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Original Article

Comprehensive investigation into the preventive and therapeutic efficacy and underlying mechanisms of phycocyanin in diabetic retinopathy in rats

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Abstract: Objective: To investigate the protective effects of phycocyanin (PC) on diabetic retinopathy (DR) in a rat model and explore its underlying mechanisms. Methods: Male Sprague-Dawley rats aged 6-8 weeks were used to establish a diabetes model through a high-fat and high-sugar diet followed by intraperitoneal injection of streptozotocin. The rats were randomly divided into the normal, model, and PC groups (n=9). The PC group received a daily oral gavage of 35 mg/kg PC, while the normal and model groups received an equal amount of drinking water. The animals were sacrificed at 8, 12, and 16 weeks after intervention to collect samples. General conditions, body weight, blood glucose levels, retinal morphology, expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and vascular endothelial growth factor (VEGF) proteins, and levels of interleukin-6 (IL-6) and tumor-necrosis factor-alpha (TNF-α) in serum were assessed. Results: PC significantly improved the symptoms and signs of diabetic rats; alleviated polydipsia, polyphagia, polyuria, and weight loss; and reduced the incidence of diabetic cataracts. After intervention, the rats in the PC group showed significant improvements in body weight and fasting blood glucose levels compared with the model group ($P<0.01$). Hematoxylin-eosin staining of retinal sections revealed that PC inhibited retinal damage, mitigated the reduction of cell numbers in the inner and outer nuclear layers, and significantly restored retinal thickness at week 16 ($P<0.01$). Immunohistochemical staining showed that PC significantly reduced the overexpression of NF-κB and VEGF in the retina ($P<0.05$). Enzyme-linked immunosorbent assay results indicated that PC significantly decreased the serum levels of IL-6 and TNF-α ($P<0.01$). Conclusion: PC exerts protective effects against DR by mitigating inflammatory responses and modulating the expression of NF-κB and VEGF, suggesting its potential as a therapeutic agent for DR.

Keywords: Phycocyanin, diabetic retinopathy, inflammatory response, streptozotocin

Introduction

Diabetic retinopathy (DR) is a common small blood vessel problem in diabetes. It is also a very common and serious eye problem [1]. DR patients have been growing in number in China in recent years. According to the World Health Organization, in 2017, the percentage of adults with diabetes in China was 11.2% [2]. The International Diabetes Federation reported in 2017 that 425 million people aged 20-79 had diabetes globally. China had 114 million of them [3]. In 2020, about 103.12 million adults globally had DR. This may rise by 25.9% to 129.84 million in 2030 and by 55.6% to 160.5 million in 2045 [4]. Long-term high blood sugar is the main cause of DR, especially in type 2 diabetes patients. When first diagnosed, 12% of people have DR. After 15 years, it rises to 63%-78%. The risk of losing their vision is 25 times higher than in non-diabetic people. DR has emerged as the leading factor of blindness in adults [5, 6]. DR is divided into nonproliferative DR (NPDR) and proliferative DR (PDR) based on its pathology [7]. NPDR is the early stage of DR. Its main problems are higher blood vessel permeability and blocked capillaries. Other pathological features of NPDR include changes in retinal blood flow, basement membrane thickening, pericyte loss, and decellularized capillary formation. The pathogenesis involves the interaction of local inflammatory responses in the retina with neural mechanisms. The main feature of PDR is pathological retinal neovascularization [8, 9].

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Phycocyanin (PC) is a natural cyanobacterial pigment complex protein synthesized by Spirulina. Relevant studies have shown that PC, by virtue of its anti-inflammatory, antioxidant, antifibrotic, and antitumor properties, exhibits great potential in the prevention and treatment of various diseases such as pulmonary fibrosis [10], chronic obstructive pulmonary disease [11], alcoholic liver cirrhosis [12], cardiovascular and cerebrovascular disorders [13], and intestinal injuries [14], and it offers new hope and possibilities in the prevention and treatment of other relevant diseases. PC can play an antifibrotic role by inhibiting the expression of cytokines such as interleukin-6 (IL-6) and tumor-necrosis factor-alpha (TNF-α), thereby inhibiting fibroblast proliferation, and initially, may play an important role in the Toll-like receptor 2 (TLR2) - myeloid differentiation factor 88 (MyD88) - nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway [10]. There have been no studies on the role of PC in DR. It can be hypothesized that PC may inhibit, delay, or arrest the onset and progression of DR by inhibiting the production of reactive oxygen species (ROS) and other components; reducing or eliminating inflammatory responses and oxidative stress; or inhibiting the NF-κB signaling pathway by downregulating or modifying Toll-like receptor 4 (TLR4), TLR2, or the molecule MyD88 pathway.

The existing clinical treatments for DR are limited and unsatisfactory, necessitating further research. The aim of the present study was to explore the potential of PC to inhibit, delay, or treat DR and to preliminarily investigate its mechanism of action.

Materials and methods

Specific Pathogen Free-grade healthy male Sprague-Dawley rats, aged 6-8 weeks and weighing 180-220 g, were obtained from Jinan Pengyue Laboratory Animal Breeding Company. The rats were acclimated to the national standard conditions for 1 week with free access to drinking water and were used for subsequent experiments after no abnormalities were observed. This experiment was approved by the Institutional Animal Care and Use Committee of Shandong Second Medical University (Approval No. 2025SDL042) and was conducted in compliance with the institutional guidelines for the care and use of laboratory animals.

PC was extracted from spirulina. Streptozotocin (STZ: S8050, Solarbio, Co., Ltd.), sodium citrate buffer (pH=4.5, C1013, Solarbio), hematoxylin and eosin (HE) staining kit (G1120, Solarbio), anti-VEGF monoclonal antibody (1:100, 216974-75-3, Solarbio); anti-NF-κB p65 rabbit polyclonal antibody (1:100, GB11997-100, Servicebio), FAS eye fixation fluid (G1109, Servicebio), rat IL-6 ELISA kit (EK0412, Servicebio), and rat TNF-α ELISA kit (EK0526, Servicebio) were purchased. The following instrumentation was used: Sanluo glucometer (Sanluo Biosensor Co., Ltd.), rotary microtome (Leica Microsystems Shanghai Trading Co., Ltd.), water bath (Shanghai Bilang Instrument Manufacturing Co., Ltd.), upright microscope (Nikon Precision Shanghai Co., Ltd.), and electrically heated convection oven (Shanghai Yiheng Scientific Instrument Co., Ltd.).

