• Basic Research •

Fructus lycii mitigates oxidative stress in dry age-related macular degeneration models *via* Nrf2/ARE pathway

Yuan Peng[1], Ling–Yan Liang[2], Han–Lei Wei[1], Qin Huang[1], Ling–Mei Yuan[1], Yu–Yan Xie[1], Qiu–Yu Tang[1], An–Qi Wang[1], Jia–Ming Li[1], Qin–Shang Guo[1], Bing–Lin Huang[3]

[1]Department of Ophthalmology, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, Jiangxi Province, China

[2]Department of Ophthalmology, Shenzhen Traditional Chinese Medicine Hospital, Shenzhen 518033, Guangdong Province, China

[3]College of Clinical Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, Jiangxi Province, China

Co-first Authors: Yuan Peng and Ling-Yan Liang

Correspondence to: Bing-Lin Huang. College of Clinical Medicine, Jiangxi University of Traditional Chinese Medicine, No.1688, Meiling Avenue, Xinjian District, Nanchang 330004, Jiangxi Province, China. huangbinglin2009@163.com

Received: 2025-09-24 Accepted: 2026-03-12

Abstract

● **AIM:** To evaluate the effect of *Fructus lycii* (FL) aqueous extract on dry age-related macular degeneration (AMD) in mice *via* the nuclear factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) signaling pathway and investigate the protective effect of FL-containing serum on hydrogen peroxide (H_2O_2)-treated human retinal pigment epithelial cells (ARPE-19) *in vitro*.

● **METHODS:** *In vivo* dry AMD mouse model was established by intraperitoneal injection of $NaIO_3$ solution and treated with aqueous extract of FL. The pathological changes of mouse retinal tissues were observed by electron microscopy; the activity of superoxide dismutase (SOD) and catalase (CAT) in mouse serum was detected by colorimetric method. *In vitro* dry AMD model was established by H_2O_2 induction of ARPE-19 cells and treated with FL-containing serum. Methylthiazolyldiphenyl-tetrazolium bromide assay and scratch assay were performed to detect cell activity and proliferation ability. Expression of Nrf2, heme oxygenase-1 (HO-1), and glutamate-cysteine ligase catalytic subunit (GCLC) in retinal tissues and ARPE-19 cells were detected by Western blot and quantitative real-time polymerase chain reaction (Q-PCR).

● **RESULTS:** The *in vivo* study revealed severe deposits under the retinal pigment epithelium and thickened Bruch's membrane in dry AMD mice. However, aqueous extract of FL reduced the formation of deposits and decreased the thickness of Bruch's membrane. SOD and CAT activities were significantly reduced in the serum of dry AMD mice, and aqueous extract of FL upregulated SOD and CAT activities. In addition, gene and protein expression of Nrf2, HO-1, and GCLC were significantly downregulated in dry AMD mice, but significantly upregulated by FL aqueous extract treatment. *In vitro* studies showed that H_2O_2 inhibited the activity and proliferative capacity of ARPE-19 cells and downregulated the protein and gene expression of Nrf2, HO-1 and GCLC. However, in H_2O_2-treated ARPE-19 cells, FL-containing serum not only increased cell activity and proliferative capacity, but also upregulated protein and gene expression of Nrf2, HO-1, and GCLC.

● **CONCLUSION:** FL reduces oxidative stress in an animal model of dry AMD through the Nrf2/ARE signaling pathway and has a protective effect on dry AMD *in vitro* and *in vivo*, providing new insights into the therapeutic use of FL for dry AMD.

● **KEYWORDS:** dry age-related macular degeneration; Nrf2/ARE pathway; *Fructus lycii* aqueous extract; oxidative stress

DOI:10.18240/ijo.2026.06.04

Citation: Peng Y, Liang LY, Wei HL, Huang Q, Yuan LM, Xie YY, Tang QY, Wang AQ, Li JM, Guo QS, Huang BL. *Fructus lycii* mitigates oxidative stress in dry age-related macular degeneration models *via* Nrf2/ARE pathway. *Int J Ophthalmol* 2026;19(6):1048-1056

INTRODUCTION

Age-related macular degeneration (AMD) is an age-related blinding degenerative fundus lesion that has become one of the top 3 causes of blindness[1]. The prevalence of AMD was 5.80% in people older than 50 years of age and 17.65% in people older than 80 years of age[2]. The prevalence of blindness due to AMD is approximately 8.7%[3]. AMD can be divided into dry and wet forms. The dry AMD is characterized by geographic atrophy and drusen, while the wet AMD has choroidal neovascularization[4]. The pathogenesis of AMD is unclear, and age is usually considered to be the greatest risk factor, which may involve oxidative stress, retinal pigment epithelium (RPE) damage, mitochondrial dysfunction, inflammation, complement pathway activation, and other aspects[5-6].

AMD mainly involves RPE and choroid in the macula[7]. RPE cells, as highly specialized polarized epithelial cells with close apical contact with photoreceptor outer segments and basolateral adhesion to Bruch's membrane, are essential for the maintenance of normal visual acuity, especially in the macula, due to their specific location in the retinal tissue and important physiological functions central visual acuity[8]. Recently, it has been reported that the nuclear factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) signaling pathway is an important defense center to protect the body from oxidative and electrophilic stress[9-10], which can neutralize reactive oxygen species (ROS) clusters and electrophiles and mitigate their cellular damage by increasing the expression of antioxidant genes. When aged RPE cells are attacked by oxidation, the Nrf2/ARE signaling pathway will be damaged, suggesting that the Nrf2/ARE signaling pathway plays a role in the pathogenesis of dry AMD and may be a potential target for the prevention and treatment of dry AMD[11]. Dysfunctional regulation of Nrf2 has been shown to contribute to the AMD-like disease development, and polymorphisms in the *Nrf2* gene have been associated with the development of AMD, emphasizing the role of oxidative stress and the function of Nrf2 as a regulator of antioxidant defense systems[12]. Therefore, Nrf2 is a promising therapeutic target for AMD.

