Ind. J. Pharm. Edu. Res., 2025; 59(3):1085-1092.
https://www.ijper.org

Original Article

Lutein and Zeaxanthin Complex 5:1 Protects Pigmented Epithelium Cells and Photoreceptors in the Retina from A2E-Mediated Phototoxicity: An *in vitro* Study

Chepattu Mohammed Abdullah Anzar, Molath Varkey Joseph, Ramachandran Sundaram, Bharadwaj Gururaj Vadiraj*, Padmanabhan Chettayil Prasad, Bineesh Earanimose, Neethumol Benny

Department of Research and Development, Olive Lifesciences Pvt. Ltd., Nelamangala, Bangalore, Karnataka, INDIA.

ABSTRACT

Background: The aging process of RPE (Retinal Pigment Epithelium cells) and the effects of blue light are caused by a high level of oxidative stress, which makes these cells susceptible to malfunctioning, cellular senescence and cell death. The accumulation of toxic byproducts of the visual pigment cycle, Bis-retinoid N-retinyl-N-retinylidene Ethanolamine (A2E), in the RPE is a major cause of visual impairment in the blue light effect. Degeneration of the retinal pigment epithelium and related photoreceptors follow photo-oxidation of A2E. **Objectives:** The objective of this study was to evaluate the protective effects of Lutein and Zeaxanthin complex 5:1 against photooxidation in A2E accumulated ARPE cells. **Materials and Methods:** ARPE -19 Cells were procured from ATCC, USA. The cell lines were subjected to Cytotoxicity evaluation against A2E and the test samples individually. A LC-MS/MS method of analysis was involved in assessing A2E levels in cells. A blue light of 480 nm was used to mediate A2E induced cytotoxicity in ARPE cells. Post treatment with Lutein and zeaxanthin and exposure to blue light, the cell viability was measured to evaluate the protective effect. Results**:** The LC-MS/MS method was refined to quantify A2E in cell lysate at 1-100 ng/mL. R^2 of >0.99 and accuracy of 93.01-109.81 across the tested concentrations indicated that the LC-MS/MS method was linear. There was no A2E carryover, and the method was unique to A2E with the detection of blank cell lysate. **Conclusion:** This study connotes that Lutein and Zeaxanthin may help in improvement of RPE cell viability against the ill effect of blue light induced A2E accumulation. This finding also supports the literature evidence that Lutein and Zeaxanthin helps protect eyes from the bad effects of blue light.

Keywords: A2E (Bis-retinoid N-retinyl-N-retinylidene ethanolamine), Blue light effect, Lutein and Zeaxanthin Complex 5:1.

Correspondence:
Mr. Bharadwaj Gururaj Vadiraj
M. Pharm (Pharmaceutics), Formulation Scientist, Department of Research and Development, Olive Lifesciences Pvt. Ltd., Bangalore-562123, Karnataka, INDIA.
Email: vadiraj@olivelifesciences.com

Received: 10-10-2024;
Revised: 21-12-2024;
Accepted: 02-04-2025.

INTRODUCTION

Age-related macular degeneration, or AMD, is the primary cause of blindness in older people in developed countries.[1] The most common type of AMD, known as dry AMD, is a slowly developing disease. The local accumulation of waste deposits produces retinal deformation in early-dry AMD. Following that, photoreceptors degenerate into smaller areas, which enlarge, resulting in advanced dry AMD (geographic atrophy). Photoreceptor death in the macula, the central part of the retina, leads to the loss of high-resolution color vision. This happens comes when the Retinal Pigment Epithelium (RPE) cells die.[2] RPE cells are important for both the phagocytosis of shed oxidized photoreceptor outer segments and the visual pigment cycle. The accumulation of A2E and associated retinal dimers is thought to be correlated with the A2E-induced disruption of RPE cell lysosomal activity, which is thought to be early stage of AMD development.

A2E and its isomers are produced by the reaction of Phosphatidyl-ethanolamine with two trans-retinal molecules.[3,4] A2E is an amphiphilic molecule that can change the characteristics of membranes[5] and interfere with lysosomal functions.[6,7] It can also increase the secretion of VEGF (Vascular Endothelial Growth Factor) *in vitro*,[8,9] activate inflammatory processes by recruiting macrophages and possibly induce neovascularization by increasing the production of Vascular Endothelial Growth Factor (VEGF).[10]

A group of yellow pigments that are fat-soluble and mostly present in fruits, green leafy vegetables and marigold flowers, carotenoids include lutein, one of the most prevalent forms of xanthophyll. Lutein is gaining a lot of attention due to its potential to prevent chronic conditions such as cataracts, Age-related Macular

DOI: 10.5530/ijper.20251039

Degeneration (AMD) and other eye diseases.[11,12] Furthermore, the strong antioxidant characteristics of lutein have elevated its significance as a functional component. For example, the pigment protects cells from photooxidation and photo destruction and functions as an active, high-energy blue light filter.[13] Strong antioxidant effects are produced by reducing the absorption of high-energy blue light, which protects the skin and macula. These advantageous biological functions are primarily ascribed to lutein's distinct molecular structure of unsaturated double bonds and hydroxyl carotenoids. Thus, lutein's antioxidant effects are closely related to its structural configuration, chemical stability and inherent properties.[14,15]

