

α -Iso-Cubebene Alleviates AMD-like Retinal Injury Through Activation of Nrf2-Mediated Antioxidant Signaling and Suppression of Inflammation

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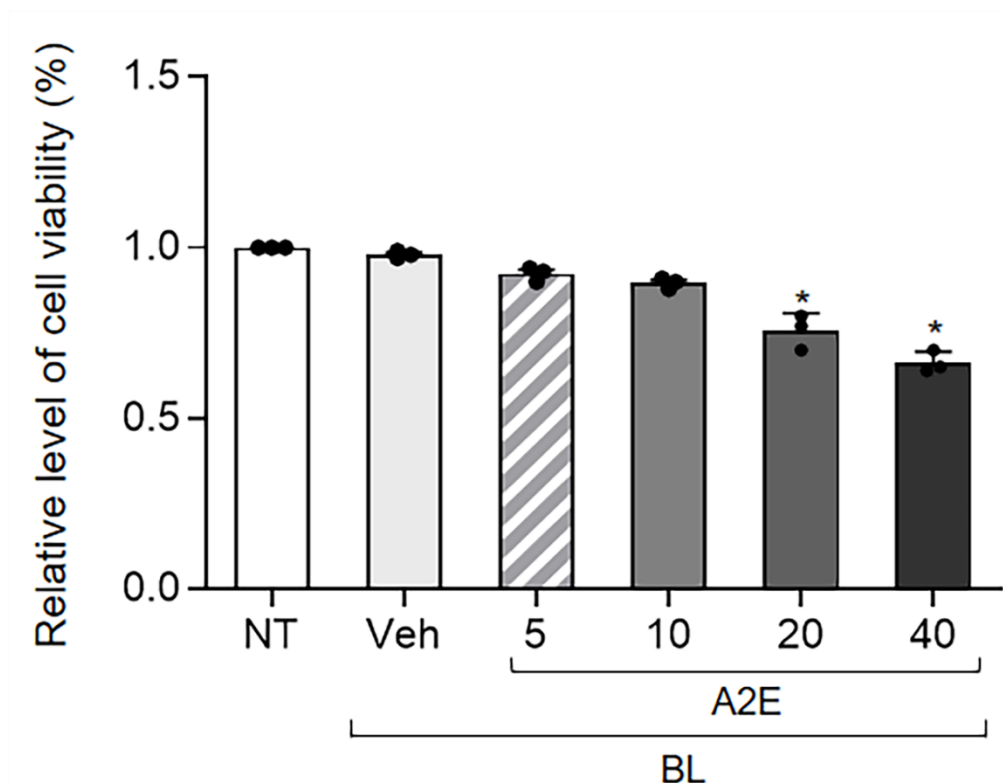


Figure S1. Determination of the optimal A2E dosage for cytotoxicity analysis. Cell viability was determined using an MTT assay. Three wells were prepared per treatment group, and the optical density was measured in triplicate. Results are reported as the mean $\pm$ SD. * $P < 0.05$ versus the NT group. Abbreviations: NT, Non-treated; Veh, Vehicle (DMSO); VitC, Vitamin C as a positive control; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BL, blue light; A2E, bis-retinoid N-retinyl-N-retinylidene ethanolamine.

