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Multi-target ocular protection of blueberry anthocyanins in elderly type 2 diabetes: An exploratory clinical investigation

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ABSTRACT: With the increasing prevalence of diabetic retinopathy (DR), there is an urgent need for interventions offering both metabolic and ocular benefits. This initial clinical exploration investigated the intervention effects of blueberry anthocyanins in elderly type 2 diabetic patients over six months. Supplementation with blueberry anthocyanin extract (BAE, 400 mg/day) showed favorable changes in ocular functions, with improved visual acuity (as reflected by negative $\Delta\log\text{MAR}$ values), reduced intraocular pressure (IOP $\sim$ 8%, $P < 0.05$), and restored retinal structural integrity, which was evidenced by decreased macular edema and retinal thickness via optical coherence tomography (OCT) imaging. Furthermore, patients reported a notable alleviation of visual fatigue symptom, such as eye soreness ($P < 0.05$) and lacrimation ($P < 0.01$). Correlating with the observed ocular protection, BAE also improved systemic metabolic parameters such as fasting blood glucose and glycated hemoglobin (HbA1c) ($P < 0.01$), insulin resistance (triglyceride-glucose index), and inflammation markers (such as neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio). These findings should be interpreted as preliminary associations, as concurrent medications were not systematically controlled. Nonetheless, they tentatively indicated the dual action of blueberry anthocyanins for simultaneously targeting metabolic dysregulation and DR-associated visual impairment, providing clinical evidence for its development as a promising functional food used in adjunct therapy for diabetic patients.

Keywords: Blueberry anthocyanins; Diabetic retinopathy; Visual acuity; Intraocular pressure; Metabolic regulation

1. Introduction

The global burden of type 2 diabetes (T2D) has escalated dramatically with population aging and lifestyle changes^[1]. Accounting for over 90% of all diabetes, T2D demonstrates strong age-dependent prevalence with the majority of cases occurring in older adults^[2-4]. According to International Diabetes Federation projections, diabetes prevalence will increase 46% by 2050, affecting 1 in 8 adults worldwide (approximately 853 million) (<https://idf.org/>). This epidemic carries severe ocular consequences, with

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24.7-37.5% of T2D patients developing retinopathy, approximately 8% progressing to vision-threatening stages^[5]. Among diabetes' systemic complications, visual impairment remains particularly devastating due to its irreversible progression and profound quality-of-life impact^[6-9]. Global data from 1990-2020 identify diabetic retinopathy (DR) as one of the four leading causes of blindness, alongside cataract, glaucoma, and age-related macular degeneration^[10].

Diabetic retinopathy is a vision-threatening microvascular complication of diabetes, caused by chronic hyperglycemia-induced damage to retinal vasculature^[11]. Characterized by vascular dilation, leakage, and microaneurysm formation, DR progresses through distinct pathological stages^[12]. The early pathological features of DR primarily involve abnormal changes in the microvascular system, including endothelial cell dysfunction, capillary hypoperfusion, and microaneurysm formation^[13-14]. As the disease progresses to vision-threatening stages, patients may develop macular edema and retinal neovascularization. Current clinical management at advanced stages relies on invasive interventions such as laser therapy, intraocular injections, and ophthalmic surgery^[15-17], which carry risks of side effects and limited efficacy^[18]. Given these therapeutic limitations and growing patient demand for safer alternatives, natural bioactive compounds have emerged as promising candidates for become an important research direction in the field of adjunctive DR prevention and management^[19].

Blueberries are a kind of berry rich in anthocyanins, bioactive compounds with significant pharmacological potential. Anthocyanins, a class of water-soluble flavonoids, exhibit a variety of biological activities, such as potent antioxidant, anti-inflammatory, microcirculation-improving, and vascular endothelial-protective effects^[20-22]. Blueberry anthocyanins demonstrate notable antioxidant properties, scavenging free radicals and mitigating oxidative stress-induced damage to retinal cells^[22]. Additionally, they suppress inflammatory factor release, alleviating retinal inflammatory response, thus preserving microvascular structure and function^[23]. Furthermore, by modulating vascular endothelial growth factor (VEGF) expression, these compounds inhibit pathological angiogenesis, reduce retinal vascular leakage, and enhance blood perfusion^[24-25]. Studies also indicate that blueberry anthocyanins protect retinal neurons by inhibiting apoptosis and promoting cell survival, thereby slowing DR progression.

Although blueberries have long been recognized for their vision-enhancing bioactivity, their precise mechanisms remain unclear^[26]. Our previous research revealed that blueberry anthocyanin extracts (BAE), particularly the predominant major components malvidin glycosides (e.g., malvidin-3-*O*-galactoside and malvidin-3-*O*-glucoside), exert protective effects on human retinal vascular endothelial cells against high glucose-induced damage, enhancing cell viability by reducing oxidative damage, improving retinal activity, and suppressing inflammation^[27]. In addition, both the extracts and their primary anthocyanins protect human retinal pigment epithelial (RPE) cells from H₂O₂-induced oxidative stress^[28]. However, clinical evidence regarding the efficacy of blueberry anthocyanins in alleviating visual fatigue and improving ocular health in diabetics remains limited. This study investigated the effects of blueberry anthocyanin intervention in diabetic patients, with a focus on its visual function, intraocular pressure, and ocular structural integrity.

By analyzing changes in glycemic control, insulin resistance, and inflammatory biomarkers, we explore the potential mechanisms by which blueberry anthocyanins may improve ocular health in elderly patients with T2D. Additionally, optical coherence tomography (OCT) was employed to evaluate retinal thickness and macular edema, providing objective structural assessments of blueberry anthocyanins-mediated protection.

2. Material and methods

2.1 Participants

This prospective clinical cohort study was conducted at the Affiliated Hospitals of Nanjing Medical University in Nanjing and 928th Hospital of PLA Joint Logistics Support Force in Haikou, China. The elderly patients with T2D over 60 years old were recruited. Participants were enrolled based on both 1999 World Health Organization diagnostic criteria for diabetes mellitus and Chinese Guidelines for Prevention and Treatment of Type 2 Diabetes (2020 edition), meeting at least one of the following criteria: fasting blood glucose (FBG) ≥ 7.0 mmol/L (126 mg/dL), random blood glucose ≥ 11.1 mmol/L, 2-hour postprandial glucose (2h-PG) ≥ 11.1 mmol/L (200 mg/dL), or glycated hemoglobin (HbA1c) $\geq 6.5\%$. The included patients should be in stable health status, able to communicate effectively, and compliant with study protocols. The exclusion criteria included cognitive impairment or inability to, severe comorbidities (e.g., cardiac, pulmonary, or renal dysfunction), autoimmune diseases or recent major surgery within the past six months. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University/Jiangsu Province Hospital (Ethics ID: 2022-SR-455) and registered on chictr.org.cn (ChiCTR2500111198). All volunteers signed an informed consent before the enrolment.

2.2. Interventions

This study was conducted as an exploratory clinical investigation embedded within the above-mentioned prospective cohort study. Given the limited clinical data on blueberry anthocyanins in diabetic ocular outcomes, a randomized placebo-controlled design was not implemented at this stage. Instead, a combination of within-subject longitudinal observation and parallel group comparison was used to obtain preliminary real-world evidence and assess feasibility. Blueberry anthocyanins were administered as a nutritional supplement (functional food ingredient), not as a replacement for standard antidiabetic therapy. All volunteers continued their prescribed antidiabetic medications throughout the study period. The blueberry anthocyanins (PureBlue One™, purity 44.56%) used in this study was provided by Zhejiang Lanmei Technology Co., whose detailed compositional analysis has been reported previously^[29].

