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Protective Effects of Bioactive Compound-Derived Nanoparticle Against Diabetic Retinopathy Through the Modulation of the NF-κB Signaling Pathway

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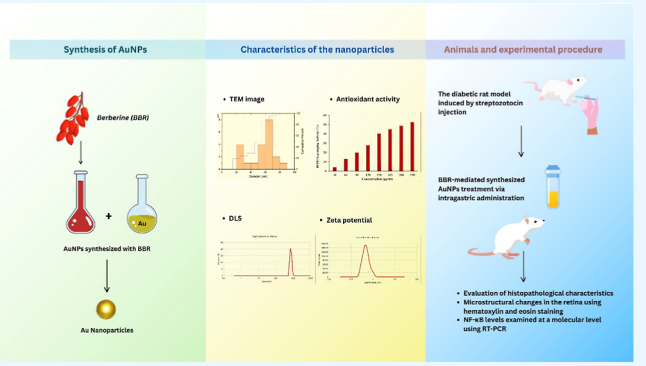
ABSTRACT: Diabetic retinopathy is a prevalent and severe microvascular complication of diabetes, often causing visual impairment and blindness in adults. This condition significantly impacts the quality of life for many diabetes patients worldwide. Berberine (BBR), a bioactive compound known for its effects on blood glucose levels, has shown promise in managing diabetic complications. However, the exact mechanism of how BBR influences the development of diabetic retinopathy remains unclear. In this study, we focused on synthesizing a formulation derived from BBR and assessing its protective effects against diabetic retinopathy. The formulation was created using a green synthesis method and thoroughly characterized. In vitro studies demonstrated the antioxidant activity of the formulation against 2,2-diphenyl-1-picryl-hydrazyl-hydrate. We also examined the NF-κB signaling pathway at a molecular level using real-time polymerase chain reaction. To mimic diabetic retinopathy in a controlled setting, a diabetic rat model was established through streptozotocin injection. The rats were divided into normal, diabetic, and treatment groups. The treatment group received the formulated treatment via intragastric administration for several weeks, while the other groups received normal saline. Evaluation of histopathological characteristics and microstructural changes in the retina using hematoxylin and eosin staining revealed that the bioactive compound-derived nanoparticle exhibited favorable biological, chemical, and physical properties. Treatment with the formulation effectively reduced oxidative stress induced by diabetes and inhibited the NF-κB signaling pathway in the diabetic rat model. Under high glucose conditions, oxidative stress was heightened, leading to mitochondria-dependent cell apoptosis in Müller cells via the activation of the NF-κB signaling pathway. The bioactive compound-derived formulation counteracted these effects by decreasing IκB phosphorylation, preventing NF-κB nuclear translocation, and deactivating the NF-κB signaling pathway. Furthermore, treatment with the bioactive compound-derived formulation mitigated retinal micro- and ultrastructural changes associated with diabetic retinopathy. These results indicate that the formulation protects against diabetic retinopathy by suppressing oxidative stress, reducing cell apoptosis, and deactivating the NF-κB signaling pathway. This suggests that the bioactive compound-derived formulation could be a promising therapeutic option for diabetic retinopathy.



1. INTRODUCTION

Diabetic retinopathy is an eye condition caused by diabetes.[1] The gradual damage occurs to the light-sensitive lining of the retina.[2] Untreated diabetic retinopathy can lead to permanent vision loss. Diabetes affects the body's sugar processing and storage, resulting in high blood sugar levels which can harm the body, particularly the eyes. Over time, diabetes causes lasting harm to the retina and tiny blood vessels across the body.[3−5] When blood vessels start leaking blood and fluids, it leads to diabetic retinopathy, causing swelling in the retinal tissue and affecting vision clarity.[6] Typically, both eyes are impacted by diabetic retinopathy, with a higher likelihood of occurrence the longer a person has had diabetes.[1] Diabetic retinopathy stands as a primary cause of preventable blindness. Prolonged high blood sugar levels in individuals with diabetes can lead to fluid accumulation in the eye's lens, impacting focus regulation.[7] This alteration in the lens curvature affects the individual's field of vision. Fortunately, stabilizing blood sugar levels can restore the lens to its usual shape and improve eyesight.[8] Patients who maintain stable blood sugar levels can slow down the development and progression of diabetic retinopathy.[9−12]

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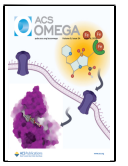

The American Optometric Association recommends that everyone with diabetes get a comprehensive dilated eye examination at least once a year.[13] However, a poll indicated that more than a third of Americans were unaware that a comprehensive eye exam is the only way to identify if a person's diabetes may cause blindness. If left untreated, diabetic retinopathy can lead to significant vision impairment. Treatment options for diabetic retinopathy vary based on the individual's condition. Patients with diabetic retinopathy may benefit from laser surgery to repair damaged blood vessels or prevent further harm.[14] Optometrists may administer eye injections to reduce inflammation or inhibit the growth of new blood vessels.[15] In advanced cases, surgical procedures, such as replacing the vitreous fluid in the back of the eye, might be necessary. Surgical intervention may also be required to repair retinal detachment when the light-sensitive lining at the back of the eye becomes detached.[16−19]

Over the past few years, nanotechnology has experienced remarkable advancement within the scientific and technological spheres.[20] Nanomaterials, renowned for their exceptional properties, have seen wide-ranging applications in biomedicine.[20] The pharmaceutical industry has shown a growing inclination toward leveraging nanotechnology for drug development. Nanomedicine has exhibited promising results in treating various life-threatening diseases, showcasing significant efficacy in their treatment.[21−23] Gold nanoparticles (AuNPs) have attracted a lot of interest because of their potential therapeutic uses (such as an anti-inflammatory, antioxidative, antiangiogenic, antiproliferative, and antidiabetic agent). Diabetic wound healing, an autistic diabetic model, and diabetic nephropathy were all helped by the antihyperglycemic, antioxidant properties of AuNPs.[24,25] Recent years have seen an uptick in research on the effects of AuNPs on a variety of diabetes problems.[26,27] Treatment with 50 nm AuNPs at a dosage of 2.5 mg/kg effectively reversed nearly all liver redox parameters in an animal model of autism and diabetes. This included restoring levels of glutathione (GSH) and oxidized glutathione (GssG), as well as enhancing the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Additionally, the oxygen radical absorbance capacity showed improvement. The study also observed a reversal of damage in pancreatic B cells and enhancements in glucose and lipid profiles, indicating regenerative potential.

