ฤทธิ์ต้านการอักเสบของสารสกัดและส่วนที่ผ่านกระบวนการย่อยภายในร่างกายของลูกหม่อนต่อเซลล์จอประสาทตามนุษย์ที่เหนี่ยวนำให้เกิดการอักเสบด้วยอินเตอร์ลิวคิน-1 เบต้า

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บทคัดย่อ

การอักเสบของเซลล์เยื่อบุจอประสาทตาเป็นปัจจัยสำคัญที่เหนี่ยวนำให้เกิดภาวะจอประสาทตาเสื่อมซึ่งนำไปสู่ความบกพร่องทางการมองเห็นและตาบอดในผู้สูงอายุ ดังนั้น การป้องกันการเสื่อมของเซลล์ดังกล่าวจากการอักเสบอาจป้องกันหรือชะลอความรุนแรงของภาวะจอประสาทตาเสื่อมได้ ลูกหม่อนอุดมไปด้วยสารออกฤทธิ์ทางชีวภาพมากมายรวมทั้งสารแอนโทไซยานิน ซึ่งมีคุณสมบัติทางชีวภาพที่หลากหลาย โดยเฉพาะฤทธิ์ต้านการอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อประเมินฤทธิ์การต้านอักเสบของสารสกัดจากลูกหม่อน (ME) และลูกหม่อนที่ผ่านกระบวนการย่อยภายในร่างกาย (BFM) ต่อเซลล์จอประสาทตามนุษย์ชนิด ARPE-19 ที่ถูกเหนี่ยวนำให้เกิดการอักเสบด้วยอินเตอร์ลิวคิน-1 เบต้า (IL-1β) เซลล์ ARPE-19 ถูกเลี้ยงด้วย ME ที่ความเข้มข้น 10-500 ไมโครกรัมต่อมิลลิลิตร หรือ BFM ที่ถูกเจือจางด้วยอาหารเลี้ยงเซลล์มาตรฐานในอัตราส่วน 1:3 เป็นเวลา 1 ชม. ก่อนถูกเหนี่ยวนำด้วย IL-1β เป็นเวลา 24 ชม. ผลการศึกษาแสดงให้เห็นว่า ME และ BFM สามารถลดระดับของ IL-6 IL-8 และ MCP-1 ในเซลล์จอประสาทตาที่ถูกเหนี่ยวนำให้เกิดการอักเสบได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) และไม่เป็นพิษต่อเซลล์ งานวิจัยนี้แสดงให้เห็นว่าลูกหม่อนสามารถปกป้องเซลล์จอประสาทตาของมนุษย์จากการกระตุ้นให้เกิดการอักเสบซึ่งเป็นสาเหตุของการเกิดภาวะจอประสาทตาเสื่อมได้ และลูกหม่อนที่ผ่านกระบวนการย่อยภายในร่างกายแล้วก็ยังคงความสามารถป้องกันการอักเสบของเซลล์จอประสาทตาได้ อย่างไรก็ตามควรมีการศึกษาผลของลูกหม่อนต่อภาวะจอประสาทตาเสื่อมโดยศึกษาในสัตว์ทดลองและในมนุษย์ต่อไป

คำสำคัญ: ลูกหม่อน การอักเสบ ภาวะจอประสาทตาเสื่อมตามสูงอายุ การจำลองการย่อยของร่างกาย เซลล์จอประสาทตามนุษย์

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Anti-Inflammatory Activity of Extraction and Bioaccessible Fraction of Mulberry against IL-1β-Induced Inflammation in Human Retinal Pigment Epithelial Cells

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Abstract

The inflammation of retinal pigmented epithelium cells (RPE) is a strong risk factor inducing age-related macular degeneration (AMD) leading to visual impairment and blindness among the elderly. Therefore, protecting RPE cells from inflammation may protect or delay the progression of AMD development. Mulberry fruits are rich in bioactive compounds including anthocyanin which exerts many biological activities, especially anti-inflammatory properties. This study aims to assess the anti-inflammatory activity of mulberry extract (ME) and bioaccessible fraction from simulated gastrointestinal digestion of mulberry (BFM) on interleukin-1β (IL-1β) induced inflammation in human RPE (ARPE-19) cells. Pre-treating ARPE-19 cells with ME at concentration 10-500 µg/ml or BFM diluted 1:3 (v/v) with basal media for 1 h prior to stimulation with IL-1β for another 24 h. Results showed that ME and BFM significantly reduced the secretion of pro-inflammatory mediators (IL-6, IL-8, and MCP-1) levels ($p < 0.05$) without toxicity in ARPE-19 cells induced inflammation. These findings indicated that ME could protect human retina cells from inflammatory induction, which causes AMD development. The portion of mulberry that underwent simulated digestion retained its ability to protect ARPE-19 cells from inflammatory induction. However, further research is necessary to investigate the impact of mulberry on AMD, incorporating both experimental animal models and human subjects.