Currently, dry AMD is mainly treated by intervening the progression of the disease and mainly administered with antioxidants. For wet AMD, the main treatment is currently anti-vascular endothelial growth factor (VEGF) drugs, and anti-VEGF therapy has been widely used in clinical practice, which can significantly inhibit neovascularization, but there are some side effects, such as the risk of RPE tear, vitreoretinal fibrosis, intravitreal hemorrhage and infection, and retinal detachment[13]. In a prior experiment we concluded that *Fructus lycii* (FL) was able to significantly reduce RPE cell damage in mice and had a better anti-AMD effect[14]. FL is a commonly used traditional Chinese herbal medicine, and in recent years, there have been numerous pharmacological studies showing that FL has significant effects such as antioxidant and delaying aging[15].

In this study, we aim to evaluate the *in vivo* effects of aqueous extract of *FL* on sodium iodate ($NaIO_3$)-induced dry AMD in mice, assess the *in vitro* effects of FL-containing serum on human retinal pigment epithelial cells (ARPE-19 cells), investigate its effects on Nrf2/ARE signaling pathway, and contribute new ideas for the prevention and treatment of dry AMD.

MATERIALS AND METHODS

Ethical Approval The study was approved by the Animal Experiment Ethics Committee of Jiangxi University of Chinese Medicine (Approval No.JZLLSC20240003). Animal production license number: SYXK (Gan) 2017-0004; use license number: SCXY (Gan) 2020-0005.

Reagents and Antibodies $NaIO_3$ were from Sigma (Shanghai, China) Trading Co., Ltd., lot number: S4007-100G. H_2O_2 (30%) were from Guoyaojituan (China). ARPE-19 cells were purchased from the cell bank of the Chinese Academy of Sciences (China). β-actin, heme oxygenase-1 (HO-1), Nrf2, horseradish peroxidase (HRP)-labeled sheep anti-rabbit IgG antibody were from Abcam (USA), RNA extraction kit were from Juhemei company (Beijing, China). First-strand cDNA synthesis kit and quantitative real-time polymerase chain reaction (Q-PCR) detection reagent were from Kangwei Century company (Beijing, China), Q-PCR primer was from BGI. Superoxide dismutase (SOD) kit was from Nanjing Jiancheng Institute of Biological Engineering (China).

Animals and Treatments C57BL/6 mice, specific pathogen free (SPF) grade, male, 6-8 weeks old (Henan Sikebes Biotechnology Co., Ltd., China), weighing 20-22 g, were fed adaptively for one week and randomly divided into 5 groups (10 mice/each group). Control group: intraperitoneal injection of saline, no treatment); model group: intraperitoneal injection of 1% $NaIO_3$ solution (30 mg/kg)[16], no treatment; low-dose group: 1.25 g/kg·d treatment, medium-dose group: 2.5 g/kg·d treatment, high-dose group: 3.75 g/kg·d treatment, for 1wk. They were kept in the animal room of the Experimental Animal Center of Jiangxi University of Traditional Chinese Medicine, and the room temperature and relative humidity were maintained at around 18℃–25℃ and around 50%–80%, respectively, and the laboratory management regulations were strictly followed.

Preparation of *Fructus lycii* Aqueous Extract The herb FL was procured from the Pharmacy Department of the Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine. FL was soaked in double-distilled water for 2h and then heated to boiling, kept at a slight boil for 2h after boiling, stirred and filtered while hot. The dregs were boiled with distilled water and heated to reflux for 2h, filtered, combined with filtrate and concentrated to obtain the aqueous extract concentrate. Then the concentrate was freeze-dried into powder as described earlier[17-18]. Each gram of the aqueous extract contained 1.36 g crude herb.

Electron Microscopy Examination After treatment, the mice were sacrificed, the eyes were removed and fixed in 2.5% glutaraldehyde for 24h. Then the cornea, lens and vitreous were removed, and 2 mm×4 mm spherical wall tissues (including retina, choroid, and sclera) were cut on both sides

Table 1 Scoring and assessment of sediment thickness and width under RPE

Score	Sediment width	Sediment thickness	Composite score calculation
0	No sediment	No sediment	Width score+1/2 thickness score
1	Localized patchy deposits	Flattened sediment	
2	<Two RPE cells	<1/4 RPE cell height	
3	≥Two RPE cells	≥1/4 RPE cell height	

RPE: Retinal pigment epithelium.

of the optic papilla and fixed with glutaraldehyde/osmic acid, routinely dehydrated, epoxy resin embedded, and sectioned, and double-stained with 3% uranyl acetate and lead citrate. Finally, transmission electron microscopy JEOL JEM-1230 (80 kV) was observed and photographed for recording.

Observation methods The scoring and evaluation of the subcellular deposit thickness and width of RPE and Bruch's membrane thickness were performed according to the method of reference[14], as shown in Table 1. Five sections of each eye were selected for observation after sectioning, and subsequently three fields of view were selected for scoring the deposit width and thickness of each section; and the thickness of Bruch's membrane was determined under electron microscopy directly (magnification ×25 000). For all these examinations, every 5 sections from 1 eyeball were observed, and 3 random fields were visualized for scoring.

Assessment of Oxidative Stress Levels After the observation period, mice were cut off both sides of their whiskers to avoid hemolysis. Blood was obtained using the method of removing the eyeball to obtain blood, which was left at room temperature for one hour and centrifuged to obtain mouse serum. The levels of SOD and catalase (CAT) activity in mouse serum were measured using the kit according to the manufacturer's instructions.

Cell Culture ARPE-19 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% phosphate buffered saline (PBS) at 37°C with 5% CO_2. Cells were divided into 5 groups, control group (normal culture), model group (H_2O_2 culture for 2h+blank serum for 24h), low dose group (H_2O_2 culture for 2h+2.5% FL-containing serum for 24h), medium dose group (H_2O_2 culture for 2h+5% FL-containing serum for 24h), and high dose group (H_2O_2 culture for 2h+10% FL-containing serum for 24h).

Preparation of *Fructus lycii*-Containing Serum SPF grade Wistar rats were acclimatized for one week. The amount of FL administered to Wistar rats was calculated based on the equivalent dose ratio converted from human to animal body surface area, resulting in a dosage of 296 mg/kg[19]. The administration was performed daily at 8 *a.m.* for one week. At 1h after the last dose, aortic blood was collected. The blood samples were then centrifuged at 3000 g for 2h to obtain the supernatant. The resulting serum was placed in 56°C water bath and inactivated for 30min, filtered and de-bacterized with a microporous filter membrane in an ultra-clean table, and then dispensed and stored at -20°C for further use.

