Original Research Article

Effect of *Zhixue Mingmu* Formula on retinal vasopermeability of streptozotocin-induced diabetic retinopathy in rats

ABSTRACT

Aims: To investigate the efficacy of *Zhixue Mingmu* Formula (ZXMM), a traditional Chinese herbal compound, for retinal vasopermeability in streptozotocin (STZ)-induced diabetic retinopathy (DR) in rats.
Materials and Methods: Sprague Dawley rats were injected with STZ (45 mg/kg, i.p.) to induce experimental DR and divided into 5 groups (n=10): a model control group (MG, vehicle); a calcium dobesilate group (CD, 135 mg/kg); and low-, moderate-, and high-dose ZXMM groups (LZXMM, 15.3 g/kg; MZXMM, 30.6 g/kg; HZXMM, 45.9 g/kg). Normal rats were used for a normal control group (NG). Drug treatments were administered daily for 4 weeks. Fasting blood glucose (FBG) levels were monitored every 2 weeks. Retinal vasopermeability was measured using an Evans blue assay. Then, serum samples and retinal tissues were harvested to assess the levels of inflammatory factors and the activity of antioxidative enzymes by enzyme-linked immunosorbent assay (ELISA). Retinal hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor-A (VEGF-A) protein levels were evaluated by ELISA.
Results: In the MG, FBG levels were significantly ($P < 0.001$) increased, and retinal vasopermeability was markedly ($P < 0.001$) elevated compared with those in the NG. After oral administration of ZXMM or calcium dobesilate, the elevated retinal vasopermeability was significantly ($P < 0.001$) decreased in DR rats. Concurrently, ZXMM treatment significantly ($P < 0.05$) attenuated the levels of the inflammatory factors interleukin-6 (IL-6) and C-reactive protein (CRP) in serum and ameliorated the activity of the antioxidative enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the retina of DR rats. ZXMM treatment significantly ($P < 0.05$) decreased HIF-1α and VEGF-A expression in retinal tissue compared with MG treatment. Furthermore, the effects of ZXMM were dose dependent.
Conclusion: ZXMM was shown to have a protective effect against retinal vasopermeability in DR comparable to that of calcium dobesilate, and its mechanism may be attributed to inhibition of the downstream pathway of glycaemia-induced hypoxia, inflammation and oxidative stress of retinal tissue.

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Keywords: Zhixue Mingmu Formula; retinal vasopermeability; diabetic retinopathy; hypoxia injury; inflammation; oxidative stress.

1. INTRODUCTION

Diabetes is a major global health concern and is often accompanied by serious complications involving numerous systems, such as diabetic oculopathy, diabetic retinopathy, cardiovascular disease and diabetic neuropathy [1,2]. Diabetic retinopathy (DR) is the most common and severe ocular complication of diabetes and is one of the main causes of visual impairment in the working-age population [3]. The pathogenesis of DR is complicated and involves diverse mechanisms.

Among the factors implicated, inflammation, oxidative stress and growth factors are considered major triggers of DR [4,5]. In diabetic patients, hyperglycaemia increases the production of inflammatory mediators and oxidation products and weakens antioxidant defence, resulting in retinal microvascular injury and blood–retinal barrier (BRB) damage [6]. Breakdown of the inner BRB and increased retinal vasopermeability occur in the early stage of DR and result in vascular leakage and subsequent retinal oedema [7-9]. In addition, occlusion of retinal capillaries in DR leads to the regulation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) and in turn induces the expression of vascular endothelial growth factor-A (VEGF-A), which promotes subsequent neovascularization [10-12]. Pathologic angiogenesis in the retina contributes to vitreous haemorrhage and tractive retinal detachment followed by serious visual impairment and even blindness [13-14].

