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Original Article

Investigating the Anti-Inflammatory, Antioxidant and Apoptotic Protein Inhibition Study of Berberine on Diabetic Retinopathy in Rats

Lihui Wang[1], Wei Fei[2], Chongjuan Qin[3], Jing Zhang[4,*]

[1]Department of Medical Imaging Center, Jinan Central Hospital, Jiefang, Lixia, Jinan, Shandong Province, CHINA.
[2]Department of Pediatrics, Jinan Central Hospital, Jiefang, Lixia, Jinan, Shandong Province, CHINA.
[3]Department of Internal Medicine, Jinan Licheng District Traditional Chinese Medicine Hospital, Hongjialou South, Licheng, Jinan, Shandong Province, CHINA.
[4]Department of Digestive, Jinan Central Hospital, Jiefang, Lixia, Jinan, Shandong Province, CHINA.

ABSTRACT

Background: Globally, Diabetic Retinopathy (DR) is a leading cause of vision loss. This disease, which impacts the microvasculature of the retina, is brought on by the oxidative stress linked to diabetes. Several studies have shown the anti-diabetic properties of the plant-derived phytochemical "Berberine". However, there is a limited amount of research available on its effects, specifically on Diabetic Retinopathy (DR). **Objectives:** The primary aim of the present investigation is to assess the impact of berberine on DR in rats by examining its underlying influence on oxidative stress, inflammation and programmed cell death. **Materials and Methods:** The current study effectively established a rat model of diabetic retinopathy utilizing Streptozotocin (STZ). The diabetic rats were treated for 12 weeks and evaluated for inflammatory markers (TNF-α, VEGF), antioxidant markers (superoxide, dismutase, catalase and glutathione) and apoptosis markers (Bcl-2, Bax, Cleaved-caspase-3). **Results:** The results demonstrated that Berberine treatment had a significant hypoglycemic index when compared to the diabetic group. Berberine therapy significantly reduced high levels of pro-inflammatory cytokines (TNF-α and VEGF) in the diabetic retina. Berberine also was effective in modulating the antioxidant enzymes and bringing their concentrations to acceptable levels. Berberine treatment also reduced the apoptosis of diabetic mice retina by reducing the elevated ROS level caused by high glucose and reduced the ratio of Bax/Bcl-2 and the expression of cleaved caspase-3. **Conclusion:** Based on the findings, it is possible to infer that Berberine has potential as a pharmacological candidate for the prevention of DR.

Keywords: Diabetic retinopathy, Apoptosis, Anti-inflammatory, Alloxan, Cytokines, Antioxidant.

Correspondence:
Dr. Jing Zhang
Department of Digestive, Jinan Central Hospital, No.105, Jiefang Road, Lixia District, Jinan City, Shandong Province, 250013, CHINA.
Email: jingzhang100@outlook.com

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INTRODUCTION

Diabetic Retinopathy (DR) is a disorder resulting from elevated blood glucose levels that impair the blood vessels in the retina, the light-sensitive tissue located at the posterior of the eye. It is a primary cause of blindness among working-age individuals and a significant contributor to global visual impairment.

Numerous studies in both humans and rodents with diabetes have shown that the disease specifically affects the neuronal and vascular components of the retina.[1,2] In addition, a large body of evidence suggests that neurons are injured soon after diabetes is developed, which can lead to vascular injury and, ultimately, DR.[3,4] It is currently difficult to determine the precise pathophysiological mechanism producing neurovascular injury in the diabetic retina. The essential factor in starting the chain reaction that results in retinal damage, according to several research reports, is oxidative assault brought on by diabetes.[5,6] Diabetes is related to changed metabolites in the body, which cause an increase in oxidative assault and damage to retinal cells. Major contributors to these detrimental effects on retinal cells include induction of cell death, activation of inflammatory responses and reduction of neurotrophic support. All these factors eventually lead to diabetes-induced retinal lesions.[7]

Reactive Oxygen Species (ROS) are unstable oxygen molecules that react with cell molecules. ROS can injure cells if their levels exceed the cell's antioxidant capability, yet they can also signal and maintain homeostasis.[8] It has also been noted that specific growth factors, transcription factors and cytokines are produced in response to the excessive formation of ROS that is