Diabetic wounds heal significantly faster when treated with AuNPs in conjunction with antioxidants.[28] Chen et al. found that 3−5 nm AuNPs synthesized without surface modifiers or stabilizers had a significant impact. They found that the synergistic effects of AuNP, EGCG, and ALA dramatically sped up the healing of diabetic cutaneous wounds by regulating angiogenesis and reducing inflammation. When applied to the skin, AuNP may also act as an adjuvant, boosting the effectiveness of antioxidants and facilitating their uptake.[29]

Rhizoma coptidis, the source of berberine (BBR), is a bioactive component known for its impact on glucose metabolism, lipid profiles, and congestive heart failure.[30] Additionally, BBR protects against damage caused by oxidative stress,[31] such as the oxidative stress seen in the early stages of DR development, when reactive oxygen species levels are elevated.[32] In addition, BBR reduces oxidative stress caused by fatty acids and glutamate, and protects cells from apoptosis caused by oxidative damage.[33,34] Therefore, BBR holds promise as a therapy for hypertension, elevated cholesterol levels, and type 2 diabetes mellitus. In the context of diabetic issues, BBR demonstrates a calming influence. When Müller cells experience stress or injury, they upregulate the expression of the structural protein glial fibrillary acidic protein. According to research by Fu et al.,[35] activation of the AMPK signaling system by BBR protects Müller cells from damage generated by modified LDL. Furthermore, BBR can prevent retinal disorders caused by oxidative stress by reducing antioxidant enzyme activities, inhibiting leukocyte-mediated death of vascular endothelium, and vice versa.[36,37] Though the findings above hint at therapeutic potential during DR, the compound's probable mechanism of action in this context remains unclear.

2. MATERIALS AND METHODS

2.1. Materials. Unless specified otherwise, all chemicals used were of reagent grade and did not require additional purification prior to utilization. Gold aurochlorate ($HAuCl_4$), ascorbic acid, diphenylpicrylhydrazyl (DPPH) ($C_{18}H_{12}N_{50}$), berberine hydrochloride (BHC), and an MTT assay kit were sourced from Sigma-Aldrich Chemicals in Shanghai. DMEM-F12, fetal bovine serum, trypsin, and penicillin−streptomycin were obtained from GIBCO/BRL Invitrogen in Carlsbad, California. The solvents were acquired from Merck Chemicals in Germany.

2.2. Synthesis and Characterization of AuNPs. A green/biogenic method was utilized for the synthesis of AuNPs. In brief, a solution of BHC (100 μL) in DMSO was mixed with DI water (15 mL) in a round-bottom flask and stirred. The pH was adjusted using K_2CO_3 (300 mM). Subsequently, $HAuCl_4{\cdot}3H_2O$ (2.5 mL, 2.5 mM) was added drop by drop to the BHC solution and stirred for 3 h. The resulting nanoparticles were purified through multiple rounds of centrifugation and washing with water and ethanol. The morphology of the synthesized nanoparticles was examined using a JEOL JEM-1400 plus transmission electron microscopy (TEM) in Peabody, MA, USA. The hydrodynamic size and zeta potential of the nanoparticles were determined using a dynamic light scattering (DLS) Zetasizer Nano-ZS from Malvern Instruments in Worcestershire, UK.

2.3. Biological Evaluations. The antioxidant activity of the synthesized nanoparticles was assessed using the DPPH assay kit. The nanoparticles were dispersed in 50 μL of deionized water at various concentrations (30, 60, 90, 120, 150, 165, 180, and 190 μg/mL) and incubated with 100 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution dissolved in methanol at a concentration of 100 μM. The nanoparticle-DPPH mixture was placed in a 96-well plate and incubated, and then the absorbance of the solution was measured to calculate the percentage of free radical scavenging activity.

2.4. Generation of Diabetic Rat Model. Based on previous research with minor adjustments, streptozotocin (STZ) was administered to induce the model. The beta cells in the mammalian pancreas are especially sensitive to the toxic effects of STZ, a compound naturally produced by *Streptomyces achromogenes*. This substance is used in medical settings for specific islet of Langerhans tumor treatments and is also employed by scientists to create a type 1 diabetes animal model. The necessary ethical approvals were obtained from the Ethical Committee and relevant authorities at our research institution (s) to conduct animal experiments, ensuring compliance with all guidelines, regulations, and ethical standards. The animal housing maintained a constant temperature of 23 °C with proper ventilation, and all animals had unrestricted access to standard feed and water throughout the

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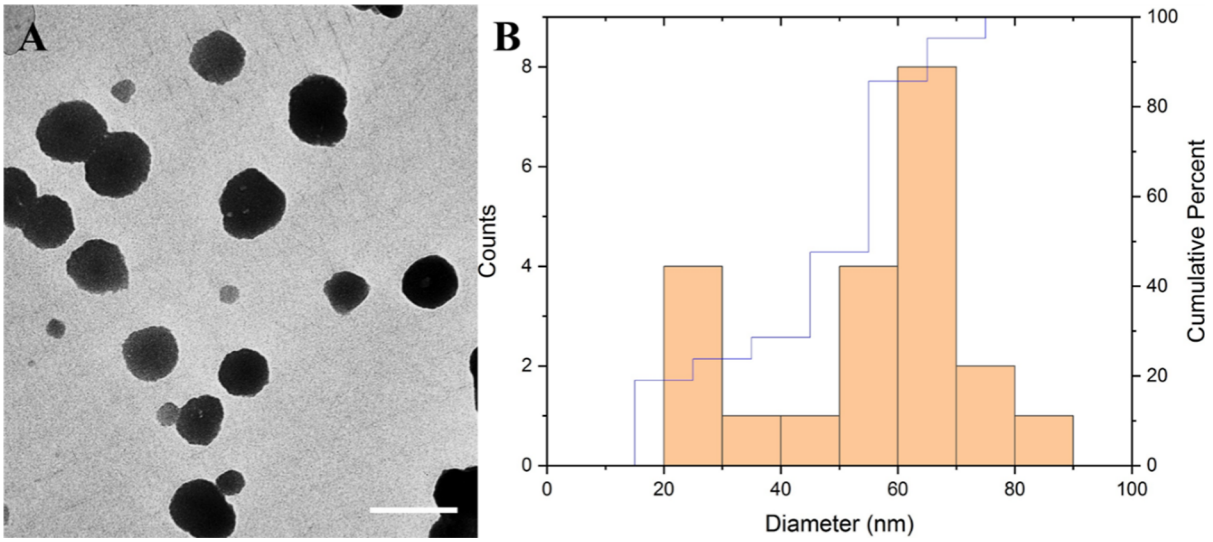