In traditional Chinese medicine (TCM), DR belongs to the categories of “Shi Zhan Hun Miao”, “Bao Mang” and “Ying Xing Man Mu”, and it is believed that DR is primarily due to Yin deficiency and blood stasis [15]. Chinese herbal medicine has been used to treat diabetic complications for centuries and has been revealed to be effective for the prevention and treatment of DR in recent clinical and experimental studies [16-18]. According to the theory of meridians in TCM, Professor Zhang Minglian, who was part of the first cohort of famous Chinese medicine doctors of Hebei Province in China, created *Zhixue Mingmu* Formula (ZXMM) for the treatment of haemorrhagic ocular diseases. It has been shown that ZXMM effectively improves the ocular microcirculation, promotes the absorption of retinal and vitreous haemorrhages and restores the visual acuity of DR patients [19]. In this study, we aimed to use a streptozotocin (STZ)-induced DR rat model to explore the effect of ZXMM on retinal vasopermeability, inflammatory factors, antioxidative enzymes and the HIF-1α/VEGF-A signalling pathway. Elucidating the underlying molecular mechanism of ZXMM may provide a basis for the clinical prevention and therapy of DR with TCM.

2. MATERIAL AND METHODS

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2.1 Chemicals

Calcium dobesilate capsules (Kangya of Ningxia Pharmaceutical Co., Ltd., Ningxia, China); Evans blue (EB), STZ (Sigma-Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China); 0.1 mol/L sodium citrate buffer, pH 4.5 (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China); chloral hydrate (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China); fluorescein sodium (Guangxi Wuzhou Pharmaceutical (Group) Co., Ltd., Guangxi, China); glucose estimation kits (Johnson & Johnson (Shanghai) Medical Devices Co., Ltd., Shanghai, China); interleukin-6 (IL-6), C-reactive protein (CRP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), HIF-1α and VEGF-A enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) were used in this study. All other chemicals and reagents used in the study were of analytical grade.

2.2 Preparation of ZXMM

ZXMM was prepared using Puhuang (Pollen Typhae), Mohanlian (Herba Ecliptae), Danshen (Salvia Miltiorrhiza), Yujin (Radix Curcumae), Chishao (Paeoniae Radix Rubra), Mudanpi (Moutan Bark), Jingjie (Schizonepeta), Xiakucao (Selfheal), Chuanqiong (Ligusticum Wallichii), Sanqi (Notoginseng), and Qiancaotan (Dasey Peat) (all from the TCM Pharmacy of Hebei Eye Hospital, Xingtai, China). The medicinal materials were purchased from Anguo Shengfang TCM Decoction Pieces Co., Ltd. (Baoding, China). The dried herbs were first soaked in 15 volumes of distilled water for 30 minutes and then boiled twice for 1.5 hours and 2 hours; during the second boil, the herbs were boiled with water at a 1:10 herb: water ratio (w/v). After filtration, the remaining solution was concentrated to final densities of 1.53, 3.06 and 4.59 g/mL and stored at 4°C for further experiments.

2.3 Animals

Male Sprague Dawley (SD) rats with a body weight of 220 ± 20 g were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) under permit no. SCXK (Jing) 2021-0006. The rats were housed under a temperature of 22 ± 1°C and a relative humidity of 55% ± 5% in a controlled environment with a 12-hour light/dark cycle in the Experimental Animal Department of Hebei Provincial Key Laboratory of Ophthalmology (Xingtai, China). The animals were handled in accordance with the approved guidelines and regulations.

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2.4 Induction of Experimental DR in the STZ-induced Model and Experimental Design

SD rats (n = 70) were randomly divided into a control group (n = 10) and a model group (n = 60). The rats in the model group were injected with 1% STZ in 0.1 mol/L sodium citrate buffer (45 mg/kg, i.p.). The control rats were injected with vehicle. Seventy-two hours after STZ injection, fasting blood glucose (FBG) levels were measured, and rats were

diagnosed with diabetes when FBG ≥16.7 mmol/L [20]. All rats in the model group met the criteria. After 4 weeks, the diabetic rats underwent fundus fluorescence angiography (FFA). Rats were anaesthetized with 10% chloral hydrate (4 mL/kg, i.p.), and their pupils were dilated with tropicamide eye drops. Then, 10% fluorescein sodium (0.5 mL/kg) was injected into the tail vein, and FFA imaging was performed with a Heidelberg Retina Angiograph (HRA; Heidelberg Engineering, Heidelberg, Germany). Eventually, retinopathy was observed in 51 diabetic rats.