Tagetes erecta L., or marigolds, are plants of the Asteraceae family that have yellow to orange flowers. They are an essential source of lutein. Research has indicated that marigold flowers contain around 70% more lutein than carotenes. Lutein and Zeaxanthin are very powerful antioxidants in the food, cosmetic and pharmaceutical industries.[16,17] Marigold petals, which contain lutein and zeaxanthin, have been used to improve the color of egg yolks.[18] Lutein is linked to eye health because it is abundant in the macular region, a crucial portion of the eye. Furthermore, lutein is present in all regions of the eyes, with particular concentrations in the choroid, ciliary body, peripheral retina and retinal pigment epithelium.[19-21]

The novel formulation of Lutein and Zeaxanthin Complex 5:1 combines the unique and individual benefits of zeaxanthin and lutein in a synergistic manner, making it a truly innovative composition that stands out from the conventional products in the market. Unlike others that contain only one of these essential components, this formulation has both lutein and zeaxanthin in a precisely balanced ratio that leads to optimal efficacy.

This study aims at evaluating the protective effects of Lutein and Zeaxanthin complex 5:1 against photooxidation of ARPE-19 cells. This study offers a mechanistic approach on evaluating positive effects Lutein and Zeaxanthin complex 5:1 in management of macular degeneration.

MATERIALS AND METHODS

MaQxan®-5:1 is a proprietary composition containing Lutein and Zeaxanthin in the ratio of 5:1 manufactured and registered by Olive Lifesciences Pvt. Ltd., Nelamangala, Bangalore and Karnataka, India.

Evaluation of cytotoxicity of A2E and Test sample

Preparation of Cell lines and culture medium

ARPE-19 cells procured from ATCC, USA were cultured in 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 medium 1 mM HEPES (4-(2-Hydroxyethyl) Piperazine-1-Ethanesulfonic acid), 2 mM Glutamine and with 10% Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with cell dissociating solution (0.05 % trypsin). The cells are centrifuged, and the viability of the cells is determined.

Sample preparation

For cytotoxicity studies, 32 mg/mL stocks of test samples were prepared in 1 mL DMSO. Serial 2-fold dilutions were prepared from 320 µg/mL to 10 µg/mL using DMEM plain media for treatment.

A2E stock was prepared in DMSO at 10 mM stock followed by 1 mM and 100 µM working stocks. From 100 µM working stocks, further 2-fold dilutions were prepared from 100 µM to 3.12 µM using DMEM plain media for treatment.

Evaluation of Cytotoxicity of A2E and test sample

The cell count was adjusted to $5.0x10^5$ cells/mL using culture media. To each well of the 96 well microtiter plate, 100 µL of the cell suspension (50,000 cells/well) was added. After 24 hr, culture media was removed and monolayers were rinsed once with 1X PBS (Phosphate Buffered Saline) and 100 µL of A2E (with blue light exposure at 480 nm; 30 mW/cm^2 for 15 min) and Test sample at various test concentrations in a 2-fold dilution range was added to pre-designated wells. The plates were then incubated at 37°C for 24 hr in 5% CO_2 atmosphere. After the incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (5 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 hr at 37°C in 5% CO_2 atmosphere. The supernatant was removed carefully and 100 µL of DMSO was added and the plates and were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage of growth inhibition was calculated using the following formula and concentration of A2E needed to inhibit cell growth by 50% (IC_{50}) is determined from the dose-response curve.

Percentage of growth inhibition= 100 − (Test OD/Non-treated OD) × 100)

Evaluation of A2E photooxidation in cells pre-treated with Lutein and Zeaxanthin Complex 5:1

The cell count was adjusted to $3x10^6$ cells/mL using culture media. To each well of the 12- well microtiter plate, 1 mL of the cell suspension was added. After 24 hr, culture media was removed and monolayer was gently rinsed once with 1X PBS and the cells were pre-treated for 2 hr with Lutein and Zeaxanthin Complex 5:1 at 25 µg/mL and 50 µg/mL followed by addition of A2E at 30 µM along with the exposure of cells with blue light at 480 nm; 30 mW/cm^2 for 15 min followed by incubation of cells in conventional culture conditions for 22 hr. After the incubation, the test solutions in the wells were discarded and cell monolayer is rinsed with 1XPBS and harvested.

Illuminated cells were harvested and added to a solution of chloroform and methanol at a 2:1 ratio. This mixture was homogenized and centrifuged at 12,000 rpm for 10 min. The supernatant was dried under argon, re-dissolved in methanol and subjected to Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis.

LC-MS/MS Analytical method

Preparations of Reagents

Preparation of Mobile Phase A

500 mL of HPLC-grade water was taken in a 1-L glass bottle and 0.5 mL of 100% Formic acid was added and sonicated for 5 min.

Preparation of Mobile Phase B

500 mL of Methanol was taken in a 1-L glass bottle and sonicated for 5 min.

Needle wash

250 mL of each, Acetonitrile, Methanol, IPA and HPLC-grade water were taken in a 1-L glass bottle, mixed properly and sonicated for a few minutes.

