Delay of cone degeneration in retinitis pigmentosa using a 12-month treatment with Lycium barbarum supplement

Henry Ho-lung Chan[a], Hang-i Lam[a], Kai-yip Choi[a], Serena Zhe-chuang Li[a], Yamunadevi Lakshmanan[a], Wing-yan Yu[a], Raymond Chuen-chung Chang[b], Jimmy Lai[c], Kwok-fai So[c,d]

[a] Laboratory of Experimental Optometry (Neuroscience), School of Optometry, The Hong Kong Polytechnic University, Hong Kong SAR, China.

[b] Laboratory of Neurodegenerative Diseases, School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, China

[c] Department of Ophthalmology, The University of Hong Kong, Hong Kong SAR, China

[d]Guangdong-Hongkong-Macau (GHM) Institute of CNS Regeneration, Jinan University, Guangzhou, China

Declarations of interest: None

Trial registration: clinicaltrials.gov Identifier NCT02244996

Corresponding author:

Dr. Henry H.L. Chan

School of Optometry,

The Hong Kong Polytechnic University,

Hung Hom, Kowloon,

Hong Kong SAR.

E-mail: henryhl.chan@polyu.edu.hk

Abstract

Ethnopharmacological relevance:

Lycium barbarum L. (also known as "Goji berry"), a traditional Chinese herbal medicine, has been a common herb in the traditional Chinese pharmacopoeia for centuries. The main active component is the Lycium barbarum polysaccharides and its antioxidative effect has been widely shown to provide neuroprotection to the eye, and it would, therefore, be interesting to determine if Lycium barbarum help delay vision deterioration in patients with retinitis pigmentosa.

Aim of the study:

Cone rescue is a potential method for delaying deterioration of visual function in Retinitis pigmentosa (RP). This study aimed to investigate the treatment effect of *Lycium barbarum* L. (LB) supplement on retinal functions and structure in RP patients after a 12-month intervention trial.

Methods:

The investigation was a double-masked and placebo-controlled clinical study. Each of forty-two RP subjects who completed the 12-month intervention (23 and 19 in the treatment and placebo groups respectively) received a daily supply of LB or placebo granules for oral administration. The primary outcome was change of best corrected visual acuity (VA) (90% and 10% contrast) from the baseline to the end of treatment. The secondary outcomes were sensitivity changes of the central visual field, amplitude of full-field electroretinogram (ffERG) (including scotopic maximal response and photopic cone response), and average macular thickness.

Results:

The compliance rates for both groups exceeded 80%. There were no deteriorations of either 90% or 10% contrast VA in the LB group compared with the placebo group ($p=0.001$). A thinning of macular layer was observed in the placebo group, which was not observed in the LB group ($p=0.008$). However, no significant differences were found in the sensitivity of visual field or in any parameters of ffERG between the two groups. No significant adverse effects were reported in the treatment group.

Conclusions:

LB supplement provides a neuroprotective effect for the retina and could help delay or minimize cone degeneration in RP.

Classifications: Clinical Studies (1.05)
Keywords: Clinical Trial (2.172), Traditional Chinese Medicine (2.592), Antioxidant (2.084),
Specific keywords: Lycium barbarum, Retinitis pigmentosa, Retinal degeneration, Neuroprotection

1. Introduction

Retinitis pigmentosa (RP) is a heredofamilial disease, characterized by progressive visual field loss, night blindness, and an abnormal electroretinogram (ERG). Patients often initially present with poor night vision, followed by deterioration in day vision. The genetic mutation associated with RP initially causes rod degeneration, with later cone degeneration. It has been reported that the cone cells in RP initially remain in a semi-stable state with preserved normal phenotype (Lin et al., 2009) and cone number and that their function can be maintained independently for a relatively long period (Chrysostomou et al., 2009). Hence, cone rescue has recently been introduced to preserve photopic vision, becoming an important strategy for treatment of RP.

There are different approaches, including pharmacological therapy, gene therapy, cell transplantation and retinal prostheses, for the management of RP. Gene therapy aims to target and replace the mutated genes using viral vectors (Dalkara et al., 2016) and cell transplantation focuses on replacement of the damaged cells by normal cells (Jones et al., 2017). Both procedures address the fundamental cause of RP, but there are limitations in their clinical application. Retinal prostheses use advanced electronic devices to overcome the vision loss (Yue et al., 2016), but this technique is still hindered by the current electronic technology. Most recently, genome editing, which rectifies the disease-causing mutation by means of TALENS or CRISPR-cas (Yanik et al. 2017), is being developed for the treatment of heredity diseases. However, although major advances have been made, such therapies are still far from being available for management of RP. Neuroprotection using antioxidants is widely employed as a pharmacological approach, which aims to delay the progression of retinal degeneration. Although it cannot correct the underlying cause of RP, it is the most acceptable and effective current therapy.

It has been hypothesized that an abnormal high oxidative load may explain damage to cones (Shen et al., 2005). Abnormal neural rewiring (i.e. ectopic synapse) in the RP retina provides evidence of a re-construction of the neural network and this rewiring between cone and rod systems has been shown to lead to an overloading activity on the cone pathway (Ng et al., 2008). It has been suggested that the presence of an ectopic synapse in RP would form a collision circuit, which may corrupt the cone pathway (Marc et al., 2007). Numerous treatments have been suggested to minimize oxidative damage to cones. For example, different mixtures of antioxidants have been suggested as treatments intended to slow the rate of degeneration, thus reducing cone death (Sanz et al., 2007). Neuronal nitric oxide synthase (NOS) has been reported to cause oxidative damage to cones in RP and an NOS-inhibitor has been suggested to reduce cone death (Komeima et al., 2008). Another therapy, using Ciliary Neurotrophic Factor (CNTF), has shown a positive result in a rat RP model, in which the thickness of the retinal layer was increased (Zeiss et al., 2006).

Lycium barbarum L. (LB) (from The Plant List, 2013) is a Solanaceous defoliated shrubbery, also known as Goji berry or Wolfberry, which can be found in Europe and China, used in a traditional Chinese herbal medicine for centuries (Lam and But, 1999). It is recognised as one of the two *Lycium* species having proven pharmacological benefit to our body health (Yao et al., 2018). Traditional use of LB fruits has been shown to help maintain the function of the eyes and replenish the liver and kidneys through balancing “Yin” and “Yang” in the body (Chang and So, 2008; Potterat, 2010), which is attributable to the presence of a very effective antioxidant. Based on recent studies using chromatography analysis, dried fruits comprised of 5-8% Lycium barbarum polysaccharides (LBP) (Jin et al., 2013; Tang et al., 2015) together with other potential beneficial substances including 0.4% flavonoids, 0.4% flavan-3-ols, 1.5% phenolic acids, 0.03% amino acids and derivatives, and 0.1% carotenoids (Protti et al., 2017). The main active antioxidative component in LB is the LBP which are a mixture of six different monosaccharides, including arabinose, galactose, glucose, mannose, rhamnose and xylose (Wang et al., 2009; Tang et al., 2015). The antioxidative effect have been widely shown to provide neuroprotection in various conditions (Chang and So, 2008). Moreover, several studies have demonstrated the beneficial effect of LBP in various animal disease models, eg., protecting the retinal function and retinal ganglion cells after partial optic nerve section (Chu et al., 2013; Li et al., 2015), and preserving the retinal vasculature from retinal ischemia/ reperfusion injury (Li et al., 2011).