DR rats (n = 50) were randomly divided into five groups: a model control group (MG), a calcium dobesilate group (CD), a low-dose ZXMM group (LZXMM), a moderate-dose ZXMM group (MZXMM) and a high-dose ZXMM group (HZXMM). Normal rats were used for a normal control group (NG). Each group comprised 10 rats. CD received calcium dobesilate (135 mg/kg, equivalent to the dose used for adult humans, p.o.), which served as the reference standard group. ZXMM at doses of 15.3, 30.6 and 45.9 g/kg (1, 2 and 3 times the adult human-equivalent dose) was administered orally to the rats of LZXMM, MZXMM and HZXMM, respectively. NG and MG received 0.9% saline. All treatments were performed daily for 4 weeks. The FBG levels of the rats were measured at the beginning of this study on day 0, and measurements were repeated 2, 4, 6 and 8 weeks after STZ injection.

2.5 Measurement of retinal vasopermeability

Retinal vasopermeability in rats was quantified after the end of drug treatment using an EB permeation test [21]. The rats were anaesthetized and injected with EB solution (45 mg/kg, i.v.). After the dye had circulated for 2 hours, the rats were reanaesthetized and perfused via the left ventricle with citrate buffer (0.05 mol/L, pH 3.5) at 37°C. Then, the retinas were dissected, dried and weighed. Formamide was added to the retinas, and the mixtures were incubated at 70°C for 18 hours and centrifuged at 12000 rpm for 15 minutes. The absorbance of the supernatant was measured at 620 and 740 nm using a microplate reader (EPOCH2 microplate reader; Bio Tek Instruments Inc., Winooski, VT, USA), and the concentration of EB dye in the retinal extract was calculated from a standard curve of the dye. Retinal vasopermeability was calculated and is reported as μg/g standardized EB leakage/retinal dry weight.

Comment [u5]: add reference source

2.6 ELISA analysis

Retinal tissues of the rats were collected in precooled phosphate-buffered saline (0.01 mol/L, pH=7.4) and homogenized with a homogenizer (TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China). Blood samples were collected via the retro-orbital vein and left to stand for 30 minutes at room temperature. Then, the retinal homogenates and the blood samples were centrifuged for 15 minutes at 5000 ×g and 1500 ×g, respectively, to obtain the supernatant. The levels of IL-6 and CRP in serum, the activity of SOD and GSH-Px and the expression of HIF-1α and VEGF-A in retina were measured with ELISA kits. All procedures were performed according to the kit instructions.

Comment [u6]: add reference source

2.7 Statistical analysis

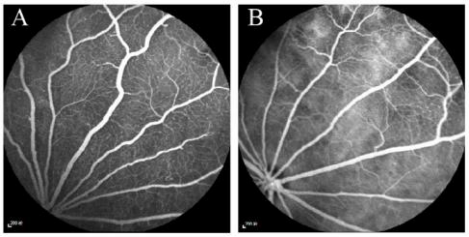
The data were analysed using SPSS 20.0 (SPSS for Windows, version 20.0; SPSS Inc., Chicago, IL, USA) and are presented as the mean ± standard deviation (SD). Comparisons between the groups were determined by one-way analysis of variance (ANOVA), as appropriate. $P < 0.05$ was considered to indicate statistical significance.

3. RESULTS

3.1 FFA Results

FFA images of normal rats exhibited a normal retinal appearance without leakage and showed that the retinal blood vessels were radially and uniformly distributed with uniform diameters (Figure 1A). The angiographic images of DR rats showed that the large vascular walls of the retina were stained, some vessels appeared tortuous and irregular with telangiectasia, and diffuse fluorescein leakage was obvious (Figure 1B).

