• Basic Research •

Epigallocatechin gallate in N-methyl-N-nitrosourea-induced retinitis pigmentosa mouse model

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Abstract

• **AIM:** To investigate the therapeutic efficacy and underlying mechanisms of epigallocatechin gallate (EGCG), a major green tea catechin with potent antioxidant properties, in an N-methyl-N-nitrosourea (MNU)-induced mouse model of retinitis pigmentosa (RP).

• **METHODS:** C57BL/6 mice were randomly divided into control (PBS), MNU-induced RP, and MNU+EGCG pretreatment groups. EGCG (50 mg/kg, intraperitoneal) was administered daily for 3 consecutive days prior to a single MNU injection (50 mg/kg). Retinal function was evaluated by scotopic electroretinography (ERG). Retinal structure was assessed using optical coherence tomography (OCT) and hematoxylin-eosin staining, with outer nuclear layer (ONL) thickness measurement. Mechanisms were explored *via* RNA sequencing, reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) validation of oxidative stress- and inflammation-related genes, and immunohistochemistry for microglial activation, astrocytic gliosis, and apoptosis markers.

• **RESULTS:** Compared with the MNU group, EGCG pretreatment significantly preserved scotopic ERG a-wave and b-wave amplitudes (P<0.001). OCT and histological analysis showed that EGCG markedly attenuated MNU-induced thinning of total retina and ONL (P<0.005, P<0.001, respectively). RNA sequencing identified 1147 differentially expressed genes modulated by EGCG, with significant upregulation of antioxidant genes (*Nrf2*, *Sod1*, *Gpx4*, *Cat1*, *Ho-1*) and downregulation of pro-inflammatory genes. Immunohistochemistry confirmed that EGCG significantly reduced microglial activation, glial fibrillary acidic protein (GFAP) expression, and cleaved caspase-3-positive cells (P<0.01 to P<0.001).

• **CONCLUSION:** EGCG exerts robust neuroprotective effects in MNU-induced RP through enhancement of antioxidant defenses, suppression of neuroinflammation, and preservation of retinal structure and function. These findings suggest EGCG as a promising candidate for adjuvant antioxidant therapy in RP.

• **KEYWORDS:** epigallocatechin gallate; retinitis pigmentosa; N-methyl-N-nitrosourea; retinal degeneration; antioxidant; anti-inflammation; mouse

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INTRODUCTION

Retinitis pigmentosa (RP) is a heterogeneous inherited retinal dystrophy characterized by progressive photoreceptor cell apoptosis which eventually results in vision loss[1]. It is reported that the prevalence of RP is approximately 1 in 4000 in Western countries, thus affecting over 1.5 million individuals[2]. While no effective long-term treatment presently exists for RP, clinical trials and experimental treatments using sophisticated methods such as gene therapy, neuroprotection, anti-apoptotic drugs, retinal transplantation, retinal prostheses, and stem cell therapy are been explored but are largely invasive and potentially inconvenient[3-4]. As such, finding a new non-invasive and effective treatment for RP and alleviating the medical burden on patients is paramount.

In various RP animal models, the mechanism of photoreceptor apoptosis induced by N-methyl-N-nitrosourea (MNU), as well as the biological mechanisms and cellular responses, are reported to be similar to that in humans[5-6], and thus considered useful in the search of treatment for human RP[7-9].

In as much as RP is known to be a hereditary condition, reactive oxygen species (ROS), *via* its ability to induce oxidative stress, have been implicated as a key factor in the pathogenesis of several retinal conditions including photoreceptor apoptosis in RP[10-13]. ROS subtypes such as hydroxyl (OH) and peroxynitrite (ONOO) radicals can induce mitochondrial DNA stress and lipid oxidation, leading to

mitochondrial membrane rupture and cytochrome C release, which promotes the activation and progression of apoptotic cascade. Studies have found that ROS can excessively increase poly ADP-ribose polymerase activity by interacting with transcription factors such as nuclear factor-kappa B and activating protein-1, leading to photoreceptor apoptosis[14]. As a result, oxidative stress is believed to play a major role in the development of RP. For example, oxidative stress is reported to contribute to the progression of MNU-induced photoreceptor cell apoptosis, while antioxidant substances such as quercetin, melatonin, curcumin, vitamin A, and carotenoids can inhibit the loss of photoreceptor cells in the MNU model[15-18].

To protect the retina against oxidative damage, microglial cells infiltrate the outer nuclear layer (ONL) of the retina in the early stages of retinal degeneration where they are responsible for clear debris of apoptotic neurons by means of phagocytosis[19-20]. However, left untreated and the underlying cause unremedied, a rapid and excessive apoptosis in RP will typically leads to retinal degeneration and visual loss, especially in patients who develop RP during adolescence or young adulthood.

Epigallocatechin gallate (EGCG), a major catechin found in green tea, has gained considerable attention for its potent antioxidant properties. EGCG has been shown to scavenge ROS, thereby reducing oxidative stress and exerting a protective effect on cells[21-24]. However, the potential of EGCG as a therapeutic agent in retinal degenerative diseases remains largely unexplored. As such, in this study, we sought to investigate the neuroprotective effects of EGCG in the retinal degenerative disease RP. We hypothesize that EGCG can mitigate oxidative stress-induced damage in retinal neurons by scavenging ROS, thereby slowing the progression of retinal degeneration and preserving visual function. The findings of this study could provide valuable insights into the development of novel therapeutic strategies for retinal degenerative diseases, potentially improving the quality of life for millions of patients worldwide.

MATERIALS AND METHODS

Ethical Approval All animal procedures were approved by the Institutional Animal Care and Use Committee of Tongji University (E547895421, granted on May/5/2023) and conducted following the guidelines of the ARVO statement for the use of animals in ophthalmic and vision research.

Animals Male C57BL/6 mice (25–30 g body weight) were procured from Modelorg (Shanghai, China) and housed in a 12-hour light/12-hour dark cycle at 25°C±1°C and 39%–45% relative humidity, with *ad libitum* access to water and food. The mice were euthanized by active exposure to CO_2 for an initial 2 to 3min followed by an additional passive exposure time of 3min, reliably resulting in irreversible euthanasia.

Mouse Model To induce MNU-induced retinal degeneration, C57BL/6 mice were intraperitoneally injected with a dose of MNU (50 mg/kg; N136701, Aladdin, Shanghai, China) and evaluated on day 1 or day 7 post-injection. Eyes were rapidly enucleated and processed for respective studies. EGCG (50 mg/kg; E107404, Tocris, Minneapolis, USA) or the vehicle control [phosphate-buffered saline (PBS)] were also administered intraperitoneally. For experiments lasting 7d, EGCG was administered daily for 3d after which MNU was administered on the next day after EGCG treatment. Drugs were stored at -20℃ in Shanghai East Hospital of Tongji University.