Eligible volunteers were enrolled and allocated into two distinct study groups. Group A (n = 42/57, eligible individuals/screened participants) underwent a controlled crossover design, serving as their own controls during the initial 3-month observation period without blueberry anthocyanin supplementation, followed by a 3-month active intervention phase with daily 400 mg blueberry anthocyanin administration, with comprehensive assessments conducted at baseline, 3 months (pre-intervention), 4 months (1-month post-intervention), and 6 months (3-month post-intervention/study completion). In parallel, Group B (n =

43/51) received continuous daily 400 mg blueberry anthocyanin supplementation from study initiation, with monitoring performed at baseline and subsequently at 1, 3, and 6 months to evaluate longitudinal effects. This dual-arm design enabled comparative analysis of both immediate and delayed intervention effects while controlling for temporal variations. The daily dose of 400 mg blueberry anthocyanins was administered before bedtime. This dose was determined based on previously published human intervention studies^[30-31], established safety ranges, and dietary reference data.

2.3. Physical examination and biochemical parameter measurement

For comprehensive assessments, samples of blood and urine were collected. The data of age, weight, height, heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were also recorded. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²). Biochemical parameters including FBG, 2h-PG, HbA1c, fasting insulin (INS), total cholesterol (CHOL), triglycerides (TG), alanine transaminase (ALT), aspartic transaminase (AST), lactate dehydrogenase (LDH), serum creatinine (CR), urea (UR), and other related indicators were measured.

2.4. Calculation of metabolic and inflammatory indices

The following indices were calculated to assess insulin resistance, metabolic status, and systemic inflammation:

Triglyceride-glucose (TyG) index is a surrogate marker of insulin resistance, calculated as:

$$TyG = \ln \left(\frac{\text{Fasting triglycerides (mg/dL)} \times \text{Fasting glucose (mg/dL)}}{2} \right)$$

Homeostatic model assessment of insulin resistance (HOMA-IR) is an estimate of insulin resistance, computed using:

$$HOMA - IR = \frac{\text{Fasting insulin}(\mu\text{IU/mL}) \times \text{Fasting glucose}(\text{mmol/L})}{22.5}$$

Neutrophil-to-lymphocyte ratio (NLR) is a systemic inflammation marker, determined by:

$$NLR = \frac{\text{Neutrophil count}(\times 10^9/\text{L})}{\text{Lymphocyte count}(\times 10^9/\text{L})}$$

Monocyte-to-lymphocyte ratio (MLR) is reflecting immune-inflammatory status, calculated as:

$$MLR = \frac{\text{Monocyte count}(\times 10^9/\text{L})}{\text{Lymphocyte count}(\times 10^9/\text{L})}$$

Platelet-to-lymphocyte ratio (PLR) is an indicator of inflammation and thrombosis risk, derived from:

$$PLR = \frac{\text{Platelet count}(\times 10^9/\text{L})}{\text{Lymphocyte count}(\times 10^9/\text{L})}$$

Systemic inflammation response index (SIRI) is a composite inflammatory score, computed as:

$$SIRI = \frac{\text{Neutrophil count}(\times 10^9/\text{L}) \times \text{Monocyte count}(\times 10^9/\text{L})}{\text{Lymphocyte count}(\times 10^9/\text{L})}$$

Systemic immune-inflammation index (SII) is integrating platelet and immune cell counts, defined by:

$$SIRI = \frac{\text{Platelet count}(\times 10^9/\text{L}) \times \text{Neutrophil count}(\times 10^9/\text{L})}{\text{Lymphocyte count}(\times 10^9/\text{L})}$$

All measurements were obtained from fasting blood samples.

2.5. Ocular examination

Ocular examinations include visual acuity, IOP, OCT, photopic persistence, and visual fatigue symptom questionnaire. Visual acuity was measured under uncorrected conditions using a standard logarithmic E chart. Raw chart readings were recorded in decimal and converted to logarithm of the minimum angle of resolution (logMAR) for analysis. Intraocular pressure was measured with a TX-20 non-contact tonometer (Canon Inc., Kawasaki, Japan). Measurements were scheduled at roughly similar times of day for each participant, and central corneal thickness was not measured. Optical coherence tomography examinations were performed using a standard macular scanning protocol on a RTVue XR OCT system (Optovue, Inc., Fremont, California, America). Central macular and regional retinal thicknesses were extracted using built-in segmentation software. The photopic persistence was measured using a specially designed 1 cm³ “Pin” diagram under controlled illumination (100-150 LX). Subjects alternately started/paused a stopwatch when the “Pin” image became clearly visible/invisible during 3-minute observation periods, with cumulative visible time recorded as photopic persistence. Visual fatigue symptoms (Table S1) were quantified using a validated questionnaire assessing eight characteristic symptoms (3-point scale).

2.6. Statistical analysis

All statistical analyses were performed using statistical product and service solutions (SPSS) software version 25.0 (SPSS Inc., Chicago, IL, US) and GraphPad Prism 9.0 (GraphPad Software, Inc., MA, US). Categorical variables in the table were summarized as numbers and percentages, while continuous variables were expressed as mean $\pm$ standard deviation (SD); bar graphs were all presented as means with 95% confidence intervals. Data normality was assessed by the Shapiro-Wilk test. For normally distributed quantitative data, intergroup differences were analyzed using independent (unpaired) *t*-tests, and intragroup comparisons were performed using one-way analysis of variance (ANOVA). For non-normally distributed data, intergroup differences were analyzed using the Mann-Whitney U test (unpaired), and intragroup comparisons were assessed using the Wilcoxon signed-rank test (paired), and intragroup comparisons were performed using the Kruskal-Wallis test. Statistical significance was considered at a level of $P < 0.05$.

3. Results

3.1. Baseline characteristics of the participants

The baseline demographics and clinical characteristics of the participants in Group A and Group B are presented in Table 1. Overall, the two groups were well-balanced and comparable at the initiation of the study. There were no statistically significant differences between the groups in key parameters including age, height, body mass index, heart rate, and blood pressure. The observed difference in body weight, which approached but did not reach statistical significance (61.12 ± 9.78 kg in Group B vs 64.93 ± 9.71 kg in Group A, $P =$

0.0544), may be partly attributable to the higher proportion of male participants in Group A (38.10%) compared to Group B (27.91%).

Table 1 Baseline demographics and clinical characteristics.

Parameters	Group A-0M	Group A -3M	P_1	Group B -0M	P_2	P_3
N	42			43		
Sex, male, n (%)	16 (38.10%)			12 (27.91%)		
Age, years (range)	69.62 ± 6.11 (60-85)			70.72 ± 5.56 (63-84)	0.3863	
Height, cm	162.12 ± 7.58			161.25 ± 7.33	0.4725	
Weight, kg	64.93 ± 9.71			61.12 ± 9.78	0.0544	
BMI, kg/m ²	24.57 ± 2.99			23.46 ± 3.06	0.0633	
Heart rate, beats/min	76.55 ± 10.56			79.41 ± 8.84	0.1461	
Systolic pressure, mmHg	133.33 ± 19.99			133.14 ± 19.26	0.1369	
Diastolic pressure, mmHg	76.80 ± 9.02			73.57 ± 10.06	0.0653	
Fasting blood glucose, mmol/L	8.33 ± 2.92	8.30 ± 2.32	0.8795	8.24 ± 1.77	0.8857	0.9216
HbA1c, %, mmol/mol	8.27 ± 1.45	8.00 ± 1.36	0.1216	8.01 ± 1.40	0.4371	0.9838
Fasting insulin, μU/ml	16.80 ± 14.42	15.09 ± 9.11	0.1888	12.75 ± 18.98	0.3274	0.5326
Triglycerides, mmol/L	1.96 ± 1.02	1.70 ± 0.76	0.0516	1.52 ± 1.37	0.1012	0.4607
Total cholesterol, mmol/L	4.96 ± 1.44	4.45 ± 1.10	0.1036	4.48 ± 1.01	0.1072	0.8905
ALT, U/L	26.09 ± 19.31	21.95 ± 9.43	0.2743	21.27 ± 13.38	0.1837	0.7904
AST, U/L	24.16 ± 14.72	23.16 ± 10.66	0.8029	22.07 ± 7.66	0.3874	0.5909
LDH, U/L	177.73 ± 34.07	168.30 ± 36.46	0.5038	187.02 ± 41.43	0.2992	0.0884

Data are presented as mean ± standard deviation (SD). N or n, number of patients; BMI, body mass index; HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase. P_1 , intragroup comparison between A-0M and A-3M by paired two-tailed student *t*-test; P_2 , intergroup comparison between A-0M and B-0M by unpaired two-tailed student *t*-test; P_3 , intergroup comparison between A-3M and B-0M by unpaired two-tailed student *t*-test.