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associated with diabetes. Thus, numerous studies point to the importance of proinflammatory cytokines in the progression of DR. The pathophysiology of DR is defined by certain cases in which oxidative assault and inflammation interact to activate mitochondrial-dependent apoptosis. Rapid neuronal cell death and reduced axonal regeneration are associated with raised expression of apoptosis markers Bax and caspase-3 in the early stages of diabetic retina. To combat DR, this research suggests ways to lower inflammation and oxidative assault.[9,10]

The past two decades have seen increasingly rapid advances in the field of pharmacology using naturally occurring medicine.[11,12] In this regard, there is growing experimental evidence that demonstrates that natural products have the tendency to reduce oxidative assault due to their antioxidant potential.[13-15] Therefore, natural products may be beneficial in preventing the damage caused to retinal tissues due to DR.[16] Berberine is an isoquinoline alkaloid derived from many medicinal plants including *Berberis lycium, Coptis chinensis* and many others. This alkaloid compound has already been reported to exhibit beneficial effects against cardiovascular problems, cancers, diabetes and diabetes-associated complications. There are numerous studies that demonstrate the anti-diabetic effects of berberine, however, studies reporting its effects on DR are very scarce.[17] Therefore, the main objective of the current study was to evaluate the effect of berberine on DR in rats by studying its underlying effects on oxidative assault, inflammation and apoptosis.

MATERIALS AND METHODS

Chemicals

Sigma (USA) provided berberine, reduced Glutathione (GSH), Streptozotocin (STZ), Thiobarbituric Acid (TBA), 5,5′-Dithiobis-(2-Nitrobenzoic; acid) (DTNB), 1,1,3,3; tetramethox propane, Sodium Dodecyl Sulphate (SDS) and 2,2-Azino-Bis-3-ethylbenzothiazoline-6-Sulfonic; acid (ABTS). All other compounds were analytical grade.

Experimental animals

Typical cage conditions were maintained, including a 12-hr light/dark cycle, for adult male Wistar rats weighing 100 to 150 g. The temperature was kept at room temperature (25ºC). All rats had unrestricted access to water and their feed consisted of a conventional pellet diet with the following ingredients: 21% crude protein, 38.1% non-fiber carbohydrate, 9.0% moisture, 5.0% crude fat, 6.0% crude ash, 3.2% crude fiber and vitamins and minerals to fulfill their nutritional requirements.

All animals were observed prior to the start of the experiment to rule out any concurrent infection. The Institutional Ethics Committee at Jinan Central Hospital.

Establishment of experimental diabetes using Streptozotocin (STZ) and animal grouping

An artificial diabetic environment was created by injecting intraperitoneally 50 mg/kg of body weight of a freshly made STZ solution in 0.1 M citrate buffer with a pH of 5. This was done to simulate the clinical conditions of diabetes. We investigated the effects of STZ treatment on hyperglycemia that was caused by diabetes over a period of one week. To carrying out the experiment, rats were selected because their blood glucose levels were either equal to or higher than 180 mg/dL. A total of 24 rats were randomly randomized to four groups, with six rats assigned to each group (N=6), as will be seen below:

Group-1: Rats that do not exhibit any abnormalities (control group).

Group-2: Normal rats administered with 50 mg/kg/day dose of berberine dissolved in water for 16 weeks.

Group-3: Diabetic control rats.

Group-4: Diabetic rats administered with 50 mg/kg/day of berberine dissolved in distilled water for 16 weeks.

Collection of samples

At the end of the experiment, rats who had fasted overnight were euthanized by decapitation while being gently sedated with ether. Serum was extracted from blood samples, which were then kept at minus 20ºC until analysis. Rapid removal of the eye globes was followed by dissection and ice-cold saline rinsing of the retinas. To measure lipid peroxidation and antioxidant defenses, retinal samples were homogenized in 0.2 M potassium phosphate buffer. For a Western blotting examination, some materials were frozen at 80ºC. To measure the amounts of sorbitol and fructose, additional retinal samples were homogenized in 6% (wt/vol) ice-cold perchloric acid and neutralized with potassium carbonate.

