Urolithin A ameliorates diabetic retinopathy via activation of the Nrf2/HO-1 pathway

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Abstract. Diabetic retinopathy (DR) is a progressive microvascular complication of diabetes mellitus and is characterised by excessive inflammation and oxidative stress. Urolithin A (UA), a major metabolite of ellagic acid, exerts anti-inflammatory and antioxidant functions in various human diseases. This study, for the first time, uncovered the role of UA in DR pathogenesis. Streptozotocin-induced diabetic rats were used to determine the effects of UA on blood glucose levels, retinal structures, inflammation, and oxidative stress. High glucose (HG)-induced human retinal endothelial cells (HRECs) were used to elucidate the anti-inflammatory and antioxidant mechanisms of UA in DR in vitro. The in vivo experiments demonstrated that UA injection reduced blood glucose levels, decreased albumin and vascular endothelial growth factor concentrations, and ameliorated the injured retinal structures caused by DR. UA administration also inhibited inflammation and oxidative damage in the retinal tissues of diabetic rats. Similar anti-inflammatory and antioxidant effects of UA were observed in HRECs induced by HG. Furthermore, we found that UA elevated the levels of nuclear Nrf2 and HO-1 both in vivo and in vitro. Nrf2 silencing reversed the inhibitory effects of UA on inflammation and oxidative stress during DR progression. Together, our findings indicate that UA can ameliorate DR by repressing inflammation and oxidative stress via the Nrf2/HO-1 pathway, which suggests that UA could be an effective drug for clinical DR treatment.

Key words: Urolithin A, Diabetic retinopathy, Inflammation, Oxidative stress, Nrf2/HO-1 pathway

DIABETIC RETINOPATHY (DR) is a common and progressive microvascular complication of diabetes mellitus (DM) [1]. Patients are generally asymptomatic in the early DR stage, and vision exacerbation, such as blindness, may appear with the development of the disease [2, 3]. Although the current DR therapies, including vitrectomy, use of anti-vascular endothelial growth factor (VEGF) drugs, or laser photocoagulation, have advanced considerably, the treatment effects remain unsatisfactory [4-6]. Therefore, exploring more effective agents on the basis of understanding the mechanism of DR is urgently required.

Vascular hyperpermeability is the initial pathological feature of DR capillaries [7]. The increased vascular permeability caused by endothelial dysfunction leads to retinal haemorrhages, exudate formation, and macular oedema, eventually resulting in vision loss [7]. Abnormal albumin and VEGF levels have been confirmed to affect the integrity of the blood-retinal barrier (BRB) [8, 9]. Moreover, it is acknowledged that DR pathogenesis is accompanied by oxidative stress and inflammation [10, 11]. Augustin et al. revealed that oxidative stress increases VEGF levels in the vitreous of DR patients, which further aggravates BRB breakdown and promotes DR progression [12]. Chang et al. demonstrated that sorbitol production from glucose in DR pathogenesis increases NADPH consumption, but decreases the concentration of GSH [13], which plays a vital role in removing free radicals [14]. Inflammation is another important pathological feature of DR progression [15]. Increased levels of various pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α, are found in the vitreous humour and retinal tissues of diabetic patients and animal models [11, 16,
17]. Hence, drugs that decrease vascular permeability, reduce oxidative stress, and repress inflammation may be useful for clinical DR treatment.

Ellagic acid (EA) is a polyphenol that is hydrolysed by ellagitannin, which is abundant in pomegranate, berries, and certain herbs, and is combined with Chinese herbal medicine for clinical diabetes treatment [18-20]. Generally, EA is poorly absorbed by human gut, but it can be further metabolised by the microflora into a series of micromolecular urolithins [21], including urolithin A (UA), B, and C [22-24]. Among these three urolithin species, UA is the major metabolite found in humans [25] and has been reported to exert antioxidant and anti-inflammatory effects in various human diseases, such as Alzheimer’s disease (AD) [26], cisplatin-induced nephrotoxicity [27], Parkinson’s disease (PD) [28], and inflammatory diseases [29]. Notably, UA has also been reported to play a crucial role in preventing cardiac dysfunction [30], pancreatic injury [31] and attenuation of insulin resistance [32] in a diabetic rat/mouse model. However, the detailed functions of UA in DR treatment remain unclear.

