Total anthocyanin in CEE

Total anthocyanin content of CEE was examined prior to all cell-based investigations. C.



nervosum var. *paniala* powder was extracted using 95% ethanol, the percentage yield was $13.56 \pm 0.87\%$, and the total anthocyanin content was 31.54 ± 0.53 mg C3G equivalent/100 g DW compared with the standard curve of C3G.

Effects of CEE and C3G on viability of ARPE-19 cells

To evaluate the effects of CEE and C3G on cell viability and toxicity in ARPE-19 cells, we firstly examined the non-cytotoxic concentrations of CEE and C3G. ARPE-19 cells were treated with CEE (50, 100, 200, 500, and 1,000 $\mu\text{g/ml}$) or C3G (6.25, 12.5, 25, 50, and 100 μM) for 24 h, and then cell viability was detected by MTT assay. The results showed that treatments of cells with CEE at concentrations of 50-500 $\mu\text{g/ml}$ did not have cytotoxic effects, whereas only at 1000 $\mu\text{g/ml}$ was it significantly toxic compared to untreated control cells (**Figure 1a**). Cell morphology was observed under a light microscope, which showed CEE at concentration of 1000 $\mu\text{g/ml}$ damaged and devastated the ARPE-19 cells (**Figure 1b**). Additionally, treated cells with C3G at concentrations of 6.25-100 $\mu\text{g/ml}$ did not show any toxic effect in ARPE-19 cells both in terms of the viability and morphology of cells (**Figures 1c and d**). Based on these results, non-toxic concentrations (50-500 $\mu\text{g/ml}$) of CEE and the 100 μM highest dose of G3G (used as positive control) were then chosen for the subsequent investigations.

Effect of IL-1 β on cell viability and production of inflammatory mediators in ARPE-19 cells

IL-1 β is a pro-inflammatory cytokine that can trigger inflammatory cascades and play a major role in retinal inflammation²⁷⁻²⁸. To

determine the effect of IL-1 β -induced inflammation on ARPE-19 cells, the viability of cells was also investigated by MTT assay. The produced inflammatory mediators (IL-6 and IL-8) were measured by ELISA assay. ARPE-19 cells were treated with IL-1 β at concentrations 0.1, 1, 2, 5, and 10 ng/ml for 24 h. Results showed that all concentrations of IL-1 β treatment did not affect the cytotoxicity of cells compared to the untreated control group (**Figure 2a**). Moreover, treated cells with IL-1 β at concentrations of 0.1-10 ng/ml could significantly induce production of inflammatory mediators, both IL-6 and IL-8, compared to the untreated control group (**Figures 2b and c**). However, based on this data, IL-1 β at 0.1 ng/ml was also selected for further experiments on the inhibition of IL-1 β -induced inflammation in ARPE-19 cells.

Effect of CEE and C3G on the productions of IL-6, IL-8, and MCP-1 and cell viability in ARPE-19 cells induced inflammation with IL-1 β

To illustrate the effects of CEE and C3G on IL-1 β -induced inflammation, the production of inflammatory mediators was measured using ELISA assay. The ARPE-19 cells were pretreated with different concentrations of CEE or C3G for 1 h before being incubated with IL-1 β for 24 h. We found that exposure of ARPE-19 cells to IL-1 β significantly produced IL-6, IL-8 and MCP-1, whereas the control cells or cells treated with extract or C3G alone had no significant effect (**Figures 3a, b, and c**). Pretreated cells with CEE inhibited IL-6, IL-8 and MCP-1 production in a dose-dependent manner when compared to the IL-1 β only treatment group. Additionally, we founded that all treatments did not show a cytotoxic effect in