Diluent for Stock and Working Solution of Standard and Internal Standard

Methanol

Preparation of Calibration Curve and cell lysate blank

Preparation of Blank Cell Lysate

To prepare the blank cell lysate, 45 µL of A2E-free cell lysate was transferred into a centrifuge tube, and 100 µL of methanol was added.

Preparation of Calibration Curve Standards

Preparation of Standard Stock Solutions

- ***Standard Stock Solution I*:** 10 mg of A2E standard was weighed and transferred into a 2 mL centrifuge tube. 1 mL of methanol was added to prepare a stock solution with a concentration of 10 mg/mL.
- ***Standard Stock Solution II*:** 10 µL of Standard Stock Solution I was transferred into a 2 mL centrifuge tube, and 990 µL of methanol was added to obtain a concentration of 100 µg/mL.

Preparation of Working Solution of Calibration Curve

To prepare the Calibration Curve, various working solutions were created from the standard stock solutions. The first solution, WS, was prepared by taking 10 µL of the standard stock II and diluting it to a final volume of 1000 µL, resulting in a concentration of 1000 ng/mL (SS5). The second solution, SS-5, was made by taking 500 µL of SS5 and diluting it to 1000 µL, giving a final concentration of 500 ng/mL (SS4). The third solution, SS-4, was prepared by diluting 200 µL of SS4 to a final volume of 1000 µL, yielding a concentration of 100 ng/mL (SS3). The fourth solution, SS-3, was made by diluting 500 µL of SS3 to 1000 µL, resulting in a concentration of 50 ng/mL (SS2). Finally, SS-2 was prepared by diluting 500 µL of SS2 to a final volume of 1000 µL, giving a concentration of 10 ng/mL (SS1).

Preparation of Calibration Standards

For the calibration curve, 45 µL of drug-free cell lysate was added to pre-labeled centrifuge tubes. To each tube, 5 µL of the appropriate calibrant was introduced to achieve the final cell lysate concentrations as listed below:

- **SS-5**: 1000 ng/mL (Calibrant ID: CC5)
- **SS-4**: 500 ng/mL (Calibrant ID: CC4)
- **SS-3**: 100 ng/mL (Calibrant ID: CC3)
- **SS-2**: 50 ng/mL (Calibrant ID: CC2)
- **SS-1**: 10 ng/mL (Calibrant ID: CC1)

10 µL of internal standard (IS) working solution were added. Subsequently, 100 µL of methanol was added, and the sample was vortexed for 10 min at 1200 rpm. The mixture was centrifuged at 15,000 rpm for 15 min. 100 µL of the supernatant was collected and analyzed by LC-MS/MS.

LC-MS/MS Analysis

2 µL each of blank cell lysate, calibration standards, QC and test samples were injected into the LC-MS/MS. The chromatograms were recorded and the responses were measured.

Acceptance Criteria of Analytical Batch Run

Linearity: A minimum of 5 non-zero calibration standards, when back calculated (Including CC5) shall fall within±15 %.

Evaluation of protective activity of test sample

The cell count was adjusted to $5.0x10^5$ cells/mL using culture media. To each well of the 96 well microtiter plate, 100 µL of the cell suspension (50,000 cells/well) was added. After 24 h, culture media was removed and monolayer was rinsed once with 1X PBS and the cells were pre-treated for 2 hr with 100 µL of test sample at various non-toxic concentrations in a two-fold dilution range followed by addition of A2E at 30 µM along with the exposure of cells with blue light at 480 nm; 30 mW/cm^2 for 15 min followed by incubation of cell in conventional culture conditions for 22 hrs. After the incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (5 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 hr at 37°C in 5% CO_2 atmosphere. The supernatant was removed carefully and 100 µL of DMSO was added and the plates and were

gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at a wavelength of 590 nm and the percentage protection/ cell viability was calculated.

Statistical evaluation

IC_{50} Value

The half maximal Inhibitory Concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, San Diego, CA, USA).

Nonlinear regression

In statistics, nonlinear regression is a form of regression analysis in which observational data are modelled by a function which is a nonlinear combination of the model parameters and depends