Keywords: Mulberry, Inflammation, Age-related macular degeneration, Simulated digestion, ARPE-19 cells

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Introduction

According to data from United Nations 2017, global citizens aged more than 60 have been increasing around 3% per year and will continue to increase[1]. Such countries are ongoing into aging society; elders will be the majority of the population which is linked to increased incidence of age-related diseases. Most of the major age-related diseases are eye diseases, many of which lead to irreversible vision impairment, including age-related macular degeneration (AMD)[2-4]. AMD is an incurable ocular disease causing loss of central and/or night vision and finally leads to blindness in the elders[5,6]. The early pathogenesis of AMD involves the accumulation of intracellular toxic lipofuscin and the build-up of lipid and protein deposits, known as retinal drusen that are signs of inflammation as they contain many inflammation-associated components that are considered clinical hallmarks of AMD[7,8]. Advanced AMD presents in two groups: dry form (geographic atrophic) is characterized by the development of drusen, and a more severe wet (neovascular) form is characterized by choroidal neovascularization (CNV)[9].

The activation of the immune system takes place during AMD progression including resident immune cell activation, the production of inflammatory mediators, and the recruitment of peripheral immune cells to the retina[10]. Interleukin (IL)-1β is a potent pro-inflammatory cytokine playing a key role in inflammatory response. Activated macrophages play a role by secreting mature IL-1β into the extracellular environment. After IL-1β stimulation, retinal pigment epithelial cells (RPE) cells further modulate inflammatory-signal molecules, thus enhancing the production of other proinflammatory mediators such as IL-6, IL-8, and monocyte chemotactic protein (MCP-1) which play a vital role in inflammatory response[11]. In human AMD patients, the level of several inflammatory cytokines and chemokines was increased in ocular fluids or tissues[12]. Therefore, the protection of RPE cells from chronic inflammation may be a potential preventive strategy against AMD development[13,14].

Currently, scientists and health concerns people are greatly interested in dietary phytochemicals which exert many biological and pharmacological properties. Numerous studies have reported that phytochemicals possess remarkable antioxidant and anti-inflammatory functions[15-17]. *Morus* spp., commonly called mulberry, is widely cultivated in many regions, especially in Asian countries. In Thailand, mulberry fruits are widely grown in the Northern and Northeast regions and are well known as an

excellent source of anthocyanins. They are used in Chinese traditional medicine due to their wide range of biological and pharmacological activities[18], including antioxidant capacity, anti-inflammation, anti-obesity, hypolipidemic, anti-diabetic, anticancer, neuroprotective effect, and protective against pancreatic and hepatic damage[18-21]. Previous studies demonstrated that mulberry fruits and their bioactive compounds had cytoprotective effects against hydrogen peroxide (H_2O_2)-induced oxidative damage pancreatic β-cells via modulation of pro-apoptotic processes[22,23]. Another study showed that mulberry extract could protect human hepatocellular carcinoma cell lines (HepG2 cells) against ethyl carbamate-induced cytotoxicity and oxidative stress[24]. A recent study reported that mulberry fruit extract suppressed lipopolysaccharide (LPS)-induced increase of inducible nitric oxide synthase (iNOS) expression and NO production in a murine macrophage cell line (RAW 264.7 cells)[25]. However, the anti-inflammatory activities of mulberry extract on RPE cells have never been reported. Therefore, this study aims to investigate the anti-inflammatory effects of the ethanol extract and bioaccessible fraction following simulated digestion treatment from mulberry extract on IL-1β induced inflammation in human retinal pigment epithelial cells.

Materials and Methods

Chemicals

Dulbecco's modified eagle's medium (DMEM) /F-12 powder and 0.25% Trypsin-EDTA (1X) were bought from Life Technologies Corporation, Grand Island, NY, USA. Fetal Bovine Serum (FBS) was purchased from PAN-Biotech (South America). Penicillin-Streptomycin was bought from Caisson Laboratories (Smithfield, UT, USA). 3-[4,5-dimethyltiazol-2-yl]- 2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Bio Basic Inc. (Markham, ON, Canada). Recombinant Human IL-1β and ELISA kits were obtained from BioLegend Way (San Diego, CA, USA). The solvents and reagents were purchased from commercial sources and used analytical grade.