Pharmacological intervention and physiological changes in rats

We selected a dose of 35 mg/kg for PC intervention. This dose demonstrated good tolerance and therapeutic effects in our preliminary experiments, without significant toxic reactions. Moreover, this dose was referenced from the effective dose ranges used in similar studies [15, 16]. After the successful establishment of the diabetic rat model, the intervention was initiated. The rats in the PC group were administered the compound via gavage at a dose of 35 mg/kg once daily; the rats in the normal and model groups were given an equal volume of drinking water via gavage. The general condition of the rats was observed daily, including fur color, mental state, activity level, food and water intake, and the degree of bedding dampness. Fasting blood glucose, fasting body weight, daily water intake, daily food intake, and daily urine volume were measured every 4 weeks to ensure the maintenance of the hyperglycemic state.

Sampling procedures

Sampling was conducted on the 8th, 12th, and 16th weeks of intervention. The rats were anesthetized with sevoflurane. The skin was incised, and the thoracic cavity was opened to fully

expose the heart. After blood was drawn from the rat's heart, the right atrial appendage was cut open. Room-temperature physiological saline was slowly injected into the left ventricle for cardiac perfusion, followed by the infusion of 100 mL of paraformaldehyde solution. The perfusion was considered successful when the blood vessels became transparent and clear fluid was observed to flow out. After the rat's tissues and organs had hardened and the limbs stopped twitching, the eyeballs were enucleated and placed in FAS eye fixation fluid for fixation for 24 to 48 hours. The rats were euthanized by cervical dislocation. The entire experimental procedure was strictly conducted in accordance with international animal welfare guidelines to ensure proper treatment of the rats, following the "3R" principles: replacing animal experiments with other methods whenever possible (Replacement), reducing the number of animals used (Reduction), and ensuring the animals were as comfortable as possible (Refinement). Before sampling, we anesthetized the rats with sevoflurane to put them into a deep sleep, so they would not feel any pain. At the end of the experiment, the euthanasia procedure was carried out carefully and swiftly by trained personnel to ensure that the rats lost consciousness immediately.

HE staining of the retina

The rat eye wall sections were dewaxed and hydrated, stained with hematoxylin for 5 minutes, immersed in a differentiation solution for 5-8 seconds, returned to blue by ammonia for 5-8 seconds, and stained with eosin for 1 minute. The sections were then dehydrated and made transparent, followed by sealing with neutral gum. The changes in retinal tissue morphology and structure in each group of rats were observed under a microscope. Namely, three fields of view were randomly selected for each section, and three positions of each field of view were selected from left to right to measure the overall thickness of the retina, and statistically analyze the changes.

Immunohistochemical staining

The rat eye sections were routinely dewaxed and hydrated. Next, 3% hydrogen peroxide was added dropwise and incubated at room temperature for 10 minutes. Then, ethylenediaminetetraacetic acid (EDTA) antigen repair was performed at 37°C for 30 minutes, followed by washing in phosphate-buffered saline (PBS) three times, for 3 minutes each time. A primary antibody against NF-κB and VEGF was added dropwise, incubated at 37°C for 2 hours, and washed in PBS three times, for 3 minutes each time. A secondary antibody was added dropwise, incubated for 1 hour at room temperature, and washed in PBS three times, for 3 minutes each time. DAB staining was then conducted under microscopic control. After restaining with hematoxylin for 1 minute, immersion in a differentiation solution for 5-8 seconds, and returning to blue by ammonia for 5-8 seconds, the sections were dehydrated, made transparent, and sealed by neutral gum. Six fields of view were selected for each section, and the mean optical density of positive NF-κB and VEGF staining in the rat retina was determined.

ELISA detection of serum levels of IL-6, TNF-α, and VEGF in rats

After taking blood from the rats' hearts, serum was centrifuged and serum levels of inflammatory factors were determined.

Statistical analysis

All experimental data were expressed as $\bar{x} \pm s$. Before doing statistical analyses, all data were tested for normality and same variance. This checked if parametric tests were okay to use. The Shapiro-Wilk test was used to check normally distributed data. The Levene's test was used to evaluate homogeneity of variance. Only data that passed both normality and variance tests used parametric analyses. For data that met strict normality and variance criteria, a Two-way ANOVA tested two factors. These were treatment group and time point on results. This method looked at main effects of both factors at the same time. It also checked for possible interactions between them. Data processing and analysis were meticulously performed using ImageJ, specifically for the measurement of rat retinal thickness and the mean optical density of NF-κB and VEGF staining positivity. All data analyses and generation of graphs were don in GraphPad Prism 10.0. This kept data interpretation precise and reliable. In all analyses, a *P* value lower than 0.05 was considered to indicate statistical significance.

