ผลการปกป้องของสารสกัดข้าวโพดหวานในเซลล์จอประสาทตามนุษย์ที่เหนี่ยวนำให้เกิดภาวะเครียดออกซิเดชันด้วยไฮโดรเจนเปอร์ออกไซด์

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บทคัดย่อ

ภาวะจอประสาทตาเสื่อมตามอายุ (age-related macular degeneration หรือ AMD) เป็นภาวะที่เกี่ยวข้องกับเซลล์จอประสาทตาซึ่งมีผลต่อบริเวณจุดภาพชัดบริเวณเรตินา เป็นสาเหตุของความบกพร่องในการมองเห็นและส่งผลให้เกิดอาการตาบอด ปัจจัยเสี่ยงของการเกิดภาวะจอประสาทตาเสื่อมตามอายุที่สำคัญ คือ การเกิดภาวะเครียดออกซิเดชัน ข้าวโพดหวานมีสารพฤกษเคมีหลายชนิดที่มีฤทธิ์ในการต้านอนุมูลอิสระ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดจากข้าวโพดหวานต้มในการป้องกันเซลล์จอประสาทตาที่เหนี่ยวนำให้เกิดการตายจากภาวะเครียดออกซิเดชันด้วยไฮโดรเจนเปอร์ออกไซด์ เซลล์จอประสาทตาถูกเลี้ยงด้วยอาหารที่มีส่วนผสมของสารสกัดจากข้าวโพดหวานต้มที่ความเข้มข้น 1, 10 และ 100 ไมโครกรัมต่อมิลลิลิตร เป็นเวลา 24 ชั่วโมง จากนั้นเหนี่ยวนำให้เซลล์เกิดการตายภาวะเครียดออกซิเดชันด้วยไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น 400 ไมโครโมลาร์ เป็นเวลา 60 นาที ผลการศึกษาพบว่า สารสกัดจากข้าวโพดหวานต้มที่ความเข้มข้น 10 และ 100 ไมโครกรัมต่อมิลลิลิตร สามารถป้องกันการตายของเซลล์จอประสาทตาที่เหนี่ยวนำให้เกิดการตายได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) โดยการลดการสร้างอนุมูลอิสระ (ROS) เพิ่มระดับของสารต้านอนุมูลอิสระภายในเซลล์ (SOD, CAT, GPx และ GSH) และลดระดับการทำงานและการแสดงออกของโปรตีนที่เหนี่ยวนำให้เซลล์เกิดการตายในแบบอะพอพโทซิส (Bax, Bcl-2, caspase-9 และ caspase-3) ซึ่งเป็นไปในแบบแปรผันตรงกับความเข้มข้นของสารสกัด อย่างไรก็ตามข้อมูลที่ได้นี้ก็เป็นเพียงการทดสอบในหลอดทดลองเท่านั้น การที่จะได้ถึงข้อมูลในด้านของประโยชน์ต่อสุขภาพโดยเฉพาะการป้องกันการเกิดภาวะจอประสาทตาเสื่อมของข้าวโพดหวานต้มที่ชัดเจนและสมบูรณ์กว่านี้ควรทำการศึกษาในสัตว์ทดลองและมนุษย์ต่อไป

คำสำคัญ: ภาวะจอประสาทตาเสื่อมตามอายุ ภาวะเครียดออกซิเดชัน เซลล์จอประสาทตามนุษย์ ข้าวโพดหวาน สารต้านอนุมูลอิสระ

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Protective Effects of Sweet Corn Extract Against H_2O_2-Induced Oxidative Stress in Human Retinal Pigment Epithelial (ARPE-19) Cells

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Abstract

Age-related macular degeneration or AMD is an eye disease from the macular area of retina, which is a cause of impaired vision and irreversible blindness in the elderly population. The risk factor of AMD is oxidative stress. Sweet corn contains many phytochemicals that are beneficial to health, which is an important source of substances in the carotenoid group. Especially lutein and zeaxanthin, which have antioxidant properties. The objective of this study was to evaluate the effect and mechanism of boiled sweet corn extract and lutein in the human retinal pigment epithelial (ARPE-19) cell induced cell death from oxidative stress with hydrogen peroxide (H_2O_2). The results showed that the effect and mechanism of boiled sweet corn extract on the ARPE-19 cell induced cell damage from oxidative stress with H_2O_2. ARPE-19 cells were treated with serum free medium contained sweet corn extract (SCE) at the concentrations of 1, 10 and 100 µg/mL for 24 h. After incubation, cells were induced with 400 µM of H_2O_2 for 60 min. The results showed that SCE at concentrations of 10 and 100 µg/mL was a significant ($p < 0.05$) effective in preventing of ARPE-19 cells induced oxidative stress by reducing ROS, increasing levels of intracellular antioxidants (SOD, CAT, GPx and GSH) and modulation of protein in the apoptosis pathway (Bax, Bcl-2, caspase-9 and -3) in a dose dependent manner. However, this study is as only *in vitro* study. Thus, the health benefits of sweet corn especially, eye health, vision and prevention of AMD of sweet corn should be further investigated in animal and human.