Sample preparation

Ripe mulberry (*Morus alba* var. Chiang Mai) fruits were harvested from farms under the royal factories representing four regions (North, Northeast, Central, and South) of Thailand. After collection, the fruits were washed with tap water twice, air-dried, and then blended with an electric kitchen blender, before lyophilization. The dried samples were ground to a fine powder. An equal amount of dry sample from each region was pooled together and thoroughly mixed before being packed in

aluminum foil bags under vacuum, then kept at -20 °C until usage.

Sample extraction from ethanol

The freeze-dried samples (1 g) were extracted with 15 ml of 70% ethanol at the ratio 1:15 weight/volume 3 times[26] before being thoroughly shaken by a vortex mixer for 1 min followed by sonication in an ultrasonic bath for 15 min and centrifugation at 4,140 g at 25 ºC for another 10 min and transferred clear supernatant to the flat- bottom flask. Extraction residues were repeated twice and collected aqueous fraction. The supernatants were pooled and evaporated until dry by using a vacuum rotary evaporator at 35 – 40 °C. The film extract in a round bottom flask was solubilized with 2 ml of absolute ethanol before being removed to an amber vial and dried with nitrogen gas. The extracts were stored in the dark at -20 °C until further used. The extraction yield of ME is 33.6 ± 0.7%.

In vitro simulated digestion

The simulated digestion of gastric and small intestinal phases of the mulberry extract was carried out according to Ferruzzi et al.[27] and Chitchumroonchokchai et al.[28]. Briefly, the gastric phase, 1.0 g of freeze-dried mulberry extract were mixed with 20 ml of 120 mM NaCl in 50 ml polypropylene tube by a homogenizer (model T25D, Germany). The mixture was adjusted to a pH of 2.0 ± 0.1 using 1 M HCl. Then, 2 ml of pepsin (40 mg/ml in 100 mM HCl) was added up the volume to 40 ml with 120 mM NaCl. The tube was blanked with nitrogen gas, tightly capped and sealed with parafilm, and incubated in a shaking incubator at 37 °C, 95 rpm for 1 h. After completion of the gastric phase, the sample was adjusted pH to 6.0 ± 0.1 with 1 M sodium bicarbonate (Na_2HCO_3). Then, 3 ml of crude bile extract (40 mg/ml in 100 mM Na_2HCO_3), 2 ml of pancreatin (10 mg/ml in 100 mM Na_2HCO_3), and 2 ml of lipase (5 mg/ml in 100 mM Na_2HCO_3) were added to the reaction tube. The mixture was finally adjusted to a pH of 6.5 ± 0.1 with 1 M Na_2HCO_3. Then, the tube was filled with nitrogen gas, tightly capped, and sealed with parafilm before putting in a shaking water bath for 2 h at 37 ºC, 95 rpm.

The solution after completed gastric and small intestinal phases digestion was centrifuged (Becton Dickinson Dynac Centrifuge, Sparks, MD, U.S.A.) at 4,000g for 1 h at room temperature for isolation of aqueous fraction (supernatant). Control digestion without mulberry extract was also conducted to assess the possible cytotoxic effects of digested compounds in the aqueous fraction. The supernatant was collected and filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter membrane (Millipore Corp., Cork, Ireland)

to obtain the bioaccessible fraction of mulberry (BFM). The BFM was used to assess anti-inflammatory activity in ARPE-19 cells.

The cell culture

Adult human retinal pigment epithelial (ARPE-19) cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were routinely maintained and cultured in complete media which consisted of Dulbecco's modified Eagle's Medium (DMEM)/F12 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), and 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2/95% air[29]. The adherence cells reached the confluency of 9 0% and were then used for subsequent experiments.

Cell viability assay

The viability of the cells was assessed by MTT assay. Briefly, Cells were seeded and treated with specific condition treatments. After incubation, the media was removed carefully and washed with phosphate-buffered saline (PBS) before adding (0.5 mg/ml in PBS) MTT solution in each well and incubated for 3-4 h. Dimethyl sulfoxide (DMSO) was added into each well and then shaken for 5 min to solubilize the purple formazan crystals from viable cells. The absorbance of soluble formazan was read at the wavelength of 540 nm by a microplate reader (BioTek® Instruments, Vermont, USA)[30]. The amount of viability of cells is directly proportionated to the intensity of optical density. The cell viability was reported as a percentage relative to the control cells.

