Research article

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

Pranee Srimard[1], Chawanphat Muangnoi[2], Siriporn Tuntipopipat[2],
Somsri Charoenkiatkul[3], Monruedee Sukprasansap[4*]

[1] Graduate student in Master of Science Program in Nutrition, Faculty of Medicine Ramathibodi Hospital and Institute of Nutrition, Mahidol University, Bangkok, Thailand.
[2] Cell and Animal Model Unit, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand,
[3] Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand.
[4] Food Toxicology Unit, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand.

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)-1β-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 µg/ml) or C3G at 100 µM for 1 h prior to co-incubation with or without IL-1β (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-1β-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

Received: 2 October 2022 Accepted: 31 October 2022 Available online: 4 November 2022

*Corresponding author's e-mail: monruedee.suk@mahidol.edu

บทความวิจัย

สารสกัดผลมะเกี๋ยงลดการอักเสบจากการเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้าในเซลล์จอประสาทตามนุษย์

ปราณี ศรีหมาด[1], ชวัลพัชร เมืองน้อย[2], ศิริพร ตันติโพธิ์พิพัฒน์[2],
สมศรี เจริญเกียรติกุล[3], มลฤดี สุขประสารทรัพย์[4*]

[1] หลักสูตรวิทยาศาสตรมหาบัณฑิตสาขาวิชาโภชนศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี และสถาบันโภชนาการ มหาวิทยาลัยมหิดล กรุงเทพฯ ประเทศไทย.
[2] หน่วยเซลล์และสัตว์ทดลองต้นแบบ สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย.
[3] สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย.
[4] หน่วยพิษวิทยาทางอาหาร สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย.

บทคัดย่อ

การอักเสบในเซลล์เยื่อบุจอตาชั้นนอก (จอประสาทตา) เป็นสาเหตุสำคัญเริ่มต้นนำไปสู่ภาวะจอตาเสื่อมหรือจุดรับภาพเสื่อมในผู้สูงอายุ ผลมะเกี๋ยงสุกอุดมไปด้วยสารพฤกษเคมีมากมายโดยเฉพาะสารแอนโทไซยานินซึ่งพบว่ามีฤทธิ์ต้านอนุมูลอิสระและต้านอักเสบได้ดี อย่างไรก็ตาม ยังไม่มีการศึกษาฤทธิ์ของผลมะเกี๋ยงสุกนี้ต่อเซลล์จอประสาทตา วัตถุประสงค์ของงานวิจัยนี้ คือ เพื่อศึกษาผลการยับยั้งของสารสกัดจากผลมะเกี๋ยงต่อการอักเสบที่ถูกเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้า ในเซลล์จอประสาทตามนุษย์ (ARPE-19) ผลมะเกี๋ยงสุกถูกสกัดด้วยเอทานอล (CEE) ปริมาณแอนโทไซยานินรวมของสารสกัดถูกวิเคราะห์โดยเทคนิคสเปกโตรโฟโตเมตรี ผลการศึกษาที่ได้พบว่าสารสกัดผลมะเกี๋ยงสุกมีปริมาณแอนโธไซยานินรวมสูง (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 ถูกเก็บเพื่อตรวจหาสารสื่ออักเสบชนิดต่างๆ ได้แก่ อินเตอร์ลิวคิน-6, อินเตอร์ลิวคิน-8 และโมโนไซต์เคโมแอดแทรกแตนท์โปรตีน-1 ทดสอบด้วยวิธี ELISA จากผลการทดสอบ พบว่า CEE และ C3G ในทุกความเข้มข้นไม่มีความเป็นพิษต่อเซลล์และมีศักยภาพที่ดีในการยับยั้งการหลั่งสารสื่ออักเสบต่างๆ ที่เกิดจากการเหนี่ยวนำด้วยอินเตอร์ลิวคิน-1 เบต้า ในเซลล์ ARPE-19 ได้อย่างมีนัยสำคัญทางสถิติ ดังนั้นข้อมูลที่ได้จากการค้นพบครั้งนี้ ชี้ให้เห็นว่าสารสกัดจากผลมะเกี๋ยงสุกและสารแอนโธไซยานินหลัก C3G มีศักยภาพออกฤทธิ์ต้านการอักเสบได้ดี ผลไม้นี้อาจถูกนำมาใช้เป็นผลิตภัณฑ์ทางเลือกจากธรรมชาติเพื่อป้องกันการเกิดภาวะจอประสาทตาเสื่อมซึ่งสัมพันธ์กับการอักเสบ

