• Basic Research •

Curcumin protects the diabetic mouse retina by modulating the Hippo-YAP signaling pathway

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Abstract

● **AIM:** To explore the protective effects and underlying mechanisms of curcumin in preventing and treating diabetic retinopathy in the C57BL/6J diabetic mouse model.

● **METHODS:** The C57BL/6J diabetic mouse models were established through streptozotocin (STZ) induction and randomly assigned into five groups: Control, Model, Cal (0.15 g/kg·d), Cur-H (0.2 g/kg·d), and Cur-L (0.05 g/kg·d; *n*=10/group). Treatment was administered by oral gavage for 12wk. Upon completion of the observation period, retinal function was evaluated by electroretinography (ERG), retinal thickness and structural changes were assessed *via* optical coherence tomography (OCT), retinal vascular density and leakage were analyzed using optical coherence tomography angiography (OCTA) and fundus fluorescein angiography (FFA), the number of acellular capillaries in retinal flat mounts was counted, histopathological changes were observed with hematoxylin and eosin (HE) staining, and protein expression levels of components involved in the Hippo signaling pathway-Yes-associated protein (Hippo-YAP) signaling pathway and endothelial-to-mesenchymal transition (EndMT) were quantified by Western blot.

● **RESULTS:** In diabetic mice, ERG amplitudes were significantly reduced, retinal thinning was observed, and the number of non-perfusion areas and acellular capillaries increased. Additionally, the phospho-large tumor suppressor kinase 1 (p-LATS1)/2/LATS1/2 and p-YAP/YAP ratios were diminished, vascular endothelial (VE)-cadherin expression was reduced, and α-smooth muscle actin (α-SMA) expression was elevated (all $P<0.05$). In the high-dose curcumin group, ERG amplitudes were significantly improved, retinal structure was restored, vascular density was increased, and acellular capillaries were reduced. Furthermore, the p-LATS1/2/LATS1/2 and p-YAP/YAP ratios were normalized, VE-cadherin expression was upregulated, and α-SMA expression was suppressed (all $P<0.05$).

● **CONCLUSION:** Curcumin offers protective effects on the retinas of diabetic mice, likely through the modulation of the Hippo-YAP signaling pathway and the inhibition of EndMT. These findings provide support for the use of curcumin as a promising adjunctive therapy for diabetic retinopathy.

● **KEYWORDS:** diabetic retinopathy; curcumin; Hippo-YAP signaling pathway; endothelial-to-mesenchymal transition
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INTRODUCTION

As diabetes mellitus progresses, patients often develop various complications. As a frequently observed microvascular complication of diabetes mellitus, diabetic retinopathy (DR) significantly contributes to the progressive deterioration of vision, particularly affecting middle-aged and elderly populations[1]. The pathogenesis of DR involves chronic hyperglycemia, which triggers microvascular damage, inflammation, and oxidative stress.

Curcumin is a natural polyphenol abundantly found in the rhizomes of *Zingiberaceae* plants such as *Curcuma longa*, *Curcuma aromatica*, and *Zingiber zerumbet*, and exhibits anti-inflammatory, antioxidant, anti-apoptotic, and anticancer

activities. It has been shown to delay the progression of diabetic nephropathy and promote wound healing by modulating oxidative stress and inflammatory pathways[2]. Curcumin has been demonstrated to mitigate inflammatory responses and oxidative stress within the vitreous humor of diabetic rats[3]. Recent studies have shown that curcumin effectively inhibits retinal vascular leakage in DR animal models[4]. Furthermore, pharmacokinetic studies of curcumin indicate that a curcumin formulation using a polyethylene glycol-pyrrolidone hydrophilic carrier can cross the blood-retinal barrier and reach retinal tissue in rabbits[5], providing theoretical support for our oral aqueous curcumin formulation. Although the protective effects of curcumin on DR have been reported, its underlying mechanisms remain incompletely understood. Recent mechanistic studies have shown that dysregulated Hippo signaling pathway-Yes-associated protein (Hippo-YAP) critically regulates endothelial function, vascular integrity, and aberrant angiogenesis in diabetes. In high-glucose environments, post-translational modifications like O-GlcNAcylation stabilize YAP, promoting its nuclear translocation and activation, thereby triggering downstream vascular dysfunction[6]. YAP, an essential downstream mediator of the Hippo pathway, participates in regulating gluconeogenesis and maintaining glucose homeostasis[7]. Recent studies have linked aberrant YAP activation in endothelial cells to endothelial-to-mesenchymal transition (EndMT) and tissue fibrosis, but its role in diabetic retinal pathology remains to be clarified[8-10]. This study, therefore, investigates the protective effects and mechanisms of curcumin in a C57BL/6J diabetic mouse model of DR.

MATERIALS AND METHODS

Ethical Approval All animal experiments adhered to the guidelines for animal care and were approved by the Experimental Animal Ethics Committee of Nanjing University of Chinese Medicine (approval number: 2023NL-KS183).

Animals Six-week-old male C57BL/6J mice (weighing 20±5 g) were purchased from Speifo Laboratory Animal Co., Ltd. (Suzhou, License No. SCXK 2022-0006). The animals were maintained in a controlled environment (temperature: 18°C–25°C; humidity: 40%–65%) and provided *ad libitum* access to standard diet and autoclaved water.