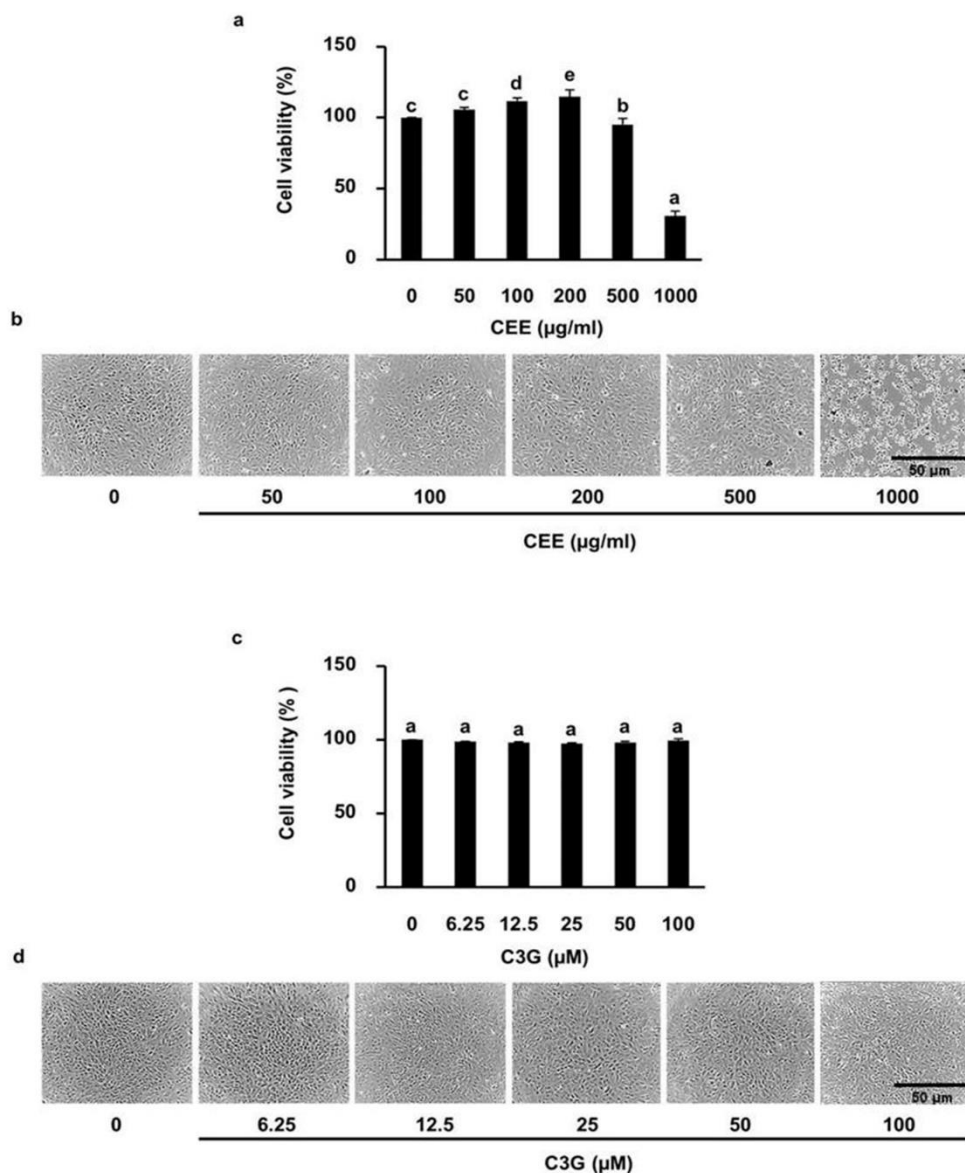


Figure 1. Effects of CEE and C3G on viability of ARPE-19 cells. Cell viability was investigated with MTT assay (**a-b**). The cells were treated with CEE at various concentrations (0-1000 µg/ml) for 24 h. (**c-d**). The cells were treated with C3G at various concentrations (0-100 µM) for 24 h. Cell morphologies were visualized by light microscopy (scale bar is 50 µM). Results are shown as mean ± SD (n=3). Different letters above the error bars indicate significant differences among treatment groups (p<0.05).

