CHAPTER ONE

Effects of *Lycium barbarum* on the Visual System

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Abstract

Lycium barbarum (wolfberry, gogi berry, *gouqizi*, 枸杞) is one of the most widely used Chinese herbal medicines (CHMs) and is also one of the most scientifically studied. Indeed, the polysaccharide component of this berry (LBP) has been shown to have antioxidant, antiinflammatory, antiexcitotoxic, and antiapoptotic properties. These

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properties make it a particularly useful treatment option for the ocular environment. Although there are a handful of studies investigating the use of LBP to treat diseases affecting the lens, the vast majority of the published literature investigating LBP in the visual system focus on the retina. In this chapter, we have described what is currently understood concerning the effects of LBP treatment on various retinal diseases, including glaucoma, ischemia/reperfusion, age-related macular degeneration, retinitis pigmentosa, and diabetic retinopathy. We then describe the functions attributed to LBP using other cellular contexts to elucidate the full mechanisms this CHM utilizes in the retina. By making connections between what is known about the function of LBP in a variety of tissues and its function as a therapy for retinal degenerative diseases, we hope to further emphasize the continued use of this CHM in clinical medicine in addition to providing a platform for additional study.

1. INTRODUCTION

Treatment with Chinese herbal medicines (CHMs) is a common aspect of the healthcare system in many Asian countries and traditionally focuses on the concept of balancing a person's yin and yang. When applied to the physiological functions and pathological changes in the human body, this theory utilizes a holistic approach, whereby various herbal supplements are administered to synergistically treat a disease. This model is in contrast to that of single synthetic drugs, which are often used in Western cultures. In fact, as the study and use of CHMs becomes increasingly accepted, more herbal treatments are being used, alone or in combination with classical drugs, to treat diseases worldwide. This has also lead to further scientific analysis, discovery, and quality control of various CHMs.

1.1 *Lycium barbarum*

Lycium barbarum, a reddish-orange berry (Fig. 1) also known as wolfberry, gogi berry, or *gouqizi* (枸杞) in Chinese, has been used as a preventative and curative agent in various forms for thousands of years (Wang et al., 2015). In fact, *L. barbarum* has been consumed as an herbal supplement in a number of forms, from tea to concentrated capsule, and has been linked to enhanced protection and nourishment of the kidney, liver, and eye (Junlin & Aicheng, 2002). It is also currently one of the most widely scientifically studied CHMs. Various active compounds have been isolated from *L. barbarum*, including various polyphenols/flavonoids and carotenoids (reviewed in Wang et al., 2015). Indeed, Zhou et al. (2016) recently identified 15 new compounds that appear to be dicaffeoylspermidine derivatives

Fig. 1 Representative images showing *Lycium barbarum* fruit in its natural (*left panel*) and dried (*right panel*) forms.

that also make up a significant portion of the active constituents in *L. barbarum*. However, the most well-studied functional moiety involved in the observed *L. barbarum*-mediated health benefits appears to be the polysaccharide (LBP) component. This group of water-soluble glycoconjugates includes rhamnose, xylose, glucose, mannose, arabinose, and galactose (Benlloch et al., 2015; Liu et al., 2012).

The health benefits of LBPs have been classically linked to their antioxidative properties (Cheng & Kong, 2011; Cui et al., 2011; He, Yang, Jiao, Tian, & Zhao, 2012; Li, 2007; Shan, Zhou, Ma, & Chai, 2011; Xiao et al., 2012; Zhang, Chen, Zhao, & Xi, 2016; Zhou et al., 2016). LBPs have also been demonstrated to influence proliferation, cell cycle arrest, and apoptosis during carcinogenesis (Jin, Huang, Zhao, & Shang, 2013; Mao et al., 2011; Zhang et al., 2005). Other protective mechanisms related to *L. barbarum* include antiexcitotoxicity (Ho et al., 2009), antiinflammation (Chen, Soo, Srinivasan, Tan, & Chan, 2009; Xiao et al., 2012), and antiapoptosis (Ho et al., 2010; Li et al., 2011; Song, Roufogalis, & Huang, 2012). This CHM was also previously shown to play a neuroprotective role and help protect cells against amyloid-β neurotoxicity (Yu et al., 2007, 2005) and enhance cognitive function (Zhang, Du, et al., 2013; Zhou et al., 2016) in Alzheimer's disease-related neurodegeneration.

While the function of LBPs appears to be multifaceted, the underlying mechanisms involved may differ to a degree depending on physiological context and the specific cell type affected. In the current review, we have focused on the mechanism of LBPs in the visual system in addition to detailing their therapeutic use to treat various vision-related diseases.

1.2 The Visual System

Notably, the visual system is exceedingly sensitive to change, including increased oxidative stress (Kruk, Kubasik-Kladna, & Aboul-Enein, 2015), altered blood flow (Flammer et al., 2013), inflammation (Perez, Saeed, Tan, Urbieta, & Cruz-Guilloty, 2013), etc., implying that the mechanisms of LBPs in this cellular context may be similar to those demonstrated systemically or in other tissues. The eye is derived from three types of tissue during embryogenesis: the surface ectoderm, which generates the lens; the mesoderm, from which the cornea and sclera are produced; and the neural ectoderm, which gives rise to the retina and retinal pigmented epithelium (RPE) (for an excellent review of general ocular development, please see Heavner & Pevny, 2012). During development, signals between the surface ectoderm and the optic vesicle, an outgrowth from the developing central nervous system that will later become the optic nerve, initiate lens placode formation, invagination, and creation of the bilayered optic cup. These tissues will then differentiate into a number of specialized cell types that are essential for vision. Although some recent work has been conducted concerning the therapeutic effects of LBPs on both the formation of senile cataracts in the human lens (Kee et al., 2013 (clinicaltrials.gov identifier: NCT01142960)) and oxidatively damaged lens epithelial cells in culture (Qi et al., 2014), the majority of published literature concerning LBPs in the visual system focus on the retina.

The cells in the outer and inner layers of the optic cup formed during development will specifically differentiate into the RPE and neural retina, respectively (Fig. 2) (reviewed in Heavner & Pevny, 2012). The latter includes various cells types and is organized into three layers: the outer nuclear layer, composed of the rod and cone photoreceptors; the inner nuclear layer, containing the amacrine, horizontal, and bipolar cells as well as Müller glia; and the ganglion cell layer, which is composed of the retinal ganglion cells (RGCs). During vision, the dense layer of posteriorly located photoreceptors converts the incoming light into electrochemical signals involving a phototransduction cascade in the outer segment of the cell and downstream changes in cation channels (reviewed in Sung & Chuang, 2010). This hyperpolarization of the light-stimulated photoreceptor then causes altered neurotransmitter release that initiates an action potential-mediated chain reaction through the cells in the inner nuclear layer to the RGCs, which then transmit the signal to the brain via the optic nerve (reviewed in Baccus, 2007). Alternatively, the outer layer-derived RPE is a monolayer of cells that forms the blood–retinal barrier (BRB) and supports the photoreceptor cells via the phagocytosis of the outer

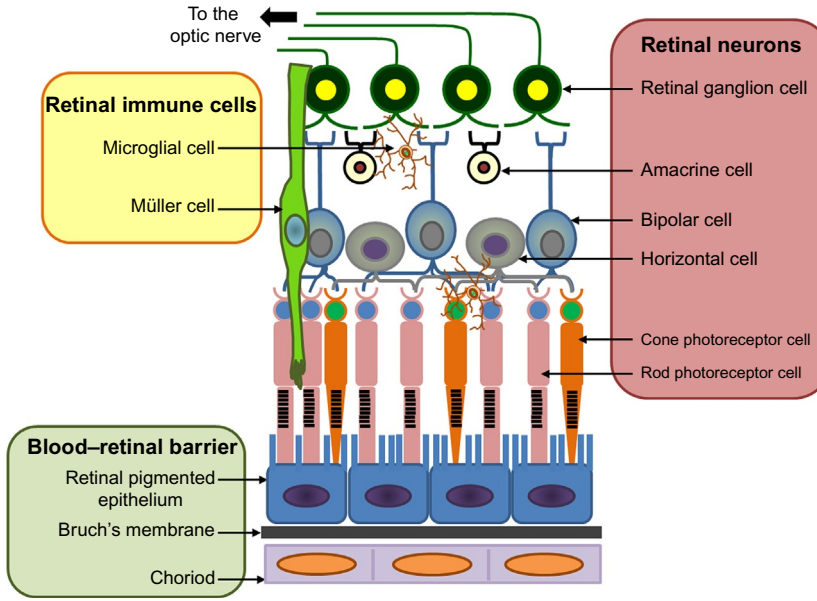

Fig. 2 Diagram highlighting the organization and basic functions of various retinal cell types.

segments and secretion of various trophic factors (Heavner & Pevny, 2012). Finally, the Müller and retinal microglial cells are the resident immune cells in this tissue and eliminate foreign substances as well as dead/dying cells.

Under normal conditions, the retina internally balances the levels of reactive oxygen species (ROS) and inflammatory molecules, while limiting aberrant apoptosis and interference from the surrounding vasculature in order to process light signals properly for vision. Unfortunately, in a diseased state, these mechanisms may be inactive or dysfunctional leading to further problems. Thus, while some drugs or herbal supplements, including LBPs, may not have an effect on the visual system under normal conditions, they may have significant therapeutic benefits on retinal function under stress or after trauma-/diseased-induced retinal cell death or dysfunction.

