ผลของสารสกัดจากข้าวไรซ์เบอร์รีหุงสุกต่อเซลล์จอประสาทตามนุษย์ชนิด ARPE-19 ที่ถูกเหนี่ยวนำให้เกิดภาวะเครียดออกซิเดชันด้วยไฮโดรเจนเปอร์ออกไซด์

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บทคัดย่อ

ภาวะจอประสาทตาเสื่อมตามอายุ (AMD) เป็นภาวะความเสื่อมของเซลล์จอประสาทตาส่งผลต่อบริเวณจุดรับภาพชัด ทำให้สูญเสียการมองเห็นได้ โดยเฉพาะในผู้สูงอายุ ซึ่งปัจจัยสำคัญที่เกี่ยวข้องคือ ภาวะเครียดออกซิเดชัน พืชที่อุดมไปด้วยสารแอนโทไซยานิน เช่น ข้าวไรซ์เบอร์รี สามารถลดความเสียหายของเซลล์จากภาวะเครียดออกซิเดชันได้ การศึกษานี้มีวัตถุประสงค์เพื่อประเมินศักยภาพของสารสกัดจากข้าวไรซ์เบอร์รีหุงสุกในการลดความเสียหายที่เกิดจากภาวะเครียดออกซิเดชันซึ่งถูกเหนี่ยวนำโดยไฮโดรเจนเปอร์ออกไซด์ในเซลล์ ARPE-19 ในการทดลองเซลล์ ARPE-19 จะถูกบ่มด้วยสารสกัดจากข้าวไรซ์เบอร์รีหุงสุกที่ความเข้มข้น 250, 500 และ 1,000 ไมโครกรัมต่อมิลลิลิตร เป็นเวลา 24 ชั่วโมง ก่อนจะกระตุ้นให้เกิดภาวะเครียดออกซิเดชันด้วยไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น 600 ไมโครโมลาร์ เป็นเวลา 2 ชั่วโมง ผลการศึกษาพบว่า สารสกัดจากข้าวไรซ์เบอร์รีหุงสุกที่ความเข้มข้น 500 และ 1,000 ไมโครกรัมต่อมิลลิลิตร สามารถลดการเกิดอนุมูลอิสระภายในเซลล์ เพิ่มการทำงานของสารต้านอนุมูลอิสระภายในเซลล์ (SOD, CAT, GPx, GSH) และยังยับยั้งการทำงานของเอนไซม์ที่เกี่ยวข้องกับกระบวนการอะพ็อพโทซิส (caspase-9 และ caspase-3) ได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในลักษณะแปรผันตรงกับความเข้มข้นของสารสกัด อย่างไรก็ตามผลการทดสอบนี้เป็นผลการทดสอบในหลอดทดลอง (*in vitro*) จำเป็นต้องมีการประเมินด้านพิษวิทยาและโภชนาการในสัตว์ทดลองและมนุษย์ (*in vivo*) เพื่อยืนยันความปลอดภัยและทำความเข้าใจกลไกในการป้องกันความเสียหายของดวงตาที่เกิดจากภาวะเครียดออกซิเดชันอย่างชัดเจนยิ่งขึ้น

คำสำคัญ: ข้าวไรซ์เบอร์รีหุงสุก เซลล์จอประสาทตามนุษย์ ภาวะจอประสาทตาเสื่อมตามอายุ ภาวะเครียดออกซิเดชัน ฤทธิ์ต้านอนุมูลอิสระ

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Effect of Cooked Riceberry Rice Extract Against Hydrogen Peroxide-Induced Oxidative Stress in Human Retinal Pigment Epithelial (ARPE-19) Cells

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Abstract

Age-related macular degeneration (AMD) is a progressive retinal disorder that primarily affects the macula, leading to irreversible vision loss, particularly among the elderly. The key factor of AMD is oxidative stress. Plant-based foods rich in anthocyanins, such as Riceberry rice, help mitigate oxidative damage. This study aims to investigate whether the antioxidant properties of cooked Riceberry rice can mitigate hydrogen peroxide (H_2O_2)–induced oxidative stress in ARPE-19 cells. ARPE-19 cells were pretreated with cooked Riceberry rice extracts (250, 500, and 1,000 µg/mL) for 24 h, followed by exposure to 600 µM hydrogen peroxide (H_2O_2) for 2 h. The results demonstrated that cooked Riceberry rice extracts at 500 and 1,000 µg/mL significantly ($p < 0.05$) attenuated oxidative stress in ARPE-19 cells by reducing intracellular reactive oxygen species (ROS) generation, enhancing intracellular antioxidant systems (SOD, CAT, GPx, GSH), and suppressing apoptosis-related enzyme activities (caspases-9 and -3) in a dose-dependent manner. However, this research is only an in vitro study. Toxicological and nutritional evaluations in animal and human models (*in vivo*) are required to ensure food safety and to elucidate its protective effects against oxidative stress–induced ocular damage.

