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Original Research Article

The efficiency of lutein and zeaxanthin in *Zea mays* Linn. on protecting human corneal epithelial cells from photo-oxidative damage

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ABSTRACT

Oxidative damage plays a vital role in the pathogenesis of age-related macular degeneration (AMD). Lutein and zeaxanthin reduce phototoxic damage to the human eye and this supplementation of these carotenoids has a protective effect against photoinduced damage to the lens and the retina. This study aimed to demonstrate the protective effects and antioxidation properties of some lutein and zeaxanthin in *Zea mays* Linn. against the UVA-induced oxidative damage to human corneal epithelial cells (HEC). The antioxidant potential of lutein and zeaxanthin in *Zea mays* Linn. was evaluated by measuring DPPH assay. Cell viability was determined by MTT assay. Levels of malonyldialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured. The results showed that the IC_{50} values of Trolox (the positive control) and the *Zea mays* Linn. extracts associated with DPPH radical scavenging activity were 0.01 and 5.91 mg/mL, respectively. The UVA radiation significantly induced oxidative stress to reduce viability of HEC cells. The *Zea mays* Linn. extract significantly ameliorated UVA-induced oxidative damage by decreasing MDA concentration and increasing antioxidant enzymes activities (SOD and CAT). This suggests that lutein and zeaxanthin in *Zea mays* Linn. are effective in preventing UVA-induced damage in HEC cells and may be suitable as protective factors for the prevention of ocular damage.

INTRODUCTION

Ultraviolet radiation induces oxidative stress because it produces reactive oxygen species and modulates the level of antioxidants (Poswig et al., 1999; Shindo et al., 1993). Oxidative stress leads to DNA, protein and lipid damage. In DNA, hydroxyl radicals have been observed to cause strand breaks, and singlet oxygen has been observed to oxidize bases (Zhang et al., 1997). UV spectrum radiation encompasses UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm). Ultraviolet radiation provokes overproduction of free radicals, which damages biological systems. This observation has stimulated research into the role of natural

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antioxidants that can mitigate photobiologic damage. Most of the studies have focused on carotenoids, a group of natural pigments that have been recently used in biological systems for their antioxidant capacity (Krinsky, 1993; Mortensen et al., 2001). It now appears that important actions can be attributed to some carotenoids, and evidence indicates that they may reduce development rates of some human cancers (Peto et al., 1981).

Lutein and zeaxanthin, two xanthophylls supposed to delay formation of age-related macular degeneration (AMD), are found in numerous new dietary supplements appearing on the international market (Johra et al., 2020; Mrowicka et al., 2022). There are antioxidants that accumulate in the lens and retina of the human eye (Bernstein et al., 2001; Billsten et al., 2003; Cristaldi et al., 2022). These antioxidants protect ocular tissues against singlet oxygen and lipid peroxide damage (Edge et al., 1997). Beginning with middle age, antioxidant protection is depleted and this leads to the formation of age-related cataracts and macular degeneration (Nolan et al., 2007). Increasing the intake of fruits and vegetables high in lutein and zeaxanthin has been found to retard age related cataracts and macular degeneration (Humphries et al., 2003; Khachik et al.,1991)

Macular degeneration, also known as age-related macular degeneration (AMD), is associated with a decline in lutein and zeaxanthin concentration in the macula, and occurs late in life, provoking loss of acute vision in the fovea (Wong et al., 2014). AMD is the main cause of blindness in developed countries (Bressler et al., 2003; Kokotas et al., 2011), affecting about 8.7% of the population worldwide over 50 years of age, and recognized as the third leading cause of blindness after cataract and glaucoma (Kokotas et al., 2011).

Despite corn (*Zea mays* Linn.) being known for its carotenoid concentration, especially of lutein and zeaxanthin (Perry et al., 2009). The lutein and zeaxanthin are the predominant carotenoids of the macular pigment of the human retina and the levels of their concentration in the retina have functional and pathological consequences (i.e., age-related macular degeneration). Individuals suffering from age-related eye disease have inferior xantophyll densities throughout their retinas, and dietary zeaxanthin and lutein levels are inversely linked to the risk of AMD as well as cataracts. Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet via consumption of fruits and vegetables (Chapman et al., 2019). It has been reported that macular pigments might prevent a wide range of human diseases, including various cancers and other conditions (Bernstein et al., 2016)

The objectives of this study were to establish the effects of UVA radiation on endogenous antioxidant enzyme activities and lipid peroxidation in cells in culture and to evaluate if the addition *Zea mays* Linn. extract would protect against UVA-induced modulation of endogenous antioxidant enzyme activities and lipid peroxidation in these cells.