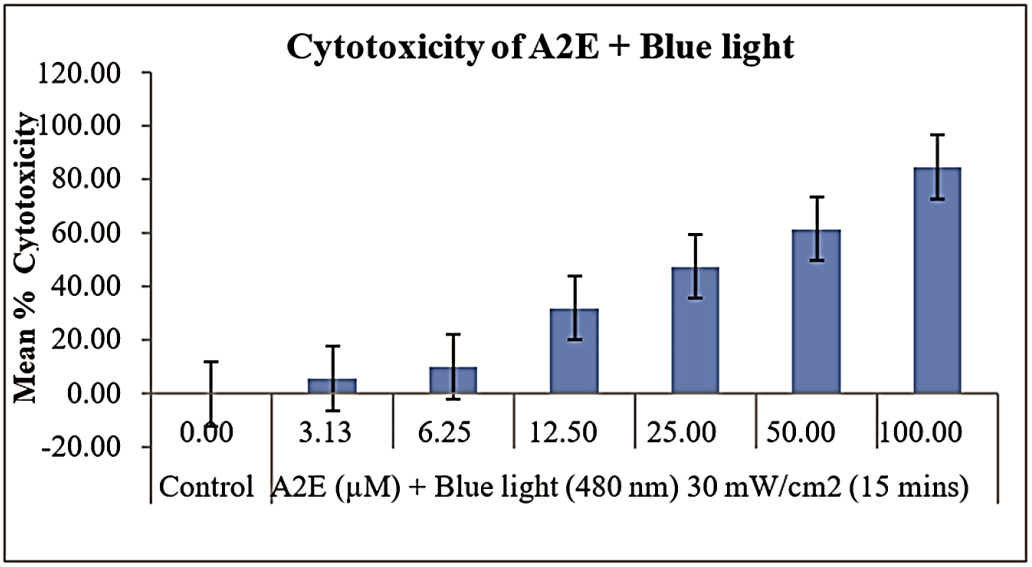

Figure 1: *In vitro* cytotoxicity of A2E in terms of percentage of cell viability against human Retinal Pigment Epithelial cell line (ARPE-19) by MTT assay.

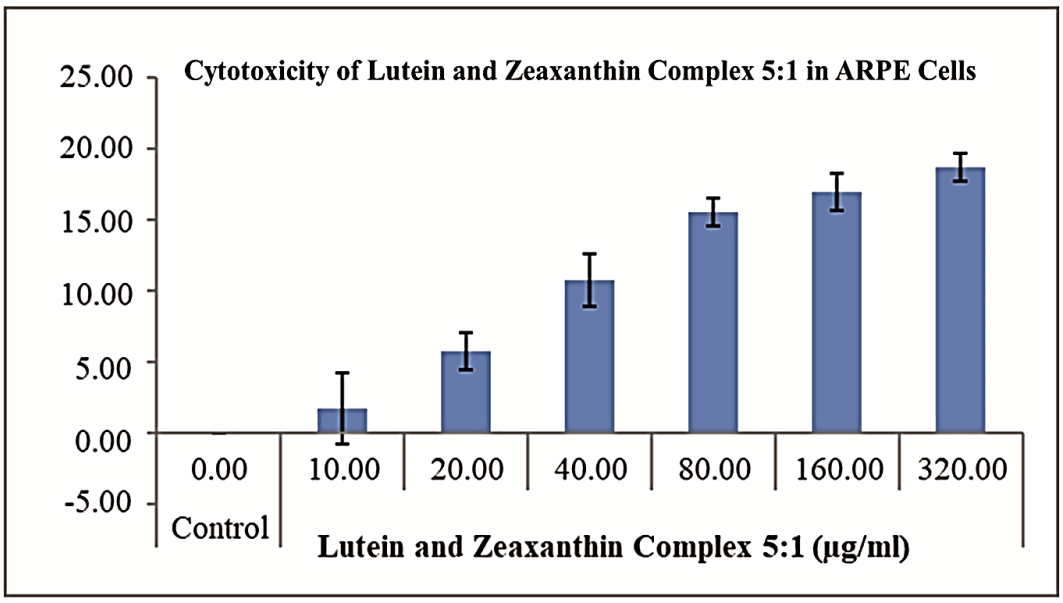

Figure 2: *In vitro* cytotoxicity of Lutein and Zeaxanthin complex 5:1 in terms of percentage of cell viability against human Retinal Pigment Epithelial cell line (ARPE-19) by assay.

Table 1: *In vitro* cytotoxicity of A2E in terms of percentage of cell viability against human Retinal Pigment Epithelial cell line (ARPE-19) by MTT assay.

Adult Retinal Pigment Epithelial Cells (ARPE									
Treatment	Conc. µM	OD at 590 nm	% of Cytotoxicity	OD at 590 nm	% of Cytotoxicity	OD at 590 nm	%of Cytotoxicity	Mean % of Cytotoxicity	SD
Control	0.00	0.695	0.00	0.730	0.00	0.715	0.00	0.00	0.00
A2E+Blue light(480 nm) 30mW/ cm^2 (15 min).	3.13	0.661	4.89	0.685	6.17	0.671	6.15	5.74	0.73
	6.25	0.625	10.07	0.659	9.73	0.640	10.49	10.10	0.38
	12.50	0.502	27.77	0.474	35.07	0.475	33.57	32.14	3.86
	25.00	0.328	52.81	0.417	42.88	0.378	47.13	47.61	4.98
	50.00	0.264	62.07	0.289	60.42	0.271	62.10	61.53	0.96
	100.00	0.090	87.05	0.129	82.33	0.111	84.48	84.62	2.37

Table 2: *In vitro* cytotoxicity of Lutein and Zeaxanthin Complex 5:1 in terms of percentage of cell viability against human Retinal Pigment Epithelial cell line (ARPE-19) by MTT assay.

Adult Retinal Pigment Epithelial Cells (ARPE)									
Treatment	Conc. µg/ mL	OD at 590 nm	% Cytotoxicity	OD at 590 nm	% Cytotoxicity	OD at 590 nm	% Cytotoxicity	Mean % Cytotoxicity	SD
Control	0.00	0.731	0.00	0.708	0.00	0.751	0.00	0.00	0.00
Lutein and Zeaxanthin Complex 5:1 (µg/mL)	10.00	0.698	4.46	0.711	-0.48	0.742	1.22	1.73	2.51
	20.00	0.684	6.37	0.678	4.18	0.701	6.64	5.73	1.35
	40.00	0.650	11.03	0.645	8.84	0.657	12.46	10.77	1.82
	80.00	0.609	16.63	0.602	14.92	0.638	15.09	15.55	0.95
	160.00	0.604	17.32	0.580	18.02	0.634	15.53	16.96	1.29
	320.00	0.590	19.23	0.571	19.29	0.619	17.59	18.71	0.97

Table 3: Summary of A2E on Lutein and Zeaxanthin Complex 5:1.