2. EFFECT OF *L. BARBARUM* POLYSACCHARIDE TREATMENT IN ANIMAL MODELS OF RETINAL DISEASE

The loss of retinal neurons, including the RGCs and photoreceptors, either directly or as a secondary effect, can lead to significant deficits in visual acuity that have been associated with a number of retinal diseases, including

glaucoma, ischemia/reperfusion (I/R), age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy (DR). Although a number of clinical/preclinical studies have been performed in healthy humans (Amagase & Nance, 2008; Amagase, Sun, & Borek, 2009; Amagase, Sun, & Nance, 2009; Cheng, Chung, Szeto, & Benzie, 2005; Paul Hsu, Nance, & Amagase, 2012), few have been conducted on patients with retinal disorders. In fact, only three studies appear to focus on *L. barbarum* as a therapy for retinal diseases in humans, one involving RP patients (Chan et al., ongoing), one involving patients with normal tension glaucoma (Lai et al., ongoing), and another on development of AMD in the elderly (Bucheli et al., 2011). The bulk of the work in the current literature makes use of various animal models, which has greatly influenced our understanding of how LBP functions as a therapeutic supplement. Here, we outline these particular retinal diseases, focusing on their global prevalence and the general effects of LBP in the various animal models of the disease.

2.1 Glaucoma

Glaucoma is a retinal degenerative disease in which RGCs forming the optic nerve are selectively lost. This loss has been shown to occur in two phases: a primary phase that occurs due to direct damage to the retinal neurons and a secondary phase that is caused by the release of toxic factors during the primary wave of cell death (Levkovitch-Verbin et al., 2001, 2003; Yoles & Schwartz, 1998). Decreased thickness of the nerve fiber layer and increased intraocular pressure (IOP) are often associated with the progressive loss of vision experienced by glaucoma patients (Calkins, 2012). Notably, glaucoma is the most common cause of irreversible blindness, second only to cataract in terms of ocular diseases globally (Pascolini & Mariotti, 2012). Furthermore, glaucoma is expected to affect approximately 111.8 million people between 40 and 80 years of age by 2040 (Tham et al., 2014). Thus, continued research investigating this disease is essential.

Glaucoma etiology has two primary aspects: progressive disruption of RGC axonal transport and retinal ischemia. Several animal models have been used to study glaucoma-related disruption of the axons, including laser photocoagulation of the limbal and episcleral veins, complete and partial optic nerve transection, and optic nerve crush. Importantly, the detrimental effects observed following photocoagulation of the episcleral and limbal veins in Sprague-Dawley rats, a model of chronic ocular hypertension, were

significantly prevented by feeding the rats LBPs for 1 week before laser treatment (Chan et al., 2007). Although the IOP was not affected in these animals, the level of RGC death was significantly decreased compared to control animals, an effect that may be modulated by various trophic factors (Chiu et al., 2010; Mi, Chiu, et al., 2012). These changes also appear to involve microglial cell function as treatment with factors that stimulate or inhibit activation altered the impact of LBP (Chiu et al., 2009). Notably, the use of this technique to model glaucoma and the effects of various drugs are continually being evaluated and advanced. In vivo manganese-enhanced magnetic resonance imaging, for example, was employed by Chan, Fu, Hui, So, and Wu (2008) to better understand the mechanisms underlying this disease. However, one aspect of glaucoma this model, as well as the complete optic nerve transection model, do not address fully is the differentiation between primary and secondary RGC loss. Fortunately, the recent development of partial optic nerve transection has allowed researchers to compare the effects of LBP treatment on these stages of progressive neuronal degradation. In fact, Li et al. (2013) administered LBPs for 1 week prior to either complete (highlighting primary degradation) or partial (highlighting secondary degradation) surgery and investigated the effects at various time points thereafter. In doing so, it appears that LBP treatment does not delay primary RGC degeneration in either model, but does decrease oxidative stress and increase the secretion of trophic factors (e.g., insulin-like growth factor 1 (IGF-1)), resulting in lower levels of secondary neuronal cell death. This decrease in secondary cell death after partial transection also appears to be related to an LBP-mediated decrease in microglial/macrophage activation in this model (Li et al., 2015). Importantly, these cellular changes modulated by LBP pretreatment were demonstrated to preserve retinal function, as highlighted by multifocal electroretinographic (ERG) analysis (Chu, Li, Chin, So, & Chan, 2013). While the aforementioned models focus on the disruption of axonal transport that occurs during glaucoma or acute optic nerve injury, various models have also been used to specifically study the ischemia-induced damaged that occurs during a number of retinal degenerative diseases.

2.2 Ischemia/Reperfusion Injury

Retinal ischemia occurs when the blood supply to the retina is inadequate, resulting in altered metabolic function due to the lack of oxygen. These changes ultimately lead to irreversible cell death. In fact, ischemia has been

shown to play a role in neuronal cell death in a number of retinal disorders, including glaucoma, DR, ischemic optic neuropathies, etc. The most widely used method to study retinal ischemia is to induce acute ocular hypertension via the anterior chamber using an elevated hydrostatic reservoir. Doing so results in a jump in the IOP of the insulted eye to a level that is higher than that of the animal's systolic blood pressure, thus limiting the blood supply to the retina. Treating a mouse model of acute ocular hypertension with LBP for 7 days prior to increasing IOP (as well as postinsult for 4 or 7 days) resulted in a decreased loss of RGCs as well as less thinning of the nerve fiber layer, decreased immunoglobulin leakage, and increased blood vessel density compared to the control treated retinas (Mi, Feng, et al., 2012). A similar model using rats also showed an LBP-mediated delay in RGC death, with more than 50% of the cells remaining viable 7 days after the insult (He et al., 2014).

Animal models of middle cerebral artery occlusion, often used to study focal cerebral ischemia in the brain (i.e., ischemic stroke), have also been used to evaluate the effects of LBP in both the brain (Wang et al., 2013; Wang, Li, et al., 2014; Yang et al., 2012) and retina (Li et al., 2011). In this study, pretreatment with LBP for 1 week prior to ischemia was shown to protect the retina from neuronal cell death, glial cell activation, oxidative stress, retinal swelling, and disruption of the BRB at 48 h after reperfusion.

2.3 Age-Related Macular Degeneration

AMD, an aging-associated disease involving the progressive loss of vision, can be classified as wet or dry, with the latter being more common (approximately 80%–85% of cases). While the wet form involves the pathological growth of blood vessels into the subretinal space and physical disruption of retinal structure, the dry form is initiated by dysfunction in Bruch's membrane leading to the degeneration of the RPE and, subsequently, the loss of photoreceptors in the macular region of the retina (reviewed in Ambati, Ambati, Yoo, Ianchulev, & Adamis, 2003; Rattner & Nathans, 2006). Dry AMD has also been associated with the presence of amyloid-β deposits known as drusen. In regards to global prevalence, an estimated 5% of blindness is caused by AMD (WHO, 2012). However, with the proportion of elderly continually increasing, it is likely that this disease may become more common.

AMD appears to involve both genetic and environmental risk factors, making it a difficult disease to mirror in animal models. In order to study

the end-point morphology of human AMD, light-induced retinal damage, which causes similar defects as those observed during advanced atrophic AMD, has been used in various rodent models (Marc et al., 2008; Organisciak & Vaughan, 2010; Zeiss, 2010). Several genetic models have also been used to mirror the symptoms associated with AMD (reviewed in Pennesi, Neuringer, & Courtney, 2012). However, while several human studies have evaluated the effects of LBP in the prevention of AMD symptoms in the elderly (Bucheli et al., 2011; Cheng et al., 2005), none of the aforementioned animal models have been used to directly test the effects of LBP treatment. Interestingly, the link between amyloid-β deposition in the brain and AMD retinas may prove to be indicative of the effects of LBPs during disease pathogenesis as cell cultures of primary cortical neurons pretreated with LBPs appear to be protected from amyloid-β-induced stress and cell death (Ho et al., 2007; Yu et al., 2007, 2005). Amyloid-β expression was also downregulated following LBP treatment in a mouse model of acute ocular hypertension (Mi, Feng, et al., 2012), a phenomena that may be mimicked during AMD.

2.4 Retinitis Pigmentosa

In RP, photoreceptors are lost due to inherited genetic mutations resulting in the loss of peripheral vision (initial loss of rod photoreceptors) followed by central vision (subsequent loss of cone photoreceptors) (Hartong, Berson, & Dryja, 2006). The disease is genetically and phenotypically heterogeneous and is estimated to affect 1 in 3000 to 1 in 7000 people worldwide (Ferrari et al., 2011). A number of animal models have been used to study RP, including P347L transgenic rabbits (which have rod-dominated, progressive photoreceptor degeneration with regional variations in the pattern of photoreceptor loss), RCS rats (which have a Merkd mutation related to RP pathogenesis), and mer^{kd} mice (which share the same mutation as the RCS rats) as well as rd1 and rd10 mice (which have mutations in the phosphodiesterase 6b gene), etc.

Similar to the effects observed for LBP treatment in other retinal diseases, treating rd1 mice with LBP extract appears to decrease photoreceptor cell degeneration via reduced oxidative stress (Miranda et al., 2010). In addition to its capacity as an antioxidant, LBP treatment in rd10 mice also demonstrated antiinflammatory and neuroprotective functions (Wang, Xiao, et al., 2014). Recent work by Ni, Wei, Yin, Liu, and Liu (2013) using RCS rats also suggests that treatment with *L. barbarum* reduces apoptosis

of photoreceptors in this model of RP; however, the authors hypothesize that this function is largely due to the carotenoid components of this CHM and additional work is necessary to evaluate these effects and mechanism.

2.5 Diabetic Retinopathy

The elevated glucose levels, or hyperglycemia, associated with systemic diabetes have been related to a number of complications/comorbidities. DR involves changes in the microvasculature of the retina leading to increased permeability of the BRB, ischemia, neovascularization, macular edema, inflammation, oxidative stress, and ultimately blindness. In 2010, DR accounted for 2.6% of blindness globally and appears to be progressively increasing compared to previous years (Leasher et al., 2016).

