

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

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

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7 ^aLaboratory of Experimental Optometry (Neuroscience), School of Optometry, The Hong Kong
8 Polytechnic University, Hong Kong SAR, China.

9 ^bLaboratory of Neurodegenerative Diseases, School of Biomedical Sciences, The University of
10 Hong Kong, Hong Kong SAR, China

11 ^cDepartment of Ophthalmology, The University of Hong Kong, Hong Kong SAR, China

12 ^dGuangdong-Hongkong-Macau (GHM) Institute of CNS Regeneration, Jinan University,
13 Guangzhou, China

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16 **Declarations of interest:** None

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20 **Corresponding author:**

21 Dr. Henry H.L. Chan

22 School of Optometry,

23 The Hong Kong Polytechnic University,

24 Hung Hom, Kowloon,

25 Hong Kong SAR.

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

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28

29 Abstract**30 Ethnopharmacological relevance:**

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

37 Aim of the study:

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

41 Methods:

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

49 Results:

50 The compliance rates for both groups exceeded 80%. There were no deteriorations of either
51 90% or 10% contrast VA in the LB group compared with the placebo group ($p=0.001$). A thinning
52 of macular layer was observed in the placebo group, which was not observed in the LB group
53 ($p=0.008$). However, no significant differences were found in the sensitivity of visual field or in
54 any parameters of ffERG between the two groups. No significant adverse effects were reported
55 in the treatment group.

56 Conclusions:

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

59

60 **Classifications:** Clinical Studies (1.05)

61 **Keywords:** Clinical Trial (2.172), Traditional Chinese Medicine (2.592), Antioxidant (2.084),

62 **Specific keywords:** Lycium barbarum, Retinitis pigmentosa, Retinal degeneration,

63 Neuroprotection

64

65

66 1. Introduction

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

75

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

90

91 It has been hypothesized that an abnormal high oxidative load may explain damage to cones
92 (Shen et al., 2005). Abnormal neural rewiring (i.e. ectopic synapse) in the RP retina provides
93 evidence of a re-construction of the neural network and this rewiring between cone and rod

94 systems has been shown to lead to an overloading activity on the cone pathway (Ng et al.,
95 2008). It has been suggested that the presence of an ectopic synapse in RP would form a
96 collision circuit, which may corrupt the cone pathway (Marc et al., 2007). Numerous treatments
97 have been suggested to minimize oxidative damage to cones. For example, different mixtures
98 of antioxidants have been suggested as treatments intended to slow the rate of degeneration,
99 thus reducing cone death (Sanz et al., 2007). Neuronal nitric oxide synthase (NOS) has been
100 reported to cause oxidative damage to cones in RP and an NOS-inhibitor has been suggested to
101 reduce cone death (Komeima et al., 2008). Another therapy, using Ciliary Neurotrophic Factor
102 (CNTF), has shown a positive result in a rat RP model, in which the thickness of the retinal layer
103 was increased (Zeiss et al., 2006).

104

105 *Lycium barbarum* L. (LB) (from The Plant List, 2013) is a Solanaceous defoliated shrubbery, also
106 known as Goji berry or Wolfberry, which can be found in Europe and China, used in a traditional
107 Chinese herbal medicine for centuries (Lam and But, 1999). It is recognised as one of the two
108 *Lycium* species having proven pharmacological benefit to our body health (Yao et al., 2018).
109 Traditional use of LB fruits has been shown to help maintain the function of the eyes and
110 replenish the liver and kidneys through balancing “Yin” and “Yang” in the body (Chang and So,
111 2008; Potterat, 2010), which is attributable to the presence of a very effective antioxidant.
112 Based on recent studies using chromatography analysis, dried fruits comprised of 5-8% *Lycium*
113 *barbarum* polysaccharides (LBP) (Jin et al., 2013; Tang et al., 2015) together with other
114 potential beneficial substances including 0.4% flavonoids, 0.4% flavan-3-ols, 1.5% phenolic acids,
115 0.03% amino acids and derivatives, and 0.1% carotenoids (Protti et al., 2017). The main active
116 antioxidative component in LB is the LBP which are a mixture of six different monosaccharides,
117 including arabinose, galactose, glucose, mannose, rhamnose and xylose (Wang et al., 2009;
118 Tang et al., 2015). The antioxidative effect have been widely shown to provide neuroprotection
119 in various conditions (Chang and So, 2008). Moreover, several studies have demonstrated the
120 beneficial effect of LBP in various animal disease models, eg., protecting the retinal function and
121 retinal ganglion cells after partial optic nerve section (Chu et al., 2013; Li et al., 2015), and
122 preserving the retinal vasculature from retinal ischemia/ reperfusion injury (Li et al., 2011).