Critically, the key metabolic indicators and liver function profiles were comparable at baseline. During the 3-month conventional therapy period without blueberry anthocyanin supplementation, Group A demonstrated no significant changes from baseline. The values at the end of this period remained comparable to those of Group B at baseline. The overall balance in baseline characteristics supports the validity of subsequent comparative analyses following intervention.

3.2. Blueberry anthocyanins improve visual acuity in T2D patients

The study revealed significant visual improvements in both diabetic patient groups following blueberry anthocyanin supplementation. In Group A, which initially received standard antidiabetic treatment for three months without BAE intervention, visual acuity remained relatively stable, with logMAR value of left eye from 0.19 ± 0.15 to 0.23 ± 0.19 ($P = 0.2074$, Fig. 1A) and right eye from 0.31 ± 0.24 to 0.33 ± 0.27 ($P = 0.0591$, Fig. 1B). In contrast, a positive trend emerged after blueberry anthocyanin intervention. Notably, after three months of BAE supplementation, right-eye visual acuity demonstrated a significant reduction in logMAR value ($P < 0.01$, Fig. 1B), whereas the change in the left eye did not reach statistical significance. Group B, which received continuous BAE supplementation for six months, exhibited a progressive decline in logMAR values during the intervention period, indicating improved visual acuity (Figs. 1C-D). The left eye showed a significant reduction in logMAR values after one month of BAE supplementation compared with baseline ($P < 0.05$, Fig. 1C), while the right eye demonstrated a significant improvement after 6 months of

intervention ($P < 0.05$, Fig. 1D). Although the magnitude of improvement varied between eyes, both eyes showed a consistent downward trend in logMAR values during BAE supplementation.

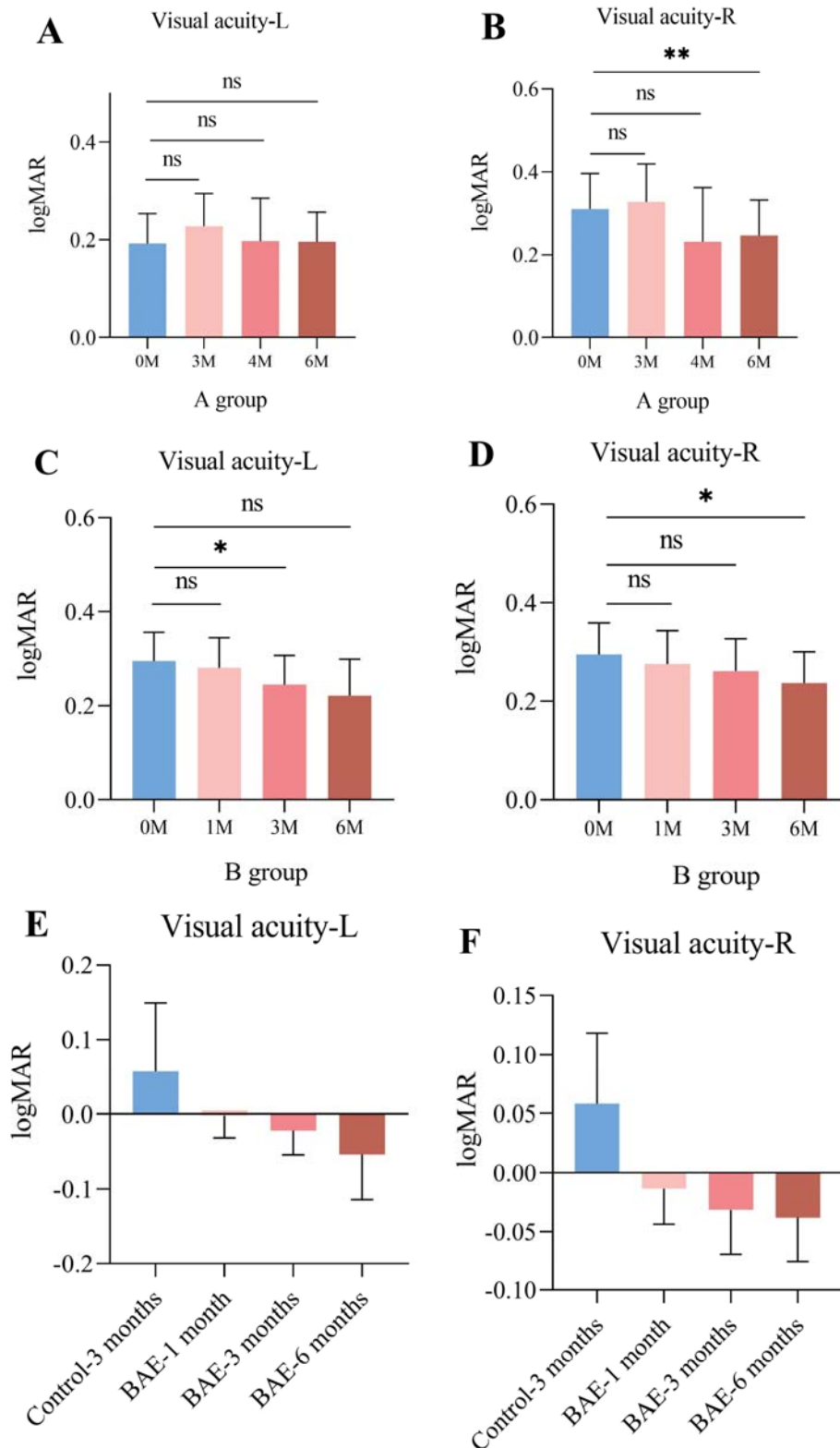


Fig. 1 Changes in the visual acuity of diabetic volunteers. The logarithm of the minimum angle of resolution (logMAR) value on visual acuity of left eyes (A) and right eyes (B) in Group A at 0M (baseline), 3M (after 3 months without intervention), 4M (after 1 month of blueberry anthocyanin extract, BAE), and 6M (after 3 months of BAE). The logMAR value on visual acuity of left eyes (C) and right eyes (D) in Group B at 0M (baseline), 1M (after 1 month of BAE), 3M (after 3 months of BAE), and 6M (after 6 months of BAE). The changes of logMAR value from baseline for left eyes (E) and right eyes (F) after 3 months without intervention, 1 month of BAE, 3 months of BAE, and 6 months of BAE. Statistical significance for intragroup comparisons (A–D) were analyzed using paired two-tailed *t*-tests, while intergroup differences (E, F) were conducted using unpaired Student's *t*-tests (ns $P > 0.05$, * $P < 0.05$, and ** $P < 0.01$).

To further evaluate the temporal pattern of visual acuity changes, the changes from baseline were analyzed at different intervention stages (Figs. 1E-F). Without BAE supplementation, visual acuity exhibited a slight decline during the initial 3-month control period (positive $\Delta\log\text{MAR}$). Following BAE intervention, this declining trend was attenuated, with visual acuity changes becoming progressively more favorable with longer supplementation duration (negative $\Delta\log\text{MAR}$). These findings suggest that blueberry anthocyanin supplementation may contribute to the maintenance and improvement of vision in elderly patients with type 2 diabetes.