Figure 1. TEM image (A) and size histogram (B) of BBR-mediated synthesized AuNPs.

study. Except for the control group, all rat groups received a single intraperitoneal injection of 60 mg/kg STZ dissolved in 0.05 mol/L citrate buffer (pH 4.5) over 2 days. Blood glucose levels above 300 mg/dL were used to confirm hyperglycemia in the animals. The rats were divided into three groups: the healthy group (untreated animals), the negative control group (diabetic animals without treatment), and the treatment group (diabetic animals treated with nanoparticles). Following isolation, rat retinas were fixed in a 4% paraformaldehyde solution. Thin sections (5 μm) of the retinal tissues were stained with E&H and examined under a surgical microscope. High-resolution images of the retina were captured using a digital camera system (C3040-AD6, Shanghai, China) connected to a desktop computer. Photos of the retina were taken at a standardized distance of 1500 mm from the optic nerve in both eyes.

2.5. Molecular Studies. *2.5.1. RNA Extraction and Complementary DNA Synthesis.* Total RNA was extracted from fresh frozen tissue biopsies using Trizol reagent (by TaKaRa Biotechnology, Dalian) and the RNeasy Mini Kit (by Qiagen, Hilden, Germany) following the manufacturer's instructions. Elution of RNA was performed using 25 μL of RNase-free water. The integrity of the RNAs was confirmed by 2% agarose gel electrophoresis, while spectrophotometry was utilized to assess the yield and quality of the RNA, which was then stored at −80 °C. DNase I (by Invitrogen) was employed to eliminate DNA contamination. A mixture of random hexamers and 1 μL of total RNA was incubated at 70 °C for 10 min, followed by cooling on ice for 2 min. Subsequently, 2 μL of 10× polymerase chain reaction (PCR) buffer, 2 μL of 25 mmol/L magnesium chloride, 1 μL of 10 mmol/L deoxyribonucleoside triphosphate mix, and 2 μL of 0.1 mol/L dithiothreitol were added in sequence. SuperScript II reverse transcriptase (200 U; by Life Technologies, Rockville, MD, USA) was added to the tubes after 5 min of incubation at 25 °C, followed by incubation at 25 °C for 10 min and 42 °C for 50 min. The reactions were halted after 15 min at 70 °C and then cooled on ice. Each sample was treated with 1 μL of RNase H and incubated at 37 °C before being stored at −20 °C for future use.

2.5.2. Real-Time PCR. Target gene expression levels, including NF-κB, SIRT1, SIRT3, FOXO3, Mn-SOD, Nrf2, NQO1, IL-6, and TNF-α, were quantified through real-time PCR using the SYBR Premix Ex *Taq*II PCR kit (by TaKaRa). The primers for these target genes were designed using NCBI Primer-BLAST and their characteristics were evaluated using Gene Runner and Oligo Analyzer 3.1 software tools. Mouse β-actin served as the housekeeping gene to standardize variations in initial RNA concentrations as an internal control. The quantitative PCRs consisted of 10 μL of 2× Premix Ex *Taq*II PCR SYBR green Mastermix, 0.4 μL of both sense and antisense primers (50 nM each), 7.2 μL of ddH_2O, and 2 μL (20 ng) of template cDNA, making up a total reaction volume of 20 μL. The real-time PCR was conducted in an optical 96-well plate (from PE Applied Biosystems, CA, USA), utilizing optical caps for fluorescence detection. The PCR steps included activating the AmpliTaq Gold polymerase at 95 °C for 10 min, followed by 40 cycles of: 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s. Comparative cycle threshold (Ct) calculations were made relative to the control group, with β-actin Ct values subtracted from gene Ct values to yield a ΔCt value. The ΔΔCt values were obtained by subtracting the average control Ct value, and the relative expression of target genes to the control was calculated using the eq 2^(−ΔΔCt). The normalized data were generated based on the average of three replicates.

2.6. Statistical Analysis. The mean and standard deviation were utilized to describe all data sets. A statistical analysis was carried out using SPSS for Windows to confirm that the data adhered to a normal distribution, ensuring homogeneity of variance and determining significant differences. Results were deemed statistically significant when the P value was below 0.05.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the Nanoparticles. In this study, we employed green chemistry to synthesize AuNPs. While chemical and physical methods have traditionally been utilized for nanoparticle synthesis, there is a growing demand in the medical field for nontoxic, environmentally friendly alternatives. Further exploration is required to develop safer synthesis techniques that utilize natural compounds as reducing agents. Plant-mediated synthesis is gaining popularity due to the ease of handling and manipulation of nanoparticles

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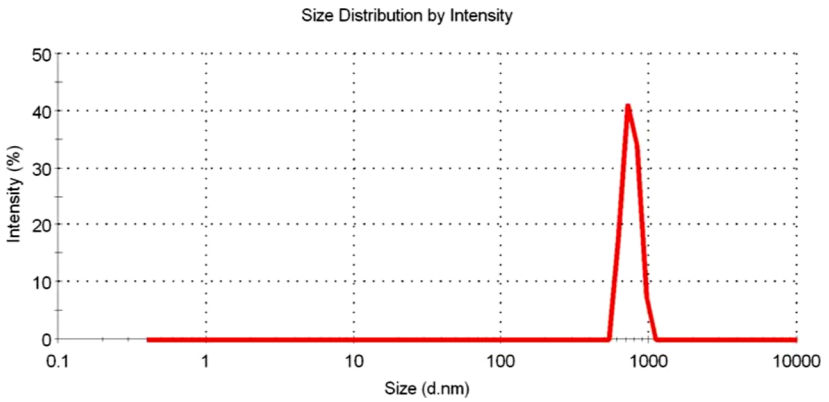

Figure 2. DLS results of the BBR-mediated synthesized AuNPs.