Keywords: Cooked Riceberry rice, Human retinal pigment epithelial cells, Age-related macular degeneration, Oxidative stress, Antioxidant activity

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Introduction

Age-related macular degeneration (AMD) is a progressive retinal disorder affecting the macula, the central region responsible for high-acuity vision, and is a leading cause of irreversible visual impairment in individuals aged 50 years and older[1]. With the global aging population, the prevalence of AMD is expected to increase substantially, with projections estimating 288–370 million affected individuals worldwide by 2040[2]. This trend highlights the growing public health burden and the need for effective prevention, early detection, and therapeutic strategies. In Thailand, as in other aging societies, the prevalence of AMD is rising alongside the expanding elderly population. Although nationwide data remain limited compared to Western countries, available evidence indicates that AMD is an emerging public health concern. One epidemiological study reported a prevalence of 12.2% among individuals aged ≥ 50 years, with 862 cases identified out of 7,043 participants[3]. AMD is broadly classified into two forms: dry (atrophic) and wet (neovascular)[4]. The dry form, accounting for approximately 80–90% of cases, is characterized by macular thinning and the accumulation of drusen beneath the retina. In contrast, the wet form is less common but more severe, involving abnormal choroidal neovascularization that leads to fluid leakage, hemorrhage, and eventual scarring[5]. Although the exact etiology of AMD remains incompletely understood, several risk factors have been identified, including aging, genetic predisposition, smoking, obesity, hypertension, and prolonged ultraviolet exposure[6]. Among these, oxidative stress is considered a central driver of AMD pathogenesis.

Oxidative stress in AMD arises from multiple factors, including aging-associated mitochondrial dysfunction, chronic exposure to visible and ultraviolet light, high oxygen consumption by retinal tissues, accumulation of lipofuscin and drusen components, and chronic inflammation. These factors promote excessive generation of reactive oxygen species (ROS), including superoxide anion, hydroxyl radicals, and hydrogen peroxide (H_2O_2), leading to oxidative damage of retinal pigment epithelial (RPE) cells. Among these ROS, H_2O_2 is relatively stable and membrane permeable, enabling it to diffuse throughout cells and induce mitochondrial dysfunction, DNA damage, lipid peroxidation, and apoptosis. Consequently, H_2O_2 is widely employed as an experimental inducer of oxidative stress in ARPE-19 cells to model oxidative injury associated with AMD[4–7]. Excessive accumulation of H_2O_2 disrupts cellular redox homeostasis and contributes to oxidative damage to cellular macromolecules, including lipids, proteins, and DNA, ultimately impairing RPE cell function and viability[8,9]. At the molecular level, oxidative stress induces mitochondrial

dysfunction, disrupting the balance between pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins. This imbalance triggers cytochrome c release, activating caspases 9 and 3 and leading to apoptotic cell death in RPE cells, thereby contributing to disease progression[9]. Numerous studies indicate that phytochemicals, particularly anthocyanins, exert protective effects against AMD[10-11]. These compounds support visual health through their antioxidant, anti-inflammatory, and anti-apoptotic properties[11]. Anthocyanins are widely distributed in deeply pigmented plant foods, including vegetables, fruits, grains, and rice varieties with black, purple, red, or pink coloration[12-13].

Riceberry rice, a hybrid of Thai jasmine rice (*Oryza sativa* L.) and black rice, has gained increasing attention due to its rich profile of bioactive compounds. Its deep purple color reflects a high anthocyanin content, along with other antioxidants such as vitamin E (tocotrienols), γ-oryzanol, and phytosterols[13]. These compounds, particularly anthocyanins, exhibit strong antioxidant properties by scavenging ROS and mitigating oxidative damage, suggesting potential protective effects in retinal pigment epithelial (RPE) cells [14]. Because Riceberry rice is typically consumed cooked rather than raw, evaluating the biological activity of cooked Riceberry rice may provide more physiologically and nutritionally relevant information about its potential health benefits. Although thermal processing may alter the levels of certain bioactive compounds, the cooked product more closely reflects actual human dietary exposure. However, the protective effects and underlying mechanisms of Riceberry rice, especially cooked Riceberry rice, against oxidative stress in ARPE-19 cells remain uninvestigated. Therefore, this study aims to investigate the effect of cooked Riceberry rice extract on H_2O_2-induced oxidative stress in ARPE-19 cells.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture reagents for ARPE-19 cells were obtained from Gibco (Grand Island, NY, USA), and all kits for antioxidant and caspase assays were supplied by Cayman Chemical (Ann Arbor, MI, USA).