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

126
127 In this double-masked, placebo-controlled clinical study, we aimed to evaluate the treatment
128 effect of LB over a 12-month period of in RP patients, by conducting several clinical ophthalmic
129 assessments in terms of functional and structural approaches, including visual acuity (VA),
130 Ganzfeld full-field electroretinogram (ffERG), Humphrey Visual Field Analysis (HFA), and
131 Spectral-domain Optical Coherent Tomography (SD-OCT).

132

133 **2. Materials and Methods**

134 *2.1 Drugs*

135 A proprietary extract of *Lycium barbarum* L. granules (batch no.: 90436) and placebo (lactose)
136 granules were prepared by a Traditional Chinese Medicine manufacturer (Eu Yan Sang (Hong
137 Kong) Ltd., Hong Kong) in Hong Kong. According to the technical bulletin, this product was
138 standardized to 3.5% LB polysaccharides as active ingredients and the remaining non-active
139 ingredients was mainly lactose for granulation. The LB of this product was the premium grade
140 “Gonqui (Wolfberry)” sourced from Ningxia province of China. To repeat the experiments of
141 this study, the LB polysaccharides could be extracted by boiling pure fruit of LB at 80°C for 30
142 minutes and followed by another 30 minutes of soaking, then concentrated into approximately
143 40% of the original mass by the soaking extracting method (Tian et al., 2017; Xu et al., 2012).
144 Each pack of LB was specified to have 5g ($\pm 7\%$) net weight of granules, and thus each 5g pack of
145 LB granules was estimated to contain about 0.175g of polysaccharides.