3.3. Blueberry anthocyanins improve intraocular pressure in T2D patients

Intraocular pressure (IOP) is a key critical indicator of ocular health, essential for assessing glaucoma risk and optic nerve integrity, with the normal range established between 10 and 21 mmHg. Values beyond this range may indicate elevated glaucoma risk. The study demonstrated that blueberry anthocyanin supplementation significantly reduced IOP in both groups. In Group A, no significant IOP changes were observed during the initial three months of standard antidiabetic therapy (Figs. 2A-B). However, one month after initiating BAE intervention, IOP decreased significantly ($P < 0.05$ for left eye while $P < 0.01$ for right eye). Although a minor rebound occurred at the three-month mark compared to the one-month measurement, IOP remained below baseline levels. Group B exhibited baseline IOP values of 16.90 ± 2.53 mmHg (left eye) and 16.59 ± 2.29 mmHg (right eye). After six months of BAE intervention, left eye IOP was reduced by 8.05% ($P < 0.05$, Fig. 2C), while right eye IOP by 8.20% ($P < 0.05$, Fig. 2D). Group B demonstrated pronounced IOP improvements, with statistically significant reductions observed in both eyes at all three time points (1, 3, and 6 months), which were most pronounced at the three-month mark ($P < 0.01$). Similar, IOP change data confirmed that without intervention, IOP increased over three months, whereas one, three, or six months of BAE supplementation consistently lowered IOP (Figs. 2E-F), further supporting the IOP-improving efficacy of blueberry anthocyanin. Although all mean IOP values remained within the clinically normal range (10–21 mmHg), BAE supplementation was associated with statistically significant reductions in IOP compared to baseline and the no-intervention period. These findings suggest that blueberry anthocyanins may lower IOP within the physiological range, which could be relevant for long-term risk reduction in diabetic patients.

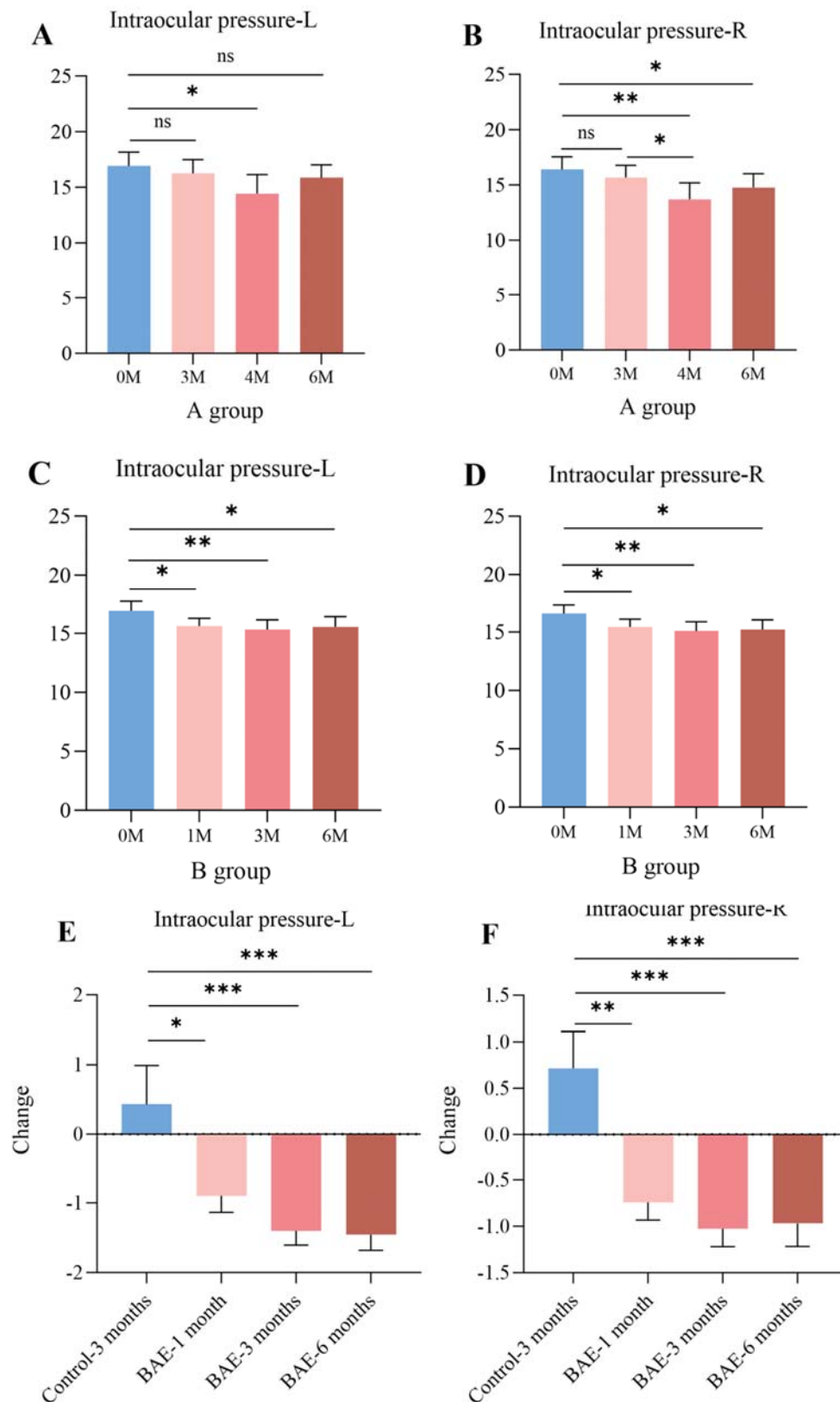
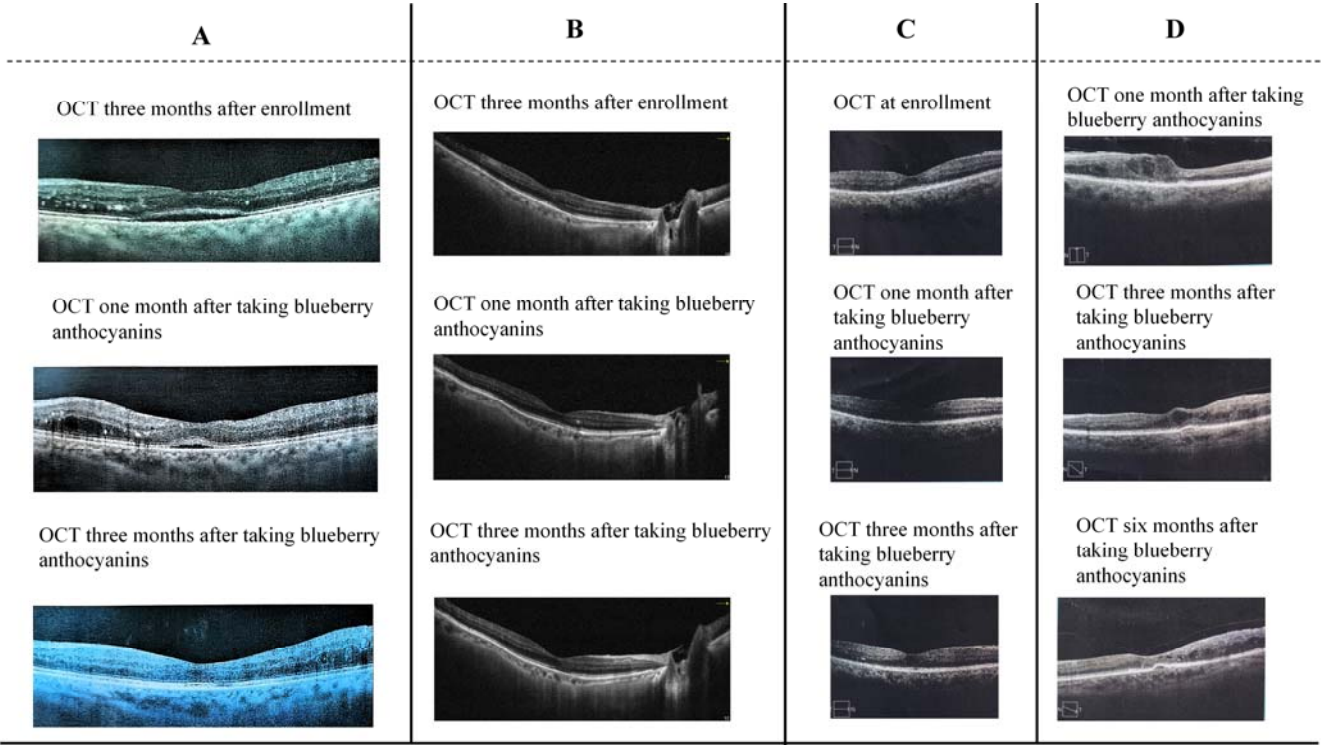