Sample preparation

Riceberry rice (*O. sativa* L. cv. Riceberry) samples were collected from rice producers in five provinces of Thailand, including Surin, Kanchanaburi, Chiang Mai, Phatthalung, and Suphan Buri. These provinces were selected to encompass geographically diverse Riceberry rice-growing regions and to provide a representative overview of Riceberry rice

produced in Thailand. The samples were rinsed twice with deionized (DI) water and cooked at a rice-to-water ratio of 1:2. After cooling, the cooked rice was lyophilized to dryness, ground into powder, vacuum-sealed in aluminum foil, and stored at −20 °C.

Sample extraction

Dried samples from the five provinces were pooled in equal proportions and homogenized. One gram of the pooled sample was extracted with 15 mL of 70% ethanol, vortexed for 1 min, and sonicated for 15 min. The mixture was centrifuged at 4,600 rpm for 15 min, and the supernatant was collected. The extraction was repeated twice, and the supernatants were combined. The pooled extract was concentrated using a vacuum evaporator at 40 °C, reconstituted in absolute ethanol, and transferred to an amber vial. The solvent was removed under a stream of nitrogen gas to obtain the cooked Riceberry rice extract (CRE), which was stored at −20 °C.

Cell culture

Human retinal pigment epithelial (ARPE-19) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM/F-12 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37 °C in a humidified atmosphere with 5% CO_2. Cells were used for experiments at > 90% confluence.

Evaluation of cytotoxicity of CRE and H_2O_2 on the cell viability of ARPE-19 cells

ARPE-19 cells were seeded at a density of 0.1 × 10^6 cells/well in 48-well plates and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. Cells were then washed once with serum-free medium. For cytotoxicity assessment of CRE, cells were treated with various concentrations (10 – 1000 µg/mL) for 24 h, with 0.5% DMSO in serum-free medium as the control. For H_2O_2-induced cytotoxicity, cells were exposed to different concentrations of H_2O_2 (200 – 1000 µM) for 1, 2, and 3 h, with serum-free medium as the control. After incubation, cells were washed with PBS and incubated with MTT solution (0.5 mg/mL in PBS) for 3 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 540 nm using a microplate reader. Cell viability was expressed as a percentage of the control, and all experiments were performed in triplicate. The highest CRE concentration that did not significantly affect cell viability, along with the H_2O_2 concentration that reduced viability to

approximately 50% ($p < 0.05$), were selected for subsequent experiments.

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

ARPE-19 cells were seeded at a density of 0.1×10^6 cells/well in 48-well plates and incubated for 24 h. Cells were then pretreated with non-cytotoxic concentrations of CRE (250, 500, and 1,000 µg/mL) for an additional 24 h. A 0.5% DMSO in serum-free medium was used as the vehicle control. After pretreatment, cells were washed with serum-free medium and exposed to 600 µM H_2O_2 for 2 h. Following incubation, cell viability was determined using the MTT assay as described above. Cell viability was expressed as a percentage of the control group.

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

ARPE-19 cells were seeded at a density of 5×10^4 cells/well in black 96-well plates and incubated for 24 h. Cells were then pretreated with non-cytotoxic concentrations of CRE (250, 500, and 1,000 µg/mL) for 24 h. A 0.5% DMSO in serum-free medium was used as the vehicle control. Following pretreatment, the cells were washed with serum-free medium and incubated with 10 µM DCFH-DA at 37°C for 30 min. After washing, the cells were exposed to 600 µM H_2O_2 for 2 h. Finally, the cells were washed twice with PBS, and intracellular ROS levels were determined using a fluorescence microplate reader at excitation and emission wavelengths of 485 and 530 nm, respectively.

