



Apocynin ameliorates diabetic retinopathy in rats: Involvement of TLR4/NF- κ B signaling pathway

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ABSTRACT

Diabetic retinopathy is a diabetes complication. During diabetic retinopathy development, the TLR4/NF- κ B pathway is up-regulated. Apocynin is a nicotinamide adenine dinucleotide phosphate oxidase blocker which can reduce the superoxide radicals. It is demonstrated that apocynin can inhibit TLR4/NF- κ B pathway in rats. We aim to figure out whether apocynin treatment is benefit for the diabetic retinopathy in rat model. The diabetes in rats was induced by streptozotocin. The treatment of apocynin (16 mg/kg/day) or vehicle in diabetic rat model was maintained for 12 weeks. The expression levels of relative genes in this research were shown through Western blot and qRT-PCR. Morphology of the retinas was shown by Hematoxylin-Eosin staining. The treatment of apocynin ameliorated biochemical indexes in diabetic rats and rescued the morphology of the retinas. After a 12 weeks apocynin treatment, the cell apoptosis, oxidative stress, and inflammatory in retina was reduced in diabetic rats. TLR4/NF- κ B signaling pathway activity in diabetic rat retina was inhibited by apocynin. Based on our study, the treatment of apocynin ameliorates diabetic retinopathy in rats. The TLR4/NF- κ B signaling pathway inhibition by apocynin is involved in this process. This result indicated a great therapeutic potential of apocynin in diabetic retinopathy treatment.

1. Introduction

As a microvascular complication of diabetes, diabetic retinopathy is a serious disease which is considered to be involved in the leading reason of preventable blindness all over the world [1]. The mechanism of diabetic retinopathy pathogenesis is still not being fully understood till now. Many different biological processes are considered to participate in diabetic retinopathy pathology, including polyol pathway activation, hexosamine pathway, inflammatory response, protein kinase C activation, and the overexpression of vascular endothelial growth factor (VEGF) [2]. The activation of Toll-like receptor 4 (TLR4)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway promotes the diabetes induced inflammatory response and participates in diabetic retinopathy pathogenesis [3]. Present therapeutic strategies for diabetic retinopathy involve the anti-VEGF drug treatment, steroids treatment, retinal laser photocoagulation, and surgical procedure [4]. Since these strategies do not have a satisfied effect, a new method in diabetic retinopathy therapy is needed.

Apocynin is a natural organic compound isolated from *Picrorhiza kurroa* which acts an antioxidant agent by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex activation inhibition and reactive oxygen species (ROS) scavenging acceleration [5]. It is

confirmed that the treatment of apocynin in rat model reduces the oxidative stress, prevents microglial activation [6], suppresses neuronal autophagy, and downregulates the inflammation cascade through inhibition of TLR4/NF- κ B pathway [7]. Apocynin shows beneficial effects in cardiac remodeling process in diabetic cardiomyopathy rats [8]. Another research demonstrated that the reduction of superoxide radicals in retina by apocynin makes a great contribution to the cone cell death prevention in retinitis pigmentosa [9]. All these data shown us the apocynin potential function in the therapy of diabetic retinopathy.

In this research, we explored whether the treatment of apocynin ameliorates diabetic retinopathy in rats and whether the inhibition of TLR4/NF- κ B pathway activity by apocynin participates in this process.

2. Methods

2.1. Animals and experimental design

Three-month-old male Wistar-NIN rats with average body weight of 230 ± 23 g were used in this experiment. All these rats were divided into three groups: 1. Control group ($n = 15$); 2. Diabetes mellitus (DM, $n = 15$); 3. Diabetes with apocynin treatment (DM + apocynin, $n = 15$).

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The intraperitoneal injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) at 32 mg/kg body weight diluted in 0.1 M citrate buffer was used for diabetes induction in rats. Meanwhile, control group was treated with only 0.1 M citrate buffer. Fasting blood glucose levels were tested 72 h after treatment. Rats with blood glucose level > 150 mg/dl were thought to have diabetes and used in this research.

During 12 weeks, 15 rats in DM group received only AIN-93 diet. The other 15 diabetic rats were fed with AIN-93 diet and 16 mg/kg/day apocynin. The dose was chosen based on the published paper [10] and our pilot study. Blood samples were obtained from the retro-orbital plexus once a week for analyzation. The retinas were dissected from eye after 12 weeks apocynin treatment. Retinas from 12 animals in each group were stored at -80°C for biochemistry analyzation and retinas from the other 3 animals were fixed for histological detection. Animal studies were reviewed and approved by the Ethical Committee in Wuxi People's Hospital Affiliated to Nanjing Medical University (#WPH2018JQ1).