Evaluation of cytotoxicity of ME

The non-toxic concentration of ME was assessed by viability test before conducting further experiments. ARPE-19 cells were seeded at a density of 1.0×10^5 cells/well in a 48-well plate and allowed to incubate for 24 h. Cell monolayers were washed with excess serum-free media, and pre-treated with ME (10, 50, 100, 200, and 500 µg/ml) in 0.5% DMSO in serum-free media for 24 h. Then, cell viability was evaluated by MTT assay.

Evaluation of suitable IL-1β concentration for inflammatory induction

ARPE-19 cells were seeded at a density of 1.0×10^5 cells/well in a 48-well plate and allowed to incubate for 24 h. Cells were washed with basal media and were added with basal media containing 0.5% DMSO. After incubation for 1 h, cells were incubated with IL-1β at different concentrations (0.1, 1, 2, 5, and 10 ng/ml) for 24 h. After IL-1β stimulation, cell culture supernatants were collected to

measure IL-6 and IL-8 levels using an ELISA kit, and cells were determined the viability using MTT colorimetric assay.

Evaluation of IL-8, IL-6 and MCP-1 levels by ELISA

Determination of the ME and BFM having the protective effect on ARPE-19 cells stimulated inflammation by IL-1β were evaluated by percentage of cell viability with MTT assay and cytokines secretion with ELISA. The cells were seeded at a density of 1.0×10^5 cells/well in a 48-well plate and incubated under 5% CO_2 in a humidified atmosphere at 37 ºC for 24 h. Then cells were washed with basal media and pre-treated with ME at 1, 10, and 100 µg/ml in 0.5% DMSO in serum-free media, or BFM diluted 1:3 (v/v) with basal media for 1 h. Then, cells were induced with appropriate IL-1β concentrations for 24 h. After IL-1β stimulation, a culture medium was collected to determine the levels of IL-6, IL-8, and MCP-1 using an ELISA kit. In brief, high-binding 96-well plates were incubated overnight with capture antibodies against IL-6, IL-8, and MCP-1 at 25°C. After washing with 0.05% Tween-20 in PBS (PBST), the unbound sites were blocked with 1% bovine serum albumin (BSA) in PBS for 1 h at 25°C. After washing with PBST, culture medium or recombinant human IL-6, IL-8, and MCP-1 standards were added to each well and incubated for 2 h at 25°C before the addition of biotinylated detecting antibodies to each well. After incubation and washing with PBST, the immune complex was detected with streptavidin HRP–tetramethylbenzidine detection system by incubating at 25 °C for 30 min. Reactions were terminated with 2M H_2SO_4 and the absorbance at 450 nm was measured by a microplate reader. The concentration of IL-6, IL-8, and MCP-1 in sample solutions was calculated by comparing absorbance with their standard curve.

Statistical analysis

All data in this study were presented as mean ± standard deviations (SD) from at least three independent experiments. One-way analysis of variance (ANOVA) when appropriate followed by Duncan's New Multiple Range Test was performed using SPSS statistical software, version 19. Differences with a *p*-value of less than 0.05 ($p<0.05$) were considered statistically significant.

Results

Evaluation of ME effect on cell viability of ARPE-19 cells

To choose the appropriate concentrations of ME that could be used without affecting ARPE-19 cell viability, the cells were treated with ME (10-500 µg/ml)

for 24 h, followed by MTT assay. The results showed that, above 100 µg/ml, ME significantly decreased cell viability compared to the vehicle control group as shown in Figure 1. Therefore, ME at 100 µg/ml was then chosen for subsequent experiments to assess their anti-inflammatory activities on ARPE-19 cells.

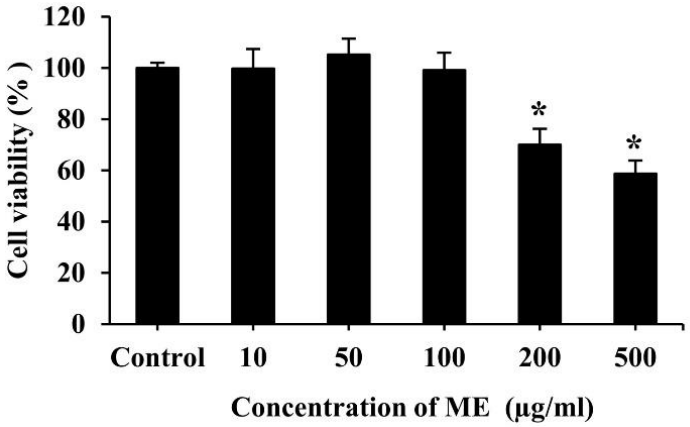

Figure 1. Effects of ME on cell viability of ARPE-19 cells. ARPE-19 cells were treated with ME at 10, 50, 100, 200, and 500 µg/ml for 24 h. Controls were cells treated with 0.5% DMSO in serum-free media. The cell viability was measured by MTT assay. The results are presented as mean ± SD of at least three independent experiments. *p<0.05 indicates significant differences from the control group.