146

147 *2.2 Subjects*

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

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

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

162

163 *2.3 Protocol*

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

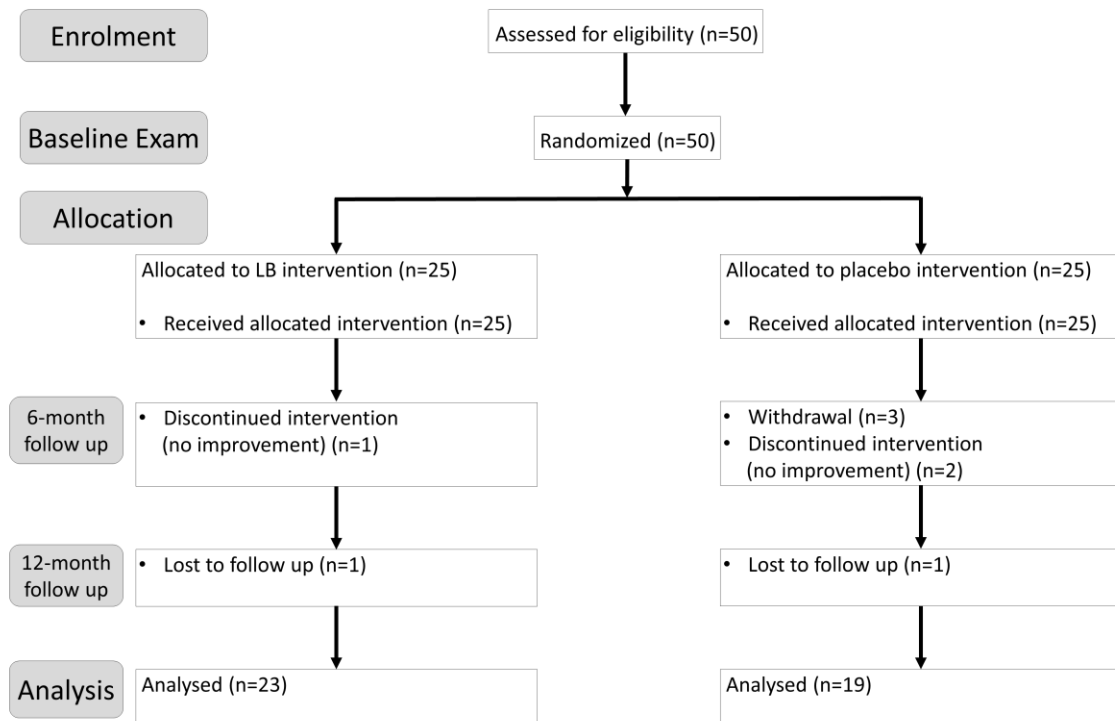
177

178 *2.4 Clinical procedures*

179 All eligible subjects had a general eye examination, including visual acuity (VA) using the Early
180 Treatment of Diabetic Retinopathy Study (ETDRS) charts with letters at 90% and 10% contrast
181 levels, refractions, tonometry, external and internal ocular health assessments, and fundus

182 photo-documentation. Three additional tests were conducted to investigate functional and
 183 structural changes in the eyes of all patients. Ganzfeld ERG (Espion E3, Diagnosys LLC, Lowell,
 184 US) was applied to assess retinal function. The ffERG measurement followed the ISCEV standard
 185 (McCulloch et al., 2015). The pupils of the tested eyes were dilated (1% tropicamide) for ffERG
 186 measurement. DTL electrodes and gold-cup electrodes were used for recording. Each subject
 187 had at least 45 minutes initial dark adaptation before commencement of ERG measurement.
 188 Two standard ffERGs (Scotopic (3.0) maximal response and Light-adapted (3.0) cone response)
 189 were measured. The 30-2 full threshold visual field test was conducted to investigate the
 190 sensitivity of the visual field using the Humphrey Visual Field Analyser (HFA) (Carl Zeiss, Dublin,
 191 US). Spectral-domain Optical Coherent Tomography (SD-OCT) (Spectralis, Heidelberg
 192 Engineering, US) was used to assess the retinal thickness at the macula.

193



194

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

198

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

203

204 *2.5 Statistical Analysis*

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

220

221 **3. Results**

222 *3.1 Clinical and Demographic Data*

223 A total of 50 eligible RP subjects were recruited and randomly allocated into treatment and
224 placebo groups. Of the original 25 subjects in each group, 23 in the LB group (age:
225 50.4 ± 12.2 year) and 19 in the placebo group (age: 47.7 ± 9.5 year) completed the 12-month
226 intervention. The compliance rates for treatment and placebo groups after 12 months were

227 88.8±8.9% and 85.5±10.2% respectively. No significant difference was found in compliance rate
 228 between the two groups ($p>0.05$). Previously reported adverse effects of taking LB were
 229 hypoglycaemic or hypolipidemic effects, sensitivity to sunlight, and allergic reaction. However,
 230 no such adverse effects were reported in this study. Only 3 cases reported side effects, including
 231 mild epistaxis (1 case from treatment group) and thirst (2 cases from placebo group). The
 232 characteristics of these 42 subjects at baseline are shown in Table 1. All the results of different
 233 outcomes at baseline, 6-month and 12-month intervention are listed in Table 2.

234

235 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) (μ V)	Range: 2.05 to 314.40	Range: 2.49 to 202.30
Photopic cone response (μ V)	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 (μ m)	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)

236

237

238 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 (μ V)	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 (μ V)	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 (μ m)	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)

