Neuroprotective effects of resveratrol on retinal ganglion cells in glaucoma: Focusing on oxidative stress, cellular, and molecular mechanisms

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Abstract
Retinal ganglion cells (RGCs) are the major cells that are damaged in glaucoma leading to vision loss and blindness. The damage to RGCs in glaucoma is caused by various mechanisms such as elevated intraocular pressure, oxidative stress, inflammation, and other neurodegenerative processes. As the disease progresses, more and more RGCs are lost, leading to a gradual loss of vision. Therefore, protecting RGCs from damage and promoting their survival is an important goal in the treatment of glaucoma. Resveratrol (RES) exerts anti-oxidant effects and slows down the evolution and progression of glaucoma. This review was prepared using databases such as Google Scholar, PubMed, Scopus, and ScienceDirect. Based on the findings of this review, RES has a protective role on RGCs in cases of ischemic injury and hypoxia as well as ErbB2 protein expression in the retina. Additionally, RES has protective effects on RGCs by promoting cell growth, reducing apoptosis, and decreasing oxidative stress in H2O2-exposed RGCs. RES was also found to inhibit oxidative stress damage in RGCs and suppress the activation of MAPK signaling pathways. Moreover, RES could alleviate retinal function impairment by suppressing the HIF-1α/VEGF and p38/p53 axis while stimulating the PI3K/Akt pathway. Hence, RES might exert potential therapeutic effects in the treatment of glaucoma by protecting RGCs from damage and promoting their survival.

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Abstract

Retinal ganglion cells (RGCs) are the major cells that are damaged in glaucoma leading to vision loss and blindness. The damage to RGCs in glaucoma is caused by various mechanisms such as elevated intraocular pressure, oxidative stress, inflammation, and other neurodegenerative processes. As the disease progresses, more and more RGCs are lost, leading to a gradual loss of vision. Therefore, protecting RGCs from damage and promoting their survival is an important goal in the treatment of glaucoma. Resveratrol (RES) exerts anti-oxidant effects and slows down the evolution and progression of glaucoma. This review was prepared using databases such as Google Scholar, PubMed, Scopus, and ScienceDirect. Based on the findings of this review, RES has a protective role on RGCs in cases of ischemic injury and hypoxia as well as ErbB2 protein expression in the retina. Additionally, RES has protective effects on RGCs by promoting cell growth, reducing apoptosis, and decreasing oxidative stress in H2O2-exposed RGCs. RES was also found to inhibit oxidative stress damage in RGCs and suppress the activation of MAPK signaling pathways. Moreover, RES could alleviate retinal function impairment by suppressing the HIF-1α/VEGF and p38/p53 axis while stimulating the PI3K/Akt pathway. Hence, RES might exert potential therapeutic effects in the treatment of glaucoma by protecting RGCs from damage and promoting their survival.

Keywords: Resveratrol, Retinal ganglion cells, Glaucoma, Oxidative stress, Ischemic-Reperfusion Injury

Abbreviations:

RGC: Retinal ganglion cells
RES: Resveratrol
POAG: Primary open-angle glaucoma
ACG: Angle-closure glaucoma
ROS: Reactive oxygen species
MAPKs: Mitogen-activated protein kinases
IOP: Intraocular pressure
SLT: Selective laser trabeculoplasty
MIGS: Micro-invasive glaucoma surgery
RES: Resveratrol
SOD: Superoxide dismutase
CAT: Catalase
GSH: Glutathione
FDA: Food and Drug Administration
EFSA: European Food Safety Authority
NTG: Normal-tension glaucoma
TM: Trabecular meshwork
HER2: human epidermal growth factor receptor 2
I/R: Ischemia/Reperfusion
GCL: Ganglion cell layer
ERKs: Extracellular signal-regulated kinases
JNKs: c-Jun N-terminal kinases
HIF-1α: Hypoxia-inducible factor-1 alpha
Sirt1: Sirtuin-1
Opa1: Optic atrophy 1
NF-H: Neurofilament heavy chain
BDNF: Brain-derived neurotrophic factor
TrkB: Tropomyosin receptor kinase B
MGCs: Müller glial cells
p-Akt: Phosphorylated-Akt
UPR: Unfolded protein response
BiP: Binding immunoglobulin Protein
CHOP: C/EBP homologous protein
XBP-1: X-box binding protein-1
ONT: Optic nerve transection

**Introduction**

Glaucoma, a progressive eye disease, is a chronic condition and a leading cause of blindness globally (1). It is characterized by increased intraocular pressure (IOP), which could impair the optic nerve over time and cause visual impairment if left untreated (2, 3). Early diagnosis and treatment of glaucoma are vital to minimize or prevent vision loss (4). According to the World Health Organization (WHO) report, glaucoma is estimated to affect around 3% of the global population aged 40 to 80 years, which amounts to approximately
76 million people (5). Regular eye examinations and early detection are crucial in managing glaucoma and preventing vision loss (6). The etiology of glaucoma is diverse and can be categorized into two main types: primary open-angle glaucoma (POAG) and angle-closure glaucoma (ACG) (7, 8). POAG, which is the most prevalent type of glaucoma, often has an unknown cause (9). However, it seems to be associated with genetic factors, age-related changes in the eye, and IOP developing from lessened aqueous humor drainage (10). On the other hand, ACG can occur when the drainage angle between the iris and the cornea becomes blocked, leading to a sudden rise in IOP (11).