In db/db mice, a common animal model of DR, *L. barbarum* appears to attenuate hypoxia and mitochondrial stress (Tang et al., 2011; Yu et al., 2013), functions which have been attributed to the carotenoids in this medicinal herb, but need to be evaluated for the polysaccharide component. A streptozotocin-induced rat model of diabetes has also been used to study the effects of *L. barbarum* (Hu, Lee, Colitz, Chang, & Lin, 2012). In this study, retinal function was assessed using ERG and histopathological studies, and it appears that *L. barbarum* protected the function and viability of the retinal cells when administered to the rats after diabetes was confirmed.

3. UNDERLYING MECHANISMS OF *L. BARBARUM* POLYSACCHARIDES IN THE RETINA

The retinal diseases described in the previous sections, while different in genetics, phenotype, and prevalence, all share some common variables, namely, changes in the local retinal neurons, inflammation, and altered vascular function in addition to the overall increase in oxidative stress in the eye/retina. While the mechanistic effects of LBP treatment on these pathogenic processes could be dependent on the unique retinal microenvironment, it is also likely that some signaling pathway changes involved may mimic those employed in other cellular contexts. In fact, these functional pathways may overlap in a number of ways. In this section, we discuss the various mechanistic effects of LBP during retinal disease (Fig. 3), linking what has observed in this tissue to what is known in other parts of the body.

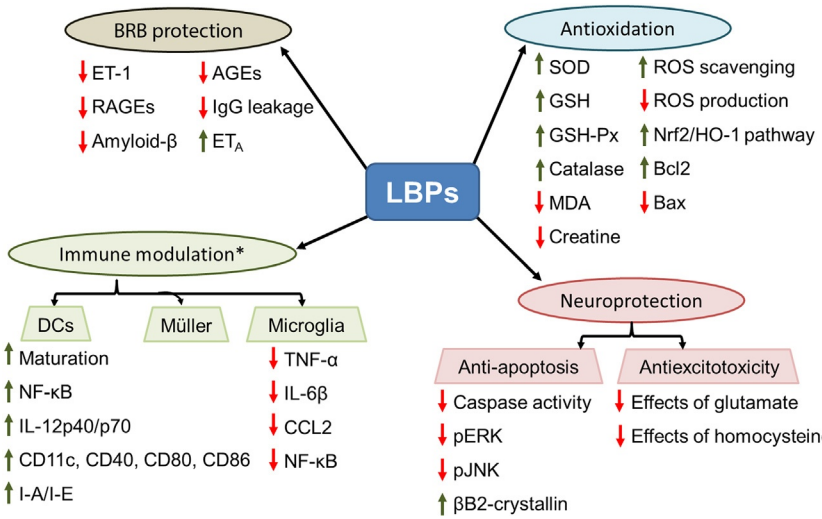

Fig. 3 Mechanisms involved in *Lycium barbarum* polysaccharide (LBP) function in the diseased retina. During retinal disease, treatment with LBPs appears to affect four primary processes: antioxidation, neuroprotection, blood–retinal barrier (BRB) protection, and immune modulation. With the exception of immune system activation, the mechanisms underlying these LBP-mediated effects appear to be similar to those in nonocular tissues. The discrepancies in immune activation are likely related to the immune privileged nature of the retinal environment. As additional work is required to determine the retina-specific immune pathways affected, this process has been marked with an *asterisk* (*). *Abbreviations*: *AGEs*, advanced glycation endproducts; *Bax*, Bcl2-associated X protein; *Bcl2*, B-cell lymphoma 2; *CCL2*, C–C motif chemokine ligand 2; *CD*, cluster of differentiation; *ET-1*, endothelin 1; ET_A, endothelin receptor A; *GSH*, glutathione; *GSH-Px*, glutathione peroxidase; *I-A/I-E*, major histocompatibility complex class II I-A/I-E subregions; *IgG*, immunoglobulin; *IL*, interleukin; *MDA*, malondialdehyde; *NF-κB*, nuclear factor kappa B; *Nrf2/HO-1*, nuclear factor erythroid 1-related factor/heme oxygenase-1; *pERK*, phosphorylated extracellular signal-related kinase; *pJNK*, phosphorylated c-Jun N-terminal kinase; *RAGEs*, receptor for advanced glycation endproducts; *ROS*, reactive oxygen species; *SOD*, superoxide dismutase; *TNF-α*, tumor necrosis factor alpha.

3.1 Antioxidant Properties

Functionally, the beneficial mechanism of LBPs can be related to a number of potential functions that have been directly or indirectly linked to their antioxidant potential in various tissues (Cheng & Kong, 2011; Li, 2007; Li et al., 2011; Shan et al., 2011; Xiao et al., 2012; Zhang, Chen, et al., 2016), whereby the polysaccharides not only donate a hydrogen to various ROS (Jin, Lu, Huang, & Wang, 2011), but also activate enzymes involved in antioxidative activities. Indeed, in a 30 day randomized, double-blind, placebo-controlled clinical study, LBP treatment was shown to significantly

increase the serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), while decreasing serum malondialdehyde (MDA) in healthy Chinese adults, supporting its role as a therapeutic antioxidant (Amagase & Nance, 2008).

Notably, the formation of ROS during normal mitochondrial metabolism is expected in most tissues, including the retina; however, under normal conditions, elimination and repair mechanisms are active (Puertas et al., 1993). When the body is under stress or affected by disease, on the other hand, these mechanisms may be dysfunctional or inactive. High fat diets, exhaustive exercise, and subhealth conditions, for example, have all been shown to activate oxidative stress pathways. Notably, treating mice on a high fat diet with LBPs has been shown to increase the activity of SOD, GSH-Px, and catalase (Wu et al., 2010). Similarly, increased SOD and GSH-Px activity along with decreased MDA and creatine kinase activity was observed in the skeletal muscle of LBP-treated rats after intense exercise (Niu, Wu, Yu, & Wang, 2008; Shan et al., 2011). In addition to altering enzyme activity, LBPs also appear to inhibit ROS-producing reactions, including ultraviolet light-induced peroxidation and free radical generation by cytochrome *c* (Wang et al., 2002), that also play a significant role in oxidative stress in various tissues. Similar changes in peroxidation and decreased antioxidant activity, which are restored by LBP treatment, have been observed during normal aging processes (Li, Ma, & Liu, 2007) as well as chronic fatigue conditions (Zhao, Cai, Shao, & Ma, 2015).

In the retina, glutathione (GSH) and other GSH-related enzymes are the primary defenses against oxidative stress (Ganea & Harding, 2006). While the entire retina can be affected by disease/trauma-induced oxidative stress, the photoreceptors appear to be one of the primary targets along with other retinal neurons and the RPE (Beatty, Koh, Phil, Henson, & Boulton, 2000; Tanito et al., 2002). In rd1 mice, a model of RP photoreceptor cell death, treatment with a mixture of antioxidants that included LBPs was shown to increase GSH-Px and GSH concentration and decrease cell death (Miranda et al., 2010). However, these effects were not observed when the antioxidants were administered individually, indicating a synergistic effect. In RGCs, treatment with LBPs alone for 1 week prior to partial optic nerve transection significantly increased the expression of SOD 1 day after surgery (Li et al., 2013). In fact, the redox status appears to be maintained in RGCs through the activation of the nuclear factor erythroid 2-related factor (Nrf2)/heme oxygenase-1 (HO-1) antioxidant pathway (He et al., 2014). Furthermore, the effect of LBPs on stressed RPE cells in culture appears

to involve the inhibition of ROS-induced downregulation of B-cell lymphoma 2 (Bcl2) and upregulation of Bcl2-associated X protein (Bax), indicating that the polysaccharides may function to not only decrease the levels of ROS via free radical scavenging and increased activity of antioxidative enzymes, but also by altering downstream gene function to prevent ROS-induced apoptosis (Liu et al., 2015).

3.2 Modulation of the Blood Vessels/Vascularization

The survival of neurons and other cells in a tissue microenvironment is, at least in part, dependent on the stability of the surrounding vasculature. The interaction between the retina and the vascular system is similar to that of the brain, whereby a cellular blood-barrier exists, known as the BRB and the blood–brain barrier (BBB), respectively. The BRB has two layers: an outer barrier formed by the RPE, Bruch's membrane, and the choriocapillaris; and an inner barrier formed by tight junctions between retinal endothelial cells, which functions similarly to the BBB. The presence of two barriers in the BRB limits the effect of vascularization on vision, allowing a clear path for light to travel to the photoreceptors. The primary function of these barriers is to regulate the transport of various molecules from the blood to the retina, functions that were recently outlined by Rizzolo (2014) and others (Steuer et al., 2005). Furthermore, disruption of the integrity of these barriers can lead to issues in the brain and retina, including leakage of serum inflammatory factors (e.g., immunoglobulin) (Cheung et al., 2005; Farrall & Wardlaw, 2009; Mi, Feng, et al., 2012), edema (Kimelberg, 1995), and neovascularization (Mi, Feng, et al., 2012).