คำสำคัญ: มะเกี๋ยง การต้านอักเสบ เซลล์จอประสาทตามนุษย์ชนิด ARPE-19

*Corresponding author's e-mail: monruedee.suk@mahidol.edu

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 cells[1]. 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 diseases[2], 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 expectancy[3]. Globally, AMD is the fourth leading cause of visual impairment and is considered to be an irreversible permanent affliction among older populations[4]. 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 colors[5]. 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 neovascularization[6]. 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 AMD[8]. Plants and natural substances with antioxidant properties are widely used to prevent or reduce inflammation[9-11]. 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 fruits[12-13]. 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 properties[14-16]. 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 studies[17-19]. *Cleistocalyx nervosum* var. *paniala* (Ma-kiang) is a berry found in Northern Thailand that, when ripe, exhibits a relatively high anthocyanin content[20]. 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 enhancement[21-24]. 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 IL1β-

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 powder[25]. Dried samples were packed in aluminum foil under vacuum and stored at -20 ºC 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 37ºC in an incubator in a humidified atmosphere of 5% CO_2.

Cell viability assay

The cytotoxic effect of the ethanolic extract of *C. nervosum* var. *paniala (*CEE), C3G or *IL-1β* 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 $1x10^5$ cells/well, and incubated at 37°C in a humidified atmosphere of 5% CO_2 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 µg/ml), C3G (6.25-100 µM), or IL-1β (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 37ºC 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-1β in a complete medium at 37ºC 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-1β 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 (H_2SO_4). 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 ± 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 ± 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 µg/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 µg/ml did not have cytotoxic effects, whereas only at 1000 µg/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 µg/ml damaged and devastated the ARPE-19 cells (**Figure 1b**). Additionally, treated cells with C3G at concentrations of 6.25-100 µg/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 µg/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-1β on cell viability and production of inflammatory mediators in ARPE-19 cells

IL-1β is a pro-inflammatory cytokine that can trigger inflammatory cascades and play a major role in retinal inflammation[27-28]. To determine the effect of IL-1β-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-1β at concentrations 0.1, 1, 2, 5, and 10 ng/ml for 24 h. Results showed that all concentrations of IL-1β treatment did not affect the cytotoxicity of cells compared to the untreated control group (**Figure 2a**). Moreover, treated cells with IL-1β 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-1β at 0.1 ng/ml was also selected for further experiments on the inhibition of IL-1β-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-1β

To illustrate the effects of CEE and C3G on IL-1β-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-1β for 24 h. We found that exposure of ARPE-19 cells to IL-1β 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-1β only treatment group. Additionally, we founded that all treatments did not show a cytotoxic effect in

