ABSTRACT

**Background:** Glaucoma is a progressive neurodegenerative disease characterized by glaucomatous optic neuropathy, including loss of retinal ganglion cells and axons. Optic neuropathy causes progressive vision loss and is managed by reducing intraocular pressure. However, even when the intraocular pressure has dropped to normal, the damage to the retinal ganglion cells may linger in certain situations. Thus, commence investigations on natural sources such as neuroprotection to prevent the loss of retinal ganglion cells.

**Methods:** This literature review compiles and elaborates on previous studies to support future experimental studies that will be conducted to comprehend the effect of intravitreal Resveratrol as a glaucoma treatment to prevent the loss of retinal ganglion cells.

**Results:** Resveratrol is a naturally occurring phenol and phytoalexin detected in the skins of grapes and berries like raspberries, blueberries, and mulberries. Resveratrol inhibits the production of reactive oxygen species (ROS) and apoptosis as well as lengthens the life span of nerve cells via multiple mechanisms. Resveratrol treatment can reverse the progression of apoptosis, preserve mitochondrial membrane potential by reducing caspase-3, and inhibit cytochrome-C release, enhancing cell survival, as shown in many previous studies. Before the systemic administration of resveratrol in eye disease, it is necessary to consider the presence of the blood-ocular barrier—about 10 out of 35 eyes detected resveratrol in the conjunctiva after oral trans-resveratrol supplementation. Several studies have administered intravitreal injections of resveratrol to increase intraocular resveratrol concentrations.

**Conclusion:** Resveratrol may be utilized as an alternative adjuvant treatment for glaucoma due to its antioxidant and antiapoptosis properties.

**Keywords:** Apoptosis, Bax, Glaucoma, Ischemic-Reperfusion Injury, Resveratrol.

INTRODUCTION

There are an estimated 285 million individuals of all ages all over the world living with some form of visual impairment. Globally, cataracts, age-related macular degeneration, diabetic retinopathy, and glaucoma are the leading causes of blindness and impaired vision. The process is driven by molecular pathways such as oxidative stress, processes associated with inflammation, and excessive angiogenesis. Glaucoma almost always results in irreversible blindness. Even after therapy, as many as twenty percent of those with glaucoma will continue to have visual loss. Both medicinal treatment and surgical procedures for glaucoma are aimed at lowering the pressure inside the eye (intraocular pressure). It is anticipated that reducing the pressure inside the eye may slow down the course of glaucoma. Even when the intraocular pressure is normal, the visual field and optic nerve continue to deteriorate gradually.

Multiple investigations have demonstrated that progressive ganglion cell damage continues to occur even after IOP has returned to normal; hence, alternative or additional therapies are necessary to prevent retinal ganglion cell damage progression. Because of this, research into the effectiveness of naturally occurring compounds as neuroprotective agents was initiated. Resveratrol is one of the neuroprotective drugs that has received the greatest attention from researchers. Resveratrol is a kind of polyphenol that may be found in red wine, grapes, almonds, and chocolate. It is also present in some berry families, such as blueberries,
bilberries, and cranberries. Resveratrol has several therapeutic applications, including anti-aging, anti-cancer, anti-diabetic, neuroprotective, cardioprotective, wound healing, and anti-depressant effects.3-5

The incidence of glaucoma without an increase in IOP and the progression of retinal ganglion cell damage that continues to occur after IOP decreases after administration of IOP-lowering drugs encourages the need to develop research that can provide additional or alternative therapies for damage to retinal ganglion cells.5,6 Based on those mentioned above, this study aims to provide a basis for using resveratrol as a natural agent that can protect against retinal ganglion cell damage, including mechanisms, also previous studies that have been carried out.

METHODS

All authors (AS, EK, WM, MD, MC, IKS) are involved in selecting literature in this review. We conducted literature searching from various databases such as PubMed, EBSCOHost, EMBASE, Cochrane, and Google Scholar, using relevant keywords such as “glaucoma”, “ischemic-reperfusion injury”, “apoptosis”, “resveratrol”, “Bax”, and “Caspase-3”. The search results were screened based on their title and abstracts. We then retrieved the full text of the related articles for further analysis.