2.2. Biochemical estimations

Twelve weeks after treatment, glucose Oxidase/peroxidase Kit (BioSystems, S.A.Costa Brava 30, Barcelona, Spain) was used for serum glucose analysis based on the manufacture's description. HbA1c level was measured by HEMOGLOBIN A1C-DIRECT (HbA1C-DIR) Kit (BioSystems, S.A.Costa Brava 30, Barcelona, Spain) based on the manufacture's description.

The levels of cholesterol, triglyceride (TG), and high-density lipoprotein (HDL) in serum were detected using standard laboratory functions. Friedwald's formula was used in calculating the level of low-density lipoprotein (LDL). Atherogenic index was calculated using the following formula: $\text{AI} = \text{Cholesterol} - \text{HDL} - \text{Cholesterol} / \text{HDL} - \text{Cholesterol}$.

2.3. Hematoxylin-eosin (HE) staining

After being fixed in paraformaldehyde, eye tissue was embedded in paraffin and cut into 5 μm thickness sections for HE staining. The retina total thickness was measured by Spot Software ver. Axiovision Rel 4.8.

2.4. Quantitative real-time PCR (qRT-PCR)

The retina total RNA was isolated by Tri-reagent based on the manufacture's instruction. High Capacity cDNA Reverse Transcription kit was used in the reverse transcription of cDNA from total RNA. qRT-PCR was performed using SYBR green master mix in triplicates. Primers used in this experiment were shown as follows:

VEGF Forward: GGC TCA CTT CCA GAA ACA CG
 VEGF Reverse: GTG CTC TTG CAG AAT CTA GTG G
 GFAP Forward: TGG CCA CCA GTA ACA TGC AA
 GFAP Reverse: CAG TTG GCG GCG ATA GTC AT
 Bax Forward: GTT TCA TCC AGG ATC GAG CAG
 Bax Reverse: CAT CTT CTT CCA GAT GGT GA
 Bcl-2 Forward: CCT GTG GAT GAC TGA GTA CC
 Bcl-2 Reverse: GAG ACA GCC AGG AGA AAT CA
 IL-6 Forward: TGC CTT CTT GGG ACT GAT GT
 IL-6 Reverse: ATA CTG GTC TGT TGT GGG TGG T
 IL-1 β Forward: CCT CTG ACA GGC AAC CAC TTA
 IL-1 β Reverse: GCA CTG GTC CAA ATT CAA TTC
 TNF- α Forward: CTG GCG TGT TCA TCC GTT CT
 TNF- α Reverse: GCC ACT ACT TCA GCG TCT CGT
 TLR4 Forward: GGA CTC TGC CCT GCC ACC ATT TA
 TLR4 Reverse: CTT GTG CCC TGT GAG GTC GTT GA
 NF- κ B(p65) Forward: GTG CAG AAA GAA GAC ATT GA
 NF- κ B(p65) Reverse: AGG CTA GGG TCA GCG TAT GG
 GAPDH Forward: ACT GGC ATT GTG ATG GAT TC

GAPDH Reverse: CAG CAC TGT GTT GGC ATA GA

2.5. Western blot

Western blot was performed with the standard method. Image J software was used in the band intensity quantification. The following antibodies were used: anti-VEGF (Cell Signaling Technology, Boston, MA, USA), anti-GFAP (Cell Signaling Technology, Boston, MA, USA), anti-Bax (Cell Signaling Technology, Boston, MA, USA), anti-Bcl-2 (Cell Signaling Technology, Boston, MA, USA), anti-TLR4 (Santa Cruz Biotechnology, Texas, TX, USA), anti-NF- κ B (p65) (Santa Cruz Biotechnology, Texas, TX, USA), p-NF- κ B (p65) (Santa Cruz Biotechnology, Texas, TX, USA), and anti- β -actin (Santa Cruz Biotechnology, Texas, TX, USA).

2.6. Measurement of superoxide dismutase (SOD) activity, malondialdehyde (MDA) level, and glutathione (GSH) level in retina

SOD activity was detected by hydroxylamine assay kit (Jiancheng Institute of Biotechnology, Nanjing, China). The concentration of MDA was determined by malondialdehyde assay kit (Jiancheng Institute of Biotechnology, Nanjing, China). The concentration of GSH was detected by Bioxytech GSH/GSSG-412 kit (#21040; OxisResearch, Beverly Hills, CA, USA).

2.7. Statistical analysis

Data were shown as mean \pm standard deviation (SD). Statistical analysis was performed by SPSS 16.0 software (version 16.0). The differences were evaluated by one-way ANOVA analysis followed by a Tukey's post hoc test.

3. Results

3.1. Apocynin reduced the blood glucose level in diabetes induced rat model

First, apocynin administration did not induce any obvious side-effects in the normal rats, including body weight loss and death. Then, we tested whether the treatment of apocynin can influence the diabetes induced by STZ in rat model. After the apocynin treatment, the elevated food intake by diabetes was significantly decreased and the declined body weight by diabetes was significantly up-regulated in rat (Fig. 1a, b). When compared to the DM group, the increased blood glucose level and HbA1c level by diabetes were both dramatically down-regulated by apocynin (Fig. 1c, d). All these changes demonstrated that apocynin treatment can rescue the symptoms caused by diabetes in rat model.