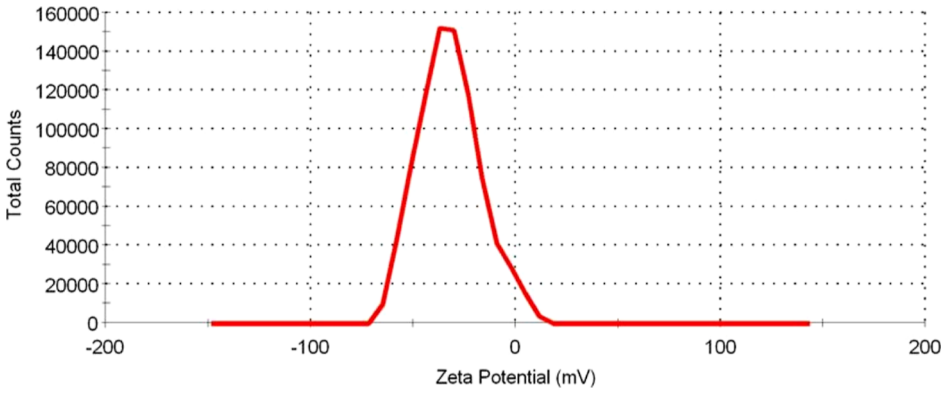

Figure 3. Zeta potential of the BBR-mediated synthesized AuNPs.

compared to microbe-assisted synthesis. Plant-based synthesis is rapid, safe, and convenient, making it achievable in a standard laboratory setting without specialized equipment. In this experiment, we used BBR as the reducing and stabilizing agent for the synthesis of AuNPs. The synthesized nanoparticles were thoroughly characterized. TEM imaging revealed that the AuNPs synthesized with BBR exhibited a predominantly spherical morphology, with an average size of 54 ± 17 nm (Figure 1A). Analysis of the size distribution, conducted using image processing software and statistical methods, indicated that approximately 90% of the BBR-mediated AuNPs fell within the size range of 60−70 nm (Figure 1B).

Apart from TEM imaging, DLS was utilized to determine the hydrodynamic size of the AuNPs synthesized with BBR. The findings (Figure 2) revealed a larger size compared to the TEM image. This discrepancy in size can be attributed to the fact that DLS measures the hydrodynamic diameter of particles, which tends to be larger than their actual size.

The zeta potential of the AuNPs synthesized with BBR was assessed, and the results indicated a value of −32.1 mV (Figure 3). This zeta potential is noteworthy as it signifies a robust electrostatic barrier surrounding the AuNPs synthesized with BBR, which helps prevent their aggregation and ensures the colloidal stability of the nanoparticles.

3.2. Biological Properties. *3.2.1. Antioxidant Activity.* Nanomaterials exhibit varied biological properties based on their origins and physicochemical characteristics. One particularly intriguing attribute that has motivated researchers to explore nanomaterials for various diseases is their antioxidant properties. While not all nanomaterials inherently possess exceptional antioxidant and radical scavenging capabilities, these traits can be imparted during nanomaterial synthesis using green chemistry techniques. Numerous studies have highlighted the robust antioxidant effects of BBR.[38−40] Moreover, its antidiabetic activities of BBR persuaded us to apply it as the reducing and capping agent of AuNPs during the synthesis process.[41−44] The DPPH assay kit was utilized to measure the antioxidant characteristics of AuNPs synthesized through BBR mediation, with the outcomes depicted in Figure 4. The findings indicated that BBR-mediated synthesized AuNPs displayed antioxidant properties that were dependent on the dosage administered.

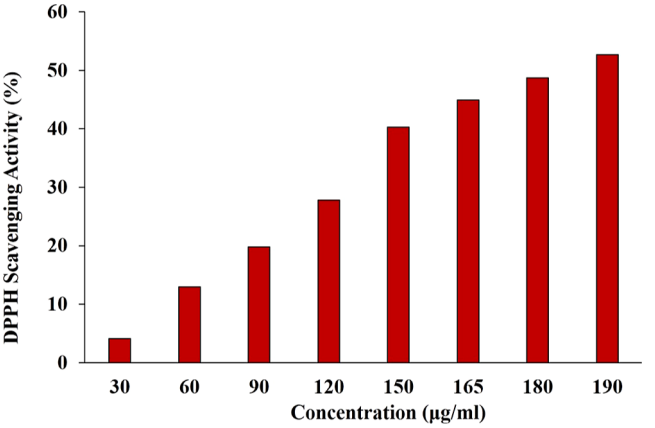

Figure 4. Antioxidant activity of the BBR-mediated synthesized AuNPs.

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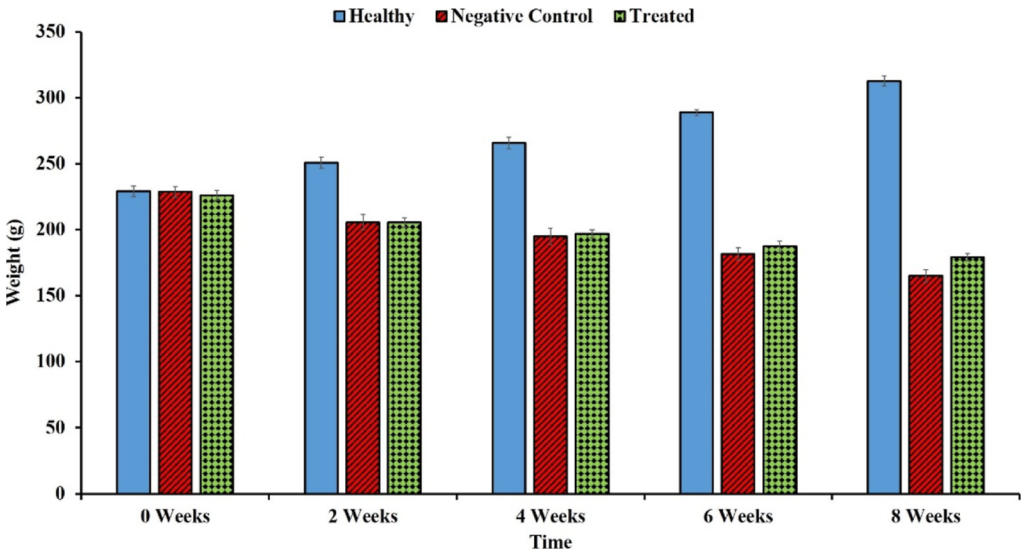

Figure 5. Animals body weight during the experiment.