Recently, LBP was also reported to protect against the degeneration of photoreceptors in animal RP models (Wang et al., 2014; Zhu et al., 2016). We believe that treatment with LB may also help prevent or delay vision deterioration in the patients with RP.

In this double-masked, placebo-controlled clinical study, we aimed to evaluate the treatment effect of LB over a 12-month period of in RP patients, by conducting several clinical ophthalmic assessments in terms of functional and structural approaches, including visual acuity (VA), Ganzfeld full-field electroretinogram (ffERG), Humphrey Visual Field Analysis (HFA), and Spectral-domain Optical Coherent Tomography (SD-OCT).

2. Materials and Methods

2.1 Drugs

A proprietary extract of *Lycium barbarum* L. granules (batch no.: 90436) and placebo (lactose) granules were prepared by a Traditional Chinese Medicine manufacturer (Eu Yan Sang (Hong Kong) Ltd., Hong Kong) in Hong Kong. According to the technical bulletin, this product was standardized to 3.5% LB polysaccharides as active ingredients and the remaining non-active ingredients was mainly lactose for granulation. The LB of this product was the premium grade "Gonqui (Wolfberry)" sourced from Ningxia province of China. To repeat the experiments of this study, the LB polysaccharides could be extracted by boiling pure fruit of LB at 80^{o}C for 30 minutes and followed by another 30 minutes of soaking, then concentrated into approximately 40% of the original mass by the soaking extracting method (Tian et al., 2017; Xu et al., 2012). Each pack of LB was specified to have 5g (±7%) net weight of granules, and thus each 5g pack of LB granules was estimated to contain about 0. 175g of polysaccharides.

2.2 Subjects

Subjects who met our inclusion criteria[#] were recruited from Retina Hong Kong (a retinal disease patient association in Hong Kong) and the Optometry Clinic at The Hong Kong Polytechnic University. All procedures adhered to the tenets of the Declaration of Helsinki and Toyko for humans, and were approved by the human ethics committee of The Hong Kong

Polytechnic University. All subjects were fully informed of the possible risks and gave written voluntary consent.

Ocular conditions: Retinitis pigmentosa diagnosed by an ophthalmologist and confirmed by visual field testing and full-field ERG. All subjects had: IOP <21 mmHg; van-Herick ratio ≤0.5; no other ocular diseases; ***Dietary conditions:*** Fruit and vegetable intake <10 servings/day; spinach or kale intake ≤1 serving/day; no daily intake of lutein supplement; no intake of cod liver oil or omega-3 capsules; dietary Wolfberry intake ≤10 fruits/week; supplement intake ≤5000 IU/day of Vitamin A and ≤30 IU/day of Vitamin E; alcoholic consumption ≤3 beverages/day; ***Other conditions:*** Age 18 years or above; no intake of any anti-coagulants (especially Warfarin), not pregnant or planning to be pregnant; no smoking; no other clinically significant systemic diseases.

2.3 Protocol

The subjects were randomly assigned (by computer-generated numbers) to either LB (treatment) group or placebo (control) group. The daily dosage by oral administration was 2 packs/day, each containing 5g net weight, for each subject. One pack of granules was ingested after mixing with 200ml of water in the morning and evening. All subjects received sufficient packs (either LB or placebo) for 12 months. A follow-up call was made to subjects to check compliance with taking the assigned treatment, to check for any side-effects 1-2 weeks after beginning the intake and to remind the requirement of dietary conditions regularly during the study. An eye examination was performed before commencing the trial, after 6 months, and at the end of the 12-month study period. At each eye examination, the remaining packs of granules were examined to counter-check compliance and the history of dietary conditions was also recorded. This ensured that all the subjects followed the treatment plan and met the dietary requirements. Figure 1 shows the study design, including when subjects were lost from the study pool.

2.4 Clinical procedures

All eligible subjects had a general eye examination, including visual acuity (VA) using the Early Treatment of Diabetic Retinopathy Study (ETDRS) charts with letters at 90% and 10% contrast levels, refractions, tonometry, external and internal ocular health assessments, and fundus photo-documentation. Three additional tests were conducted to investigate functional and structural changes in the eyes of all patients. Ganzfeld ERG (Espion E3, Diagnosys LLC, Lowell, US) was applied to assess retinal function. The ffERG measurement followed the ISCEV standard (McCulloch et al., 2015). The pupils of the tested eyes were dilated (1% tropicamide) for ffERG measurement. DTL electrodes and gold-cup electrodes were used for recording. Each subject had at least 45 minutes initial dark adaptation before commencement of ERG measurement. Two standard ffERGs (Scotopic (3.0) maximal response and Light-adapted (3.0) cone response) were measured. The 30-2 full threshold visual field test was conducted to investigate the sensitivity of the visual field using the Humphrey Visual Field Analyser (HFA) (Carl Zeiss, Dublin, US). Spectral-domain Optical Coherent Tomography (SD-OCT) (Spectralis, Heidelberg Engineering, US) was used to assess the retinal thickness at the macula.