Experimental Design After one week of acclimatization, fasting blood glucose was measured to screen for normoglycemic mice. Following 12h of fasting, mice received intraperitoneal injections of streptozotocin (STZ; 50 mg/kg·d) for five consecutive days. From day 6, random blood glucose was monitored; mice with glucose levels >16.7 mmol/L (Yuwell Blood Glucose Meter, Jiangsu, China) for three consecutive days were considered successfully diabetic mice were randomly allocated into five experimental groups (n=10 per group), including a control group (vehicle, 10 mL/kg·d), a diabetic model group (vehicle, 10 mL/kg·d), a positive control group (*Calcium dobesilate*, Cal, 0.15 g/kg·d), a high-dose curcumin group (Cur-H, 0.2 g/kg·d), and a low-dose curcumin group (Cur-L, 0.05 g/kg·d). All treatments were administered by oral gavage daily for 12 consecutive weeks. It is important to note that the low dose of 0.05 g/kg·d was defined based on previous studies[11], and the high dose was set as four times this amount. Using the standard dose conversion formula between humans and mice[12], the dose for the Cur-H group in this study falls within the safe human intake range[13]. Additionally, we measured random blood glucose every two weeks. The control and model groups received sterile water as a vehicle, whereas the positive control group was administered calcium dobesilate. All experimental groups, excluding the control group, received a diet rich in fat and sugar.

Electroretinography Following 12h of overnight dark adaptation, mice were anesthetized with an intraperitoneal injection of 1.25% tribromoethanol (0.2 mL/10 g; AIBI Bio-Technology, Nanjing, China)[14]. Upon confirmation of anesthesia, compound tropicamide eye drops were applied for mydriasis, followed by topical anesthetic drops to suppress ocular reflexes. Once fully anesthetized, a coupling gel was applied to the cornea, and mice were positioned in front of the electroretinography (ERG) recording system (Celeris, d430-P-10, Austria). Under both scotopic and photopic conditions, retinal responses to light stimuli of increasing intensities were recorded using the Celeris ERG system.

Optical Coherence Tomography and Angiography Following pupil dilation, mice were anesthetized *via* intraperitoneal injection. Once fully anesthetized, they were positioned in front of an optical coherence tomography (OCT) system (VG200D, Vimaging Tech Co., Henan, China). Using the Angio 9×9 scanning mode (software version V3.0116), bilateral OCT/optical coherence tomography angiography (OCTA) images were acquired by targeting the central posterior pole of the retina. Focus was adjusted to obtain clear, artifact-free vascular images for assessment of retinal capillary plexus density. For each group, five mice were randomly selected, and retinal thickness as well as the average superficial vascular density (1–9 mm) were quantified using the system's built-in software.

Fundus Fluorescein Angiography After completing bilateral OCT/OCTA imaging, medical sodium hyaluronate gel was applied to the cornea. Mice were then administered an intraperitoneal injection of fluorescein sodium (0.05 mL/mouse) and covered with a coverslip. Retinal angiography was performed using a confocal scanning laser ophthalmoscope (Spectralis HRA, HEIDELBERG, Germany), with immediate image acquisition for both eyes followed by a second capture

Table 1 Blood glucose levels of mice in each group over 12wk mean±SD, mmol/L

Time	Control (*n*=10)	Model (*n*=10)	Cal (*n*=10)	Cur-L (*n*=10)	Cur-H (*n*=10)
1wk	10.24±1.50	22.80±5.66[b]	24.57±3.98	23.00±2.62	26.79±3.18
2wk	11.03±1.28	28.24±4.57[b]	26.44±5.13	25.60±5.17	27.94±4.76
4wk	9.37±0.71	30.25±2.55[b]	24.27±6.64[e]	21.10±3.19[f]	19.67±5.98[f]
6wk	9.55±0.71	30.27±2.70[b]	25.42±4.91[f]	21.76±3.47[f]	21.98±3.59[f]
8wk	11.17±1.31	30.16±3.25[b]	25.42±5.09	20.31±6.64[f]	20.94±8.06[f]
10wk	9.79±2.08	26.17±6.01[b]	26.33±3.42	22.36±8.52	23.97±8.67
12wk	10.39±1.53	27.10±7.11[b]	29.78±3.74	22.36±6.10	26.69±5.82

SD: Standard deviation. [b]*P*<0.01 compared with control; [e]*P*<0.05, [f]*P*<0.01 compared with model.

at 5min post-injection. Fundus fluorescein angiography (FFA) images were required to show a centered optic disc and clearly defined vascular networks.

Retinal Vascular Flat-Mount with PAS Staining After sacrificing the animals, ocular globes were collected and fixed in 4% paraformaldehyde overnight. The retinas were then isolated, incubated in phosphate-buffered saline (PBS) for 30–120min, and treated with type A digestion buffer at 37°C for 1–2h. After PBS washing, tissues were further digested with trypsin for 10–60min to remove nonvascular components. Retinal vasculature was mounted on slides and air-dried. Dried samples were rehydrated, stained sequentially with oxidizing solution, Schiff reagent, and hematoxylin, with PBS rinses between steps. After ethanol dehydration and xylene clearing, slides were sealed with neutral resin. For quantification, five retinas per group and ten fields per retina were analyzed to determine the average number of acellular capillaries.