Oxidative stress has a notable impact on the evolution of glaucoma and can cause damage and dysfunction of retinal ganglion cells (RGCs) (12). The production of reactive oxygen species (ROS), mainly hydrogen peroxide (H$_2$O$_2$), can induce apoptosis of RGCs (13). This apoptosis is one of the main causes of the development and progression of glaucoma (14). Oxidative stress can also impair the antioxidant enzyme activity, leading to a reduced ability to neutralize ROS and protection against oxidative damage (15, 16). This further exacerbates the damage to RGCs and contributes to the progression of glaucoma (17). Furthermore, oxidative stress could initiate various signaling axes like apoptosis-associated signals and mitogen-activated protein kinases (MAPKs), which are involved in cell death and inflammation-related cascades (18). These pathways would result in RGC death and development of glaucoma (18). Therefore, protecting RGCs against ROS-induced apoptosis and mitigating oxidative stress are important strategies in the management and prevention of glaucoma (18).

The choice of glaucoma treatment is related to various factors, such as the patient’s overall health, the type and stage of glaucoma, and their ability to adhere to the treatment plan (19, 20). Regular monitoring and follow-up are essential to assess the effectiveness of treatment and stopping further vision loss (21). Medical therapy is usually the first choice of treatment and involves the administration of eye drops or oral medications to lower IOP (22). Selective laser trabeculoplasty (SLT), is a type of laser treatment used to lower IOP in patients with glaucoma (23). Surgical interventions, such as trabeculectomy or micro-invasive glaucoma surgery (MIGS), are typically reserved for patients who are unresponsive to or cannot take medical or laser treatment (24). On the other hand, natural compounds, including antioxidants, have shown potential in the treatment of glaucoma (25). Antioxidants have a crucial role in combating oxidative stress, which is a major risk factor in the initiation and advancement of glaucoma (26). Studies have indicated that antioxidants such as resveratrol (RES), curcumin, as well as vitamins C and E, and carotenoids (lutein and zeaxanthin), have protective functions on RGCs and the optic nerve (27-31). RES is found in cereals, fruits, vegetables, and red wine (32, 33). It has been approved that RES has antioxidant, anti-inflammatory, and anti-apoptotic effects which may help to keep RGCs from damage caused by oxidative stress and inflammation (31, 34). Additionally, it prevents hypoxia-induced RGC death by downregulating apoptosis and the expression of ErbB2, a protein involved in cell survival and proliferation (35).

This review article focuses on the in vitro experimental findings that demonstrate the effectiveness of RES in protecting RGCs from damage. The review also provides insights into the cellular and molecular mechanisms of action of resveratrol and its potential as an adjuvant treatment for glaucoma. Resveratrol’s antioxidant and anti-apoptotic properties contribute to its therapeutic potential in mitigating the effects of glaucoma.

**Overview of resveratrol**

RES was originally discovered by Takaoka in 1939, who isolated the compound from the root of the Veratrum grandiflorum plant (36). The structure of RES is a natural polyphenol with the chemical formula C$_{14}$H$_{12}$O$_3$ (37). It consists of a stilbene backbone, which is a double-bonded phenyl group connected to another phenyl group by a double bond (38). RES has three hydroxyl groups (-OH) attached to the phenyl rings, located at positions 3, 5, and 4’ (39). The structure of RES allows it to exist in different geometric arrangements, including cis and trans isomers (Figure 1) (40).

**Figure 1. Structures of trans- RES and cis- RES (41).**

RES is abundant in grapes, especially in the skins and seeds (42). Red grapes tend to have a higher amount of RES than green grapes (43). Red wine, made from fermented grapes, contains RES due to the presence of
grape skins during the fermentation process (44). RES is also present in peanuts and peanut products, such as peanut butter (45). However, the levels may vary depending on the processing and roasting methods.