Cell Viability Assay ARPE-19 cells were inoculated in 96-well plates at a density of 5×10^3 cells/well. Subsequently, the cells were treated with H_2O_2 at concentrations of 0, 100, 200, 400, 500, and 600 μmol/L, followed by treatment with varying concentrations (1%, 2.5%, 5%, 10%, 15%) of FL-containing serum. Control cells were maintained in normal culture conditions. The original culture medium was discarded, and methylthiazolyldiphenyl-tetrazolium bromide solution (5 mg/mL) was added under light-protected conditions. The plates were then incubated for 3-4h. After incubation, the supernatant was aspirated and replaced with dimethyl sulfoxide (DMSO). The plates were shaken for 10min, and absorbance was measured at 490 nm using a microplate reader.

Cell Scratch Assay The density of ARPE-19 cells was adjusted to 5×10^5 cells/mL, and 2 mL/well was inoculated into a 6-well plate, which was then placed in a 37°C constant-temperature cell culture incubator. When 80% cell density was reached, the medium was replaced with serum-free medium, and incubation was continued for 2h. A uniform scratch was introduced into the cell monolayer using a 200 μL pipette tip. The medium was discarded, and cells along the scratch were washed with PBS solution. Images were acquired under a microscope, and the scratch wound width was recorded. Serum was added to the cultures according to the groupings, and incubation was resumed. Images of the same location in each group were captured at 12 and 24h, the scratch width was recorded, and cell migration distance was observed. The area covered by the cell scratch was calculated using ImageJ.

Western Blot Analysis Proteins were extracted from mouse retinal tissue samples or ARPE-19 cell samples in protein lysis solution. The protein content was quantified using the bicinchoninic acid (BCA) kit. Samples from all experimental groups were electrophoresed simultaneously on the same gel to ensure comparable conditions. Specifically, protein samples from each group were loaded alongside pre-stained protein molecular weight markers (Thermo, Cat. 26616) on a 12% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel to allow accurate molecular weight determination and inter-group comparisons. Following electrophoresis, proteins were transferred to a single polyvinylidene fluoride (PVDF) membrane under standard transfer conditions. The membrane was then cut into strips according to the molecular weight ranges of the target proteins to optimize incubation conditions and minimize non-specific binding. All membrane strips were processed in parallel under identical conditions. β-actin was detected on the same membrane strips corresponding to the mid-molecular-weight region, ensuring that the internal control and target proteins were derived from the same membrane. PVDF membranes were incubated with 5% bovine serum protein (BSA; Solarbio, Cat.A8020, Lot.120O0516) for 1.5h. The membranes were washed three times using Tris-buffered saline with Tween-20 (TBST) and incubated overnight at 4℃ in antibodies against Nrf2 (proteintech, Cat.16396-1-AP, Lot.00119425), HO-1 (abcam, Cat.28477, Lot.GR3449458-3), glutamate-cysteine ligase catalytic subunit (GCLC; proteintech, Cat.12601-1-AP, Lot.00096028), and β-actin (proteintech, Cat.20536-1-AP, Lot.00114542). After three washes with TBST, PVDF membranes were incubated with HRP-coupled secondary antibody (Beyotime, Cat. ab205718, Lot.GR3366929-3, 1:5000) for 2h. The blots were visualized using a luminescence detection kit. All Western blot experiments were performed in triplicate. Quantification was performed using Image J software.

Real-time Quantitative Fluorescence Q-PCR Analysis Total RNA was extracted from mouse retinal tissue samples or ARPE-19 cell samples using the RNA extraction kit. the RNA concentration was determined. The total RNA was then reverse transcribed into cDNA using a reverse transcription kit. Q-PCR was performed on this cDNA with reaction conditions of 94℃ for 5min, 94℃ for 10s, 60℃ for 20s, 72℃ for 30s, followed by 40 cycles of 72℃ for 150s, 40℃ for 90s, followed by melting from 60℃ to 94℃, 25℃ for 60-120s. Q-PCR primer was listed in Table 2.

Statistical Analysis The data were analyzed using SPSS 25 and GraphPad Prism software. Data are expressed as mean±standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). $P<0.05$ was the threshold for statistical significance.

RESULTS

Aqueous Extract of *Fructus lycii* Reduces RPE Deposition and Bruch's Membrane Thickness in AMD Mice The histopathological results showed that in the control group, the morphology of the mitochondrial cristae of the RPE cells was normal and the mitochondria were full in size. In contrast, mitochondria in the model group exhibited disruption of mitochondrial cristae and vacuolization, and their number was significantly reduced. Our observations are consistent with previous study[20]. In the FL-treated groups: mitochondria in the low-dose group showed disruption of mitochondrial cristae and vacuolization with a reduced count, while those in the medium- and high-dose groups displayed largely normal mitochondrial cristae morphology with slightly relatively large mitochondria, and the number was greater than that in the low-dose group (Figure 1A). In Figure 1B, there were no obvious deposits under the RPE in the control group; in the model group, a large number of continuous plate-like deposits can be seen under the RPE, and the inner folds of the base are widened, indicating that the dry AMD model has been successfully established. However, the RPE deposits were reduced after treatment with different doses of FL aqueous extract. The higher the concentration of FL aqueous extract, the lower the RPE deposits (Figure 1C). Bruch's membrane in the control group was slightly thickened with good uniformity and significantly thickened and uneven in the model group (Figure 1B). FL aqueous extract significantly reduced Bruch's membrane thickness in a dose-dependent manner (Figure 1D).

Table 2 The primers used in the study for Q-PCR analysis

Primer name	Primer sequence (5'-3')
Mouse	
GAPDH	F: CCCAGCTTAGGTTCATCAGG
	R: CCAAATCCGTTCACACCGAC
NRF2	F: TGTCTTAATACCGAAAACAAGCAGC
	R: GACCACAGTTGCCCACTTCTTTT
HO-1	F: GCTAAGACCGCCTTCCTGCT
	R: ACGAAGTGACGCCATCTGTGA
Human	
GAPDH	F: TGGTCACCAGGGCTGCTTTTA
	R: CATCGCCCCACTTGATTTTG
NRF2	F: AGCCCAGCACATCCAGTCAG
	R: AAACGTAGCCGAAGAAACCTCA
HO-1	F: ATGCCCCAGGATTTGTCAGAG
	R: GGAAGTAGACAGGGGCGAAGAC

Q-PCR: Quantitative real-time polymerase chain reaction; *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase; *NRF2*: Nuclear factor erythroid 2-related factor 2; *HO-1*: Heme oxygenase-1.