Hematoxylin and Eosin Hematoxylin and eosin (HE) staining was used to assess retinal histopathology. Mouse eyeballs were fixed in FAS ocular fixative for 24h prior to paraffin embedding. Eyes were then sectioned into continuous 4 μm slices using a microtome. After drying, sections were stained with HE. Histological alterations in retinal architecture were visualized using light microscopy in all experimental groups.

Western Blotting Tissues were lysed, and protein levels were quantified with a BCA assay kit. Equal protein quantities were electrophoretically separated using SDS-PAGE and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were then blocked with 5% skim milk at room temperature for 1h, followed by overnight incubation at 4°C with primary antibodies specific for large tumor suppressor kinase 1/2 (LATS1/2; Affinity Biosciences, DF7517), Phospho-LATS1/2 (p-LATS1/2, Affinity Biosciences, AF8163), YAP (Affinity Biosciences, AF6328), Phospho-YAP (p-YAP; Affinity Biosciences, AF3328), vascular endothelial (VE)-cadherin (Affinity Biosciences, AF6265), α-smooth muscle actin (α-SMA; Affinity Biosciences, AF1032), and β-actin (Abcam, ab8227). After thorough washing, membranes were incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibodies (Abcam, ab6721) for 2h at 4°C. Protein bands were visualized by enhanced chemiluminescence using Tanon ECL reagent, detected using a Tanon 5200 imaging system, and quantified with ImageJ software.

Statistical Analysis Statistical analyses were performed using GraphPad Prism version 8.0. All data are presented as mean±standard deviation (SD). Comparisons among multiple groups were conducted *via* one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Blood Glucose Parameters During the 12wk of oral administration, random blood glucose levels were measured every two weeks, and the results are shown in Table 1. The blood glucose levels of the control group remained stable between 8–12 mmol/L throughout the experiment. Compared to the control group, all diabetic groups (model, Cal, Cur-L, and Cur-H) showed a significant increase in blood glucose (P<0.01), with levels consistently >16.7 mmol/L. Compared to the model group, the treatment groups exhibited a gradual reduction in blood glucose between weeks 4 and 8. Although curcumin treatment did not restore blood glucose levels to normal, it demonstrated a certain degree of blood glucose control.

Curcumin Improves Retinal Function in Diabetic Mice Under scotopic conditions (Figure 1A, Scotopic), the model group exhibited a marked reduction in both a-wave and b-wave amplitudes compared to the control group. After treatment, the amplitudes increased in both the positive control and Cur-H groups relative to the model group. Under photopic conditions (Figure 1A, Photopic), the model group similarly showed decreased and more variable a-wave and b-wave amplitudes compared to the control group. Post-treatment, these amplitudes were elevated in both the positive control and Cur-H groups. Notably, in both scotopic and photopic assessments, a-wave and b-wave amplitudes in the Cur-H group more closely approximated those of the control group than those in the positive control group. These findings suggest that curcumin improves retinal function in diabetic mice. Line

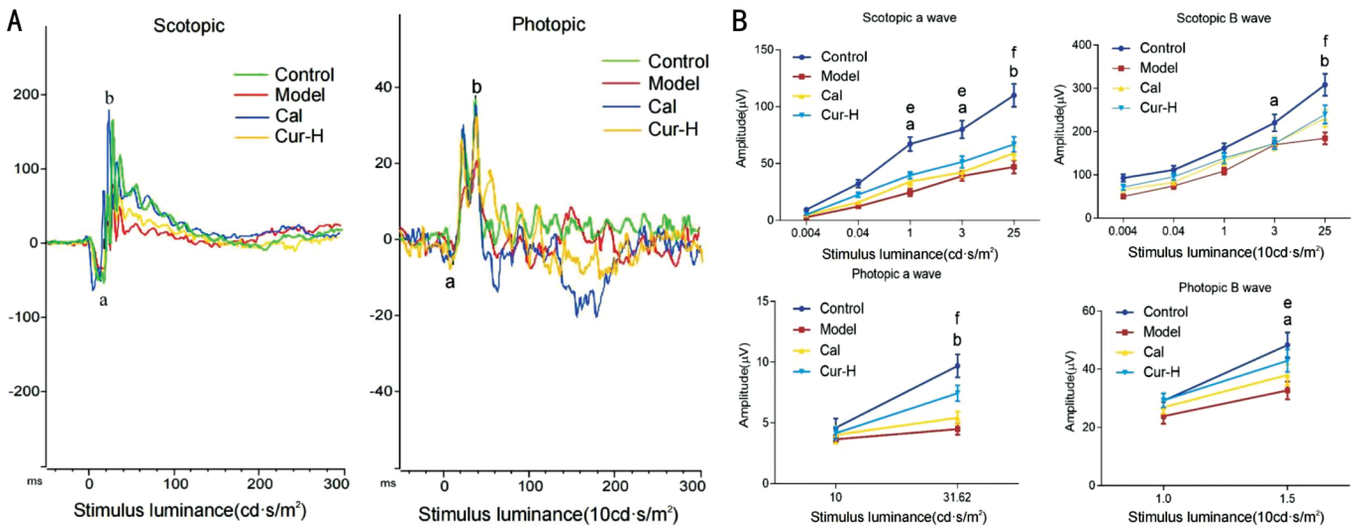

Figure 1 Retinal functional changes induced by curcumin in diabetic mice A: Representative ERG waveforms under scotopic and photopic conditions in control, model, calcium dobesilate, and high-dose curcumin groups; B: Line graphs depicting a-wave and b-wave amplitudes under both scotopic and photopic conditions in each experimental group. Results are expressed as mean±SD, n=5 per group. [a]P<0.05, [b]P<0.01 compared with control; [e]P<0.05, [f]P<0.01 compared with model. ERG: Electroretinography; SD: Standard deviation.