In this study, the possible role of UA in oxidative stress and inflammation and the downstream regulatory pathways were preliminarily investigated. The findings may provide an effective therapeutic method for DR.

Materials and Methods

Reagents

Cell culture and transfection-related reagents were purchased from Invitrogen (Carlsbad, CA, USA). Commercial enzyme-linked immunosorbent assay (ELISA) kits for inflammatory cytokines were obtained from EseBio (Shanghai, China). Sigma-Aldrich (St. Louis, MO, USA) provided streptozotocin (STZ) and commercial kits for the measurement of SOD, GSH, MDA, LDH, albumin, and VEGF. Western blotting analysis-related reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA), and the primary antibodies (Nrf2, Lamin B, HO-1, and β-actin) and HRP-conjugated secondary antibody were procured from Abcam (Cambridge, UK). Nuclear Extract kit and TransAM® Nrf2 DNA-Binding ELISA kits were purchased from Active Motif (Shanghai, China). The apoptosis detection kit was obtained from Thermo Fisher Scientific. Nrf2-siRNA (si-Nrf2) and the negative control (si-NC) were acquired from Sangon Biotech (Shanghai, China).

Cell culture and treatment

Human retinal endothelial cells (HRECs) were cultured in endothelial cell medium, containing 10% foetal bovine serum, 1% penicillin/streptomycin, and normal glucose (NG; 5.5 mmol/L), at 37°C and 5% CO₂. To mimic DR in vitro, NG was replaced with high glucose (HG; 25 mmol/L). Transfection experiments were performed using Lipofectamine 3000 according to the manufacturer’s instructions. Following transfection for 48 h, the cells were collected for the subsequent experiments.

Cell viability assay

HG-induced HRECs were cultured in 96-well plates (2 × 10⁴) for 24 h, followed by incubation with different concentrations of UA (0, 0.5, 2.5, 10, 20, and 40 μM) for another 24 h. Thereafter, approximately 150 μL MTT was added for a 2 h incubation at 37°C. The absorbance values were measured using a microplate reader (Molecular Devices, Shanghai, China) at a wavelength of 570 nm.

Cell apoptosis assay

Cell apoptosis was assessed using flow cytometry. Briefly, the collected cells (1 × 10⁶ cells/mL) were cultured in 96-well plates for 24 h and then stained with V-FITC and propidium iodide, using an apoptosis detection kit, at 25°C for 20 min in the dark. Apoptotic cells were measured using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Diabetic rat model

Thirty 10-week-old male Sprague-Dawley (SD) rats (220–240 g) were purchased from EseBio, Co., Ltd. (Shanghai, China). The rats were initially allowed to adapt to the laboratory environment for one week prior to testing. Thereafter, the rats were assigned to three groups (n = 10): the control group, diabetic control (DC) group, and diabetes + UA (DC + UA) group. STZ dissolved in citrate buffer (10 mM) was intraperitoneally (i.p.) injected into the rats to induce DR, while rats injected with an equal volume of citrate buffer served as the control group. After one week, the levels of fasting blood glucose in STZ-induced rats were detected, and more than 16.7 mmol/L was included in the experimental group. Subsequently, rats in the DC + UA group were administered UA (2.5 mg/kg/day; i.p.) for 12 weeks. All rats were euthanised with an overdose of pentobarbital sodium (200 mg/kg; i.p.), and retinal tissues were collected for further experiments. All animal experiments in this study were performed in strict accordance with the protocols stated in the Guide for the Care and Use of Laboratory Animals and were approved by the ethical committee of Wuyi Hospital of Traditional Chinese Medicine.