ANATOMY AND HISTOLOGY RETINA

The retina is 0.2 mm thin and developed from the neuroectoderm. Vision begins with retinal sensory neurons. From the inside out, the 10 sensory retina layers are (1) the internal limiting membrane; (2) the retinal nerve fiber layer made of ganglion cell axons; (3) the ganglion cell layer made of ganglion cell bodies; (4) the inner plexiform layer; (5) inner nuclear layer; (6) outer plexiform layer; (7) outer nuclear layer; (8) the external limiting membrane; and (9) the photoreceptor layer; and (10) the retinal pigment epithelium (RPE). Bruch’s membrane resides underneath the RPE, separating the retina and RPE from the underlying choroid; if this membrane is defective, choroidal vessels will develop into the subretinal space.6,7

Retinal ganglion cells (RGC), photoreceptors (cone and rod cells), amacrine cells, bipolar cells, and horizontal cells are among the five kinds of neurons found in the retina.7 Long axons of RGC, which combine to form the optic nerve, transmit light and visual signals from the retina to the brain. There are approximately 1.5 million RGC in the human retina. Most of these cells are found in the ganglion cell layer, although some can also be seen in the inner nuclear layer.8

GLAUCOMA

Gluacoma is a progressive neurodegenerative disease characterized by cupping of the optic nerve head, loss of retinal ganglion cells and their axons, and loss of vision. A visual field disturbance that progresses from the periphery to the center. The cause of glaucoma is multifactorial, including genetic and environmental factors. Intraocular pressure (IOP) is the key risk factor for the development of glaucoma. In most glaucoma patients, lowering intraocular pressure will either stop or reduce the visual field loss. However, optic nerve damage can still worsen in certain eyes even when the intraocular pressure is within the normal range. Other risk factors for glaucoma include advanced age, a glaucoma family history, black race, and systemic or topical corticosteroid usage.1,2,9

More than 70 million individuals globally are affected by glaucoma, which accounts for 10% of bilateral blindness, making it the main cause of irreversible blindness. According to WHO data from 2010, it is projected that glaucoma blindness affects up to 3.2 million individuals globally. According to surveys, 10-50% of the population is unaware of the condition of glaucoma. According to the findings of the 2007 Basic Health Research, 0.46% of respondents in Indonesia.1,2,10

Gluacoma pathogenesis constitutes a complex mechanism. The progressive loss of RGC serves as a potential mechanism. RGCs, like central nervous system cells, do not regenerate after cell death. Since the retina has a high metabolic activity, it is vulnerable to eye disorders such as prolonged hyperglycemia and elevated IOP both of which cause oxidative stress. Oxidative stress and reactive oxygen species (ROS) have been associated with the etiology of glaucoma.11-13 Under oxidative stress circumstances, hydroxyl radicals (OH) can develop, particularly reactive to cell membranes and easily enter the cell membrane system. OH will cause DNA fragmentation after transfer to the nucleus, and activation of P53 will trigger cell death.14

The apoptosis mechanism consists of two pathways, the extrinsic and intrinsic pathways, which can interact and influence one another. The extrinsic route, the death receptor pathway, is triggered by death ligands and receptors, such as tumor necrosis factor (TNF). This cell death trigger complex activates the protease Caspase-8, resulting in the cleavage of Caspase-3 and the proteolytic death of injured cells. Hypoxia, radiation, or cellular contaminants activate the intrinsic route, also known as the mitochondriald pathway, which disrupts the integrity of the mitochondrial membrane, activating the pro-apoptotic Bcl-2 family and initiating the apoptotic cascade.15-18

Bax is a molecule that belongs to the Bcl-2 family. The Bcl-2 family is classified into three distinct subfamilies, which are as follows: (1) members of the Bcl-2 family that promote survival (Pro-survival), such as Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and others. This protein is located in the outer membrane of the mitochondria and prevents cytochrome C from being released from the mitochondria; (2) BH3-only proteins (Bad, Bid, Noxa, and Puma); and (3) pro-apoptotic Bcl-2 family proteins (Bax, Bak). The release of cytochrome C is brought about by the activation of Bax, which increases the permeability of the mitochondrial outer membrane.19,20 The apoptosisosome is created when cytochrome C interacts with apoptotic protease activating factor 1, also known as APAF-1, in the cytoplasm. Caspases-9 and caspase-3 are both activated by the apoptosisosome. Caspase-3 activation is ultimately responsible for cell death.15,16,21