Fig. 1. FFA images of normal rats and DR rats

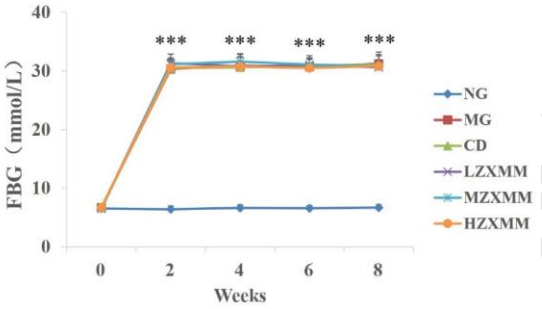


A: control group; B: model group.

3.2 FBG Levels

The FBG levels in NG remained within a physiological range throughout the experiment (Fig. 2). Compared with those in NG, there were no significant differences in the blood glucose values in MG, CD, LZXMM, MZXMM and HZXMM at the beginning of the study. After 2, 4, 6 and 8 weeks of treatment, the FBG levels in the MG, CD, LZXMM, MZXMM and HZXMM remained significantly ($P < 0.001$) higher than those in the NG, which showed that the rat model of diabetes was stable and that ZXMM did not affect the blood glucose levels of diabetic rats.

Fig. 2. FBG levels of rats every 2 weeks before and during treatment

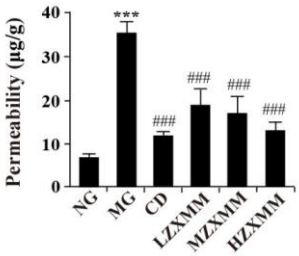


*Data are expressed as the mean ± SD (n=10); ***P < 0.001 compared to NG. NG: normal control group; MG: model control group; CD: calcium dobesilate group (135 mg/kg); LZXMM: low-dose ZXMM group (15.3 g/kg); MZXMM: moderate-dose ZXMM group (30.6 g/kg); HZXMM: high-dose ZXMM group (45.9 g/kg); ZXMM: Zhixue Mingmu Formula.*

3.3 Effect of ZXMM on Retinal Vasopermeability in DR Rats

Retinal vasopermeability was markedly ($P < 0.001$) elevated in MG compared with NG (Fig. 3). Meanwhile, vasopermeability was significantly ($P < 0.001$) reduced in CD, LZXMM, MZXMM and HZXMM compared with MG. Additionally, HZXMM and MZXMM were superior to LZXMM, suggesting the benefits of higher doses of ZXMM.

Fig. 3. Effect of ZXMM on retinal vasopermeability in DR rats

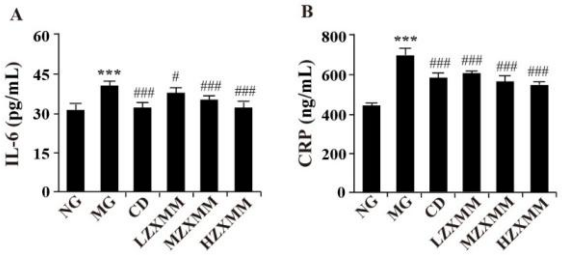


*Data are expressed as the mean ± SD (n=4); ***P < 0.001 compared to NG; ### P < 0.001 compared to MG. NG: normal control group; MG: model control group; CD: calcium dobesilate group (135 mg/kg); LZXMM: low-dose ZXMM group (15.3 g/kg); MZXMM: moderate-dose ZXMM group (30.6 g/kg); HZXMM: high-dose ZXMM group (45.9 g/kg); ZXMM: Zhixue Mingmu Formula.*

3.4 Effect of ZXMM on IL-6 and CRP levels in the serum of DR rats

Serum levels of IL-6 and CRP were significantly (P < 0.001) increased in MG compared with those in NG (Fig. 4). Administration of ZXMM or calcium dobesilate resulted in significant (P < 0.05) downregulation of IL-6 and CRP levels in DR rats, and a higher dose of ZXMM exhibited better effects.