Effect of IL-1β on cell viability and pro-inflammatory secretion of ARPE-19 cells

IL-1β is a major inflammatory mediator cytokine that is implicated in retinal inflammation[31]. Thus, IL-1β was used to induce inflammation of ARPE-19 cells in this study. The cells were treated with IL-1β at concentrations 0.1, 1, 2, 5, and 10 ng/ml for 24h. The data showed that all concentrations of IL-1β had a non-cytotoxic effect on ARPE-19 cells (Figure 2A), and also IL-1β could significantly induce the secretion of IL-6 and IL-8 when compared to the control group (Figure 2B and 2C). We therefore used the lowest concentration of IL-1β (0.1 ng/ml) for IL-1β induced inflammation in ARPE-19 cells.

Effects of ME and BFM on IL-6, IL-8 and MCP-1 secretion in IL-1β-induced ARPE-19 cells

It is known that pro-inflammatory mediators, mainly IL-6, IL-8, and MCP-1 are secreted by RPE cells during the inflammatory process[32]. We next measured the levels of inflammatory mediators (IL-6, IL-8, and MCP-1) in cultured ARPE-19 cells following different treatment by ELISA. Exposure of ARPE-19 cells to IL-1β significantly produced IL-6, IL-8, and MCP-1 compared to the control (p<0.05)

(Figure 3). However, pre-incubation with ME could suppress IL-1β-induced IL-6, IL-8, and MCP-1 secretion in a dose-dependent manner when compared to the IL-1β group (ARPE-19 cells treated with IL-1β alone) (p<0.05) (Figure 3A) without cytotoxicity (Figure 4A). Also, the pre-treatment of BFM (diluted 1:3 (v/v) with basal media) could significantly decrease the level of IL-6, IL-8, and MCP-1 when compared to the IL-1β group (p<0.05) (Figure 3B) without toxicity (Figure 4B). These results indicated that ME could protect ARPE-19 cells from inflammatory induction in a dose-dependent manner without exhibiting toxicity. Moreover, the portions of mulberry that endured *in vitro* simulated digestion continued to protect ARPE-19 cells from inflammation without causing toxicity**.**