Caspase is a cysteine aspartate protease that serves a crucial role in the control
of the process of cell death. Depending on their function, caspases can either be the initiator or effector caspases. The caspase cascade begins with activating the initiator caspase, which then divides and activates the effector caspase. In the end, the caspases begin to break down DNA and cause cell death. Caspase-3 is an effector caspase, like caspases 6 and 7. Both internal and extrinsic apoptotic mechanisms trigger caspase-3. Despite the lack of understanding about caspases 6 and 7, it is known that suppressing caspase-3 inhibits apoptosis. However, suppressing caspases 6 and 7 does not affect the apoptotic process.8,17,20

GLAUCOMA TREATMENT

The primary objective of glaucoma treatment is to reduce the progression of optic nerve damage and maintain patients' quality of life, which necessitates early diagnosis and treatment. IOP reduction is a validated glaucoma treatment for reducing optic nerve damage. A 30% or greater drop in IOP can avoid additional harm. If the damage is more severe, IOP reduction with a larger target can be carried out.22,23

Medication, laser therapy, and surgery are among the treatment options for glaucoma. Medications are usually the first line of treatment. Glaucoma treatment often begins with one kind of eye drop for one eye, which is then reviewed 3 to 6 weeks later to determine efficacy. Medical therapy options for glaucoma include prostaglandin analogs (Latanoprost 0.005%, Bimatoprost 0.01% or 0.03%, Travoprost 0.004%, Tafuriloprost 0.0015%), beta-blockers (Levobunolol or Timolol 0.25%, 0.5%), selective a2-adrenergic agonist (brimonidine 0.1%, 0.15%, or 0.2%), topical carbonic anhydrase inhibitors (CAIs) (Dorzolamide 2% or Brinzolamide 1%), Miotics (pilocarpine), systemic CAIs. Glaucoma surgical therapy lowers IOP by lowering resistance to aqueous humor outflow or reducing aqueous humor production. The reasons for surgical operations include progress or further damage, even though the medical therapy given has been maximized. Currently, available surgical treatments for glaucoma include trabeculectomy, trabeculectomy, goniotomy, glaucoma drainage devices, minimally invasive glaucoma surgeries (MIGS), and cyclodestruction.23,24

NEUROPROTECTION IN GLAUCOMA

IOP-lowering therapy has been shown to reduce RGC damage. Still, there is also a high incidence of glaucoma without increased IOP or normal-tension glaucoma and ongoing disease progression in IOP conditions that IOP-lowering drugs have controlled. As a result, neuroprotective treatment can be utilized in addition to lowering IOP.22,25

Many studies have been performed on the effect of natural ingredients as neuroprotective against glaucoma. Ginkgo biloba is one of the natural substances that has been investigated. Ginkgo biloba is a member of the Ginkgoaceae family. It has been demonstrated that extracts increase the likelihood of retinal ganglion cells surviving oxidative stress. Lycium barbarum, often known as goji berry, is widely used in China as a herbal cure and anti-aging agent. Lycium barbarum supplementation has been demonstrated to preserve retinal ganglion cells, maintain retinal function in hypertension oculars, and decrease ROS generation. Resveratrol, an antioxidant neuroprotective, can inhibit damage in glaucoma and suppress the development of pro-inflammatory and pro-apoptotic TM indicators. Resveratrol's neuroprotective properties have been studied in experimental animal models of glaucoma using oral and systemic Resveratrol supplementation.12,26

RESVERATROL

Resveratrol (3,5,4'-trihidroxy-trans-stilbene) is a polyphenol generated by some plants in response to damage or when the plant is attacked by a pathogen such as bacteria or fungus. Grapes, grape skins, nuts, cocoa, and Vaccinum species berries such as blueberries, raspberries, and mulberries are dietary sources of resveratrol.1,3

The major advantages of resveratrol have been connected to its ability to act as an antioxidant, reduce inflammation, and affect many targets in a range of diseases, including coronary heart disease, cancer, inflammatory sickness, and age-related degenerative disease. Consequently, research into the effects of resveratrol on age-related ocular illnesses such as age-related macular degeneration (AMD), cataract, retinal degeneration, optic neuritis, glaucoma, and retinoblastoma has been conducted. Resveratrol is advantageous because of its capability of stimulating sirtuin 1 (Sirt1) activity.1

The activation of SIRT 1 plays a significant part in the pathway that initiates apoptosis, autophagy, and insulin sensitivity, as well as in a low-calorie diet. Resveratrol is known to increase endothelial nitric oxide synthase (eNOS) activity at a concentration of 0.1-1 μmoles/L in the endothelial cell layer after two minutes of incubation and to increase the activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) via SIRT 1 in human vascular muscle at a concentration of 3 μmoles/L.3