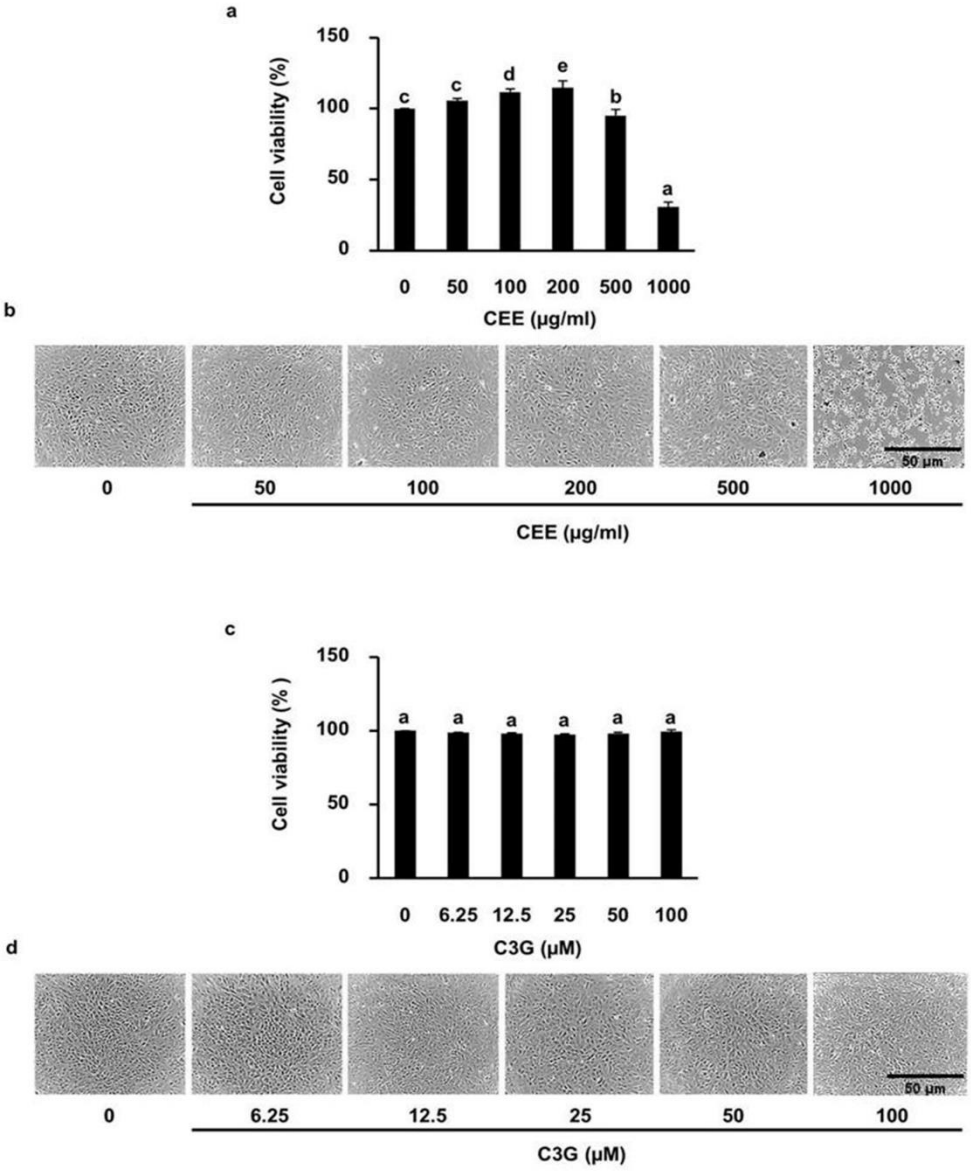

Figure 1. Effects of CEE and C3G on viability of ARPE-19 cells. Cell viability was investigated with MTT assay (**a-b**). The cells were treated with CEE at various concentrations (0-1000 μg/ml) for 24 h. (**c-d**). The cells were treated with C3G at various concentrations (0-100 μM) for 24 h. Cell morphologies 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 ($p<0.05$).