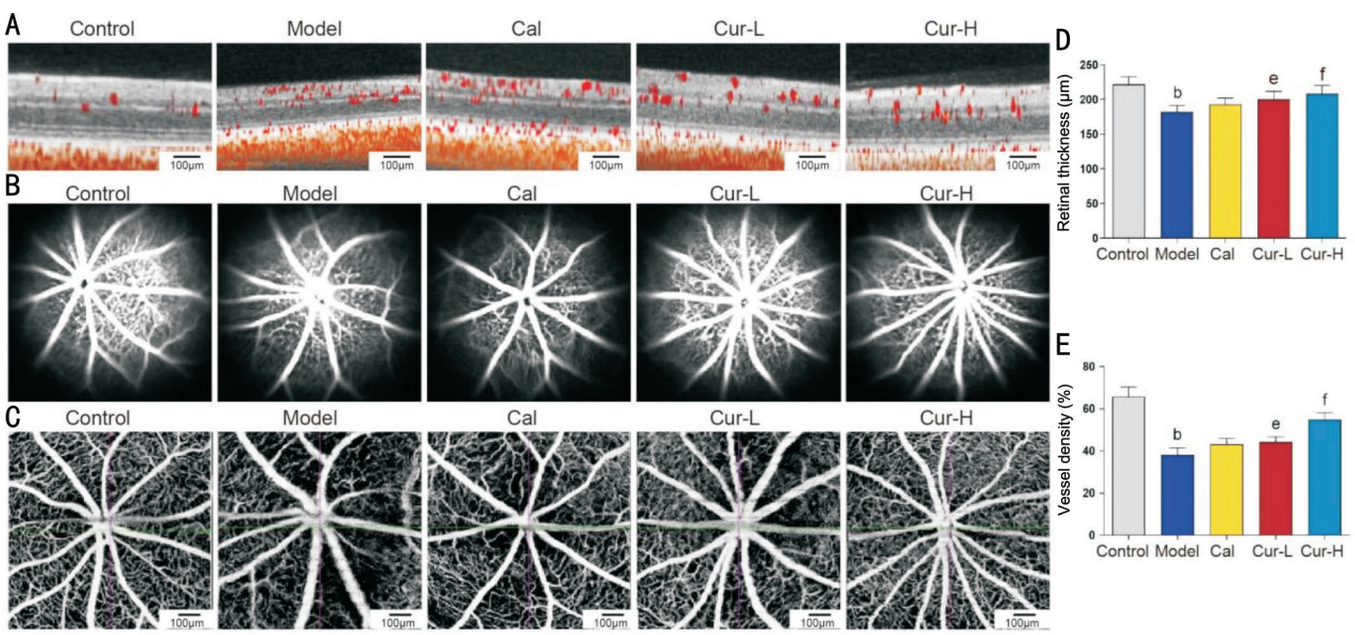

Figure 2 The effect of curcumin on retinal pathological changes A: Representative OCT scans from mice in the control, model, Cal, Cur-L, and Cur-H groups; B: Representative FFA images from in the control, model, Cal, Cur-L, and Cur-H groups; C: Representative OCTA images from in the control, model, Cal, Cur-L, and Cur-H groups; D: Quantification of retinal thickness across groups; E: Quantification of mean retinal vessel density across the 1–9 mm retinal region in each group. Results are expressed as mean±SD, n=5 per group, scale bar=100 μm. [b]P<0.01 compared with control; [e]P<0.05, [f]P<0.01 compared with model. OCT: Optical coherence tomography; FFA: Fundus fluorescein angiography; OCTA: Optical coherence tomography angiography; SD: Standard deviation.

graphs of ERG a-wave and b-wave amplitudes are shown in Figure 1B.

Curcumin Alleviated Retinal Pathological Changes in Diabetic Mice OCT was used to assess the average retinal thickness in diabetic mice (Figure 2A, 2D). In the control group, the retinal structure was clear, with distinct layers. Compared to the control group, the model group showed a significant decrease in average retinal thickness, a loss of continuity in the ellipsoid zone, and less defined retinal layers. In contrast to the model group, retinal thickness was increased in the Cal, Cur-L, and Cur-H treatment groups. Notably, the Cur-L and Cur-H groups exhibited a statistically significant reversal of retinal thinning compared to the model group, with relatively clearer layer structure. In the control group, the retinal vasculature exhibited a uniform network of capillaries. FFA (Figure 2B) revealed that, compared to the control group,