MATERIALS AND METHODS

Cell culture

The human corneal epithelial (HCE) cells were received from American Type Culture Collection (Cat No. CRL-11135, USA). Cells were grown in defined K-SFM medium supplemented with 12.5 µg/mL bovine pituitary extract, 1.25 µg/mL bovine insulin, and 1.25 ng/mL EGF, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37 °C in a humidified incubator with 5% CO_2.

Ethanol extracts of *Zea mays* Linn.

Zea mays Linn. was dried and ground to small particle size. Fifty grams of each dried powdered was extracted with 300 mL of 95% ethanol by using a sonicator for 5 min. the supernatants were filtered, concentrated under reduced pressure, and evaporated to dryness. The *Zea mays* Linn. extracts were collected and stored at 4°C. Extraction and quantification of the *Zea* mays Linn. extracts followed the method of Kingkaew et al. (2022).

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The free radical-scavenging activity of the mixed plant extracts was evaluated using a modification of a previously published method (Chandrasekar et al., 2006). Aliquots of sample extracts at various concentrations were each mixed with ethanol and then with 100 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in EtOH to a final concentration of 100 µM. The mixture was vigorously shaken and left to stand at room temperature for 30 minutes in the dark. The absorbance of the reaction solution was spectrophotometrically measured at 517 nm with microplate reader (Tecan Infinite 200 Pro, USA). The percentages of DPPH decolorization of the samples were calculated according to the equation: % decolorization = $[1 – (ABS_{sample}/ABS_{control})] \times 100$. The IC_{50} value was the effective concentration at which 50% of the DPPH radicals were scavenged, and butylated hydroxytoluene (BHT) was used as a positive control. All tests were performed in triplicate.

UVA irradiation and cell viability

Human corneal epithelial cells ($2x10^5$ cells/well) were seeded into 96-well plates and were irradiated with UVA irradiation. UVA irradiation exposure was performed which emitted wavelengths between 320–400 nm. The dose intensity was fixed at 5 J/cm^2 as measured with a power-meter when the lamp was placed 5 cm over the cell layer. The effects of *Zea mays* Linn. extracts on the cell viability of cultured HCE were tested by using MTT assay. For each experiment, cells were seeded into 96- well plates at a density of $2x10^5$ cells/well and were irradiated with UVA irradiation. The *Zea mays* Linn. extract was dissolved in DMSO and added to the medium at different levels 24 h later. After incubation for 24 h, MTT (50 μL/well of 1 mg/mL) was added and cells were incubated for another 4 h. DMSO at 100 μL/well was added after the removal of the culture medium. The optical density as the parameter of cell viability was measured at 570 nm with a microplate reader (Tecan Infinite 200 Pro, USA). All experiments were performed in triplicate.

Antioxidant enzyme activities

Superoxide dismutase activity was measured using an SOD assay kit (SOD Assay Kit-WST, Kumamoto, Japan). Superoxide dismutase activity values were calculated using the following equation: SOD activity (inhibition rate %) = $[(A_{blank1} – A_{blank3}) – (A_{sample} – A_{blank2})]/(A_{blank1} – A_{blank3}) \times 100$. Catalase activity was

measured by monitoring the enzymatic decomposition of hydrogen peroxide spectrophotometrically at 540 nm, using a CAT assay kit (Cayman Chemical Company, USA). Catalase activity values were calculated using the following equation: CAT activity = µmol of sample/20 min x sample dilution =nmol/min/mL TBARS (nmol MDA/mg protein) were measured as an index of lipid peroxidation (Wu et al., 2011). To prepare cell sonicates for measurement of *Zea mays* Linn. extract the incubation medium was rapidly aspirated and 0.5 mL of the appropriate chromatographic solvent was added at 0°C to the cell layer. The cells were removed from the dishes by scraping and placed on ice.