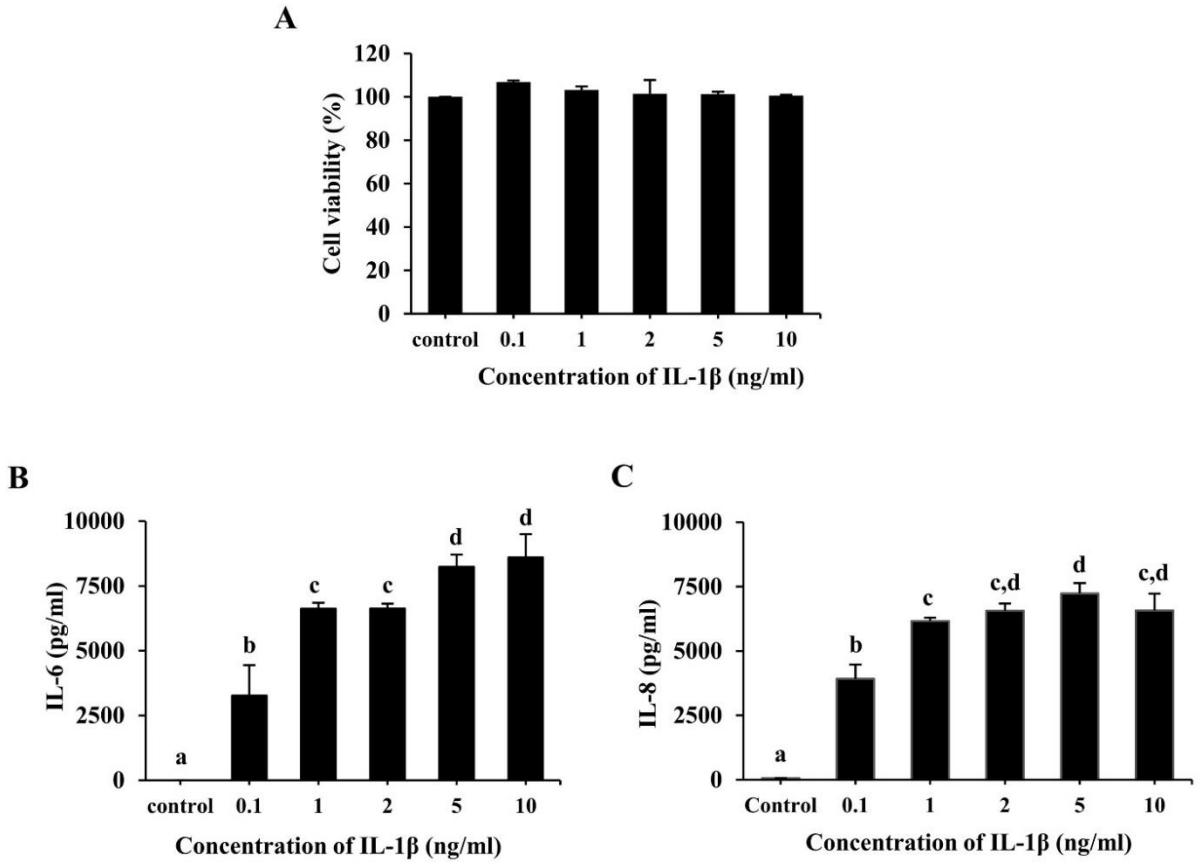

Figure 2. Effect of IL-1β on cell viability and pro-inflammatory secretion of ARPE-19 cells. ARPE-19 cells were treated with 0.1-10 ng/ml IL-1β for 24 h. Controls were cells treated with 0.5% DMSO in serum-free media. (A) The cell viability was measured by MTT assay. (B) IL-6 and (C) IL-8 levels in supernatants from the experiment were measured by ELISA. The results are presented as mean ± SD of at least three independent experiments. Different superscripts above the error bar (a-d) indicate statistically significant differences at p<0.05 from each column.