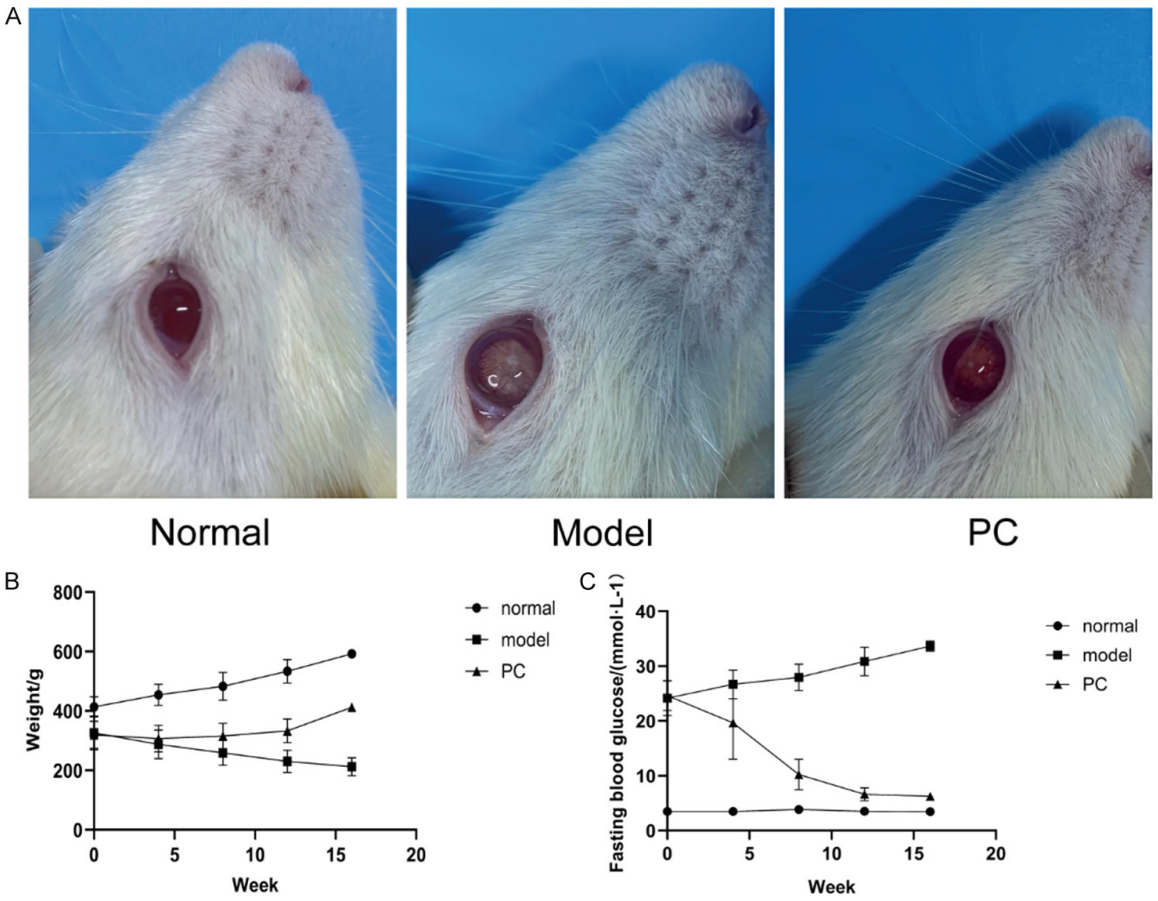

Figure 1. Phycocyanin (PC) can alleviate the symptoms and signs of rats with diabetic retinopathy. Note: A: The eyes of rats in each group were compared after 16 weeks of drug intervention; B and C: Analysis of changes in fasting blood glucose and body weight of rats in each group, n=3-9.

Results

PC alleviates the symptoms and signs of DR in rats

The rats in the normal group exhibited rapid and stable growth, with smooth, shiny, milky-white fur, normal dietary intake, regular bowel movements, and a robust physique. In contrast, the rats in the model group displayed slow growth, yellowish and sparse fur, excessive consumption of food and water, increased urination, loose stools, significant weight loss, and pronounced symptoms of polyphagia, polydipsia, polyuria, and weight loss, accompanied by abdominal distension. As the disease progressed, the lens opacity gradually worsened, leading to the development of diabetic cataracts in the model group by the 12th and 16th weeks. The rats in the PC group also exhibited slower growth, yellowish and sparse fur, increased food and water intake, frequent urination, loose stools, and weight loss, but their overall condition was better than that of the model group, with fewer instances of abdominal distension. The incidence of diabetic cataracts was lower in the PC group compared with the model group, with 2 and 1 cases at the 12th week and 3 and 1 cases at the 16th week, respectively (**Figure 1A**).

At weeks 0 and 4 of drug intervention, both the model group and the PC group had lower body weight than the normal group, while their fasting blood glucose levels were higher than those of the normal group (**Table 1**; **Figure 1B**). Throughout the experimental period, the fasting blood glucose levels of both the model group and the PC group were significantly higher than those of the normal group. At weeks 8, 12, and 16, the fasting blood glucose levels of the PC group were lower than those of the model group (**Table 2**; **Figure 1C**).

Table 1. Analysis of changes in body weight of rats in each group (x±s), n=3-9

Group	Weight/g				
	Week 0	Week 4	Week 8	Week 12	Week 16
Normal control	413.8±34.26	454.3±35.24	483.1±46.95	533.3±39.88	592.1±13.82
Model group	326.5±57.11[a]	287.8±48.19[a]	259.1±42.07[a]	230.1±37.6[a]	212.3±29.87[a]
Phycocyanin group	319.8±45.76[a]	307.2±44.91[a]	316±43.2[a]	333.3±40.09[a,b]	412.6±9.903[a,b]

Data are expressed as the mean ± standard error of three replicate determinations. One-way ANOVA (analysis of variance) was used for comparisons between groups, where a *P*-value<0.01 was considered significant. Values or bars with different letters of the alphabet are significantly (P<0.01) different. Specifically, "a" represents a statistically significant difference compared to the normal control group (P<0.01); "b" represents a statistically significant difference compared to the model group (P<0.01).

Table 2. Analysis of changes in fasting blood glucose of rats in each group (x±s), n=3-9

Group	Fasting blood glucose/(mmol·L-1)				
	Week 0	Week 4	Week 8	Week 12	Week 16
Normal Control	3.49±0.66	3.49±0.27	3.87±0.60	3.52±0.75	3.43±0.21
Model group	24.14±3.18[a]	26.69±2.62[a]	27.94±2.40[a]	30.83±2.62[a]	33.63±0.96[a]
Phycocyanin group	24.61±2.7[a]	19.63±6.68[a,b]	10.21±2.75[a,b]	6.6±1.19[b]	6.23±0.49[b]

Data are expressed as the mean ± standard error of three replicate determinations. One-way ANOVA (analysis of variance) was used for comparisons between groups, where a *P*-value<0.01 was considered significant. Values or bars with different letters of the alphabet are significantly (P<0.01) different. Specifically, "a" represents a statistically significant difference compared to the normal control group (P<0.01); "b" represents a statistically significant difference compared to the model group (P<0.01).