Electroretinography Electroretinography (ERG) measurements were taken following previous reports with slight modification[25-26]. Briefly, the mice were anesthetized, and their pupils dilated with 1% tropicamide and 1% atropine. ERG measurements were conducted using RetiMINER-C system (IRC Medical Equipment, Chongqing, China) from the corneal surface using a pair of platinum loop electrodes, along with a ground electrode placed in the tail and a reference electrode in the anterior scalp between the eyes. A drop of Genteal Gel (Alcon,Texas, USA) was applied to the cornea to keep it moist. For scotopic ERG, mice were dark-adapted overnight and stimulated with flashes of steadily increasing light intensity (0.1, 1, 10 cd•s/m^2). The band-pass filter was set between 0.3 and 300 Hz, and responses were averaged from 10 single flashes. Data analysis was conducted using RetiMINER4.0.

Quantitative Polymerase Chain Reaction Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was performed using specific primers for eight genes in mouse eyeball samples as shown in Table 1. Primers were synthesized by Accurate Biotechnology (Hunan) Co., Ltd (Shanghai, China). Total RNA from mouse eyeball tissues was extracted using the TaKaRa MiniBEST Universal RNA extraction kit. RNA concentration was measured using a NanoDrop microspectrophotometer, and RNA integrity was assessed by agarose gel electrophoresis. RNA was transcribed into cDNA using the TaKaRa PrimeScript Reverse Transcription Master Kit and stored at -80°C for later use. Real-time fluorescent qPCR was performed following the instructions of the PowerUP SYBR Green Master Mix reagent and Applied Biosystems Q3 quantitative PCR system. The qPCR amplification program consisted of an initial hold at 50°C for 2min, followed by activation of DNA polymerase at 95°C for 2min, and 40 cycles of target DNA fragment amplification (95°C for 15s, 60°C for 60s) to obtain an SYBR green fluorescence signal. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference, and relative quantitative analysis was conducted using the $2^{-\Delta\Delta Ct}$ method.

Table 1 Target genes and primer sequences

Genes	Forward (5′-3′)	Reverse (5′-3′)
Nrf2	ACCAAGGGGCACCATATAAAAG	CTTCGCCGAGTTGCACTCA
Keap1	TGCCCCTGTGGTCAAAGTG	GGTTCGGTTACCGTCCTGC
Sod1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG
Gsh	TATCAGAGGCGGGAAATCTCTT	ATTCTTGCTTCGGCCACATAC
Gpx4	GATGGAGCCCATTCCTGAACC	CCCTGTACTTATCCAGGCAGA
Cat1	GAAGTGGCGGAGACTTTTAGG	TGGTCAGTAAACGTAGCTCCA
HO-1	GTCTTCCGTGTGTCCTCGG	CCTTACACTTCTTGTGGGTCTTG
Gapdh	TGGATTTGGACGCATTGGTC	TTGCACTGGTACGTGTTGAT

Optical Coherence Tomography Spectral-domain optical coherence tomography (SD-OCT) imaging was used to non-invasively acquire cross-sectional tomographic images of the retina, allowing for the monitoring of retinal morphology in live experimental animals. The Micron IV intraocular imaging system (Phoenix Research Labs, Pleasanton, USA) was utilized as previously described[7]. Optical coherence tomography images were analyzed using InSight XL software (Phoenix Research Laboratories) to measure total retinal thickness and the thickness of different retinal layers.

Immunohistochemistry Mice were anesthetized and subjected to perfusion with 4% paraformaldehyde, after which their eyes were enucleated. Retinas were carefully dissected with forceps and then post-fixed in 4% paraformaldehyde for 30min. Following removal of the lens, the retinas were immersed in 30% sucrose at 4°C overnight and then embedded in optical coherence tomography compound (Leica, Hesse, Germany) at room temperature for 2h. Subsequently, the embedded retinas were cryo-sectioned at a thickness of 20 μm using a Leica CM1950. Slides containing retinal sections were washed with PBS and incubated with a blocking buffer comprising 5% normal donkey serum (Jackson ImmunoResearch, PA, USA), 0.1% Triton X-100, and 0.1% NaN_3 in PBS for 1h at room temperature. After overnight incubation with primary antibodies at 4°C, sections were washed three times with PBS and then incubated with secondary antibodies in a humidified dark box for 2h at room temperature. Nuclei were counterstained with DAPI (Sigma-Aldrich, MO, USA), and sections were mounted with Fluoromount G (SouthernBiotech, AL, USA).

Hematoxylin and Eosin Staining Retinas were isolated from enucleated eyes and fixed in 4% paraformaldehyde for 30min. After dehydration using increasing concentrations of ethanol (70%, 95%, and 100%) and incubation in xylene, retinas were embedded in paraffin and sectioned at a thickness of 5 μm using a Leica microtome. Following deparaffinization and rehydration, slides containing retinal sections were stained with hematoxylin and eosin (HE) and mounted with Canada balsam (Sigma-Aldrich, MO, USA). Images were captured using an Olympus IX83 microscope.

RNA Sequencing

RNA-seq analysis of retina epithelium Total RNA was extracted from the retina tissue using the TRIzol® reagent (Invitrogen, CA, USA). Genomic DNA contamination was eliminated with DNase I obtained (Takara, Tokyo, Japan). RNA integrity was assessed using the Bioanalyser 2100 (Agilent Technologies, Santa Clara, USA), and quantified *via* the ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). Only RNA samples of superior quality (with an OD260/280 ratio of 1.8 to 2.2, an OD260/230 ratio greater than or equal to 2.0, an RIN value of at least 6.5, and a quantity exceeding 10 μg) were selected for the construction of the RNA-seq libraries.

A quantity of 5 μg of RNA was utilized to prepare the RNA-seq library with the TruSeq™ RNA sample preparation Kit (Illumina, San Diego, USA), following the polyA selection method for mRNA isolation and subsequent fragmentation. Complementary DNA (cDNA) was synthesized in a double-stranded format as per the protocol of the SuperScript double-stranded cDNA synthesis kit by Invitrogen, CA, USA. The cDNA library was quantified using the TBS380 fluorometer, and the paired-end RNA-seq library was sequenced on the Illumina HiSeq X Ten platform with a read length of 2×150 bp.

Post-quality control measures, the cleaned reads were aligned to the Mus_musculus reference genome (GRCm38) employing TopHat2 software, which can be found at http://ccb.jhu.edu/software/tophat/index.shtml. The gene expression levels across samples were estimated using RSEM, accessible at http://deweylab.github.io/RSEM/, and were quantified in terms of transcripts per million (TPM). Comparative analysis of gene expression was performed using DESeq2 to identify genes with differential expression.