Group	Sample Name	Analyte Retention Time (min)	Analyte Peak Area (counts)	Calculated Concentration (ng/ mL)	Mean A2E±SD (ng/mL)
Normal control	G1-C-1	0.88	103	< 0	0±0
	G1-C-2	0.86	20	< 0	
A2E+Blue light	G2-IC-1	0.85	57	7361.37	7336.87±34.65
	G2-IC-2	0.91	102199	7312.37	
	G4-T2-2	0.93	166250	11701.5	
Lutein and Zeaxanthin Complex 5:1 (25 µg/ mL)+A2E+blue light.	G5-T3-1	0.93	162338	11710.6	11660.89±70.3
	G5-T3-2	0.91	162464	11611.18	
Lutein and Zeaxanthin Complex 5:1 (50 µg/ mL)+A2E+blue light.	G6-T4-1	0.91	161086	12900.88	12876.65±34.27
	G6-T4-2	0.91	178957	12852.42	

on one or more independent variables. The data are fitted by a method of successive approximation.

RESULTS

Evaluation of Cytotoxicity of A2E and Lutein and Zeaxanthin Complex 5:1

The results of cytotoxicity A2E+Blue light in terms of percentage of cell viability against ARPE-19 cells by MTT assay is tabulated in Table 1 and Figure 1. Based on this the IC_{50} was found to be 29.49µM. The results of cytotoxicity of Lutein and Zeaxanthin complex 5:1 in terms of percentage of cell viability against ARPE-19 cells by MTT assay is tabulated in Table 2 and Figure 2.

A2E levels in ARPE cells

The analysis of A2E in all the samples of study is tabulated in Table 3 and represented in Figure 3.

Evaluation of protective activity of Lutein and Zeaxanthin Complex 5:1

The percentage of protective effect of Lutein and Zeaxanthin Complex 5:1 against A2E+Blue light treated cells is tabulated in Table 4 and Figure 4.

DISCUSSION

The third most common cause of blindness in the elderly is AMD, which causes the macula to deteriorate. While numerous approaches to treating neovascular AMD have been investigated, no strategy has been shown to be sufficiently successful in treating or preventing dry AMD. Antioxidants derived from plants, such as lutein and zeaxanthin, have been shown to have protective effects against ARPE-19 cell damage caused by A2E.[22,23] Previous research has indicated that *Tagetes erecta* is a source of antioxidants.

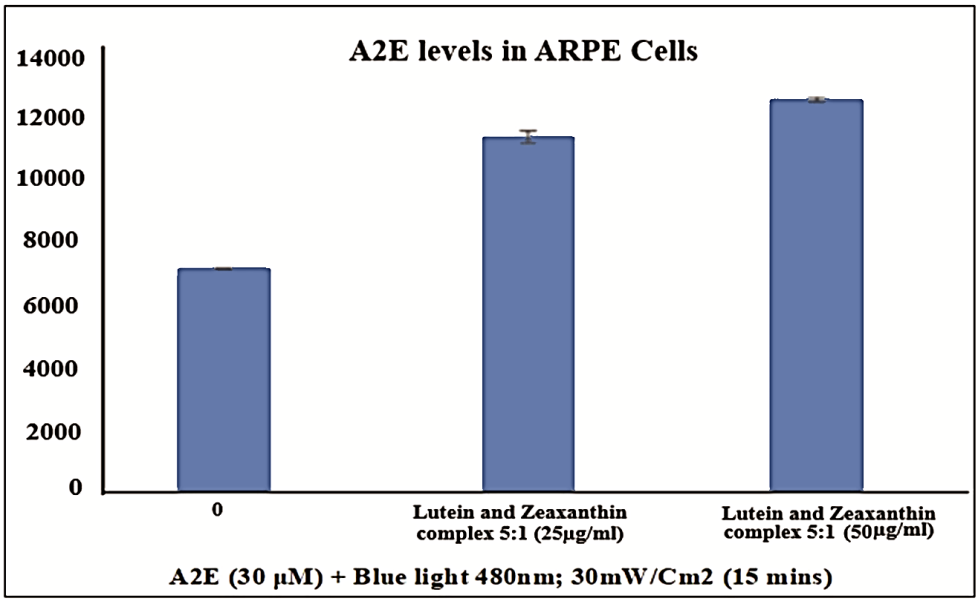

Figure 3: A2E levels in ARPE cells.

Table 4: Percentage of Protective effect of Lutein and Zeaxanthin Complex 5:1 against A2E+Blue light treated cells.