ELISA

The levels of inflammatory cytokines (IL-6, IL-1β, and TNF-α), the endothelial dysfunction marker LDH,
and oxidative stress-related factors (SOD, GSH, and MDA) in HG-induced HRECs and/or retinal tissues of diabetic rats were measured via ELISA using the corresponding commercial kits according to the manufacturer’s instructions.

Nuclear Extraction assay

Subcellular nuclei were extracted from HG-induced HRECs and the retinal tissues of diabetic rats using a Nuclear Extract kit. Briefly, the retinal tissues or HRECs were homogenised in HEPES buffer (20 mM), containing phenylmethylsulphonyl fluoride (1 mM), MgCl₂ (1.5 mM), KCl (10 mM), leupeptin (10 μg/mL), EDTA (0.5 mM), aproamin (10 μg/mL), sucrose (250 mM), and EGTA (0.5 mM), to prepare the cytosolic fraction. The homogenate was centrifuged at 750 g for 10 min at 4°C to separate the nuclei and cytosol.

Nrf2 activity detection assay

Nrf2 activity was assessed using the TransAM® Nrf2 DNA-Binding ELISA kit according to the manufacturer’s instructions. Briefly, an antioxidant response element sequence oligonucleotide was pre-coated in a 96-well plate, followed by adding the nuclear protein (15 μg) for incubation. After 1 h, anti-Nrf2 and secondary antibodies were added. The results were assessed at 450 and 655 nm using a spectrophotometer.

Western blotting

Protein concentrations were determined using a BCA Protein Assay Kit (Thermo Fisher Scientific). Next, the protein products were separated via 10% SDS-PAGE and transferred onto PVDF membranes. Following blocking, the membranes were incubated with the primary antibodies at 4°C overnight and then with the secondary antibody for 1 h at room temperature. Lamin B or β-actin was used as an internal control. Immunoblotting was performed using an ECL detection kit (Amersham Biosciences, Amersham, UK).

Vascular Leakage test

Albumin from the vitreous humour of diabetic rats and VEGF from retinal tissues of diabetic rats were measured using the corresponding commercial kits according to the manufacturer’s instructions. The vitreous humour extraction procedures were strictly performed according to the method of Deliyanti et al. [33].

Quantitative real time PCR (qRT-PCR)

The mRNA expression of VEGF, IL-6, IL-1β, and TNF-α was detected using RT-qPCR. In brief, total RNA extracted from the cells and retinal tissues of diabetic rats was used for cDNA synthesis and then for RT-qPCR analysis. The 2−ΔΔCt method was used to calculate mRNA expression, and GAPDH was used as the internal control.

Haematoxylin-eosin (HE) staining

The retinal tissues of diabetic rats were fixed in 4% paraformaldehyde for 24 h, followed by embedding in paraffin sectioned at 5 μm thickness. All sections were immediately stained with HE and observed via light microscopy.

Statistical analysis

Data are presented as mean ± standard deviation. The SPSS software (version 20.0; Chicago, IL, USA) was used for statistical analysis. Student’s t-test and a one-way ANOVA, followed by Tukey’s multiple comparisons test, were used to assess the data. The difference was considered statistically significant when the p-value was <0.05.

Results

UA promotes the viability of HG-induced HRECs

Different UA concentrations were used to assess the toxic effects on HRECs (Fig. 1A). We found that cell viability began to decrease at 20 μM UA (p < 0.05) and further decreased at 40 μM UA (p < 0.01), which suggested that 0.5–10 μM UA may be the relatively safe concentration. Thereafter, we demonstrated that HG treatment significantly reduced cell viability (Fig. 1B, p < 0.01). Meanwhile, both 2.5 μM UA and 10 μM UA partially eliminated the inhibitory effect of HG on cell viability (p < 0.01). Considering that there were no significant differences between the effects of the two UA concentrations on HG-induced HREC viability, 10 μM UA was selected for subsequent experiments. LDH is an enzyme that can induce endothelial dysfunction [34]. As expected, HG treatment elevated LDH levels in HRECs; however, addition of UA restrained the release of LDH caused by HG (Fig. 1C, p < 0.01).