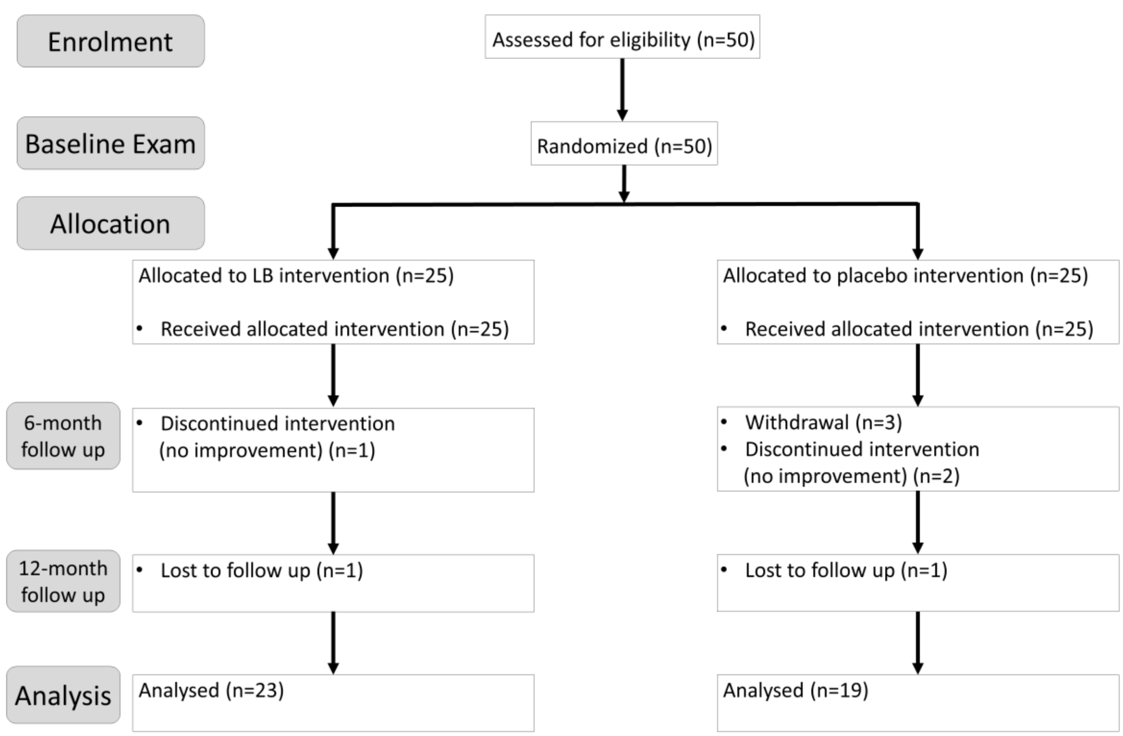

Figure 1. Study design of the clinical study. Visual acuity, visual field, electroretinogram and macular thickness measurements were conducted at baseline examination and two follow up visits at 6 months and 12 months.

The primary outcomes were the best-corrected visual acuities (VA) in 90% contrast (HCVA) and in 10% contrast (LCVA)). The secondary outcomes were the sensitivity in central 30-degree of visual field, the amplitudes of scotopic maximal ERG and photopic cone ERG response, and the average macular thickness.

2.5 Statistical Analysis

The better eye (in terms of HCVA) from each subject at the first visit was selected for the analysis. The HCVA and LCVA in LogMAR, as the primary outcomes, were compared between treatment and placebo groups from the baseline to the first post-treatment (6-month), then the second post-treatment (12-month) visits using repeated-measures Two-way ANOVA with Bonferroni post-hoc adjustment. Similar analysis was also conducted for the secondary outcomes, including scotopic b-wave amplitude, photopic a- and b-wave amplitudes of ffERG, mean defect (MD) of central 30-2 Humphrey visual field test, and macular thickness by SD-OCT. Furthermore, non-parametric partial correlation was used to assess the relationship between the treatment type (treatment group vs. placebo group) and change of the clinical outcomes from baseline to 6- and 12-month follow-up visits respectively, adjusted for the baseline value of that particular test as the co-variate. Because of the multiple comparisons, a statistical correction was made for the partial correlation, in which $p \leq 0.0125$ was considered as statistically significant. For comparison of the compliance between the two groups, $p \leq 0.05$ was considered as statistically significant. All statistical analyses were performed using SPSS (IBM, ver. 22, United States).

3. Results

3.1 Clinical and Demographic Data

A total of 50 eligible RP subjects were recruited and randomly allocated into treatment and placebo groups. Of the original 25 subjects in each group, 23 in the LB group (age: 50.4±12.2year) and 19 in the placebo group (age: 47.7±9.5year) completed the 12-month intervention. The compliance rates for treatment and placebo groups after 12 months were

88.8±8.9% and 85.5±10.2% respectively. No significant difference was found in compliance rate between the two groups ($p>0.05$). Previously reported adverse effects of taking LB were hypoglycaemic or hypolipidemic effects, sensitivity to sunlight, and allergic reaction. However, no such adverse effects were reported in this study. Only 3 cases reported side effects, including mild epistaxis (1 case from treatment group) and thirst (2 cases from placebo group). The characteristics of these 42 subjects at baseline are shown in Table 1. All the results of different outcomes at baseline, 6-month and 12-month intervention are listed in Table 2.

Table 1. Demographic information of the subjects

	Treatment (n = 23)	**Placebo (n = 19)**
Male: Female	9:14	5:14
Age (years)	50.4 (SD: 12.2) (Range: 26-69)	47.7 (SD: 9.5) (Range: 32-57)
Visual Acuity (ETDRS)	HCVA: Range: -0.10 to 1.68 LCVA: Range: 0.06 to 0.86	HCVA: Range: -0.12 to 1.04 LCVA: Range: 0.12 to 1.18
Visual Field (MD) (dB)	Range: -3.15 to -32.06	Range: -7.43 to -32.25
Scotopic maximal response (b-wave) (µV)	Range: 2.05 to 314.40	Range: 2.49 to 202.30
Photopic cone response (µV)	a-wave: Range: 0.50 to 13.22 b-wave: Range: 3.11 to 41.75	a-wave: Range: 0.90 to 12.35 b-wave: Range: 3.06 to 58.56
Macular thickness (µm)	Range: 118 to 360	Range: 96 to 244
Compliance (after 12 months) (%)	88.8 (SD: 8.9)	85.5 (SD: 10.2)
Reported side effect(s) (within 12 months)	Mild epistaxis (1 case)	Thirst (2 cases)

Table 2. Results of the different outcomes at 6-month and 12-month interventions

		Treatment (n = 23)	**Placebo (n = 19)**
Visual Acuity (ETDRS)	baseline	HCVA: 0.30 (SD: 0.44) LCVA: 0.34 (SD: 0.25)	HCVA: 0.50(SD: 0.34) LCVA: 0.67 (SD: 0.29)
	6-month intervention	HCVA: 0.26 (SD: 0.43) LCVA: 0.30 (SD: 0.22)	HCVA: 0.61 (SD: 0.30) LCVA: 0.74 (SD: 0.37)
	12-month intervention	HCVA: 0.25 (SD: 0.45) LCVA: 0.27(SD: 0.21)	HCVA: 0.63 (SD: 0.33) LCVA: 0.75 (SD: 0.38)
Visual Field (MD) (dB)	baseline	-25.84 (SD: 7.75)	-29.06 (SD: 7.20)
	6-month intervention	-25.62 (SD: 8.66)	-29.63 (SD: 6.10)
	12-month intervention	-25.52 (SD: 8.91)	-29.01 (SD: 7.07)
Scotopic maximal response (µV)	baseline	35.85 (SD: 72.8)	23.60 (SD: 59.34)
	6-month intervention	39.03 (SD: 76.47)	23.31 (SD: 57.30)
	12-month intervention	37.30 (SD: 80.72)	27.06 (SD: 62.50)
Photopic cone response (µV)	baseline	a-wave: 3.92 (SD: 6.11) b-wave: 13.20 (SD: 19.07)	a-wave: 3.36 (SD: 3.51) b-wave: 9.48 (SD: 16.58)
	6-month intervention	a-wave: 5.00 (SD: 4.22) b-wave: 14.44 (SD: 21.76)	a-wave: 3.16 (SD: 7.60) b-wave: 8.10 (SD: 12.83)
	12-month intervention	a-wave: 4.91 (SD: 7.59) b-wave: 16.27 (SD: 24.46)	a-wave: 2.72 (SD: 3.83) b-wave: 8.07 (SD: 12.35)
Macular thickness (µm)	baseline	226.52 (SD: 52.68)	158.29 (SD: 47.12)
	6-month intervention	228.57 (SD: 53.43)	155.88 (SD: 47.19)
	12-month intervention	229.14 (SD: 54.86)	161.54 (SD: 36.18)