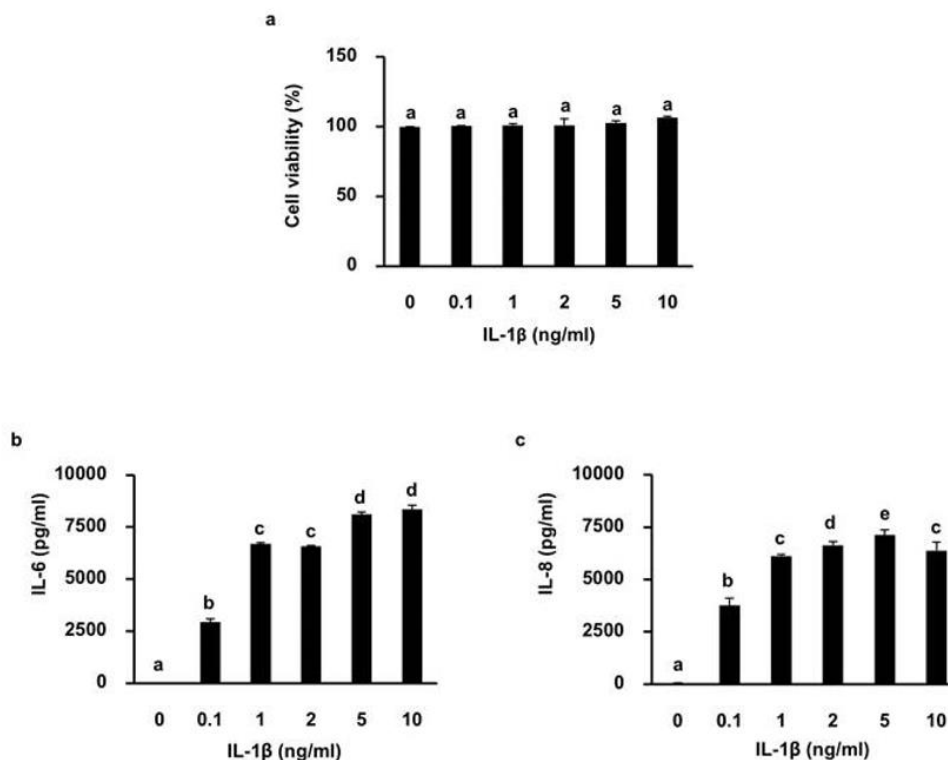


Figure 2. Effect of IL-1 β on cell viability and production of inflammatory mediators in ARPE-19 cells (a). The cells were treated with IL-1 β at various concentrations (0-10 ng/ml) for 24 h, and cell viability was assessed by MTT assay (b-c). The cells were treated with IL-1 β at various concentrations (0-10 ng/ml) for 24 h. IL-6 and IL-8 were detected using ELISA assay. Data are shown as mean $\pm$ SD (n=3). Different letters above the error bars indicate significant differences among treatment groups ($p < 0.05$).

ARPE-19 cells (**Figure 3d**). Moreover, the highest dose (500 μ g/ml) of CEE that could suppress the production of all inflammatory mediators was similar to C3G (100 μ M). These results suggest that CEE exerts a preventive effect against IL-1 β -induced inflammation in ARPE-19 cells.

Discussion

C. nervosum var. *paniala* is in the berry plant family and its ripe fruit is a rich source of anthocyanin that gives a dark-purple pigment color^{24,29}. Ripe fruit of *C. nervosum* var. *paniala* is widely used in food products, such as jams, wines, and juices²⁴. Several research studies

have revealed that one of the main bioactive compounds of *C. nervosum* var. *paniala* was C3G-anthocyanin^{22-24,30-32}. The present study showed that CEE was plentiful in total anthocyanin content (31.54 ± 0.53 mg C3G equivalent/100 g DW) that was calculated as equivalent to C3G-major anthocyanin in CEE. This result was lower than data from Sukprasansap and colleagues (2017)²⁴, which was reported to be 50.49 ± 0.64 mg C3G equivalent/100 g DW, due to the *C. nervosum* var. *paniala* fruits being collected in a different year and location. This fruit-derived anthocyanin compound provides various health benefits,