Keywords: Age-related macular degeneration, Oxidative stress, Human pigment epithelial cell, Sweet corn, Antioxidant

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Introduction

Age-related macular degeneration or AMD is an eye disease from the macular area of retina, which is a major cause of vision loss and irreversible blindness in the population in the developing world, especially elderly population[1]. The prevalence of AMD is increasing which has been estimated to 288 million in 2040 because of the increasing number of population aging[2]. Moreover, the amount of Asia' population which is more than half of the population of world, therefore the amount of AMD cases is expected to reach in 2040 about 113 million[3]. AMD is characterized by loss of central and sharp vision due to the progressive of the macula degeneration[4]. AMD has two forms: the dry or anthropic AMD and the wet or neo-vascular AMD. The dry AMD is characterized by a progressive dysfunction and death of the retinal pigment epithelium (RPE) leading to dysfunction of retinal and photoreceptors degeneration, which does not involve blood or serum leakage[4-5]. The wet AMD is characterized by the presence of choroidal neovascularization which involve blood or serum leakage. Although AMD is a major problem of health, the therapeutics that exist for AMD are restricted and limited to the wet form, with no current suitable treatment for the dry form of AMD. Therefore, the development and discovery of effective treatments are critically needed to delay or prevent AMD development. Even though the pathogenesis and development of AMD is not completely understood, dysfunction of the RPE plays an importance role in the AMD progression and is a major feature of AMD development and pathogenesis.

Several studies proved that the cause of cell dysfunction and death in RPE cells as oxidative stress, which lead to AMD progression and development[6-8]. Oxidative stress is characterized by the increasing of reactive oxygen species (ROS), caused by an imbalance between production and accumulation of ROS in cells and the efficacy of a biological system to eradicate ROS. These ROS exert damaging effects on lipids, proteins and nucleic acids, which subsequently cause dysfunction and death of cells. RPE cells have a high metabolic rate and exist in an environment that is profuse in endogenous ROS, such as OH^- and H_2O_2[9-10]. The accumulation of oxidative damage in long-term leads to dysfunction and death of RPE cells and increases their susceptibility to oxidative stress. The ROS production causes activation of apoptosis pathway results in the dysfunction and death of RPE cells via apoptosis-associated proteins in the mitochondria, namely, Bax (pro-apoptotic), Bcl-2 (anti-apoptotic), cytochrome C, caspase-9 and caspase-3 proteins. Therefore, the protection of RPE cells from

oxidative damage may be an effective therapeutic strategy against AMD development[11-13]. The normal physiological conditions, the endogenous antioxidant defense mechanisms can neutralize ROS which cause of cellular damage. RPE cells also contain a wide range of antioxidant enzymes and non-enzymatic antioxidant include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH), which can eradicate the ROS[7,10]. Aging of cell, the ability of RPE cells to scavenge ROS decreases resulting in oxidative stress in RPE cells[14].

Dietary bioactive compounds, especially polyphenol group have emerged as potential bioactive molecules with the capacity to exert a positive action against AMD[15, 16]. Previous studies found that regular consumption of dietary carotenoids; lutein and zeaxanthin could reduce the AMD development via their antioxidant property[17, 18]. Human cannot synthesize lutein and zeaxanthin, therefore we need to consume from diet only[19]. Several studies have shown that high lutein and zeaxanthin intake, either through diet or as nutritional supplement, relief sign and symptom of eye diseases by improving AMD[20, 21].

Corn, especially sweet corn (*Zea Mays L.* ssp. saccharata Sturt) which contains several bioactive compounds that are beneficial to health. Many studies showed that sweet corn as a good source of bioactive compound in carotenoid group, especially lutein and zeaxanthin which can exert antioxidant activity[22, 23]. Lutein have been shown antioxidant properties higher than other bioactive compound in carotenoid group by the antioxidant activities of lutein are almost 15- and 10-folds of those of lycopene and beta-carotene, respectively[24]. Moreover, lutein is forms human macular pigments with zeaxanthin in the retina, decreasing noxious blue light into retina and contributing to strengthening of the antioxidant defense of RPE cells[25, 26]. However, there is no study examined the effect of sweet corn extract, which is dietary extracts rich carotenoids, against human retinal pigment epithelial cells induced cell damage from oxidative stress. Therefore, the present study aimed to evaluate the effect of sweet corn extract on oxidative stress induced by H_2O_2 in ARPE-19 cells and explored the underlying mechanisms.

Materials and Methods

Chemicals

All chemical and reagents were purchased from Sigma Chemical (St. Louis, MO) unless indicated otherwise. Fetal bovine serum was obtained from Hyclone (Cramlington, USA). DMEM/F12 and penicillin were bought from Gibco (Invitrogen, CA). Anti-Bax and anti-Bcl-2

were bought Cell signaling (Cell Signalling, Danvers, USA).