Adult Retinal Pigment Epithelial Cells (ARPE)									
Treatment	Conc. µg/mL	OD at 590 nm	% of Protection	OD at 590 nm	% of Protection	OD at 590 nm	% of Protection	Mean % of Protection	SD
Control	0	0.729	100.00	0.719	100.00	0.736	100.00	100.00	0.00
Lutein and Zeaxanthin Complex 5:1+A2E (30µM)+Blue light 480 nm; 30 mW/cm2 (15 min)	0	0.307	0.00	0.268	0.00	0.319	0.00	0.00	0.00
	1.56	0.318	2.49	0.270	0.40	0.321	0.59	1.16	1.16
	3.13	0.357	11.88	0.301	7.34	0.345	6.32	8.51	2.96
	6.25	0.392	20.19	0.360	20.38	0.399	19.18	19.92	0.64
	12.50	0.440	31.48	0.442	38.61	0.468	35.73	35.28	3.59
	25.00	0.531	53.18	0.532	58.58	0.532	51.13	54.30	3.85
	50.00	0.610	71.88	0.577	68.55	0.611	70.14	70.19	1.67

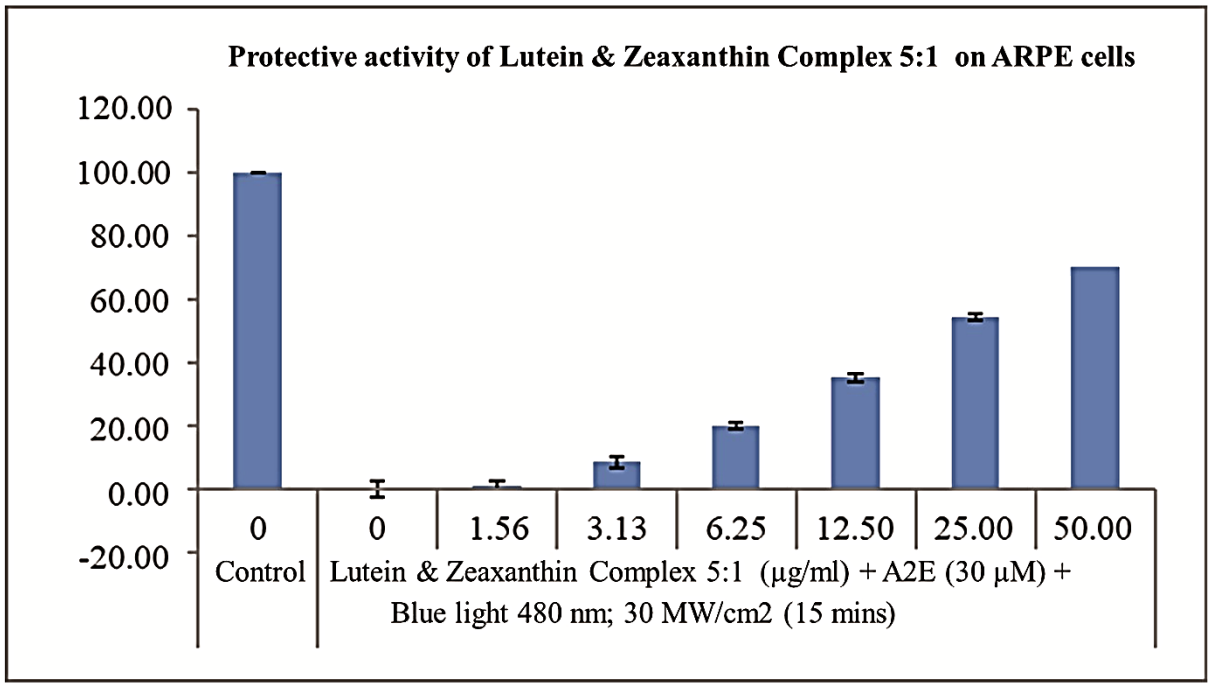

Figure 4: Percentage of protective effect of Lutein and Zeaxanthin complex 5:1 against A2E +Blue light treated cells.

Photochemical damage to the retina can result from exposure to high-energy radiation.[24] Photo-oxidative stress is caused in the retina during this phase by an important rise in superoxide anion generation and phagocytosis of the outer segment of photoreceptors.[25,26] Therefore, one therapeutic approach to prevent or slow down the evolution of early AMD is the administration of ROS scavengers.[27]

LC-MS/MS method was optimized for quantification of 1-100 ng/mL of A2E in cell lysate. LC-MS/MS method was linear with R^2 of >0.99 and accuracy of 93.01-109.81 across the tested concentration. The method was specific to A2E with detection of blank cell lysate and no carryover of A2E was observed. ARPE-19 cells were treated with Lutein and Zeaxanthin complex 5:1 at various test concentrations ranging from 1.56 µg/mL to 50µg/mL in a two-fold dilution range to evaluate its Protective activity of test sample in blue light mediated A2E induced cytotoxicity in ARPE cells. The results showed that at the highest concentration of Lutein and Zeaxanthin complex 5:1 at 50 µg/mL has shown protective activity of 70.19±1.67 % protection.

The purpose of this study was to evaluate the effectiveness of Lutein and Zeaxanthin Complex 5:1, in *in vitro* models of retinal degeneration induced by blue light. A2E can become extremely toxic to ARPE-19 cells after photooxidation, even at modest levels that are normally non-toxic to RPE cells.[28] Therefore, we used the A2E and BL (blue light) combination as a suitable model to mimic the progression of dry AMD *in vitro*. Lutein and Zeaxanthin Complex 5:1, interestingly, inhibited RPE cells from dying when exposed to A2E and BL (blue light).