Biochemical analysis

Oral Glucose Tolerance Test (OGTT)

Blood samples were collected from the lateral tail veins of control and diabetic rats that had been fasted the night before killing. Then, 40, 80, 120 and 160 min after administering the 2 g;/kg body weight glucose solution, sequential blood samples were obtained. After allowing blood samples to coagulate, serum was separated with a centrifuge. Using a reagent kit, serum glucose concentration was determined using Trinder's method.[18]

Estimation of fructosamine, insulin and glycosylated Hemoglobin (HBA_{1c})

Serum insulin levels were tested using specialist ELISA kits acquired from R&D Systems (USA) and according to the manufacturer's recommendations. Serum fructosamine levels

were determined using the Baker *et al.*[19] technique and a reagent kit (Spinreact, Spain).

Using reagent kits obtained from (Stanbio Company, Texas, USA), blood samples from each rat were collected on ethylenediaminetetraacetic acid solutions and used to determine HBA_{1c}% in accordance with Abraham *et al.*[20]

Determination of oxidative assault and antioxidant status

Oxidative assault was assessed by detecting lipid peroxidation in retinal homogenates and calculating Malondialdehyde (MDA) using a previously described method.[21] GSH content, antioxidant enzyme Catalase (CAT) activity, Superoxide: Dismutase (SOD) activity and glutathione peroxidase activity were determined using the Beutler *et al.* technique,[22] Cohen *et al.* method,[23] Marklund and Marklund method,[24] and Matkovics methods.[25]

Estimation of cytokines like IL-1β, PKCβ and TNF-α

ELISA kits were used to measure levels of IL-1β, PKCβ and TNF-α in retinal homogenates, following manufacturer instructions. The concentrations of various cytokine tests were determined using a spectrophotometer set to 450 nm. Standard curves were built using standard IL-1β, PKCβ and TNF-α and the amounts of the unknown samples were determined from the plots.

Western blot assay

The frozen retinas were homogenized using an ice-cold lysis buffer. To remove insoluble particles, the samples were centrifuged at 10,000 g for 10 min. The Bradford method was used to calculate protein concentrations. Using 10% SDS polyacrylamide gel electrophoresis, identical amounts of proteins were electrophoresed and transferred to nitrocellulose membranes. Over an hour at room temperature, the membranes were blocked with PBST, a solution of 5% skim milk powder in PBS with 0.01% Tween 20. The membranes were next inoculated with diluted antibodies against VEFG, Bax, Bcl2, active caspase-3 and -actin (Santa Cruz Biotechnology, USA), which were mixed 1:1000 in blocking buffer. The membranes were rinsed and then treated with secondary antibodies that matched their primary colour for an additional hour at room temperature. Following this, they were washed one more and finally developed.

Statistical analysis

All statistical comparisons were made using: the one-way ANOVA test, followed by Tukey's test *post hoc* analysis with the data analysis software GraphPad Prism 5. A p value of 0.05 was considered significant and the findings were reported as mean, SD, or SEM.

RESULTS

Berberine improves hyperglycaemic effect in diabetic rats

When compared to control rats, STZ-induced diabetic rats had significantly higher blood glucose levels at fasting and 40, 80, 120 and 160 min after oral glucose loading (Figure 1a). Berberine therapy effectively reduced blood glucose levels in STZ-induced diabetic mice across all OGTT time points. STZ diabetic rats demonstrated significantly larger OGTT Areas Under the Curve (AUCs) than the control group (p<0.01). Berberine therapy may reduce OGTT AUC in diabetic rats compared to control rats (Figure 1b, p<0.01).

Berberine reduces protein glycation and improves insulin release

Table 1 summarizes berberine's effects on blood insulin, fructosamine and Hb A1c% levels. In STZ-induced diabetic rats, blood insulin levels were significantly lower (p<0.001) than in control rats. Berberine was administered orally to STZ-induced diabetic mice and serum insulin levels were dramatically improved afterward. Diabetic rats had significantly higher levels of serum fructosamine and blood HbA;1c% (p<0.001) compared to control or berberine-treated groups. Berberine treatment significantly reduced serum fructosamine and blood HbA_{1c}% in diabetic rats (p<0.001) but had no detectable influence in normal rats.