Sample preparation

Raw sweet corns were purchased from main wholesale market (Talad Thai). They were bought from 4 dealers representing four regions of Thailand to achieve the diversity of the sample's cultivated agricultural sites. The part of peel and corn straw were removed prior to thoroughly wash. Water was heated until boiled. Then, the raw sweet corns were boiled in boiling water for 15 mins and cooled down in cold water for 2 mins. Corn kernels (the edible portion) were sliced from the cob, ground and lyophilized until dry. The freeze dry sample was packed in vacuum-aluminum foil and kept at -20°C until use.

Sample extraction

The equal amount of dry samples from each region were pooled and homogenized. One gram of freeze dried sample were extracted with 15 ml of hexane: acetone: ethanol (2:1:1) and centrifuged at 4,500 x *g* for 10 min at room temperature and evaporated by rotary evaporator under vacuum at 35-40° C until dryness. The percent extraction yield of SCE was 15%. The dried film of extract was stored at -20°C until used. Dried film was dissolved with DMSO before serially diluted with serum free medium for addition to ARPE-19 cells (final concentration of 0.5% DMSO in serum free medium). Sweet corn extract was called SCE.

Cell culture

Human retinal pigment (ARPE-19) cells (ATCC, Rockville, MD, USA) were maintained and cultured with complete medium contained DMEM/F-12, 10% (v/v) heat-inactivated FBS and 1% (v/v) penicillin-streptomycin.

Cell viability assay

Cell viability was determined using the 3-(4, 5-dimethlthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide tetrazolium (MTT) assay. The MTT substrate is changed into a formazan product with purple color, which is proportional to the number of live cells. After cells reached their respective experimental time points, media was removed and washed one time with phosphate buffer saline (PBS) and incubated with PBS containing 0.5 mg/ml of MTT at 37° C for 4 h. The MTT solution was removed from the cells and DMSO was added to solubilize formazan crystal produced from MTT by viable cells. The absorbance was measured at 540 nm using a microplate reader (SPECTROstarNano, BMG LABTECH, Germany).

Evaluation of Cytotoxicity of SCE and H_2O_2

ARPE-19 cells were seeded at a density of 0.1 x 10^6 cells/well in 48 well plate for 24 h. Cells were washed with one time of serum free media. For cytotoxicity of SCE, cells were incubated with different SCE concentrations (1, 10, 100, 500 and 1000 µg/ml) for 24 h. A 0.5% DMSO as a vehicle control. For cytotoxicity of H_2O_2, cells were incubated with different H_2O_2 concentrations (200, 400, 600, 800 and 1000 µM) for 30, 60 and 120 min. A serum free media as a control group. Cell viability was measured by MTT assay.

Evaluation of the effect of SCE on cell viability in ARPE-19 cells induced oxidative stress with H_2O_2

ARPE-19 cells were seeded at a density of 0.1 x 10^6 cells/well in 48 well plate for 24 h. Cells were washed with one time of serum free media and pre-incubated with three concentrations of SCE without toxicity in serum-free media for 24 h. A 0.5% DMSO in serum free media was used as a control. After incubation, cells were washed with one time of serum free media and then incubated with H_2O_2 in serum free medium at suitable concentrations and time. Cell viability was measured by MTT assay.

Evaluation of the effect of SCE on ROS production in ARPE-19 cells induced oxidative stress with H_2O_2

DCFH-DA was used to detect the intracellular ROS. ARPE-19 cells were seeded into clear bottom, black 96-well plates (Corning, NY, USA) with a density of 30,000 cells/well and incubated for 24 h. Cells were washed with serum free media and pre-incubated with three concentrations of SCE without toxicity in serum free media for 24 h. A 0.5% DMSO was used as a control. After incubation, cells were washed with serum free media and incubated with 10 µM DCFH-DA in serum free media at 37°C for 20 mins. The cells were washed with 2-3 times of serum free media followed by incubation with H_2O_2 in serum free media at suitable concentration and time. Finally, cells were washed twice with PBS, and production of ROS was determined in the plates using the Fluostar Optima plate reader (BMG Labtech, Aylesbury, UK) with the excitation and emission settings of 485 nm and 530 nm, respectively.

Evaluation of the effect of SCE on SOD, CAT and GPx activities and GSH level in ARPE-19 cells induced oxidative stress with H_2O_2

ARPE-19 cells were seeded at a cell density of 1.0 x 10^6 cells/well in 6 well plate for 24 h. Cells were washed with one time of serum free media. Cells were pre-treated

with three concentrations of SCE without toxicity and induced with H_2O_2. Cells were scraped with PBS containing 0.5% (v/v) triton-x100. The cell solution was sonicated in an ultrasonic sonicator at 4°C for 10 min. Cell lysates were centrifuged at 14,000 x g at 4°C for 10 min. The supernatants were collected to determine SOD, GPx and CAT activities, and GSH levels using the respective assay kits (Cayman Chemical, Michigan, USA).