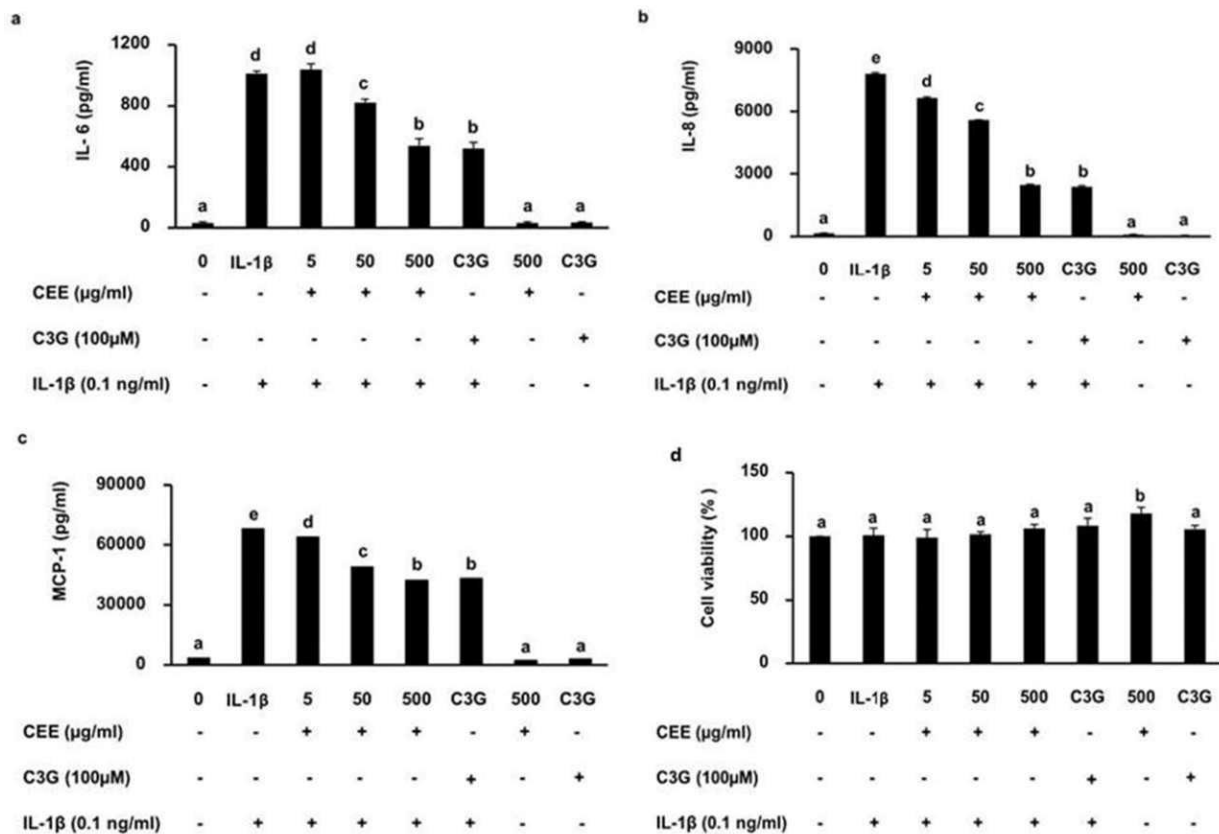


Figure 3. Effects of CEE or C3G on IL-1 β -induced inflammatory mediators (IL-6, IL-8 and MCP-1) and cell viability in ARPE-19 cells. ARPE-19 cells were pretreated with 0, 50 and 500 μ M of CEE or 100 μ M of C3G for 1 h prior to stimulation with 0.1 ng/ml IL-1 β for 24 h. (a) IL-6, (b) IL-8 and (c) MCP-1 concentrations in culture media determined by ELISA assay. (d) Cell viability was assessed by MTT assay. Results are presented as mean $\pm$ SD (n=3). Different letters above the error bars indicate significant differences among treatment groups ($p < 0.05$).