RES has a relatively high absorption rate through the small intestine, likely due to its small, non-polar structure. When taken orally, trans-RES is well absorbed by the human body (46). However, its bioavailability is fairly low due to its rapid metabolism, which results in the formation of various metabolites, such as RES glucurónides and RES sulfates (47). According to a study, the absorption of RES can be significantly increased when it is in micronized form (48). It showed a four-fold increase in plasma concentration, which is a doubling (49). Earlier research has indicated that RES is efficiently absorbed by the body when taken orally, with approximately 75% of the dose being absorbed (50). Once absorbed, RES and its metabolites enter the systemic circulation and are disseminated to peripheral tissues, including adipose tissue (51). To increase the absorption of RES, one can consume it with a meal containing fat (52). The absorption of RES occurs through a rapid process of passive diffusion, as observed in Caco-2 cells (53). Transepithelial diffusion is responsible for approximately 70% to 75% of the absorption of RES (54). Micronized RES has been investigated to have a notably increased absorption rate than non-micronized RES (55). The major metabolites of RES include RES-3-O-glucurónide, RES-3-O-sulfate, and RES-4′-O-glucurónide (56). In addition, other metabolites such as RES diglucurónide, RES sulfoglucurónide isomers, RES glucurónide isomers, and RES sulfate have also been identified in some studies (57, 58). Extensive phase-II metabolism occurs in both the intestine and liver following oral administration of RES, leading to the formation of various metabolites (57). The main metabolites of RES that have been recognized in plasma and urine are RES glucurónides and sulfates (59).

RES has antioxidant activity by scavenging free radicals and reducing oxidative damage to cells and tissues (60). It could directly neutralize ROS and inhibit lipid peroxidation, which is a common marker of oxidative stress (54). Additionally, RES has been shown to upregulate the activity of different antioxidant enzymes like superoxide dismutase (SOD) and catalase, which help to counteract oxidative stress (32). RES stimulates the SOD, catalase (CAT), and glutathione (GSH), in RGC-5 cells (18). These enzymes help to counteract oxidative stress and keep cells from damage (61, 62). Moreover, RES reveals anti-inflammatory properties by preventing the formation of pro-inflammatory molecules and modulating inflammatory signaling pathways (63). This can help to reduce inflammation and promote overall health. RES has been shown to have metabolic regulatory effects, such as improving mitochondrial function and energy metabolism (64). It can enhance nutrient utilization and metabolic efficiency, leading to improved growth performance and overall metabolic health (65). According to a systematic review of experimental in vivo and in vitro models of Parkinson’s disease, RES has the potential to pass the blood-brain barrier (BBB) and prevent or slow the development of the disease (66). The findings of the review suggest that RES may have neuroprotective effects by reducing oxidative stress and inflammation in the brain, which are key factors in the development and progression of Parkinson’s disease.

The recommended daily intake of RES has not been established by regulatory authorities such as the Food and Drug Administration (FDA) or the European Food Safety Authority (EFSA) (67). Since RES is considered a dietary supplement rather than a medication, it does not have an official recommended daily allowance (RDA) or dietary reference intake (DRI) at this time. Nevertheless, studies have suggested that a typical daily dose of resveratrol ranges from 150 mg to 500 mg for potential health benefits (68, 69). Individual needs may be different, and before embarking on any supplementation regimen, it is recommended to seek the advice of a healthcare professional or a registered dietitian. RES is generally regarded as safe and well-tolerated in humans at doses of up to 5g per day (70). Nevertheless, some studies have reported potential adverse effects of RES, particularly at high doses (71, 72). These effects include gastrointestinal symptoms like nausea, diarrhea, and abdominal pain, as well as potential interactions with certain medications (73, 74). In addition, some animal studies have reported potential toxic effects of RES on the liver and kidneys at high doses (75, 76). In general, RES seems to be safe and well-tolerated at typical doses. However, additional research is required to gain a full understanding of the potential advantages and drawbacks of high-dose RES supplementation.

**Overview of Glaucoma**
Glaucoma comprises a group of eye diseases that could cause optic nerve damage, potentially leading to vision loss and even blindness if left untreated (77). Typically, glaucoma is linked with elevated IOP, though it could also develop with normal or low intraocular pressure (78). Glaucoma encompasses different forms, such as POAG, ACG, normal-tension glaucoma, and secondary glaucoma (79). POAG is the most prevalent type and usually progresses slowly over time, often without noticeable symptoms in the early stages (80). In contrast, ACG can lead to acute symptoms like intense eye pain, headaches, blurred vision, and nausea (81). The primary factor that contributes to the development of POAG is increased pressure within the eye, also known as IOP (82). Elevated IOP is intensely associated with all forms of glaucoma except normal-tension glaucoma (NTG) (83). In POAG, there is a slow progression of optic disc atrophy, loss of peripheral visual field, and a characteristically excavated optic disc (84). Regular eye examinations and early detection are crucial for diagnosing and managing glaucoma (85). The treatment approach for glaucoma may involve a combination of therapies, including medications in the form of eye drops, laser treatment, or surgery (86). The specific treatment modality depends on several factors such as the type and severity of glaucoma, the overall health status of the patient, and the presence of any underlying medical conditions (87). It is important to note that while treatment can help slow down the progression of glaucoma, any vision loss that has already occurred is irreversible. Below are some typical interventions that may be used to manage glaucoma:

1. Medications: Eye drops or oral medications are often prescribed to decrease IOP (88). The primary purpose of these medications is to regulate the amount of fluid in the eye (aqueous humor), either by reducing its production or increasing its outflow, to manage IOP (89). Medications may need to be used long-term and require regular monitoring.