Evaluation of the effect of CRE on apoptotic-related markers and intracellular antioxidant defenses in H_2O_2-induced oxidative stress in ARPE-19 cells

ARPE-19 cells were seeded at a density of 1×10^6 cells/well in 6-well plates and incubated for 24 h. Cells were then pretreated with non-cytotoxic concentrations of CRE (250, 500, and 1,000 µg/mL) for 24 h. A 0.5% DMSO in serum-free medium was used as the vehicle control. Following pretreatment, the cells were washed with serum-free medium and exposed to 600 µM H_2O_2 for 2 h. After incubation, the cells were lysed in PBS containing 1% (v/v) Triton X-100, and lysates were collected by scraping. The cell suspensions were sonicated at 4°C for 10 min and centrifuged at 13,500 × g for 10 min at 4°C. The resulting supernatants were collected for determination of apoptosis-related markers (caspase-9 and caspase-3 activities) and intracellular antioxidant defenses (SOD, CAT, and GPx activities and GSH levels) using commercial assay kits.

Statistical analysis

All data will be presented as mean ± standard deviation (SD) from three independent experiments. Results were analyzed using SPSS 18.0 software. One-way ANOVA will evaluate the significance of the difference with the post hoc Duncan's test. A value of $p < 0.05$ is considered to be statistically significant.

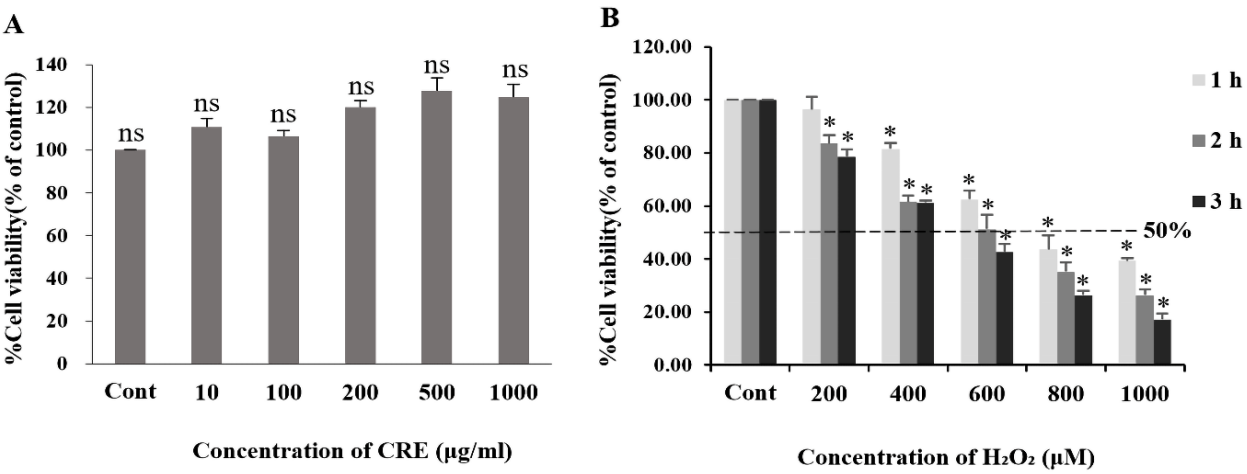


Figure 1. Effect of CRE and H_2O_2 on the cell viability of ARPE-19 cells. (A) ARPE-19 cells were treated with CRE (10, 100, 200, 500, and 1000 µg/ml) in serum-free medium for 24 h. A 0.5% DMSO was used as a vehicle control. **(B)** ARPE-19 cells were exposed to various concentrations of H_2O_2 (200, 400, 600, 800, and 1,000 µM) in serum-free medium for 1, 2, and 3 h**.** Serum-free medium served as the vehicle control. Cell viability was measured using the MTT assay. Data are represented as mean ± SD, n = 3. "ns" above the error bars indicates no significant differences among treatment groups ($p > 0.05$). The symbol * indicates significant differences compared to the control group ($p < 0.05$).

Results

Effect of CRE and H_2O_2 on the cell viability of ARPE-19 cells

ARPE-19 cells were incubated with CRE at 10, 100, 200, 500, and 1,000 µg/ml for 24 h to evaluate cytotoxicity or with H_2O_2 at 200, 400, 600, 800, and 1,000 µM for 1–3 h to establish conditions to induce oxidative stress. The results showed that all tested CRE concentrations (10–1,000 µg/mL) were non-cytotoxic, with cell viability comparable to that of the control (Figure 1A). In contrast, H_2O_2 induced a concentration- and time-dependent decrease in cell viability, with 600 µM for 2 h reducing viability to approximately 50% of the control (Figure 1B). Based on these findings, 1,000 µg/mL CRE and 600 µM H_2O_2 for 2 h were selected for subsequent experiments. Based on the cytotoxicity results, the highest non-cytotoxic concentration of CRE (1,000 µg/mL) and two lower concentrations (500 and 250 µg/mL)

were selected for subsequent experiments to evaluate concentration-dependent protective effects.