UA represses the inflammatory reactions and oxidative stress caused by HG in HRECs

It is widely accepted that both inflammation and oxidative damage play pivotal roles in DR progression [15, 35]. Therefore, we investigated the effects of UA on inflammatory cytokines and oxidative stress. As illustrated in Fig. 2A, B, HG significantly elevated the protein levels and mRNA expression of IL-6, IL-1β, and TNF-α in HRECs, while UA treatment partly eliminated these promoting effects (p < 0.01). In terms of oxidative stress, HG remarkably inhibited the release of SOD and GSH, but facilitated MDA levels (Fig. 2C, p < 0.01). Interestingly, UA treatment attenuated the HG-induced oxidative stress in HRECs (p < 0.01).
UA elevates the levels of nuclear Nrf2 and HO-1 in HG-induced HRECs

The Nrf2/HO-1 pathway has been reported to be involved in DR pathogenesis [36, 37]. Notably, we found that HG treatment reduced the nuclear Nrf2 levels, which was significantly reversed by the addition of UA (Fig. 3A, \( p < 0.01 \)). Consistent with the decrease in Nrf2 expression in HG-induced HREC nuclei, the DNA-binding activity of Nrf2 was also subnormal in the HG group, although it was promoted in the HG + UA group (Fig. 3B, \( p < 0.01 \)). Similar data were observed in the western blotting results for HO-1, a downstream target of
Nrf2 (Fig. 3C, \( p < 0.01 \)), which suggested that HO-1 protein levels may be positively correlated with nuclear Nrf2 levels.

**Nrf2 knockdown reverses the protective effects of UA on HG-induced HRECs**

To investigate the interaction between UA and the Nrf2/HO-1 pathway in DR progression in vitro, the transfection efficiency of si-Nrf2 was determined. As shown in Fig. 4A, Nrf2 protein levels were remarkably reduced in si-Nrf2-transfected HRECs (\( p < 0.01 \)). In HG-induced HRECs, UA treatment promoted cell viability and elevated the levels of GSH and SOD, but suppressed LDH levels, MDA levels, apoptosis, and the levels of IL-6, IL-1\( \beta \), and TNF-\( \alpha \) (Fig. 4B–G, \( p < 0.01 \)). Interestingly, we found that Nrf2 silencing reversed the influence of UA treatment on HG-induced HRECs (\( p < 0.05 \)).

**Injection of UA relieves retinal injury in STZ-induced diabetic rats**

Diabetic rat models were established to further explore the therapeutic effect of UA on DR in vivo. Compared to those of the control group, the fasting blood glucose levels of the DC group were significantly high (Fig. 5A, \( p < 0.01 \)). Unsurprisingly, the glucose levels in rats of the DC + UA group were remarkably lower than those in the DC group after 12 weeks (\( p < 0.01 \)). As presented in Fig. 5B, the control group showed an intact retinal structure. A decrease in retinal thickness was observed in diabetic rats (\( p < 0.01 \)), whereas injection of UA alleviated such morphological changes (\( p < 0.05 \)). The integrity of the BRB is crucial for maintaining vision, while BRB breakdown and subsequent vascular leakage may lead to retinopathy and vision loss [8]. Albumin, a known representative marker of BRB injury, was then detected. We found that albumin levels were higher in the DC group than in the control group (Fig. 5C, \( p < 0.01 \)), and UA treatment distinctly reduced albumin levels in diabetic rats (\( p < 0.05 \)). VEGF is known to be the primary factor affecting vascular permeability in DR pathogenesis [9]. Both the mRNA expression and protein levels of VEGF in the DC group rats were significantly increased; however, they were decreased following UA injection (Fig. 5D, E; \( p < 0.01 \)).