239

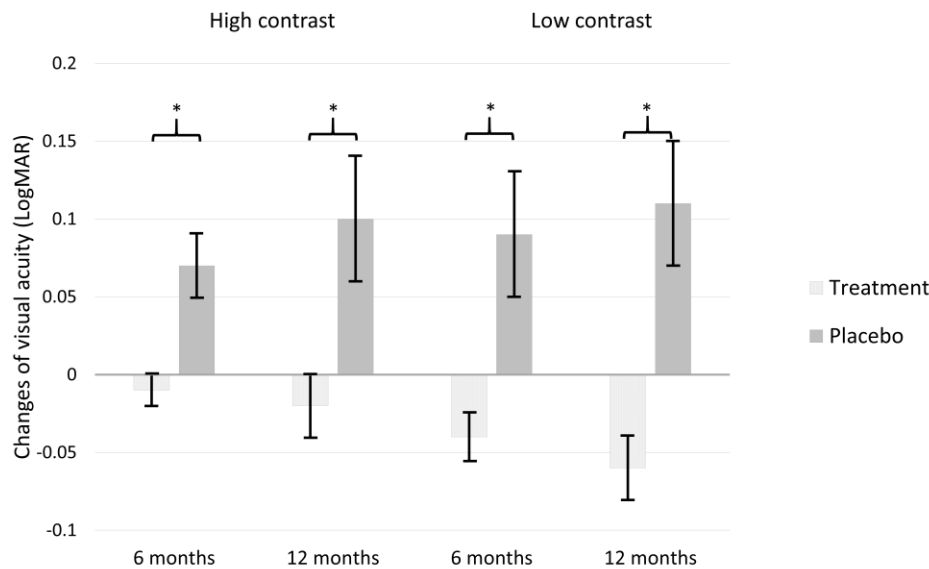
240 *3.2 Primary Outcome*241 *3.2.1 Visual Acuity*

242 The treatment group showed less reduction in visual acuity, compared to the placebo group
243 after both 6-month and 12-month intervention. (Figure 2) Overall, the ANOVA analysis revealed
244 significant differences for both HCVA [Pillai's trace=0.229, $F(2)=5.79$, $p=0.006$] and LCVA [Pillai's
245 trace=0.374, $F(2)=8.36$, $p=0.001$]. Interaction effect between time and treatment type was
246 significant [HCVA: $F(1,36)=7.59$, $p=0.004$; LCVA: $F(1,28)=12.62$, $p<0.001$]. Pairwise comparison

247 did not show a significant time effect in neither treatment nor placebo groups in either HCVA or
 248 LCVA (all $p>0.99$), nor a significant difference between the baseline values of treatment and
 249 placebo groups ($p=0.11$). However, the treatment group had significantly better HCVA (6-month
 250 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-
 251 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
 252 placebo group at both 6- and 12-month follow-ups. (Note: the smaller the LogMAR VA is, the
 253 better the vision is.)

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

261



262

263 Figure 2. Changes of HCVA and LCVA of the treatment and placebo group after the 6-month and
 264 12-month interventions. The error bars are the standard errors of the mean. (* $p<0.0125$)

265

266 3.3 Secondary Outcomes

267 3.3.1 Visual Field

268 Two-way ANOVA did not reveal any significant effect of time nor treatment type on MD of
269 central 30-2 [Pillai's trace=0.006, $F(2)=0.09$, $p=0.91$]. There was a slight difference in change in
270 MD between treatment and placebo groups after 6-month ($0.22\pm 3.02\text{dB}$ vs. $-0.02\pm 0.52\text{dB}$) and
271 12-month ($0.32\pm 2.93\text{dB}$ vs. $-0.16\pm 0.94\text{dB}$) intervention, but these did not reach statistical
272 significance [6-month $\rho(34)=-0.07$, $p=0.693$; 12-month $\rho(30)=-0.10$, $p=0.573$].