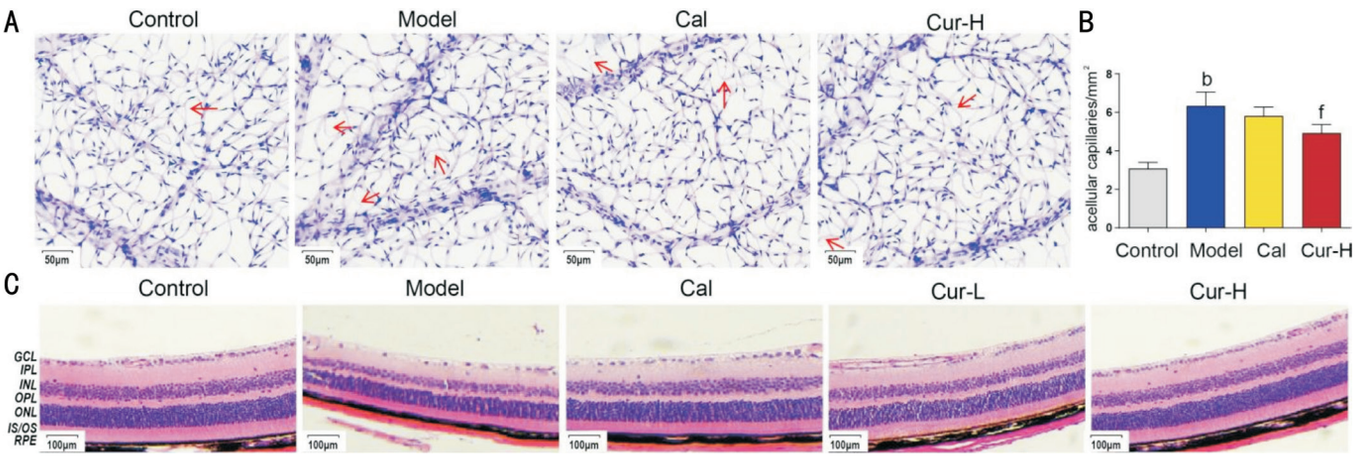

Figure 3 Curcumin alleviates retinal vascular injury and improves retinal structure in diabetic mice A: Representative PAS-stained retinal flat-mount images from the control, model, Cal, and Cur-H groups. Red arrows highlight acellular capillary regions in the retina (×400). B: Quantification of acellular capillaries in the control, model, Cal, Cur-L, and Cur-H groups. Results are expressed as mean±SD, n=3 per group. ^{b}P<0.01 compared with control; ^{f}P<0.01 compared with model. C: Representative HE-stained retinal sections from the control, model, Cal, and Cur-H groups. HE staining (×200), scale bar=100 μm, n=5 per group. In the control group, retinal layers are well-defined, with orderly cellular arrangement. In the model group, there is significant ganglion cell loss, and the INL and ONL are markedly thinned and disorganized. In the high-dose curcumin group, ganglion cell numbers increased, and the INL and ONL thicknesses were restored, with retinal structure becoming more organized. PAS: Periodic acid-Schiff; HE: Hematoxylin and eosin; SD: Standard deviation; GCL: Ganglion cell layer; IPL: Inner plexiform layer; INL: Inner nuclear layer; OPL: Outer plexiform layer; ONL: Outer nuclear layer; IS/OS: Photoreceptor inner/outer segments layers; RPE: Retinal pigment epithelium.

the model group showed areas of non-perfusion between some retinal vessels. In contrast, the treatment groups displayed significant improvements in retinal vascular morphology, with no obvious leakage or non-perfusion areas. These results suggest that curcumin increased retinal vascular density and improved non-perfusion areas in diabetic mice. OCTA (Figure 2C, 2E) showed that, compared to the control group, the model group developed small non-perfusion areas in the retina, with a significant decrease in the average retinal vascular density (1–9 mm). Compared to the model group, the treatment groups exhibited improvements in vascular patterning, increased vessel spacing, and reduced microvascular dropout. Notably, the high-dose curcumin group showed the most significant improvement in average retinal vascular density.

Curcumin Attenuates Retinal Microvascular Injury and Preserves Retinal Structure in Diabetic Mice Retinal vascular flatmounts (Figure 3A) were used to count acellular capillaries. In the control group, the retinal capillaries exhibited continuous morphology, uniform density, and consistent thickness, with few acellular capillaries observed. In contrast, the model group showed irregular and tortuous vessels, with a significant increase in the number of acellular capillaries. Compared to the model group, the treatment groups exhibited more regular vascular structures and fewer acellular capillaries. Notably, the Cur-H group showed a significant reduction in acellular capillaries (Figure 3B), indicating the most prominent protective effect against diabetic microvascular damage. Retinal structural alterations were evaluated using HE staining (Figure 3C). In the control group, the retinal layers were well-organized, with clear structure and densely arranged cell nuclei. Compared to the control group, the model group exhibited a significant reduction in the number of retinal ganglion cell layer nuclei, with sparse nuclear arrangement in the inner and outer nuclear layers and decreased cell density. The density of the photoreceptor inner/outer segment layer was also notably reduced. In comparison to the model group, the Cal group showed a slight increase in the number of retinal ganglion cell layer nuclei, with higher cell density in the inner and outer nuclear layers. The Cur-H group demonstrated a significant increase in the number of retinal ganglion cell layer nuclei compared to the model group, with a marked increase in both the inner and outer nuclear layer cell numbers. Additionally, the retinal structure was more organized in the Cur-H group than in the model group.