Evaluation of the effect of SCE on caspase-9 and caspase-3 activities in ARPE-19 cells induced oxidative stress with H_2O_2

ARPE-19 cells were seeded at a cell density of 1.0 x 10^6 cells/well in 6 well plate for 24 h. Cells were washed with one time of serum free media. Cells were pre-treated with three concentrations of SCE without toxicity and induced with H_2O_2. Cells were homogenized in a hypotonic buffer[27] to obtain the part of supernatant. The supernatant was added with a specific substrate (*N*-acetyl-Leu-Glu-His-Asp-*p*-nitroanilide or *N*-acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide for caspase-9 or caspase-3, respectively) at the concentration of 100 µmol/L. The mixture was then incubated at 37 °C for 1 h prior to absorbance measurement at 450 nm using the microplate reader.

Evaluation of the effect of SCE on Bax and Bcl-2 expression in ARPE-19 cells induced oxidative stress with H_2O_2

ARPE-19 cells were seeded at a cell density of 1.0 x 10^6 cells/well in 6 well plate for 24 h. Cells were washed with one time of serum free media. Cells were pre-treated with three concentrations of SCE without toxicity and induced with H_2O_2. Cell protein lysates were prepared in cell lysis buffer (RIPA buffer) and centrifuged at 14,000 x g 4°C for 10 min. The supernatants were collected and determined protein concentration using the BCA protein assay kits (Thermo Scientific Company, Rockford, USA). Equal amounts (40 µg) of protein samples were separated by 10% SDS-PAGE; the resolved proteins were then transferred to nitrocellulose membrane after which the membrane was blocked with 5% dry milk. The membrane was incubated with 1: 1000 of anti-Bax or anti-Bcl-2 for overnight. The membrane was washed with TBST, the membrane was incubated with 1: 2000 of the species-specific horseradish peroxide conjugated secondary antibody for 2 h. Then, the membrane was exposed to X-ray film. The densities of target bands were quantified by the Image J program. The results were expressed as a relative band intensity ratio of the target proteins against that of beta-actin.

Statistical analysis

All experiments were conducted at least in four replications. Statistical analyses were performed using SPSS (version 13.0) (SPSS Inc., USA). Data are presented as mean ± standard deviation (SD). Means were compared by One-way Analysis of Variance (ANOVA) with Duncan's post hoc test. Difference is considered significant at $p < 0.05$.

Results

Evaluation of SCE on cell viability of ARPE-19 cells

Cytotoxicity against ARPE-19 cells was determined before evaluating the effects of SCE against oxidative stress to obtain an optimal concentration that without toxicity on viability of ARPE-19 cells. Cells were treated with SCE (1, 10, 100, 500 and 1000 µg/ml) for 24 h. The results showed that concentrations of SCE more than 100 µg/ml significantly ($p < 0.05$) decreased cell viability of ARPE-19 cells (Figure 1A). The result indicated that the concentration of SCE more than 100 µg/ml had toxic on cell viability of ARPE-19 cells. Therefore, the highest concentration of SCE at 100 µg/ml was used for further study.

Evaluation of H_2O_2 on cell viability of ARPE-19 cells

To evaluate the concentration and time of H_2O_2 required to cause approximately 50% reduction in ARPE-19 cell viability. ARPE-19 cells were treated with various concentrations of H_2O_2 (200–1000 µM) for 30, 60 and 120 min. The results indicated that the treatment of H_2O_2 decreased cell viability of ARPE-19 cells in a concentration and time-dependent manner (Figure 1B). The data showed that treatment of H_2O_2 for 60 min at a concentration of 400 µM was sufficient for a 50% reduction in cell viability (Figure 1B). We therefore used the above-determined concentration and time point for H_2O_2 treatment.

Effect of SCE on cell viability and ROS production in ARPE-19 cells induced oxidative stress with H_2O_2

We determined the protective effects of SCE on ARPE-19 cells induced oxidative stress. Pre-treatment of cells with SCE at the concentration 1, 10 and 100 µg/ml for 24 h and followed oxidative stress induction with H_2O_2 at the concentration 400 µM for 60 min. We found that H_2O_2 showed 50% cell viability reduction in comparison with the control group (Figure 2A). SCE at the concentrations of 10 and 100 µg/ml was a significant ($p < 0.05$) protected cell viability of ARPE-19 cells when compared with H_2O_2 group (cells were treated only H_2O_2) in a dose dependent manner (Figure 2A). The part of ROS production, we found that H_2O_2 showed significantly ($p < 0.05$)

increased ROS production in comparison with the control group (Figure 2B). SCE at the concentrations of 10 and 100 µg/ml was a significant (p < 0.05) decreased ROS production when compared with H_2O_2 group in a dose dependent manner (Figure 2B). These results indicated that the SCE at the concentrations of 10 and 100 µg/ml could protect ARPE-19 induced oxidative stress by decreasing of ROS production in a dose dependent manner.