273

274 3.3.2 Full-field Electroretinogram

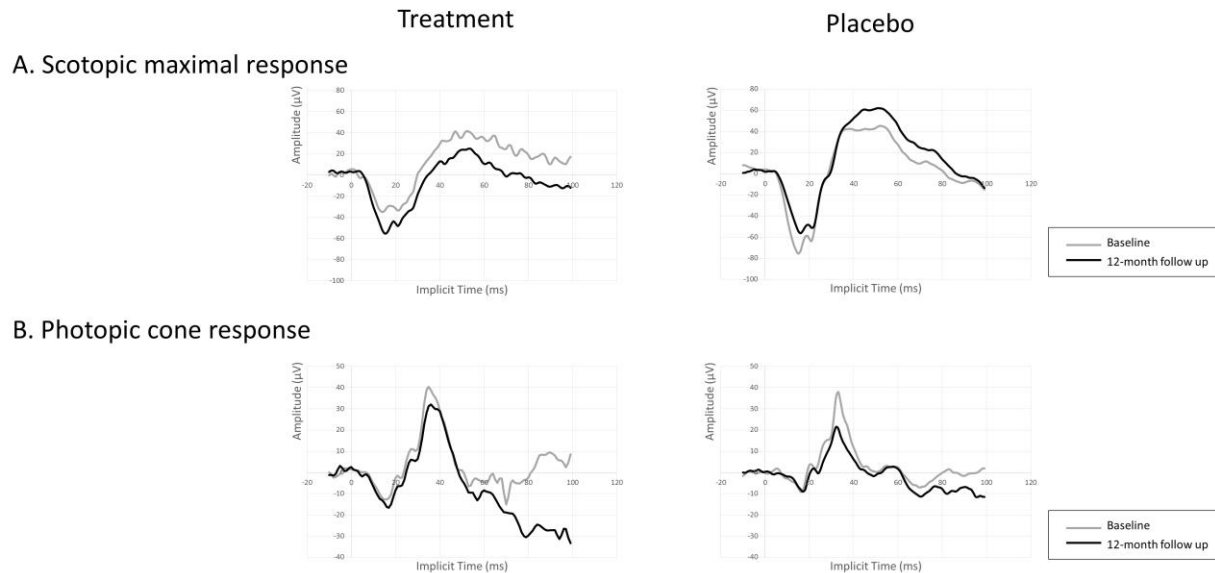
275 The two-way ANOVA did not show a significant result for the scotopic maximal response [Pillai's
276 trace=0.10, $F(2)=1.36$, $p=0.28$], photopic a-wave [Pillai's trace=0.12, $F(2)=1.76$, $p=0.19$], nor
277 photopic b-wave [Pillai's trace=0.16, $F(2)=2.39$, $p=0.11$].

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

283

284 The changes of photopic cone response after 6-month intervention in a-wave amplitude for the
285 treatment and placebo groups were $1.06\pm 2.3\mu\text{V}$ and $-0.20\pm 1.19\mu\text{V}$ respectively, whilst changes
286 in b-wave amplitude were $1.24\pm 3.85\mu\text{V}$ and $-1.42\pm 3.96\mu\text{V}$ respectively. Neither of these
287 changes significantly differed between the treatment and placebo groups [a-wave: $\rho(30)=-0.27$,
288 $p=0.14$; b-wave: $\rho(30)=0.22$, $p=0.23$]. Similarly, at 12-month, although changes for both in a-
289 wave and b-wave amplitudes differed between the treatment and placebo groups (a-wave:
290 $0.96\pm 2.44\mu\text{V}$ vs. $-0.91\pm 2.87\mu\text{V}$; b-wave: $2.75\pm 9.17\mu\text{V}$ vs. $-4.00\pm 7.38\mu\text{V}$), they did not reach
291 statistical significance [a-wave: $\rho(26)=-0.29$, $p=0.13$; b-wave: $\rho(26)=0.41$, $p=0.03$]. (Figure 3B)

292



293

294 Figure 3. (A) The scotopic maximal responses of typical RP subjects from treatment or placebo
 295 group were similar in terms of b-wave amplitude between the baseline and 12-month follow up.

296 (B) The photopic cone responses of a typical RP subject from treatment group were similar in
 297 terms of a-wave and b-wave amplitudes between the baseline and 12-month follow up; while
 298 the response from another subject from the placebo group showed a mild reduction of b-wave
 299 amplitude at 12-month follow up.