Fig. 2 Changes in the intraocular pressure of diabetic volunteers. The intraocular pressure of left eyes (**A**) and right eyes (**B**) in Group A at 0M (baseline), 3M (after 3 months without intervention), 4M (after 1 month of blueberry anthocyanin extract, BAE), and 6M (after 3 months of BAE). The intraocular pressure of left eyes (**C**) and right eyes (**D**) in Group B at 0M (baseline), 1M (after 1 month of BAE), 3M (after 3 months of BAE), and 6M (after 6 months of BAE). The changes of intraocular pressure from baseline for left eyes (**E**) and right eyes (**F**) after 3 months without intervention, 1 month of BAE, 3 months of BAE, and 6 months of BAE. Statistical significance for intragroup comparisons (**A–D**) were analyzed using paired two-tailed *t*-tests, while intergroup differences (**E, F**) were conducted using unpaired Student's *t*-tests (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

3.4. Blueberry anthocyanins improve the retinal structure in T2D patients

Optical coherence tomography, a reliable diagnostic tool for ocular diseases, enables direct visualization of retinal changes and provides critical insights for clinical management. Observation via OCT revealed that blueberry anthocyanin supplementation was associated with improvements in retinal structure in T2D patients.

One representative OCT at three months post-enrollment revealed hypo-reflective areas between retinal layers in the right eye, indicating fluid accumulation and possible edema (Fig. 3A). Although retinal edema appeared to worsen after one month of BAE intervention, manifested as localized RPE elevation and subchoroidal fluid accumulation, prolonged supplementation led to marked improvements. By three months, the foveal contour flattened, retinal thickness decreased, and intraretinal cystoid spaces, hyperreflective foci, and subretinal fluid showed partial resolution, suggesting reduced edema. Retinal thickness maps (Fig. 3E) further confirmed edema reduction, with decreased red areas indicating fluid attenuation. In older diabetic patients with comorbid age-related macular degeneration (AMD), blueberry anthocyanin intervention was associated with reduced atrophic areas (blue regions on retinal thickness maps, Fig. 3F) after three months, suggesting a potential amelioration of AMD progression. Although OCT changes on were subtle, trends indicated gradual foveal structural recovery (Fig. 3B).



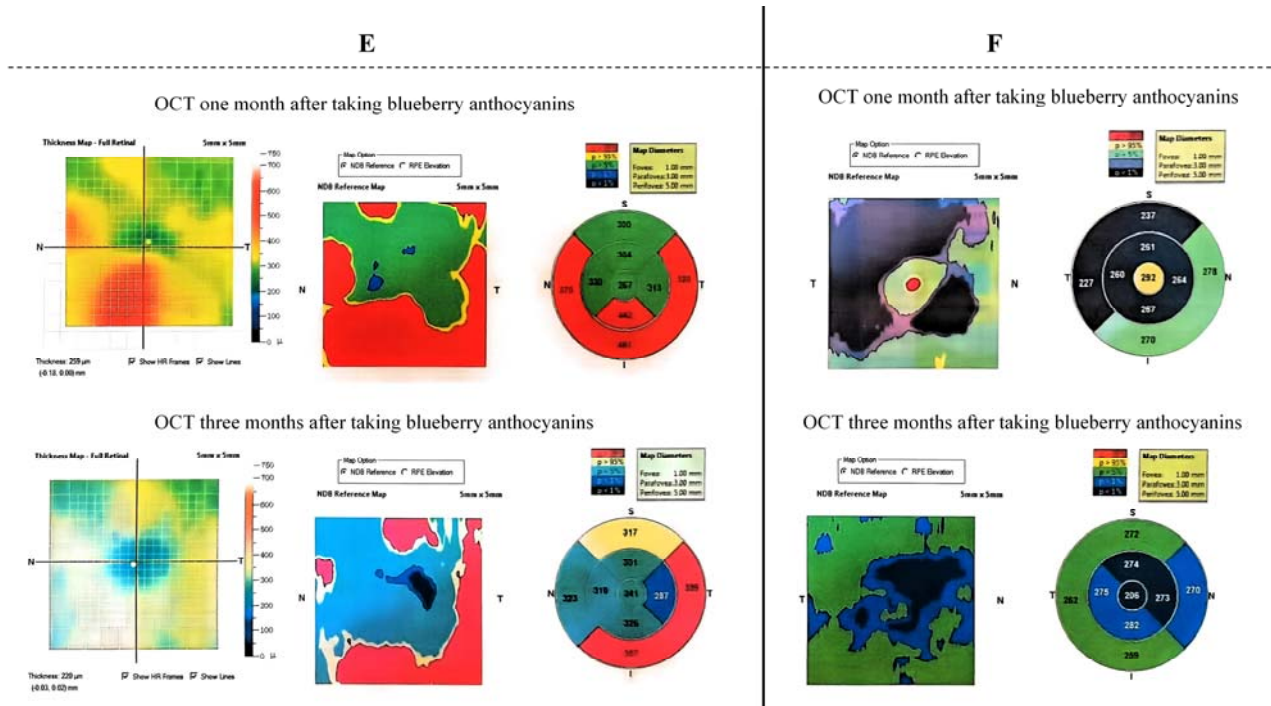


Fig. 3 Changes in retinal structure of representative diabetic volunteers under optical coherence tomography (OCT) detection. The macular thickness B-scans of volunteer #3's left eye (A) and volunteer #4's right eye (B) from Group A after 3 months without intervention, 1 month of blueberry anthocyanin extract (BAE) and 3 months of BAE. The macular thickness B-scans of volunteer #15's right eye (C) from Group B after enrollment (baseline), 1 month of BAE, and 3 months of BAE. The macular thickness B-scans of volunteer #22's left eye (D) from Group B after 1 month of BAE, 3 months of BAE, and 6 months of BAE. The thickness maps of volunteer #3' left eye (E) and volunteer #4' right eye (F) from Group A at 1 month of BAE, and 3 months of BAE.

In some cases, the initial OCT images appeared blurred, likely due to ocular opacities (e.g., cataracts). After three months of BAE intervention, improved retinal visibility was observed in certain patients (Fig. 3C). However, improved OCT clarity does not necessarily indicate reduced lens opacity. Further investigation with appropriate ophthalmic assessments is needed to determine whether blueberry anthocyanins benefit lens opacification in diabetic patients with concurrent ocular conditions. One representative case of diabetic macular edema (DME) exhibited characteristic features at baseline, primarily manifested as foveal thickening with intraretinal cystoid edema. At the one-month mark, OCT imaging revealed discontinuity of the ellipsoid zone (EZ) in the outer retina, irregular hyporeflective cystic cavities in the inner nuclear layer, and loss of the normal foveal contour. Continued BAE intervention led to reduced cystoid edema, partial EZ restoration, and gradual reappearance of the foveal pit (Fig. 3D). Regarding the observed improvement in diabetic macular edema, we acknowledge that spontaneous fluctuation cannot be fully excluded as a contributing factor. Furthermore, while OCT images suggested partial ellipsoid zone restoration, quantitative analysis of EZ integrity (e.g., EZ restoration ratio) was not performed. Future studies should incorporate quantitative EZ metrics as gold-standard endpoints for evaluating retinal repair.