Resveratrol's biological activities include: antioxidative effects caused by the induction of superoxide dismutase, catalase, and glutathione peroxidase-1, which inhibit reactive oxygen species (ROS); Anti-angiogenesis via the expression of leukotriene B4 and matrix metalloproteinase (such as MMP9), causing inhibition of tumor growth, cell migration, invasion, and metastasis; anti-inflammatory by activating SIRT1 reducing DNA damage and increasing IL-6, TNF a.3

RESVERATROL AND GLAUCOMA

Several studies have found that resveratrol has the potential to inhibit the progression of eye diseases such as glaucoma, cataracts, age-related macular degeneration, and diabetic retinopathy through biological actions such as anti-oxidative, anti-apoptotic, anti-tumourigenic, anti-inflammatory, anti-angiogenic, and vasodilator. Resveratrol pre- or post-treatment significantly reduced DNA damage in glaucoma animal models.1,17

A study investigated the effect of resveratrol on trabecular meshwork in mice subjected to prolonged oxidative stress. Resveratrol was administered every three days for 15 days, then incubated under conditions of oxidative stress (40% oxygen) demonstrated that resveratrol
treatment dramatically decreased endogenous ROS levels. The level of intracellular ROS in resveratrol-treated cells was the same as in non-stressed control cells. Resveratrol protects cells from apoptosis following acute oxidative stress.\textsuperscript{15}

Several further studies have found that resveratrol activates SIRT1. SIRT1 is a sirtuin (SIRT), a nicotinamide adenine dinucleotide (NAD\textsuperscript{+})-dependent histone deacetylase known to regulate cell life span. Mammalian sirtuins (SIRT 1-7) are located in various sites, tissues, and target activities. All sirtuins, except for SIRT5, are expressed in the human retina. All normal eye components, including the cornea, lens, iris, ciliary body, and retina, contain SIRT1. This sirtuin has been the subject of the most investigation in their cell nuclei and cytoplasm. SIRT1 has been connected to several disease-related processes, such as cell death, inflammation, and stress response. According to Luo et al., resveratrol-induced enhancement of SIRT1 expression significantly lowered SGR apoptosis, increased p-Akt expression, and decreased Bax expression.\textsuperscript{17} Akt is a protein kinase that participates in cellular development, metabolism, proliferation, and other functions.\textsuperscript{16,27,28} Increased Akt protects against ischemia-reperfusion injury to the retina and central nervous system. According to Singh et al., activating SIRT1 improves the antioxidant response to aging and ROS-mediated mitochondrial dysfunction. SIRT1 function is mediated by redox-related transcription factors such as FOXO3a and p53. SIRT1 modulates SOD2 and CAT to boost the antioxidant response via FOXO3a deacetylase. Resveratrol activation of SIRT1 inhibits H\textsubscript{2}O\textsubscript{2}-induced cell death, lowers cell proliferation, and slows degeneration. SIRT1 also suppresses sirtinol and nicotinamide, which can promote H\textsubscript{2}O\textsubscript{2}-induced cell death. Several studies suggest that SIRT1 is vital in oxidative protection in diverse ways.\textsuperscript{16,27,28}

The significance of mitochondrial dysfunction in the etiology of glaucoma neurodegeneration has been widely researched. A previous study investigated the effect of resveratrol in mitochondrial biogenesis in preventing retinal ganglion cell death.\textsuperscript{16} Apoptosis is induced by serum depletion because mitochondrial function is impaired. Resveratrol therapy can reverse apoptosis, preserve mitochondrial membrane potential, lower caspase-3, and block cytochrome-C release, increasing cell survival. By maintaining mitochondrial integrity, resveratrol may shield retinal ganglion cells from harm from serum deprivation, as indicated by decreased caspase-3 expression.\textsuperscript{3}

In an experimental study with a glaucoma model of ischemia-reperfusion injury, a previous study demonstrated that resveratrol administration before and after induction showed a neuroprotective effect associated with Bax-caspase-3 inhibition reduced inflammation related to retinal gliosis in ischemia-reperfusion injury.\textsuperscript{17} In this study, ischemia-reperfusion injury enhanced Bax protein regulation from the first to the third day, which could be greatly avoided by administering resveratrol on the first day after ischemia-reperfusion injury. Furthermore, on the third day following ischemic reperfusion damage, resveratrol can suppress caspase-3 cleavage. Similar results were found in previous studies that administrered intraperitoneal injections of resveratrol to an animal model of ischemia-reperfusion injury and found that the injection group had decreased expression of the pro-apoptotic proteins Bax and caspase-3.\textsuperscript{3,17,19}