In both the brain and retina, there is a strong relationship between the microvasculature, neurons, and associated glial cells (Yu et al., 2010). The cross talk between the astrocytes and vascular cells, for example, plays a role in maintaining barrier function and integrity (Zhang & Stone, 1997), whereby changes in blood flow/pressure to the tissue are modulated by altered expression of endothelin-1 (ET-1) and its associated receptors, ET_A (which is expressed on vascular smooth muscle cells and is known to mediate blood vessel contraction) and ET_B (which has been shown to be expressed in the endothelium and astrocytes and dilates the blood vessels) (Masaki, Vane, & Vanhoutte, 1994; Narayan, Prasanna, Krishnamoorthy, Zhang, & Yorio, 2003). During ischemic insult and disruption of the blood-barrier, the concentration of ET-1 increases along with that of ET_B (Mi, Chiu, et al., 2012). Moreover, disruption of the BRB during

ocular hypertension has been associated with overexpression of glial fibrillary acidic protein (GFAP) and aquaporin-4 (AQP4) (Kaur et al., 2007), as well as increased expression of receptor for advanced glycation endproducts (RAGEs), amyloid-β, and advanced glycation endproducts (AGEs) during ischemia (Mi, Feng, et al., 2012). Notably, pretreatment with LBP for 1 week prior to disrupting the BRB ameliorated these changes and prevented BRB disruption (Li et al., 2011). In fact, expression of ET-1, RAGEs, amyloid-β, and AGEs along with immunoglobulin leakage were all decreased during ocular hypertension after LBP treatment, while the expression of the ET_A receptor was increased (Mi, Chiu, et al., 2012; Mi, Feng, et al., 2012). Thus, it appears that one of the primary mechanisms underlying the ocular health benefits of LBP is related to its ability to protect the structure and function of the retinal vasculature and BRB while also maintaining retinal homeostasis. This mechanism can also be related to LBP's neuroprotective qualities as retinal neuron viability and function is preserved.

3.3 Neuroprotection

In addition to balancing retinal homeostasis and limiting the effects of oxidative stress, apoptosis, and inflammation, LBPs have also been shown to have antiexcitotoxicity properties that directly protect neurons. Excitotoxicity occurs when receptors in the neurons are over activated by a surplus of extracellular neurotransmitters, such as glutamate, leading to the influx of ions into the cell and prolonged, excessive depolarization. These neurotoxic changes can in turn flood the tissue with more neurotransmitters, exacerbating the levels of cellular damage and death. Neuroprotection ultimately means saving the neuronal cells from apoptosis and preserving synapse function.

Glutamate excitotoxicity in particular has been identified as a possible pathogenic mechanism during various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease (Arundine & Tymianski, 2004; Coyle & Puttfarcken, 1993; Ho et al., 2009; Won, Kim, & Gwag, 2002). Glutamate is also the primary neurotransmitter found in mammalian brain and retinal neurons. Thus, increased levels in these tissues often lead to over excitation. In the brain, LBP treatment appears to significantly reduce neuronal cell death caused by excess glutamate in a manner similar to that observed for conventional drugs such as *N*-methyl-D-aspartate receptor antagonists (Ho et al., 2009). Interestingly, ischemia, oxidative stress, inflammation, and accumulation of other

neurotoxic amino acids and substances can also result in downstream glutamate excitotoxicity. For example, amyloid-β and homocysteine, both of which have been linked to neuronal damage in various diseases, have been shown to induce downstream glutamate excitotoxicity (Ho, Ortiz, Rogers, & Shea, 2002). Treatment with LBPs appears to reduce the levels of amyloid-β (Ho et al., 2007) and the effects of homocysteine in primary cortical neurons (Ho et al., 2010), while also reducing the downstream elevation in proapoptotic phosphorylated extracellular signal-related kinase (pERK) and c-Jun N-terminal kinase (pJNK) (Li et al., 2013) along with caspase-mediated apoptosis (Ho et al., 2007). In terms of neuronal function, Zhang et al. (2012) showed that LBP treatment prevented corticosterone-induced changes in the expression of postsynaptic density protein 95, an important protein in synapse maintenance, in the hippocampus.

In the retina, LBP treatment has been shown to decrease the phosphorylation of both JNK3 and c-Jun after partial optic nerve transection (Li et al., 2013). Furthermore, Chiu et al. (2010) previously identified multiple changes in the protein expression of various crystallins in LBP fed rats following induced ocular hypertension. While this family of proteins is well known for their structural role in the ocular lens, in other tissues they often act as molecular chaperones (please see Slingsby, Wistow, & Clark, 2013 and Thanos et al., 2014 for a review of crystallin function in the lens and other ocular tissues). It appears that LBP-mediated neuroprotection may, at least in part, function via the upregulation of these chaperones, specifically βB2-crystallin, during retinal stress (Chiu et al., 2010). Other studies investigating other retinal insults, including oxidative stress, ischemia, and inflammation, have also indicated decreases in neuronal cell death and restoration of function following LBP treatment.

3.4 Immunomodulation

LBPs have been show to induce a variety of changes in immune function, targeting dendritic cells, T- and B-lymphocytes, macrophage, and natural killer (NK) cells (for a review of these various target cell types, please see Zhang, Zhou, & Zhang, 2015). The eye is an immune privileged tissue, meaning that many of the cell types responsible for the cellular and humoral immune responses in the rest of the body are unable to participate in ocular immunity. Indeed, many of the classical cell types and signaling pathways involved in the inflammatory immune response to foreign antigens are suppressed or completely inhibited in this tissue. This is in part a consequence of the BRB, which

acts as a physical barrier to a number of cell types and immune molecules. RPE cells have also been demonstrated to suppress T-cell activation and induce T regulatory cell production via surface-bound and secreted factors, including programmed death 1 (PD-1) (Sugita et al., 2009; Usui et al., 2008), FasL (Kaplan, Leibole, Tezel, & Ferguson, 1999), galectins, transforming growth factor β (TGFβ) (Sugita et al., 2008; Tanihara, Yoshida, Matsumoto, & Yoshimura, 1993), pigment epithelial-derived factor (PEDF) (Zamiri, Masli, Streilein, & Taylor, 2006), thrombospondin (Zamiri, Masli, Kitaichi, Taylor, & Streilein, 2005), and others (Ishida, Panjwani, Cao, & Streilein, 2003; Sugita, 2009). Furthermore, the RPE also appears to activate local macrophage in addition to suppressing inflammatory activity (Lau & Taylor, 2009).

The task of protecting the retina from foreign substances largely falls to the retinal Müller and microglial cells, although a small population of dendritic cells has been found and may respond to injured retinal neurons (Heuss, Lehmann, Norbury, McPherson, & Gregerson, 2012; Lehmann, Heuss, McPherson, Roehrich, & Gregerson, 2010; McPherson, Heuss, Pierson, & Gregerson, 2014; Xu, Dawson, Forrester, & Liversidge, 2007). These cells are found in all of the layers of the neural retina, with their morphology being largely associated with their function (Jonas et al., 2012; Saijo & Glass, 2011). In fact, a recent study using a mathematical modeling program further quantified retinal microglial cell morphology and orientation after optic nerve transection, indicating that changes in morphology are essential for function along with altered gene expression and cytokine secretion (Yuan, Liang, Peng, Lin, & So, 2015; Zhang, Peng, et al., 2016).

Recent work suggests that LBP treatment promotes maturation and antigen presentation in dendritic cells, specifically those derived from bone marrow (BMDCs), and increases the levels of interleukin (IL)-12p40, IL-12p70, cluster of differentiation (CD) 11c, CD40, CD80, CD86, and major histocompatibility complex class II I-A/I-E subregions (Chen, Lu, Srinivasan, Tan, & Chan, 2009; Zhu, Zhao, Zhao, & Chen, 2007). While the effects of LBPs on the small population of dendritic cells in the retina remain unstudied, it is possible that the mechanism of action is related to similar changes in cytokine secretion. Notably, in primary BMDC culture, LBPs appear to directly activate nuclear factor kappa B (NF-κB) via toll-like receptor (TLR)-2 and TLR4 (Zhu, Zhang, Shen, Zhou, & Yu, 2013).

Although the various roles of Müller cells in retinal disease have been well documented (reviewed in Bringmann & Wiedemann, 2012), to date, the effects of LBPs specifically on retinal Müller cell morphology and activity are largely unstudied. However, in an in vitro spinal cord injury model,

LBPs do not appear to directly affect astrocytes, which are phenotypically and functionally similar to Müller cells and astrocytes in the retina (Zhang, Wang, et al., 2013). In this same study, the authors sought to explain their findings by further investigating the role of microglial activation in their model. Retinal microglia, like those in the brain and spinal cord, are derived from myeloid cells and are the resident macrophage of this tissue. Their activation state, either M1 (proinflammatory) or M2 (tissue repair), has been associated with the pathogenesis of multiple retinal diseases (Gupta, Brown, & Milam, 2003; Zeiss & Johnson, 2004; Zeng et al., 2005). In fact, activated retinal microglia have also been demonstrated to interact with Müller cells (Fernandez-Bueno, Pastor, Gayoso, Alcalde, & Garcia, 2008; Wang, Ma, Zhao, Fariss, & Wong, 2011), leading to what is likely a synergistic signaling cascade involved in the immunomodulatory effects that occur following retinal insult.

It is important to note that the effect of LBPs on microglia/macrophage appears to vary depending on the cellular context, disease state/stimulus, and activation state of the cell at the time of treatment. For example, various studies indicate that LBPs enhance immunity by increasing the phagocytotic capabilities of the activated macrophages, as well as secretion of tumor necrosis factor (TNF)-α, nitric oxide, and other cytotoxic factors (Chen, Soo, et al., 2009). In the retina, on the other hand, LBPs have been shown to prevent microglial cell activation in rd10 mouse retinas, resulting in decreased production of inflammatory mediators such as TNF-α, IL6β, and C–C motif chemokine ligand 2 (CCL2) via the reduced expression of NF-κB (Wang, Xiao, et al., 2014). This neuroprotective state was also observed by Chiu et al. (2009) in a rat model of ocular hypertension after pretreatment with 1–100 mg/kg of LBPs. In this study, changes in the activation state of the microglia were highlighted by injecting either lipopolysaccharide or macrophage/microglia inhibitory factor immediately after the first laser treatment, leading to the stimulation or inhibition of microglial activation, respectively. The morphology of the LBP-activated retinal microglia also appears different from that induced by cytotoxic stimuli and ocular insult (Li et al., 2015). Thus, it is likely that LBPs may manipulate microglial activation in a way that increases activity in general, of both states, and it is the balance of these two pathways that leads to beneficial mechanisms and protection of the retinal neurons (Li, Lu, Tay, Moochhala, & He, 2007).