3.2. Apocynin ameliorated biochemical indexes in diabetic rats

Diabetes in connected with variations in several different biochemical parameters, such as cholesterol, TG, and HDL. In rat, diabetes induced by STZ resulted in the up-regulated levels of cholesterol and TG and down-regulated level of HDL (Fig. 2a-c). But when apocynin was applied for diabetic rats, the cholesterol level and TG level in blood were significantly decreased and the HDL level was elevated (Fig. 2a-c). The calculation results demonstrated that the diabetes related elevation in LDL level and atherogenic index were declined by apocynin treatment (Fig. 2d, e). It was shown that the metabolic disorder caused by diabetes can be rescued by apocynin in rat model.

3.3. Apocynin attenuated VEGF and GFAP expressions in retinas of diabetic rats

The hyperglycemia-mediated up-regulation of VEGF stimulates neovascularization and participate the diabetic retinopathy pathogenesis. Glial acidic fibrillary protein (GFAP), a gliosis marker, is also

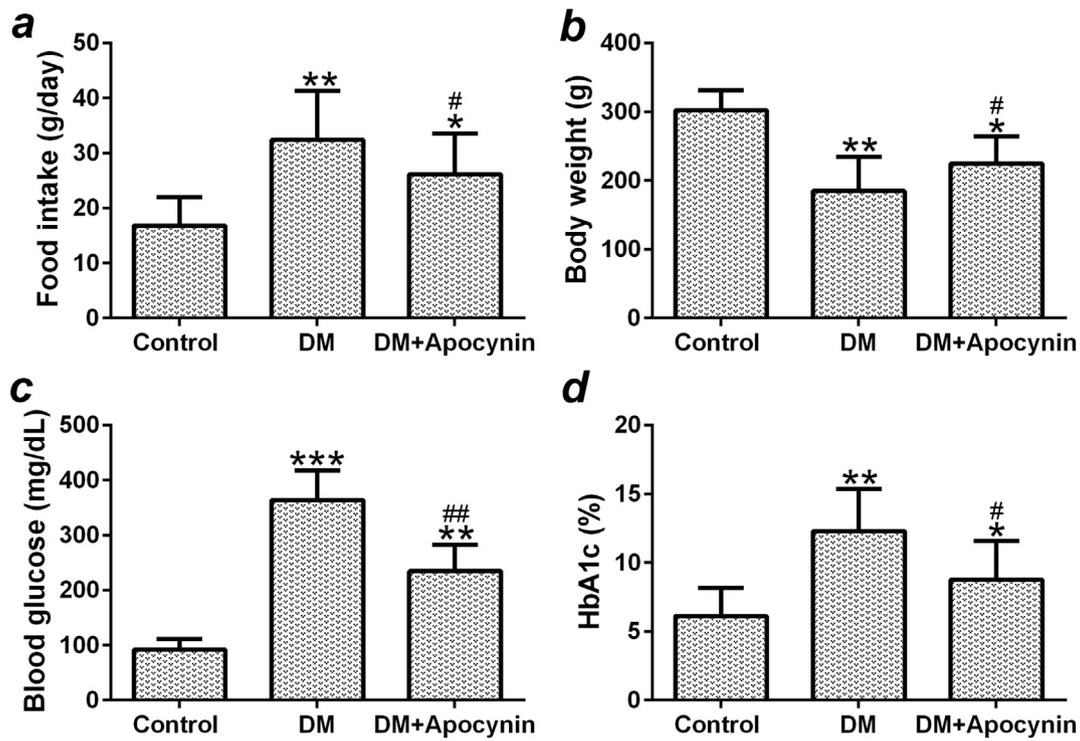


Fig. 1. Average food intake, body weight, blood glucose and glycosylated hemoglobin data from experimental rats. Average food intake (a), body weight (b), blood glucose (c) and glycosylated hemoglobin (d) during the whole experimental period among different groups. Data were collected every week and presented as mean ± SD. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to control group, #p < 0.05 and ##p < 0.01 compared to DM group.