During the experimental period, the daily water intake, food intake, and urine output of both the model group and the PC group were significantly higher than those of the normal group. After 4 weeks of the drug intervention, the daily water intake, food intake, and urine output of the model group were significantly higher than those of the PC group (**Figure 2**).

Effect of PC on the retina of DR rats

In the normal group, the retinal morphology and structure were intact, with clearly defined and neatly arranged layers, including the ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, and outer nuclear layer. Ganglion cell detachment was minimal. In contrast, the model group exhibited significant retinal structural disorganization, with loosely arranged cells across all layers. The inner and outer nuclear layers showed a clear reduction in cell count, accompanied by substantial ganglion cell loss and altered retinal thickness. In the PC group, the retinal structure demonstrated notable improvement, with more normalized cell arrangement and increased cell numbers in the inner and outer nuclear layers compared with the model group. The layered structure was more distinct, and ganglion cell counts were higher. These findings indicate that PC effectively inhibits retinal damage and mitigates the reduction in cell numbers within the inner and outer nuclear layers, preserving retinal integrity (**Figure 3A**).

Compared with the normal group, the retinal thickness of the model group increased significantly at 8 weeks of the intervention, but subsequently decreased, becoming significantly lower than that of the normal group and the PC group at 12 and 16 weeks. By the 16th week, there was no statistically significant difference in retinal thickness between the normal and PC groups. These results demonstrate that PC intervention promotes a gradual recovery of retinal thickness, highlighting its significant positive regulatory role in retinal structural repair and functional improvement (**Figure 3B**).

PC reduces the expression levels of NF-κB and VEGF in the retina of DR rats

NF-κB and VEGF proteins were expressed across most layers of the rat retinal tissue. Throughout the intervention period, the model group exhibited significantly higher expression levels of NF-κB and VEGF compared with the normal group. However, following PC treatment, the expression of NF-κB and VEGF in the retina was significantly reduced at all time points

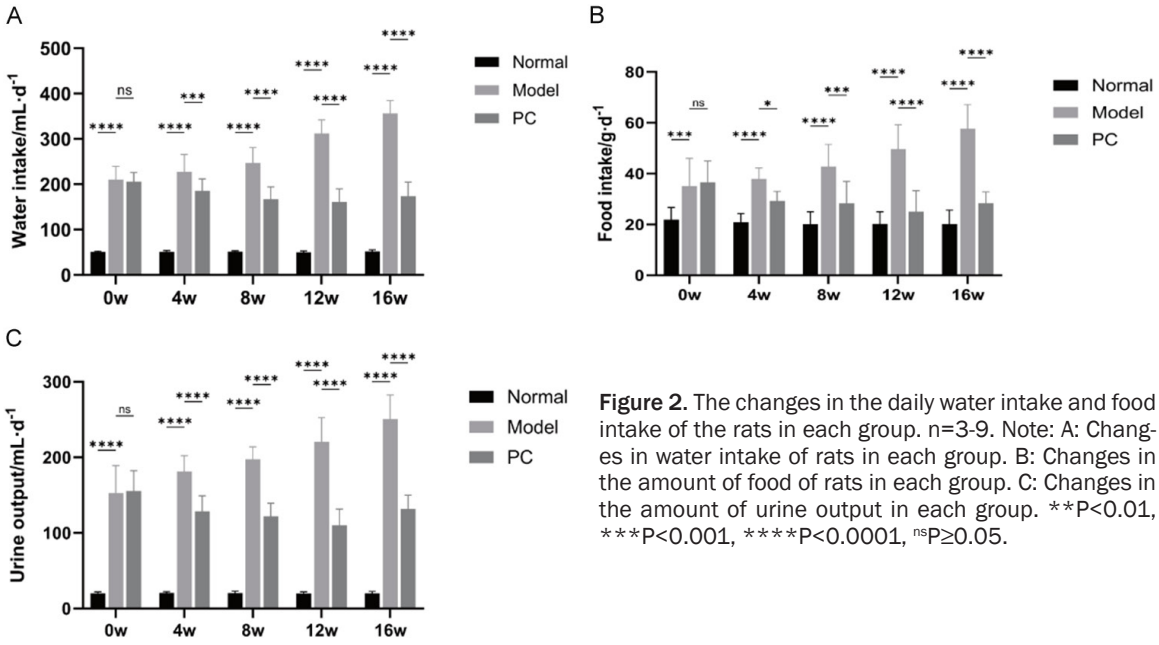

Figure 2. The changes in the daily water intake and food intake of the rats in each group. n=3-9. Note: A: Changes in water intake of rats in each group. B: Changes in the amount of food of rats in each group. C: Changes in the amount of urine output in each group. **P<0.01, ***P<0.001, ****P<0.0001, ^{ns}P≥0.05.

compared with the model group. These findings suggest that PC effectively suppresses the overexpression of NF-κB and VEGF, which contributes to its protective effects against retinal damage (**Figure 4**).

PC reduces the expression of inflammatory factors in DR rats

Throughout the drug intervention period, compared with the normal group, the levels of IL-6, TNF-α, and VEGF in the model group rats were significantly increased. Compared with the model group, after treatment with PC, the levels of IL-6, TNF-α, and VEGF were significantly decreased (**Figure 5**).

Discussion

DR develops due to the activation of inflammatory cells, such as macrophages and monocytes, in a hyperglycemic state, causing them to secrete a large number of inflammatory factors, such as IL-1, IL-6, TNF-α, IL-8, and monocyte chemotactic protein-1 [9, 17]. The increase of these inflammatory factors leads to increased permeability of vascular endothelial cells and retinal tissue edema; it leads to leukocyte adhesion to vascular endothelial cells, formation of microthrombi, and obstruction of retinal microvessels, which further aggravates retinal ischemia and hypoxia and leads to rupture of the blood-retina barrier [8, 18]. Hyperglycemia causes production of a large amount of ROS, such as superoxide anions and hydrogen peroxide, in retinal cells, leading to oxidative stress, and ROS can act as signaling molecules to activate the NF-κB pathway [19]. Meanwhile, a high-glucose environment can induce TLR4 activation and activate the NF-κB pathway. Activated NF-κB initiates the transcription of inflammatory factor genes, further aggravates oxidative damage, stimulates retinal pigment epithelial cells and vascular endothelial cells to secrete VEGF, and promotes neovascularization, thereby promoting the development of DR. Neovascularization can be an important indicator of DR progression and is closely related to the inflammatory response. If the TLR4/NF-κB pathway is inhibited, the level of proinflammatory factors in the retinal endothelial cells decreases, and neovascularization is reduced [20].