Genes were classified as differentially expressed (DE) when they met the criteria of a false discovery rate (FDR) of less than 0.05, employing the Benjamini-Hochberg procedure for multiple test corrections, and exhibited an absolute fold change (FC) exceeding 2. Functional annotation and enrichment analysis of the DE genes (DEGs) were conducted using DAVID (version 6.8), available at https://david.ncifcrf.gov/tools.jsp. Genes were associated with Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, with those having an FDR of less than 0.05 considered to be significantly enriched. Dataset details (DOI: 10.6084/m9.figshare.26044690).

Statistics To ensure rigor and reduce bias, mice were randomly assigned to PBS, MNU, and MNU+EGCG groups *via* a computer-generated table; investigators for sample handling, data acquisition [ERG, OCT, immunohistochemistry (IHC)], and statistical analysis were blinded to group assignments using anonymous coding (automated software

RetiMINER4.0 for ERG, InSight XL for OCT reduced subjective bias). Analyses used GraphPad Prism 9.0 (general) or R 4.3.0 (RNA-seq): normality was verified *via* Shapiro-Wilk test; two-group comparisons used unpaired *t*-test, three-group *via* one-way ANOVA with Tukey's post-hoc test; RNA-seq differential expression *via* DESeq2 (FDR<0.05, |FC|>2). Data are mean±standard error of the mean (SEM), significance denoted as $P<0.05$.

RESULTS

EGCG Protects Against MNU-Induced Retinal Electrophysiological Impairment The selection of this EGCG regimen (50 mg/kg, 3d pre-MNU) was based on two key points: First, prior mouse pharmacokinetic studies showed intraperitoneal 50 mg/kg EGCG achieves peak retinal concentrations (~1.2 μmol/L) within 24h, with sustained levels (>0.5 μmol/L) for 72h-concentrations proven to scavenge ROS and inhibit retinal pro-inflammatory pathways[27]. Second, 3-day pre-treatment aligns with Tsuruma *et al*[18], who found this window suffices to upregulate retinal antioxidant genes (*e.g.*, *Nrf2*, *Sod1*) before MNU exposure, optimizing protection. Preliminary tests confirmed 50 mg/kg EGCG alone is non-toxic, with no changes in mouse body weight or retinal morphology. The mice were observed 7d post-MNU injection using OCT and fundus photography (Figure 1). ERG was employed to assess changes in retinal electrical signals in EGCG+MNU and MNU groups (n=5 for PBS group and n=10 for other two groups). Seven days after the establishment of the model, mice treated with MNU exhibited significantly reduced b-wave amplitudes at various light intensities compared to the untreated group. Concurrently, mice pre-treated with EGCG displayed significantly less ERG signal damage following MNU treatment, indicating a protective effect (Figure 2).

Retinal Structural Preservation by EGCG in MNU-Induced RP Model We further explored the impact of EGCG on the retinal structure in the mouse model with MNU-induced RP. A total of 15 mice were randomly assigned to three groups (n=5 mice per group): PBS control group, MNU-treated group, and MNU+EGCG intervention group. OCT revealed that, compared to the control group treated with PBS, the MNU-treated group exhibited increased reflectivity in the ONL on day 3 (D3) post-MNU administration, indicating a significant reduction in retinal thickness. In contrast, OCT images of EGCG-treated mice showed that ONL reflectivity returned to the level of the control group by D3, suggesting that EGCG treatment improved the retinal structure (Figure 3A and 3B).

Further statistical analysis confirmed that the ONL thickness in the MNU-treated group was significantly reduced compared to the control group ($P<0.001$). In comparison, the ONL thickness in the EGCG intervention group was increased, although still lower than the control group, but not significantly different from the MNU-treated group ($P>0.05$). Nonetheless, the improvement in ONL thickness in the EGCG treated group indicates that EGCG could exhibits a protective function against MNU-induced thinning of the retina. Additionally, HE staining of retinal sections revealed characteristics of retinal structural disarray and ONL thinning in the MNU-treated group. In contrast, the retinas of EGCG intervention group mice were relatively intact with thicker ONL. Measurement results confirmed that the total retinal and ONL thickness in the EGCG intervention group were significantly higher than those in the MNU-treated group ($P<0.001$) but comparatively lower than the control group ($P<0.005$). The ability for EGCG to ensure the retainment of the total retinal and ONL thickness after MNU administration further confirms its potential therapeutic efficacy in protecting the retina from MNU-induced damage (Figure 3C and 3D).

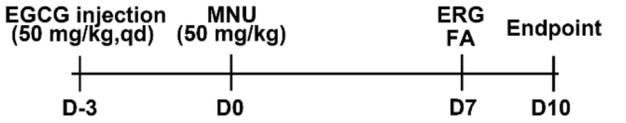

Figure 1 Schematic representation of the experimental protocol The experiment included a control group treated with PBS, MNU-exposed (50 mg/kg) group, and EGCG (50 mg/kg) with MNU-exposed group. EGCG: Epigallocatechin gallate; MNU: N-methyl-N-nitrosourea; qd: *Quaque die*; ERG: Electroretinogram; FA: Fluorescein angiography; PBS: Phosphate-buffered saline; D: Day.

EGCG Modulates Gene Expression and Ameliorates MNU-Induced Retinal Degeneration We further investigated the effects of EGCG on MNU-induced gene expressions and their relation to retinal degeneration or restoration. For this analysis, 6 mice were randomly allocated to two groups (n=3 mice per group): PBS control group, MNU-treated group, and MNU+EGCG intervention group. Through RNA sequencing and hierarchical clustering analysis, we observed significant changes in the expression patterns of DEGs between EGCG-treated and MNU-treated mice. The heatmap showed that there were 1147 dysregulated mRNAs in the EGCG+MNU group compared to the MNU-treated group, with red gene bars indicating upregulated genes and green bars indicating downregulated genes (Figure 4A). These data revealed that EGCG treatment can modulate gene expression changes induced by MNU. Figure 4B shows a comparison of mRNA expression levels between the EGCG-treated group and the control group (where red, black, and blue dots represented mRNAs that were increased, equivalent, or decreased after EGCG treatment, respectively). This result suggests that EGCG has a potential alleviating effect on MNU-induced retinal degeneration, which can be achieved by regulating the expression of related genes.