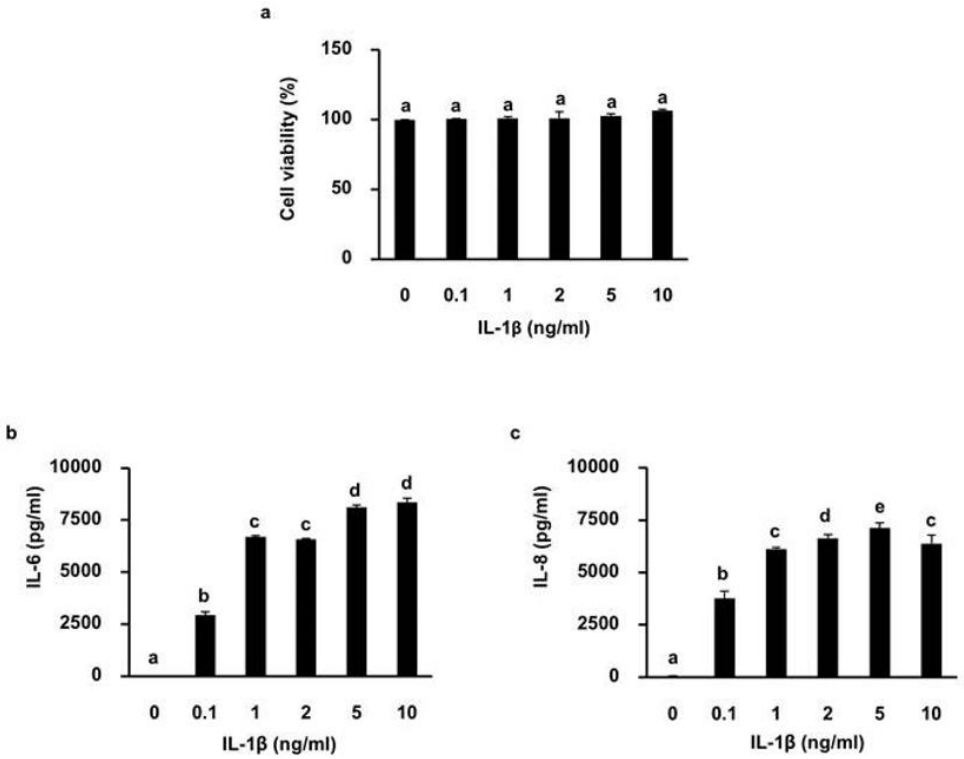

Figure 2. Effect of IL-1β on cell viability and production of inflammatory mediators in ARPE-19 cells (**a**). The cells were treated with IL-1β at various concentrations (0-10 ng/ml) for 24 h, and cell viability was assessed by MTT assay (**b-c**). The cells were treated with IL-1β at various concentrations (0-10 ng/ml) for 24 h. IL-6 and IL-8 were detected using ELISA assay. Data are shown as mean ± 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 µg/ml) of CEE that could suppress the production of all inflammatory mediators was similar to C3G (100 µM). These results suggest that CEE exerts a preventive effect against IL-1β-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 juices[24]. 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,