300

301 3.3.4 Macular thickness

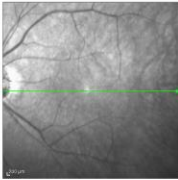
302 Overall, the two-way ANOVA analysis was significant for macular thickness [Pillai's trace=0.29,
 303 $F(2)=6.19$, $p=0.005$]. There was significant interaction effect between time and treatment type
 304 [$F(1.58)=7.95$, $p=0.002$]. Pairwise comparison did not show significant time effect on the
 305 macular thickness within group (all $p>0.99$) but showed effect of treatment type after 6-month
 306 ($228.57\pm 53.42\mu\text{m}$ vs. $155.88\pm 47.19\mu\text{m}$, $p=0.008$) and 12-month ($229.14\pm 54.86\mu\text{m}$ vs.
 307 $161.54\pm 36.18\mu\text{m}$, $p=0.002$) intervention. However, there was a significant difference between
 308 groups at baseline ($226.52\pm 52.68\mu\text{m}$ vs. $158.29\pm 47.12\mu\text{m}$, $p=0.006$).

309

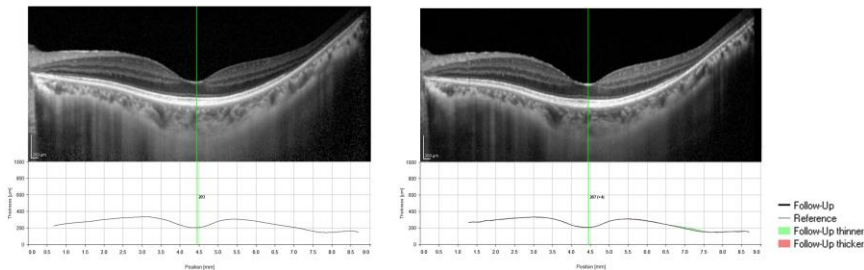
310 Figure 4 illustrates that the 12-month LB treatment maintained the macular thickness in a
 311 subject as compared with another subject in the placebo group whose macular thickness

312 reduced. A significant difference in the change of macular thickness was found between the
 313 treatment types ($2.62 \pm 8.81 \mu\text{m}$ vs. $-6.36 \pm 11.91 \mu\text{m}$) after 12-month intervention [$p(31)=0.45$,
 314 $p=0.008$]. There was also a slight difference in change of macular thickness between the
 315 treatment types ($2.05 \pm 4.49 \mu\text{m}$ vs. $-1.83 \pm 8.41 \mu\text{m}$) after 6-month intervention but it did not
 316 reach statistical significance [$p(35)=0.25$, $p=0.132$] (Figure 5).
 317