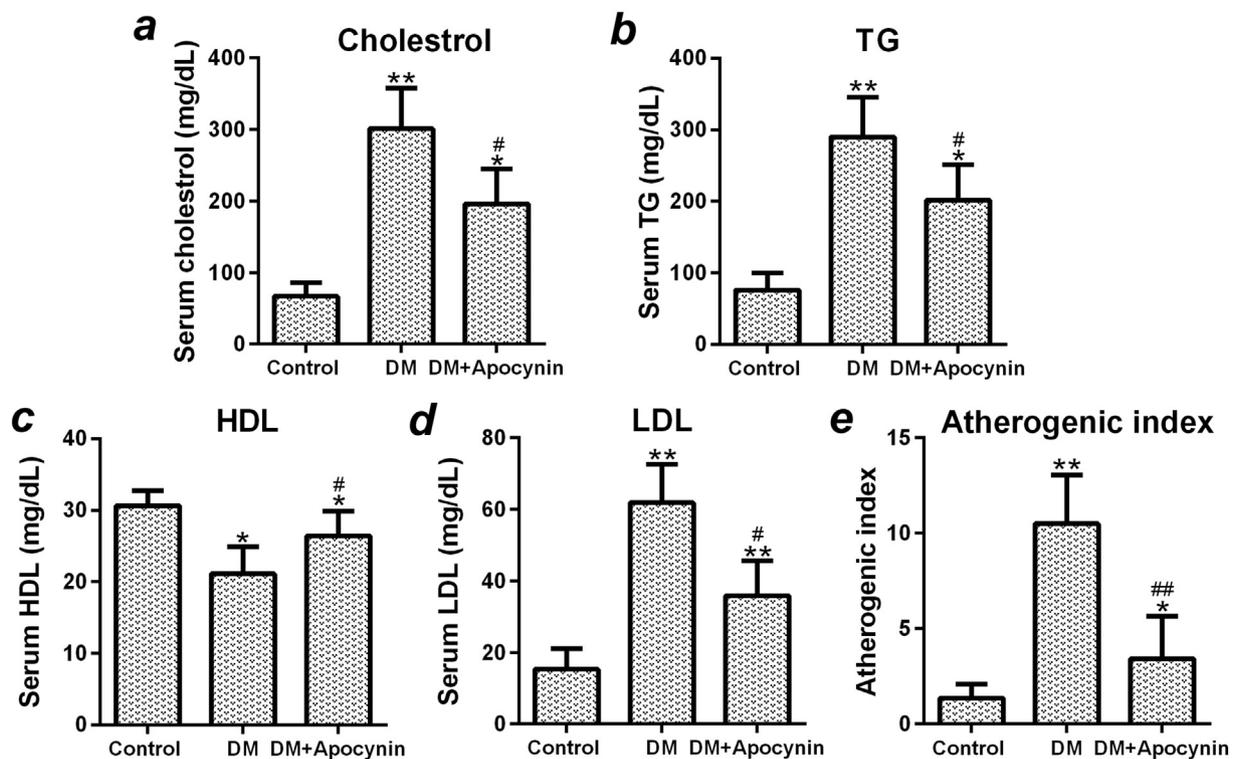


Fig. 2. Apocynin ameliorated biochemical indexes in diabetic rats after 12 weeks treatment. Serum cholesterol (a), serum triglyceride (TG) (b), serum high-density lipoprotein (HDL) (c), serum low-density lipoprotein (LDL) (d) and atherogenic index (e) were tested at the end of the treatment. Data were presented as mean ± SD. *p < 0.05, **p < 0.01 compared to control group, #p < 0.05 and ##p < 0.01 compared to DM group.