such as antioxidant, anti-inflammatory, anti-aging, and neuroprotective activities^{22,25,33}. Prolonged inflammatory processes cause many chronic and degenerative diseases. This condition has been reported to be involved in the pathophysiology of various retinal diseases, including AMD, polypoidal choroidal vasculopathy, diabetic retinopathy, and retinal vein occlusion³⁴⁻³⁶. Retinal pigment epithelial cells have been shown to secrete inflammatory mediators *in vitro* after stimulation with IL-1 β ⁸. The IL-1 β is a pro-inflammatory cytokine that can trigger an inflammatory cascade and play a major role in

retinal inflammation²⁷⁻²⁸. MCP-1 belongs to the chemokine family. It stimulates and attracts monocytes and lymphocytes, resulting in monocyte/macrophage infiltration^{8,37}. IL-8 belongs to the chemokine family and is a chemoattractant for eosinophils and neutrophils³⁸. Previous studies have demonstrated that IL-6, IL-8 and MCP-1 not only initiate inflammatory responses but also promote angiogenesis, thereby stimulating AMD progression³⁹⁻⁴¹.

Consequently, our present research focused on the effect of CEE on IL-1 β -induced inflammation in ARPE-19 cells. We found that



CEE was able to prevent the inflammatory effect caused by IL-1 β without non-cytotoxicity. Moreover, we also observed the production and secretion of inflammatory mediators, namely IL-6 and IL-8 (**Figures 2b and c**), in response to IL-1 β treatment, and mediating inflammation in ARPE-19 cells. This suggests that these cytokines and chemokines play a crucial part in retinal pigment epithelial inflammation⁴¹. Remarkably, pretreatment of CEE or C3G could inhibit all inflammatory mediators, including cytokine (IL-6) and chemokines (IL-8 and MCP-1), induced by IL-1 β (**Figure 3**). This anti-inflammatory effect was related to the potent anthocyanin property of this berry's extract. In addition, previous studies have shown IL-1 β -stimulated increases in the production of IL-6, IL-8, and MCP-1 in retinal pigment epithelial cells⁴²⁻⁴⁴. Cells treated with aronia berry extract reduced the expression of these inflammatory mediators, which suggests that the abundant anthocyanins in this berry extract can also affect the inflammatory responses in vascular endothelial cells⁴⁵. One of the most widely reported signaling pathways in many cell systems is the MAPK signaling pathway, in which inflammatory stimulants contribute to the activation of MAPKs, followed by increased release of cytokines and chemokines⁴⁶⁻⁴⁸. Anthocyanin has been reported to exhibit anti-inflammatory effects by inhibiting the activation of the inflammatory pathway in a number of different cell lines treated with different inflammatory stimulants⁴⁹⁻⁵¹. Furthermore, several reports revealed that anthocyanin compounds, especially C3G, or anthocyanin-rich plant extracts inhibit the expression of inflammatory cytokines/chemokines, adhesion molecules, and the adhesion of monocytes to various vascular

endothelial cells⁵²⁻⁵⁴. However, other phytochemicals in this *C. nervosum* var. *paniala* berry may contribute to these synergistic capabilities together with specific bioactive anthocyanin for anti-inflammation in ARPE-19 cells.

Conclusion

This research indicates that CEE can inhibit IL-1 β -induced inflammation in human retinal pigment epithelial ARPE-19 cells by suppressing the production of inflammatory mediators namely, IL-6, IL-8, and MCP-1. However, further studies are needed to identify the anthocyanin profiles in CEE and also clarify the underlying mechanisms of CEE on anti-inflammatory signaling pathway in ARPE-19 cells. Our findings provide information about the *C. nervosum* var. *paniala* berry fruit and its major anthocyanin C3G as a good anti-inflammatory agent. It might be applied as a natural alternative product to reduce inflammation-associated AMD risks.

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Conflict of interests

The authors have no conflicts of interest to declare.

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