**UA treatment inhibits inflammation and oxidative stress in STZ-induced diabetic rats via activation of the Nrf2/HO-1 pathway**

We further explored the relationship between UA and the Nrf2/HO-1 pathway in a diabetic rat model. Significantly increased levels of IL-6, IL-1\( \beta \), TNF-\( \alpha \), and MDA and decreased SOD and GSH levels were observed in diabetic rats (Fig. 6A–F; \( p < 0.01 \)). Similar to the inhibitory effects of UA on HG-induced HRECs, UA-injected rats exhibited relatively low inflammatory reactions and oxidative stress injury (\( p < 0.01 \)). Additionally, we found that the levels of nuclear Nrf2 and HO-1 were distinctly reduced in diabetic rats (Fig. 6G, H; \( p < 0.01 \)). As expected, both nuclear Nrf2 and HO-1 levels were elevated in the DC + UA group relative to those in the DC group.

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**Fig. 3** UA elevates the levels of nuclear Nrf2 and HO-1 in HG-induced HRECs. (A) The protein level of nuclear Nrf2 in HG-induced HRECs was determined by western blotting. (B) The activity of nuclear Nrf2 in HG-induced HRECs was measured by a commercial kit. (C) The protein level of HO-1 in HG-induced HRECs was determined by western blotting. **\( p < 0.01 \) vs. the NG group. **\( p < 0.01 \) vs. the HG group.
Fig. 4  Nrf2 knockdown reverses the protective effects of UA on HG-induced HRECs. (A) The protein level of nuclear Nrf2 in HRECs after transfection of si-Nrf2/NC was determined by western blotting. ** p < 0.01 vs. the si-NC group. (B) The viability of HG-induced HRECs transfected with si-Nrf2/NC was measured by MTT assay. (C) The level of LDH in HG-induced HRECs transfected with si-Nrf2/NC was measured by a commercial kit. (D) The apoptosis of HG-induced HRECs transfected with si-Nrf2/NC was measured by flow cytometric analysis. (E) The level of IL-6 in HG-induced HRECs transfected with si-Nrf2/NC was measured by ELISA assay. (F) The level of IL-β in HG-induced HRECs transfected with si-Nrf2/NC was measured by ELISA assay. (G) The level of TNF-α in HG-induced HRECs transfected with si-Nrf2/NC was measured by ELISA assay. (H) The level of MDA in HG-induced HRECs transfected with si-Nrf2/NC was measured by a commercial kit. (I) The level of GSH in HG-induced HRECs transfected with si-Nrf2/NC was measured by a commercial kit. (J) The level of SOD in HG-induced HRECs transfected with si-Nrf2/NC was measured by a commercial kit. ** p < 0.01 vs. the HG group. * p < 0.05, ** p < 0.01 vs. the HG + UA + si-NC group.
(p < 0.01), suggesting that the Nrf2/HO-1 pathway may be activated by UA treatment.

**Discussion**

DR is a severe microvascular complication of (DM) due to uncontrolled HG levels [1]. It has been reported that approximately one-third of (DM) patients undergo various degrees of DR [38]. The clinical management of DR remains challenging, and there is still no satisfactory clinical medicine to protect against DR [4-6]. Therefore, exploring more effective and satisfactory agents for DR treatment is urgently required. Our findings, for the first time, indicate that UA may attenuate DR effectively through repression of inflammation and oxidative stress via the Nrf2/HO-1 pathway.

An increasing number of studies have demonstrated that UA plays a protective role against various human diseases, such as AD [26], cisplatin-induced nephrotoxicity [27], and PD [28]. Excessive inflammatory response and oxidative stress generally occur during the progression of these disorders [26-28]. In the present study, a HG-induced HREC model was initially used to mimic DR in vitro. We demonstrated that UA treatment remarkably decreased the elevated inflammatory cytokine levels and excessive peroxidation product formation caused by HG induction. Furthermore, the levels of LDH, an endothelial dysfunction marker, have been reported to increase under HG conditions, while the viability of retinal endothelial cells is repressed [36, 39].