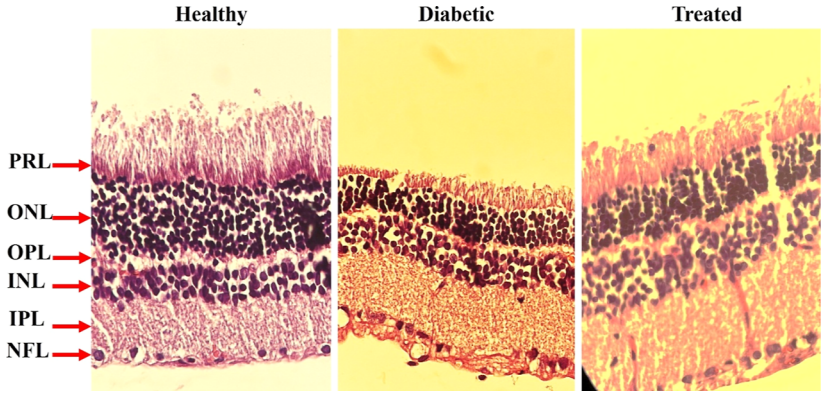

Figure 6. Effects of BBR-mediated synthesized AuNPs on histopathological changes in the diabetic rat retinas.

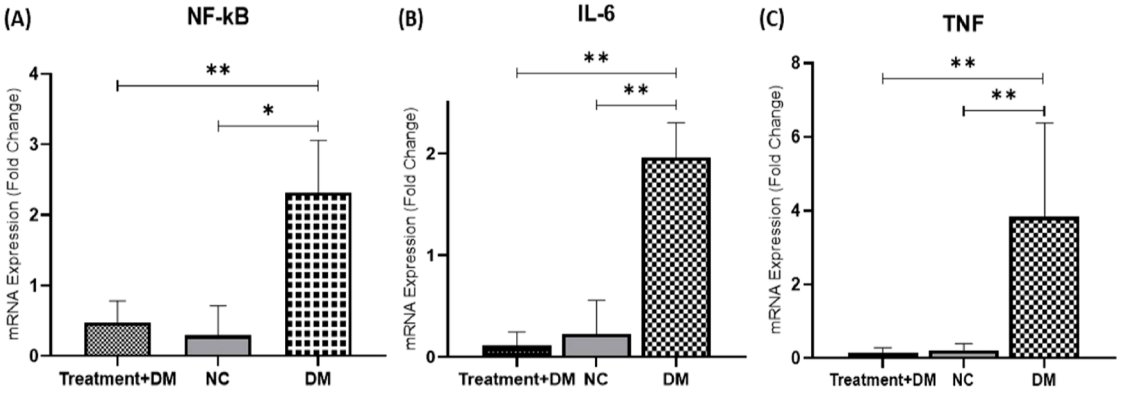

Figure 7. Effects of BBR-mediated synthesized AuNPs on the gene expression of (A) NF-κB, IL-6 (B), and TNF (C), in the study groups (data are presented as mean ± SEM). The results are shown as means ± SD from experiments. *P < 0.05, **P < 0.01, and ***P < 0.001.

3.3. Animal Studies. Animal studies were carried out on rats with STZ-induced diabetic retinopathy. For over the past three decades, STZ has been commonly employed to induce diabetes in various animal species, aiding in the screening of hypoglycemic medications. STZ treatment in animals leads to diabetic symptoms akin to those in human diabetes cases. Consequently, diabetogenic mechanisms and the preclinical assessment of potential antidiabetic drugs have been investigated in mice treated with STZ. Throughout the experiment, we monitored the animals' body weights, with the results illustrated in Figure 5. The findings revealed a significant decrease in body weight in the negative control group (diabetic rats without treatment), a consequence of diabetes. Treatment with BBR-mediated synthesized AuNPs marginally mitigated weight loss, although the difference was not statistically significant compared to the negative control group (Figure 5).

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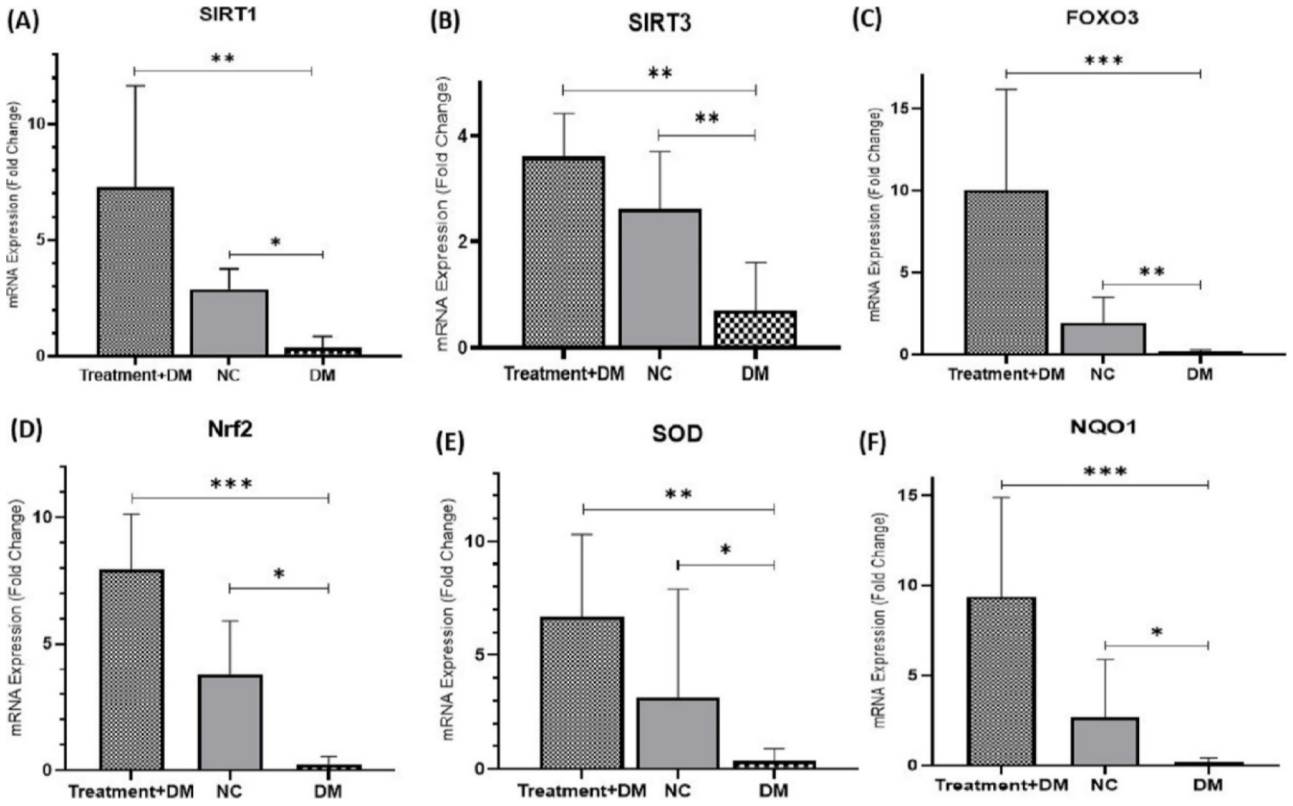