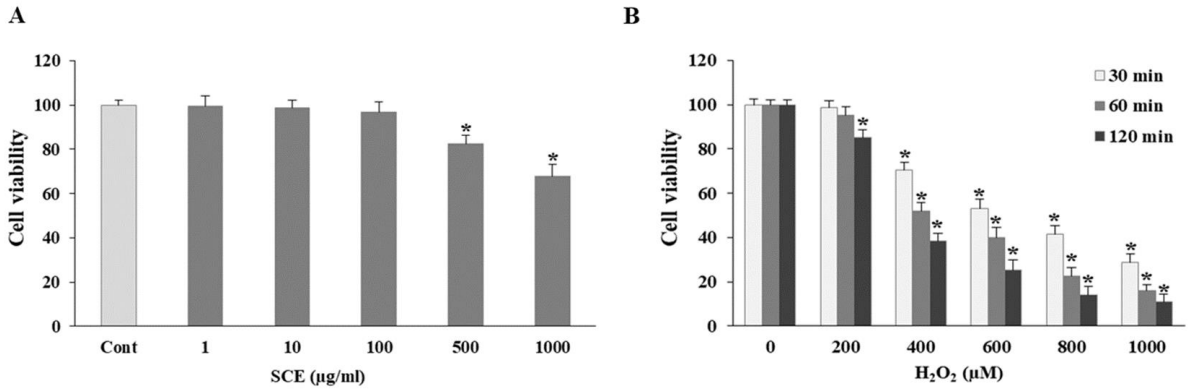

Figure 1. Effects of SCE and H_2O_2 on cell viability of ARPE-19 cells. (A) ARPE-19 cells were incubated with various concentrations of SCE in serum free medium for 24 h. The cell controls were treated with 0.5% DMSO in serum free medium. (B) ARPE-19 cells were incubated with varies concentrations of H_2O_2 in serum free medium for 30, 60, and 120 min. Controls were cells treated with serum free medium. The cell viability of ARPE-19 cells were investigated using MTT assay. Results are reported as mean ± SD of at least in four replications. $^*p$ < 0.05 compared with the control group.

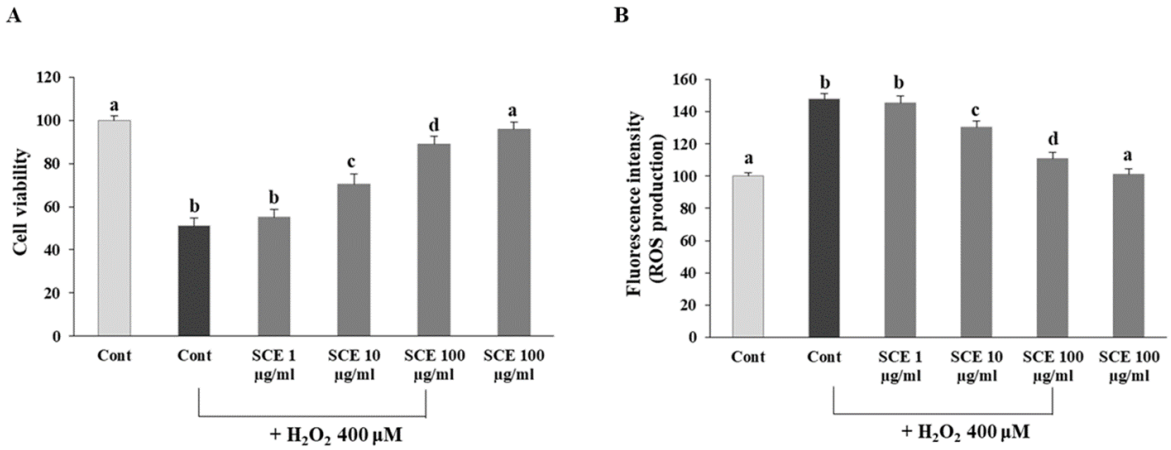

Figure 2. Effects of SCE on cell viability and ROS production in ARPE-19 cells induced oxidative stress with H_2O_2. ARPE-19 cells were pre-treated with SCE at the concentration 1, 10 and 100 µg/ml for 24 h. After incubation, cells were induced oxidative stress with H_2O_2 at the concentration 400 µM

for 60 min. (A) Cells viability and (B) ROS production were determine using MTT and DCFH-DA assay, respectively. Results are reported as mean ± SD of at least in four replications. Different superscript letters indicate significant differences between values in the column ($p < 0.05$).

Effect of SCE on SOD, CAT, and GPx activities, and GSH levels in ARPE-19 cells induced oxidative stress with H_2O_2

SOD, CAT, and GPx activities and GSH level showed that respective H_2O_2 treatment significantly ($p < 0.05$) decreased the activity and level of these antioxidants compared to the control group (Figure 3A, 3B, 3C and 3D). However, pre-treatment with SCE at the concentration 10 and 100 µg/ml significantly improved SOD, CAT and GPx activities and GSH level in ARPE-19 cells subjected to oxidative stress in a dose dependent manner (Figure 3A, 3B, 3C and 3D). Interestingly, we observed that the only treatment of SCE at the concentration 100 µg/ml significantly increased SOD, CAT, and GPx activities and a GSH level compared to the control group (Figure 3A, 3B, 3C and 3D). The results suggest that pre-treatment of SCE can improve the antioxidant system of the RPE in order to be armed against potential oxidative stress inducers.