Regulatory Effects of Curcumin on the Hippo-YAP Pathway and EndMT in Diabetic Mouse Retina As illustrated in Figure 4, compared to the control group, diabetic mouse retinas exhibited decreased p-LATS1/2 to LATS1/2 and p-YAP to YAP ratios, downregulation of VE-cadherin expression, and upregulation of α-SMA protein levels. In contrast, both the Cur-H and Cal groups showed increased p-LATS1/2/LATS1/2 and p-YAP/YAP ratios, upregulation of VE-cadherin, and reduced α-SMA expression compared to the model group.

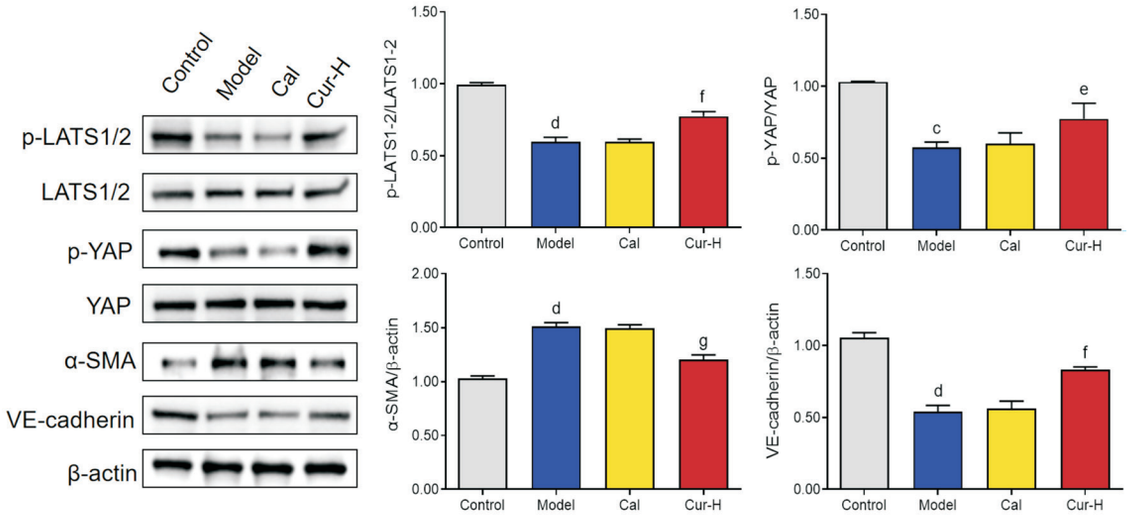

Figure 4 Protein expression levels in the control, model, Cal, and Cur-H groups (*n*=3) [c]*P*<0.001, [d]*P*<0.0001 compared with control; [e]*P*<0.05, [f]*P*<0.01, [g]*P*<0.001 compared with model. YAP: Yes-associated protein; p-YAP: Phospho-yes-associated protein; LATS1/2: Large tumor suppressor kinase 1/2; p-LATS1/2: Phospho-large tumor suppressor kinase 1/2; α-SMA: α-smooth muscle actin; VE-cadherin: Vascular endothelial-cadherin.

DISCUSSION

Evaluation of STZ-Induced Diabetic Mice Model Six-week-old male C57BL/6J mice were utilized to induce diabetes through intraperitoneal administration of STZ[15]. STZ administration leads to a diabetic condition through targeted destruction of pancreatic β-cells, causing insulin deficiency[16]. Various STZ-based protocols have been developed for mice, and regardless of dosage, hyperglycemia typically appears within 2wk and may persist for up to 22mo. Previous studies have shown that in STZ-induced diabetic mice, astrocyte activation and gliosis are detectable approximately 4 to 5wk after the onset of hyperglycemia. Retinal ganglion cell loss typically begins by week 6, followed by progressive thinning of the inner nuclear layer (INL) and outer nuclear layer (ONL) around week 10. Pathological neovascularization tends to appear by week 16, while loss of capillaries and pericytes becomes evident at approximately 6mo[17]. In the experimental model of the present study, curcumin failed to exert a significant improving effect on overall metabolic parameters, including blood glucose levels. All diabetic groups remained in a marked hyperglycemic state at the study endpoint. Existing studies have confirmed that curcumin directly targets retinal endothelial cells, exerting protective effects through the regulation of specific signaling pathways, independent of improvements in systemic metabolism[5].

Curcumin Attenuates Retinal Neurofunctional Impairment in Diabetic Mice ERG is used to assess retinal function through the detection of light-evoked electrical signals from the retina[18-19]. Dysfunction of retinal neurons can disrupt the blood–retinal barrier, thereby exacerbating inflammation and oxidative stress, ultimately leading to irreversible vascular damage[20]. Ahmadieh *et al*[21] demonstrated that in individuals with non-proliferative DR, both a-wave and b-wave amplitudes were diminished, with the b-wave showing a more substantial reduction and a notably prolonged implicit time. Similarly, Dorofeeva *et al*[22] observed that, 14wk after STZ induction, diabetic mice showed markedly reduced a- and b-wave amplitudes relative to non-diabetic controls. In the present study, diabetic mice showed marked reductions in both a- and b-wave amplitudes, indicating functional impairment of both the outer and inner retinal layers. Curcumin intervention restored ERG amplitudes, suggesting its neuroprotective effect on retinal function in diabetic mice.