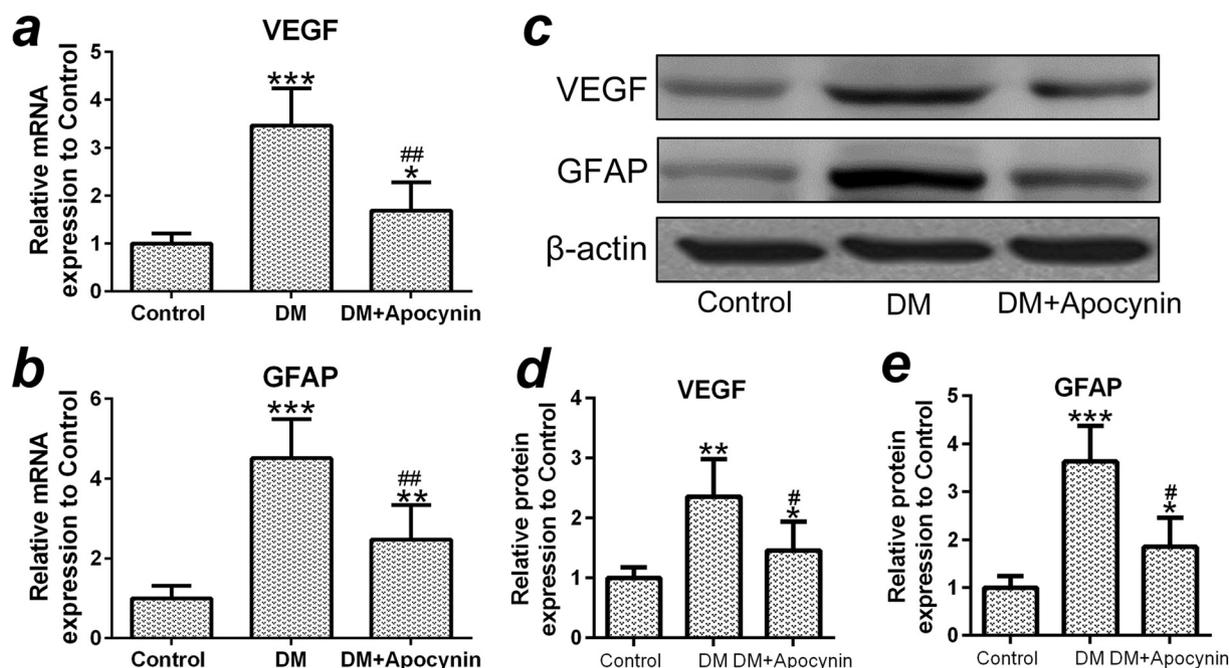


Fig. 3. Apocynin attenuated VEGF and GFAP expressions in retinas of diabetic rats after 12 weeks treatment.

a, b. qRT-PCR results of VEGF and GFAP mRNA expressions in retinas of rats among different groups. Relative expression pattern was analyzed by comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to control group. c. Representative immunoblots of VEGF and GFAP in retinas of rats among different groups. β -actin used as loading control. d, e. Quantification of immunoblots of VEGF and GFAP. Expressions were normalized to control group. Data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control group, # $p < 0.05$ and ## $p < 0.01$ compared to DM group.

confirmed to be up-regulated in retinas of diabetic rat. To confirm whether apocynin has effect on diabetic retinopathy, VEGF and GFAP mRNA levels in diabetic rat retinas were measured by qRT-PCR. Fig. 3a, b illustrated that the up-regulated mRNA levels of VEGF and GFAP in retinas caused by diabetes were dramatically decreased by apocynin treatment. Then the protein levels of VEGF and GFAP were also detected by western blot. Based on the western blot result, diabetic rats treated with apocynin shown an obvious lower VEGF and GFAP protein levels when compared with those diabetic rats with no apocynin treatment (Fig. 3c-e). The changes in the expression of VEGF and GFAP indicated that apocynin treatment can rescue the pathogenic events in retina caused by diabetes.

3.4. Apocynin rescued the abnormal retina morphology in diabetic rats

The retina morphology was detected by HE-staining. The HE-staining results shown a decreased retina overall thickness in diabetes induced rats (Fig. 4a, b). The diabetic rats treated with apocynin shown

a rescued retina morphology since the retina overall thickness is elevated when compared with DM group (Fig. 4a, b).

3.5. Apocynin attenuated cell apoptosis in diabetes induced rat retina

The expression levels of pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2 were detected using western blot and qRT-PCR. Bax mRNA level was increased and Bcl-2 mRNA level was down-regulated in retinas of diabetic rats (Fig. 5a, b). But in apocynin treated diabetic rats, a depressed Bax expression and an enhanced Bcl-2 expression were observed when compared with DM group (Fig. 5a, b). We also checked the retina Bax and Bcl-2 protein levels by western blot (Fig. 5c). The ratio of Bax to Bcl-2 was shown in Fig. 5d and elevated Bax/Bcl-2 ratio in retina of diabetic rat was dramatically declined after the treatment of apocynin. The changed expression levels of Bax and Bcl-2 illustrated the function of apocynin in the inhibition of apoptosis in diabetic retinopathy.

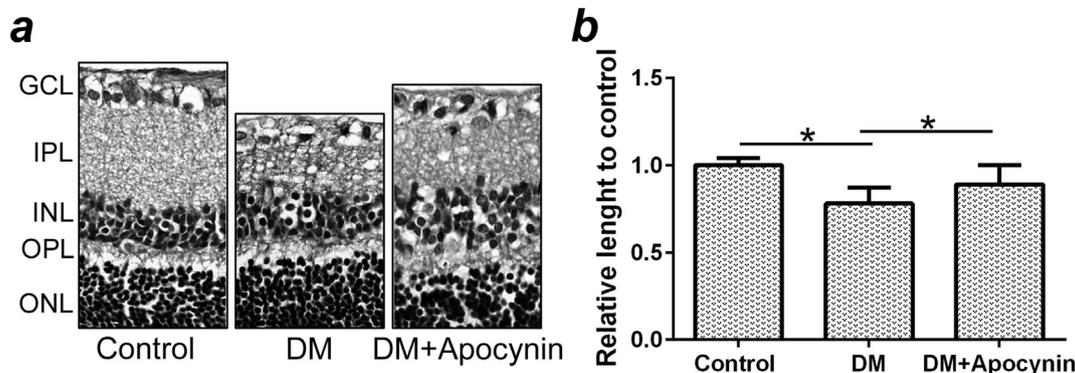


Fig. 4. Effect of apocynin on the morphology of the retinas in diabetic rats after 12 weeks treatment.

a. Representative images of HE-stained sections of retinas of the rats among different groups. Magnification $\times 400$. b. The thickness of the overall retina was normalized to control group. Data were presented as mean \pm SD. * $p < 0.05$.