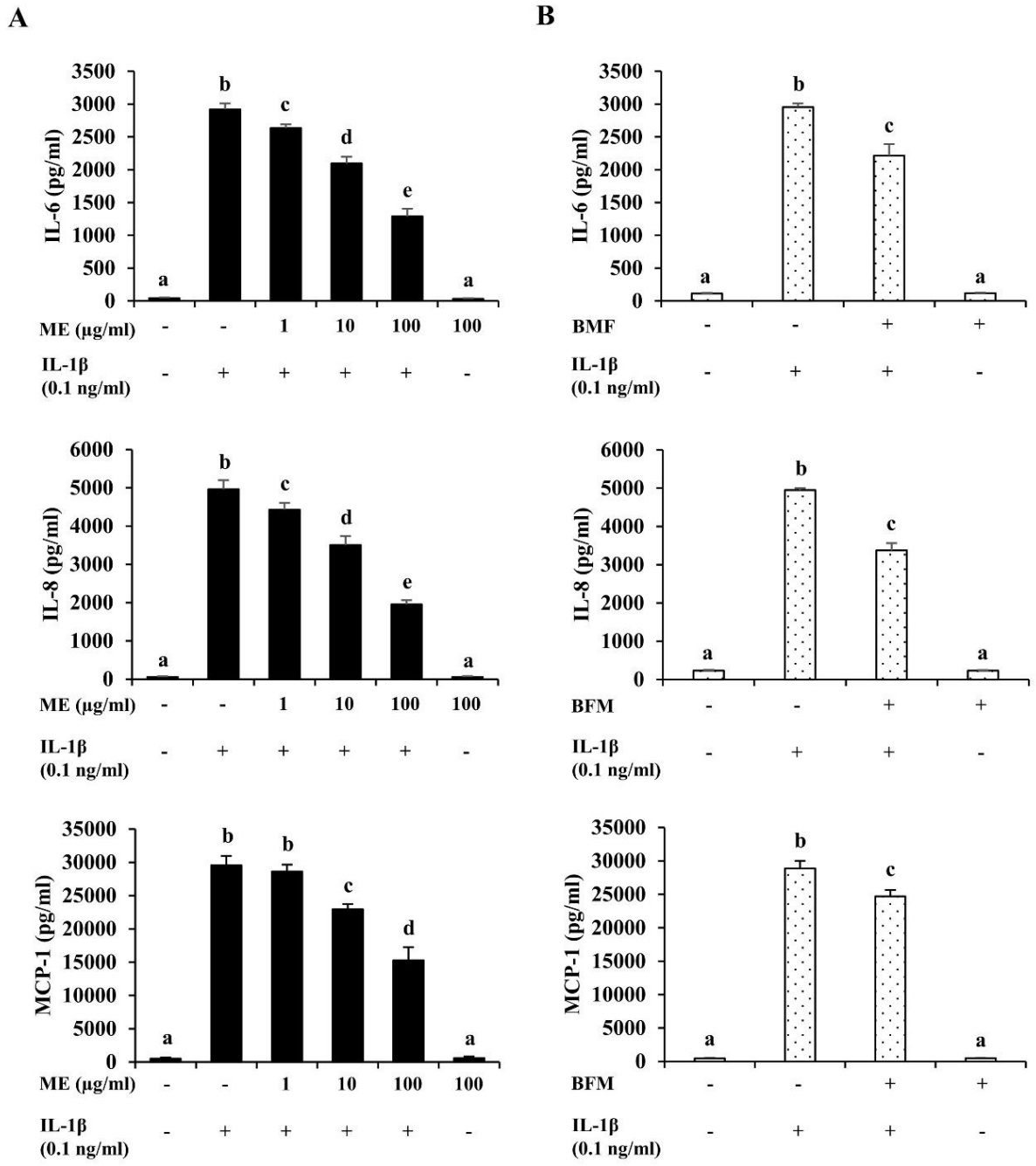

Figure 3. Effects of ME and BFM on IL-6, IL-8, and MCP-1 secretion from IL-1β-induced inflammation in ARPE-19 cells. ARPE-19 cells were treated with (A) 1, 10, 100 µg/ml of ME or (B) BFM diluted 1:3 (v/v) with basal media for 1 h, followed by IL-1β 0.1 ng/ml for 24 h. Controls were cells treated with 0.5% DMSO and bioaccessible fraction without ME for ME and BFM, respectively. The concentrations of IL-6, IL-8, and MCP-1 in supernatants were detected by ELISA. The results are presented as mean ± SD of at least three independent experiments. Different superscripts above the error bar (a-e) indicate statistically significant differences at $p<0.05$ from each column.

Discussion

Prolonged inflammation or chronic inflammation may be developed after a recurrent or persistent acute inflammatory process which is involved in the pathological development of many age-related degenerative diseases, such as AMD[33]. During ocular inflammation, IL-1β is an early inflammatory cytokine secreted by immune functioning cells that triggers RPE by binding IL-1β with its receptor on the RPE cell surface to produce other proinflammatory mediators and recruit remote inflammatory cells into the retina. They may contribute to RPE cells and local ocular cell damage resulting in retina impairment and degeneration[11,12,34]. Additionally, high IL-1β levels have been detected in the vitreous humor of AMD patients[35] and contribute to macular degeneration in different animal models[36]. In the present study, IL-1β was also used to stimulate inflammatory damage in ARPE-19 cells. Numerous studies have reported that human RPE cells stimulated by IL-1β were found with an increase of inflammatory mediator (IL-6, IL-8, MCP-1) releases[12,34,37-39] via activation of NF-κB and MAPK signaling pathways [(12,34)]. Previous *in vivo* studies found IL-6[40], IL-8[41,] and MCP-1[42] in the ocular fluids, and their levels correlated with the severity of ocular inflammatory diseases[43]. Thus, the reduction of IL-6, IL-8, and MCP-1 levels might be an alternative approach to protect or delay the pathological progression of AMD development. Nowadays, scientists and health-conscious people are greatly interested in antioxidant and anti-inflammatory-rich diets that possess various biological and pharmacological properties to use as natural compounds instead of synthetic supplements and drugs to avoid undesirable adverse side effects. A growing body of evidence coming from *in vitro* and *in vivo* studies suggested the essential role of dietary anthocyanins, the plant-food phytochemicals, in the prevention and treatment of eye health[44-47]. *Morus alba* L., commonly known as mulberry, is widely planted in the Northern and Northeast of Thailand[48]. Many scientific studies have reported that mulberry fruit has high nutritive value and is rich in anthocyanins. Anthocyanins in mulberry fruit have been hypothesized as a phytotherapeutic agent due to their antioxidant and anti-inflammatory activities[49-51]. However, to be beneficial in human, these components must be able to exert their biological activities following digestion.