3.2 Primary Outcome

3.2.1 Visual Acuity

The treatment group showed less reduction in visual acuity, compared to the placebo group after both 6-month and 12-month intervention. (Figure 2) Overall, the ANOVA analysis revealed significant differences for both HCVA [Pillai's trace=0.229, $F(2)=5.79$, $p=0.006$] and LCVA [Pillai's trace=0.374, $F(2)=8.36$, $p=0.001$]. Interaction effect between time and treatment type was significant [HCVA: $F(1.36)=7.59$, $p=0.004$; LCVA: $F(1.28)=12.62$, $p<0.001$]. Pairwise comparison did not show a significant time effect in neither treatment nor placebo groups in either HCVA or LCVA (all $p>0.99$), nor a significant difference between the baseline values of treatment and placebo groups ($p=0.11$). However, the treatment group had significantly better HCVA (6-month 0.26±0.43 vs. 0.61±0.30, $p=0.01$; 12-month 0.25±0.45 vs. 0.64±0.33, $p=0.004$) and LCVA (6-month 0.30±0.22 vs. 0.74±0.37, $p=0.03$; 12-month 0.27±0.21 vs. 0.75±0.38, $p=0.004$) than the placebo group at both 6- and 12-month follow-ups. (Note: the smaller the LogMAR VA is, the better the vision is.)

For the change from baseline, the treatment group showed significantly less reduction in HCVA than the placebo group after both 6-month [-0.01±0.05 vs. 0.07±0.10, $\rho(39)=-0.58$, $p=0.001$], and 12-month intervention (-0.02±0.09 vs. 0.11±0.17, $\rho(39)=-0.63$, $p=0.001$) after adjusting for the baseline value. Similarly, the treatment group showed significantly less reduction in LCVA than the placebo group after both 6-month [-0.04±0.07 vs. 0.09±0.15, $\rho(29)=-0.59$, $p=0.001$], and 12-month intervention (-0.06±0.08 vs. 0.11±0.16, $\rho(28)=-0.71$, $p=0.001$) after adjusting for the baseline value. (Figure 2)

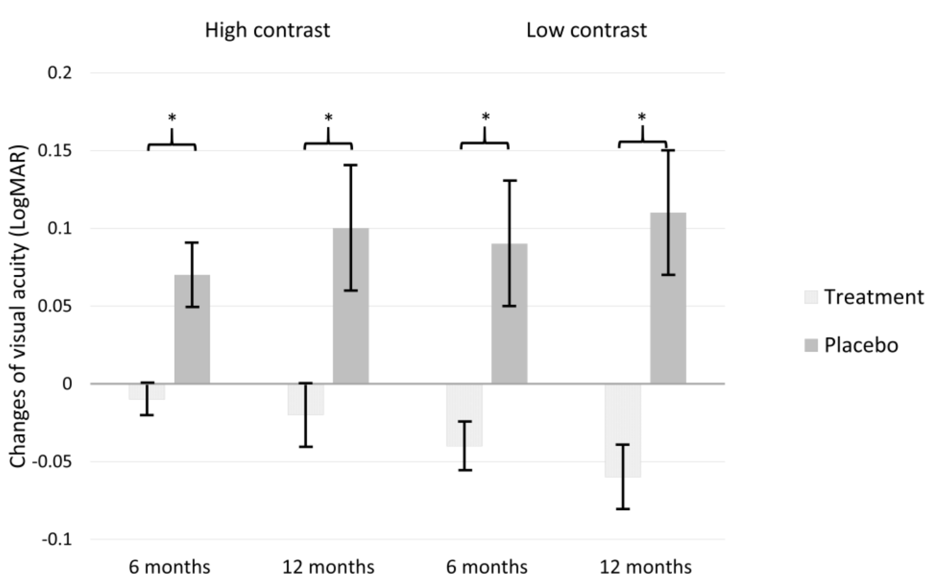

Figure 2. Changes of HCVA and LCVA of the treatment and placebo group after the 6-month and 12-month interventions. The error bars are the standard errors of the mean. (*$p<0.0125$)

3.3 Secondary Outcomes

3.3.1 Visual Field

Two-way ANOVA did not reveal any significant effect of time nor treatment type on MD of central 30-2 [Pillai's trace=0.006, $F(2)=0.09$, $p=0.91$]. There was a slight difference in change in MD between treatment and placebo groups after 6-month (0.22±3.02dB vs. -0.02±0.52dB) and 12-month (0.32±2.93dB vs. -0.16±0.94 dB) intervention, but these did not reach statistical significance [6-month $\rho(34)=-0.07$, p=0.693; 12-month $\rho(30)=-0.10$, $p=0.573$].

3.3.2 Full-field Electroretinogram

The two-way ANOVA did not show a significant result for the scotopic maximal response [Pillai's trace=0.10, $F(2)=1.36$, $p=0.28$], photopic a-wave [Pillai's trace=0.12, $F(2)=1.76$, $p=0.19$], nor photopic b-wave [Pillai's trace=0.16, $F(2)=2.39$, $p=0.11$].

A non-statistically significant improvement in scotopic maximal response, was observed in the b-wave amplitude after 6-month intervention in the treatment group compared to the placebo group [3.17±4.91μV vs. -0.28±3.89μV, $\rho(29)=0.41$, $p=0.02$), which was similar but less pronounced after 12-month intervention [0.08±12.15μV vs. -3.80±7.22μV, $\rho(25)=0.32$, $p=0.11$]. (Figure 3A)

The changes of photopic cone response after 6-month intervention in a-wave amplitude for the treatment and placebo groups were 1.06±2.3μV and -0.20±1.19μV respectively, whilst changes in b-wave amplitude were 1.24±3.85μV and -1.42±3.96μV respectively. Neither of these changes significantly differed between the treatment and placebo groups [a-wave: $\rho(30)=-0.27$, $p=0.14$; b-wave: $\rho(30)=0.22$, $p=0.23$]. Similarly, at 12-month, although changes for both in a-wave and b-wave amplitudes differed between the treatment and placebo groups (a-wave: 0.96±2.44μV vs. -0.91±2.87μV; b-wave: 2.75±9.17μV vs. -4.00±7.38μV), they did not reach statistical significance [a-wave: $\rho(26)=-0.29$, $p=0.13$; b-wave: $\rho(26)=0.41$, $p=0.03$]. (Figure 3B)