***Fructus lycii* Upregulates Nrf2 Pathway and Alleviates Retinal Oxidative Damage in Mice** SOD and CAT are indicators of oxidative stress and antioxidant activity. We examined whether the expressions of SOD and CAT in the eye tissues was modulated by treatment with FL aqueous extract. The results showed that SOD and CAT levels were significantly lower in the model group compared with the control group ($P<0.01$) and were significantly increased after FL treatment compared to the model group ($P<0.05$; Figure 2A, 2B).

Next, we detected the expression levels of Nrf2 (cytoplasm and nucleus) and Nrf2/ARE pathway downstream genes HO-1 and GCLC proteins in mouse retinal tissues. The results showed that $NaIO_3$ significantly reduced the expression level

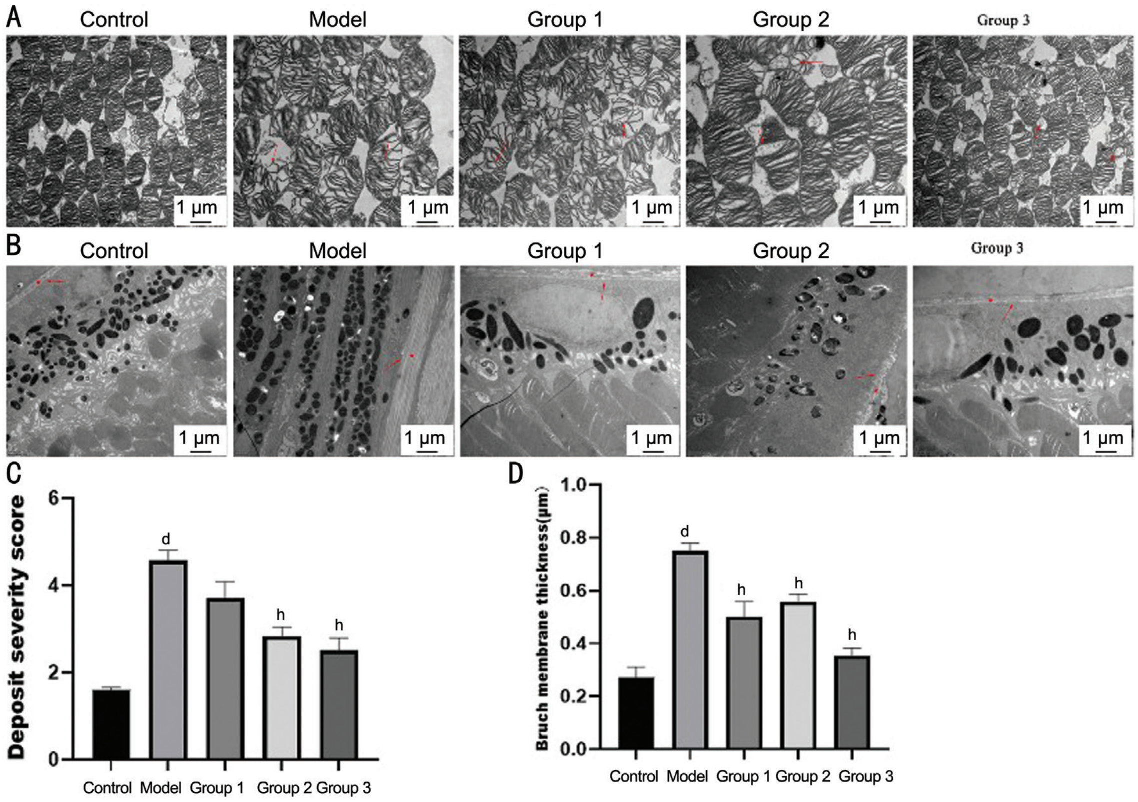

Figure 1 Effects of *Fructus lycii* aqueous extract on the histopathology in AMD mice Control group: Intraperitoneal injection of saline, no treatment; Model group: Intraperitoneal injection of $NaIO_3$ solution, no treatment; Group 1: Low dose group (intraperitoneal injection of $NaIO_3$ solution, 1.25 g/kg treatment); Group 2: Moderate dose group (intraperitoneal injection of $NaIO_3$ solution, 2.50 g/kg treatment); Group 3: High dose group (intraperitoneal injection of $NaIO_3$ solution, 3.75 g/kg treatment). A: Electron microscopy examination of the eye tissues (magnification ×9300). The red arrows indicate the RPE cells. B: Electron microscopy examination of the Bruch's membrane (magnification ×9300). The red arrows indicate the RPE sediment. The asterisks indicate the Bruch's membrane. C: Comprehensive scoring on the severity of RPE sediment. The total score=width score+1/2 thickness score. D: Determination of Bruch's membrane thickness. Data are expressed as mean±SD (n=15). Statistical analysis was performed using one-way ANOVA. $^{d}P<0.0001$ *vs* control group; $^{h}P<0.0001$ *vs* model group. AMD: Age-related macular degeneration; RPE: Retinal pigment epithelium; SD: Standard deviation; ANOVA: Analysis of variance.

of Nrf2 in the cytoplasm compared with the control group. FL aqueous extract treatment upregulated the expression of Nrf2 in the cytoplasm (Figure 2C). However, the nuclear expression of Nrf2 in the model group was significantly higher than that in the control group, and the Nrf2 expression in the FL group was significantly lower than that in the model group (Figure 2D). In addition, compared with the control group, the expression of HO-1 and GCLC in the model group was significantly down-regulated, while the expression levels in FL group were significantly higher than those in the model group. We next detected the mRNA expression levels of Nrf2 and HO-1. The results showed that compared with the control group, the expression of Nrf2 and HO-1 in the model group was significantly down-regulated, and the expression in the FL group was significantly up-regulated compared with the model group (Figure 2E, 2F).