2. Laser therapy: Laser trabeculoplasty and laser peripheral iridotomy are two common laser procedures used to treat glaucoma (90). Laser trabeculoplasty helps improve the drainage of fluid from the eye, while laser peripheral iridotomy forms a small hole in the iris to improve the flow of fluid and relieve pressure (91, 92).

3. Surgical procedures: In cases where medications and laser therapy are not effective, surgical interventions may be necessary. Trabeculectomy, in which a new drainage channel is created, and drainage implant surgery are examples of surgical procedures used to lower IOP (93, 94). MIGS procedures are novel surgical techniques that aim to reduce IOP with minimal trauma and faster recovery compared to traditional surgeries (95). These procedures involve the use of tiny devices or implants to improve the drainage of fluid from the eye (96).

Several molecular mechanisms have been proposed to cause glaucoma such as oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress (97-99). Oxidative stress has been identified as a potential factor in the development and progression of a range of optic diseases like glaucoma (100). Disruption of the balance between free radical generation and antioxidant defenses can result in oxidative stress and damage to cells and tissues (15). Oxidative damage to trabecular meshwork (TM) cells would cause RGCs apoptosis followed by trabecular dysgenesis and IOP rise due to aqueous flow impairment (101). Mitochondrial function dysregulation has been correlated with the pathophysiology of glaucoma and is considered a major factor in its development (102). In glaucoma, dysfunctional mitochondria could decrease ATP synthesis, oxidative stress, and impaired cellular metabolism (103). Mitochondria play a significant role in the generation of ROS. However, when mitochondrial function is compromised, it disrupts the balance between ROS production and antioxidant defenses, resulting in oxidative stress (103). Mitochondrial dysfunction can trigger apoptotic pathways, leading to the death of RGCs, which are the primary cells affected in glaucoma (104). In addition, studies have depicted that genetic factors have an important part in the pathophysiology of glaucoma. Mutations in MYOC, OPTN, WDR36, and CYP1B1 are among the most commonly identified genetic risk factors for the disease (105, 106). These genes are responsible for multiple cellular processes, including regulation of IOP, oxidative stress response, and extracellular matrix remodeling.

**Neuroprotective effects of resveratrol on RGCs in glaucoma**

RGCs have a crucial role in the onset and advancement of glaucoma, a prevalent cause of permanent vision loss across the globe (107). Understanding the roles of RGCs in glaucoma is essential for developing effective therapeutic strategies to protect and preserve these cells. Targeting the underlying mechanisms of RGC damage, such as reducing IOP, promoting neuroprotection, and inhibiting inflammation, can help stop or
lessen the progression of glaucoma and preserve visual function. Several mechanisms are responsible for the pathogenesis of glaucoma which eventually effect on RGCs and result in vision loss. In this regard, RES has multiple protective cellular and molecular impacts on RGCs (Figure 2).

Figure 2. Μεταβολισμοί στις αστικές οδούς του ΕΡΕΣ για τη διατήρηση της ευεξίας της ΡΝΚ. Α: ΕΡΕΣ συγκεκριμένες της αστικής οδού του ΠΝΚ, Π38, Π53 σημαίνοντας στήριξη της ένδυσης βηχάδας έκφρασης ζώνης στην ηλεκτρονική. Β: ΕΡΕΣ συγκεκριμένες της προστασίας της ΕΡΕΣ και των ΣΙΡΤ1. Γ: ΡΕΣ ασπασμοί ΑΜΠΚ υποδεικνύουν τη ΣΙΡΤ1 ασπασμό άσπρων και θαλαμικούς της τρανσλειτρισμού ανα-ανοιγμένης γενεάς ανάλογα με την ευεξία της ΣΟΔ. Δ: ΡΕΣ απενεργοποιεί την ΗΙΦ-1α/ΕΓΦ βηχάδας ζώνης Π3Κ/Ακτ.