Berberine reduced the oxidative assault brought on by hyperglycaemia in the retina of diabetic rats

Figure 2a shows that STZ-induced diabetic rats had significantly (p<0.001) greater MDA levels in their retinas compared to normal controls, indicating lipid peroxidation. Berberine significantly (P<0.001) reduced retinal MDA levels in STZ-induced diabetic mice. STZ injection significantly reduced GSH concentration in

Table 1: Impact of berberine on serum insulin, fructosamine and blood glycated hemoglobin levels.

	Insulin (µlU/mL)	Fructosamine (µ mol/L)	HbA_{1c} (%)
Control	26.7±2.4	181.33±15.22	6.34±0.76
Control +Berberine	26.82±2.4	193.21±12.5	5.85±0.65
Diabetic	7.43±0.51**	493.33±42.2**	18.77±1.52**
Diabetic + Berberine	15.22±2.4 #	139.74±13.44 ##	11.32±0.66 ##
F-prob.	p<0.001	p<0.001	p<0.001

Note: Data expressed in M± SD (N=6); ** p<0.001 versus control and # p<0.05 and ## p<0.001 versus diabetic group.

diabetic rats' retinas ($p<0.01$) compared to the healthy control group. The oral administration of berberine to diabetic rats significantly enhanced retinal GSH content, as seen in Figure 2b. Figure 3a shows that GPx activity reduced significantly ($p<0.01$) in the retina of STZ-induced diabetic rats compared to the control group. Similarly, STZ-induced diabetic rats showed considerably lower retinal SOD and CAT activity (Figure 3b). Berberine therapy significantly increased the activity of GPx ($p<0.001$), SOD ($p<0.05$) and CAT ($p<0.05$) in diabetic rats' retinas. Normal rats treated with berberine showed no significant changes in retinal lipid peroxidation or antioxidant defenses.

DISCUSSION

DR is a significant public health issue, as it is the leading cause of blindness in industrialized nations. DR is a complication of diabetes mellitus that affects both the neuronal and vascular components of the retina.[26] Studies in diabetic humans and rodent models have established that the onset of diabetes can cause early neuronal damage, which subsequently leads to vascular impairment and the development of DR.[27,28] A critical factor implicated in retinal damage in diabetes is oxidative assault. Diabetes alters body metabolites, leading to increased oxidative assault, which damages retinal cells.[29]

This oxidative assault induces cell death, activates inflammatory responses and reduces neurotrophic support, culminating in diabetes-induced retinal lesions.[30] Additionally, the excessive formation of ROS in diabetes triggers the production of specific growth factors, transcription factors and cytokines, which play crucial roles in the progression of DR. Particularly, proinflammatory cytokines are pivotal in DR progression.[31,32]

An increase of mitochondrial-dependent apoptosis, which is characterized by higher expression of apoptosis markers Bax and caspase-3, is a result of the interaction between oxidative