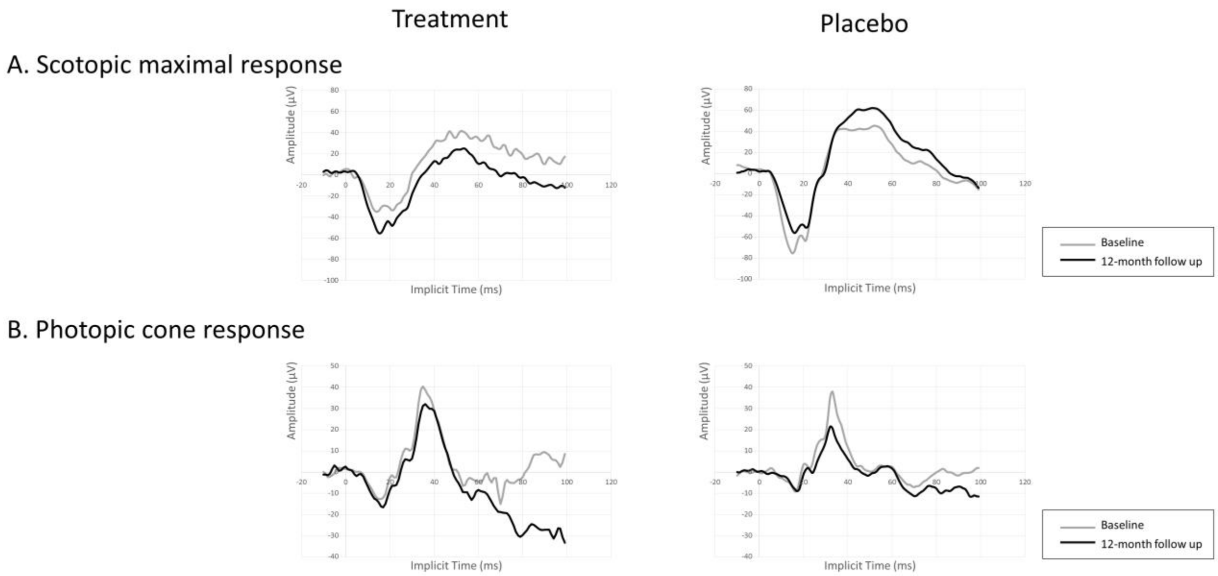

Figure 3. (A) The scotopic maximal responses of typical RP subjects from treatment or placebo group were similar in terms of b-wave amplitude between the baseline and 12-month follow up. (B) The photopic cone responses of a typical RP subject from treatment group were similar in terms of a-wave and b-wave amplitudes between the baseline and 12-month follow up; while the response from another subject from the placebo group showed a mild reduction of b-wave amplitude at 12-month follow up.

3.3.4 Macular thickness

Overall, the two-way ANOVA analysis was significant for macular thickness [Pillai's trace=0.29, F(2)=6.19, p=0.005]. There was significant interaction effect between time and treatment type [F(1.58)=7.95, p=0.002]. Pairwise comparison did not show significant time effect on the macular thickness within group (all *p*>0.99) but showed effect of treatment type after 6-month (228.57±53.42um vs. 155.88±47.19um, *p*=0.008) and 12-month (229.14±54.86um vs. 161.54±36.18um, *p*=0.002) intervention. However, there was a significant difference between groups at baseline (226.52±52.68um vs. 158.29±47.12um, *p*=0.006).

Figure 4 illustrates that the 12-month LB treatment maintained the macular thickness in a subject as compared with another subject in the placebo group whose macular thickness reduced. A significant difference in the change of macular thickness was found between the treatment types (2.62±8.81um vs. -6.36±11.91um) after 12-month intervention [ρ(31)=0.45, *p*=0.008]. There was also a slight difference in change of macular thickness between the treatment types (2.05±4.49um vs. -1.83±8.41um) after 6-month intervention but it did not reach statistical significance [ρ(35)=0.25, *p*=0.132] (Figure 5).

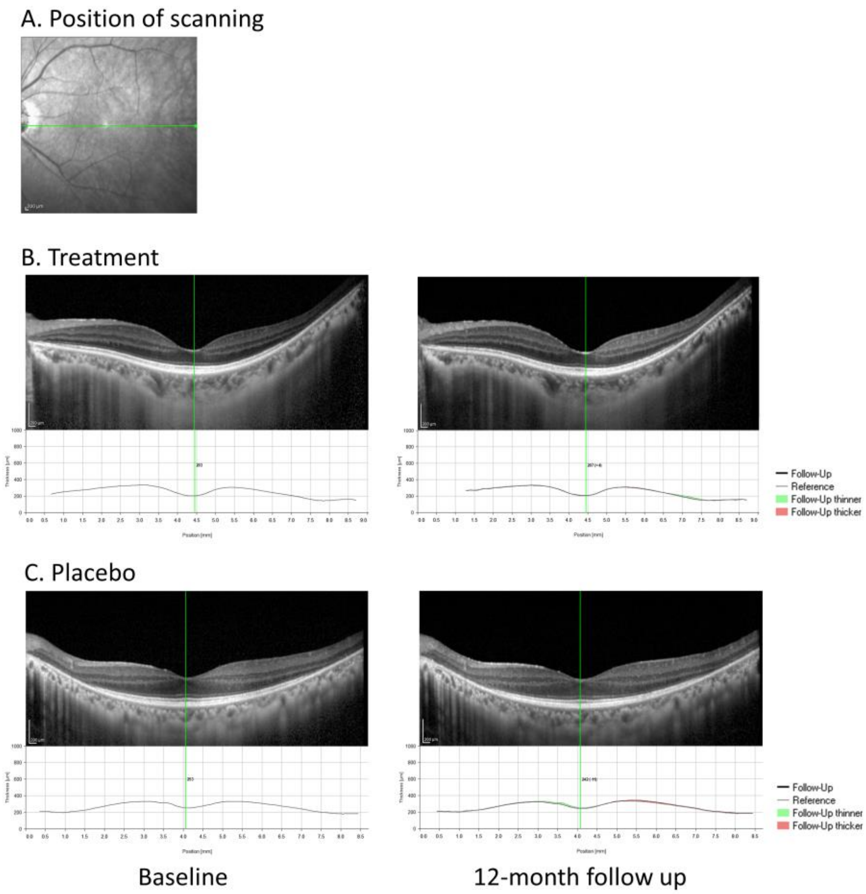

Figure 4. (A) The green line illustrated the position of scanning for the measurement of retinal thickness. (B) In the treatment group, a typical RP subject was seen to maintain the macular retinal thickness between the baseline and 12-month follow up. (C) In the placebo group, a typical RP subject displayed thinning of macular retinal thickness between the baseline and 12-month follow up.