ErbB2, also referred to as human epidermal growth factor receptor 2 (HER2), is a member of the ErbB family of receptor tyrosine kinases (108). It is an essential component in regulating cell growth, survival, and differentiation (109). ErbB2 is implicated in different cellular processes like the regulation of the nervous system, heart, and mammary glands (110-112). Seong et al. explored how RES affects hypoxia-induced RGC death in ErbB2. RES was found to effectively suppress retinal cell death induced by I/R injury in the ganglion cell layer (GCL) of the retina. This was demonstrated by TUNEL staining, which showed a significantly higher number of TUNEL-positive cells in the I/R group compared to the control group. However, the I/R+RES group exhibited a significantly lower number of TUNEL-positive cells compared to the I/R group. The study revealed that RES effectively decreased the expression of ErbB2 in the retina following I/R injury. These results highlight the potential of RES as a therapeutic intervention to prevent hypoxia-induced retinal ganglion cell death associated with ErbB2 (35).

Apoptosis, a programmed cell death process, involves the activation of caspase-3 and cleaved caspase-9 enzymes (113). In the process of apoptosis, caspase-3 is a crucial enzyme that belongs to the caspase family of proteases (113). This family of enzymes regulates and executes apoptosis (113). Upon receiving signals that initiate programmed cell death, such as DNA damage, cellular stress, or signaling from other caspases, it becomes activated (114). Once activated, caspase-3 cleaves and activates specific cellular proteins, leading to the characteristic changes associated with apoptosis (115). These changes include DNA fragmentation, cytoskeletal breakdown, nuclear condensation, and membrane blebbing (116). Caspase-9, a member of the caspase family of proteases, is an important component in the process of apoptosis, and is considered an initiator caspase because it plays a notable role in initiating the apoptotic cascade (117, 118). Activation of caspase-9 primarily occurs via the intrinsic or mitochondrial pathway of apoptosis, which is triggered by cellular stresses such as DNA damage or cellular damage (119). The formation of the apoptosome complex, which includes cytochrome c, Apaf-1, and ATP, are involved in activating caspase-9 in this pathway (120). MAPKs are a group of enzymes that are critical for numerous cellular processes, like cell proliferation, differentiation, survival, and response to external signals (121). MAPKs participate in signal transduction pathways that transmit signals from the cell membrane to the nucleus, resulting in alterations in gene expression and cellular function (122). The MAPK family consists of multiple components including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPKs (123, 124). Activation of each component of the MAPK family is triggered by distinct upstream kinases responding to different stimuli (125). ERKs are primarily responsible for cell growth, differentiation, and survival (126). They are activated by growth factors, hormones, and mitogen, and their activation leads to the regulation of Genome activity and cellular proliferation (127, 128). JNKs have a role in stress responses, inflammation, and apoptosis (129, 130). They are activated by various stress signals, including UV radiation, heat shock, and pro-inflammatory cytokines (129, 131). p38 MAPKs are also involved in stress responses and inflammation (132, 133). p38 MAPK activation can regulate gene expression, cell cycle progression, and inflammatory responses (134). Ye and Meng found that RES exhibited protective effects against H2O2-induced apoptosis in RGCs. The study indicated that H2O2 raised the levels of cleaved caspases-3 and -9, which are responsible for initiating and driving apoptosis, while RES was found to counteract this effect. This finding indicates that RES exhibits strong antioxidant properties against H2O2-induced damage in RGCs. Additionally, the researchers noted that RES was able to suppress ERK, JNK, and p38 signaling pathways in RGC-5 cells.
induced by H$_2$O$_2$. These three MAPKs contribute to intracellular metabolism regulation and response to external stress. The findings of the study imply that resveratrol’s protective effects may be attributed to the inhibition of these MAPK pathways (18).

The retinal I/R injury model is a method used to induce retinal damage in mice (135). In this model, the induction of retinal I/R injury was achieved by briefly cannulating the anterior chambers of both eyes of adult male C57BL/6 J mice with a stainless needle (136). In this study of Luo et al., one eye of the mice was exposed to increased IOP above the systolic blood pressure for a duration of 60 minutes, while the other eye served as the control and maintained a normal IOP. After the 60-minute period, the needle was removed from the eye, and the mice were sacrificed at different time points (0, 1, 3, and 7 days) following the I/R injury. The aim of this model was to induce retinal I/R injury in mice, which was then used to investigate the effects of RES on the loss of RGCs and impairment of retinal function (137).