PC has remarkable anti-inflammatory, antioxidant, and ROS-scavenging properties, and possesses the ability to protect against oxidative stress [21, 22]. PC can reduce liver inflammation and decrease liver injury by inhibiting the synthesis and release of inflammatory factors such as TNF-α and interferon-γ, and by increasing the activities of catalase and superoxide dismutase [13, 23, 24]. PC reduces the expression of NF-κB in a mouse model of bleomycin-induced pulmonary fibrosis, inhibits the pro-

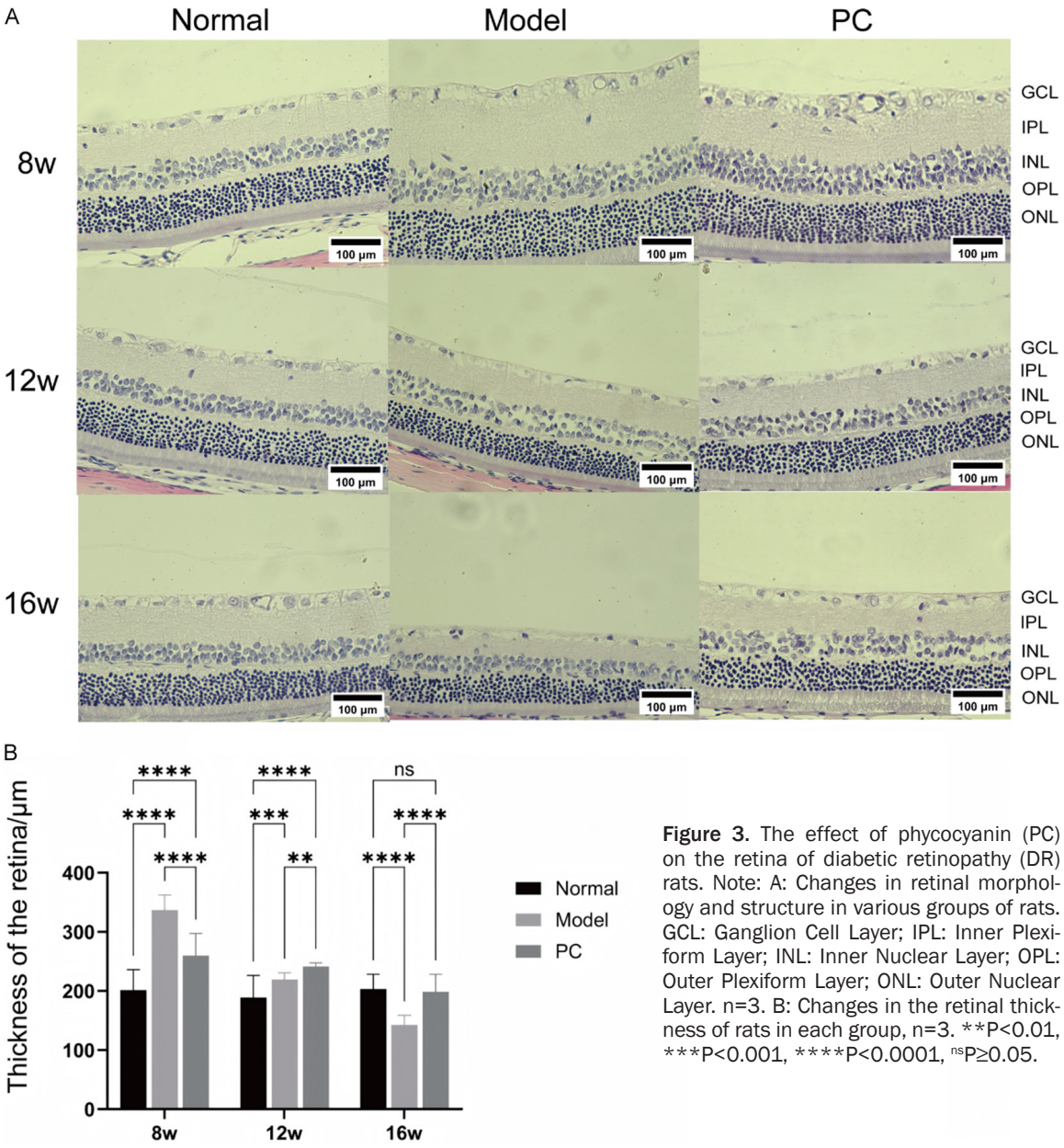

Figure 3. The effect of phycocyanin (PC) on the retina of diabetic retinopathy (DR) rats. Note: A: Changes in retinal morphology and structure in various groups of rats. GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Layer. n=3. B: Changes in the retinal thickness of rats in each group, n=3. **P<0.01, ***P<0.001, ****P<0.0001, ^{ns}P≥0.05.

duction of proinflammatory factors, and exerts an antifibrotic effect [10]. The above studies provide a theoretical basis for exploring the potential effect and mechanism of PC in the prevention and treatment of DR.

This study's results show strong proof that PC helps diabetic symptoms in DR rats. It also improves their body measurements. The hallmark symptoms of diabetes, characterized by polyphagia, polydipsia, polyuria, and weight loss, were prominently displayed in the model group. However, these symptoms were markedly alleviated in the PC group. The PC group had better fur color and mental state. They grew better and had fewer problems like a bloated stomach. Also, the PC group had fewer diabetic cataracts than the model group. This means PC can help prevent diabetes problems.