CONCLUSION

These findings support that Lutein and Zeaxanthin Complex 5:1, inhibits BL-induced retinal degeneration by lowering visual impairment caused by blue light and preventing photooxidation of intracellular A2E. The purpose of this study was to determine whether Lutein and Zeaxanthin Complex 5:1 exhibited any protective effects against retinal damage caused by BL and to identify the underlying mechanisms of this action *in vitro*. Lutein and Zeaxanthin Complex 5:1 inhibited BL-induced RPE cell death in a concentration-dependent manner. Photooxidation of A2E was found to be one of the reasons for cellular death. Treating such cells with Lutein and Zeaxanthin complex 5:1 increased the cell viability, suggesting the protective actions of Lutein and Zeaxanthin. These findings pave ways for development of Lutein and Zeaxanthin based prophylactic and therapeutic regimens for AMD and associated eye disorders.

ACKNOWLEDGEMENT

The authors are grateful to Skanda Life Sciences Pvt. Ltd., Bangalore, who supported us in carrying out this *in vitro* study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

This is an *in vitro* study; no animals were used in the study hence ethical statement is not applicable.

ABBREVIATIONS

RPE: Retinal pigment epithelium; **A2E:** Bis-retinoid N-retinyl-N-retinylidene ethanolamine; **DMSO:** Dimethyl sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **HEPES:** (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid); **FBS:** Fetal Bovine Serum **PBS:** Phosphate buffered saline; **MTT:** 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay; **BL:** Blue light; **AMD:** Age-related macular degeneration.

SUMMARY

The study demonstrates the effects of Lutein and Zeaxanthin complex 5:1 in blue light induced photooxidation of A2E in ARPE-19 cells. Results indicate that this complex inhibits cell death in a concentration-dependent manner. These findings suggest that Lutein and Zeaxanthin Complex 5:1 may serve as a potential therapeutic or prophylactic agent to mitigate the detrimental effects of blue light exposure, thereby addressing various eye disorders and contributing to better vision.