Fig. 4. Effect of ZXMM on IL-6 and CRP levels in the serum of DR rats

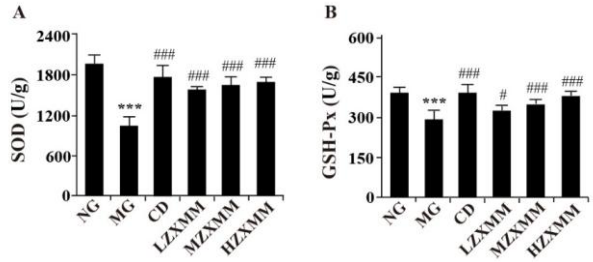


*A: IL-6 (pg/mL); B: CRP (ng/mL). Data are expressed as the mean ± SD (n=6); ***P < 0.001 compared to NG; # P < 0.05 compared to MG; ### P < 0.001 compared to MG. NG: normal control group; MG: model control group; CD: calcium dobesilate group (135 mg/kg); LZXMM: low-dose ZXMM group (15.3 g/kg); MZXMM: moderate-dose ZXMM group (30.6 g/kg); HZXMM: high-dose ZXMM group (45.9 g/kg); ZXMM: Zhixue Mingmu Formula.*

3.5 Effect of ZXMM on SOD and GSH-Px activity in the retina of DR rats

In MG, SOD and GSH-Px activity levels in retina were obviously (P < 0.001) decreased when compared with those in NG (Fig. 5). After treatment with calcium dobesilate or different doses of ZXMM, the retinal activity of SOD and GSH-Px in DR rats significantly (P < 0.05) increased. Clearly, the upregulation effect of high-dose ZXMM was much better than that of low-dose ZXMM.

Fig. 5. Effect of ZXMM on SOD and GSH-Px activity in the retina of DR rats

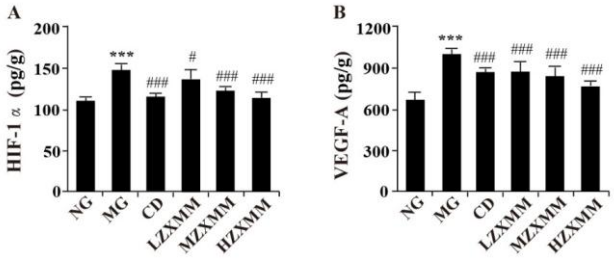


*A: SOD (U/g); B: GSH-Px (U/g). Data are expressed as the mean ± SD (n=6); ***P < 0.001 compared to NG; # P < 0.05 compared to MG; ### P < 0.001 compared to MG. NG: normal control group; MG: model control group; CD: calcium dobesilate group (135 mg/kg); LZXMM: low-dose ZXMM group (15.3 g/kg); MZXMM: moderate-dose ZXMM group (30.6 g/kg); HZXMM: high-dose ZXMM group (45.9 g/kg); ZXMM: Zhixue Mingmu Formula.*

3.6 Effect of ZXMM on HIF-1α and VEGF-A levels in the retina of DR rats

The expression levels of HIF-1α and VEGF-A in MG were significantly ($P < 0.001$) higher than those in NG (Fig. 6). Furthermore, CD, LZXMM, MZXMM and HZXMM showed significantly ($P < 0.05$) lower HIF-1α and VEGF-A expression, demonstrating the inhibitory effect of ZXMM on the HIF-1α/VEGF-A pathway. Similar to the previous results, HZXMM was superior to MZXMM and LZXMM.

Fig. 6. Effect of ZXMM on HIF-1α and VEGF-A levels in the retina of DR rats



*A: HIF-1α (pg/g); B: VEGF-A (pg/g). Data are expressed as the mean ± SD (n=6); ***P < 0.001 compared to NG; # P < 0.05 compared to MG; ### P < 0.001 compared to MG. NG: normal control group; MG: model control group; CD: calcium dobesilate group (135 mg/kg); LZXMM: low-dose ZXMM group (15.3 g/kg); MZXMM: moderate-dose ZXMM group (30.6 g/kg); HZXMM: high-dose ZXMM group (45.9 g/kg); ZXMM: Zhixue Mingmu Formula.*

4. DISCUSSION

DR is a common retinal microvascular complication of diabetes that manifests as macular oedema, retinal haemorrhage, exudation and neovascularization, consequently reducing vision [22]. According to recent data published by the International Diabetes Federation, the global diabetes prevalence is estimated to be nearly half a billion people, and the number is projected to reach 700 million by 2045 [23]. Furthermore, more than one-third of diabetic patients live with DR [24]. As a leading cause of blindness in adults, DR has already become an urgent public health problem worldwide, and it severely impacts the vision and eye health of patients with diabetes. Therefore, it is important to develop medical treatment options other than glycaemic control for the prevention and treatment of DR [25]. In this study, we evaluated the effect of the traditional Chinese herbal compound ZXMM on the treatment of DR in a rat model. Our research revealed that ZXMM alleviated vascular leakage in the retina by suppressing inflammation, enhancing antioxidation ability and suppressing the HIF-1α/VEGF-A pathway.