Effect of SCE on caspase-9 and caspase-3 activities in ARPE-19 cells induced oxidative stress with H_2O_2

To evaluate whether the anti-apoptotic property of SCE in oxidative stress-induced in ARPE-19 cells, the caspase-9 and caspase-3 activities were investigated. H_2O_2 treatment significantly ($p < 0.05$) increased the activity of caspase-9 and caspase-3 compared to the control group (Figure 4A and 4B). However, pre-treatment with SCE at the concentration 10 and 100 µg/ml significantly reduced caspase-9 and caspase-3 activities in a dose dependent manner (Figure 4A and 4B). The results indicated that SCE could improve H_2O_2-induced apoptosis in ARPE-19 cells by via modulation of caspase-9 and caspase-3 activities in the apoptosis pathway.

Effect of SCE on Bax and Bcl-2 expression in ARPE-19 cells induced oxidative stress with H_2O_2

To evaluate whether the anti-apoptotic property of SCE in oxidative stress-induced in ARPE-19 cells, Bax and Bcl-2 expression were investigated. H_2O_2 treatment significantly ($p < 0.05$) increased the expression of Bax protein which is pro-apoptotic protein, whereas of significantly ($p < 0.05$) decreased the expression of Bcl-2 protein which is anti-apoptotic protein, compared to the control group (Figure 5A and 5B). However, pre-treatment with SCE

at the concentration 10 and 100 µg/ml significantly (p < 0.05) decreased the expression of Bax protein whereas of significantly (p < 0.05) increased the expression of Bcl-2 protein in a dose dependent manner (Figure 4A and 4B). The results indicated that SCE could improve H_2O_2-induced apoptosis in ARPE-19 cells via modulation of the Bax and Bcl-2 in the apoptosis pathway.