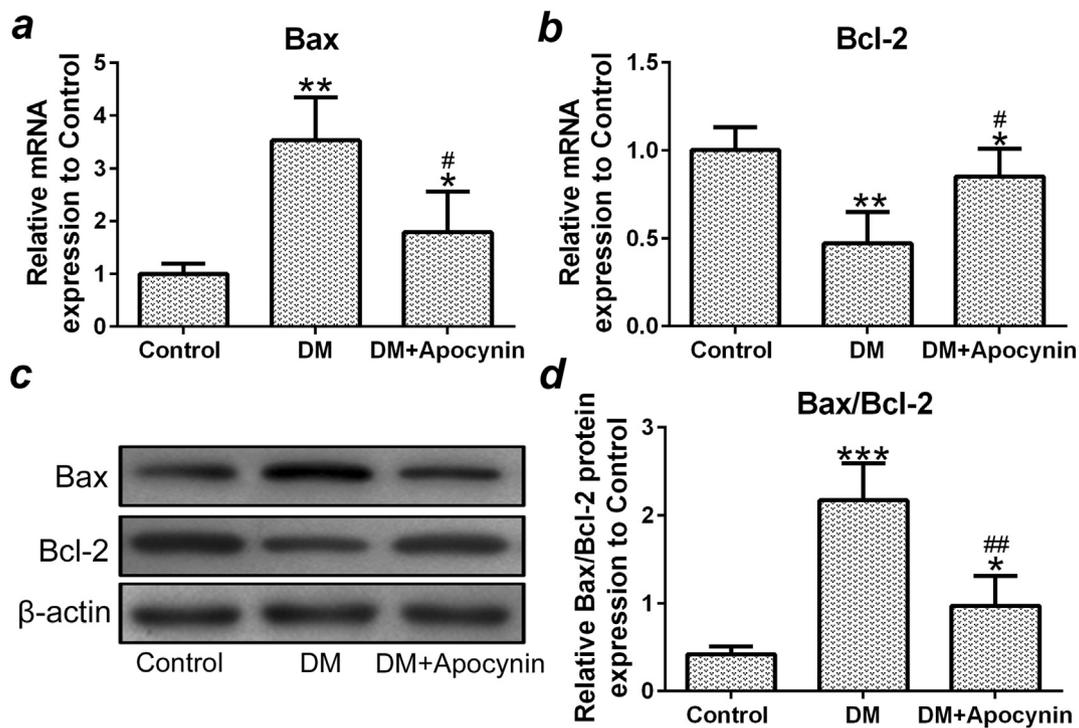


Fig. 5. Apocynin attenuated cell apoptosis in retinas of diabetic rats after 12 weeks treatment.

a, b. qRT-PCR detection of Bax and Bcl-2 mRNA expressions in retinas of rats among different groups. Relative expression pattern was analyzed by comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to control group. c. Representative immunoblots of Bax and Bcl-2 in retinas of rats among different groups. β -actin used as loading control. d. Quantification of immunoblots of Bax/Bcl-2. Data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control group, # $p < 0.05$ and ## $p < 0.01$ compared to DM group.

3.6. Apocynin attenuated inflammatory response and oxidative stress in diabetic retinopathy

Since oxidative stress makes a great contribution to diabetic retinopathy development, we check the levels of MDA, SOD, and GSH in retina by ELISA. The MDA level was elevated and SOD and GSH levels were depressed in retina by diabetes (Fig. 6a-c). After the treatment of apocynin, the elevated MDA level was declined and the decreased SOD and GSH levels were up-regulated (Fig. 6a-c). These data demonstrated the function of apocynin in reducing the oxidative stress of diabetic retinopathy. Inflammatory response also participates in the diabetic retinopathy development. So we check the mRNA levels of TNF- α , IL-1 β , and IL-6 in retina by qRT-PCR. Fig. 6d-f illustrated that the levels of these three inflammatory cytokines were up-regulated in diabetic retinopathy. A significant decrease in TNF- α , IL-1 β , and IL-6 mRNA levels was observed after the treatment of apocynin when compared with DM group (Fig. 6d-f). The declined levels of inflammatory cytokines shown the function of apocynin in reducing the inflammatory response of diabetic retinopathy.