REFERENCES

1. Congdon NO, Colmain B, Klaver CC, Klein R, Muñoz B, Friedman DS, *et al.* Causes and prevalence of visual impairment among adults in the United States. Arch Ophthalmol. 2004;122:477485. https://doi.org/10.1001/archopht.122.4.477
2. Kinnunen K, Petrovski G, Moe MC, Berta A, Kaarniranta K. Molecular mechanisms of retinal pigment epithelium damage and development of age-related macular degeneration. Acta Ophthalmol. 2012;90:299309. https://dx.doi.org/10.1111/j.1755-3768.2011.02179.x
3. Sparrow JR, Fishkin N, Zhou J, Cai B, Jang YP, Krane S, *et al.* A2E, a by-product of the visual cycle. Vision Res. 2003;43:29832990. https://doi.org/10.1016/s0042-6989(03)00475-9
4. Washington I, Saad L. The Rate of Vitamin A Dimerization in Lipofuscino genesis, Fundus Autofluorescence, Retinal Senescence and Degeneration. Adv Exp Med Biol. 2016;347353. https://doi.org/10.1007/978-3-319-17121-0_46
5. De S, Sakmar TP. Interaction of A2E with model membranes. Implications to the pathogenesis of age related macular degeneration. J Gen Physiol. 2002;120:147157. https://doi.org/10.1085/jgp.20028566
6. Eldred GE. Lipofuscin fluorophore inhibits lysosomal protein degradation and may cause early stages of macular degeneration. Gerontology. 1995;41:1528. https://doi.org/10.1159/000213722
7. Finnemann SC, Leung LW, Rodriguez-Boulan E. The lipofuscin component A2E selectively inhibits phagolysosomal degradation of photoreceptor phospholipid by the retinal pigment epithelium. Proc Natl Acad Sci USA. 2002;99:38422847. https://doi.org/10.1073%2Fpnas.052025899
8. Anderson OA, Finkelstein A, Shima DT. A2E induces IL-1β production in retinal pigment epithelial cells via the NLRP3 inflammasome. PLoSONE. 2013;8(6):e67263. https://doi.org/10.1371/journal.pone.0067263
9. Zhang J, Bai Y, Huang L, Qi Y, Zhang Q, Li S, *et al.* Protective effect of autophagy on human retinal pigment epithelial cells against lipofuscin fluorophore A2E: implications for age-related macular degeneration. Cell Death Dis. 2015;6:e1972. https://doi.org/10.1038/cddis.2015.330
10. Iriyama A, Inoue Y, Takahashi H, Tamaki Y, Jang W-D, Yanagi Y. A2E, a component of lipofuscin is pro-angiogenic *in vivo*. J Cell Physiol. 2009;220:469475. https://doi.org/10.1002/jcp.21792
11. Sajilata MG, Singhal RS, Kamat MY. The carotenoid pigment zeaxanthin - a review. Compr Rev Food Sci Food Saf. 2008;7:29-49. https://doi.org/10.1111/j.1541-4337.2007.00028.x
12. Jiao Y, Zheng X, Chang Y, Li D, Sun X, Liu X. Zein-derived peptides as nanocarriers to increase the water solubility and stability of lutein. Food Funct. 2018;9:117-123. https://doi.org/10.1039/c7fo01652b
13. Kijlstra A, Tian Y, Kelly ER, Berendschot TT. Lutein: more than just a filter for blue light. Prog Retin Eye Res. 2012;31:303-315. https://doi.org/10.1016/j.preteyeres.2012.03.002
14. Li LH, Lee JC, Leung HH, Lam WC, Fu Z, Lo ACY. Lutein supple mentation for eye diseases. Nutrients. 2020;12:1721. https://doi.org/10.3390/nu12061721
15. Nwachukwu ID, Udenigwe CC, Aluko RE. Lutein and zeaxanthin: production technology, bioavailability, mechanisms of action, visual function and health claim status. Trends Food Sci Technol. 2016;49:74-84. http://dx.doi.org/10.1016/j.tifs.2015.12.005
16. Fu XQ, Ma N, Sun WP, Dang YY. Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (*Tagetes erecta* L.) flower. Ind Crops Prod. 2018;123:296-302. http://dx.doi.org/10.1016/j.indcrop.2018.06.087
17. Indrawati R, Kurniawan JM, Wibowo AA, Juliana Gunawan IA, Heriyanto Brotosudarmo TH. Integrated solvent-free extraction and encapsulation of lutein from marigold petals and its application. CyTA-J Food. 2019;17:121-127. http://dx.doi.org/10.1080/19476337.2018.1544591
18. Hasin BM, Ferdaus AJ, Islam MA, Uddin MJ, Islam MS. Marigold and orange skin as egg yolk color promoting agents. Int J Poult Sci. 2006;5:979-987. https://doi.org/10.3923/ijps.2006.979.987
19. Koushan K, Rusovici R, Li W, Ferguson LR, Chalam KV. The role of lutein in eye-related disease. Nutrients. 2013;5:1823-1839. https://doi.org/10.3390%2Fnu5051823
20. The Age-Related Eye Disease Study 2 (AREDS2) Research Group. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 Report No. 3. JAMA Ophthalmol. 2014;132:142-149. https://doi.org/10.1001/jamaophthalmol.2013.7376
21. Buscemi S, Corleo D, Di Pace F, Petroni ML, Satriano A, Marchesini G. The effect of lutein on eye and extra-eye health. Nutrients. 2018;10:1321. https://doi.org/10.3390%2Fnu10091321
22. Kim J, Jin HL, Jang DS, Jeong KW, Choung SY. Quercetin-3-O-alpha-l-arabinopyranoside protects against retinal cell death via blue light-induced damage in human RPE cells and Balb-c mice. Food Funct. 2018;9:2171-2183. https://doi.org/10.1039/c7fo01958k
23. Sparrow JR, Vollmer-Snarr HR, Zhou J, Jang YP, Jockusch S, Itagaki Y, *et al.* A2E-epoxides damage DNA in retinal pigment epithelial cells Vitamin E and other antioxidants inhibit A2E-epoxide formation. J Biol Chem. 2003;278:18207-18213. https://doi.org/10.1074/jbc.m300457200
24. Sparrow JR, Nakanishi K, Parish CA. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. Investig Ophthalmol Vis Sci. 2000;41, 1981-1989.
25. Jang YP, Zhou JL, Nakanishi K, Sparrow JR. Anthocyanins protect against A2E photooxidation and membrane permeabilization in retinal pigment epithelial cells. Photochem Photobiol. 2005;81:529-536. https://doi.org/10.1562%2F2004-12-14-RA-402
26. Fernández-Sánchez L, Lax P, Noailles A, Angulo A, Maneu V, Cuenca N. Natural compounds from saffron and bear bile prevent vision loss and retinal degeneration. Molecules. 2015;20:13875-13893. https://doi.org/10.1562%2F2004-12-14-RA-402
27. Ramkumar HL, Tuo J, Shen DF, Zhang J, Cao X, Chew EY, *et al.* Nutrient supplementation with n3 polyunsaturated fatty acids, lutein and zeaxanthin decrease A2E accumulation and VEGF expression in the retinas of Ccl2/Cx3cr1-deficient mice on Crb1(rd8) background. J Nutr. 2013;143:1129-1135. https://doi.org/10.3945%2Fjn.112.169649
28. Jin HL, Lee SC, Kwon YS, Choung SY, Jeong KW. A novel fluorescence-based assay for measuring A2E removal from human retinal pigment epithelial cells to screen for age-related macular degeneration inhibitors. J Pharm Biomed Anal. 2016;117:560-567. https://doi.org/10.1016/j.jpba.2015.10.010

Cite this article: Anzar CMA, Joseph MV, Sundaram R, Vadiraj BG, Prasad PC, Earanimose B, *et al*. Lutein and Zeaxanthin Complex 5:1 Protects Pigmented Epithelium Cells and Photoreceptors in the Retina from A2E-Mediated Phototoxicity: An *in vitro* Study. Indian J of Pharmaceutical Education and Research. 2025;59(3):1085-92.