Consistent with these previous data, our findings confirmed that HG induction triggered LDH secretion and inhibited HREC viability. Interestingly, UA ameliorated the adverse effects of HG stimulation in HRECs. Based on the above experimental data, we speculated that UA can reduce inflammation and oxidative stress injury in HG-induced HRECs, which suggests that UA may be an effective clinical drug for DR treatment.

We then confirmed the therapeutic effects of UA in vivo. STZ-induced animal models are generally used to explore the effects of novel clinical drugs on diabetes and its complications [40-42]. In this study, relatively high fasting blood glucose levels and reduced retinal tissue thickness were observed in diabetic rats, suggesting that the DR rat model was successfully established. Shi et al. recently investigated the effect of palbinone on STZ-induced DR rats and found that palbinone-treated diabetic rats exhibited significantly reduced blood glucose levels and relatively normal retinal structures compared to those of non-treated diabetic rats, suggesting that palbinone may be useful for improving STZ-induced hyperglycaemia and pathological changes in retinal tissues [42]. Based on this innovative research, we speculated that UA may also exert similar functions in STZ-induced diabetic rats. Interestingly, we further found that UA administration decreased glucose levels and promoted the retinal structures of diabetic rats to get right towards normal retinas, which validated our assumption. Meanwhile, we found that the UA-injected diabetic rats had attenuated inflammatory reactions and decreased oxidative

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**Fig. 5** Injection of UA relieves retinal injury in STZ-induced diabetic rats. (A) Effects of UA on fasting blood glucose. (B) Effects of UA on histology of retina and retina thickness. (C) Effects of UA on albumin level in retina of diabetic rat. (D) Effects of UA on mRNA expression of VEGF in retina of diabetic rat. (E) Effects of UA on the level of VEGF in retina of diabetic rat. **p < 0.01 vs. the control group. *p < 0.05, **p < 0.01 vs. the DC group.**
stress levels, which further confirmed our in vitro experimental data. It has been demonstrated that peroxidation products are closely associated with increased VEGF levels in DR patients [12]. VEGF and albumin serve as representative markers for BRB breakdown, while BRB disruption is responsible for retinopathy and vision loss [8, 9]. Therefore, we speculated that UA administration may be involved in regulating BRB integrity via inhibition of VEGF and albumin. As expected, we found that UA reversed the promoting effects of diabetes on albumin and VEGF levels, which suggested that UA contributes considerably to the improvement of BRB breakdown.

Nrf2 is a redox-sensitive transcription factor that is generally silent [43]. A previous study revealed that the activation of Nrf2 signalling ameliorates (DM) by protecting pancreatic beta cells and suppressing gluconeogenesis-related gene expression [44], suggesting that the Nrf2 system is a critical target for preventing the onset of (DM). In the current study, the hypoglycaemic mechanism of UA was investigated. According to the aforementioned results, the high fasting blood glucose levels in diabetic rats were significantly reduced upon