During the experiment, model and PC groups had much lower body weight than the normal group. At 12 and 16 weeks, weight in the PC group was higher than that in the model group. This shows that PC can reduce diabetes weight loss. Also, the PC group had much lower fasting blood glucose at weeks 8, 12, 16 than the

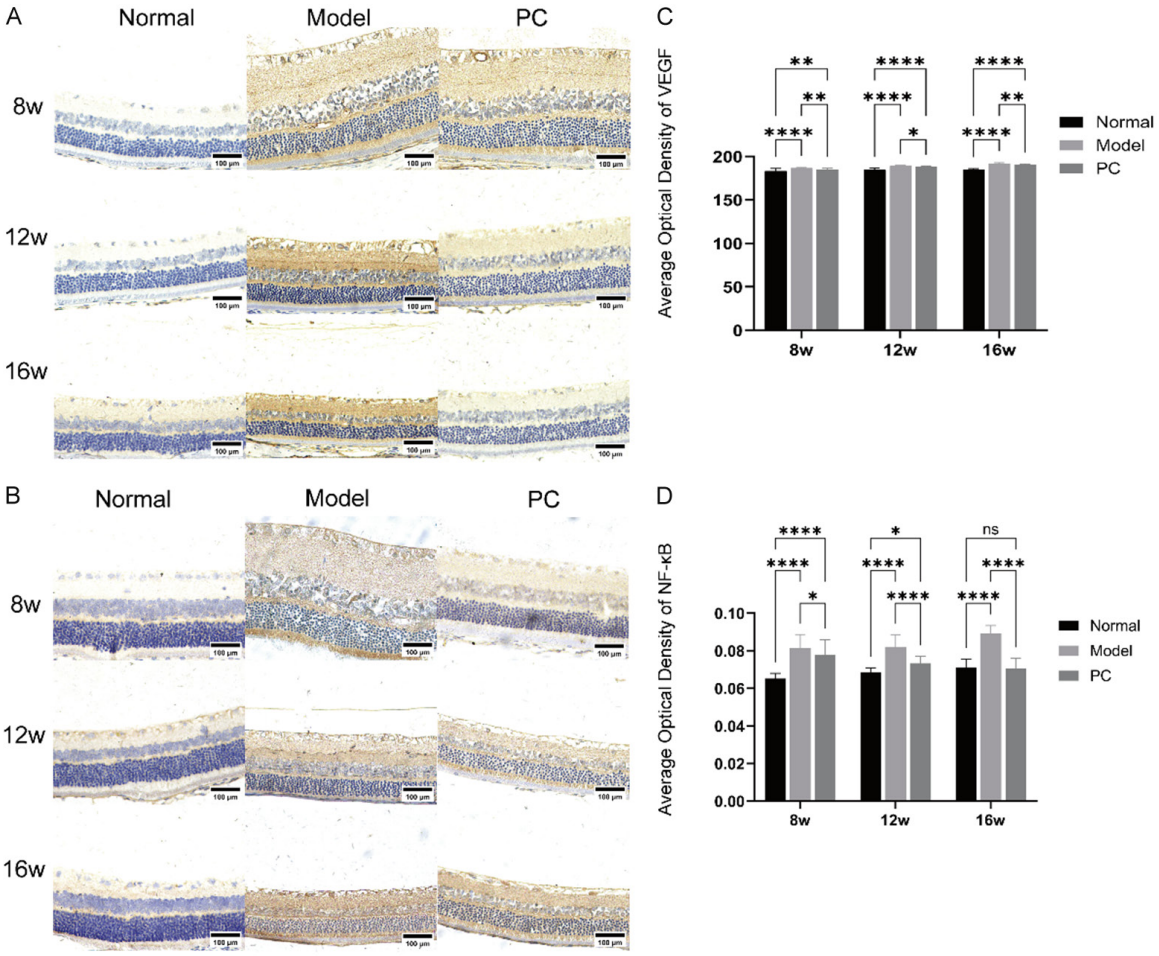

Figure 4. Phycocyanin can reduce the expression levels of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Vascular Endothelial Growth Factor (VEGF) in the retina of DR rats. Note: A: Immunohistochemical staining results of NF-κB in the retina in each group (400×), n=3. B: Average optical density of NF-κB in each group of rats. *P<0.05, ****P<0.0001. C: Immunohistochemical staining results of retinal VEGF in each group (400×), n=3. D: Average optical density of VEGF in each group of rats. *P<0.05, **P<0.01, **P<0.001, ****P<0.0001, ^{ns}P≥0.05.

model group. Therefore, PC lowers blood glucose and helps prevent high blood glucose in rats. PC greatly reduced the production of inflammatory cytokines like TNF-α and IL-6. These cytokines affect diabetes. They disrupt the insulin signaling pathway and lead to insulin resistance. Blocking these cytokines helps PC normalize insulin signaling, which improves insulin resistance. Diabetic rats had much higher water intake, food consumption, and urine volume than normal rats. This was due to osmotic diuresis and metabolic problems from hyperglycemia. After PC treatment, these measures were much lower in the PC group than the model group. This shows that PC can control metabolic processes, and it can also alleviate polydipsia, polyphagia, and polyuria.

DR is a major complication of diabetes. The model group had serious retinal structure disorders. There were fewer cells and changes in retinal thickness. The PC group had much better retinal structure. Cells were arranged more orderly. Cell numbers increased. Retinal thickness gradually returned close to normal levels. These results indicate that PC can effectively inhibit retinal damage, reduce cell loss, and maintain retinal integrity, which all help to protect against DR.

NF-κB and VEGF [25] were found to be expressed in rat retinal tissues. This suggests they help keep the retina working normally. During the drug treatment, the model group had higher NF-κB and VEGF expression than the normal group. This may be related to dis-