3.5. Blueberry anthocyanins improve visual fatigue in T2D patients

The analysis of the volunteer questionnaire scores revealed a notable reduction in visual fatigue symptoms after a six-month intervention with blueberry anthocyanins (Fig. 4). In the absence of BAE intervention, symptoms of visual fatigue either persisted or worsened over time. For instance, blurred vision

scores were significantly higher at baseline (0M) compared to three months prior (-3M, $P < 0.01$, Fig. 4D), highlighting a natural progression of symptom severity without treatment. Following BAE supplementation, visual fatigue symptoms improved progressively with the duration of intervention, reaching optimal alleviation at the three-month mark. A slight recurrence of symptoms was observed at six months, possibly due to tolerance development. Total scores exhibited a declining trend, indicating overall improvement (Fig. 4I). Specifically, ocular symptom such as eye soreness ($P < 0.05$, 3M vs 0M/1M, Fig. 4B) and lacrimation ($P < 0.01$, 3M vs 0M/1M, Fig. 4G) were significantly alleviated. Notably, systemic discomfort also improved markedly, with statistically significant reductions observed at both three- ($P < 0.05$) and six-month marks ($P < 0.01$, Fig. 3H). These findings suggest that blueberry anthocyanins may mitigate visual fatigue through multiple mechanisms, including antioxidant activity, anti-inflammatory effects, and microcirculation enhancement, supporting their potential as an adjunct therapy.

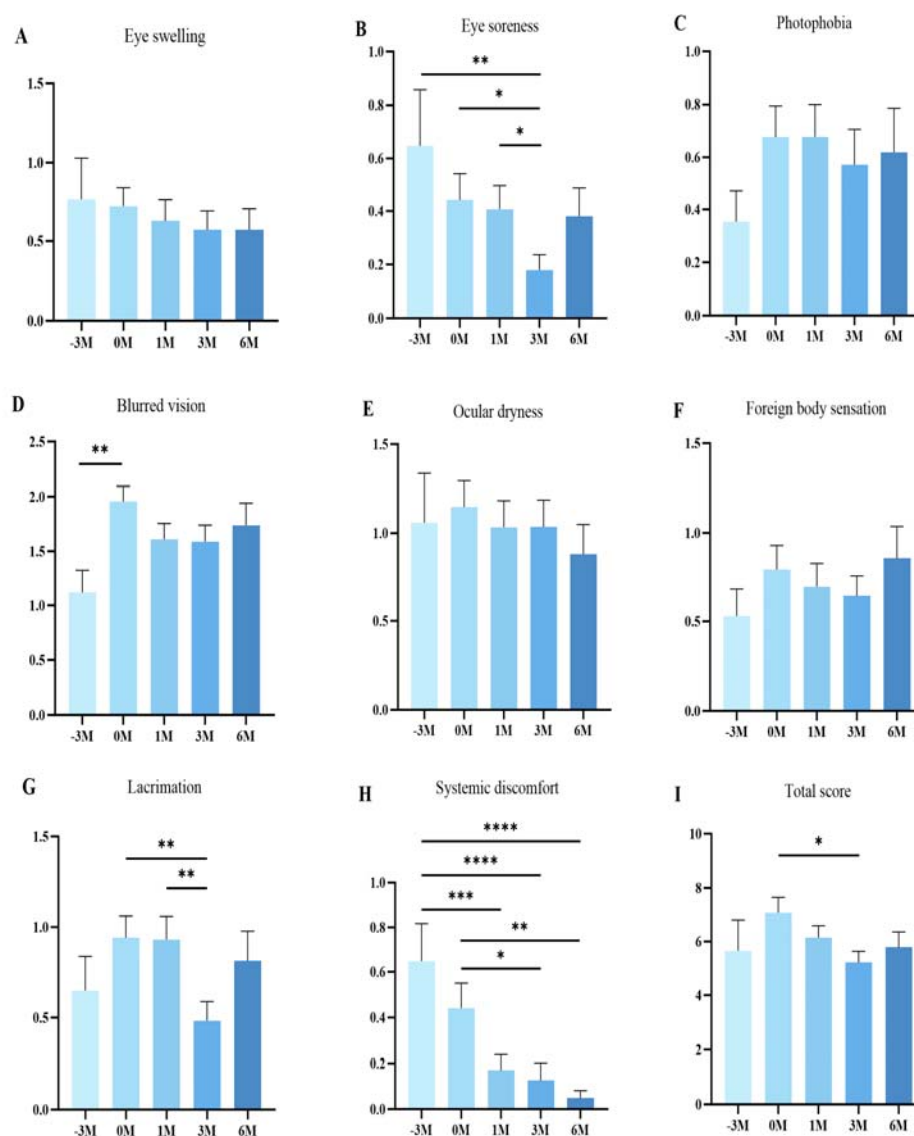


Fig. 4 Changes in the visual fatigue questionnaire results of diabetic volunteers. The score of eye swelling (a), eye soreness (b), photophobia (c), blurred vision (d), ocular dryness (e), foreign body sensation (f), lacrimation (g), systemic discomfort (h), and the total (i) of diabetic volunteers at -3M (before 3 months without intervention, A-0M), 0M (baseline, A-3M and B-0M), 1M (after 1 month of blueberry anthocyanin extract (BAE), A-4M and B-1M), 3M (after 3 months of BAE, A-6M and B-3M), 6M (after 6 months of BAE, B-6M). Statistical significance for intergroup/intragroup comparisons (a-i) was analyzed using unpaired/paired two-tailed Student's *t*-tests (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$)

Photopic persistence, a benchmark defined as the ratio of photopic time to total fixation time, was also used to evaluate visual fatigue. This parameter is an integrative indicator of visual and mental function. The duration of photopic persistence showed no statistically significant changes before and after BAE intervention, with values fluctuating between 160.7 ± 25.2 s and 172.9 ± 19.4 s (Fig. S1A, $P > 0.05$). However, a time-dependent tendency toward prolonged and stabilized photopic duration was observed following BAE supplementation (Fig. S1B, $P > 0.05$), indicating a potential clinically relevant benefit of BAE for visual sustainability over time.

3.6. Blueberry anthocyanins improve glycemic control and insulin sensitivity in T2D patients

Grouped analysis (Table 2) based on the duration of blueberry anthocyanin intervention showed that FBG, 2h-PG, and HbA1c levels consistently decreased among the volunteers. The most pronounced reduction was observed at six-month mark, with significant difference compared to baseline ($P < 0.05$). In contrast, INS levels remained largely unchanged, which may be consistent with improved insulin sensitivity rather than increased insulin secretion. Further supporting this mechanism, the TyG index significantly decreased during BAE intervention ($P < 0.01$), albeit with considerable individual variations, and although the reduction in HOMA-IR did not reach statistical significance ($P > 0.05$), a trend toward improved insulin resistance was observed. Additionally, BAE supplementation significantly improved inflammatory profiles. Markers including NLR, MLR, and SIRI declined markedly within three months ($P < 0.05$) and were sustained through six months, whereas changes in PLR and SII were modest. Due to the large standard deviations observed for fasting insulin and HOMA-IR, we additionally calculated median values and interquartile ranges (IQR) values for these parameters at each time point (Table S3). During the 6-month blueberry anthocyanin intervention, fasting insulin levels and HOMA-IR showed a downward trend. These results may be consistent with improved insulin sensitivity and amelioration of inflammation-induced insulin resistance, although this interpretation is limited by high inter-individual variability in insulin and HOMA-IR values, as well as by the incomplete standardization of concomitant antidiabetic medications.