Effect of CRE on the cell viability and ROS production of ARPE-19 cells under H_2O_2-induced oxidative stress

To evaluate the protective effect of CRE on H_2O_2-induced cytotoxicity and ROS production in ARPE-19 cells, the cells were pre-incubated with CRE at 250, 500, and 1000 µg/mL, based on prior experimental findings. After incubation for 24 h, the cells were exposed to 600 µM H_2O_2 for 2 h, and cell viability and ROS levels were assessed using the MTT and DCFH-DA assays, respectively. As shown in Figures 2A and 2B, H_2O_2 significantly decreased cell viability and increased ROS levels compared to the control ($p < 0.05$), indicating that H_2O_2 effectively induces oxidative stress in ARPE-19 cells. Pre-treatment with CRE at 500 and 1,000 µg/mL significantly increased cell viability and attenuated ROS levels compared to the H_2O_2-treated group ($p < 0.05$), in a concentration-dependent manner, whereas no significant effects were observed at 250 µg/mL (Figures 2A and 2B). CRE alone showed no cytotoxicity and did not affect ROS levels. Based on the results, indicate that CRE protects ARPE-19 cells against H_2O_2-induced oxidative stress.

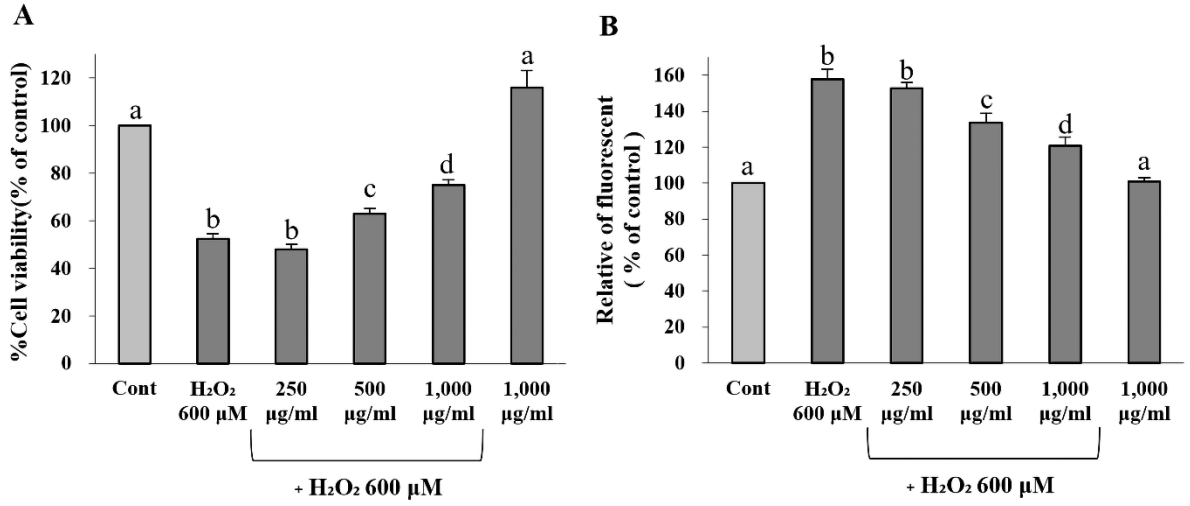


Figure 2. Effect of CRE on the cell viability and ROS production in ARPE-19 cells under H_2O_2-induced oxidative stress. ARPE-19 cells were pre-incubated with CRE at 250, 500, and 1000 µg/mL for 24 h. After incubation, the cells were exposed to 600 µM H_2O_2 for 2 h. **(A)** Cell viability and **(B)** ROS levels were measured using the MTT and DCFH-DA assays, respectively. Data are represented as mean ± SD, n = 3. Different letters above the error bars indicate significant differences among treatment groups ($p < 0.05$).