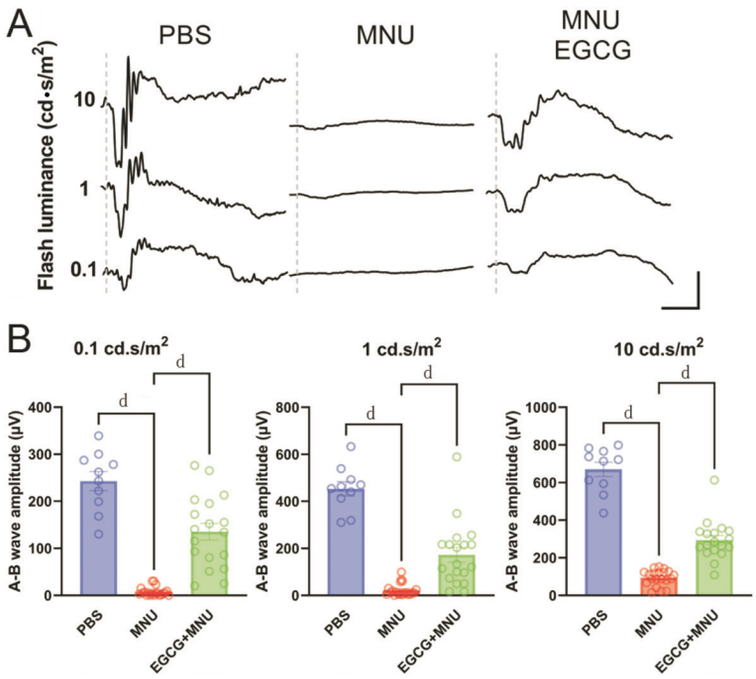

Figure 2 EGCG-induced effects on scotopic ERGs of MNU-treated mice A: Representative waveforms of scotopic ERGs of vehicle (PBS), MNU or EGCG combine MNU treated groups from 0.1, 1 and 10 cd•s/m^2; B: Quantitative analysis of scotopic ERG a-b wave amplitudes. Each column represents the mean±SEM of 5 mice (PBS treated group) or 10 mice (other two groups). Compared to the control group, the amplitude of scotopic a-b waves decreased in the MNU and partially recovered in MNU with EGCG groups. Statistical comparison was performed by ANOVA and post-hoc test. EGCG: Epigallocatechin gallate; MNU: N-methyl-N-nitrosourea; ERG: Electroretinogram; SEM: Standard error of the mean; ANOVA: Analysis of variance; PBS: Phosphate-buffered saline. $^d P<0.001$ *vs* MNU-treated group.

To further understand the retinal regulatory genes that were affected by the treatments, we focused on genes related to oxidative stress in five retina tissues, including *Nrf2*, *Keap1*, *Sod1*, *gsh*, *gpx4*, *cat1*, and *ho-1*. After MNU treatment, the expression levels of these genes were observed to have significantly changed. However, EGCG treatment significantly upregulated the expression of *Nrf2* and *Sod1*, consistent with the enhancement of the antioxidant defense mechanism. Additionally, the expression of *Gpx4* and *Cat1* was also upregulated, which may have helped reduce oxidative stress. In contrast, the expression of *Keap1* was downregulated after EGCG treatment which may have allowed the *Nrf2* to escape ubiquitination and proteasome degradation, thereby accumulating within the cell and translocating to the nucleus to promote the transcription of antioxidant genes, thus suggesting that EGCG may enhance the antioxidant capacity of cells by regulating the Keap1-Nrf2 pathway. Furthermore, the upregulation of *gsh* expression may be related to enhancing the reducing potential within cells and defending the cells against oxidative stress. These results indicate that EGCG combats MNU-induced oxidative stress and retinal degeneration by regulating the expression of key oxidative stress related genes (Figure 4C).

EGCG Suppresses Microglial Activation and Neuroinflammatory Markers in MNU-Treated Retinas We further explored the inhibitory effect of EGCG on retinal degeneration in MNU-treated mice. Using DAPI (blue) and Iba1 (purple), glial fibrillary acidic protein (GFAP; purple), cleaved caspase 3 (green), or CD68 (red) staining, we observed retinal sections from PBS-treated, MNU-treated, and EGCG-intervened MNU-treated mice. The results showed that microglial cells in the retinas of MNU-treated mice were in an active state and distributed throughout the retina, which may indicate an early response to retinal injury.

For quantitative analysis, we measured the fluorescence intensity of Iba1, the intensity of GFAP neurites, and the number of cleaved caspase 3 or CD68 puncta. Statistical analysis indicated that compared to the PBS-treated group, the fluorescence intensity of Iba1, the intensity of GFAP neurites, and the number of cleaved caspase 3 and CD68 puncta in the MNU-treated group mice was significantly increased ($P<0.001$). However, in the EGCG intervention group, these increases were significantly reduced, demonstrating that EGCG effectively inhibits MNU-induced retinal degeneration. Specifically, the fluorescence intensity of Iba1 was significantly reduced in the EGCG intervention group ($P<0.001$), and the intensity of GFAP neurites was also weakened ($P<0.005$), while the number of cleaved caspase 3 and CD68 puncta decreased similarly ($P<0.01$). These results confirm the potential protective role of EGCG in inhibiting MNU-induced retinal degeneration (Figure 5).