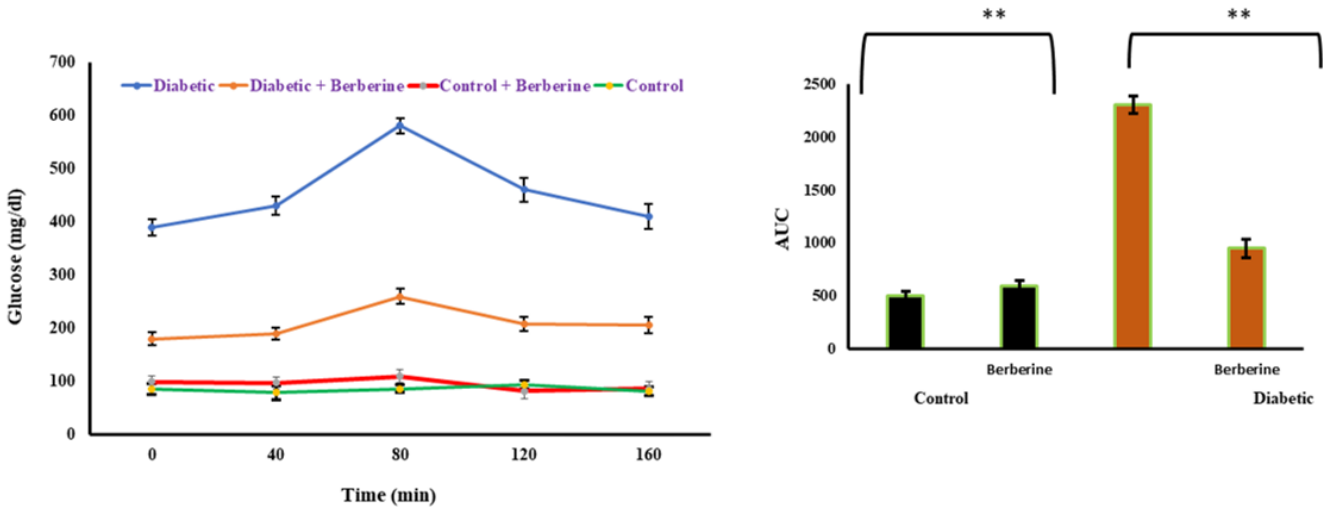

Figure 1: (a) Effect of Berberine administration on glucose tolerance in control and diabetic rats. (b) Comparison of diabetic rats with Control, OGTT oral glucose tolerance test, Area Under Curve {AUC} (**$p<0.01$).

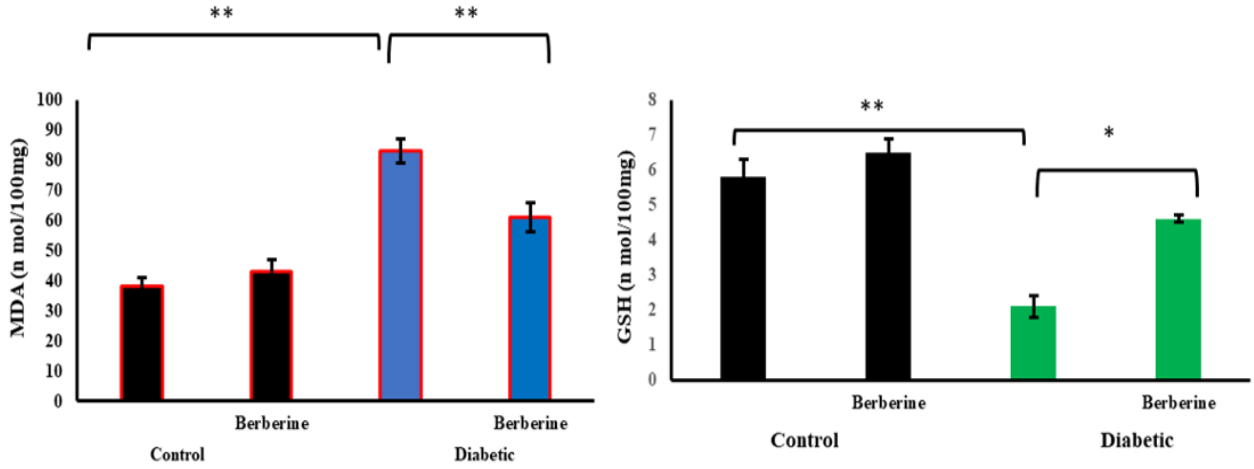

Figure 2: (a) Effect of Berberine on MDA in control and diabetic rats. (b) Effect of Berberine on GSH in control and diabetic rats.