Effect of CRE on caspase activities in ARPE-19 cells under H_2O_2-induced oxidative stress

To investigate apoptosis modulation in ARPE-19 cells, the activities of caspase-9 and caspase-3 were measured using specific assay kits. Cells were pretreated with CRE at 250, 500, and 1,000 µg/mL for 24 h, followed by exposure to 600 µM H_2O_2 for 2 h. As shown in Figures 3A and 3B, H_2O_2 treatment significantly increased caspase-9 and caspase-3 activities compared with the control ($p < 0.05$), indicating that H_2O_2 induces apoptosis in ARPE-19 cells. Pre-treatment with CRE at 500 and 1,000 µg/mL significantly reduced caspase-9 and caspase-3 activities compared to the H_2O_2-treated group ($p < 0.05$), in a concentration-dependent manner, whereas no significant effects were observed at 250 µg/mL (Figures 3A and 3B). CRE alone showed no cytotoxicity and did not affect caspase-9 and caspase-3 activities. These findings indicate that CRE effectively suppresses H_2O_2-induced apoptotic cell death.

Effect of CRE on the intracellular antioxidant system in ARPE-19 cells under H_2O_2-induced oxidative stress

Enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH), play critical roles in cellular defense against H_2O_2-induced oxidative damage. To evaluate the effect of CRE on these antioxidant systems in ARPE-19 cells, cells were pretreated with CRE at 250, 500, and 1,000 µg/mL for 24 h, followed by exposure to 600 µM H_2O_2 for 2 h. As shown in Figures 4A–D, H_2O_2 treatment significantly reduced SOD, CAT, and GPx activities, as well as GSH levels, compared with the control ($p < 0.05$), indicating that H_2O_2 impaired the cellular antioxidant defense system.

Pre-treatment with CRE at 500 and 1,000 µg/mL significantly improved these antioxidant parameters compared with the H_2O_2-treated group ($p < 0.05$), in a concentration-dependent manner (Figures 4A–D). Interestingly, CRE alone at 1,000 µg/mL significantly increased intracellular antioxidant activity compared with the control

group ($p < 0.05$), indicating that CRE enhances cellular antioxidant capacity and redox homeostasis. Collectively, these findings indicate that CRE enhances both enzymatic and non-enzymatic antioxidant defenses, thereby mitigating H_2O_2-induced oxidative stress and cytotoxicity in ARPE-19 cells.

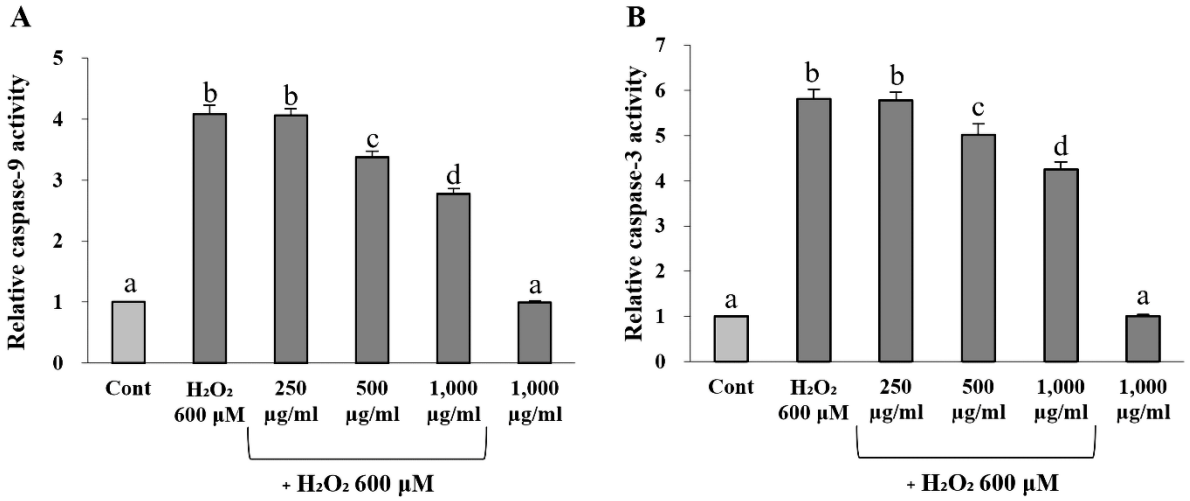


Figure 3. Effect of CRE on caspase activities in ARPE-19 cells under H_2O_2-induced oxidative stress. ARPE-19 cells were pre-incubated with CRE at 250, 500, and 1000 µg/mL for 24 h. After incubation, the cells were exposed to 600 µM H_2O_2 for 2 h. **(A)** Caspase-9 and **(B)** caspase-3 activities were measured using the specific assay kits. Data are represented as mean ± SD, n = 3. Different letters above the error bars indicate significant differences among treatment groups ($p < 0.05$).