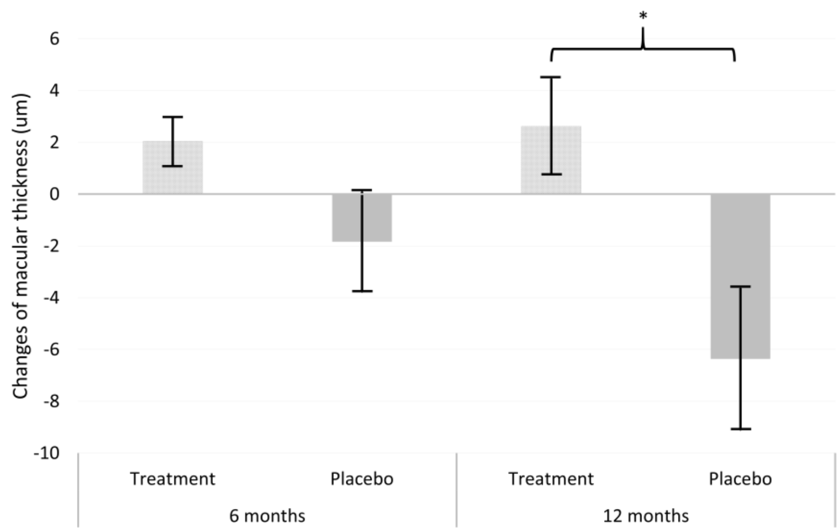

Figure 5. The changes of macular thickness of the treatment and placebo group after the 6-month and 12-month interventions. The error bars are the standard errors of the mean. (*p<0.0125)

4. Discussion

Different neuroprotective agents have been shown to be able to delay the retinal degeneration of RP. They include neurotrophic agents (eg. ciliary neurotrophic factor (CNTF)) (Sahni et al., 2011), anti-inflammatory agents (eg. fluocinolone acetonide) (Glybina et al., 2009) and antioxidants (eg. vitamin A, lutein, DHA, etc.) (Berson et al., 1993, 2010; Hoffman et al., 2015). As many plants, including traditional Chinese herbs, contain antioxidative substances, the use of such herbs has become a popular approach for treatment of RP. In addition, the use of antioxidative supplements can provide a beneficial therapy until the development of other techniques come to fruition as well as offering a relatively inexpensive therapy for use in developing countries where more advanced techniques would be unlikely to be available. Our results suggested that treating RP patients with the extract of *Lycium barbarum* L. over a 12-month period delayed deterioration of vision and retinal thinning in the macular region. The preservation of VA was first detected at the 6 months follow up examination. Cone rescue in the early stage of RP thus appears to be a possible way to preserve vision, especially photopic vision.

Oxidative challenge is a suggested explanation for cone damage in RP. The isomers of lutein and zeaxanthin, which are antioxidants, were recently reported to lower the oxidative stress thereby protecting the photoreceptors in a mouse RP model (Yu et al., 2018). A mixture of anti-oxidants has been even reported to reduce the death of photoreceptors in a mouse RP model with retinal degeneration (Sanz et al., 2007) which is similar to the degeneration observed in RP in humans. RP patients have been reported to have a reduced anti-oxidative status in their eyes (Martínez-Fernández de la Cámara et al., 2013). Collectively, the evidence suggests that oxidative stress is indeed one of the key factors in cone degeneration in RP and reduction of oxidative stress may help delay or minimize cone degeneration, thereby preserving vision in RP patients. Lutein which is an antioxidant, has been reported to slow mid-peripheral visual field loss in RP patients (Berson et al., 2010). LB contains active components, including LBP, which has also been widely reported to have strong antioxidative effects. This further suggests that the LB granules may provide sufficient antioxidative effects to have a beneficial in RP patients.

Our findings reveal that the VA (both high and low contrast) was significantly better preserved in the treatment group than the placebo group. As cone degeneration in RP would cause deterioration of VA, the observed preservation of VA in the treatment group implies that intake of LB provided a neuroprotective effect for the cone cells. Such a protective effect was also evident in the maintenance of electrophysiological responses in the treatment group. Although change in central visual field sensitivity were not significant, general preservation of sensitivity in this region after the LB treatment was observed. Importantly, retinal thickness at the macular region did not show any deterioration after treatment, further illustrating the protective effect of LB, in slowing retinal thinning in RP patients.

The results of our study are comparable with those from other clinical studies (Berson et al., 1993, 2010, 2012; Rotenstreich et al., 2013; Hoffman et al., 2015), which employed treatment with other supplements, including DHA, β-Carotene, Omega 3, Lutein, and Vitamin A or E as antioxidants. Two of our four outcome measures showed significant effects. This suggests that the neuroprotective effect of LB is effective in delaying the deterioration of vision in RP patients.

Lycium barbarum polysaccharide, the most effective antioxidant in LB, has been shown to preserve the photoreceptors against degeneration through anti-oxidative, anti-inflammatory, and anti-apoptotic mechanisms in a mouse RP model (Wang et al., 2014). It appears that LBP can elicit anti-oxidative effects in the eye regardless of the blood–brain barrier or blood–retina barrier (Ho et al., 2007). Apart from the above mentioned properties, LB has also been proposed to act in other ways against cell degeneration. Increase of reactive oxidative species (ROS) has been shown in RP retina (Oka et al., 2008) and *Lycium chinensis* has been reported to reduce cell death by attenuating ROS generation and increasing the antioxidative defence capacity (Olatunji et al., 2016). In addition, changes in the insulin/mechanistic target of rapamycin (mTOR) pathway have been found to delay cone death in RP (Punzo et al., 2009). As LBP has been found to improve insulin resistance activity (which is related to the mTOR pathway) (Zhao et al., 2016). Recently, LBP was reported to reduce the protein levels of procaspase and increase the poly (ADP-ribose) polymerase (PARP) which would attenuate the apoptosis of photoreceptor cells (Zhu et al., 2016). All above findings indicate that LB can protect the cone cells and also contributes to delay of cone death.

The findings from this study indicate that LB is a useful as a supplement to effectively preserve the photopic vision of RP patients, helping to maintain their quality of life. There were, however, several limitations in this study. As the sample size was relatively small, the results may not reflect the experience of the whole RP population, and, as the duration of the treatment was only 12 months, it was not possible to show persistent long-term treatment effects. If the study could be extended to follow up the cases to 6 or 12 months who had chosen not to continue, this will help to confirm the beneficial changes. In addition, as the dose of the active ingredients in the LB granules remains unclear, it is better to repeat the study using the pure compound of those active components to reconfirm the findings. Lastly, the changes in VA and macular retinal thickness though reaching statistical significance were and may not be clinically significant. The genotypes of the RP subjects were also not identified in this study and thus any differences in the treatment effect of LB between genotypes could not be determined. Hence, a large-scale longitudinal study is necessary to further investigate the long-term protective effect of LB in RP patients with different genotypes.