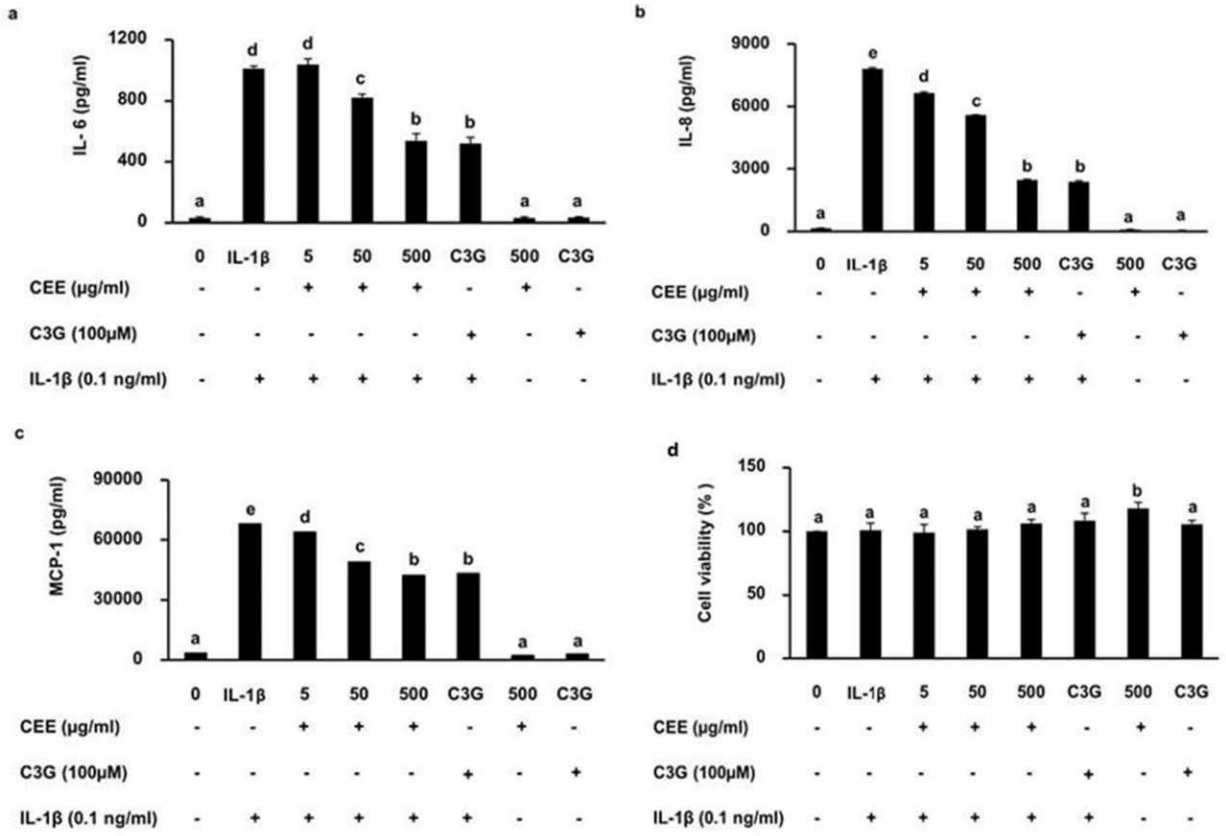

Figure 3. Effects of CEE or C3G on IL-1β-induced inflammatory mediators (IL-6, IL-8 and MCP-1) and cell viability in ARPE-19 cells. ARPE-19 cells were pretreated with 0, 50 and 500 μM of CEE or 100 μM of C3G for 1 h prior to stimulation with 0.1 ng/ml IL-1β for 24 h. (a) IL-6, (b) IL-8 and (c) MCP-1 concentrations in culture media determined by ELISA assay. (d) Cell viability was assessed by MTT assay. Results are presented as mean ± 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 occlusion[34-36]. Retinal pigment epithelial cells have been shown to secrete inflammatory mediators *in vitro* after stimulation with IL-1β[8]. The IL-1β is a pro-inflammatory cytokine that can trigger an inflammatory cascade and play a major role in retinal inflammation[27-28]. 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 neutrophils[38]. Previous studies have demonstrated that IL-6, IL-8 and MCP-1 not only initiate inflammatory responses but also promote angiogenesis, thereby stimulating AMD progression[39-41].

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

CEE was able to prevent the inflammatory effect caused by IL-1β 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-1β treatment, and mediating inflammation in ARPE-19 cells. This suggests that these cytokines and chemokines play a crucial part in retinal pigment epithelial inflammation[41]. 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-1β (**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-1β-stimulated increases in the production of IL-6, IL-8, and MCP-1 in retinal pigment epithelial cells[42-44]. 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 cells[45]. 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 chemokines[46-48]. 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 stimulants[49-51]. 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 cells[52-54]. 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-1β-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.

Acknowledgements

This work was financially supported by a grant from National Research Council of Thailand (NRCT) (695259 and 889918). The authors gratefully appreciate the cooperation of the Plant Genetic Conservation Project under the Royal initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG). We also thank Assistant Professor Dr. Thaya Jenjittikul for identifying the plant species. The authors would like to thank the Institute of Nutrition, Mahidol University, Thailand, for supporting and providing the location and instruments used in this study.

Conflict of interests

The authors have no conflicts of interest to declare.