Discussion

AMD is a progressive retinal disorder and a leading cause of irreversible vision loss among older adults worldwide[1]. Oxidative stress is widely recognized as a major contributor to AMD pathogenesis, particularly through damage to RPE cells, which are essential for maintaining retinal homeostasis and photoreceptor function. Due to their high metabolic activity, oxygen consumption, and continuous exposure to light, RPE cells are especially susceptible to oxidative injury, leading to cellular dysfunction and eventual retinal degeneration[2,15]. In the present study, CRE effectively protected ARPE-19 cells against H_2O_2-induced oxidative stress. The protective effects observed can be attributed to the abundance of antioxidant compounds (anthocyanins, γ-oryzanol, tocopherols, tocotrienols, phytosterols, and phenolic compounds) in Riceberry rice[13,16]. Previous studies have reported that diets rich in antho-cyanin-containing fruits and

vegetables are associated with a reduced risk of AMD, highlighting the potential role of these phytochemicals in retinal protection[12,17]. In addition to anthocyanins, γ-oryzanol and vitamin E derivatives have been shown to scavenge ROS and inhibit lipid peroxi-dation, whereas phenolic compounds may enhance endogenous antioxidant defense systems through the regulation of antioxidant enzymes[18-19]. Therefore, the protective effects of CRE are likely mediated by the combined actions of multiple bioactive constituents rather than a single compound alone. Consistent with this hypothesis, CRE significantly enhanced intracellular antioxidant defenses, as evidenced by increased activities of SOD, CAT, and GPx, together with elevated intracellular GSH levels. These findings suggest that CRE contributes to maintaining cellular redox homeostasis and enhances the antioxidant capacity of RPE cells under oxidative stress. Excessive accumulation of H_2O_2 has been reported to disrupt cellular redox homeostasis, leading to oxidative damage, mitochondrial dysfunction, and cellular injury in RPE cells[8, 20]. Therefore, CRE's ability to restore intracellular antioxidant defenses may play an important role in protecting RPE cells against oxidative stress-induced damage. Moreover, CRE not only possesses antioxidant activity but also exhibits anti-apoptotic effects by downregulating the activities of caspase-9 and caspase-3, key mediators in the intrinsic mitochondrial apoptotic pathway [21]. Since apoptosis of RPE cells is closely associated with the development of AMD, inhibition of caspase activation may represent an additional mechanism by which CRE promotes retinal cell survival. Overall, the present study demonstrated that CRE protected ARPE-19 cells against H_2O_2-induced oxidative stress by enhancing intracellular antioxidant defenses and inhibiting caspase-mediated apoptosis. These findings suggest that CRE attenuates oxidative stress-induced cellular damage in RPE cells and may have potential as a functional ingredient for preventing AMD. Several limitations of this study should be acknowledged. First, although CRE enhanced intracellular antioxidant defenses and reduced apoptosis in H_2O_2-treated ARPE-19 cells, the molecular mechanisms responsible for these protective effects were not fully characterized. The expression of key regulatory proteins and signaling pathways involved in oxidative stress responses and apoptosis, including Bax, Bcl-2, Akt, and Nrf2, was not directly examined. Therefore, the precise molecular mechanisms underlying the observed protective effects remain unclear and require further experimental validation[11,14,22–23]. Second, the individual bioactive compounds responsible for the observed protective effects were not identified or quantified.

Although Riceberry rice is known to contain anthocyanins, γ-oryzanol, tocotrienols, and other phenolic compounds, the relative contributions of these compounds to the observed biological activities remain unknown. Future studies should focus on identifying the active constituents of CRE, elucidating their molecular targets, and confirming the involvement of key antioxidant and apoptosis-related signaling pathways in mediating CRE's protective effects against oxidative stress-induced retinal injury.