In addition to their focus on immune function, Wang, Xiao, et al. (2014) also demonstrated antioxidative mechanisms. Interestingly, these mechanisms may overlap as NF-κB and other transcription factors are redox sensitive and

can be activated by oxidants. These activated signaling molecules can then, in turn, alter immune cell function. Immune cells themselves are also sensitive to oxidative stress as their membranes contain a high percentage of polyunsaturated fatty acids, and the presence of excessive ROS can alter the cell's redox state (Bennett & Griffiths, 2012). Taken together, these data indicate that the effect of LBP on immune cell function may also be linked to its antioxidative properties and vice versa.

3.5 Links Between the Visual System and Internal Organ Health in CHM

When discussing CHMs it is important to mention the connection between the liver and the eyes. Indeed, various systemic diseases affect both the liver and numerous ocular tissues (O'Neill, 1992), and improvements in hepatic function have been shown to heavily affect ocular health (Fatrai et al., 2015). Recent studies have demonstrated the effects of LBPs in acute liver injury, alcoholic liver disease, and nonalcoholic fatty liver disease, which are modulated through similar antioxidant, antiinflammatory, and neuroprotective mechanisms as those described for the retina (Cheng & Kong, 2011; Xiao, Fai So, Liong, & Tipoe, 2013; Xiao et al., 2012; Xiao, Wang, et al., 2014; Xiao, Xing, et al., 2014). Furthermore, diseases affecting the kidney and the eye also appear to have common pathogenesis in terms of genetic and phenotypic changes (Wong, Wong, Cheng, & Sabanayagam, 2014). Similar mechanistic functions are also apparent in kidney tissues following LBP treatment (Du, Hu, Kou, Zhang, & Zhang, 2016; Li, 2007). The connection between these organs likely plays a significant role in the similar mechanisms employed by LBPs during treatment. In traditional Chinese medicine, this interconnected pathway between the internal organs, particularly the liver and kidneys, and the eyes is described by Meridian Theory (O'Brien & Xue, 2016). This holistic philosophy explains that eye disorders are caused by liver blood deficiencies, liver heat, and internal liver wind as well as dysfunctional kidney function.

4. *L. BARBARUM* POLYSACCHARIDE AS A TREATMENT AND PREVENTATIVE MEDICINE: FUTURE PERSPECTIVES AND CONCLUSIONS

In Chinese medicine, a disease is traditionally treated as an issue of balance in the entire body. While the use of homeopathic compounds is becoming more mainstream worldwide, further scientific analysis is required in order to determine the mechanisms underlying the associated health

benefits as well as to evaluate any detrimental side effects. As one of the most well-studied CHMs, *L. barbarum* has been used to treat a variety of diseases, including those of the retina, and a vast amount of literature is available outlining its mode of action as well as the affected downstream signaling pathways. These signaling pathways, which involve antioxidative, antiinflammatory, and neuroprotective facets, are in many ways linked together, whereby the effects of treatment are modulated by overlapping and synergistic changes in both the local tissue as well as in the global health of the patient/animal. With the exception of minor cases of allergic reactions, treatment with appropriate levels of *L. barbarum* does not appear to have any significant adverse effects. Therefore, as the quality and quantity of studies published concerning its use increases, in our laboratories and others, it is likely that *L. barbarum* will continue to be explored as a reliable, effective treatment option for retinal diseases.

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REFERENCES

Amagase, H., & Nance, D. M. (2008). A randomized, double-blind, placebo-controlled, clinical study of the general effects of a standardized *Lycium barbarum* (Goji) Juice, GoChi. *Journal of Alternative and Complementary Medicine*, *14*(4), 403–412.

Amagase, H., Sun, B., & Borek, C. (2009a). *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nutrition Research*, *29*(1), 19–25.

Amagase, H., Sun, B., & Nance, D. M. (2009b). Immunomodulatory effects of a standardized *Lycium barbarum* fruit juice in Chinese older healthy human subjects. *Journal of Medicinal Food*, *12*(5), 1159–1165.

Ambati, J., Ambati, B. K., Yoo, S. H., Ianchulev, S., & Adamis, A. P. (2003). Age-related macular degeneration: Etiology, pathogenesis, and therapeutic strategies. *Survey of Ophthalmology*, *48*(3), 257–293.

Arundine, M., & Tymianski, M. (2004). Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cellular and Molecular Life Sciences*, *61*(6), 657–668.

Baccus, S. A. (2007). Timing and computation in inner retinal circuitry. *Annual Review of Physiology*, *69*, 271–290.

Beatty, S., Koh, H., Phil, M., Henson, D., & Boulton, M. (2000). The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of Ophthalmology*, *45*(2), 115–134.

Benlloch, M., Muriach, M., Castellano, G., Sancho-Pelluz, F. J., Gonzalez-Garcia, E., Flores-Bellver, M., et al. (2015). Role of *Lycium barbarum* extracts in retinal diseases.

In R. C. C. Chang & K. F. So (Eds.), Lycium barbarum *and human health*. The Netherlands: Springer.
Bennett, S. J., & Griffiths, H. R. (2012). Regulation of T-cell functions by oxidative stress. In M. J. Alcaraz, O. Gualillo, & O. Sanchez-Pernaute (Eds.), *Studies on arthritis and joint disorders*. New York: Springer.
Bringmann, A., & Wiedemann, P. (2012). Muller glial cells in retinal disease. *Ophthalmologica*, *227*(1), 1–19.
Bucheli, P., Vidal, K., Shen, L., Gu, Z., Zhang, C., Miller, L. E., et al. (2011). Goji berry effects on macular characteristics and plasma antioxidant levels. *Optometry and Vision Science*, *88*(2), 257–262.
Calkins, D. J. (2012). Critical pathogenic events underlying progression of neurodegeneration in glaucoma. *Progress in Retinal and Eye Research*, *31*(6), 702–719.
Chan, H. H. L., So K.F., Chang R.C.C., Lai S.M., Do C.W., Benzie I.F.F., et al., (ongoing). Cone rescue in retinitis pigmentosa by the treatment of *Lycium barbarum*. Estimated completion date: March 2017. Clinicaltrials.gov identifier: NCT02244996.
Chan, H. C., Chang, R. C., Koon-Ching Ip, A., Chiu, K., Yuen, W. H., Zee, S. Y., et al. (2007). Neuroprotective effects of *Lycium barbarum* Lynn on protecting retinal ganglion cells in an ocular hypertension model of glaucoma. *Experimental Neurology*, *203*(1), 269–273.
Chan, K. C., Fu, Q. L., Hui, E. S., So, K. F., & Wu, E. X. (2008). Evaluation of the retina and optic nerve in a rat model of chronic glaucoma using in vivo manganese-enhanced magnetic resonance imaging. *NeuroImage*, *40*(3), 1166–1174.
Chen, Z., Lu, J., Srinivasan, N., Tan, B. K., & Chan, S. H. (2009). Polysaccharide-protein complex from *Lycium barbarum* L. is a novel stimulus of dendritic cell immunogenicity. *Journal of Immunology*, *182*(6), 3503–3509.
Chen, Z., Soo, M. Y., Srinivasan, N., Tan, B. K., & Chan, S. H. (2009). Activation of macrophages by polysaccharide-protein complex from *Lycium barbarum* L. *Phytotherapy Research*, *23*(8), 1116–1122.
Cheng, C. Y., Chung, W. Y., Szeto, Y. T., & Benzie, I. F. (2005). Fasting plasma zeaxanthin response to Fructus barbarum L. (wolfberry; Kei Tze) in a food-based human supplementation trial. *The British Journal of Nutrition*, *93*(1), 123–130.
Cheng, D., & Kong, H. (2011). The effect of *Lycium barbarum* polysaccharide on alcohol-induced oxidative stress in rats. *Molecules*, *16*(3), 2542–2550.
Cheung, A. K., Fung, M. K., Lo, A. C., Lam, T. T., So, K. F., Chung, S. S., et al. (2005). Aldose reductase deficiency prevents diabetes-induced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of db/db mice. *Diabetes*, *54*(11), 3119–3125.
Chiu, K., Chan, H. C., Yeung, S. C., Yuen, W. H., Zee, S. Y., Chang, R. C., et al. (2009). Erratum: Modulation of microglia by Wolfberry on the survival of retinal ganglion cells in a rat ocular hypertension model. *Journal of Ocular Biology, Diseases, and Informatics*, *2*(3), 127–136.
Chiu, K., Zhou, Y., Yeung, S. C., Lok, C. K., Chan, O. O., Chang, R. C., et al. (2010). Up-regulation of crystallins is involved in the neuroprotective effect of wolfberry on survival of retinal ganglion cells in rat ocular hypertension model. *Journal of Cellular Biochemistry*, *110*(2), 311–320.
Chu, P. H., Li, H. Y., Chin, M. P., So, K. F., & Chan, H. H. (2013). Effect of *Lycium barbarum* (wolfberry) polysaccharides on preserving retinal function after partial optic nerve transection. *PLoS One*, *8*(12), e81339.
Coyle, J. T., & Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, *262*(5134), 689–695.
Cui, B., Liu, S., Lin, X., Wang, J., Li, S., & Wang, Q. (2011). Effects of *Lycium barbarum* aqueous and ethanol extracts on high-fat-diet induced oxidative stress in rat liver tissue. *Molecules*, *16*(11), 9116–9128.

Du, M., Hu, X., Kou, L., Zhang, B., & Zhang, C. (2016). *Lycium barbarum* polysaccharide mediated the antidiabetic and antinephritic effects in diet-streptozotocin-induced diabetic Sprague Dawley rats via regulation of NF-kappaB. *BioMed Research International*, *2016*, 3140290.