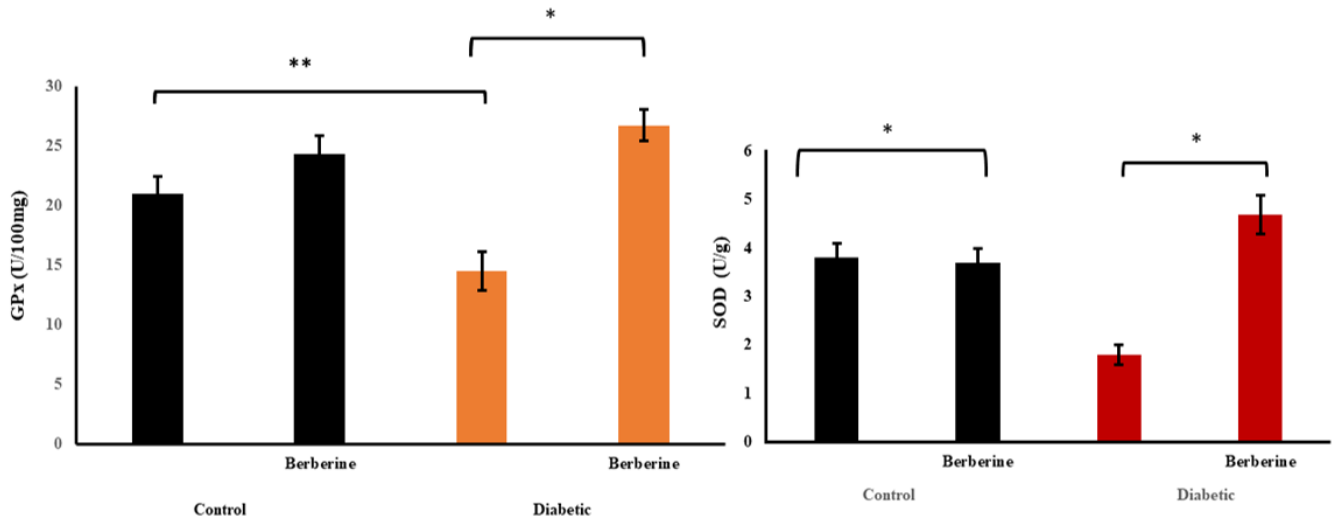

Figure 3: (a) Effect of Berberine on GPx in control and diabetic rats. (b) Effect of Berberine on SOD in control and diabetic rats.

assault and inflammation in Diabetic Retinopathy (DR).[33,34] This interaction contributes to the rapid death of neuronal cells and a reduction in axonal regeneration during diabetic retinal development.[35] The purpose of this experiment was to investigate the potential mechanisms for lowering DR as well as the impact that berberine has on the amounts of glucose contained in the blood. After receiving berberine treatment at each time point in the OGTT, diabetic rats that had been induced with STZ had significantly reduced blood glucose levels, according to the findings of our scientific research.

On top of that, it made diabetic animals' AUC for OGTT smaller than that of control rats. The reduction in DR that berberine produces may be due to several different processes. The anti-inflammatory and oxidative assault-reducing effects of berberine are important in DR. Because of this decrease, it is more probable that blood glucose levels and retinal health are both improved. Berberin reduces cell death and inflammation in the retina by blocking the NF-κB signaling cascade, which in turn stops DR from happening.[36]

As part of its neuroprotective actions in DR, berberine protects retinal ganglion cells by activating GABA-alpha receptors. Aside from that, berberine slows down DR progression and new blood vessel formation by blocking insulin-induced retinal endotheliocyte activation. Additionally, it reduces cell mortality and promotes survival via improving autophagy and the AMPK/mTOR signaling cascade in retinal Müller cells, which contributes to its therapeutic effects. These results are in line with earlier studies that showed berberine has anti-diabetic benefits.[37,38]

Berberine improves insulin sensitivity, boosts insulin production and reduces glucose absorption in the stomach, all of which contribute to a reduction in blood glucose levels after consumption. According to the findings of the study, the administration of berberine led to significant reductions in both serum fructosamine and blood HbA_{1c} levels, which indicates that effective glycemic management was brought about. The way that berberine acts to relieve DR is consistent with what we know about how it works so far. Berberine stimulates the production of insulin by pancreatic beta cells, which results in an increase in the amount of insulin present in the blood. Insulin sensitivity can be raised by increasing the binding of insulin to its receptors, which results in improved glucose uptake into cells and lower blood glucose levels. This is accomplished by enhancing insulin's ability to attach to its receptors. Through its ability to prevent gluconeogenesis, which is the process of producing glucose from precursors that are not carbohydrates, berberine makes an additional contribution to lowering blood glucose levels,[39] a Berberine is responsible for activating the AMPK pathway, which is one of the most important pathways for managing the metabolism of glucose and lipids.[40] At the same time that AMPK activation increases insulin sensitivity, it also increases gluconeogenesis.[41]