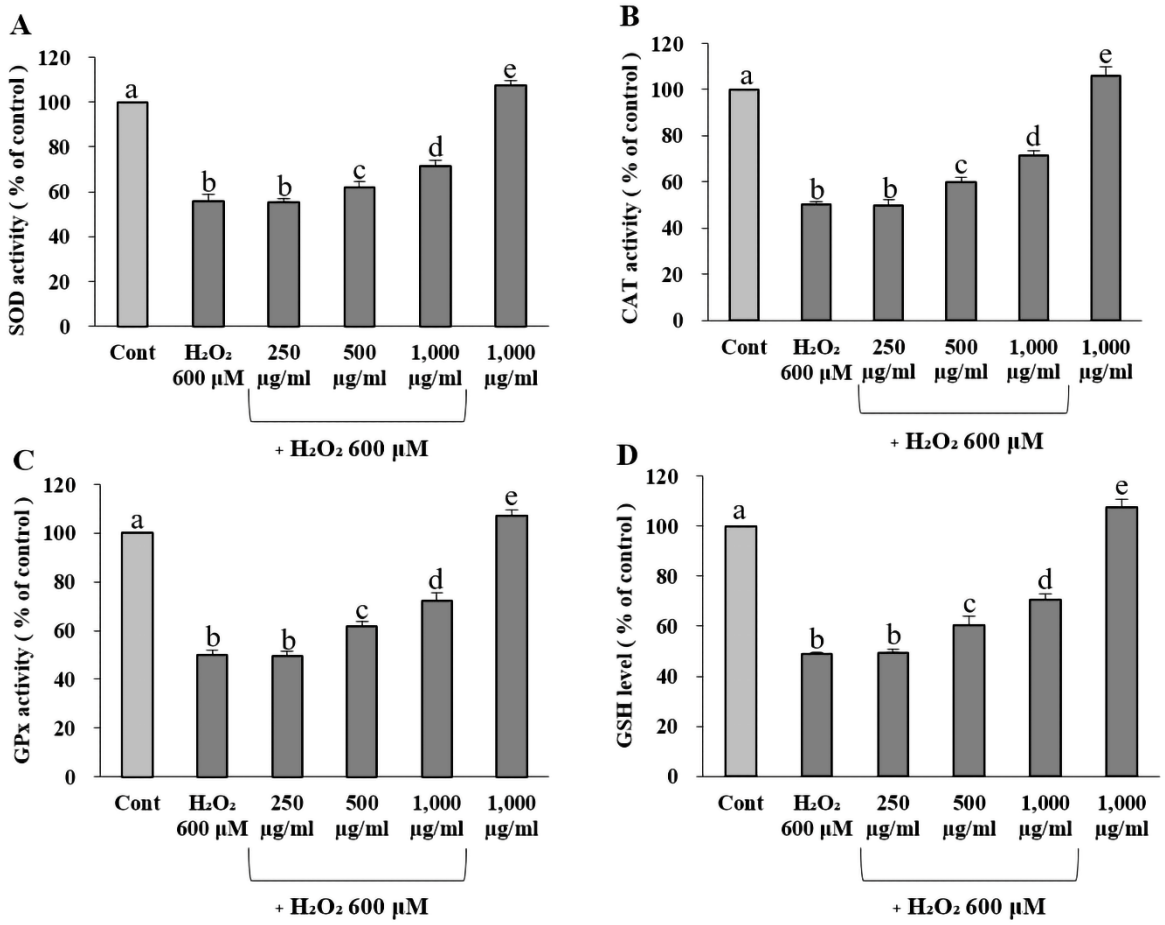


Figure 4. Effect of CRE on the intracellular antioxidant system in ARPE-19 cells under H_2O_2-induced oxidative stress. ARPE-19 cells were pre-incubated with CRE at 250, 500, and 1000 µg/mL for 24 h. After incubation, the cells were exposed to 600 µM H_2O_2 for 2 h. Cells were lysed and incubated in PBS containing 0.5% (v/v) Triton X-100. The supernatant was collected to determine **(A)** SOD, **(B)** CAT, and **(C)** GPx activities, and **(D)** GSH level using the specific assay kits. Data are represented as mean ± SD, n = 3. Different letters above the error bars indicate significant differences among treatment groups ($p < 0.05$).

Conclusion

In summary, CRE exerted protective effects against oxidative stress-induced cellular damage in ARPE-19 cells by scavenging ROS, inhibiting apoptosis (caspases-3 and -9), and enhancing both enzymatic antioxidants (SOD, CAT, and GPx) and the non-enzymatic antioxidant GSH. These findings highlight the potential health benefits of CRE in attenuating retinal oxidative stress, which may reduce risk factors associated with retinal degenerative disorders, including AMD. The results also suggest that Riceberry rice may help maintain eye health. However, the precise molecular mechanisms underlying these protective effects remain unclear because key antioxidant- and apoptosis-related signaling pathways were not directly investigated in the present study. Future studies should identify the bioactive compounds responsible for these effects and elucidate the roles of molecular targets and signaling pathways involved in oxidative stress and apoptosis. In addition, further in vivo and clinical studies are required to confirm its efficacy, safety, and protective effects against oxidative stress-induced ocular damage.

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Conflict of interest

The authors declare no conflict of interest.

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