References

1. Brooks P. Inflammation as an important feature of osteoarthritis. Bulletin of the World Health Organization. 2003;81(9):689-90.
2. Sun K, Tordjman J, Clément K, Scherer PE. Fibrosis and adipose tissue dysfunction. Cell metabolism. 2013;18(4):470-7.
3. Zou M, Zhang Y, Chen A, Young CA, Li Y, Zheng D, et al. Variations and trends in global disease burden of age‐related macular degeneration: 1990‐2017. Acta Ophthalmol. 2021;99(3):e330-e5.
4. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. The Lancet. 2018;392(10153):1147-59.
5. Nowak JZ. Age-related macular degeneration (AMD): pathogenesis and therapy. Pharmacol Rep. 2006;58(3):353.
6. Arya M, Sabrosa AS, Duker JS, Waheed NK. Choriocapillaris changes in dry age-related macular degeneration and geographic atrophy: a review. Eye and Vision. 2018;5(1):1-7.
7. Juhn SK, Jung M-K, Hoffman MD, Drew BR, Preciado DA, Sausen NJ, et al. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. Clin Exp Otorhinolaryngol. 2008;1(3):117-38.
8. Cheng S-C, Huang W-C, S. Pang J-H, Wu Y-H, Cheng C-Y. Quercetin inhibits the production of IL-1β-induced inflammatory cytokines and chemokines in ARPE-19 cells via the MAPK and NF-KB signaling pathways. Int J Mol Sci. 2019;20(12):2957.
9. Škrovánková S, Mišurcová L, Machů L. Antioxidant activity and protecting health effects of common medicinal plants. Adv Food Nutr Res. 2012;67:75-139.
10. Diniz do Nascimento L, Moraes AABd, Costa KSd, Pereira Galúcio JM, Taube PS, Costa CML, et al. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: New findings and potential applications. Biomolecules. 2020;10(7):988.
11. Dzoyem JP, Eloff JN. Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. J Ethnopharmacol. 2015;160:194-201.
12. Azarpazhooh E, Sharayei P, Zomorodi S, Ramaswamy HS. Physicochemical and phytochemical characterization and storage stability of freeze-dried encapsulated pomegranate peel anthocyanin and in vitro evaluation of its antioxidant activity. Food Bioproc Tech. 2019;12(2):199-210.
13. Jezek M, Zorb C, Merkt N, Geilfus C-M. Anthocyanin management in fruits by fertilization. J Agr Food Chem. 2018;66(4):753-64.
14. Meydan I, Kizil G, Demir H, Ceken Toptanci B, Kizil M. In vitro DNA damage, protein oxidation protective activity and antioxidant potentials of almond fruit (Amygdalus trichamygdalus) parts (hull and drupe) using soxhlet ethanol extraction. Adv Trad Med. 2020;20(4):571-9.

15. Shahreza FD. Oxidative stress, free radicals, kidney disease and plant antioxidants. Immunopathologia Persa. 2016;3(2):e11.
16. Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: Source of Antioxidants and Role in Disease Prevention. BoD–Books on Demand. 2018.
17. Kizawa Y, Sekikawa T, Kageyama M, Tomobe H, Kobashi R, Yamada T. Effects of anthocyanin, astaxanthin, and lutein on eye functions: a randomized, double-blind, placebo- controlled study. J Clin Biochem Nutr. 2021:20-149.
18. Cherlet A. The disease-fighting power of berries. Life Extension magazine. 2008;9:1-2.
19. Yang J, Cui J, Chen J, Yao J, Hao Y, Fan Y, et al. Evaluation of physicochemical properties in three raspberries (*Rubus idaeus*) at five ripening stages in northern China. Scientia Horticulturae. 2020;263:109146.
20. Tantratian S, Balmuang N, Krusong W. Phenolic enrichment of Ma-Kieng seed extract using absorbent and this enriched extract application for safety control of fresh-cut cantaloupe. LWT. 2019;106:105-12.
21. Prasansuklab A, Brimson JM, Tencomnao T. Potential Thai medicinal plants for neurodegenerative diseases: A review focusing on the anti-glutamate toxicity effect. J Tradi Compl Med. 2020;10(3):301-8.
22. Prasanth MI, Brimson JM, Chuchawankul S, Sukprasansap M, Tencomnao T. Antiaging, stress resistance, and neuroprotective efficacies of *Cleistocalyx nervosum* var. *paniala* fruit extracts using *Caenorhabditis elegans* model. Oxidative Med Cell Long. 2019;2019.
23. Brimson JM, Prasanth MI, Isidoro C, Sukprasansap M, Tencomnao T. *Cleistocalyx nervosum* var. *paniala* seed extracts exhibit sigma-1 antagonist sensitive neuroprotective effects in PC12 cells and protects *C. elegans* from stress via the SKN-1/NRF-2 pathway. Nutr Healthy Aging. 2021;6(2):131-46.
24. Sukprasansap M, Chanvorachote P, Tencomnao T. *Cleistocalyx nervosum* var. *paniala* berry fruit protects neurotoxicity against endoplasmic reticulum stress-induced apoptosis. Food Chem Tox. 2017;103:279-88.
25. Nantacharoen W, Baek SJ, Plaingam W, Charoenkiatkul S, Tencomnao T, Sukprasansap M. *Cleistocalyx nervosum* var. *paniala* Berry Promotes Antioxidant Response and Suppresses Glutamate-Induced Cell Death via SIRT1/Nrf2 Survival Pathway in Hippocampal HT22 Neuronal Cells. Molecules. 2022;27(18):5813.
26. Abdel‐Aal ES, Hucl P. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem. 1999;76(3):350-4.
27. Da Cunha A, Zhang Q, Prentiss M, Wu X, Kainz V, Xu Y, et al. The hierarchy of proinflammatory cytokines in ocular inflammation. Curr Eye Res. 2018;43(4):553-65.
28. Beaudry-Richard A, Nadeau-Vallée M, Prairie É, Maurice N, Heckel É, Nezhady M, et al. Antenatal IL-1-dependent inflammation persists postnatally and causes retinal and sub-retinal vasculopathy in progeny. Sci Rep. 2018;8(1):1-13.