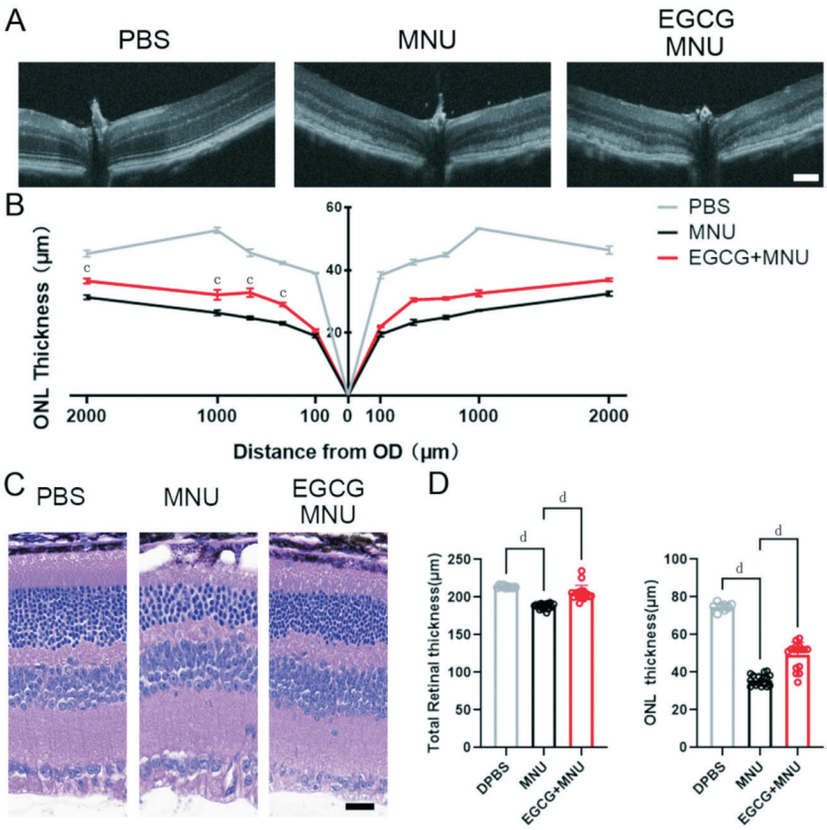

Figure 3 Effect of EGCG intervention on retinal structure in MNU-induced RP mice A: Retinal OCT images on day 3 (D3) after MNU administration; B: Statistical analysis of ONL thickness across all groups. On D3, the ONL in the EGCG intervention group showed restored hyporeflectivity similar to the PBS group, and outer retinal thickness was significantly higher than that in the MNU group; C: HE staining of retinal sections showing total retinal and ONL thickness 3d after MNU administration; D: Both whole retina and ONL thickness in the EGCG intervention group were lower than those in the PBS group, but higher than those in the MNU-treated group, with statistically significant differences. Scale bar: 100 μm; $^{c}P<0.005$; $^{d}P<0.001$. n=5 mice per group. EGCG: Epigallocatechin gallate; MNU: N-methyl-N-nitrosourea; RP: Retinitis pigmentosa; OCT: Optical coherence tomography; ONL: Outer nuclear layer; PBS: Phosphate-buffered saline; HE: Hematoxylin and eosin.

DISCUSSION

RP refers to a broad category of hereditary retinal dystrophies characterized by vision loss as a result of progressive retinal apoptosis[28]. While the condition results from inherited genetic changes, its occurrence may be aggravated by secondary factors such as oxidative stress and inflammation which promotes retinal photoreceptor cell apoptosis[29]. Despite its prevalence, no effective treatment regimen has been found and therefore management practices have included gene therapy, neuroprotection, antibody therapy, optogenetics, retinal transplantation, retinal prostheses, and stem cell therapy, with a number of therapeutic drugs currently in clinical trial phase[30]. In spite of the current challenges in identifying efficacious treatment, some research work has focused on traditional raw materials with potent active ingredients for RP treatment. A recent study has reported on the protective effect of Zhangyanmin Tablet (ZYMT), prepared from the formulation of several distinct plant parts, on RP as it preserves the ultrastructure of the retina, improves the thickness of the ONL, decreases the rate of apoptosis and regulates the expression of antioxidant and pro-apoptotic factors[31]. Other studies have reported a similar functional property of Chinese herbal medicine such as, Bujing Yishi tablets which prevented the thickness reduction of ONL, decreased apoptosis level in retinal cells and inhibited the levels of inflammatory factors[32], and extracts of *Fructus lycii* and *Salvia miltiorrhiza* which regulated the Nrf2/HO-1 pathway to inhibit oxidative reaction[33]. In these studies, however, the tablets were made from the formulation of many active ingredients and thus may be difficult to identify the biological and therapeutic role of the constituent ingredients in the treatment process. In the present study however, the therapeutic effects of EGCG, a major catechin and a single active ingredient derived from green tea, on MNU-induced RP in C57BL/6 mouse model was investigated. The MNU-induced model exhibits characteristics akin to human RP and is therefore considered a valuable tool for exploring RP treatment strategies. It was observed that, the RP stimulation by MNU led to a reduction in photoreceptor cells in the ONL and a significant decrease in central retinal thickness.

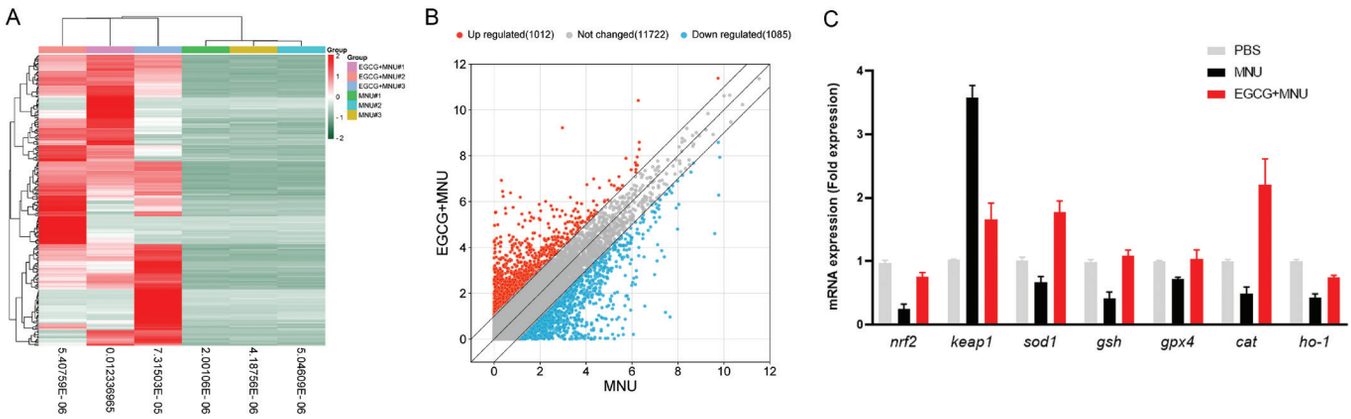

Figure 4 EGCG alleviated MNU-induced retinal degeneration A: Hierarchical clustering of differentially expressed mRNAs. Expression levels of RNA-related DEGs were visualized in a heatmap; red bars indicate up-regulated genes and green bars indicate down-regulated genes. Comparisons between EGCG+MNU samples and MNU-treated samples (MNU1-3) were based on 1147 dysregulated mRNAs (*n*=3). Red represents upregulated mRNAs, while green represents downregulated mRNAs. B: Red, gray, and blue dots represent mRNAs with increased, unchanged, or decreased expression between the EGCG-treated and control groups (*n*=5). C: Effects of different treatments on mRNA expression levels of related genes in mouse eyeball tissue: *nrf2*, *keap1*, *sod1*, *gsh*, *gpx4*, *cat1* and *ho-1*. EGCG: Epigallocatechin gallate; MNU: N-methyl-N-nitrosourea; DEGs: Differentially expressed genes; PBS: Phosphate-buffered saline.