5. Conclusions

Our results demonstrated that a 12-month treatment of RP patients with *Lycium barbarum* L. was able to preserve visual acuity and macular structure. Its neuroprotective effect is believed to delay or minimize the deterioration of central visual function. Treatment with *Lycium barbarum* L. is believed to be a potential supplement to protect retinal functions in RP patients helping to and thereby maintain photopic vision.

Acknowledgement: This work was supported by Health and Medical Research Fund (01121876, Department of Health, HKSAR) and PolyU Internal Grants (G-YBBS, G-YBGS, Z-0GF). We thank for Retina Hong Kong for the help in the recruitment of subjects; Eu Yan Sang (Hong Kong) Ltd. for supporting the Lycium barbarum supplement; Dr. CP Leung (Chinese Medical Practitioner) for providing the advice/consultation in the use of Traditional Chinese Medicine.

Authors' contributions: Chan HHL, So KF, Chang RCC and Lai J designed the study. Lam HI recruited and selected the subjects. Lai J confirmed the ocular condition of each subject. Lam HI, Choi KY, Li SZC and Yu WY conducted the eye examinations. Chan HHL and Lakshmanan Y allocated the supplement of LB and placebo to subjects. Lam HI checked the compliance and all the data obtained. Choi KY performed the statistical analysis. Chan HHL and Yu WY prepared the manuscript.

Glossary:

CNTF	Ciliary Neurotrophic Factor
DHA	Docosahexaenoic acid
ERG	Electroretinogram
ETDRS	Early Treatment of Diabetic Retinopathy Study
ffERG	Full-field electroretinogram
HFA	Humphrey Visual Field Analyzer

IOP	Intra-ocular pressure
LB	Lycium barbarum
mTOR	Insulin/mechanistic target of rapamycin
NOS	Nitric oxide synthase
OCT	Optical Coherence Tomography
PARP	Poly (ADP-ribose) polymerase
ROS	Reactive oxidative species
RP	Retinitis Pigmentosa
VA	Visual acuity
VF	Visual field

References

Berson, E.L., Rosner, B., Sandberg, M.A., Hayes, K.C., Nicholson, B.W., Weigel-DiFranco, C., Willett, W.C. 1993. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol* 111, 761-772.

Berson, E.L., Rosner, B., Sandberg, M.A., Weigel-DiFranco, C., Brockhurst, R.J., Hayes, K.C., Johnson, E.J., Anderson, E.J., Johnson, C.A., Gaudio, A.R., Willett, W.C., Schaefer, E.J. 2010. Clinical trial of lutein in patients with retinitis pigmentosa receiving vitamin A. *Arch Ophthalmol* 128, 403-411.

Berson, E.L., Rosner, B., Sandberg, M.A., Weigel-DiFranco, C., Willett, W.C. 2012. ω-3 intake and visual acuity in patients with retinitis pigmentosa receiving vitamin A. *Arch Ophthalmol* 130, 707-711.

Chang, R.C., So, K.F. 2008. Use of anti-aging herbal medicine, Lycium barbarum, against aging-associated diseases. What do we know so far? *Cell Mol Neurobiol* 28, 643-652.

Chrysostomou, V., Stone, J., Valter, K. 2009. Life history of cones in the rhodopsin-mutant P23H-3 rat: evidence of long-term survival. *Invest Ophthalmol Vis Sci* 50, 2407-2416.

Chu, P.H.W., Li, H.Y., Chin, M.P., So, K.F., Chan H.H.L. 2013. Effect of *Lycium Barbarum* (Wolfberry) *Polysaccharides* on Preserving Retinal Function after Partial Optic Nerve Transection. *PLoS One* 8, e81339.

Dalkara, D., Goureau, O., Marazova, K., Sahel, J.A. 2016. Let there Be light: gene and cell thera-py for blindness. *Hum Gene Ther* 27, 134–147.

Glybina, I.V., Kennedy, A., Ashton, P., Abrams, G.W., Iezzi, R. 2009. Photoreceptor neuroprotection in RCS rats via low-dose intravitreal sustained-delivery of fluocinolone acetonide. *Invest Ophthalmol Vis Sci* 50, 4847–4857.

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 Res* 1158, 123-134.

Hoffman, D.R., Hughbanks-Wheaton, D.K., Spencer, R., Fish, G.E., Pearson, N.S., Wang, Y.Z., Klein, M., Takacs, A., Locke, K.G., Birch, D.G. 2015. Docosahexaenoic Acid Slows Visual Field Progression in X-Linked Retinitis Pigmentosa: Ancillary Outcomes of the DHAX Trial. *Invest Ophthalmol Vis Sci* 56, 6646-6653.

Jin, M.L., Huang, Q.S., Zhao, K., Shang, P. 2013. Biological activities and potential health benefit effects of polysaccharide isolated from Lycium barbarum L. *Int J Biol Macromol* 54, 16–23.

Jones, M.K., Lu, B., Girman, S., Wang, S. 2017. Cell-based therapeutic strategies for replacement and preservation in retinal degenerative diseases. *Prog Retin Eye Res* 58, 1–27.

Komeima, K., Usui, S., Shen, J., Rogers, B.S., Campochiaro, P.A. 2008. Blockade of neuronal nitric oxide synthase reduces cone cell death in a model of retinitis pigmentosa. *Free Radic Biol Med* 45, 905-912.

Lam, K.W., But, P. 1999. The content of zeaxanthin in Gou Qi Zi, a potential health benefit to improve visual activity. *Food Chem* 67, 173-176.

Li, H.Y., Ruan, Y.W., Kau, P.W.F., Chiu, K., Chang, R.C., Chan, H.H., So, K.F. 2015. Effect of *Lycium Barbarum* (wolfberry) on Alleviating Axonal Degeneration after Partial Optic Nerve Transection. *Cell Transplant* 24, 403-417.

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

Lin, B., Masland, R.H., Strettoi, E. 2009. Remodeling of cone photoreceptor cells after rod degeneration in rd mice. *Exp Eye Res* 88, 589-599.

Marc, R.E., Jones, B.W., Anderson, J.R., Kinard, K., Marshak, D.W., Wilson, J.H., Wensel, T., Lucas, R.J. 2007. Neural reprogramming in retinal degeneration. *Invest Ophthalmol Vis Sci* 48, 3364-3371.

Martínez-Fernández de la Cámara, C., Salom, D., Sequedo, M.D., Hervás, D., Marín-Lambíes, C., Aller, E., Jaijo, T., Díaz-LLopis, M., Millán, J.M., Rodrigo, R. 2013. Altered antioxidant-oxidant status in the aqueous humor and peripheral blood of patients with retinitis pigmentosa. *PLoS One* 8, e74223.