Statistical analysis

Results are presented as mean values and standard errors of the means. Data were analysed by one-way analysis of variance (ANOVA). The level of statistical significance was taken as $P<0.05$.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Assay

From previous study, the lutein (25.59 mg/100g) and zeaxanthin (17.74 mg/100g) content were found in the *Zea mays* Linn. extract (Kingkaew et al., 2022). To investigate the antioxidant activity of *Zea mays* Linn. extract, we evaluated their ability to scavenge the stable free radical DPPH. The scavenging activity of various concentrations of the extracts and the control compound, BHT, was analyzed, and the concentration required to inhibit each radical by 50% (IC_{50}) was measured. The IC_{50} values of trolox (the positive control) and the extracts associated with DPPH radical scavenging activity were 0.01 and 5.91 mg/mL, respectively. These results indicate that lutein and zeaxanthin in *Zea mays* Linn. extract has a profound effect on the antioxidant defense system. However, there was statistically significant difference in the IC_{50} value between Trolox and *Zea mays* Linn. extract of antioxidant capacity, as measured by the DPPH assay. Sindhu et al. (2010) reported that the antioxidant activity of lutein and zeaxanthin may be attributed to its unique chemical structure. Lutein and zeaxanthin not only have conjugated double bonds but also has two hydroxyl groups on both ends making it stronger antioxidant as compared to other carotenoids.

Cell death induced by UVA light exposure in HCE cells and protective role of antioxidants

UVA light triggered a dramatic apoptotic cell death after the cells were exposed for 5 min to a final dose of 5 J/cm^2. Cell viability of HCE cells were evaluated after irradiation with the UVA lamp. Survival of HCE cell progressively decreased to 62%. The *Zea mays* Linn. extract did not affect the survival of HCE cells at doses 50, 100 and 125 µg/mL (data not shown). The morphology of HEC cells did not change under 125 µg/mL of *Zea mays* Linn. extract (Figure 1.) The shielding and anti-oxidant power of different doses (50, 100 and 125 µg/mL) of *Zea mays* Linn. Extract in this photo-stress model was tested at the irradiation energy of 5 J/cm^2, which gave an intermediate response in terms of toxicity.

With regard to SOD activity, the inhibition rate of the control was 28.63%, whereas the inhibition rates of the extracts were 45.29% at 50 µg/mL ($P<0.05$ versus the control), 58.66% at 100 µg/mL ($P<0.05$ versus the control) and 63.17% at 125 µg/mL ($P<0.05$ versus the control), respectively (Figure 1). In addition, CAT activity also increased with the addition of the extracts (Figure 2). SOD and CAT activity were significantly increased ($P<0.05$) compared to control cells following exposure to UVA light and a significant increase ($P<0.05$) in TBARS level occurred. Supplementation with *Zea mays* Linn. extract also prevented the UVA-induced increase in TBARS (Table 1).

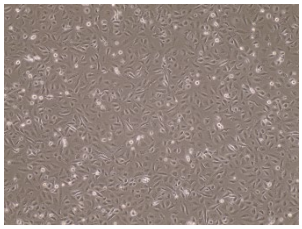

Figure 1. Cell morphology of HEC cells under 125 µg/mL of *Zea mays* Linn. extract

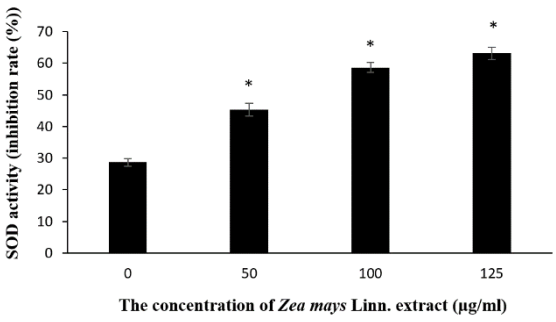

Figure 2. SOD activity after addition of the *Zea mays* Linn. extract (50, 100 and 125 µg/mL), as measured by SOD assay kits. *$P <$ 0.05 compared with baseline.