Curcumin Ameliorates Retinal Structural Alterations in Diabetic Mice Previous studies have shown that in diabetic rats treated with STZ for 7.5mo, retinal histological analysis revealed a 22% reduction in inner plexiform layer thickness and a 14% reduction in inner nuclear layer thickness[23]. It is important to note that, on the one hand, the small size and relatively spherical lens structure of mouse eyes limit the effective imaging window for OCT/OCTA. On the other hand, the VG200D ophthalmic OCT scanner used in this study exhibits a significant decrease in image resolution in the Angio 12 mm×12 mm scan mode and lacks a stable automated vascular density quantification algorithm. Therefore, this study employed the Angio 9 mm×9 mm scan mode centered on the optic disc, which was used to assess vascular density in the central retina and the peripapillary region. In this study, OCT imaging revealed that diabetic mice exhibited retinal thinning, blurred retinal layer demarcation, and ellipsoid zone disruption compared to controls. Curcumin treatment partially ameliorated these abnormalities and alleviated the

trend of retinal thinning. HE staining further confirmed the OCT findings. In the model group, disorganization and loss of ganglion cells were observed, along with thinning of the INL, ONL, and retinal nerve fiber layer. In contrast, curcumin treatment reduced ganglion cell loss, restored more orderly cellular arrangement in the INL and ONL, and increased overall retinal thickness. The retinal architecture appeared clearer and more intact. Taken together, these results indicate that curcumin confers significant protection against retinal structural damage in diabetic mice and helps preserve retinal anatomical integrity.

Curcumin Reduces Retinal Capillary Damage in Diabetic Mice OCTA enables visualization of the capillary networks across all retinal layers, with vessel density considered a key indicator of visual function[24-25]. Studies have shown that reduced capillary plexus density is positively correlated with the severity of non-proliferative DR. In addition[26], FFA can detect early microvascular damage, ischemic areas, and assess the extent of vascular impairment[27]. In this study, both OCTA and FFA were used to comprehensively assess retinal perfusion from static and dynamic perspectives. OCTA revealed a significant reduction in retinal vessel density and the formation of non-perfused areas in diabetic mice. Software-assisted analysis further confirmed a marked decrease in superficial vessel density compared to the control group, likely due to capillary dropout and reduced perfusion. FFA results corroborated these findings, showing capillary loss and insufficient perfusion in the model group. To further validate microvascular injury, PAS staining was performed on retinal flat-mounts. Diabetic mice exhibited a significant increase in acellular capillaries, along with disorganized and tortuous vascular morphology, indicating severe microangiopathy. Following curcumin treatment, retinal vessel density significantly improved, non-perfusion areas were reduced, and the number of acellular capillaries markedly decreased. Collectively, these results demonstrate that curcumin alleviates retinal vascular occlusion and improves impaired perfusion in diabetic mice.

Regulatory Effects of Curcumin on the Hippo-YAP Signaling Pathway Increasing evidence suggests that dysregulated Hippo-YAP signaling is a key contributor to diabetic microvascular complications. For example, in DR models, post-translational modifications of YAP protein, such as O-GlcNAcylation, inhibit its phosphorylation, promoting its accumulation in the nucleus and exacerbating vascular dysfunction[6]. Nuclear YAP/transcriptional coactivator with PDZ-binding motif (TAZ) acts as co-activators and, together with TEA domain transcription factor (TEAD) transcription factors, induces the expression of pro-fibrotic and pro-angiogenic genes, such as connective tissue growth factor and cysteine-rich angiogenic inducer 61. These genes are known to play a role in endothelial cell proliferation, migration, and vascular remodeling[28]. It is noteworthy that in other diabetic vascular injury models, interactions between YAP/TAZ and classical pro-fibrotic pathways, such as transforming growth factor-β/Smad signaling, have been observed. Their synergistic activation promotes EndMT and the expression of fibrosis-related genes[29].

EndMT is defined by decreased expression of endothelial markers (*e.g.*, CD31, VE-cadherin) and concurrent upregulation of mesenchymal proteins like α-SMA and vimentin, promoting cell migration and matrix synthesis[30]. Evidence suggests that EndMT contributes to fibrosis in multiple organs[31-32]. Similarly, in DR, EndMT is thought to promote fibrotic changes and exacerbate vascular abnormalities. Proliferative DR features pathological neovascularization and fibrotic changes. Hyperglycemia has been shown to induce EndMT in retinal endothelial cells, a phenomenon reproduced in diabetic animal models[33]. Mechanistically, curcumin activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and upregulates dimethylaminohydrolase 1 expression, thereby counteracting transforming growth factor-β1-induced EndMT[34]. The role of the Hippo-YAP signaling pathway in DR is not fully consistent in the existing literature. While some studies suggest it exacerbates the pathology, others indicate a potential protective or compensatory role. Dysregulation of this kinase cascade is thought to contribute to pathological angiogenesis in DR. For instance, a study found that *Acacia catechu* extract inhibits pathological neovascularization and improves retinal structure in DR rats by modulating Hippo pathway-related proteins[35], suggesting that abnormal Hippo-YAP signaling is involved in DR progression and could serve as a potential therapeutic target. Hao *et al*[36] observed decreased levels of phosphorylated Mammalian Sterile 20-like kinase (p-MST) and p-YAP in the retinas of diabetic rats, while downstream effectors TAZ and TEAD were upregulated, indicating suppression of the upstream Hippo kinase cascade and enhanced YAP/TAZ transcriptional output. These changes coexist with DR lesions, supporting the notion that “sustained YAP activation plays a pathological role in DR”. In contrast, Du *et al*[37], under different experimental conditions, reported that YAP inhibits aberrant angiogenesis by downregulating vascular endothelial growth factor receptor 2 (VEGFR2) expression, suggesting that YAP may also exert anti-angiogenic or compensatory protective effects in certain contexts. These seemingly contradictory results are likely due to differences in experimental models and intervention timing. For instance, variations in disease progression, blood glucose levels, and the inflammatory microenvironment among different DR models may lead to differing pathway activity. Additionally, the role