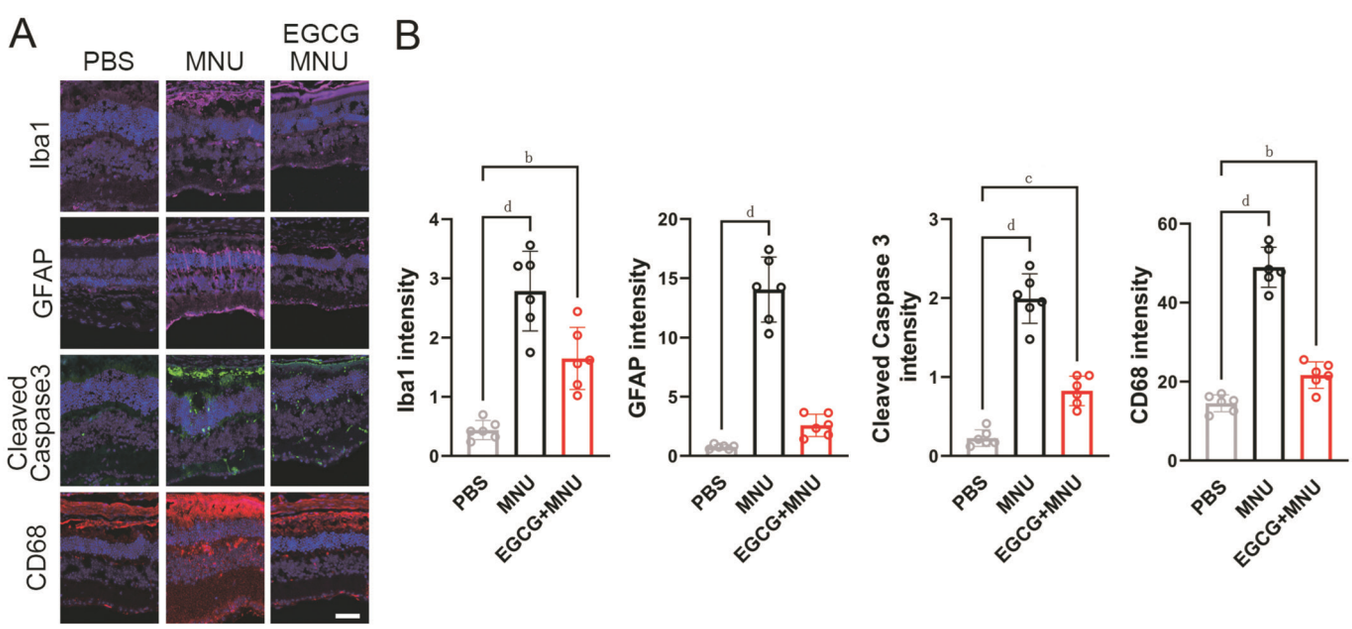

Figure 5 EGCG inhibits the retinal degeneration in MNU treated mice A: Retinal sections stained with DAPI (blue) and for Iba1 (purple), GFAP (purple), cleaved caspase 3 (green) or CD68 (red) in PBS, MNU-treated or EGCG intervention mice. In the retina, microglial cells presented as active state and distributed throughout the whole retina; B: The fluorescence intensity of Iba1, the GFAP neurite intensity and the puncta of cleaved caspase 3 or CD68 in mouse retinas were quantified. $^{b}P<0.01$, $^{c}P<0.005$, $^{d}P<0.001$, one-way ANOVA. Three mice and six eyes for each group. EGCG: Epigallocatechin gallate; MNU: N-methyl-N-nitrosourea; PBS: Phosphate-buffered saline; DAPI: 4',6-diamidino-2-phenylindole; Iba1: Ionized calcium-binding adaptor molecule 1; GFAP: Glial fibrillary acidic protein; Cleaved caspase-3: Cleaved cysteine-aspartic protease 3; CD68: Cluster of differentiation 68; ANOVA: Analysis of variance.

EGCG, known for its potent antioxidant, anti-inflammatory, and anticancer properties, has been extensively studied for its health benefits[34]. We found that EGCG treatment significantly ameliorated the MNU-induced pathological changes of the retina by increasing the thickness of the ONL which approached that of the control group. ERG tests also demonstrated significant improvements in the EGCG-treated group. Biochemical analysis revealed that EGCG elevated the expression of genes involved in the ROS pathway, indicating its antioxidant role. Notably, this regulatory effect of EGCG on gene expression and tissue pathological protection is not limited to retinal models. Al-Awaida *et al*[35] in their study on water-pipe smoke-induced toxicity found that EGCG could modulate gene expression profiles and

alleviate histopathological damage, which is consistent with our findings that EGCG reverses MNU-induced retinal gene dysregulation and structural impairment. This cross-model consistency further supports the broad therapeutic potential of EGCG in counteracting oxidative stress-related tissue damage, providing additional evidence for its applicability in RP treatment. IHC staining showed activation of GFAP in MNU-treated mice, which was mitigated by EGCG, potentially due to its anti-inflammatory effects.

In RP patients, alterations in the expression of several ROS-related genes are observed, including *IL2*, *IL18*, *NRF2*, and *SOD*, which play crucial roles in the pathogenesis of RP[36-39]. *NRF2*, a key transcription factor regulating the cellular antioxidant defense system, may become dysregulated in RP, leading to a dysfunction in the antioxidant defense system[40]. As a potent antioxidant, EGCG can regulate ROS levels in RP patients through various mechanisms, including neutralizing free radicals, enhancing the antioxidant defense system, modulating BCL2 expression to inhibit apoptosis, and suppressing inflammation. B-cell lymphoma 2 (BCL2) is a protein that regulates apoptosis, and its dysregulation can lead to excessive cell death, a characteristic feature of RP. By inhibiting BCL2 expression, EGCG can help prevent the apoptosis of photoreceptor cells, thus preserving retinal function[41]. Furthermore, Keap1-Nrf2 signaling pathway is a primary target for EGCG's action[42]. By binding to Keap1, EGCG prevents its inhibitory effect on Nrf2, thereby enhancing Nrf2 activity and promoting the transcription of genes that encode for antioxidant enzymes. This upregulation of antioxidant enzymes strengthens the retina's defense against ROS, which is crucial for the survival of photoreceptor cells in RP patients. Moreover, EGCG's influence on intracellular signaling pathways, such as PI3K/Akt and MAPK, further modulates Nrf2's nuclear translocation and activity, enhancing the overall antioxidant response[42-43].