29. Jansom C, Bhamarapravati S, Itharat A. Major anthocyanin from ripe berries of *Cleistocalyx nervosum* var. *paniala*. Thammasat Med J. 2008;8(3):364-70.
30. Charoensin S, Taya S, Wongpornchai S, Wongpoomchai R. Assessment of genotoxicity and antigenotoxicity of an aqueous extract of *Cleistocalyx nervosum* var. *paniala in vitro* and *in vivo* models. Interdiscip Toxicol. 2012;5(4):201.
31. Pipattanamomgkol P, Lourith N, Kanlayavattanakul M. The natural approach to hair dyeing product with *Cleistocalyx nervosum* var. *paniala*. Sustain Chem Pharm. 2018;8:88-93.
32. Chariyakornkul A, Juengwiroj W, Ruangsuriya J, Wongpoomchai R. Antioxidant extract from *Cleistocalyx nervosum* var. *paniala* pulp ameliorates acetaminophen-induced acute hepatotoxicity in rats. Molecules. 2022;27(2):553.
33. Prasanth MI, Sivamaruthi BS, Sukprasansap M, Chuchawankul S, Tencomnao T, Chaiyasut C. Functional properties and bioactivities of *Cleistocalyx nervosum* var. *paniala* berry plant: a review. Food Sci Technol. 2020;40:369-73.
34. Jonas JB, Wei WB, Xu L, Wang YX. Systemic inflammation and eye diseases. The Beijing Eye Study. PLoS One. 2018;13(10):e0204263.
35. Hu W-W, Huang Y-K, Huang X-G. Comparison of peripheral blood inflammatory indices in patients with neovascular age-related macular degeneration and haemorrhagic polypoidal choroidal vasculopathy. Ocul Immunol Inflamm. 2022:1-5.
36. Pan M, Zhou P, Liu Z, Guo J, Du L, Jin X. Peripheral complete blood cell count indices and serum lipid levels in polypoidal choroidal vasculopathy. Clin Exp Optom. 2022:1-6.
37. Yoshimura T. The production of monocyte chemoattractant protein-1 (MCP-1)/CCL2 in tumor microenvironments. Cytokine. 2017;98:71-8.
38. Osuka K, Ohmichi Y, Ohmichi M, Nakura T, Iwami K, Watanabe Y, et al. Sequential Expression of Chemokines in Chronic Subdural Hematoma Fluids after Trepanation Surgery. J Neurotrauma. 2021;38(14):1979-87.
39. Sato T, Takeuchi M, Karasawa Y, Enoki T, Ito M. Intraocular inflammatory cytokines in patients with neovascular age-related macular degeneration before and after initiation of intravitreal injection of anti-VEGF inhibitor. Sci Rep. 2018;8(1):1-10.
40. Terao N, Koizumi H, Kojima K, Yamagishi T, Yamamoto Y, Yoshii K, et al. Distinct aqueous humour cytokine profiles of patients with pachychoroid neovasculopathy and neovascular age-related macular degeneration. Sci Rep. 2018;8(1):1-10.
41. Tan W, Zou J, Yoshida S, Jiang B, Zhou Y. The role of inflammation in age-related macular degeneration. Int J Biol Sci. 2020;16(15):2989.
42. Shukla R, Pandey V, Vadnere GP, Lodhi S. Role of flavonoids in management of inflammatory disorders. Bioactive food as dietary interventions for arthritis and related