Effects of *Fructus lycii*-Containing Serum on Migration and Oxidative Damage in ARPE-19 Cells Cell viability assays showed that H_2O_2 dose-dependently reduced ARPE-19 cell proliferation and inhibited cell viability by 50% at a concentration of 500 μmol/L (Figure 3A). Therefore, we chose H_2O_2 at 500 μmol/L to establish the oxidative stress-induced injury of ARPE-19 cells. Subsequent experiments showed that FL-containing serum ameliorated the H_2O_2-induced decrease in cell viability in a dose-dependent manner (Figure 3B), and concentrations of 2.5%, 5%, and 10% were selected as the optimal drug concentrations of FL-containing serum for the next experiments.

The scratch test was used to detect the migration ability of ARPE-19 cells treated with H_2O_2 and FL-containing serum. The results showed that the cell migration ability of the model group was reduced compared with the control group. However, compared with the model group, FL-containing serum groups improved the cell migration ability, and the improvement was concentration-dependent (Figure 3C-3E). This shows that FL-containing serum can increase the migration ability of cells and improve the oxidative damage of ARPE-19 cells caused by H_2O_2.

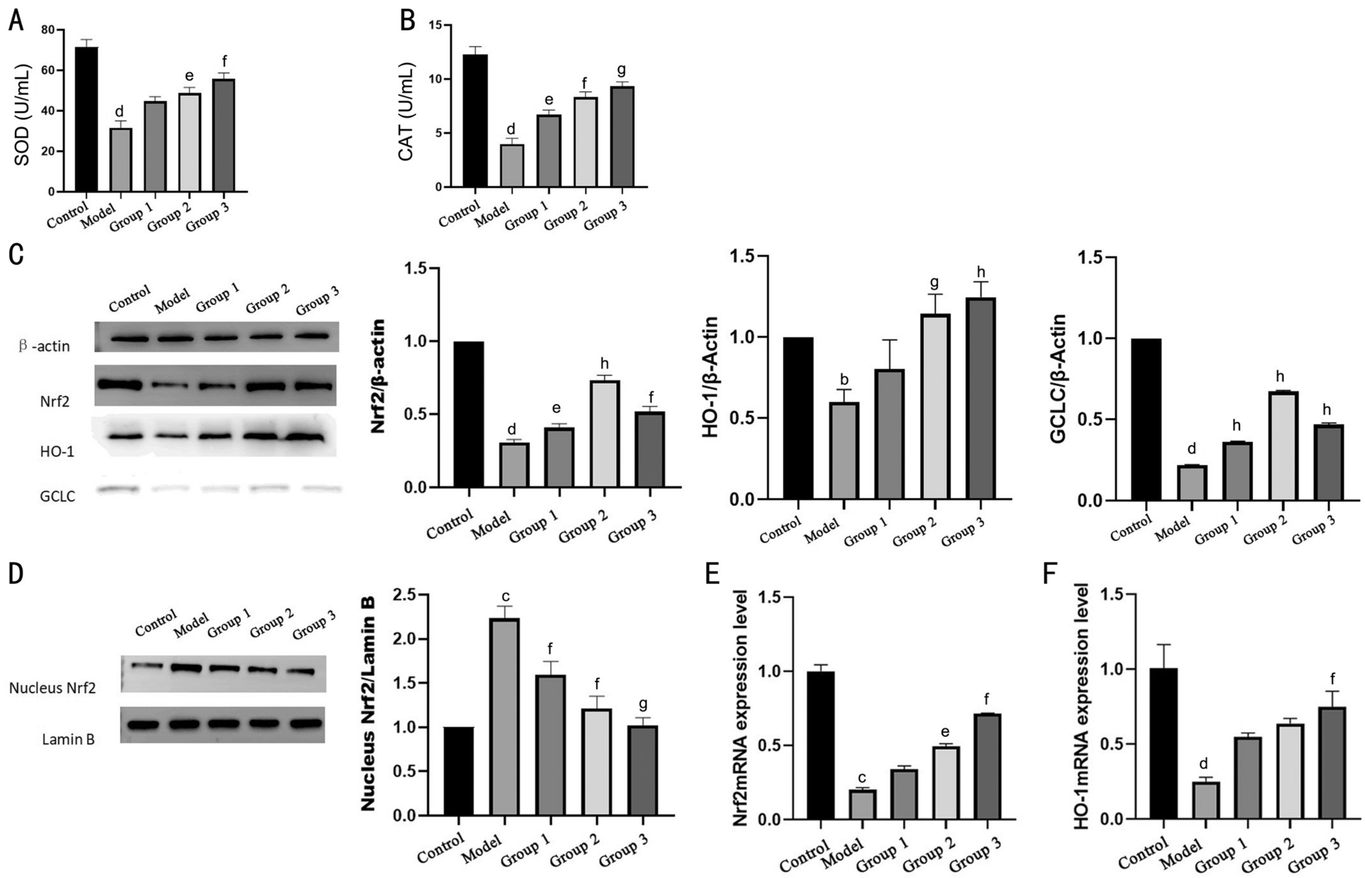

Figure 2 Effects of *Fructus lycii* aqueous extract on Nrf2 pathway expression and oxidative stress in AMD mice A, B: Results of SOD and CAT expression in mouse serum by colorimetric method; C, D: Western blot observation of Nrf2, HO-1 and GCLC protein expression results in mouse retinal tissues; E, F: Fluorescence quantitative PCR detection of Nrf2 and HO-1 mRNA expression in the retinal tissues of experimental mice. Data are expressed as mean±SD (*n*=3). Statistical analysis was performed using one-way ANOVA. ^{b}*P*<0.01, ^{c}*P*<0.001, ^{d}*P*<0.0001 *vs* control group; ^{e}*P*<0.05, ^{f}*P*<0.01, ^{g}*P*<0.001, ^{h}*P*<0.0001 *vs* model group. AMD: Age-related macular degeneration; SOD: Superoxide dismutase; CAT: Catalase; Nrf2: Nuclear factor erythroid 2-related factor 2; HO-1: Heme oxygenase-1; GCLC: Glutamate-cysteine ligase catalytic subunit; PCR: Polymerase chain reaction; SD: Standard deviation; ANOVA: Analysis of variance.