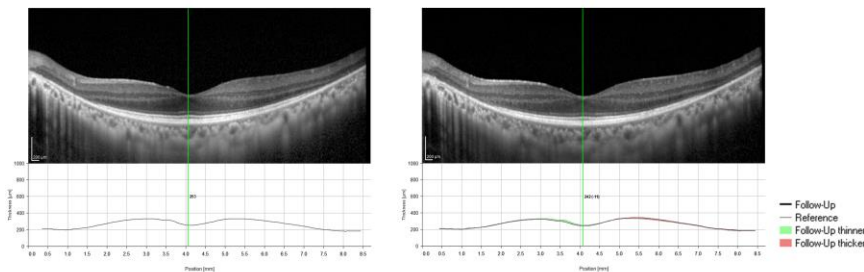
A. Position of scanning



B. Treatment



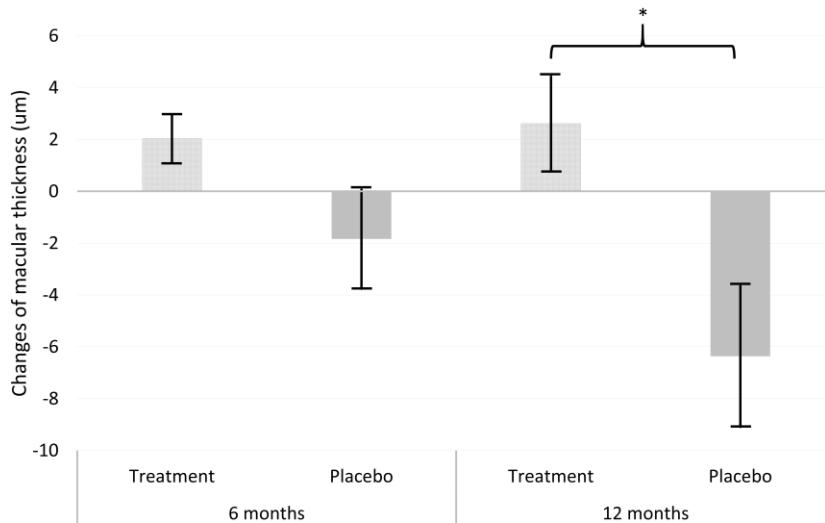
C. Placebo



Baseline

12-month follow up

318
 319 Figure 4. (A) The green line illustrated the position of scanning for the measurement of retinal
 320 thickness. (B) In the treatment group, a typical RP subject was seen to maintain the macular
 321 retinal thickness between the baseline and 12-month follow up. (C) In the placebo group, a
 322 typical RP subject displayed thinning of macular retinal thickness between the baseline and 12-
 323 month follow up.
 324



325

326 Figure 5. The changes of macular thickness of the treatment and placebo group after the 6-
 327 month and 12-month interventions. The error bars are the standard errors of the mean.

328 (* $p < 0.0125$)

329

330 4. Discussion

331 Different neuroprotective agents have been shown to be able to delay the retinal degeneration

332 of RP. They include neurotrophic agents (eg. ciliary neurotrophic factor (CNTF)) (Sahni et al.,

333 2011), anti-inflammatory agents (eg. fluocinolone acetonide) (Glybina et al., 2009) and

334 antioxidants (eg. vitamin A, lutein, DHA, etc.) (Berson et al., 1993, 2010; Hoffman et al., 2015).

335 As many plants, including traditional Chinese herbs, contain antioxidative substances, the use of

336 such herbs has become a popular approach for treatment of RP. In addition, the use of

337 antioxidative supplements can provide a beneficial therapy until the development of other

338 techniques come to fruition as well as offering a relatively inexpensive therapy for use in

339 developing countries where more advanced techniques would be unlikely to be available. Our

340 results suggested that treating RP patients with the extract of *Lycium barbarum* L. over a 12-

341 month period delayed deterioration of vision and retinal thinning in the macular region. The

342 preservation of VA was first detected at the 6 months follow up examination. Cone rescue in the

343 early stage of RP thus appears to be a possible way to preserve vision, especially photopic vision.

344 Oxidative challenge is a suggested explanation for cone damage in RP. The isomers of lutein and
345 zeaxanthin, which are antioxidants, were recently reported to lower the oxidative stress
346 thereby protecting the photoreceptors in a mouse RP model (Yu et al., 2018). A mixture of anti-
347 oxidants has been even reported to reduce the death of photoreceptors in a mouse RP model
348 with retinal degeneration (Sanz et al., 2007) which is similar to the degeneration observed in RP
349 in humans. RP patients have been reported to have a reduced anti-oxidative status in their eyes
350 (Martínez-Fernández de la Cámara et al., 2013). Collectively, the evidence suggests that
351 oxidative stress is indeed one of the key factors in cone degeneration in RP and reduction of
352 oxidative stress may help delay or minimize cone degeneration, thereby preserving vision in RP
353 patients. Lutein which is an antioxidant, has been reported to slow mid-peripheral visual field
354 loss in RP patients (Berson et al., 2010). LB contains active components, including LBP, which
355 has also been widely reported to have strong antioxidative effects. This further suggests that
356 the LB granules may provide sufficient antioxidative effects to have a beneficial in RP patients.
357

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

367
368 The results of our study are comparable with those from other clinical studies (Berson et al.,
369 1993, 2010, 2012; Rotenstreich et al., 2013; Hoffman et al., 2015), which employed treatment
370 with other supplements, including DHA, β -Carotene, Omega 3, Lutein, and Vitamin A or E as
371 antioxidants. Two of our four outcome measures showed significant effects. This suggests that
372 the neuroprotective effect of LB is effective in delaying the deterioration of vision in RP patients.

373

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

389

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

402 longitudinal study is necessary to further investigate the long-term protective effect of LB in RP
403 patients with different genotypes.

404

405 5. Conclusions

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

411

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417

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

424

425 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

426

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- 540

541 **Figures/Table Captions**

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

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

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

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

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

561 Table 1. Demographic information of the subjects

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

563