3.7. Apocynin inhibited TLR4/NF- κ B signaling pathway in retinas of diabetic

Since TLR4-NF- κ B signal pathway activation promotes inflammation response, we checked whether apocynin influences TLR4-NF- κ B signal pathway activity in diabetic retinopathy. First, we tested TLR4 and NF- κ B mRNA levels in retina through qRT-PCR. Fig. 7a, b indicated that the up-regulated TLR4 and NF- κ B mRNA levels in diabetic retinopathy were decreased after apocynin treatment. We also performed western blot to checked TLR4, NF- κ B, and phosphorylated NF- κ B protein levels in retina. Western blot result also shown that that the up-regulated protein levels in diabetic retinopathy were decreased after apocynin treatment. (Fig. 7c, d). It was proved that apocynin plays a

role in the inhibition of TLR4/NF- κ B signaling pathway activity in diabetic retinopathy.

4. Discussion

In our research, we found several new evidences of the inhibition function of apocynin in the pathology of diabetic retinopathy. The result of this work provide us a potential therapeutic strategy for diabetic retinopathy.

Apocynin belongs to methoxy-substituted catechol which is extracted from the root of a medicinal herb called *Picrorhiza kurroa* [11]. The main function of apocynin is being a NADPH oxidase inhibitor [12]. It is reported that the elevated oxidative stress in diabetic mice or rabbit is reduced by apocynin through the inhibition of NADPH oxidase activity [13,14]. In a diabetic cardiomyopathy rat model, the treatment of apocynin was proved to attenuate the myocardial and ventricular abnormal by the inhibition of ROS production and cell apoptosis [8]. Another research reported that the cell death of cone cell in retinitis pigmentosa is rescued by NADPH oxidase inhibitor apocynin through reduction of oxidative stress in retina [9]. As an ordinary diabetes complication, diabetic retinopathy caused microglia and neurons degeneration in retina [15]. The pathology of diabetic retinopathy was connected with oxidative damage, activation of inflammation response, and neuronal dysfunction [16]. The function of apocynin in diabetic cardiomyopathy and retinitis pigmentosa indicated the existence of potential function of apocynin in diabetic retinopathy. So we explored whether the treatment of apocynin can influence the pathology of diabetic retinopathy in diabetes induced rat model.

In our research, the diabetes in rat model was induced by intraperitoneal injection of STZ. To illustrate the diabetes symptom induced by STZ in rats, the body weight, food intake, and several biochemical parameters including blood glucose, glycosylated hemoglobin, cholesterol, triglyceride, and high-density lipoprotein