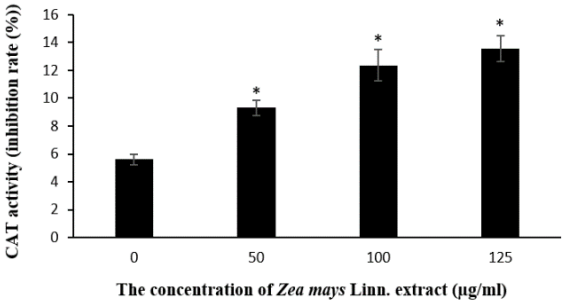

Figure 3. CAT activity after addition of the *Zea mays* Linn. extract (50, 100 and 125 µg/mL), as measured by CAT assay kits. *$P <$ 0.05 compared with baseline.

Cellular phototoxicity induced by UVA radiation has been reported in different cell lines (De Leeuw et al., 2001). UVA induced cellular alterations occur when ROS production during photosensitization generates oxidative stress (Marrot et al., 1998; Shen et al., 2017). Membrane leakage, DNA strand breaks,

pyrimidine dimers and adducts as well as damage to proteins and enzymes have been observed as consequences of UVA exposure (Didier et al., 2001). Lutein, together with its stereoisomer zeaxanthin, is the only carotenoid present in the human retina with the highest amount concentrated in the macula, where lutein dominates in the periphery, while zeaxanthin become predominant in the center. Moreover, the superoxide and hydrogen peroxide scavenging ability of lutein and zeaxanthin, carotenoids that are correlated with the function of the human retina, and of astaxanthin, chosen because its structure is very close to that of the other two molecules. The enhancement of antioxidant capacity in the lens via an elevation of lutein and zeaxanthin concentrations in the lens may contribute to the reduced risk of cataract in individuals with higher dietary lutein or zeaxanthin intake (Chasan-Taber et al., 1999; Hu et al., 2008). Since the only source of lutein and zeaxanthin in the body is dietary intake, it is reasonable to hypothesize that the concentrations of these nutrients in the lens and other tissues are related to status of long-term dietary intake.

Table 1 Protective effect of *Zea mays* Linn. extract against UVA light-induced modulations in TBARS levels in HCE cells in culture

***Zea mays* Linn. extract (µg/mL)**	**TBARS (nmol MDA/mg protein)**
Control*	3.29 ± 0.12
0	8.36 ± 0.32
50	6.21 ± 0.24
100	5.26 ± 0.19
125	4.02 ± 0.55

Control* Control cells not exposed to UVA light and not supplemented with *Zea mays* Linn. extract

A battery of antioxidant enzymes is essential in balancing excessive ROS build-up and maintaining the cellular redox balance. Key antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) are crucial for antioxidant defense in the eye and skin (Filip et al., 2011; Hsueh et al. 2022). SOD converts superoxide anions (O_2^-) into H_2O_2 and oxygen through a disproportionation reaction, and CAT further catalyze the conversion of H_2O_2 into water and O_2 (Obana et al., 2019). Lutein and zeaxanthin may lower ROS levels by eliminating them and by increasing the activity of SOD antioxidant enzymes, which are typically activated in response to ROS accumulation. Treatment with *Zea mays* Linn. extract increased SOD and CAT activity, indicating that it acted as a ROS-independent SOD activator. This was consistent with our observation that the extract induced SOD activity even without other stimuli, suggesting that lutein directly activated SOD activity. Lutein and zeaxanthin have multiple conjugated double bonds in its molecular structure that can eliminate free radicals in animals and increase SOD activity. This allows for quick and effective removal of free radicals in the body, protecting important cellular components such as proteins, lipids, and nucleic acids. This pathway also improves the body's immunity.

Increased the concentration of *Zea mays* Linn. extract by the HCE cells from lutein and zeaxanthin in *Zea mays* Linn. extract enriched growth media improved their ability to withstand UVA light-induced modulations in the antioxidant enzymes CAT and SOD. In this study the potential of lutein and zeaxanthin in *Zea mays* Linn. extract was more effective in restoring the UVA light-induced alteration in antioxidant enzyme activities and lipid peroxidation. These findings suggest that the *Zea mays* Linn. extracts used in this study can protect the corneal epithelium from UVA light–induced oxidative stress through the antioxidant enzymatic defense system.

CONCLUSIONS

In summary, the potential effects of lutein and zeaxanthin in *Zea mays* Linn. extracts may protect HCE cells from oxidative stress by restoring the inhibition rate of antioxidant enzymes SOD and CAT, which in turns largely reduces the cell oxidative damage markers of lipid peroxidation of membranes.

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