The preclinical findings of this study highlight EGCG's potential as a non-invasive therapeutic option for RP, addressing the long-standing clinical need for less invasive treatments compared to existing approaches like retinal transplantation or prostheses. However, transitioning EGCG from preclinical research to clinical application faces several critical challenges[44-47]. In terms of safety, while EGCG is generally recognized as safe in dietary doses, high-dose administration—likely necessary for therapeutic efficacy in RP—may trigger adverse effects. Clinical observations from other studies have reported gastrointestinal discomfort (*e.g.*, nausea, diarrhea, abdominal pain) in individuals taking high-dose EGCG supplements, and there is also a risk of interactions with commonly used medications[48-49]. For example, EGCG may interfere with the absorption of iron or the metabolism of anticoagulants, which could be problematic for RP patients who may have comorbidities and take multiple drugs[49-50]. Additionally, the optimal dosing regimen for EGCG in RP remains unclear. In our mouse model, a 50 mg/kg intraperitoneal dose showed efficacy, but translating this to human doses requires consideration of species-specific differences in pharmacokinetics, such as bioavailability. Oral administration, the most feasible route for long-term RP treatment, results in low bioavailability of EGCG due to poor absorption and rapid metabolism in the gut and liver. Developing formulations to enhance oral bioavailability (*e.g.*, lipid-based delivery systems) or exploring local delivery routes (*e.g.*, intravitreal injections) may be necessary but also present technical and safety challenges. Long-term efficacy is another key concern: RP is a progressive disease that requires decades of intervention, yet preclinical studies only evaluate short-term effects (7d in our study). It remains unknown whether EGCG can sustain its protective effect over extended periods, or if tolerance or cumulative side effects may emerge.

Furthermore, it is essential to acknowledge the potential limitations of EGCG treatment beyond the translation challenges. As mentioned earlier, high-dose EGCG can cause gastrointestinal side effects, which may reduce patient compliance, especially for long-term use. EGCG also has potential effects on liver function; although rare, cases of hepatotoxicity have been reported in individuals taking high-dose EGCG supplements, which requires close monitoring in clinical trials[51-52]. Special populations, such as pregnant or lactating women, or patients with liver or kidney dysfunction, may need to be excluded from initial trials due to insufficient safety data. Additionally, EGCG's antioxidant effects may not be universally effective across all RP subtypes. RP is a genetically heterogeneous disease, and while oxidative stress is a common secondary factor, the primary genetic defect varies among patients. It is possible that EGCG may be more effective in subsets of RP patients with more prominent oxidative stress, requiring stratified analysis in clinical trials to identify responders.

Our study indicates that the antioxidant property of EGCG can protect the retina from ROS-induced damage, providing a new theoretical basis for RP treatment. Nevertheless, further research is required to address the challenges of clinical translation, including optimizing dosing and delivery, evaluating long-term safety and efficacy, and identifying patient subsets most likely to benefit. Future studies could focus on developing improved EGCG formulations to enhance bioavailability, conducting phase I/II clinical trials to assess safety and preliminary efficacy, and exploring combination therapies (*e.g.*, EGCG with other antioxidants or anti-inflammatory agents) to synergistically enhance retinal

protection. Only by addressing these gaps can EGCG realize its potential as a clinically viable treatment for RP.

In conclusion, this study demonstrates the therapeutic potential of EGCG in mitigating MNU-induced RP in mice. EGCG administration significantly improved ERG outcomes and preserved retinal structure, indicating its protective effect against photoreceptor cell degeneration. The findings suggest EGCG's promise as a novel therapeutic agent for RP, highlighting its antioxidant properties and potential to modulate gene expression related to oxidative stress and inflammation. Further research is, however, warranted to explore EGCG's clinical application in RP treatment.

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REFERENCES

1 Parmeggiani F, Sorrentino FS, Ponzin D, *et al*. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genom* 2011;12(4):238-249.

2 Bongomin F, Gago S, Oladele R, *et al*. Global and multi-national prevalence of fungal diseases—estimate precision. *JoF* 2017;3(4):57.

3 Dias MF, Joo K, Kemp JA, *et al*. Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. *Prog Retin Eye Res* 2018;63:107-131.

4 Hu YS, Song H, Li Y, *et al*. Whole-exome sequencing identifies novel mutations in genes responsible for retinitis pigmentosa in 2 nonconsanguineous Chinese families. *Int J Ophthalmol* 2019;12(6):915-923.

5 Reisenhofer M, Balmer J, Zulliger R, *et al*. Multiple programmed cell death pathways are involved in N-methyl-N-nitrosourea-induced photoreceptor degeneration. *Graefes Arch Clin Exp Ophthalmol* 2015;253(5):721-731.

6 Lin JL, Wang YD, Ma Y, *et al*. Protective effects of naringenin eye drops on N-methyl-N-nitrosourea-induced photoreceptor cell death in rats. *Int J Ophthalmol* 2014;7(3):391-396.

7 Lin FL, Lin CH, Ho JD, *et al*. The natural retinoprotectant chrysophanol attenuated photoreceptor cell apoptosis in an N-methyl-N-nitrosourea-induced mouse model of retinal degenaration. *Sci Rep* 2017;7:41086.

8 Tsubura A, Lai YC, Miki H, *et al*. Review: animal models of N-Methyl-N-nitrosourea-induced mammary cancer and retinal degeneration with special emphasis on therapeutic trials. *In Vivo* 2011;25(1):11-22.

9 Chen YY, Liu SL, Hu DP, *et al*. N-methyl- N-nitrosourea-induced retinal degeneration in mice. *Exp Eye Res* 2014;121:102-113.

10 Carmody RJ, Cotter TG. Signalling apoptosis: a radical approach. *Redox Rep* 2001;6(2):77-90.

11 Usui S, Oveson BC, Lee SY, *et al*. NADPH oxidase plays a central role in cone cell death in retinitis pigmentosa. *J Neurochem* 2009;110(3):1028-1037.

12 Yu TZ, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci U S A* 2006;103(8):2653-2658.

13 Yu DY, Cringle SJ, Balaratnasingam C, *et al*. Retinal ganglion cells: energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. *Prog Retin Eye Res* 2013;36:217-246.

14 Shen JK, Yang XR, Dong AL, *et al*. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol* 2005;203(3):457-464.

15 Mladenov M, Lubomirov L, Grisk O, *et al*. Oxidative stress, reductive stress and antioxidants in vascular pathogenesis and aging. *Antioxidants* 2023;12(5):1126.

16 Emoto Y, Yoshizawa K, Kinoshita Y, *et al*. Green tea extract suppresses N-methyl-N-nitrosourea-induced photoreceptor apoptosis in Sprague-Dawley rats. *Graefes Arch Clin Exp Ophthalmol* 2014;252(9):1377-1384.

17 Pinilla I, Maneu V, Campello L, *et al*. Inherited retinal dystrophies: role of oxidative stress and inflammation in their physiopathology and therapeutic implications. *Antioxidants* 2022;11(6):1086.

18 Tsuruma K, Yamauchi M, Inokuchi Y, *et al*. Role of oxidative stress in retinal photoreceptor cell death in N-methyl-N-nitrosourea–treated mice. *J Pharmacol Sci* 2012;118(3):351-362.

19 Rashid K, Akhtar-Schaefer I, Langmann T. Microglia in retinal degeneration. *Front Immunol* 2019;10:1975.

20 Striebel JF, Race B, Williams K, *et al*. Microglia are not required for prion-induced retinal photoreceptor degeneration. *Acta Neuropathol Commun* 2019;7(1):48.