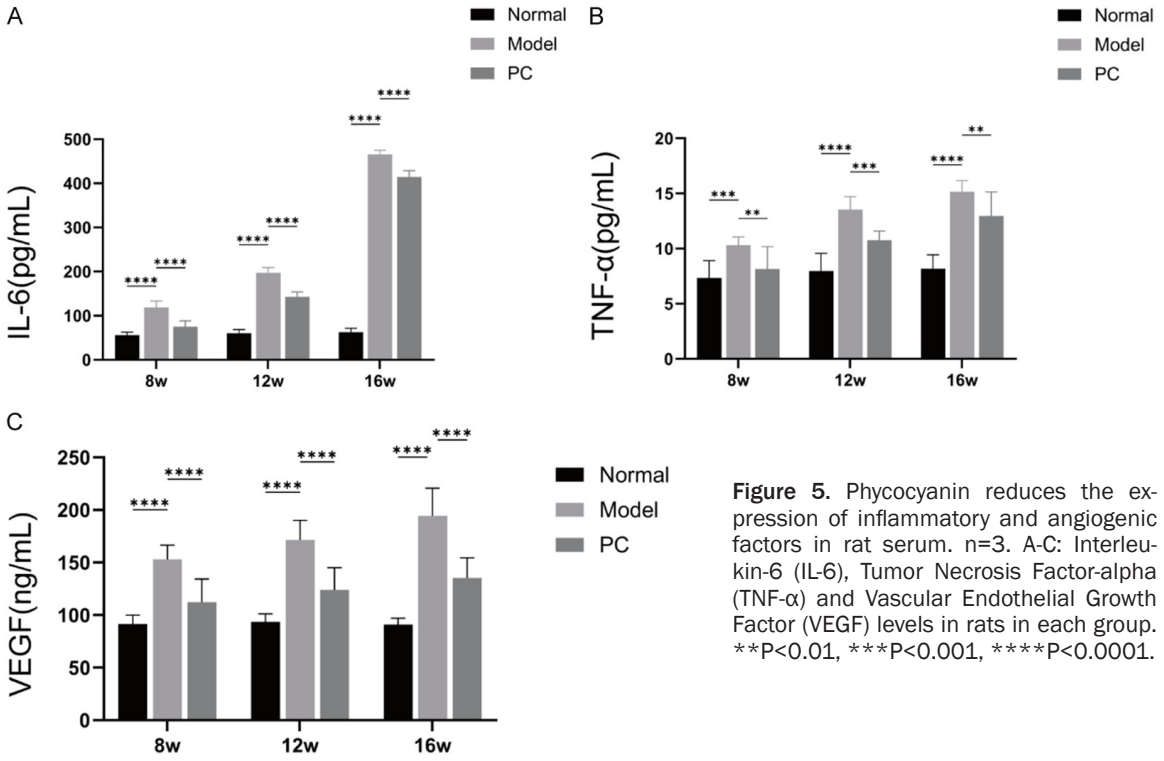

Figure 5. Phycocyanin reduces the expression of inflammatory and angiogenic factors in rat serum. n=3. A-C: Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) and Vascular Endothelial Growth Factor (VEGF) levels in rats in each group. **P<0.01, ***P<0.001, ****P<0.0001.

ease development, like inflammation and abnormal blood vessel growth [26, 27]. After PC treatment, NF-κB and VEGF levels in rat retinas were much lower than the model group at all time points. Serum VEGF levels also went down. PC stopped NF-κB and VEGF from being overexpressed in DR rats. It also controlled related pathological processes. NF-κB is a key inflammatory factor, and its lower expression shows that PC may reduce eye inflammation. Lower VEGF levels mean PC may inhibit abnormal blood vessel growth. This helps improve eye problems in DR, like abnormal blood vessel growth.

The significant elevation in IL-6 and TNF-α levels, key inflammatory mediators, indicates a robust inflammatory response in the DR disease model. This strong inflammatory state likely disrupts retinal organization, leading to retinal edema, increased microvascular permeability, and vascular occlusion. These changes impede normal retinal cell function, and make DR worse [28]. These results match earlier findings of high NF-κB and VEGF in the model group. Together, they show how inflammation and abnormal blood vessel growth are linked in DR. After PC treatment in diabetic rats, IL-6 and TNF-α levels were much lower than the model group. This shows that PC can inhibit inflammation in DR rats. PC may diminish production and release of inflammatory factors, by controlling related signaling pathways, reducing eye damage from inflammation. PC was found to lower NF-κB and VEGF levels before. This adds to evidence that PC affects multiple DR processes. It not only inhibits the inflammatory response, but also regulates angiogenesis-related factors, which helps improve eye problems in DR.

This study has some limitations. For example, more research is needed on better PC intervention timing and dosing for DR treatment. DR symptoms got worse with longer PC treatment, showing that PC can slow, but not stop, disease progression. The specific regulatory mechanism of PC in DR and the upstream and downstream factors of NF-κB need to be studied more in depth.

Conclusion

In short, the study highlights that PC has many different positive effects on rats with diabetes. PC ameliorates physiological parameters such as body weight and blood glucose levels, attenuates retinal morphological damage, regulates retinal thickness, inhibits the overexpression

of NF-κB and VEGF, and reduces the levels of inflammatory cytokines. These actions collectively contribute to the potential preventive and therapeutic effects of PC against DR. This research not only highlights the potential therapeutic benefits of PC in improving diabetic symptoms and complications, particularly DR, but also provides a strong basis for further investigation into its underlying mechanisms and clinical applications. Future studies should continue to explore the potential clinical applications of PC in the management of diabetes and its associated complications, and elucidate the mechanisms behind its therapeutic effects.

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Disclosure of conflict of interest

None.

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References

[1] Hammes HP, Feng Y, Pfister F and Brownlee M. Diabetic retinopathy: targeting vasoregression. Diabetes 2011; 60: 9-16.

[2] Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, Shi B, Sun H, Ba J, Chen B, Du J, He L, Lai X, Li Y, Chi H, Liao E, Liu C, Liu L, Tang X, Tong N, Wang G, Zhang JA, Wang Y, Xue Y, Yan L, Yang J, Yang L, Yao Y, Ye Z, Zhang Q, Zhang L, Zhu J, Zhu M, Ning G, Mu Y, Zhao J, Teng W and Shan Z. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. BMJ 2020; 369: m997.

[3] Sabanayagam C, Yip W, Ting DS, Tan G and Wong TY. Ten emerging trends in the epidemiology of diabetic retinopathy. Ophthalmic Epidemiol 2016; 23: 209-222.

[4] Teo ZL, Tham YC, Yu M, Chee ML, Rim TH, Cheung N, Bikbov MM, Wang YX, Tang Y, Lu Y, Wong IY, Ting DSW, Tan GSW, Jonas JB, Sabanayagam C, Wong TY and Cheng CY. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. Ophthalmology 2021; 128: 1580-1591.