Figure 8. Effects of BBR-mediated synthesized AuNPs on antioxidative-related genes. Gene expression of (A) SIRT1, (B) SIRT3, (C) FOXO3, (D) Nrf2, (E) SOD, and (F) NQO1 from each group were measured after treatment of AU. The results are shown as means ± SD from experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

The retinal microstructure and possible ultrastructural changes during the hyperglycemia and under the treatment. Normal rat retinas were found to be smooth upon H and E staining. The cells in each stratum were uniformly distributed and neatly structured (Figure 6 healthy), presenting a clear and complete structure. Retinal cells in the diabetic rat group seemed disorganized, there was swelling in the nerve fiber layer, and the number of capillaries increased (Figure 6 diabetic). The BBR-mediated synthesized AuNPs treatment reversed the degenerative alterations caused by diabetes (Figure 6 treated). The thickness of the retina was also measured and statistically analyzed. The BBR-mediated synthesized AuNPs enhanced retinal thickness in the diabetic group, although the effect was not statistically significant.

3.4. Gene Expression Findings. Real-time PCR was employed to evaluate the impact of treatment using BBR-mediated synthesized AuNPs on the expression levels of NF−B, SIRT1, SIRT3, FOXO3, Mn-SOD, Nrf2, NQO1, IL-6, and TNF- in the whole blood across multiple groups. The research results indicated that the administration of BBR-mediated synthesized AuNPs to diabetic rats brought about significant changes in the expression of all the specified genes when compared to the rats in the normal control group ($P < 0.05$) (Figures 7 and 8). Compared to the healthy control rats, diabetic rats exhibited significantly elevated levels of NF-kB mRNA expression ($P < 0.001$). Following treatments with BBR-mediated synthesized AuNPs, the expression of NF-kB was notably reduced in comparison to the diabetic control rats ($P < 0.05$). Interestingly, the normal group (NC) rats displayed notably lower NF-kB expression levels than diabetic rats (Figure 7A). NF-kB serves as a crucial transcription factor involved in regulating inflammatory responses. Hence, we evaluated the expression levels of two inflammatory cytokines, IL-6 and TNF. As per the qRT-PCR findings, the diabetic group showcased significantly higher levels of TNF- (See Figure 7B) and IL-6 (Figure 7C) expression as opposed to the NC ($P < 0.05$). Our results indicate that the treatment with BBR-mediated synthesized AuNPs could potentially reverse TNF- and IL-6 expression through the down-regulation of NF-kB. Overall, these results suggest that BBR-mediated synthesized AuNPs may protect against diabetes by inhibiting the production of pro-inflammatory cytokines via the inhibition of NF-κB activation.

To explore the potential antioxidant properties of BBR-mediated synthesized AuNPs, we investigated their ability to regulate the expression of key genes involved in combating oxidative stress, namely SIRT1, SIRT3, FOXO3, and Nrf2. Following treatment with BBR-mediated synthesized AuNPs, the Nrf2 gene was found to be upregulated in diabetic rats compared to the untreated control group, suggesting that BBR-mediated synthesized AuNPs could enhance the transcriptional activity of Nrf2. Comparing diabetic control rats to normal control rats, we observed a decrease in the gene expression of SIRT1, SIRT3, and FOXO3 (see Figure 8A−D). Subsequently, the treatment of diabetic rats with BBR-mediated synthesized AuNPs resulted in a significant increase in the mRNA expression of SIRT1, SIRT3, and FOXO3 compared to diabetic control rats ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively). Furthermore, we examined the gene expression of SOD and NQO1 to better understand the impact of BBR-mediated synthesized AuNPs on the downstream target genes of FOXO3 and Nrf2. Diabetes led to a significant reduction in

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the mRNA levels of NQO1 and SOD compared to the NC ($P < 0.05$ for both). However, treatment with BBR-mediated synthesized AuNPs significantly boosted the gene expression of SOD and NQO1 ($P < 0.01$ and $P < 0.001$, respectively) (Figure 8E,F). Overall, our findings suggest that BBR-mediated synthesized AuNPs possess potential antioxidant effects by enhancing the production of antioxidant enzymes through the upregulation of Nrf2 mRNA levels. Additionally, it is plausible that BBR-mediated synthesized AuNPs may offer protection against diabetic retinopathy by activating the Sirt1/Sirt3-FOXO3-mediated antioxidant defense system.

4. CONCLUSIONS

In summary, we showcased the green synthesis of AuNPs using BBR without the need for hazardous reductants. It is vital to thoroughly characterize AuNPs before usage to ensure safety and avoid potential toxicity. The formulation was evaluated for its ability to combat 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) through antioxidant activity assessment. The NF-κB signaling pathway was analyzed at the molecular level using real-time PCR. We propose the use of AuNPs for treating diabetes and its microvascular complications due to their ability to disrupt disease-causing proteins implicated in diabetic complications. The therapy with a bioactive compound-based formula decreased oxidative stress caused by diabetes and blocked the NF-κB signaling pathway in a rat model of diabetic retinopathy. This formula reversed the harmful effects of high glucose levels by reducing IκB phosphorylation, stopping NF-κB from moving into the nucleus, and deactivating the NF-κB pathway. Additionally, treatment with the bioactive compound-based formula improved both micro- and ultrastructural changes in the retina. Our findings suggest that AuNPs synthesized with BBR possess antioxidant properties by enhancing antioxidant enzyme production through upregulation of Nrf2 mRNA levels. Moreover, these AuNPs may offer protection against diabetic retinopathy possibly via the Sirt1/Sirt3-FOXO3-mediated antioxidant defense system. While AuNPs exhibit promise in managing diabetes, further systematic studies are required to determine optimal size and dosage.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kropp, M.; Golubnitschaja, O.; Mazurakova, A.; Koklesova, L.; Sargheini, N.; Vo, T.-T. K. S.; de Clerck, E.; Polivka, J.; Potuznik, P.; Polivka, J.; et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications-risks and mitigation. *EPMA J.* **2023**, *14* (1), 21−42.