During the experiment, the diabetic rats continuously displayed markedly higher levels of blood glucose than the normal rats, and significant retinopathy was observed by FFA, indicating that the rat model of DR was successfully and stably established. In the early stage of DR, impairment of vascular cells in the inner BRB causes vascular leakage and macular oedema, subsequently leading to visual impairment [26]. Administration of ZXMM significantly reduced the increased retinal vasopermeability in DR rats, and the effects were dose dependent, indicating that ZXMM exerted a protective effect against retinopathy in diabetic rats.

ZXMM decreased IL-6 and CRP levels in serum and upregulated SOD and GSH-Px activity in the retina of DR rats, especially the high dose of ZXMM, suggesting that the protective effect of ZXMM against retinal damage was associated with the reduction in inflammatory factors and the enhancement of antioxidation in rats with DR. At present, the pathogenesis of DR has not been fully elucidated, but hyperglycaemia-induced inflammation and oxidative stress are considered major triggers [5]. It has been suggested that the release of inflammatory cytokines and the abnormal adherence of leukocytes to capillaries are initial events in the development of DR, which lead to vascular leakage and capillary occlusion [27]. IL-6 is an important proinflammatory interleukin and modulates inflammatory and immune responses [28]. Elevated IL-6 concentrations have been detected in patients with DR and found to be positively correlated with the degree of DR [29-30]. CRP is an acute inflammatory protein, and serum CRP has been utilized as a marker of inflammation and vascular events [31]. The levels of CRP are significantly higher in young individuals with diabetes than in healthy controls [32]. Our results demonstrated that the serum concentrations of IL-6 and CRP were significantly higher in DR rats than in normal rats, and the increases were effectively suppressed after treatment with ZXMM. In addition, ZXMM upregulated retinal SOD and GSH-Px activity in DR rats. Diabetes-associated hyperglycaemia results in an increase in free radical generation and a decrease in antioxidant defence function, contributing to the development of DR [33]. SOD and GSH-Px are important endogenous antioxidant enzymes: SOD catalyses superoxide radicals into hydrogen peroxide, and GSH-Px further catalyses hydrogen peroxide into water and hydroxyl compounds to scavenge

free radicals [34]. Therefore, the increased SOD and GSH-Px activity indicated that the DR rats treated with ZXMM possessed a stronger capacity to scavenge free radicals, which alleviated retinal oxidative stress and damage.

Treatment with ZXMM also downregulated the retinal expression of HIF-1α and VEGF-A in DR rats, indicating a novel role for ZXMM in modulating vascular function in DR. HIF-1α is the oxygen-sensitive subunit of HIF-1, a transcriptional activator that is upregulated by hypoxia in cells [35]. In diabetes, hyperglycaemia-induced oxidative stress promotes the activation of HIF-1α, resulting in the overexpression of proangiogenic genes and other downstream genes [5]. Among these proangiogenic cytokines, VEGF-A is considered a key stimulator of physiological and pathological angiogenesis and acts specifically on the vascular endothelium, inducing endothelial cell proliferation and new microvessel formation [36]. The role of VEGF-A is not limited to the formation of blood vessels; it also includes the regulation of vascular permeability and leukocyte and endothelial responses [37]. VEGF-A is known to bind to its receptors vascular endothelial growth factor receptor, urokinase plasminogen activator receptor and neuropilin-1 to increase the permeability of endothelial cells in vitro and vascular leakage in vivo [38-39]. Previous studies have proven that HIF-1α expression in the retinas of DR rats and VEGF-A levels in the vitreous bodies and serum of DR patients are all increased [40-41]. Furthermore, intravitreal injection of anti-VEGF agents can reduce retinal oedema and alleviate neovascularization [42]. In HIF-1α-knockout mice, significant decreases in HIF-1α in the retinas attenuate the overproduction of VEGF and inflammatory factors and the increases in retinal vascular leakage under diabetic conditions [43]. Thus, the HIF-1α/VEGF signalling pathway is regarded as a prominent therapeutic target for DR [44]. Consistent with previous reports, our study revealed that HIF-1α and VEGF-A expression was markedly higher in the retinas of DR rats than in those of normal rats. ZXMM significantly depressed retinal expression of HIF-1α and VEGF-A, especially high-dose ZXMM. These results help elucidate the mechanism by which ZXMM reduces retinal vasopermeability and prevents the progression of DR.