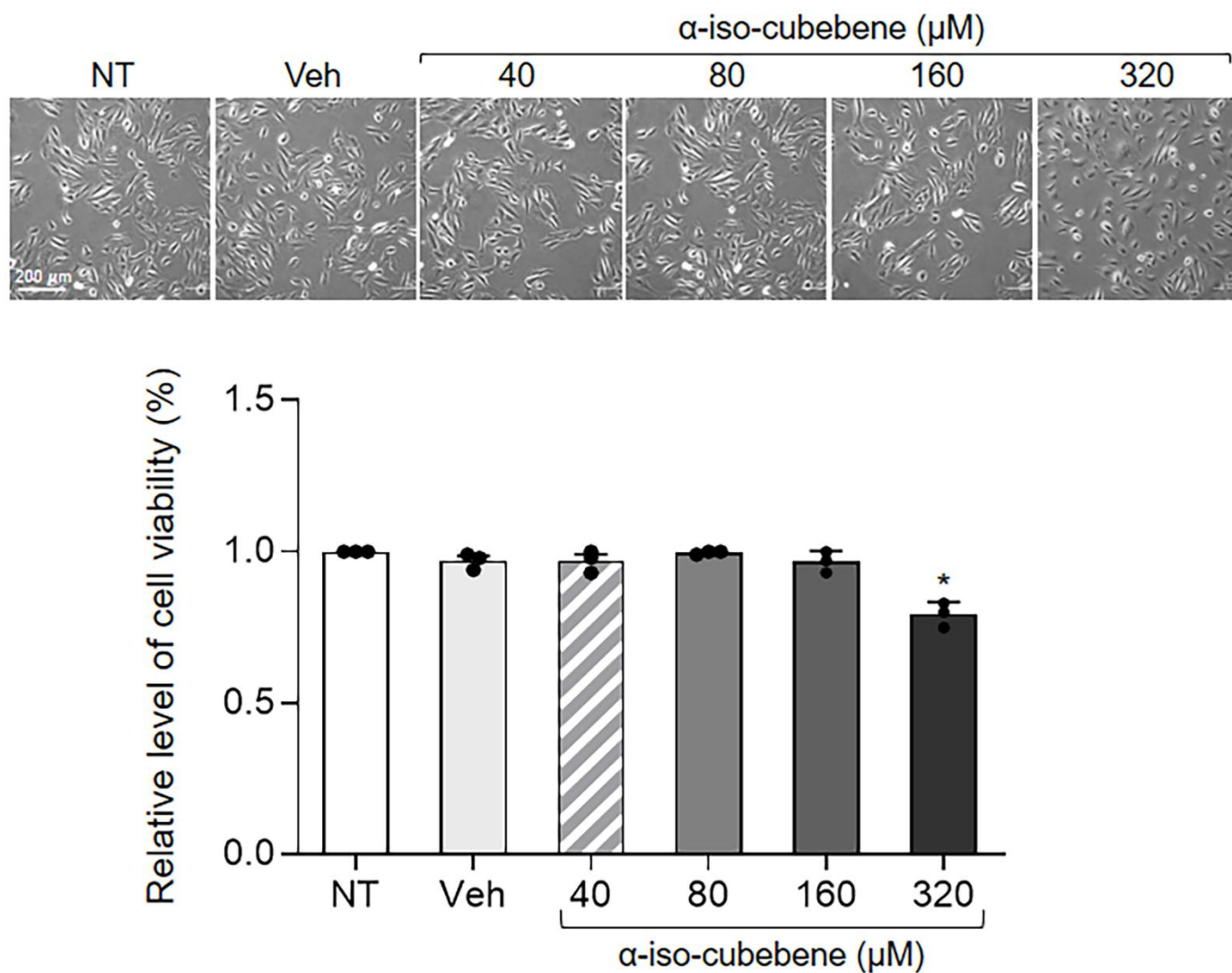


Figure S2. Determination of the optimal α -iso-cubebene concentration in ARPE-19 cells for anti-AMD effects. Morphological changes were observed under a microscope at 200 $\times$ magnification. Cell viability was determined using an MTT assay. Three wells were prepared per treatment group, and the optical density was measured in triplicate. Results are reported as the mean $\pm$ SD. * $P < 0.05$ versus the NT group. Abbreviations: NT, Non-treated; Veh, Vehicle (DMSO); VitC, Vitamin C as a positive control; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