Oxidative stress occurs when the synthesis of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) exceeds the body's ability to remove them. Antioxidants are substances that neutralize or eliminate free radicals, thereby protecting the body from oxidative stress.[42]

The antioxidant characteristics of berberine also aid in lowering the oxidative stress that comes with diabetes. It is possible that improved glycemic management and increased insulin sensitivity might result from this decrease in oxidative assault.[43,44] Previous research in both animals and humans has shown that berberine greatly enhances glycemic control.[45,46] Treatment with berberine for three months decreased HbA_{1c} levels from 9.5%±0.5% to 7.5%±0.4% ($p<0.01$) in individuals with type 2 diabetes mellitus, which is similar to the effect of metformin. In the case of poorly controlled diabetes, another study found that HbA_{1c} decreased from 8.1%±0.2% to 7.3%±0.3% ($p<0.001$). Blood glucose levels, both fasting and postprandial, were also markedly reduced

by berberine. Reducing DR requires these enhancements to glycemic control. The formation and progression of DR are primarily influenced by elevated blood glucose levels. This condition causes damage to the retina through various mechanisms, including inflammation, oxidative assault and the generation of VEGF. The lowering of blood glucose levels by berberine affects these processes. By improving insulin sensitivity and increasing glucose transport, it reduces blood sugar levels independently of insulin. This two-pronged approach lowers the risk of DR by promoting better glycemic management. The results provide credence to berberine's status as a potential treatment tool for diabetes management and the prevention of diabetic retinopathy and other related problems.

In this work, we looked at DR in rats that had been induced with STZ by measuring the levels of MDA, GSH and antioxidant enzyme activities in the retina. Oxidative assault leads to increased lipid peroxidation, which is indicated by elevated MDA levels.[47]

The MAPK pathway, specifically the JNK and p38 MAPK pathways, come into play when this oxidative assault activates several downstream cascades. Inflammatory cytokines, adhesion molecules and VEFG are produced because of these activations, which in turn cause damage to the retina and the advancement of DR. GSH levels indicate compromised antioxidant defenses, leading to an antioxidant deficit. Damage to this homeostasis triggers the Nrf2 pathway, which controls the production of antioxidant enzymes. Nevertheless, impaired Nrf2 activation can worsen oxidative damage in the retina due to hyperglycemia-induced oxidative assault in diabetes. The lessened activity of antioxidant defense systems is shown by GPx, SOD and CAT. This shortcoming triggers redox-sensitive transcription factors such as NF-κB and AP-1, which in turn elevate inflammation, malfunction in the retinal blood vessels and neuronal death.[48-51]

Our findings demonstrated that berberine boosted GPx, SOD and CAT activity in diabetic rat retinas while decreasing retinal MDA levels and increasing GSH content. Based on these findings, berberine might shield diabetic rats' retinas against oxidative assault and lipid peroxidation. The preventive effects of berberine are most likely due to its antioxidant capabilities. Reducing MDA levels, berberine may scavenge free radicals and lessen the generation of lipid peroxides. Berberine may also boost the activity of antioxidant enzymes including GPx, SOD and CAT, which play a crucial role in preserving the retina's oxidative balance and neutralizing free radicals. Based on these findings, berberine may have a role in reducing diabetic retinopathy-related retinal damage caused by oxidative assault.

Diabetes and its consequences continue to be a serious global public health concern. Animal models play an important role in advancing our understanding of DR pathophysiology, development and etiology. No model has been developed to simulate the progression of DR in humans, from early cellular and vascular abnormalities to proliferative stage and retinal detachment due to persistent hyperglycemia. Because of their small size and tendency to acquire retinopathies quickly, rats have been often employed in DR studies. However, most diabetic rodent models only showed early DR symptoms, limiting their use in mechanistic research and drugs screening. After diabetic induction, several higher-order animals developed sophisticated retinopathies such neovascularization, but they could not mimic human DR. Future progress of DR animal models is essential since DR is a complicated disease with vascular and neurologic components that are affected by both heredity and environmental factors. While there is no one animal model that encompasses the complete DR pathophysiology, we see opportunity in expanding our understanding of genetic models and developing new high-order animal models. To this aim, ocular medicines for DR treatments have a promising future.