HIF-1α is a transcription factor that is essential for maintaining cellular homeostasis in response to oxygen deprivation caused by tissue ischemia (138). HIF-1α is predominantly found in the nucleus and cytoplasm of cells (139). The HIF-1 complex is created by the dimerization of HIF-1α and HIF-1β, which in turn triggers the activation of genes involved in erythropoiesis, neuroprotection, angiogenesis, VEGF, apoptosis, and necrosis (140, 141). VEGF, a signaling protein, plays a crucial role in the process of angiogenesis (142). VEGF promotes the growth of endothelial cells, which are the building blocks of blood vessels (143). It participates in several physiological mechanisms, such as embryonic development, wound healing, and the formation of new blood vessels in response to tissue ischemia (144). However, excessive or dysregulated expression of VEGF can contribute to pathological conditions, such as retinal neovascularization and vascular leakage (145). The research conducted by Ji et al. has revealed that RES possesses the ability to protect RGCs by suppressing the HIF-1α/VEGF and p38/p53 signaling axis as well as stimulating the PI3K/Akt pathway. The HIF-1α/VEGF axis is associated with retinal ischemic injury, and the p38/p53 pathway is responsible for RGC apoptosis. Additionally, the PI3K/Akt pathway boosts cell survival and prevents apoptosis. RES administration was shown to suppress the overexpression of the HIF-1α/VEGF and p38/p53 pathways following I/R injury while activating the downregulation of the PI3K/Akt pathway. This led to an improvement in retinal function after the injury-induced functional damage, indicating that RES may have the potential to alleviate retinal ischemic injury-induced RGC loss and retinal function impairment (136).

Sirt1 is a member of the Sir2 family of proteins which are NAD(+)-dependent deacetylases (146). Sirt1 has a role in various cellular processes, including anti-apoptosis, anti-aging, and regulation of genome activity and metabolism (147, 148). It is considered a therapeutic target for the treatment of neurodegenerative disorders like Alzheimer’s disease, Parkinson’s disease, and polyglutamine disease (149, 150). According to Wu et al., RES has a positive impact on RGCs and their axons after retinal I/R injury. The research indicates that RES can protect RGC axons from injuries by suppressing the phosphorylation of JNK proteins via Sirt1. The study also discovered that Sirt1 and JNK have an interdependent association, but the specific mechanism requires further investigation. These results revealed that RES may have therapeutic implications in optic nerve degeneration. Nevertheless, more research is necessary to realize the molecular basis of this effect (34).

R28 cells are retinal neuronal-like cells that were originally derived from postnatal day 1 rat retina and have been characterized as a model for RGCs (151). R28 cells have been used in various studies to explore the mechanisms of RGC death and to evaluate potential therapeutic agents for RGC damage (152). Optic atrophy 1 (Opal) is a protein that plays a crucial role in mitochondrial fusion and remodeling of cristae structures (153). Opal is inherent in the inner mitochondrial membrane and participates in the maintenance of mitochondrial morphology, function, and dynamics (154). The function of Opal is to regulate mitochondrial fusion by promoting the fusion of the inner mitochondrial membrane, which is essential for the maintenance of mitochondrial activity and cristae structure (155). SOD is an enzyme that performs a vital function in antioxidant defense mechanisms within cells (15). SOD helps to neutralize harmful superoxide radicals, which are extremely reactive molecules that can induce oxidative harm to cells (156). By converting superoxide radicals into less harmful molecules, SOD lowers oxidative stress and contributes to the preservation
of cellular health (54). Pang et al. indicated that RES exhibits protective effects on retinal injury induced by I/R and serum deprivation. The administration of RES enhanced the viability of retinal cells (R28 cells) and partially alleviated apoptosis, as observed by the researchers. Additionally, the researchers found that RES modulated the Opa1 expression and the activity of SOD, both of which are thought to promote the protective effects of RES on RGCs. Still, more studies are required to establish a clearer understanding of the relationship between apoptosis, Opa1, and SOD activity in association with glaucoma (31).

Brn-3a is a transcription factor that belongs to the brain-specific homeobox/POU domain protein family (157). This protein is essential for the development and survival of RGCs by regulating the gene expression that is crucial for RGC development and function (158). Brn-3a is selectively expressed in RGCs, where it is essential for their development and function (158). SMI-32, a monoclonal antibody, specifically targets a non-phosphorylated epitope of the neurofilament heavy chain (NF-H) (159). This antibody is commonly employed as a marker for mature neurons, such as RGCs (160). Brain-derived neurotrophic factor (BDNF) plays a crucial part in the growth, survival, and differentiation of neurons in the brain and peripheral nervous system (161). BDNF is a member of the neurotrophin family of growth factors and participates in developing and maintaining the nervous system (162). Tropomyosin receptor kinase B (TrkB), a receptor protein also referred to as tropomyosin receptor kinase B, gets activated through binding with BDNF and other neurotrophins (163). Activation of TrkB signaling pathways promotes cell survival, synaptic plasticity, and neuroprotection (164). The results of the Cao et al. investigation suggested that RES, administered intravitreally, protected RGCs from high IOP-induced cell death through various mechanisms, making it a promising therapeutic intervention for glaucoma. Specifically, they observed that the intravitreal injection of 30-μM RES significantly elevated the expression of Sirt1 and reduced the generation of ROS in RGCs, improved the Brn-3a and SMI-32 expression, and caused a marked reduction in TUNEL-positive cells in RGCs. The effects of RES on BDNF in Müller glial cells (MGCs) and TrkB expression in RGCs were investigated in the mentioned study. It was found that RES administration caused the upregulation of BDNF gene expression in MGCs. Specifically, exposure to 140-μM RES led to a remarkable upregulation of BDNF gene expression, unlike the control group. Additionally, the study confirmed the upregulation of TrkB expression in RGCs following RES treatment. These findings suggested that RES increases BDNF-TrkB signaling between MGCs and RGCs, which is vital for the protection and survival of RGCs (165).