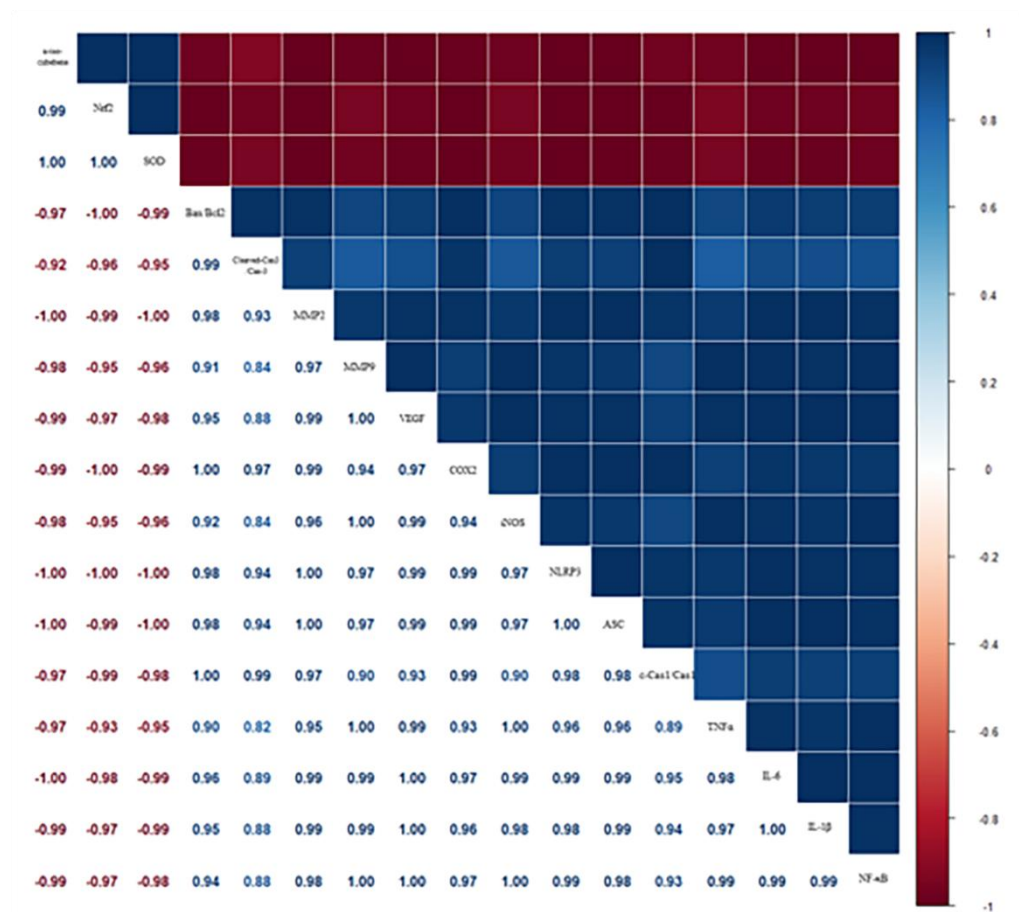


Figure S3. Correlogram drawn in Corrplot for the significance of correlative relationship between α -iso-cubebene concentration and the measured parameters from ARPE-19 cells. The coloring degree is scaled from strongly positive (dark blue) to strongly negative (dark red). The numbers and colors represent the same values in a symmetrical structure based on the factors located on the diagonal.

Table S1. List of antibodies for western blot analyses.

Name	Host	Company	Dilution
Anti-Nrf2	Rabbit	Abcam	1:1000
Anti-SOD	Rabbit	Abcam	1:1000
Anti-Bax	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-Bcl-2	Rabbit	Invitrogen	1:1000
Anti-Cas-3	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-MMP2	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-MMP9	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-VEGF	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-iNOS	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-COX-2	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-ACS	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-caspase-1	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-cleaved caspase-1	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti-NLRP3	Rabbit	Cell Signaling Technology Inc.	1:1000
Anti- β -actin	Rabbit	Sigma-Aldrich	1:3000

Table S2. List of primer for RT-qPCR analyses.

Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
IL-6	CCGGGAGCCTCTTGATACAG	AGCCCCTCTCAAAGTCACACA
IL-1 β	GCAGATTTGCTGGTTGGATT	GGCCATATAGCCTCAAACATGAT
TNF- α	CTGAGGCCCTCCCACATCT	GGAAAGGAACAAGGCCAACA
NF- κ B	GTAAC AGCAG GACCC AAGGA	AGCCC CTAAT ACACG CCTCT
β -actin	ACGGCCAGGTCATCACTATTG	CAAGAAGGAAGGCTGGAAAAGA