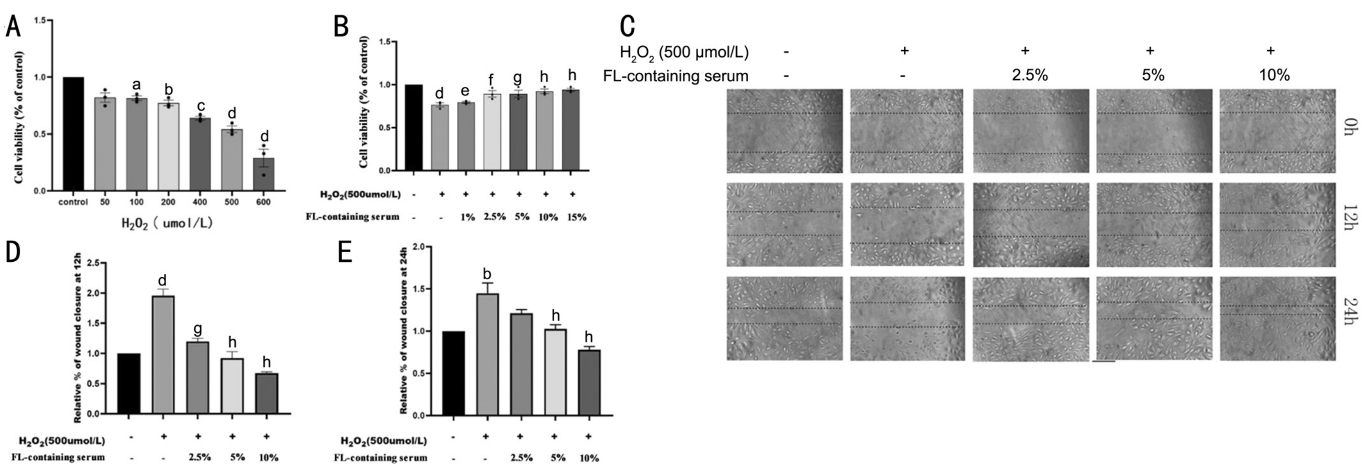

Figure 3 Effects of FL-containing serum on cell viability and cell migration in ARPE-19 cells treated with H_2O_2 A, B: Results of methylthiazolyldiphenyl-tetrazolium bromide assay on the effect of H_2O_2 and FL-containing serum on ARPE-19 cell survival rate; C-E: Scratch method to detect the effect of FL-containing serum on cell migration ability. Data are expressed as mean±SD (*n*=3). Statistical analysis was performed using one-way ANOVA. ^{a}*P*<0.05, ^{b}*P*<0.01, ^{c}*P*<0.001, ^{d}*P*<0.0001 *vs* control group; ^{e}*P*<0.05, ^{f}*P*<0.01, ^{g}*P*<0.001, ^{h}*P*<0.0001 *vs* model group. AMD: Age-related macular degeneration; FL: *Fructus lycii*; SD: Standard deviation; ANOVA: Analysis of variance; ARPE-19: Human retinal pigment epithelial cells.

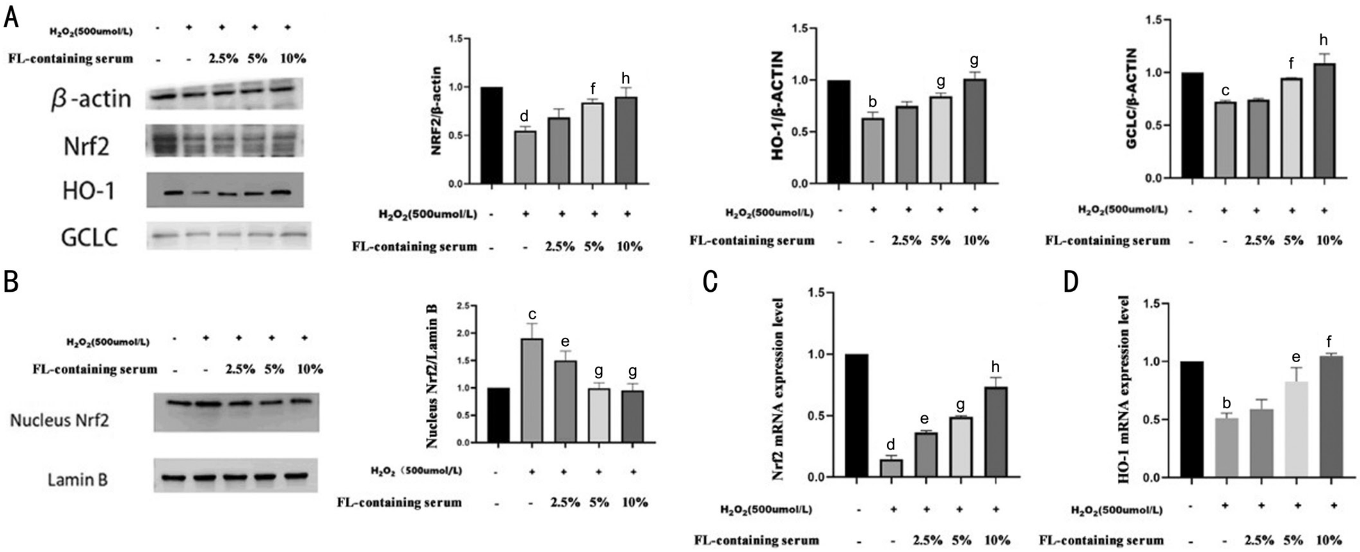

Figure 4 Effects of FL-containing serum on Nrf2/ARE signaling pathway in ARPE-19 cells treated with H_2O_2 A, B: Western blot observation of Nrf2, HO-1, and GCLC protein expression results in ARPE-19 cells; C, D: Fluorescence quantitative PCR results of Nrf2 and HO-1 mRNA expression in ARPE-19 cells. Data are expressed as mean±SD (n=3). Statistical analysis was performed using one-way ANOVA. ^bP<0.01, ^cP<0.001, ^dP<0.0001 *vs* control group; ^eP<0.05, ^fP<0.01, ^gP<0.001, ^hP<0.0001 *vs* model group. FL: *Fructus lycii*; Nrf2: Nuclear factor erythroid 2-related factor 2; ARE: Antioxidant response element; ARPE-19: Human retinal pigment epithelial cells; HO-1: Heme oxygenase-1; GCLC: Glutamate-cysteine ligase catalytic subunit; PCR: Polymerase chain reaction; SD: Standard deviation; ANOVA: Analysis of variance.