The stimulation of Bax leads to irreversible injury to mitochondria, modulates the release of apoptotic factors, and ultimately triggers caspase activation to initiate the apoptotic cascade (166). The intrinsic apoptotic axis begins with the activation of Bax, followed by pore formation in the mitochondrial outer membrane that arises from its activation, dimerization, and oligomerization (167). This results in the release of signaling molecules like cytochrome c, which eventually triggers the initiation of caspases, including cleaved caspase-3 (168). Phosphorylated-Akt (p-Akt), the active form of the protein Akt, is a serine/threonine kinase that has a crucial part in cell survival, growth, and various cellular processes (169). Akt can be activated by phosphorylation, leading to its activation and subsequent signaling cascade (170). Phosphorylation of Akt at specific sites like serine 473 and threonine 308, results in its activation and enables it to phosphorylate downstream targets associated with cell survival and growth pathways (171). Sirt1 has been identified by Luo et al. as a necessary component for the neuroprotective properties of RES on RGCs following retinal I/R injury in mice. The most effective concentration of RES was found to be 100 μM. The increase in Sirt1 levels induced by RES was found to have a significant impact on RGCs apoptosis, leading to elevated levels of phospho-Akt and decreased expressions of Bax and cleaved caspase-3 (137).

Inflammation has a major role in glaucoma (172). It has been observed that glaucoma is linked to chronic low-grade inflammation in the retina and optic nerve (173). Inflammation can contribute to the progression of glaucoma through various mechanisms:

1. Stimulation of immune cells: In reaction to injury or stress, immune cells such as microglia and astrocytes in the retina and optic nerve become activated and release pro-inflammatory mediators (IL1-β, IL6, TNFα) (174, 175). This activation can lead to the production of cytokines, chemokines, and ROS, which can contribute to neuronal injury and cell death (176).
2. Gliosis: Gliosis refers to the reactive reaction of glial cells, such as astrocytes and Müller cells, to injury or stress (177). In glaucoma, gliosis is characterized by the proliferation and hypertrophy of glial cells (178). Gliosis can lead to the formation of a glial scar, which can impair the regeneration and function of neurons (178).

3. Excitotoxicity: Inflammation can release excitatory neurotransmitters, such as glutamate, which can cause excitotoxicity and neuronal death (179). Excitotoxicity is believed to be a major contributor to RGC death in glaucoma (180).

Overall, inflammation in glaucoma can link to the loss of RGCs and optic nerve damage. Targeting inflammation and its associated pathways may hold therapeutic potential for treating glaucoma.

Luo et al. demonstrated that treatment with RES can effectively inhibit RGC death and mitigate inflammation associated with gliosis following I/R injury. The anti-apoptotic properties of RES were confirmed through reduced TUNEL staining, prevention of the early increase in the pro-apoptotic protein Bax, and subsequent decrease in cleaved caspase-3 levels. The results of this study indicate that RES has the ability to prevent RGC death by inhibiting the Bax-caspase-3-dependent apoptotic pathway. These findings suggest that RES has potential therapeutic efficacy in treating I/R injury-induced glaucoma (181).

Activation of the unfolded protein response (UPR) is a cellular stress response pathway that occurs when there is an aggregation of unfolded or misfolded proteins in the ER (182). The primary objective of the UPR is to restore ER homeostasis and facilitate cell survival (183). UPR proteins are a group of molecular chaperones and transcription factors that have a vital part in the UPR pathway. Some of the UPR proteins include:

1. Binding immunoglobulin protein (BiP, also known as GRP-78): BiP is an ER chaperone that assists in protein folding and prevents protein aggregation (184). It is upregulated during ER stress and helps properly fold unfolded proteins (185).

2. The transcription factor C/EBP homologous protein (CHOP), known as DNA damage-inducible transcript 3 or growth and DNA damage protein-153, is induced during ER stress (186). CHOP is responsible for regulating the gene expression responsible for ER stress signaling and apoptosis (187).

3. X-box binding protein-1 (XBP-1) is a transcription factor that is activated during endoplasmic reticulum (ER) stress and plays a crucial role in regulating genes associated with protein folding, ER-associated degradation, and lipid metabolism (188).