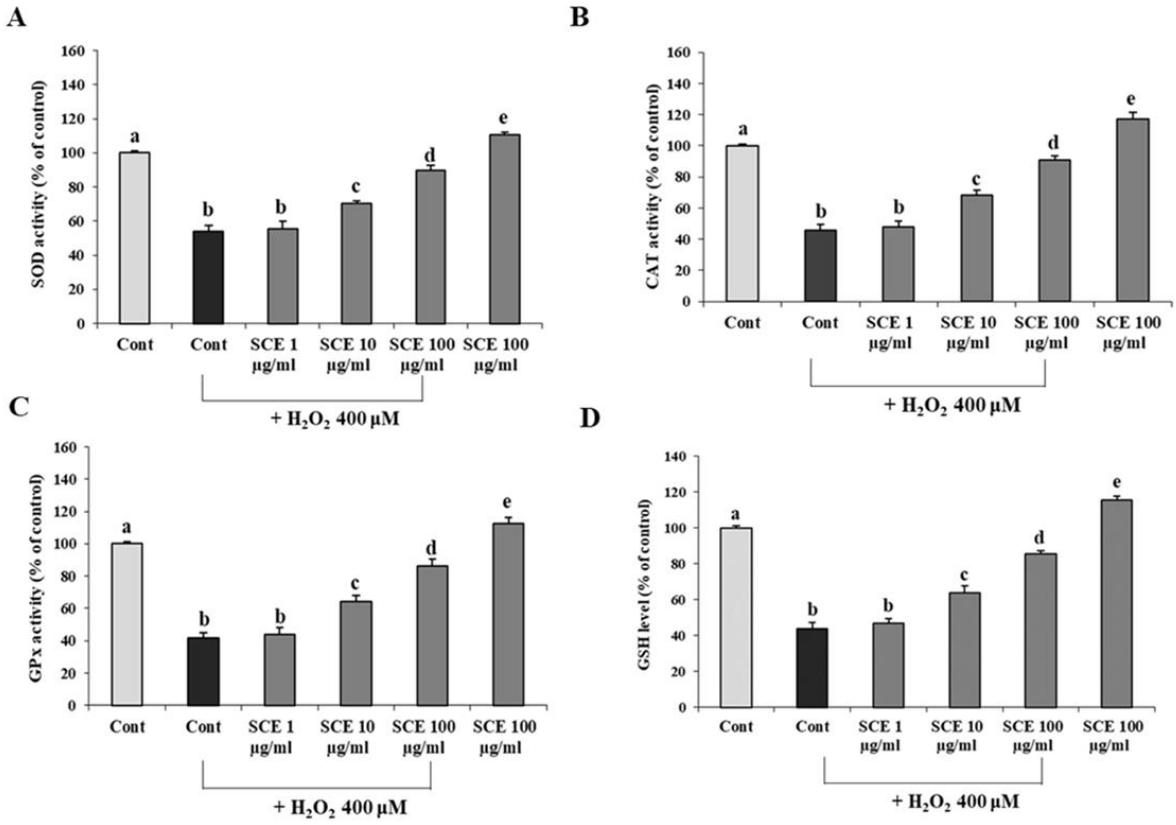

Figure 3. Effect of SCE on SOD, CAT, and GPx activities, and GSH levels in ARPE-19 cells induced oxidative stress with H_2O_2. ARPE-19 cells were pre-treated with SCE at the concentration 1, 10 and 100 µg/ml for 24 h. After incubation, cells were induced oxidative stress with H_2O_2 at the concentration 400 µM for 60 min. Cells were lysed and incubated with PBS containing 0.5% (v/v) triton-x100. The supernatant was collected to determine (A) SOD, (B) CAT and (C) GPx activities, and (D) GSH levels using the respective assay kits. Results are reported as mean ± SD of at least in four replications. Different superscript letters indicate significant differences between values in the column (p < 0.05).

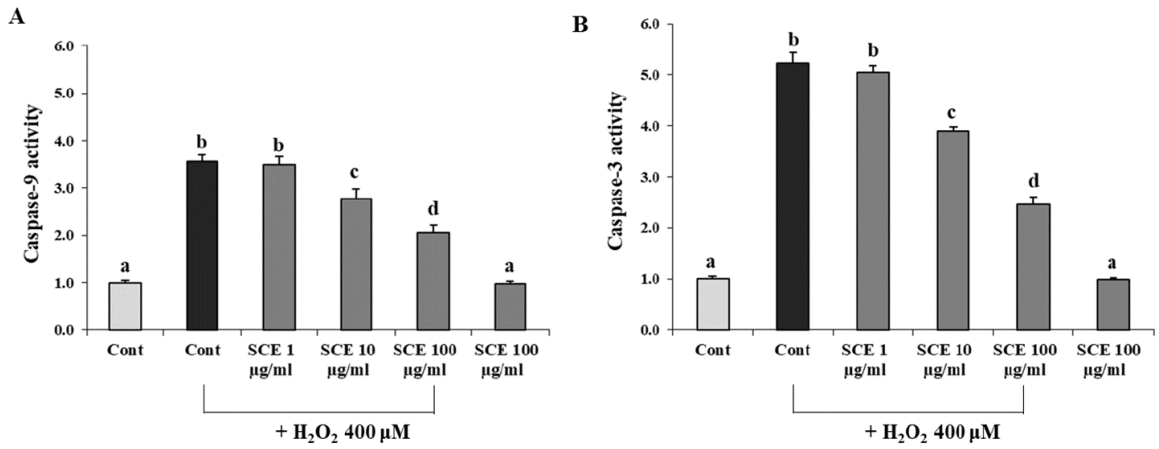

Figure 4. Effect of SCE on caspase-9 and caspase-3 activities in ARPE-19 cells induced oxidative stress with H_2O_2. ARPE-19 cells were pre-treated with SCE at the concentration 1, 10 and 100 µg/ml for 24 h. After incubation, cells were induced oxidative stress with H_2O_2 at the concentration 400 µM for 60 min. The treated cells were homogenized in a hypotonic buffer to obtain the part of supernatant. The supernatant was determined (A) caspase-9 and (B) caspase-3 activities Results are reported as mean ± SD of at least in four replications. Different superscript letters indicate significant differences between values in the column ($p < 0.05$).