CONCLUSION

In developed countries, DR is the main cause of blindness, making it a major concern in public health. Retinal vascular impairment results from early neuronal injury, which impacts both the neuronal and vascular components of the retina. Retinal lesions caused by cell death, inflammation and diminished neurotrophic support are all consequences of oxidative stress, a key component of diabetic macular degeneration. Several growth factors, transcription factors and cytokines are activated by diabetes-related elevated ROS generation, which in turn accelerates DR progression. Axonal regeneration is impaired and neuronal cell death is quickened by oxidative stress and proinflammatory cytokines. By lowering blood glucose levels and enhancing retinal function, berberine shows potential in reducing DR through its anti-inflammatory and antioxidant actions. Berberine prevents cell death and inflammation by inhibiting the NF-κB signaling cascade and it protects retinal ganglion cells by activating GABA-alpha receptors. Additionally, it improves cell viability by inhibiting insulin-induced activation of retinal endotheliocytes and by increasing autophagy and the AMPK/mTOR pathway. Clinical trials have shown that berberine can enhance glycemic control by lowering blood glucose, serum fructosamine and HbA1c levels. Berberine is a great candidate for the treatment of diabetic retinopathy and its consequences since it increases insulin sensitivity, decreases oxidative assault and blocks gluconeogenesis.

ETHICAL STATEMENT

All animal experiments are designed and carried out in accordance with international and national ethical norms and legislation. The Ethics Committee of the Jinan Central Hospital has authorized our research (Ethics code: 2023-017).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

Lihui Wang and Jing Zhang collaborated on the design of this study, Lihui Wang, Wei Fei and Jing Zhang conducted the statistical analysis. Lihui Wang, Chongjuan Qin and Jing Zhang conducted the study and gathered background information. Lihui Wang wrote the manuscript. All authors reviewed and approved the final paper.

ABBREVIATIONS

DR: Diabetic Retinopathy; **TNF-α:** Tumor Necrosis Factor-α; **VEGF:** Vascular endothelial growth factor; **ROS:** Reactive oxygen species; **Bax:** Bcl-2-associated X protein; **Bcl-2:** B-cell lymphoma protein-2; **GSH:** Glutathione; **STZ:** Streptozotocin; **TBA:** Thiobarbituric acid; **DTNB:** 5,5′-Dithiobis-(2-nitrobenzoic; acid); **SDS:** Sodium dodecyl sulphate; **ABTS:** 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic; acid; **OGTT:** Oral glucose tolerance test; **HBA1c:** Glycated haemoglobin; **SOD:** Superoxide: dismutase; **CAT:** Catalase; **MDA:** Malondialdehyde; **ELISA:** Enzyme Linked Immunosorbent Assay; **IL-1β:** Interleukin-1β; **PKCβ:** Protein kinase Cβ; **GABA:** γ-Aminobutyric acid; **AMPK:** 5'-adenosine monophosphate-activated protein kinase.

SUMMARY

Diabetic Retinopathy (DR) causes most eyesight loss worldwide. Diabetes-related oxidative stress causes this retinal microvasculature disorder. Plant-derived phytochemical "Berberine" has anti-diabetic characteristics, according to several research. Few studies have examined its effects on diabetic retinopathy. This study examines how Berberine affects oxidative stress, inflammation and programmed cell death in rats to determine its effect on DR. Diabetes rats were treated for 12 weeks and assessed for inflammatory indicators (TNF-α, VEGF), antioxidant markers (superoxide, dismutase, catalase, glutathione) and apoptotic markers (B:cl-2, Bax, Cleaved-caspase-3). Compared to the diabetic group, Berberine therapy exhibited a significant hypoglycemic index. Treatment with berberine dramatically lowered TNF-α and VEGF levels in the diabetic retina. Berberine modulated antioxidant enzymes and raised their levels. Berberine also reduced diabetic mice retinal apoptosis by lowering high glucose-induced ROS, Bax/Bcl-2 ratio and cleaved caspase-3 expression. Based on the findings, Berberine may prevent DR pharmacologically.

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