ZXMM consists of Puhuang (Pollen Typhae), Mohanlian (Herba Ecliptae), Danshen (Salvia Miltiorrhiza), Yujin (Radix Curcumae), Chishao (Paeoniae Radix Rubra), Mudanpi (Moutan Bark), Jingjie (Schizonepeta), Xiakucao (Selfheal), Chuanqiong (Ligusticum Wallichii), Sanqi (Notoginseng) and Qiancaotan (Dasey Peat). Among these components, Puhuang and Sanqi have been shown to have protective effects on blood vessels via anti-inflammatory, antiviral and immunomodulatory activity, which is related to the fact that they promote the activation of protein kinase B (AKT1) and inhibit the levels of IL-6 and VEGF-A [45]. Mohanlian has been proven to delay the process of DR in rats and reduce the vascular complications caused by diabetes by regulating oxidative stress, inflammation and glucose and lipid metabolism [46]. Danshen and Chuanqiong inhibited the production of inflammatory mediators and the oxidative stress response in the treatment of diabetic complications [47]. Additionally, Chishao has a wide range of pharmacological effects; for example, it stabilizes the microcirculation, scavenges free radicals and attenuates toxic damage to retinal neural cells to treat diabetes through multiple signalling pathways, including the HIF-1/VEGF-A signalling pathway [48]. Hence, previous studies have presented evidence that ZXMM has specific benefits for regulating vascular function and resisting oxidation and inflammation.

Calcium dobesilate, a well-known vasculoprotective drug, is clinically used for the treatment of DR or diabetic nephropathy in patients to improve microcirculation disorders and stave off disease progression [49]. According to previous studies, calcium dobesilate exhibits anti-inflammatory and antioxidative properties and improves vascular endothelial dysfunction [50-51]. Therefore, this synthetic compound was administered to the positive control group in our work. Our findings not only revealed the effects of ZXMM on retinal damage in DR but also verified the pharmacological effects of calcium dobesilate, which were consistent with those in previous reports. In addition, ZXMM showed satisfactory benefits in protecting the inner BRB and attenuating retinal vascular damage resembling or rivalling those of calcium dobesilate. However, there were several limitations in this study. Additional in vitro experiments on human retinal vascular endothelial cells are needed to support the conclusion that ZXMM prevents DR and to elucidate its pharmacological mechanism in more detail. Moreover, ZXMM inhibited the upregulation of VEGF-A, but the precise effects of ZXMM on retinal neovascularization have not yet been clarified; these effects need to be further verified in animal experimental models of DR.

5. CONCLUSION

The present study concludes that ZXMM, a traditional Chinese herbal compound, has a significantly protective effect against retinal vasopermeability in DR rats at 15.3, 30.6 and 45.9 g/kg, comparable to that of calcium dobesilate, and a higher dose of ZXMM exhibits better effects. Its mechanism may be attributed to inhibition of the downstream pathway of glycaemia-induced hypoxia, inflammation and oxidative stress in retinal tissue. These findings provide a theoretical basis for understanding the protective effect of ZXMM against DR.

Comment [u7]: add implication of this study

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Ethics Committee of Hebei Provincial Eye Hospital (approval no. 2020KY029).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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