Farrall, A. J., & Wardlaw, J. M. (2009). Blood-brain barrier: Ageing and microvascular disease—Systematic review and meta-analysis. *Neurobiology of Aging*, *30*(3), 337–352.

Fatrai, A., Hempen, C.-H., Han, C., Leonhardy, H., Hummelsberger, J., Uhrig, S., et al. (2015). *Chinese ophthalmology: Acupuncture, herbal therapy, dietary therapy, tuina and qigong*. Wiesbaden, Germany: Tipani-Verlag.

Fernandez-Bueno, I., Pastor, J. C., Gayoso, M. J., Alcalde, I., & Garcia, M. T. (2008). Muller and macrophage-like cell interactions in an organotypic culture of porcine neuroretina. *Molecular Vision*, *14*, 2148–2156.

Ferrari, S., Di Iorio, E., Barbaro, V., Ponzin, D., Sorrentino, F. S., & Parmeggiani, F. (2011). Retinitis pigmentosa: Genes and disease mechanisms. *Current Genomics*, *12*(4), 238–249.

Flammer, J., Konieczka, K., Bruno, R. M., Virdis, A., Flammer, A. J., & Taddei, S. (2013). The eye and the heart. *European Heart Journal*, *34*(17), 1270–1278.

Ganea, E., & Harding, J. J. (2006). Glutathione-related enzymes and the eye. *Current Eye Research*, *31*(1), 1–11.

Gupta, N., Brown, K. E., & Milam, A. H. (2003). Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. *Experimental Eye Research*, *76*(4), 463–471.

Hartong, D. T., Berson, E. L., & Dryja, T. P. (2006). Retinitis pigmentosa. *Lancet*, *368*(9549), 1795–1809.

He, M., Pan, H., Chang, R. C., So, K. F., Brecha, N. C., & Pu, M. (2014). Activation of the Nrf2/HO-1 antioxidant pathway contributes to the protective effects of *Lycium barbarum* polysaccharides in the rodent retina after ischemia-reperfusion-induced damage. *PLoS One*, *9*(1), e84800.

He, N., Yang, X., Jiao, Y., Tian, L., & Zhao, Y. (2012). Characterisation of antioxidant and antiproliferative acidic polysaccharides from Chinese wolfberry fruits. *Food Chemistry*, *133*(3), 978–989.

Heavner, W., & Pevny, L. (2012). Eye development and retinogenesis. *Cold Spring Harbor Perspectives in Biology*, *4*(12), a008391.

Heuss, N. D., Lehmann, U., Norbury, C. C., McPherson, S. W., & Gregerson, D. S. (2012). Local activation of dendritic cells alters the pathogenesis of autoimmune disease in the retina. *Journal of Immunology*, *188*(3), 1191–1200.

Ho, P. I., Ortiz, D., Rogers, E., & Shea, T. B. (2002). Multiple aspects of homocysteine neurotoxicity: Glutamate excitotoxicity, kinase hyperactivation and DNA damage. *Journal of Neuroscience Research*, *70*(5), 694–702.

Ho, Y. S., Yu, M. S., Lai, C. S., So, K. F., Yuen, W. H., & Chang, R. C. (2007). Characterizing the neuroprotective effects of alkaline extract of *Lycium barbarum* on beta-amyloid peptide neurotoxicity. *Brain Research*, *1158*, 123–134.

Ho, Y. S., Yu, M. S., Yang, X. F., So, K. F., Yuen, W. H., & Chang, R. C. (2010). Neuroprotective effects of polysaccharides from wolfberry, the fruits of *Lycium barbarum*, against homocysteine-induced toxicity in rat cortical neurons. *Journal of Alzheimer's Disease*, *19*(3), 813–827.

Ho, Y. S., Yu, M. S., Yik, S. Y., So, K. F., Yuen, W. H., & Chang, R. C. (2009). Polysaccharides from wolfberry antagonizes glutamate excitotoxicity in rat cortical neurons. *Cellular and Molecular Neurobiology*, *29*(8), 1233–1244.

Hu, C. K., Lee, Y. J., Colitz, C. M., Chang, C. J., & Lin, C. T. (2012). The protective effects of *Lycium barbarum* and Chrysanthemum morifolum on diabetic retinopathies in rats. *Veterinary Ophthalmology*, *15*(Suppl. 2), 65–71.

Ishida, K., Panjwani, N., Cao, Z., & Streilein, J. W. (2003). Participation of pigment epithelium in ocular immune privilege. 3. Epithelia cultured from iris, ciliary body, and retina suppress T-cell activation by partially non-overlapping mechanisms. *Ocular Immunology and Inflammation*, *11*(2), 91–105.

Jin, M., Huang, Q., Zhao, K., & Shang, P. (2013). Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *International Journal of Biological Macromolecules*, *54*, 16–23.

Jin, M., Lu, Z., Huang, M., & Wang, Y. (2011). Sulfated modification and antioxidant activity of exopolysaccahrides produced by Enterobacter cloacae Z0206. *International Journal of Biological Macromolecules*, *48*(4), 607–612.

Jonas, R. A., Yuan, T. F., Liang, Y. X., Jonas, J. B., Tay, D. K., & Ellis-Behnke, R. G. (2012). The spider effect: Morphological and orienting classification of microglia in response to stimuli in vivo. *PLoS One*, *7*(2), e30763.

Junlin, L., & Aicheng, W. (2002). *Gou qi*. Beijing: Beijing Science and Technology Press.

Kaplan, H. J., Leibole, M. A., Tezel, T., & Ferguson, T. A. (1999). Fas ligand (CD95 ligand) controls angiogenesis beneath the retina. *Nature Medicine*, *5*(3), 292–297.

Kaur, C., Sivakumar, V., Yong, Z., Lu, J., Foulds, W. S., & Ling, E. A. (2007). Blood-retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: The beneficial effect of melatonin administration. *The Journal of Pathology*, *212*(4), 429–439.

Kee, C. S., Do, C. W., Cheong, M. Y., Wong, S. W., Law, K. M., Wong, C. Y., et al. (2013). Effects of *Lycium barbarum* supplement on the progression of senile cataract in a Hong Kong Chinese population (Abstract). In *Inaugural Symposium of HEALED Research Group "Health for Life"*.

Kimelberg, H. K. (1995). Current concepts of brain edema. Review of laboratory investigations. *Journal of Neurosurgery*, *83*(6), 1051–1059.

Kruk, J., Kubasik-Kladna, K., & Aboul-Enein, H. Y. (2015). The role oxidative stress in the pathogenesis of eye diseases: Current status and a dual role of physical activity. *Mini Reviews in Medicinal Chemistry*, *16*(3), 241–257.

Lai, J.S.M., So, K.F., Chang, R.C.C., Lee, J.W.Y., Chan, J.C.H., Lo, A., et al. (ongoing). Randomized control trial on the neuroprotective effects of oral Wolfberry supplement in the treatment of normal tension glaucoma. Estimated completion date: December 2018.

Lau, C. H., & Taylor, A. W. (2009). The immune privileged retina mediates an alternative activation of J774A.1 cells. *Ocular Immunology and Inflammation*, *17*(6), 380–389.

Leasher, J. L., Bourne, R. R., Flaxman, S. R., Jonas, J. B., Keeffe, J., Naidoo, K., et al. (2016). Global estimates on the number of people blind or visually impaired by diabetic retinopathy: A meta-analysis from 1990 to 2010. *Diabetes Care*, *39*(9), 1643–1649.

Lehmann, U., Heuss, N. D., McPherson, S. W., Roehrich, H., & Gregerson, D. S. (2010). Dendritic cells are early responders to retinal injury. *Neurobiology of Disease*, *40*(1), 177–184.

Levkovitch-Verbin, H., Quigley, H. A., Kerrigan-Baumrind, L. A., D'Anna, S. A., Kerrigan, D., & Pease, M. E. (2001). Optic nerve transection in monkeys may result in secondary degeneration of retinal ganglion cells. *Investigative Ophthalmology & Visual Science*, *42*(5), 975–982.

Levkovitch-Verbin, H., Quigley, H. A., Martin, K. R., Zack, D. J., Pease, M. E., & Valenta, D. F. (2003). A model to study differences between primary and secondary degeneration of retinal ganglion cells in rats by partial optic nerve transection. *Investigative Ophthalmology & Visual Science*, *44*(8), 3388–3393.

Li, X. M. (2007). Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats. *International Journal of Biological Macromolecules*, *40*(5), 461–465.

Li, H., Liang, Y., Chiu, K., Yuan, Q., Lin, B., Chang, R. C., et al. (2013). *Lycium barbarum* (wolfberry) reduces secondary degeneration and oxidative stress, and inhibits JNK pathway in retina after partial optic nerve transection. *PLoS One*, *8*(7), e68881.

Li, L., Lu, J., Tay, S. S., Moochhala, S. M., & He, B. P. (2007). The function of microglia, either neuroprotection or neurotoxicity, is determined by the equilibrium among factors released from activated microglia in vitro. *Brain Research*, *1159*, 8–17.

Li, X. M., Ma, Y. L., & Liu, X. J. (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology*, *111*(3), 504–511.

Li, H. Y., Ruan, Y. W., Kau, P. W., Chiu, K., Chang, R. C., Chan, H. H., et al. (2015). Effect of *Lycium barbarum* (Wolfberry) on alleviating axonal degeneration after partial optic nerve transection. *Cell Transplantation*, *24*(3), 403–417.

Li, S. Y., Yang, D., Yeung, C. M., Yu, W. Y., Chang, R. C., So, K. F., et al. (2011). *Lycium barbarum* polysaccharides reduce neuronal damage, blood-retinal barrier disruption and oxidative stress in retinal ischemia/reperfusion injury. *PLoS One*, *6*(1), e16380.