21 Glei M, Pool-Zobel BL. The main catechin of green tea, (–)-epigallocatechin-3-gallate (EGCG), reduces bleomycin-induced DNA damage in human leucocytes. *Toxicol Vitro* 2006;20(3):295-300.

22 Lin SY, Kan JY, Lu CC, *et al*. Green tea catechin (-)-epigallocatechin-3-gallate (EGCG) facilitates fracture healing. *Biomolecules* 2020;10(4):620.

23 Lung HL, Ip WK, Wong CK, *et al*. Anti-proliferative and differentiation-inducing activities of the green tea catechin epigallocatechin-3-gallate (EGCG) on the human eosinophilic leukemia EoL-1 cell line. *Life Sci* 2002;72(3):257-268.

24 Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. *Biochem Pharmacol* 2011;82(12):1807-1821.

25 Hu SH, Chen YX, Xie DC, *et al*. Nme_2Cas9-mediated therapeutic editing in inhibiting angiogenesis after wet age-related macular degeneration onset. *Clinical & Translational Med* 2023;13(8):e1383.

26 Qin H, Zhang WL, Zhang SY, *et al*. Vision rescue *via* unconstrained *in vivo* prime editing in degenerating neural retinas. *J Exp Med* 2023;220(5):e20220776.

27 Kuang FM, Liu J, Tang DL, *et al*. Oxidative damage and antioxidant defense in ferroptosis. *Front Cell Dev Biol* 2020;8:586578.

28 Liu YS, Pan JQ, Wan JF, *et al*. A novel missense mutation of RPGR identified from retinitis pigmentosa affects splicing of the ORF15 region and causes loss of transcript heterogeneity. *Biochem Biophys Res Commun* 2020;531(2):172-179.

29 García-Arroyo R, Domènech EB, Herrera-Úbeda C, *et al*. Exacerbated response to oxidative stress in the retinitis pigmentosa Cerkl mouse model triggers retinal degeneration pathways upon acute light stress. *Redox Biol* 2023;66:102862.

30 Cross N, van Steen C, Zegaoui Y, *et al*. Current and future treatment of retinitis pigmentosa. *Clin Ophthalmol* 2022;16:2909-2921.

31 Huang ZY, Huang QL, Xu K, *et al*. Protective effect of ZYMT, a traditional Chinese patent medicine in a mouse model of retinitis pigmentosa. *Biomed Pharmacother* 2023;162:114580.

32 Qi YL, Liu L, Liang D, *et al*. Bujing Yishi tablets alleviate photoreceptor cells death *via* the P2X7R/CX3CL1/CX3CR1 pathway in retinitis pigmentosa rats. *Phytomedicine* 2023;115:154828.

33 Ou C, Jiang PF, 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.

34 Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. Beneficial properties of green tea catechins. *Int J Mol Sci* 2020;21(5):1744.

35 Al-Awaida W, Goh KW, Al-Ameer HJ, *et al*. Assessing the protective role of epigallocatechin gallate (EGCG) against water-pipe smoke-induced toxicity: a comparative study on gene expression and histopathology. *Molecules* 2023;28(22):7502.

36 Chan TC, Wilkinson Berka JL, Deliyanti D, *et al*. The role of reactive oxygen species in the pathogenesis and treatment of retinal diseases. *Exp Eye Res* 2020;201:108255.

37 Murakami Y, Nakabeppu Y, Sonoda KH. Oxidative stress and microglial response in retinitis pigmentosa. *Int J Mol Sci* 2020;21(19):7170.

38 Gallenga CE, Lonardi M, Pacetti S, *et al*. Molecular mechanisms related to oxidative stress in retinitis pigmentosa. *Antioxidants* 2021;10(6):848.

39 Remigante A, Morabito R. Cellular and molecular mechanisms in oxidative stress-related diseases. *Int J Mol Sci* 2022;23(14):8017.

40 Xiong WJ, MacColl Garfinkel AE, Li YQ, *et al*. NRF2 promotes neuronal survival in neurodegeneration and acute nerve damage. *J Clin Invest* 2015;125(4):1433-1445.

41 Nir I, Kedzierski W, Chen J, *et al*. Expression of Bcl-2 protects against photoreceptor degeneration in retinal degeneration slow (rds) mice. *J Neurosci* 2000;20(6):2150-2154.

42 Wang J, Zhao J, Cui X, *et al*. The molecular chaperone sigma 1 receptor mediates rescue of retinal cone photoreceptor cells *via* modulation of NRF2. *Free Radic Biol Med* 2019;134:604-616.

43 Chen WJ, Wu CY, Xu ZH, *et al*. Nrf2 protects photoreceptor cells from photo-oxidative stress induced by blue light. *Exp Eye Res* 2017;154:151-158.

44 Li DM, Cao DH, Sun YL, *et al*. The roles of epigallocatechin gallate in the tumor microenvironment, metabolic reprogramming, and immunotherapy. *Front Immunol* 2024;15:1331641.

45 Han XW, Xu RL, Xia Y, *et al*. Self-assembled EGCG nanoparticles with enhanced intracellular ROS scavenging for skin radioprotection. *Int J Nanomed* 2024;19:13135-13148.

46 Sun ML, Wu Y, Zhou ZY, *et al*. Co-delivery of EGCG and melittin with self-assembled fluoro-nanoparticles for enhanced cancer therapy. *Aging* 2023;15(11):4875-4888.

47 Khandal J, Dohare S, Dongsar TS, *et al*. Gelatin nanocarriers in oncology: a biocompatible strategy for targeted drug delivery. *Int J Biol Macromol* 2025;310:143244.

48 Yan RK, Cao YL. The safety and efficacy of dietary epigallocatechin gallate supplementation for the management of obesity and non-alcoholic fatty liver disease: recent updates. *Biomedicines* 2025;13(1):206.

49 Capasso L, De Masi L, Sirignano C, *et al*. Epigallocatechin gallate (EGCG): pharmacological properties, biological activities and therapeutic potential. *Molecules* 2025;30(3):654.

50 Talib WH, Awajan D, Alqudah A, *et al*. Targeting cancer hallmarks with epigallocatechin gallate (EGCG): mechanistic basis and therapeutic targets. *Molecules* 2024;29(6):1373.

51 Zhang Y, Xu Q, Hu JP, *et al*. Green tea and epigallocatechin gallate (EGCG) for cancer prevention: a systematic review and meta-analysis. *Am J Chin Med* 2025;53(6):1755-1784.

52 Ferrari E, Naponelli V. Catechins and human health: breakthroughs from clinical trials. *Molecules* 2025;30(15):3128.