Fructus lycii-Containing Serum Upregulates Nrf2, HO-1 and GCLC Expressions Suppressed by H_2O_2 in ARPE-19 Cells

H_2O_2 significantly down-regulated the protein expression of Nrf2 cytoplasm, HO-1, and GCLC compared with the control group, but FL-containing serum up-regulated these proteins in a dose-dependent manner. H_2O_2 upregulated the nuclear expression of Nrf2, while FL-containing serum downregulated the nuclear expression of Nrf2 in a dose-dependent manner (Figure 4A, 4B).

Then we detected the Nrf2 and HO-1 mRNA expression levels in ARPE-19 cells (Figure 4C, 4D). Compared with the control group, the expression levels of Nrf2 and HO-1 mRNA in the model group were significantly down-regulated, and both were up-regulated after treatment with FL-containing serum. These results suggest that FL-containing serum can ameliorate H_2O_2-induced oxidative damage in ARPE-19 cells.

DISCUSSION

AMD is a leading cause of vision loss in the elderly population, with its dry form characterized by the accumulation of retinal deposits and enhanced thickness of Bruch's membrane[21]. Oxidative stress is critically involved in the pathogenesis of AMD. The retina, particularly the macula, is exceptionally vulnerable to oxidative damage due to its high oxygen consumption, concentration of polyunsaturated fatty acids, and exposure to light. An age-related decline in endogenous antioxidant defenses coupled with increased ROS production creates a pro-oxidant milieu, leading to lipid peroxidation, protein modification, and ultimately, RPE dysfunction and cell death[22]. Our study successfully established a dry AMD model in mice using $NaIO_3$, an oxidative stress inducer. This model recapitulated key pathological features: significant sub-RPE deposits and Bruch's membrane thickening were observed *via* electron microscopy. Concurrently, systemic antioxidant capacity was compromised, as evidenced by significantly reduced activities of the critical antioxidant enzymes SOD and CAT in serum.

Our central finding is that the aqueous extract of FL effectively counteracted these changes. Histopathological assessment demonstrated that FL treatment markedly reduced the severity of sub-RPE deposits and decreased Bruch's membrane thickness. Furthermore, FL administration restored systemic antioxidant defense, upregulating the activities of SOD and CAT. These results provide direct evidence that FL alleviates the core structural pathology and systemic oxidative imbalance in a murine model of dry AMD.

To dissect the potential molecular mechanism, we focused on the Nrf2/ARE pathway, a master regulator of cellular antioxidant response[23]. Previous studies have reported that oxidative stress leads to alterations in the Nrf2 pathway and promotes the development of AMD[24-25]. Under oxidative stress, Nrf2 dissociates from its cytoplasmic repressor Keap1, translocates to the nucleus, and binds to ARE, driving the transcription of a battery of cytoprotective genes[24]. Key among these is HO-1, NAD(P)H quinone oxidoreductase 1 (NQO1) and GCLC[26]. In our model, Nrf2 protein expression decreased in the cytoplasm and increased in the nucleus, while its mRNA expression was reduced. These changes, consistent with previous reports on Nrf2 expression in AMD[24], indicated that the cells were under oxidative stress, and corresponding alterations were observed in the expression of Nrf2 downstream genes. Treatment with FL reversed the expression patterns of Nrf2 in both the cytoplasm and nucleus, suggesting that FL treatment alleviated the cellular oxidative stress state. However, the specific mechanisms involving the Nrf2 pathway require further investigation.

To further confirm the beneficial effects of FL on dry AMD, we examined the *in vitro* effects of FL-containing serum on H_2O_2-treated ARPE-19 cells, which could mimic the damage caused by oxidative stress in the pathogenesis of dry AMD *in vitro*. We found that FL-containing serum ameliorated oxidative damage in H_2O_2-treated ARPE-19 cells, indicating that FL-containing serum could protect ARPE-19 cells from oxidative stress in the pathogenesis of dry AMD. Furthermore, the relevance of the Nrf2/ARE signaling pathway to dry AMD was investigated. In H_2O_2-treated ARPE-19 cells, we observed increased Nrf2 protein expression in the nucleus accompanied by decreased expression in the cytoplasm, indicating nuclear translocation, while the expression of its downstream target genes HO-1 and GCLC was downregulated. However, treatment with FL-containing serum reversed the H_2O_2-induced alterations in Nrf2 subcellular distribution and restored the expression of HO-1 and GCLC, indicating a significant association between FL treatment and modulation of the Nrf2/ARE signaling pathway.

However, our study has several limitations. First, in the *in vitro* experiments, the use of drug-containing serum, while a common pharmacological method for evaluating herbal extracts, presents a confounding variable. The absence of a parallel control group treated with “blank” serum (from vehicle-administered animals) means we cannot definitively rule out potential modulating effects of other serum constituents on ARPE-19 cell resilience or baseline Nrf2 activity. Second, while our data demonstrate a strong associative link between FL treatment, Nrf2 pathway activation, and phenotypic improvement, they do not establish direct causality. Third, FL is a phytochemically complex substance. Although our results point to the involvement of the Nrf2 pathway, the specific active compound(s) (*e.g.*, polysaccharides, carotenoids, betaine) responsible for this activity remain unidentified. The crude aqueous extract contains a mixture, and the protective effect may be synergistic.

Based on these limitations, several directions for future research are warranted. First, subsequent studies should incorporate a blank serum control group and employ specific chemical inhibitors to isolate FL-specific effects and address the confounding influence of serum components. Second, to establish direct causality, experiments utilizing Nrf2-specific inhibitors or siRNA-mediated knockdown are necessary to confirm whether the protective effects of FL are strictly dependent on this pathway. Third, activity-guided fractionation should be undertaken to isolate and identify the precise Nrf2-activating constituents within FL, followed by investigation of their potential synergistic interactions.

In conclusion, our results suggest that FL has a protective effect against oxidative damage in mouse retinal tissues and ARPE-19 cells. This suggests that FL may be involved in the up-regulation of antioxidant enzymes downstream of Nrf2 and their activity *via* the Nrf2/ARE signaling pathway. Despite the noted limitations, the results of this experimental study tentatively suggest that FL is a potential drug with therapeutic value for dry AMD.

ACKNOWLEDGEMENTS

Authors’ Contributions: Huang BL, Peng Y, and Liang LY contributed to the conception of the study; Peng Y and Liang LY performed most experiments and data analyses. Wei HL, Huang Q, Yuan LM, Xie YY, Tang QY, Wang AQ, Li JM, and Guo QS contributed significantly to the data analyses; Peng Y, Liang LY and Huang BL contributed to the manuscript preparation.

