Supplementary Materials


Supplementary Figure Legends

**Figure S1.** Carnosic acid (CA)-induced upregulation of anti-oxidant and ARE-related genes by microarray analysis. ARPE-19 cell lines were treated with CA or vehicle, and total RNA was extracted and used for expression analysis of ARE-related genes. Fold-change after CA treatment compared to vehicle is plotted.

**Figure S2.** Effect of CA on Nrf2 translocation into the nucleus of ARPE-19 cells. Retinal cells were treated with vehicle (A) or CA (B) for 24 hours. Cells were then fixed, processed for immunostaining, and Nrf2 protein visualized by FITC-conjugated secondary antibody. DNA was counterstained with Hoechst.

**Figure S3.** Concentration of CA in plasma and retina after intraperitoneal (i.p.) injection in rats. Liquid chromatography/mass spectrometry (LC/MS/MS) analysis of carnosic acid in plasma (A) and neural retina (B). Rats received one or two doses of CA at a concentration of 25 mg/kg/d by i.p. injection. Then, 24 hours after the last injection, plasma and neural retina were collected and analyzed for CA (n = 3 for each group).
**Figure S4.** Retinal morphology in control animals treated with CA but not exposed to light. (A) Retinal ONL thickness in rats not exposed to light damage (LD) or drug. Values are mean ± SEM. (B) ONL thickness for 3 groups of control rats: no LD-no treatment (n = 4), no LD-Vehicle treatment (n = 4), and no LD-CA treatment (n = 4). Values represent mean ± SEM. Groups were compared by two-way ANOVA and found not to be significantly different (P > 0.05).

**Figure S5.** Cell death detected by TUNEL analysis in the ONL of rat retinas. (A and B) Photomicrographs of retinal sections from rats exposed to damaging light and treated with vehicle (A) or CA (B). Apoptotic TUNEL-positive cells (red), DNA stained with Hoechst (blue). (C) Bar graph of Tunel-positive cells in the retinal ONL. Rats were divided into the following groups: (i) not exposed to light damage (LD) and non-treated, (ii) not exposed to LD and treated with CA, (iii) exposed to LD and treated with CA, and (iv) exposed to LD and treated with vehicle (n = 8 total). TUNEL-positive cells were counted in 14 targeted fields within each retina chosen to cover the area most susceptible to light damage. Values represent mean ± SEM (****P < 0.0001 by one-way ANOVA). GraphPad Prism 5 software was used for data analysis and graphical representation.
Figure S1

![Gene expression (fold change) graph]

Figure S2

A

B
Figure S3

A

![Graph A with CA Concentration (μM) on the Y-axis, and 1 Dose and 2 Doses on the X-axis.]

B

![Graph B with CA Concentration (μM) on the Y-axis, and 1 Dose and 2 Doses on the X-axis.]

Figure S4

A

![Graph A with Thickness of Outer Nuclear Layer (μm) on the Y-axis, and various concentrations on the X-axis.]

B

![Graph B with Mean ONL Thickness (μm) on the Y-axis, and Inferior and Superior on the X-axis with different treatments represented by bars.]

- No LD - No Treatment
- No LD-Diluent
- No LD-CA
Figure S5