Liu, H., Fan, Y., Wang, W., Liu, N., Zhang, H., Zhu, Z., et al. (2012). Polysaccharides from *Lycium barbarum* leaves: Isolation, characterization and splenocyte proliferation activity. *International Journal of Biological Macromolecules*, *51*(4), 417–422.

Liu, L., Lao, W., Ji, Q. S., Yang, Z. H., Yu, G. C., & Zhong, J. X. (2015). *Lycium barbarum* polysaccharides protected human retinal pigment epithelial cells against oxidative stress-induced apoptosis. *International Journal of Ophthalmology*, *8*(1), 11–16.

Mao, F., Xiao, B., Jiang, Z., Zhao, J., Huang, X., & Guo, J. (2011). Anticancer effect of *Lycium barbarum* polysaccharides on colon cancer cells involves G0/G1 phase arrest. *Medical Oncology*, *28*(1), 121–126.

Marc, R. E., Jones, B. W., Watt, C. B., Vazquez-Chona, F., Vaughan, D. K., & Organisciak, D. T. (2008). Extreme retinal remodeling triggered by light damage: Implications for age related macular degeneration. *Molecular Vision*, *14*, 782–806.

Masaki, T., Vane, J. R., & Vanhoutte, P. M. (1994). International Union of Pharmacology nomenclature of endothelin receptors. *Pharmacological Reviews*, *46*(2), 137–142.

McPherson, S. W., Heuss, N. D., Pierson, M. J., & Gregerson, D. S. (2014). Retinal antigen-specific regulatory T cells protect against spontaneous and induced autoimmunity and require local dendritic cells. *Journal of Neuroinflammation*, *11*, 205.

Mi, X. S., Chiu, K., Van, G., Leung, J. W., Lo, A. C., Chung, S. K., et al. (2012). Effect of *Lycium barbarum* polysaccharides on the expression of endothelin-1 and its receptors in an ocular hypertension model of rat glaucoma. *Neural Regeneration Research*, 7(9), 645–651.

Mi, X. S., Feng, Q., Lo, A. C., Chang, R. C., Lin, B., Chung, S. K., et al. (2012). Protection of retinal ganglion cells and retinal vasculature by *Lycium barbarum* polysaccharides in a mouse model of acute ocular hypertension. *PLoS One*, 7(10), e45469.

Miranda, M., Arnal, E., Ahuja, S., Alvarez-Nolting, R., Lopez-Pedrajas, R., Ekstrom, P., et al. (2010). Antioxidants rescue photoreceptors in rd1 mice: Relationship with thiol metabolism. *Free Radical Biology & Medicine*, *48*(2), 216–222.

Narayan, S., Prasanna, G., Krishnamoorthy, R. R., Zhang, X., & Yorio, T. (2003). Endothelin-1 synthesis and secretion in human retinal pigment epithelial cells (ARPE-19): Differential regulation by cholinergics and TNF-alpha. *Investigative Ophthalmology & Visual Science*, *44*(11), 4885–4894.

Ni, T., Wei, G., Yin, X., Liu, X., & Liu, D. (2013). Neuroprotective effect of *Lycium barbarum* on retina of Royal College of Surgeons (RCS) rats: A preliminary study. *Folia Neuropathologica*, *51*(2), 158–163.

Niu, A. J., Wu, J. M., Yu, D. H., & Wang, R. (2008). Protective effect of *Lycium barbarum* polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats. *International Journal of Biological Macromolecules*, *42*(5), 447–449.

O'Brien, K. A., & Xue, C. C. (2016). The theoretical framework of Chinese medicine. In P.-C. Leung (Ed.), *A comprehensive guide to Chinese medicine*. Singapore: World Scientific Publishing Co. Pte. Ltd.

O'Neill, D. P. (1992). The eye and liver disorders. *Eye (London, England)*, *6*(Pt. 4), 366–370.

Organisciak, D. T., & Vaughan, D. K. (2010). Retinal light damage: Mechanisms and protection. *Progress in Retinal and Eye Research*, *29*(2), 113–134.
Pascolini, D., & Mariotti, S. P. (2012). Global estimates of visual impairment: 2010. *The British Journal of Ophthalmology*, *96*(5), 614–618.
Paul Hsu, C. H., Nance, D. M., & Amagase, H. (2012). A meta-analysis of clinical improvements of general well-being by a standardized *Lycium barbarum*. *Journal of Medicinal Food*, *15*(11), 1006–1014.
Pennesi, M. E., Neuringer, M., & Courtney, R. J. (2012). Animal models of age related macular degeneration. *Molecular Aspects of Medicine*, *33*(4), 487–509.
Perez, V. L., Saeed, A. M., Tan, Y., Urbieta, M., & Cruz-Guilloty, F. (2013). The eye: A window to the soul of the immune system. *Journal of Autoimmunity*, *45*, 7–14.
Puertas, F. J., Diaz-Llopis, M., Chipont, E., Roma, J., Raya, A., & Romero, F. J. (1993). Glutathione system of human retina: Enzymatic conjugation of lipid peroxidation products. *Free Radical Biology & Medicine*, *14*(5), 549–551.
Qi, B., Ji, Q., Wen, Y., Liu, L., Guo, X., Hou, G., et al. (2014). *Lycium barbarum* polysaccharides protect human lens epithelial cells against oxidative stress-induced apoptosis and senescence. *PLoS One*, *9*(10), e110275.
Rattner, A., & Nathans, J. (2006). Macular degeneration: Recent advances and therapeutic opportunities. *Nature Reviews. Neuroscience*, 7(11), 860–872.
Rizzolo, L. J. (2014). Barrier properties of cultured retinal pigment epithelium. *Experimental Eye Research*, *126*, 16–26.
Saijo, K., & Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nature Reviews. Immunology*, *11*(11), 775–787.
Shan, X., Zhou, J., Ma, T., & Chai, Q. (2011). *Lycium barbarum* polysaccharides reduce exercise-induced oxidative stress. *International Journal of Molecular Sciences*, *12*(2), 1081–1088.
Slingsby, C., Wistow, G. J., & Clark, A. R. (2013). Evolution of crystallins for a role in the vertebrate eye lens. *Protein Science*, *22*(4), 367–380.
Song, M. K., Roufogalis, B. D., & Huang, T. H. (2012). Reversal of the caspase-dependent apoptotic cytotoxicity pathway by taurine from *Lycium barbarum* (goji berry) in human retinal pigment epithelial cells: Potential benefit in diabetic retinopathy. *Evidence-based Complementary and Alternative Medicine*, *2012*, 323784.
Steuer, H., Jaworski, A., Elger, B., Kaussmann, M., Keldenich, J., Schneider, H., et al. (2005). Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. *Investigative Ophthalmology & Visual Science*, *46*(3), 1047–1053.
Sugita, S. (2009). Role of ocular pigment epithelial cells in immune privilege. *Archivum Immunologiae et Therapiae Experimentalis*, *57*(4), 263–268.
Sugita, S., Horie, S., Nakamura, O., Futagami, Y., Takase, H., Keino, H., et al. (2008). Retinal pigment epithelium-derived CTLA-2alpha induces TGFbeta-producing T regulatory cells. *Journal of Immunology*, *181*(11), 7525–7536.
Sugita, S., Usui, Y., Horie, S., Futagami, Y., Aburatani, H., Okazaki, T., et al. (2009). T-cell suppression by programmed cell death 1 ligand 1 on retinal pigment epithelium during inflammatory conditions. *Investigative Ophthalmology & Visual Science*, *50*(6), 2862–2870.
Sung, C. H., & Chuang, J. Z. (2010). The cell biology of vision. *The Journal of Cell Biology*, *190*(6), 953–963.
Tang, L., Zhang, Y., Jiang, Y., Willard, L., Ortiz, E., Wark, L., et al. (2011). Dietary wolfberry ameliorates retinal structure abnormalities in db/db mice at the early stage of diabetes. *Experimental Biology and Medicine (Maywood, N.J.)*, *236*(9), 1051–1063.
Tanihara, H., Yoshida, M., Matsumoto, M., & Yoshimura, N. (1993). Identification of transforming growth factor-beta expressed in cultured human retinal pigment epithelial cells. *Investigative Ophthalmology & Visual Science*, *34*(2), 413–419.

Tanito, M., Nishiyama, A., Tanaka, T., Masutani, H., Nakamura, H., Yodoi, J., et al. (2002). Change of redox status and modulation by thiol replenishment in retinal photooxidative damage. *Investigative Ophthalmology & Visual Science*, *43*(7), 2392–2400.

Tham, Y. C., Li, X., Wong, T. Y., Quigley, H. A., Aung, T., & Cheng, C. Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology*, *121*(11), 2081–2090.

Thanos, S., Bohm, M. R., Meyer zu Horste, M., Prokosch-Willing, V., Hennig, M., Bauer, D., et al. (2014). Role of crystallins in ocular neuroprotection and axonal regeneration. *Progress in Retinal and Eye Research*, *42*, 145–161.

Usui, Y., Okunuki, Y., Hattori, T., Kezuka, T., Keino, H., Ebihara, N., et al. (2008). Functional expression of B7H1 on retinal pigment epithelial cells. *Experimental Eye Research*, *86*(1), 52–59.

Wang, Y., Chen, H., Wu, M., Zeng, S., Liu, Y., & Dong, J. (2015). Chemical and genetic diversity of wolfberry. In R. C. C. Chang & K. F. So (Eds.), Lycium barbarum *and human health*. The Netherlands: Springer.

Wang, H. B., Li, Y. X., Hao, Y. J., Wang, T. F., Lei, Z., Wu, Y., et al. (2013). Neuroprotective effects of LBP on brain ischemic reperfusion neurodegeneration. *European Review for Medical and Pharmacological Sciences*, *17*(20), 2760–2765.