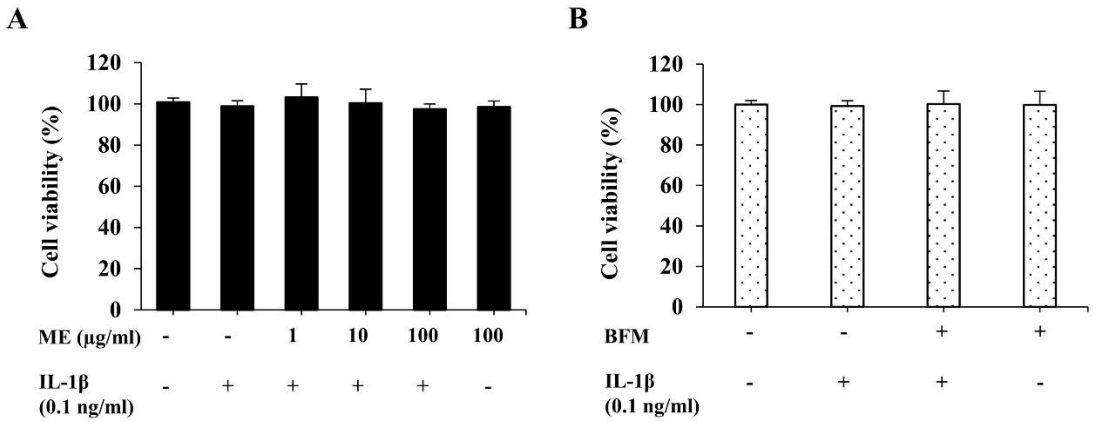

Figure 4. Effects of ME and BFM on cell viability in ARPE-19 cells induced inflammation with IL-1β. ARPE-19 cells were treated with (A) 1, 10, 100 µg/ml of ME or (B) BFM diluted 1:3 (v/v) with basal media for 1 h, followed by IL-1β 0.1 ng/ml for 24 h. Controls were cells treated with 0.5% DMSO and bioaccessible fraction without ME for ME and BFM, respectively. The cell viability was measured by MTT assay. The results are presented as mean ± SD of at least three independent experiments.

This study showed that pretreatment of ARPE-19 cells with an ethanol extract of mulberry extract or bioaccessible fraction of mulberry extract significantly attenuated IL-1β-induced pro-inflammatory mediators IL-6, IL-8, and MCP-1 production. These results were consistent with a recent publication demonstrating that the ethanol extract from mulberry fruits exerted an anti-inflammatory effect on LPS-induced NO production and iNOS expression in the murine macrophage cell line (RAW 264.7)[25]. Anthocyanin from mulberry extract significantly decreased the expression of the inflammatory genes including IL-6, TNF-α, iNOS, and NF-κB in high-fat diet (HFD) induced obese mice[52]. Importantly, anthocyanin can be found in the eye meaning that it can pass through the gastro-intestinal tract and the blood-retina barrier as they can be detected in both rats and rabbit's ocular tissues after oral administration of blackcurrant extract[47]. There is evidence that anthocyanin-rich bilberry extract may suppress the rhodopsin reduction and inhibit the production of IL-6 and NF-κB p65 in the mouse model of retinal inflammation[44]. Similar findings were reported using an *in vivo* model of retinal damage whereby bilberry extract was shown to inhibit visible light-induced increase of levels of IL-6 protein and NF-κB mRNA[53]. Interestingly, some previous studies found that cyanidin-3 glucoside from blackcurrant can regenerate visual pigment (rhodopsin) in frog retina[54]. A randomized, double-blind, placebo-controlled study reported that daily intake of bilberry anthocyanin-rich extract improved eye fatigue by relaxing the muscle

in the pupillary response[55] and ciliary muscle[56]. Therefore, the present study indicates that mulberry may serve as an alternative dietary supplement to mitigate or decelerate the transition from dry to wet AMD. However, additional research is necessary to elucidate the precise mechanisms through which mulberry affects IL-1β-induced ARPE-19 cells**.**

Conclusion

In conclusion, the present study demonstrated that both the extract and the bioaccessible fraction of mulberry exhibit anti-inflammatory effects in human retinal cells with induced inflammation, which contributes to the development of AMD. These effects are characterized by the reduced production of IL-6, IL-8, and MCP-1, without inducing toxicity. This suggests that daily consumption of mulberry may help preserve and improve eye health. Although *in vitro* evidence supports the beneficial effects of mulberry on ocular health, further *in vivo* research on patients with inflammation-induced ocular damage is necessary to fully understand their biological functions. Additionally, more comprehensive studies are required, including those involving both experi-mental animal models and human subjects, to investigate the impact of mulberry on AMD further.

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Conflict of Interest

The authors declare no conflict of interest.

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