McCulloch, D.L., Marmor, M.F., Brigell, M.G., Hamilton, R., Holder, G.E., Tzekov, R., Bach, M. 2015. ISCEV standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol* 130, 1-12.

Ng, Y.F., Chan, H.H., Chu, P.H., To, C.H., Gilger, B.C., Petters, R.M., Wong, F. 2008. Multifocal Electroretinogram in Rhodopsin P347L Transgenic Pigs. *Invest Ophthalmol Vis Sci* 49, 2208-2215.

Oka, S., Ohno, M., Tsuchimoto, D., Sakumi, K., Furuichi, M., Nakabeppu, Y. 2008. Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. *EMBO J* 27, 421-432.

Olatunji, O.J., Chen, H., Zhou, Y. 2016. Lycium chinensis Mill attenuates glutamate induced oxidative toxicity in PC12 cells by increasing antioxidant defense enzymes and down regulating ROS and Ca(2+) generation. *Neurosci Lett* 616, 111-118.

Potterat, O. 2010. Goji (Lycium barbarum and L. chinense): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Med* 76, 7–19

Protti, M., Gualandi, I., Mandrioli, R., Zappoli, S., Tonelli, D., Mercolini, L. 2017. Analytical profiling of selected antioxidants and total antioxidant capacity of goji (Lycium spp.) berries. *J Pharm Biomed Anal* 143, 252-260.

Punzo, C., Kornacker, K., Cepko, C.L. 2009. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci* 12, 44-52.

Rotenstreich, Y., Belkin, M., Sadetzki, S., Chetrit, A., Ferman-Attar, G., Sher, I., Harari, A., Shaish, A., Harats, D. 2013. Treatment with 9-cis β-carotene-rich powder in patients with retinitis pigmentosa: a randomized crossover trial. *JAMA Ophthalmol* 131, 985-992.

Sahni, J.N., Angi, M., Irigoyen, C., Semeraro, F., Romano, M.R., Parmeggiani, F. 2011. Therapeutic challenges to retinitis pigmentosa: from neuroprotection to gene therapy. *Curr Genomics* 12, 276–284.

Sanz, M.M., Johnson, L.E., Ahuja, S., Ekström, P.A., Romero, J., van Veen, T. 2007. Significant photoreceptor rescue by treatment with a combination of antioxidants in an animal model for retinal degeneration. *Neurosci* 145, 1120-1129.

Shen, J., Yang, X., Dong, A., Petters, R.M., Peng, Y.W., Wong, F., Campochiaro, P.A. 2005. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol* 203, 457-464.

Tang, H.L., Chen, C., Wang, S.K., Sun, G.J. 2015. Biochemical analysis and hypoglycemic activity of a polysaccharide isolated from the fruit of Lycium barbarum L. *Int J Biol Macromol* 77, 235–242.

Tian, X.J., Jing, B.Y., Wang, C.X., Lu, H.N., Chen S.E. 2017. Research progress on extraction methods of Lycium barbarum polysaccharides. *J Food Saf Qual* 8, 439-445.

The Plant List, 2013. (www.theplantlist.org)

Wang, C.C., Chang, S.C., Chen, B.H. 2009. Chromatographic determination of poly-saccharides in Lycium barbarum Linnaeus. *Food Chem* 116, 595–603.

Wang, K., Xiao, J., Peng, B., Xing, F., So, K.F., Tipoe, G.L., Lin, B. 2014. Retinal structure and function preservation by polysaccharides of wolfberry in a mouse model of retinal degeneration. *Sci Rep* 4, 7601.

Xu, C., Guo, W., Zhao, Y., Tan, Y., You, Y., Zhou, H. 2012. Extraction and decolouration technology for polysaccharide from Lycium barbarum in XinJiang. *J Anhui Agric Sci* 40, 2887-2889.

Yanik, M., Muller, B., Song, F., Gall, J., Wagner, F., Wende, W., Lorenz, B., Stieger, K. 2017. In vivo genome editing as a potential treatment strategy for inherited retinal dystrophies. *Prog Retin Eye Res* 56, 1–18.

Yao, R., Heinrich, M., Weckerle, C.S. 2018. The genus Lycium as food and medicine: A botanical, ethnobotanical and historical review. *J Ethnopharmacol* 212, 50-66.

Yu, M., Yan, W., Beight, C. 2018. Lutein and zeaxanthin isomers reduce photoreceptor degeneration in the $Pde6b^{rd10}$ mouse model of retinitis pigmentosa. *Biomed Res Int* 2018:4374087.

Yue, L., Weiland, J.D., Roska, B., Humayun, M.S. 2016. Retinal stimulation strategies to restore vision: fundamentals and systems. *Prog Retin Eye Res* 53, 21–47.

Zeiss, C.J., Allore, H.G., Towle, V., Tao, W. 2006. CNTF induces dose-dependent alterations in retinal morphology in normal and rcd-1 canine retina. *Exp Eye Res* 82, 395-404.

Zhao, R., Gao, X., Zhang, T., Li, X. 2016. Effects of Lycium barbarum polysaccharide on type 2 diabetes mellitus rats by regulating biological rhythms. *Iran J Basic Med Sci* 19, 1024-1030.

Zhu, Y., Zhao, Q., Gao, H., Peng, X., Wen, Y., Dai, G. 2016. Lycium barbarum polysaccharides attenuates N-methy-N-nitrosourea-induced photoreceptor cell apoptosis in rats through regulation of poly (ADP-ribose) polymerase and caspase expression. *J Ethnopharmacol* 191, 125-134.

Figures/Table Captions

Figure 1. Study design of the clinical study. Visual acuity, visual field, electroretinogram and macular thickness measurements were conducted at baseline examination and two follow up visits at 6 months and 12 months.

Figure 2. Changes of HCVA and LCVA from the treatment and placebo group after the 6-month and 12-month interventions. The error bars are the standard errors of the mean. (*$p<0.0125$)

Figure 3. (A) The scotopic maximal responses of typical RP subjects from treatment or placebo group were similar in terms of b-wave amplitude between the baseline and 12-month follow up. (B) The photopic cone responses of a typical RP subject from treatment group were similar in terms of a-wave and b-wave amplitudes between the baseline and 12-month follow up; while the response from another subject from placebo group showed a mild reduction of b-wave amplitude at 12-month follow up.

Figure 4. (A) The green line illustrated the position of scanning for the measurement of retinal thickness. (B) In the treatment group, a typical RP subject was found to maintain the macular retinal thickness between the baseline and 12-month follow up. (C) In the placebo group, a typical RP subject was found to have the thinning of macular retinal thickness between the baseline and 12-month follow up.

Figure 5. The changes of macular thickness from the treatment and placebo group after the 6-month and 12-month interventions. The error bars are the standard errors of the mean. (*$p<0.0125$)

Table 1. Demographic information of the subjects

Table 2. Results of the different outcomes at 6-month and 12-month interventions