Wang, T., Li, Y., Wang, Y., Zhou, R., Ma, L., Hao, Y., et al. (2014). *Lycium barbarum* polysaccharide prevents focal cerebral ischemic injury by inhibiting neuronal apoptosis in mice. *PLoS One*, *9*(3), e90780.

Wang, M., Ma, W., Zhao, L., Fariss, R. N., & Wong, W. T. (2011). Adaptive Muller cell responses to microglial activation mediate neuroprotection and coordinate inflammation in the retina. *Journal of Neuroinflammation*, *8*, 173.

Wang, K., Xiao, J., Peng, B., Xing, F., So, K. F., Tipoe, G. L., et al. (2014). Retinal structure and function preservation by polysaccharides of wolfberry in a mouse model of retinal degeneration. *Scientific Reports*, *4*, 7601.

Wang, Y., Zhao, H., Sheng, X., Gambino, P. E., Costello, B., & Bojanowski, K. (2002). Protective effect of Fructus Lycii polysaccharides against time and hyperthermia-induced damage in cultured seminiferous epithelium. *Journal of Ethnopharmacology*, *82*(2–3), 169–175.

WHO. (2012). *Global data on visual impairments 2010*. World Health Organization. Available from http://www.iapb.org/vision-2020/global-facts.

Won, S. J., Kim, D. Y., & Gwag, B. J. (2002). Cellular and molecular pathways of ischemic neuronal death. *Journal of Biochemistry and Molecular Biology*, *35*(1), 67–86.

Wong, C. W., Wong, T. Y., Cheng, C. Y., & Sabanayagam, C. (2014). Kidney and eye diseases: Common risk factors, etiological mechanisms, and pathways. *Kidney International*, *85*(6), 1290–1302.

Wu, H. T., He, X. J., Hong, Y. K., Ma, T., Xu, Y. P., & Li, H. H. (2010). Chemical characterization of *Lycium barbarum* polysaccharides and its inhibition against liver oxidative injury of high-fat mice. *International Journal of Biological Macromolecules*, *46*(5), 540–543.

Xiao, J., Fai So, K., Liong, E. C., & Tipoe, G. L. (2013). Recent advances in the herbal treatment of non-alcoholic fatty liver disease. *Journal of Traditional and Complementary Medicine*, *3*(2), 88–94.

Xiao, J., Liong, E. C., Ching, Y. P., Chang, R. C., So, K. F., Fung, M. L., et al. (2012). *Lycium barbarum* polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation. *Journal of Ethnopharmacology*, *139*(2), 462–470.

Xiao, J., Wang, J., Xing, F., Han, T., Jiao, R., Liong, E. C., et al. (2014). Zeaxanthin dipalmitate therapeutically improves hepatic functions in an alcoholic fatty liver disease model through modulating MAPK pathway. *PLoS One*, *9*(4), e95214.

Xiao, J., Xing, F., Huo, J., Fung, M. L., Liong, E. C., Ching, Y. P., et al. (2014). *Lycium barbarum* polysaccharides therapeutically improve hepatic functions in non-alcoholic steatohepatitis rats and cellular steatosis model. *Scientific Reports*, *4*, 5587.

Xu, H., Dawson, R., Forrester, J. V., & Liversidge, J. (2007). Identification of novel dendritic cell populations in normal mouse retina. *Investigative Ophthalmology & Visual Science*, *48*(4), 1701–1710.

Yang, D., Li, S. Y., Yeung, C. M., Chang, R. C., So, K. F., Wong, D., et al. (2012). *Lycium barbarum* extracts protect the brain from blood-brain barrier disruption and cerebral edema in experimental stroke. *PLoS One*, 7(3), e33596.

Yoles, E., & Schwartz, M. (1998). Degeneration of spared axons following partial white matter lesion: Implications for optic nerve neuropathies. *Experimental Neurology*, *153*(1), 1–7.

Yu, P. K., Balaratnasingam, C., Morgan, W. H., Cringle, S. J., McAllister, I. L., & Yu, D. Y. (2010). The structural relationship between the microvasculature, neurons, and glia in the human retina. *Investigative Ophthalmology & Visual Science*, *51*(1), 447–458.

Yu, M. S., Lai, C. S., Ho, Y. S., Zee, S. Y., So, K. F., Yuen, W. H., et al. (2007). Characterization of the effects of anti-aging medicine Fructus lycii on beta-amyloid peptide neurotoxicity. *International Journal of Molecular Medicine*, *20*(2), 261–268.

Yu, M. S., Leung, S. K., Lai, S. W., Che, C. M., Zee, S. Y., So, K. F., et al. (2005). Neuroprotective effects of anti-aging oriental medicine *Lycium barbarum* against beta-amyloid peptide neurotoxicity. *Experimental Gerontology*, *40*(8–9), 716–727.

Yu, H., Wark, L., Ji, H., Willard, L., Jaing, Y., Han, J., et al. (2013). Dietary wolfberry upregulates carotenoid metabolic genes and enhances mitochondrial biogenesis in the retina of db/db diabetic mice. *Molecular Nutrition & Food Research*, *57*(7), 1158–1169.

Yuan, T. F., Liang, Y. X., Peng, B., Lin, B., & So, K. F. (2015). Local proliferation is the main source of rod microglia after optic nerve transection. *Scientific Reports*, *5*, 10788.

Zamiri, P., Masli, S., Kitaichi, N., Taylor, A. W., & Streilein, J. W. (2005). Thrombospondin plays a vital role in the immune privilege of the eye. *Investigative Ophthalmology & Visual Science*, *46*(3), 908–919.

Zamiri, P., Masli, S., Streilein, J. W., & Taylor, A. W. (2006). Pigment epithelial growth factor suppresses inflammation by modulating macrophage activation. *Investigative Ophthalmology & Visual Science*, *47*(9), 3912–3918.

Zeiss, C. J. (2010). Animals as models of age-related macular degeneration: An imperfect measure of the truth. *Veterinary Pathology*, *47*(3), 396–413.

Zeiss, C. J., & Johnson, E. A. (2004). Proliferation of microglia, but not photoreceptors, in the outer nuclear layer of the rd-1 mouse. *Investigative Ophthalmology & Visual Science*, *45*(3), 971–976.

Zeng, H. Y., Zhu, X. A., Zhang, C., Yang, L. P., Wu, L. M., & Tso, M. O. (2005). Identification of sequential events and factors associated with microglial activation, migration, and cytotoxicity in retinal degeneration in rd mice. *Investigative Ophthalmology & Visual Science*, *46*(8), 2992–2999.

Zhang, M., Chen, H., Huang, J., Li, Z., Zhu, C., & Zhang, S. (2005). Effect of *Lycium barbarum* polysaccharide on human hepatoma QGY7703 cells: Inhibition of proliferation and induction of apoptosis. *Life Sciences*, *76*(18), 2115–2124.

Zhang, Q., Chen, W., Zhao, J., & Xi, W. (2016). Functional constituents and antioxidant activities of eight Chinese native goji genotypes. *Food Chemistry*, *200*, 230–236.

Zhang, Q., Du, X., Xu, Y., Dang, L., Xiang, L., & Zhang, J. (2013). The effects of Gouqi extracts on Morris maze learning in the APP/PS1 double transgenic mouse model of Alzheimer's disease. *Experimental and Therapeutic Medicine*, *5*(5), 1528–1530.

Zhang, Y., Peng, B., Wang, S., Liang, Y. X., Yang, J., So, K. F., et al. (2016). Image processing methods to elucidate spatial characteristics of retinal microglia after optic nerve transection. *Scientific Reports*, *6*, 21816.

Zhang, Y., & Stone, J. (1997). Role of astrocytes in the control of developing retinal vessels. *Investigative Ophthalmology & Visual Science*, *38*(9), 1653–1666.

Zhang, Y. K., Wang, J., Liu, L., Chang, R. C., So, K. F., & Ju, G. (2013). The effect of *Lycium barbarum* on spinal cord injury, particularly its relationship with M1 and M2 macrophage in rats. *BMC Complementary and Alternative Medicine*, *13*, 67.

Zhang, E., Yau, S. Y., Lau, B. W., Ma, H., Lee, T. M., Chang, R. C., et al. (2012). Synaptic plasticity, but not hippocampal neurogenesis, mediated the counteractive effect of wolfberry on depression in rats(1). *Cell Transplantation*, *21*(12), 2635–2649.

Zhang, X., Zhou, W., & Zhang, Y. (2015). Immunoregulation and *Lycium barbarum*. In R. C. C. Chang & K. F. So (Eds.), Lycium barbarum *and human health*. The Netherlands: Springer.

Zhao, R., Cai, Y., Shao, X., & Ma, B. (2015). Improving the activity of *Lycium barbarum* polysaccharide on sub-health mice. *Food & Function*, *6*(6), 2033–2040.

Zhou, Z. Q., Fan, H. X., He, R. R., Xiao, J., Tsoi, B., Lan, K. H., et al. (2016). Lycibarbarspermidines A-O, new dicaffeoylspermidine derivatives from wolfberry, with activities against Alzheimer's disease and oxidation. *Journal of Agricultural and Food Chemistry*, *64*(11), 2223–2237.

Zhu, J., Zhang, Y., Shen, Y., Zhou, H., & Yu, X. (2013). *Lycium barbarum* polysaccharides induce toll-like receptor 2- and 4-mediated phenotypic and functional maturation of murine dendritic cells via activation of NF-kappaB. *Molecular Medicine Reports*, *8*(4), 1216–1220.

Zhu, J., Zhao, L. H., Zhao, X. P., & Chen, Z. (2007). *Lycium barbarum* polysaccharides regulate phenotypic and functional maturation of murine dendritic cells. *Cell Biology International*, *31*(6), 615–619.