3.7. Safety and tolerability

Overall, BAE supplementation at 400 mg/day was generally well tolerated throughout the 6-month intervention period. No serious adverse events or hypoglycemic episodes were reported in either group.

Hepatic safety assessment showed that, rather than remaining neutral, both ALT and AST significantly decreased during BAE intervention (Table 2; $P < 0.05$ for ALT and AST at 1M; $P < 0.01$ for ALT at 3M and 6M). Notably, in a subset of patients with baseline hepatic enzyme abnormalities, several individuals were able to reduce or discontinue concomitant hepatoprotective medications while maintaining normal ALT/AST levels, suggesting an ancillary hepatoprotective benefit. Additionally, LDH was measured as a general marker of cellular integrity, though its non-specific tissue distribution limits liver-specific interpretation.

Table 2 The biochemical index data of diabetic volunteers changed with the intervention time of blueberry anthocyanins.

Parameters	-3M (A-0M)	0M (A-3M/B-0M)	P_0	1M (A-4M/B-1M)	P_1	3M (A-6M/B-3M)	P_2	6M (B-6M)	P_3	P_4
Fasting blood glucose, mmol/L	8.33 ± 2.92	8.18 ± 2.08	0.9351	7.13 ± 1.67	0.0268*	7.22 ± 1.36	0.0224*	7.11 ± 1.60	0.0057**	0.0024**
2-hour postprandial blood glucose, mmol/L	13.68 ± 4.94	16.01 ± 4.52	0.0520	13.65 ± 7.14	0.0246*	12.52 ± 4.42	0.0011**	13.87 ± 2.90	0.0192*	0.0043**
Fasting insulin, μU/ml	16.80 ± 14.42	13.25 ± 13.04	0.4682	14.88 ± 8.19	0.0423*	14.67 ± 9.36	0.1814	11.49 ± 17.32	0.1237	0.0111*
HbA1c, %	8.27 ± 1.45	8.00 ± 1.37	0.4170	7.71 ± 1.49	0.9081	7.39 ± 0.97	0.0019**	7.33 ± 0.91	0.0160*	0.0023**
ALT, U/L	26.09 ± 19.31	24.37 ± 14.07	0.8280	22.24 ± 14.35	0.0461*	20.83 ± 10.22	0.0004***	17.23 ± 11.78	0.0011**	0.0109*
AST, U/L	24.16 ± 14.72	23.57 ± 9.99	0.1534	22.07 ± 10.72	0.0111*	23.03 ± 13.51	0.1177	21.58 ± 8.23	0.2828	0.5141
LDH, U/L	177.73 ± 34.07	184.06 ± 42.34	0.8767	177.55 ± 33.70	0.0148*	175.94 ± 32.98	0.0043**	176.48 ± 29.37	0.4486	0.8581
UR, mmol/L	6.13 ± 1.58	5.94 ± 1.75	0.7206	6.04 ± 1.57	0.0588	6.03 ± 1.33	0.1417	5.32 ± 1.18	0.0605	0.1919
CR, μmol/L	69.58 ± 16.27	73.48 ± 16.66	0.2936	69.93 ± 15.56	0.0077**	69.71 ± 14.69	0.0073**	59.28 ± 11.56	0.0001***	0.0029**
TyG	9.28 ± 0.94	9.05 ± 0.68	0.1446	8.78 ± 0.56	0.0217*	8.83 ± 0.49	0.0402*	8.73 ± 0.45	0.0307*	0.0008***
HOMA-IR	4.71 ± 3.67	3.68 ± 2.95	0.1807	3.69 ± 1.89	0.2897	3.51 ± 2.29	0.0115*	2.35 ± 1.55	0.0250*	0.0141*
NLR	2.76 ± 2.36	2.21 ± 1.03	0.2974	1.98 ± 0.92	0.0147*	1.97 ± 0.81	0.0029**	1.67 ± 0.45	0.0065**	0.0141*
MLR	0.23 ± 0.08	0.21 ± 0.11	0.1040	0.20 ± 0.10	0.3771	0.19 ± 0.07	0.0195*	0.17 ± 0.04	0.0098**	0.0048**
PLR	123.43 ± 38.90	112.29 ± 38.02	0.1451	107.53 ± 42.06	0.2829	109.80 ± 45.38	0.5299	100.23 ± 23.42	0.1797	0.0738
SIRI	0.95 ± 0.62	0.86 ± 0.61	0.2631	0.78 ± 0.53	0.0489*	0.76 ± 0.41	0.0386*	0.63 ± 0.27	0.0187*	0.0447*
SII	488.51 ± 262.07	413.82 ± 168.15	0.2963	389.05 ± 178.89	0.3075	385.32 ± 145.40	0.0184*	366.23 ± 133.92	0.1609	0.2810

Data are presented as mean ± standard deviation (SD). The data were pooled according to the duration of blueberry anthocyanin extract (BAE), resulting in a combined sample size (Group A and B). HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; UR, urea; CR, creatinine; TyG, triglyceride-glucose index; HOMA-IR, homeostatic model assessment of insulin resistance; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SIRI, systemic inflammation response index; SII, systemic immune-inflammation index. P_0 , intergroup comparison between 0M (A-3M and B-0M) and -3M (A-0M) by unpaired two-tailed student *t*-test; P_1 , intragroup comparison between 0M (A-3M and B-0M) and 1M (A-4M and B-1M) by paired two-tailed student *t*-test; P_2 , intragroup comparison between 0M (A-3M and B-0M) and 3M (A-6M and B-3M) by paired two-tailed student *t*-test; P_3 , intergroup comparison between 0M (A-3M and B-0M) and 6M (B-6M) by unpaired two-tailed student *t*-test; P_4 , intergroup comparison among groups after blueberry anthocyanins extract were analyzed using either one-way ANOVA (for normally distributed data) or the Kruskal-Wallis test (for non-normally distributed data). (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$)

Renal function was evaluated by serial measurements of serum creatinine and urea, with data now presented in Table 2. Notably, CR exhibited a biphasic pattern: during the initial 3-month observation period without BAE (Group A), CR levels showed an increasing trend, consistent with the expected age- and diabetes-related decline in renal function. In contrast, following BAE supplementation, CR levels decreased in both groups ($P < 0.01$ at 1M and 3M; $P < 0.001$ at 6M vs baseline), suggesting a potential renoprotective effect. Meanwhile, UR remained stable throughout the study.

Taken together, these findings suggest that blueberry anthocyanins exert multifaceted protective effects beyond glycemic control, with favorable impacts on hepatic and renal function in this elderly diabetic cohort. Given the favorable tolerability profile observed, these preliminary data support the potential of blueberry anthocyanins as a safe, multi-organ-targeting adjunctive strategy for metabolic and organ protection in type 2 diabetes.

4. Discussion

Blueberry anthocyanins are well-characterized bioactive compounds with multifaceted physiological functions, primarily attributed to their potent free radical scavenging and antioxidant capacities^[20,23]. These properties underpin their ability to mitigate hyperglycemia-induced oxidative stress, which arises through multiple pathways including the polyol flux, accumulation of advanced glycation end products (AGEs), and excessive reactive oxygen species (ROS) generation from mitochondrial overactivity^[32-34]. Beyond improving systemic glycemic control, blueberry anthocyanins exert anti-inflammatory, microcirculation-promoting, and vascular endothelial-protective effects^[27,35]. These mechanisms support retinal cell function and vascular integrity, offering a rational basis for their ocular protective effects under diabetic conditions. This clinical study demonstrates that daily supplementation with 400 mg blueberry anthocyanins markedly improves ocular function and retinal structural integrity in elderly patients with T2D. These ocular benefits are accompanied by improvements in systemic metabolic and inflammatory markers, suggesting a multifactorial mechanism underlying the visual protective effects.