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**Fig. 6** UA treatment inhibits inflammation and oxidative stress in STZ-induced diabetic rats via activation of Nrf2/HO-1 pathway. (A) The level of IL-6 in retina of diabetic rat after injection of UA was measured by ELISA assay. (B) The level of IL-β in retina of diabetic rat after injection of UA was measured by ELISA assay. (C) The level of TNF-α in retina of diabetic rat after injection of UA was measured by ELISA assay. (D) The level of SOD in retina of diabetic rat after injection of UA was measured by a commercial kit. (E) The level of GSH in retina of diabetic rat after injection of UA was measured by a commercial kit. (F) The level of MDA in retina of diabetic rat after injection of UA was measured by a commercial kit. (G) The protein level of nuclear Nrf2 in retina of diabetic rat after injection of UA was determined by western blotting. (H) The protein level of HO-1 in retina of diabetic rat after injection of UA was determined by western blotting. **p < 0.01 vs. the control group. ## p < 0.01 vs. the DC group.
UA administration. We believe that UA may directly activate the Nrf2 system to exert hypoglycaemic effects. Additionally, activated Nrf2 enters the cell nucleus and exerts antioxidant effects through its interaction with downstream antioxidant genes such as HO-1 [45]. The Nrf2/HO-1 pathway and its transcriptional products have been confirmed to play crucial roles in the prevention of oxidative stress and inflammation in various human diseases, such as AD [46], spinal cord injury [47], and diabetic cardiomyopathy [48]. In this study, decreased levels Nrf2 and HO-1 levels were found in HG-induced HRECs and the retinal tissues of diabetic rats. Similarly, Zhong et al. observed reduced nuclear Nrf2 levels in the retinas of diabetic rats [37], and Deliyanti et al. believed that HO-1 protein levels were repressed in HG-treated retinal endothelial cells from SD rats [33]. These results imply that the Nrf2/HO-1 pathway is involved in DR progression, which is consistent with a report by Song et al. [40]. Based on the above-mentioned results, we speculated that UA may interact with the Nrf2/HO-1 pathway to modulate oxidative damage in DR pathogenesis. It is demonstrated that using Nrf2 signaling activator (sulforaphane) can protect reduced nuclear Nrf2 levels in the retinas of diabetic rats [37], and Deliyanti et al. believed that HO-1 protein levels were repressed in HG-treated retinal endothelial cells from SD rats [33]. The Nrf2/HO-1 pathway is involved in DR progression, suggesting that loss of Nrf2 may elevate the transcriptional activity of Nrf2 requires further investigation. Second, this study only focused on the interaction between UA and the Nrf2/HO-1 pathway at the cellular level, and further in vivo validation experiments are required. Third, in addition to the Nrf2/HO-1 pathway, numerous signalling pathways, such as p38-MAPK [62], TLR4/NF-κB [63], PI3K/Akt/mTOR [64], and PTEN/Akt/P-Akt [65], are also involved in DR progression. Thus, the interaction between UA and these pathways should be investigated.

In summary, the current study revealed that UA alleviates DR via interaction with the Nrf2/HO-1 pathway both in vitro and in vivo. These findings clarify the action mechanism of UA in the development of DR and may provide an effective clinical drug for DR therapy.

Declarations

Ethics approval and consent to participate: This study was conducted after obtaining the First Affiliated Hospital of Guangzhou University of Chinese Medicine’s ethical committee approval. (No. TCMF1-2019007)

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests.

Funding

1. The Science and Technology Plan Project of Guangdong Province (no. 2016ZC0103);
2. The Science and Technology Plan Project of Guangzhou (no: 201604020105);
3. Diabetic Retinopathy Treatment Research Team (2017TD04);
4. High-level hospital construction of Guangzhou University of Chinese Medicine (211010010709);
5. The Science and Technology Plan Project of Guangzhou (no.202102010499).

Authors’ Contributions

Zepeng Xu planned the study.
Ni Tian, Songtai Li, Kunmeng Li and Xiaoyu Wang conducted a survey.
Zepeng Xu, Ni Tian, Songtai Li, Kunmeng Li, and Xiaoyu Wang performed the data analyses and wrote the manuscript.
Xiaojie Li, Meixia An, Xiaoyi Yu, Ruizying Zhong, and Qiuqiong Liu performed the data analyses and revised the manuscript.
Xinguang Long assisted with the experimental work, helped to perform the data analyses and wrote the manuscript in the revising of the manuscript.
Xiaochuan Wang and Yan Yang helped perform the analysis with constructive.
All the authors read and agreed to submit the manuscript.
Ni Tian submitted the study.

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