(2) Bouzianas, D. Autologous Adipose Tissue Stem Cell-Derived Terminally Differentiated Retinal Pigment Epithelium Monolayer on a Biocompatible Scaffold, to Restore Vision in Age-Related Macular Degeneration Patients. In *Technologies in Cell Culture—A Journey From Basics to Advanced Applications*; IntechOpen, 2024.

(3) Saaddine, J. B.; Honeycutt, A. A.; Narayan, K. V.; Zhang, X.; Klein, R.; Boyle, J. P. Projection of diabetic retinopathy and other major eye diseases among people with diabetes mellitus: United States, 2005−2050. *Arch. Ophthalmol.* **2008**, *126* (12), 1740−1747.

(4) Fong, D. S.; Aiello, L.; Gardner, T. W.; King, G. L.; Blankenship, G.; Cavallerano, J. D.; Ferris, F. L.; Klein, R.; Association, A. D. Retinopathy in diabetes. *Diabetes Care* **2004**, *27* (suppl_1), s84−s87.

(5) Vujosevic, S.; Aldington, S. J.; Silva, P.; Hernández, C.; Scanlon, P.; Peto, T.; Simó, R. Screening for diabetic retinopathy: new perspectives and challenges. *Lancet Diabetes Endocrinol.* **2020**, *8* (4), 337−347.

(6) Gupta, A.; Bansal, R.; Sharma, A.; Kapil, A. Macular Oedema. In *Ophthalmic Signs in Practice of Medicine*; Springer, 2024; pp 271−317.

(7) Srivastava, N.; Chandra, M.; Nitesh. Diabetes and the retinal changes in the eye: a threat to the sight. *Int. J. Community Med. Public Health* **2024**, *11* (2), 1030−1037.

(8) Xolmatova, Y. N. m. Diabetic Angioretinopathy And Treatment Methods. *Procedia of Engineering and Medical Sciences*; SJIF, 2024; Vol. *8*(1), pp 118−125.

(9) Arora, M.; Pandey, M. *Deep Neural Network for Diabetic Retinopathy Detection, 2019 International Conference on Machine Learning, Big Data, Cloud and Parallel Computing (COMITCon)*; IEEE: 2019; pp 189−193.

(10) Leley, S. P.; Ciulla, T. A.; Bhatwadekar, A. D. Diabetic retinopathy in the aging population: a perspective of pathogenesis and treatment. *Clin. Interv. Aging* **2021**, *16*, 1367−1378.

(11) Feldman-Billard, S.; Larger, E.; Massin, P. Early worsening of diabetic retinopathy after rapid improvement of blood glucose control in patients with diabetes. *Diabetes Metab.* **2018**, *44* (1), 4−14.

(12) Aiello, L. P.; Cahill, M. T.; Wong, J. S. Systemic considerations in the management of diabetic retinopathy. *Am. J. Ophthalmol.* **2001**, *132* (5), 760−776.

(13) Silva, P. S.; Cavallerano, J. D.; Sun, J. K.; Tolson, A. M.; Tolls, D.; Abrahamson, M. J.; Aiello, L. M.; Aiello, L. P. Disparities Between Teleretinal Imaging Findings and Patient-Reported Diabetic Retinopathy Status and Follow-Up Eye Care Interval: A 10-Year Prospective Study. *Diabetes Care* **2024**, *47*, 970−977.

(14) Tan, T.-E.; Wong, T. Y. Diabetic retinopathy: Looking forward to 2030. *Front. Endocrinol.* **2023**, *13*, 1077669.

(15) Yiallouridou, C.; Acton, J. H.; Banerjee, S.; Waterman, H.; Wood, A. Pain related to intravitreal injections for age-related macular degeneration: a qualitative study of the perspectives of patients and practitioners. *BMJ Open* **2023**, *13* (8), No. e069625.

(16) Puent, B. D.; Nichols, K. K. Patients' perspectives on noncompliance with diabetic retinopathy standard of care guidelines. *J. Am. Optom. Assoc.* **2004**, *75* (11), 709−716.

(17) Jones, S. L.; Nichols, K. K. Diabetic eye examination report. *J. Am. Optom. Assoc.* **2007**, *78* (11), 588−595.

(18) Whitehead, M.; Wickremasinghe, S.; Osborne, A.; Van Wijngaarden, P.; Martin, K. R. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. *Expet Opin. Biol. Ther.* **2018**, *18* (12), 1257−1270.

(19) Duh, E. J.; Sun, J. K.; Stitt, A. W. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight.* **2017**, *2* (14), No. e93751.

(20) Haleem, A.; Javaid, M.; Singh, R. P.; Rab, S.; Suman, R. Applications of nanotechnology in medical field: a brief review. *Global Health J.* **2023**, *7* (2), 70−77.