The most striking ocular findings include significant enhancements in visual acuity and reductions in intraocular pressure. These changes are clinically relevant, as both visual impairment and elevated IOP are common in diabetic patients and contribute to the burden of diabetic eye disease^[36]. Notably, vision improved in a time-dependent manner, with the most substantial gains observed after three to six months of BAE intervention. Concurrently IOP was significantly reduced, which was sustained throughout the entire intervention period. Intraocular pressure is physiologically regulated by the balance between aqueous humor production and drainage, with potential contributions from choroidal blood volume and corneal edema^[37]. Although previous studies have linked SBP fluctuations to IOP variations^[38], Braha et al. found no association between IOP and metabolic markers, hypertensive comorbidities, or antidiabetic treatments^[39]. Nevertheless, accumulating evidence supports a direct correlation between diabetes progression and IOP elevation. Our findings imply that blueberry anthocyanins may attenuate this rise through improved glycemic control. Mechanistically, these benefits may arise from enhanced nitric oxide (NO) bioavailability

by blueberry anthocyanins, which promotes vasodilation, ameliorates retinal ischemia, and improves ocular microcirculation and function^[27].

Optical coherence tomography findings from this study revealed structural improvements in the retina. Intervention with blueberry anthocyanins resulted in a reduced retinal thickness and attenuated macular edema, indicating a protective effect of anthocyanins on the blood-retinal barrier (BRB) and demonstrating multi-target therapeutic potential for retinal diseases such as AMD and DME^[11,18]. Blueberry anthocyanins demonstrate potential in mitigating early pathological progression of DR *in vitro* by reducing VEGF expression and suppressing apoptosis in retinal-related cells^[27]. This is achieved through dual antioxidant effects, direct ROS scavenging and upregulation of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase, coupled with inhibition of NADPH oxidase 4-derived ROS generation in certain contexts^[27]. Additionally, blueberry anthocyanins exert anti-inflammatory effects via suppression of the nuclear factor kappa-B (NF-κB) pathway and modulate Akt and extracellular regulated protein kinases 1/2 (ERK1/2) signaling pathways^[31]. A key underlying mechanism involves ameliorating oxidative stress. Reactive oxygen species react with NO to form peroxynitrite, reducing NO bioavailability and impairing NO-dependent vasodilation^[40]. This diminished endothelium-dependent vasodilation contributes to vascular dysregulation^[41]. Hyperglycemia-induced metabolic alterations promote vascular endothelial damage and BRB disruption, playing a direct role in the pathogenesis of DR and AMD^[42]. While DR originates from inner BRB disruption, neovascular AMD involves impairment of outer BRB, and DME represents a direct consequence of BRB breakdown^[43]. By scavenging ROS and enhancing NO signaling, blueberry anthocyanins may improve retinal perfusion and reduce vascular leakage.

The amelioration of visual fatigue symptoms, including reduced eye soreness, lacrimation, and systemic discomfort, coupled with prolonged photopic persistence, further supports the functional benefits of blueberry anthocyanins in diabetic patients. These ocular improvements were supported by systemic metabolic enhancements, such as reductions in FBG, HbA1c, and the TyG index, indicating improved glycemic control and insulin sensitivity^[44], which are critical for preventing diabetic microvascular complications. Concurrently, blueberry anthocyanin supplementation significantly reduced systemic inflammatory markers, including NLR, MLR, and SIRI, reinforcing their role in mitigating chronic low-grade inflammation, a key driver in DR progression^[45-47]. Hyperglycemia promotes activation of inflammatory pathways such as NF-κB, leading to release of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which in turn exacerbate oxidative stress and impair antioxidant defenses^[48]. This vicious cycle of oxidative stress and inflammation aggravates insulin resistance and accelerates DR development^[49]. Thus, the ability of blueberry anthocyanins to simultaneously improve visual function, metabolic parameters, and inflammatory responses highlights their multi-target potential in managing diabetic ocular complications. Although the exact molecular mechanisms and temporal sequence of these effects require further elucidation, our findings provide preliminary clinical evidence supporting the direct ocular benefits of blueberry anthocyanins in diabetic patients. The concordant improvement in both

systemic metabolism and ocular outcomes suggests a synergistic mode of action that targets core pathological processes underlying diabetic eye disease. These findings strengthen the premise for using natural compounds as complementary interventions in retinal disorders.

However, the present study has several limitations. First, the absence of randomization and placebo control means that potential confounding factors, including time effects and concurrent treatments, cannot be fully excluded. Medication use is a major source of potential confounding in this real-world study with a long follow-up period, as treatment regimens were not fully standardized or systematically recorded. Although we retrospectively collected information on major antidiabetic, antihypertensive, and lipid-lowering medication classes (Table S2) and confirmed that no participant received ocular interventions during the study, complete medication records were unavailable for all participants. Participants were encouraged to maintain stable therapies throughout the six-month intervention. Among the available records, no evidence of treatment intensification was identified, while several participants reduced or discontinued hypoglycemic agents. Although these observations suggest that the reported improvements were unlikely to be attributable solely to intensified pharmacological management, unrecorded medication adjustments cannot be fully excluded as contributing factors. Given that glycaemic and insulin resistance indices are highly sensitive to changes in antidiabetic therapy, this limitation warrants particular caution. Additionally, while participants were advised to maintain their usual dietary habits and avoid excessive intake of anthocyanin-rich foods or supplements, the lack of quantitative dietary recording means that residual confounding from other dietary sources cannot be excluded. Second, we acknowledge that all IOP measurements remained within the clinically normal range; therefore, the observed reductions should be interpreted as favorable directional changes within the physiological range rather than correction of pathological elevation. The clinical significance of these within-normal-range reductions, particularly for long-term glaucoma risk in diabetic patients, warrants further investigation. Furthermore, although both eyes were included as separate observations in our analyses of visual acuity, IOP, and OCT parameters, we acknowledge that measurements from the two eyes of the same individual are inherently correlated, and we did not formally correct for inter-eye correlation using mixed-effects models or equivalent approaches. This represents a methodological limitation of the present exploratory study. Nonetheless, the consistently parallel changes observed between left and right eyes across the key endpoints suggest that the overall conclusions are robust. Future prospective studies with adequate statistical power should account for inter-eye correlation in the analytical framework. Given these limitations, the present findings should be interpreted as preliminary associations rather than definitive evidence of efficacy. Future large-scale, randomized, double-blind, placebo-controlled trials are needed to validate these results, along with studies incorporating structured dietary assessments to control for background anthocyanin consumption, and further investigations to clarify the precise molecular mechanisms and optimize treatment protocols for long-term ocular benefits.

5. Conclusion

Blueberry anthocyanins demonstrated potential associations with improved ocular and metabolic parameters in elderly patients with T2D. Clinical observation results showed that blueberry anthocyanin supplementation improved visual acuity, reduced intraocular pressure, alleviated retinal structural damage, such as reducing central foveal thickness and macular edema, and effectively alleviated visual fatigue symptoms, including eye soreness, dryness, and lacrimation. These benefits were most evident after three or six months of intervention, suggesting a cumulative and time-dependent therapeutic effect. However, due to the lack of systematic medication control, these results should be considered preliminary. The findings supported the role of blueberry anthocyanins as a promising nutraceutical or functional food ingredients for vision preservation and retinal protection in diabetics. Notably, their dual capacity to enhance metabolic profiles and directly protect ocular structures underscores potential as an adjunct therapy for diabetic retinopathy and associated visual impairment.

Data availability statement

The data are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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