Availability of Data and Materials: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Foundations: Supported by the National Natural Science Foundation of China Youth Science Fund Project (No.82104935; No.82305321); the Natural Science Foundation of Jiangxi Province (No.20252BAC240449; No.20202BAB206074; No.20192BAB215053); the Science and Technology Research Project of Jiangxi Provincial Department of Education (No.GJJ190647).

Conflicts of Interest: **Peng Y,** None; **Liang LY,** None; **Wei HL,** None; **Huang Q,** None; **Yuan LM,** None; **Xie YY,** None; **Tang QY,** None; **Wang AQ,** None; **Li JM,** None; **Guo QS,** None; **Huang BL,** None.

REFERENCES

1 Keenan TDL, Cukras CA, Chew EY. In: Chew EY, Swaroop A (eds.). *Age-Related Macular Degeneration: From Clinic To Genes And Back To Patient Management* 2021;1256:1-31.

2 Rosenblatt TR, Vail D, Saroj N, *et al*. Increasing incidence and prevalence of common retinal diseases in retina practices across the United States. *Ophthalmic Surg Lasers Imaging Retina* 2021;52(1):29-36.

3 GBD 2021 Global AMD Collaborators. Global burden of vision impairment due to age-related macular degeneration, 1990-2021, with forecasts to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Glob Health* 2025;13(7):e1175-e1190.

4 Dohl J, Burns G, Singh M. The intersection of mitochondria, lipids, and ferroptosis: a new avenue for dry age-related macular degeneration. *Apoptosis* 2025;30(11-12):2526-2546.

5 Deng Y, Qiao L, Du M, *et al*. Age-related macular degeneration: Epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy. *Genes Dis* 2022;9(1):62-79.

6 Guymer RH, Campbell TG. Age-related macular degeneration. *Lancet* 2023;401(10386):1459-1472.

7 Fleckenstein M, Keenan TDL, Guymer RH, *et al*. Age-related macular degeneration. *Nat Rev Dis Primers* 2021;7:31.

8 Wang Y, Zhang YC, Ding ZQ, *et al*. Feedback regulation between histone lactylation and ALKBH3-mediated glycolysis regulates age-related macular degeneration pathology. *Proc Natl Acad Sci U S A* 2025;122(24):e2416046122.

9 Hayes JD, Dayalan Naidu S, Dinkova-Kostova AT. Regulating Nrf2 activity: ubiquitin ligases and signaling molecules in redox homeostasis. *Trends Biochem Sci* 2025;50(3):179-205.

10 Chen CL, Carrillo ND, Chen M, *et al*. Regulation of NRF2 by stably associated phosphoinositides and small heat shock proteins in response to stress. *J Biol Chem* 2025;301(7):110367.

11 Liang Y, Jia X, Zheng F, *et al*. SUMOylation inhibitors exert a protective effect on oxidative damage in retinal pigment epithelial cells through the Keap1/Nrf2/ARE signaling pathway. *Curr Mol Med* 2025;25(9):1181-1190.

12 Zhang X, Ma W, Liu H, *et al*. Daphnetin protects neurons in an Alzheimer disease mouse model and normal rat neurons by inhibiting BACE1 activity and activating the Nrf2/HO-1 pathway. *J Neuropathol Exp Neurol* 2024;83(8):670-683.

13 Stahl A. The diagnosis and treatment of age-related macular degeneration. *Dtsch Arztebl Int* 2020;117(29-30):513-520.

14 Huang BL, Ding SH, Hang L, *et al*. Prevention and treatment of age-related macular degeneration by extract of Fructus lycii and its constituents lutein/zeaxanthin: an in vive and in vitro experimental research. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2013;33(4):531-537.

15 Ou C, Jiang P, Tian Y, *et al*. Fructus Lycii and Salvia miltiorrhiza Bunge extract alleviate retinitis pigmentosa through Nrf2/HO-1 signaling pathway. *J Ethnopharmacol* 2021;273:113993.

16 Nie Q, Gong X, Gong L, *et al*. Sodium iodate-induced mouse model of age-related macular degeneration displayed altered expression patterns of sumoylation enzymes E1, E2 and E3. *Curr Mol Med* 2018;18(8):550-555.

17 Cao C, Lu Y, Li B, *et al*. Lycii Fructus, a medicine food homology material, attenuates retinal damage in sodium iodate-induced retinal degeneration mice and inhibits oxidation-induced apoptosis of Müller cells *via* regulating Nrf2-HO1 signaling pathway. *J Funct Foods* 2023;108:105756.

18 Han SY, Choi H, Jo EH, *et al*. Inhibitory effects of Lycii fructus water extracts and its active ingredient, zeaxanthin, on receptor activator of nuclear factor κB ligand-induced osteoclast differentiation. *Food Sci Biotechnol* 2025;34(14):3363-3374.

19 Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharma* 2016;7(2):27.

20 Hyttinen JMT, Viiri J, Kaarniranta K, *et al*. Mitochondrial quality control in AMD: does mitophagy play a pivotal role *Cell Mol Life Sci* 2018;75(16):2991-3008.

21 Fleckenstein M, Schmitz-Valckenberg S, Chakravarthy U. Age-related macular degeneration: a review. *JAMA* 2024;331(2):147-157.

22 Zhang L, Yu J, Fan Z, *et al*. Regulated cell death in age-related macular degeneration: Regulatory mechanisms and therapeutic potential. *J Pharm Anal* 2025;15(11):101285.

23 Liu S, Pi J, Zhang Q. Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. *Redox Biol* 2022;54:102389.

24 Hu ZL, Wang YX, Lin ZY, *et al*. Regulatory factors of Nrf2 in age-related macular degeneration pathogenesis. *Int J Ophthalmol* 2024;17(7):1344-1362.

25 Mimura T, Noma H. Title oxidative stress in age-related macular degeneration: from molecular mechanisms to emerging therapeutic targets. *Antioxidants* (*Basel*) 2025;14(10):1251.

26 Rezaie T, McKercher SR, Kosaka K, *et al*. Protective effect of carnosic acid, a pro-electrophilic compound, in models of oxidative stress and light-induced retinal degeneration. *Invest Ophthalmol Vis Sci* 2012;53(12):7847.