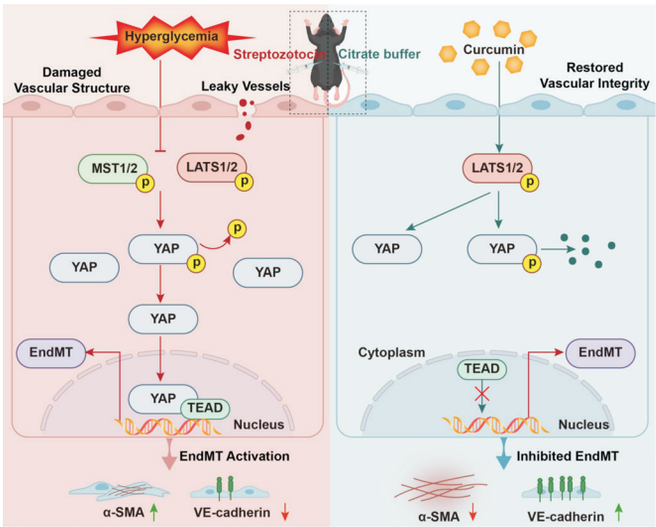

Figure 5 Proposed mechanism of curcumin regulating the Hippo-YAP pathway to inhibit EndMT in diabetic retinopathy Hyperglycemia suppresses the phosphorylation of MST1/2 and LATS1/2, leading to decreased YAP phosphorylation, YAP nuclear translocation, and its binding to TEAD. This process activates the transcription of EndMT-related genes. Curcumin treatment enhances the phosphorylation of LATS1/2, increases YAP phosphorylation, and prevents YAP nuclear accumulation, thereby blocking the YAP-TEAD-mediated transcription and inhibiting EndMT progression. EndMT: Endothelial-to-mesenchymal transition; YAP: Yes-associated protein; TEAD: TEA domain transcription factor; LATS1/2: Large tumor suppressor kinase 1/2; MST1/2: Mammalian sterile 20-like kinase 1/2; α-SMA: α-smooth muscle actin; VE: Vascular endothelial. Created with Adobe Illustrator 2019.

of the pathway may vary dynamically between the early and late stages of DR. In this study, a significant decline in the p-LATS1/2/LATS1/2 and p-YAP/YAP ratios was observed in the model group by Western blotting, implying YAP nuclear accumulation and induction of fibrosis-related genes. Curcumin treatment restored these ratios, indicating a potential retinal protective effect through modulation of the Hippo/YAP pathway (Figure 5). In summary, curcumin exhibits significant protective effects on retinal injury in diabetic mice, potentially through modulation of the Hippo-YAP signaling pathway and inhibition of EndMT, offering a novel therapeutic approach for DR. Current treatment options, including laser photocoagulation, intravitreal injection of anti-vascular endothelial growth factor (VEGF) agents, and corticosteroids, are primarily used in the advanced stages of DR, but they are limited by the need for repeated administration, high cost, and potential side effects. Compared to existing therapies, curcumin shows significant potential. It possesses multiple properties, including anti-inflammatory, antioxidant, and anti-fibrotic effects, and can provide long-term treatment through oral administration with minimal side effects. Although curcumin has low bioavailability, existing studies have proposed various strategies to improve its absorption and bioavailability, such as using water-soluble formulations, nanoparticles, or combining it with piperine[38]. Moreover, as an adjunctive therapy, curcumin offers advantages of low cost and high safety, particularly in diabetic patients requiring long-term management, providing additional protective effects and reducing the reliance on conventional therapies. Our study supports the potential of curcumin as a therapeutic agent for DR. However, this study has certain limitations. On the one hand, curcumin has poor water solubility and lacks chemical stability. We did not directly assess its pharmacokinetic properties or retinal distribution *in vivo*. Future studies should further explore curcumin formulation improvements and combination therapy strategies to maximize its clinical efficacy in DR treatment. On the other hand, the precise molecular mechanisms by which curcumin regulates EndMT *via* the Hippo-YAP pathway remain unclear and warrant further investigation. These analyses will be prioritized in future studies to further clarify the therapeutic mechanisms. Despite these limitations, our study validates the potential of curcumin in protecting the retinas of diabetic mice and highlights its role as a promising candidate for DR treatment.

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