(21) Khan, A. S. *Ethics and Nanotechnology, 2014 IEEE International Symposium on Ethics in Science, Technology and Engineering*; IEEE, 2014; pp 1−14.
(22) Beumer, K. Nation-building and the governance of emerging technologies: the case of nanotechnology in India. *Nanoethics* **2019**, *13*, 5−19.
(23) Anjum, S.; Ishaque, S.; Fatima, H.; Farooq, W.; Hano, C.; Abbasi, B. H.; Anjum, I. Emerging applications of nanotechnology in healthcare systems: Grand challenges and perspectives. *Pharmaceuticals* **2021**, *14* (8), 707.
(24) Lee, K. X.; Shameli, K.; Yew, Y. P.; Teow, S.-Y.; Jahangirian, H.; Rafiee-Moghaddam, R.; Webster, T. J. Recent developments in the facile bio-synthesis of gold nanoparticles (AuNPs) and their biomedical applications. *Int. J. Nanomed.* **2020**, *15*, 275−300.
(25) Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. Gold nanoparticles for biology and medicine. *Angew. Chem., Int. Ed.* **2010**, *49* (19), 3280−3294.
(26) BarathManiKanth, S.; Kalishwaralal, K.; Sriram, M.; Pandian, S. R. K.; Youn, H.-s.; Eom, S.; Gurunathan, S. Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. *J. Nanobiotechnol.* **2010**, *8* (1), 16.
(27) Dhas, T. S.; Kumar, V. G.; Karthick, V.; Vasanth, K.; Singaravelu, G.; Govindaraju, K. Effect of biosynthesized gold nanoparticles by Sargassum swartzii in alloxan induced diabetic rats. *Enzyme Microb. Technol.* **2016**, *95*, 100−106.
(28) Hosseinzadeh, A.; Zamani, A.; Johari, H. G.; Vaez, A.; Golchin, A.; Tayebi, L.; Vafa, E.; Abbasi, M.; Amani, A. M.; Chelliapan, S.; et al. Moving beyond nanotechnology to uncover a glimmer of hope in diabetes medicine: Effective nanoparticle-based therapeutic strategies for the management and treatment of diabetic foot ulcers. *Cell Biochem. Funct.* **2023**, *41* (5), 517−541.
(29) Chen, S.-A.; Chen, H.-M.; Yao, Y.-D.; Hung, C.-F.; Tu, C.-S.; Liang, Y.-J. Topical treatment with anti-oxidants and Au nanoparticles promote healing of diabetic wound through receptor for advance glycation end-products. *Eur. J. Pharm. Sci.* **2012**, *47* (5), 875−883.
(30) Mana, T.; Devi, O. B.; Singh, Y. D. Therapeutic Application of Berberine: a Consolidated Review. *Curr. Pharmacol. Rep.* **2023**, *9* (5), 329−340.
(31) He, X.-F.; Zhang, L.; Zhang, C.-H.; Zhao, C.-R.; Li, H.; Zhang, L.-F.; Tian, G.-F.; Guo, M.-F.; Dai, Z.; Sui, F.-G. Berberine alleviates oxidative stress in rats with osteoporosis through receptor activator of nuclear factor κ B/receptor activator of nuclear factor kappa-B ligand/osteoprotegerin (RANK/RANKL/OPG) pathway. *Bosn. J. Basic Med. Sci.* **2017**, *17* (4), 295.
(32) Kowluru, R. A.; Kowluru, A.; Veluthakal, R.; Mohammad, G.; Syed, I.; Santos, J. M.; Mishra, M. TIAM1-RAC1 signalling axis-mediated activation of NADPH oxidase-2 initiates mitochondrial damage in the development of diabetic retinopathy. *Diabetologia* **2014**, *57*, 1047−1056.
(33) Sun, Y.; Yuan, X.; Zhang, F.; Han, Y.; Chang, X.; Xu, X.; Li, Y.; Gao, X. Berberine ameliorates fatty acid-induced oxidative stress in human hepatoma cells. *Sci. Rep.* **2017**, *7* (1), 11340.
(34) Sadeghnia, H. R.; Kolangikhah, M.; Asadpour, E.; Forouzanfar, F.; Hosseinzadeh, H. Berberine protects against glutamate-induced oxidative stress and apoptosis in PC12 and N2a cells. *Iran. J. Basic Med. Sci.* **2017**, *20* (5), 594.
(35) Fu, D.; Yu, J. Y.; Connell, A. R.; Yang, S.; Hookham, M. B.; McLeese, R.; Lyons, T. J. Beneficial effects of berberine on oxidized LDL-induced cytotoxicity to human retinal Müller cells. *Invest. Ophthalmol. Visual Sci.* **2016**, *57* (7), 3369−3379.
(36) Song, D.; Song, J.; Wang, C.; Li, Y.; Dunaief, J. L. Berberine protects against light-induced photoreceptor degeneration in the mouse retina. *Exp. Eye Res.* **2016**, *145*, 1−9.
(37) Tian, P.; Ge, H.; Liu, H.; Kern, T. S.; Du, L.; Guan, L.; Su, S.; Liu, P. Leukocytes from diabetic patients kill retinal endothelial cells: effects of berberine. *Mol. Vis.* **2013**, *19*, 2092.
(38) Li, Z.; Geng, Y.-N.; Jiang, J.-D.; Kong, W.-J. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evid. base Compl. Alternative Med.* **2014**, *2014*, 1−12.
(39) Liu, Y.; Long, S.; Zhang, S.; Tan, Y.; Wang, T.; Wu, Y.; Jiang, T.; Liu, X.; Peng, D.; Liu, Z. Synthesis and antioxidant activities of berberine 9-O-benzoic acid derivatives. *RSC Adv.* **2021**, *11* (29), 17611−17621.
(40) Rajasekhar, K.; Samanta, S.; Bagoband, V.; Murugan, N. A.; Govindaraju, T. Antioxidant berberine-derivative inhibits multifaceted amyloid toxicity. *Iscience* **2020**, *23* (4), 101005.
(41) Cicero, A. F.; Tartagni, E. Antidiabetic properties of berberine: from cellular pharmacology to clinical effects. *Hosp. Pract.* **2012**, *40* (2), 56−63.
(42) Zhao, H.-L.; Sui, Y.; Qiao, C.-F.; Yip, K. Y.; Leung, R. K.; Tsui, S. K.; Lee, H.-M.; Wong, H. K.; Zhu, X.; Siu, J. J.; et al. Sustained antidiabetic effects of a berberine-containing Chinese herbal medicine through regulation of hepatic gene expression. *Diabetes* **2012**, *61* (4), 933−943.
(43) Ni, W.-J.; Ding, H.-H.; Tang, L.-Q. Berberine as a promising anti-diabetic nephropathy drug: An analysis of its effects and mechanisms. *Eur. J. Pharmacol.* **2015**, *760*, 103−112.
(44) Gulfraz, M.; Mehmood, S.; Ahmad, A.; Fatima, N.; Praveen, Z.; Williamson, E. Comparison of the antidiabetic activity of Berberis lyceum root extract and berberine in alloxan-induced diabetic rats. *Phytother Res.* **2008**, *22* (9), 1208−1212.

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