Multiple studies have documented strategies to achieve the neuroprotective and therapeutic benefits of resveratrol in glaucoma by peritoneal injection or oral. Other investigations have found that oral administration of resveratrol has a bioavailability of less than 1%. Before it enters the systemic circulation, free resveratrol is significantly reduced by presystemic metabolism. Furthermore, the blood-ocular barrier must be considered when administering resveratrol systemically to treat eye disease. Resveratrol was only found in the conjunctiva of 10 of 35 eyes following oral trans-resveratrol administration. As a result, Luo et al. performed intravitreal injection of resveratrol at a dosage of 100μM to raise intraocular resveratrol concentration. The blood-ocular barrier is established by the difference in composition of plasma and aqueous humor, which creates barriers to the transmission of substances between plasma and aqueous humor.\textsuperscript{7,18}

The administration of a high dose of (trans-) resveratrol to experimental animal models did not reveal any adverse consequences. However, there are not that many studies done on humans. At dosage concentrations of roughly 2.5 to 5g, short-term research found gastrointestinal side effects ranging from mild to moderately severe. These symptoms included nausea, stomach discomfort, flatulence, and diarrhea.\textsuperscript{3}

**GLAUCOMA ANIMAL MODEL**

Glaucoma is difficult to study directly in humans. The difficulty in getting samples with the same RGC damage is one of the factors. Glaucoma experimental animal models are essential for understanding the disease’s pathophysiology and developing the most recent treatments to prevent blindness. Like other animal models, glaucoma models must be simple to produce, efficient, and demonstrate pathophysiological and therapeutic responses similar to those found in humans. Because IOP is known to have a role in the etiology of glaucoma, animal models that induce RGC degeneration through elevated IOP are commonly employed.\textsuperscript{7,8}

The majority of glaucoma research has been conducted on mice. Several characteristics are comparable between the eyes of rats and humans, including the architecture of the drainage structure of the anterior chamber and the physiology of the aqueous humor dynamics, which may be adjusted experimentally to elevate IOP. This animal model enables testing of intraocular pressure-lowering and neuroprotective medicines in various combinations and dosages before further larger animal research and human clinical trials. However, the rat eyeball’s tiny size restricts several treatments and manipulations and makes them technically more complex than those of bigger species.\textsuperscript{7,9}

**ISCHEMIC-REPERFUSION INJURY MODEL**

Over the years, animal models of induced glaucoma have been produced. This model
can explain the disease's development, pathophysiological course, and treatment methods. Ischemic reperfusion injury is frequently employed as a glaucoma research model. Ischemic reperfusion injury lowers retinal blood flow, creating retinal hypersensitivity to oxygen and other nutrients and producing further oxidative and inflammatory damage to retinal ganglion cells when circulation is restored (reperfusion). Glaucoma may be caused by increasing IOP and limiting aqueous humor outflow by injecting microbeads, hydroxypropyl methylcellulose, and hyaluronic acid into the anterior chamber. Injection of hypertonic saline into the episcleral vein and cauterization or laser photocoagulation of the episcleral or limbal veins would result in scarring of the trabecular meshwork, an increase in resistance to aqueous humor drainage and then an increase in IOP. Compared to alternative ischemia-reperfusion injury approaches, such as retinal artery ligation, ischemia-reperfusion damage induced with higher IOP has anatomical specificity and simplicity of application, making it valuable in pathogenesis studies and treatment.2,6,30,31

The limitation of this literature review is that it only covers the resveratrol effect in the apoptotic pathway for the loss of retinal ganglion cells; other possible mechanisms are not included. It is known that death may occur in retinal ganglion cells by various mechanisms. This allows for additional research or reviews that could investigate the resveratrol effect in other pathways and mechanisms that underlie the loss of retinal ganglion cells.

CONCLUSION
Resveratrol may be utilized as an alternative adjuvant treatment for glaucoma due to its antioxidant and antiapoptosis properties. Thus, larger-scale studies can be performed on larger animals and in human clinical trials.

CONFLICT OF INTEREST
The author reports no conflicts of interest in this work.

ETHICAL CLEARANCE
Not applicable as this is a review article.

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AUTHOR CONTRIBUTION
All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

REFERENCES