Lindsey et al. described that extended dietary intake of RES could prevent RGC dendrite loss following optic nerve injury. Furthermore, the expression of certain UPR proteins, such as BiP, CHOP, and XBP-1, was altered in response to optic nerve crush (ONC), but this effect was diminished in mice that received dietary RES. These findings suggest that prolonged dietary RES supplementation can shield against RGC dendrite loss and regulate the unfolded protein response in the retina after optic nerve injury (189).

Optic nerve transection (ONT) is a surgical procedure performed in animal models to simulate optic nerve damage. In this procedure, the optic nerve is intentionally severed or cut, typically in a controlled and standardized manner (190). The purpose of ONT is to create a model that mimics optic nerve injury or degeneration, allowing researchers to study the impacts of such damage on RGCs and explore potential neuroprotective interventions (191). During ONT, an incision is made in the lateral conjunctiva, and the optic nerve is exposed through blunt dissection. A longitudinal incision is then made in the optic nerve sheath, and a cross-section of the optic nerve is created, with caution taken to avoid damaging the surrounding blood supply. Following the procedure, the incision is closed with sutures, and appropriate ophthalmic ointment is applied (191). According to another research Kim et al., the neuroprotective efficacy of RES was examined in an ONT model. The results revealed that RES when given at a concentration of 3.1 uM or higher, exhibited a significant neuroprotective effect for RGCs than the control group. The number of RGCs in eyes treated with RES was notably higher than those treated with a control substance (PBS) in transected rats. The
study depicted that resveratrol may have a therapeutic effect in treating optic nerve diseases by activating the Sirt1 pathway and exerting its neuroprotective properties (192).

Riluzole is known to inhibit glutamate release and block voltage-gated sodium channels (193, 194). These actions of riluzole help to reduce excitotoxicity and prevent the degeneration of RGCs (195). In addition, riluzole has been shown to have antioxidant properties and could save RGCs against oxidative stress-induced injury to the optic nerve (195). Pirhan et al. indicated that both riluzole and RES, when used alone or in combination, significantly delayed the degeneration of RGC in the experimental glaucoma model. According to this study, RES therapy was found to have a significant effect on RGC density in the experimental glaucoma model. The RGC density in the group receiving RES therapy was found to be the highest among all groups (195).

Limitations and Suggestions

While RES has shown potential in protecting RGCs and preventing vision loss in certain eye diseases, such as glaucoma, more evidence is needed to fully understand how it works and its potential clinical applications. Researchers are still investigating the mechanisms of action that make RES effective in protecting RGCs, and more studies are considered necessary to establish the optimal dosage and administration method. Additionally, the optimal dosage, bioavailability, and long-term effects of RES supplementation are still areas of ongoing investigation. However, it is important to note that the review article primarily focuses on experimental studies using animal models and in vitro cell cultures. Further research, including well-designed clinical studies, is necessary to determine the safety and efficacy of RES in the treatment of glaucoma. Clinical studies would involve evaluating the effects of RES on glaucoma-related parameters, such as IOP, RGC function, and optic nerve integrity, in human subjects with glaucoma. We presented a summary of the study results in Table 1.

Table 1. Studies are consistent with the purpose of this study.

Conclusion

Several antioxidants have been investigated for their potential therapeutic effects in the treatment of glaucoma. These include natural antioxidants such as RES, and curcumin, as well as vitamins C and E, and carotenoids like lutein and zeaxanthin. These antioxidants have been found to have neuroprotective effects on RGCs and might be beneficial in the prevention and treatment of glaucoma by protecting RGCs from damage and promoting their survival. RES is a natural polyphenol compound found in different plants like grapes and berries. The findings of this review showed that 1) RES has cellular and molecular impacts on RGCs by promoting cell growth, reducing apoptosis, and decreasing oxidative stress in H2O2-exposed RGCs, 2) RES can protect retinal ganglion cell axons by preventing the phosphorylation of JNK proteins through SIRT1, 3) RES has the potential to reduce the loss of RGCs and impairment of retinal function caused by IR injury. This effect is achieved by inhibiting the HIF-1α/VEGF and p38/p53 pathways, which are involved in cell death and inflammation, while activating the PI3K/Akt pathway, which promotes cell survival and growth, 4) RES inhibited oxidative stress damage in RGCs and suppress the activation of MAPKs signaling axis. In this study, we investigated the therapeutic potential of RES in protecting RGCs from cell death induced by glaucoma. Our findings demonstrate that intravitreal administration of RES effectively protected RGCs from high IOP-induced cell death through multiple pathways. These results suggest that RES may have therapeutic potential in the treatment of glaucoma. However, further investigations are required to fully evaluate the efficacy of RES as a treatment for retinal cell death in glaucoma.

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**Conflict of Interest Statement**
The authors declare no conflict of interest.

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