[5] Mohamed Q, Gillies MC and Wong TY. Management of diabetic retinopathy: a systematic review. JAMA 2007; 298: 902-16.

[6] Reddy SK, Ballal AR, Shailaja S, Seetharam RN, Raghu CH, Sankhe R, Pai K, Tender T, Mathew M, Aroor A, Shetty AK, Adiga S, Devi V, Muttigi MS and Upadhya D. Small extracellular vesicle-loaded bevacizumab reduces the frequency of intravitreal injection required for diabetic retinopathy. Theranostics 2023; 13: 2241-2255.

[7] Liao YL, Fang YF, Sun JX and Dou GR. Senescent endothelial cells: a potential target for diabetic retinopathy. Angiogenesis 2024; 27: 663-679.

[8] Wang W and Lo ACY. Diabetic retinopathy: pathophysiology and treatments. Int J Mol Sci 2018; 19: 1816.

[9] Yue T, Shi Y, Luo S, Weng J, Wu Y and Zheng X. The role of inflammation in immune system of diabetic retinopathy: molecular mechanisms, pathogenetic role and therapeutic implications. Front Immunol 2022; 13: 1055087.

[10] Li C, Yu Y, Li W, Liu B, Jiao X, Song X, Lv C and Qin S. Phycocyanin attenuates pulmonary fibrosis via the TLR2-MyD88-NF-κB signaling pathway. Sci Rep 2017; 7: 5843.

[11] Li W, Li Y, Wang Q, Liu R, Lu J, Lu W and Qin S. Therapeutic effect of phycocyanin on chronic obstructive pulmonary disease in mice. J Adv Res 2024; 66: 285-301.

[12] Sarumathi A, Sethupathy S and Saravanan N. The protective efficacy of spirulina against bacterial endotoxin potentiated alcoholic liver disease. J Funct Foods 2014; 9: 254-263.

[13] AlFadhly NKZ, Alhelfi N, Altemimi AB, Verma DK, Cacciola F and Narayanankutty A. Trends and technological advancements in the possible food applications of spirulina and their health benefits: a review. Molecules 2022; 27: 5584.

[14] Lu L, Li W, Sun C, Kang S, Li J, Luo X, Su Q, Liu B and Qin S. Phycocyanin ameliorates radiation-induced acute intestinal toxicity by regulating the effect of the gut microbiota on the TLR4/Myd88/NF-κB pathway. JPEN J Parenter Enteral Nutr 2020; 44: 1308-1317.

[15] Liu RZ, Li WJ, Zhang JJ, Liu ZY, Li Y, Liu C and Qin S. The inhibitory effect of phycocyanin peptide on pulmonary fibrosis in vitro. Mar Drugs 2022; 20: 696.

[16] Jin H, He Y, Liu T, Yang T, Sun X, Chen Y and Shen F. Ligustrazine alleviates spinal cord injury-induced neuropathic pain by inhibiting the TLR4/NF-κB signaling pathway. Am J Transl Res 2024; 16: 3557-3571.

[17] Dharmarajan S, Carrillo C, Qi Z, Wilson JM, Baucum AJ 2nd, Sorenson CM, Sheibani N and Belecky-Adams TL. Retinal inflammation in murine models of type 1 and type 2 diabetes with diabetic retinopathy. Diabetologia 2023; 66: 2170-2185.

[18] Tu Y, Li L, Zhu L, Guo Y, Du S, Zhang Y, Wang Z, Zhang Y and Zhu M. Geniposide attenuates hyperglycemia-induced oxidative stress and inflammation by activating the Nrf2 signaling pathway in experimental diabetic retinopathy. Oxid Med Cell Longev 2021; 2021: 9247947.

[19] Kang Q and Yang C. Oxidative stress and diabetic retinopathy: molecular mechanisms, pathogenetic role and therapeutic implications. Redox Biol 2020; 37: 101799.

[20] Xue L, Hu M, Zhu Q, Li Y, Zhou G, Zhang X, Zhou Y, Zhang J and Ding P. GRg1 inhibits the TLR4/NF-kB signaling pathway by upregulating miR-216a-5p to reduce growth factors and inflammatory cytokines in DR. Mol Biol Rep 2023; 50: 9379-9394.

[21] Lissi EA, Pizarro M, Aspee A and Romay C. Kinetics of phycocyanine bilin groups destruction by peroxyl radicals. Free Radic Biol Med 2000; 28: 1051-1055.

[22] Li Y, Li X, Liang ZP, Chang XY, Li FT, Wang XQ and Lian XJ. Progress of microencapsulated phycocyanin in food and pharma industries: a review. Molecules 2022; 27: 5854.

[23] Grover P, Bhatnagar A, Kumari N, Narayan Bhatt A, Kumar Nishad D and Purkayastha J. C-Phycocyanin-a novel protein from Spirulina platensis- in vivo toxicity, antioxidant and immunomodulatory studies. Saudi J Biol Sci 2021; 28: 1853-1859.

[24] Liu R, Qin S and Li W. Phycocyanin: anti-inflammatory effect and mechanism. Biomed Pharmacother 2022; 153: 113362.

[25] Zhu J, Sun H, Kang X, Zhu H and Yan X. Acidic polysaccharides from *Buddleja officinalis* inhibit angiogenesis *via* the Nrf2/ARE pathway to attenuate diabetic retinopathy. Food Funct 2022; 13: 9021-9031.

[26] Dongare S, Gupta SK, Mathur R, Saxena R, Mathur S, Agarwal R, Nag TC, Srivastava S and Kumar P. Zingiber officinale attenuates retinal microvascular changes in diabetic rats via anti-inflammatory and antiangiogenic mechanisms. Mol Vis 2016; 22: 599-609.

[27] Yang F, Yu J, Ke F, Lan M, Li D, Tan K, Ling J, Wang Y, Wu K and Li D. Curcumin alleviates diabetic retinopathy in experimental diabetic rats. Ophthalmic Res 2018; 60: 43-54.

[28] Jiang T, Chang Q, Cai J, Fan J, Zhang X and Xu G. Protective effects of melatonin on retinal inflammation and oxidative stress in experimental diabetic retinopathy. Oxid Med Cell Longev 2016; 2016: 3528274.