inflammatory diseases: Elsevier; 2019. p. 293-322.

43. Valdez JC, Bolling BW. Anthocyanins and intestinal barrier function: A review. J Food Bioact. 2019;5:18-30.
44. Stromsnes K, Correas AG, Lehmann J, Gambini J, Olaso-Gonzalez G. Anti-inflammatory properties of diet: Role in healthy aging. Biomedicines. 2021;9(8):922.
45. Iwashima T, Kudome Y, Kishimoto Y, Saita E, Tanaka M, Taguchi C, et al. Aronia berry extract inhibits TNF-α-induced vascular endothelial inflammation through the regulation of STAT3. Food & Nutr Res. 2019;63.
46. Islam SU, Lee JH, Shehzad A, Ahn E-M, Lee YM, Lee YS. Decursinol angelate inhibits LPS-induced macrophage polarization through modulation of the NFΚB and MAPK signaling pathways. Molecules. 2018;23(8):1880.
47. Jayasinghe AMK, Kirindage KGIS, Fernando IPS, Han EJ, Oh G-W, Jung W-K, et al. Fucoidan isolated from sargassum confusum suppresses inflammatory responses and oxidative stress in tnf-α/ifn-γ-stimulated hacat keratinocytes by activating nrf2/ho-1 signaling pathway. Mar Drugs. 2022;20(2):117.
48. Chen Y, Shou K, Gong C, Yang H, Yang Y, Bao T. Anti-inflammatory effect of geniposide on osteoarthritis by suppressing the activation of p38 MAPK signaling pathway. BioMed Rres Int. 2018;2018.
49. Qiu T, Sun Y, Wang X, Zheng L, Zhang H, Jiang L, et al. Drum drying-and extrusion-black rice anthocyanins exert anti-inflammatory effects via suppression of the NF-ΚB/MAPKs signaling pathways in LPS-induced RAW 264.7 cells. Food BioSci. 2021;41:100841.
50. Jung S, Lee M-S, Choi A-J, Kim C-T, Kim Y. Anti-inflammatory effects of high hydrostatic pressure extract of mulberry (Morus alba) fruit on LPS-stimulated RAW264. 7 cells. Molecules. 2019;24(7).
51. Amin FU, Shah SA, Badshah H, Khan M, Kim MO. Anthocyanins encapsulated by PLGA@ PEG nanoparticles potentially improved its free radical scavenging capabilities via p38/JNK pathway against Aβ1–42-induced oxidative stress. J Nanobiotechnol. 2017;15(1):1-16.
52. Aboonabi A, Aboonabi A. Anthocyanins reduce inflammation and improve glucose and lipid metabolism associated with inhibiting nuclear factor-kappaB activation and increasing PPAR-γ gene expression in metabolic syndrome subjects. Free Radical Biology and Medicine. 2020;150:30-9.
53. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61(1):1361779.
54. Krga I, Tamaian R, Mercier S, Boby C, Monfoulet L-E, Glibetic M, et al. Anthocyanins and their gut metabolites attenuate monocyte adhesion and transendothelial migration through nutrigenomic mechanisms regulating endothelial cell permeability. Free Radic Biol Med. 2018;124:364-79.