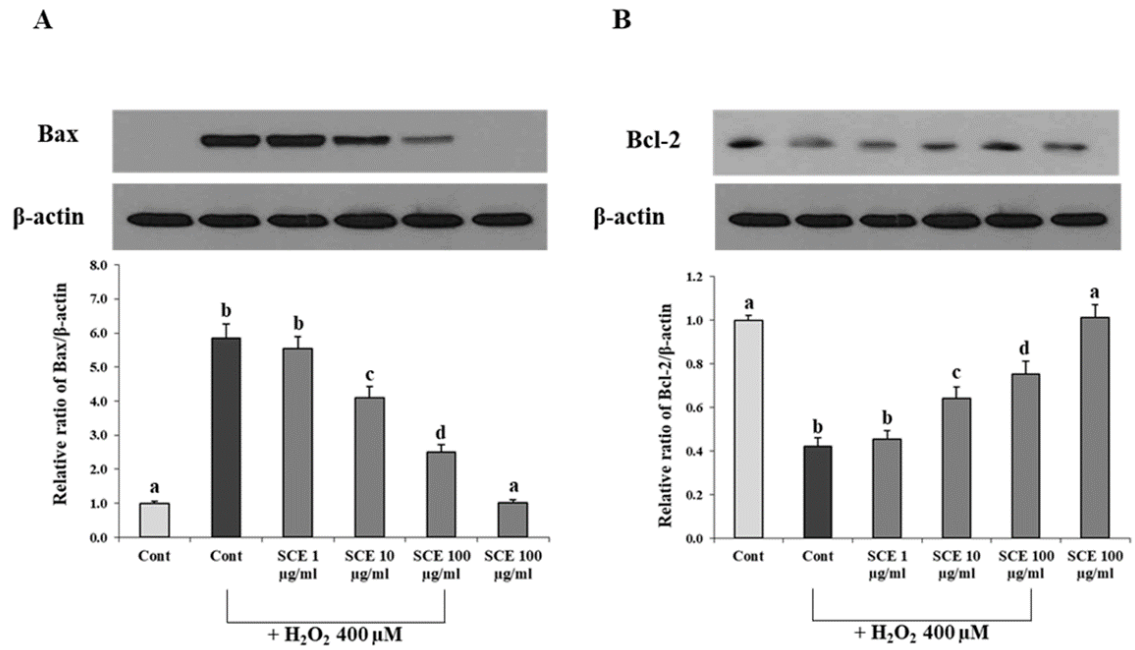

Figure 5. Effect of SCE on Bax and Bcl-2 expression in ARPE-19 cells induced oxidative stress with H_2O_2. ARPE-19 cells were pre-treated with SCE at the concentration 1, 10 and 100 µg/ml for 24 h. After incubation, cells were induced oxidative stress with H_2O_2 at the concentration 400 µM for 60 min. The treated cells were homogenized in a hypotonic buffer to obtain the part of supernatant. The supernatant was determined (A) Bax and (B) Bcl-2 proteins expression. Results are reported as mean ±

SD of at least three independent experiments. Different superscript letters indicate significant differences between values in the column ($p < 0.05$).

Discussion

AMD is an eye disease affecting the macular in retina area, which is a cause of vision loss and irreversible blindness in the elderly population. Numerous studies showed that the oxidative stress as a major cause of cell dysfunction and death in RPE cells, which lead to AMD progression and development. Therefore, the protection of RPE cells from oxidative damage may be an effective therapeutic strategy against AMD development. Many studies have indicated that the variety of bioactive compounds, including carotenoids, are able to stimulate translocation of Nrf2 into nucleus , which switches on gene expression of a number of antioxidant enzymes and phase I and II detoxification enzymes which could protect AMD development from oxidative stress[7, 28] . Several studies have found that regular consumption of dietary carotenoids such as lutein and zeaxanthin could reduce the AMD development via their antioxidant property[17, 18]. In addition, the human cannot synthesize lutein and zeaxanthin, therefore we need to consume from diet only[19]. Corn, especially sweet corn (*Zea Mays* L. ssp. saccharata Sturt) which contains several bioactive compounds that are beneficial to health. Many studies indicated that sweet corn as a good source and rich of natural bioactive compound in carotenoid group, especially lutein and zeaxanthin which can exert antioxidant activity[23-25, 29]. Moreover, several studies showed that lutein and zeaxanthin could activate Nrf2 transcription factor in protection of cell death[30-32].

The present study indicated, for the first study to our knowledge, the protective and mechanisms effects of SCE against oxidative stress in human RPE cells. At the mechanism level, we showed that SCE exert their protective effects through modulation of the key apoptotic-signaling proteins, Bax and Bcl-2 expression, and caspase-9 and caspase-3 activities. In addition, SCE was also able to protect against oxidative damage by increasing the activity of enzymatic antioxidants (SOD, CAT and GPx) and non-enzymatic (GSH) antioxidant systems in human RPE cells. Therefore, SCE is an interesting extract to be used in food and supplement development for AMD prevention.

The signaling mechanisms that regulate the SOD, CAT and GPx and the level of GSH were not investigated in this present study. Previous studies reported that the increasing of SOD, CAT and GPx activities and GSH level could protect the oxidative stressed in RPE cells through activation of the Akt/Nrf2 signaling

pathway, which this pathway involves the translocation of Nrf2 into the nucleus, which results in the expression of several antioxidants system in the body[33, 34]. Some studies have shown that lutein exerts its protective effects against oxidative stress by activating the Nrf2 signaling[35]. In addition, the amount of bioactive compounds, especially the bioactive compound in carotenoid group from SCE were not determined. From studied of Song et al.[36] found that sweet corn grains had carotenoid contents from 8.42 to 39.71 μg/g dry weight by the major identified carotenoids were all-trans-lutein, all-trans-zeaxanthin, and all-trans-α- cryptoxanthin.

Conclusion

In summary, the present study indicates that the effect of SCE on the apoptosis-related proteins is oxidative stress-dependent in the H_2O_2-induced oxidative stress in human ARPE-19 cells. SCE showed the protective effect against oxidative damage to ARPE-19 cells through reduction of ROS levels. The molecular mechanisms by which SCE showed its effects were through specific modulation of apoptotic signaling (Bax, Bcl-2, caspase-9 and caspase-3) as well as enhancement of antioxidant system (SOD, CAT, GPx and GSH). However, this study is as only in vitro study. Thus, the health benefits of sweet corn especially, eye health, vision and prevention of AMD of sweet corn should be further investigated in animal and human.

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Conflict of Interest

The authors declare no conflict of interest.

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