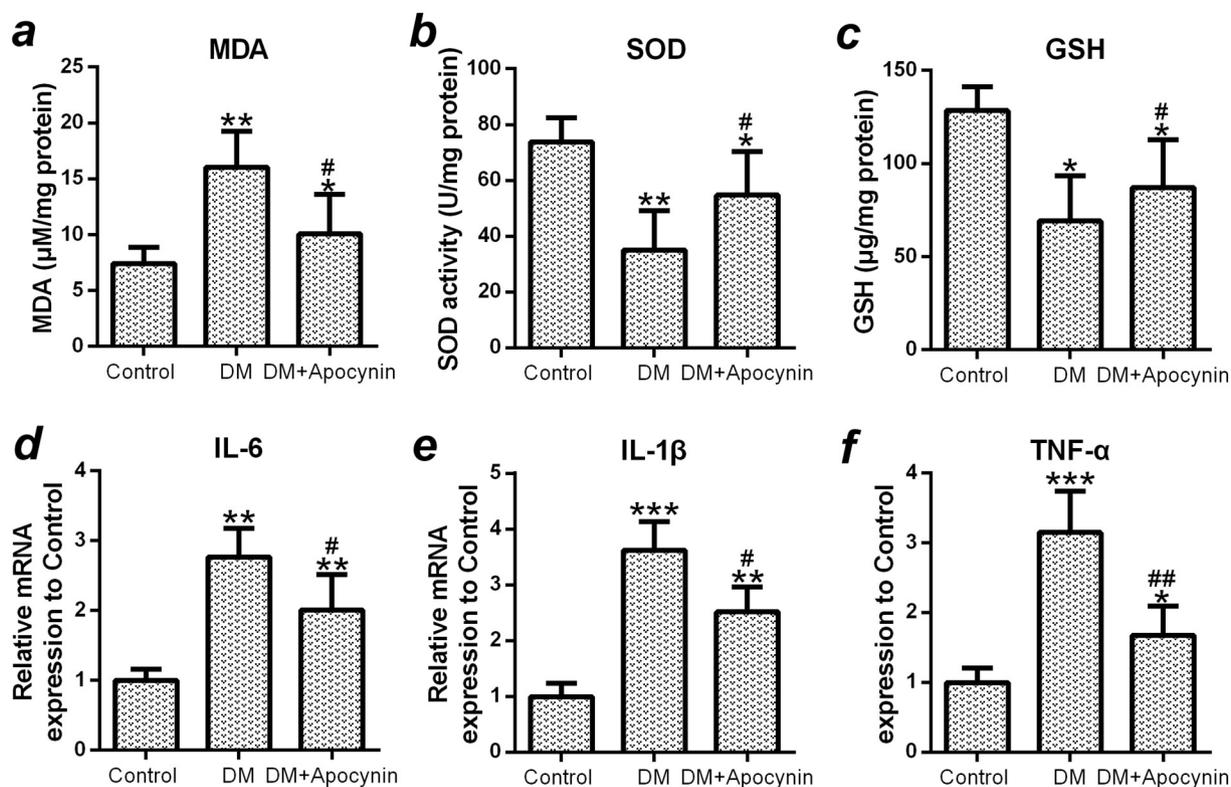


Fig. 6. Apocynin attenuated oxidative stress and inflammatory in retinas of diabetic rats after 12 weeks treatment.

Malondialdehyde (MDA) (a), Superoxide dismutase (SOD) (b) and glutathione (GSH) (c) in retinas among different groups were tested by ELISA. d-f. qRT-PCR analysis of IL-6, IL-1 β and TNF- α mRNA expressions in retinas of rats among different groups. Relative expression pattern was analyzed by comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to control group. Data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control group, # $p < 0.05$ and ## $p < 0.01$ compared to DM group.

were estimated every seven days. When compared with control group, abnormal metabolism in DM group was shown by the changes in these biochemical parameters. When the diabetic rats are treated with apocynin, the declined body weight in diabetic rats was elevated and the increased blood glucose and glycosylated hemoglobin diabetic rats were down-regulated. Meanwhile, the variation in other biochemical parameters such as cholesterol, TG, HDL, LDL, and atherogenic index also indicated an alleviated diabetes symptom in DM + Apocynin group. The treatment of apocynin in diabetic rats was proved to alleviate diabetes symptom in our research.

The injuries of microvascular in retina, such as dysfunction of blood barrier, dropout of capillary, and microaneurysms, were confirmed to be the main features of diabetic retinopathy [17]. In retinal vascular cells, advanced glycation end products generated from diabetes promoted the pathogenic events by the increased expression of GFAP and VEGF [18]. Another research also reported that the accumulation of AGE in diabetic retina induced the apoptotic cell death through the activation of VEGF expression [19]. In our research, the elevated expression levels of VEGF and GFAP in retinas of diabetic rats were detected. Based on the result from DM + Apocynin group, the treatment of apocynin depressed the enhanced VEGF and GFAP expression in diabetic retinopathy. It has been reported that the overall thickness of retina in diabetic retinopathy is declined. The systematic morphology examination of diabetic retina [20]. In our research, the reduced overall thickness of retina in diabetic retinopathy was illustrated through systematic morphology examination. But after the apocynin treatment, the reduced thickness of retina was remarkably rescued when compared with the DM group. These results illustrated that apocynin participates in the alleviation of diabetic retinopathy symptoms in rats.

The enhanced neural retinal cell apoptosis is a crucial component of diabetic retinopathy and has been reported in both human and rats

[21]. The apoptosis-promoting factor Bax plays an important function in retinal neuronal cell apoptosis [22]. The Bcl-2 protein enhances the cell survival through its cell apoptosis blockage function [23]. Since Bax to Bcl-2 ratio is crucial for cell apoptosis activation, the increased Bax to Bcl-2 ratio is proved to be critical in retinal neural cell apoptosis [24]. Our work proved that apocynin treatment is efficient in the reduction of elevated Bax to Bcl-2 ratio in diabetic retinopathy. The apoptosis of retinal neural cells in diabetic retinopathy can be rescued by apocynin. During the pathology of diabetic retinopathy, both the enhanced oxidative stress and the activated inflammatory response are thought to be involved in the process. The inhibited SOD activity and reduced levels of GSH in retina reflected the enhanced oxidative stress generated by diabetic retinopathy. The influence of apocynin in the reduction of oxidative stress in retinas was proved by the enhanced SOD activity and elevated GSH level when compared to the DM group. The activated inflammatory response was shown by the up-regulated expression of inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 in retina. The changes in the level of these inflammatory cytokines demonstrated that the inflammatory response activity is significantly reduced by the apocynin treatment in retinas of diabetic rats.

During the systemic inflammation process, the expression of inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 is induced by the activation of TLR4/NF- κ B signaling pathway [25]. It has been reported that the reduction of inflammatory response through the inhibition of the TLR4/NF- κ B pathway is benefit for the therapy of diabetic retinopathy [26]. In a rat traumatic brain injury model, the function of apocynin neuronal autophagy modulation was demonstrated to be connected with the inhibition of the TLR4/NF- κ B pathway [7]. All these research indicated that the apocynin function in diabetic retinopathy may be relayed to the inhibition of the TLR4/NF- κ B pathway. Based on our results, the up-regulated expression levels of TLR4 and NF- κ B were

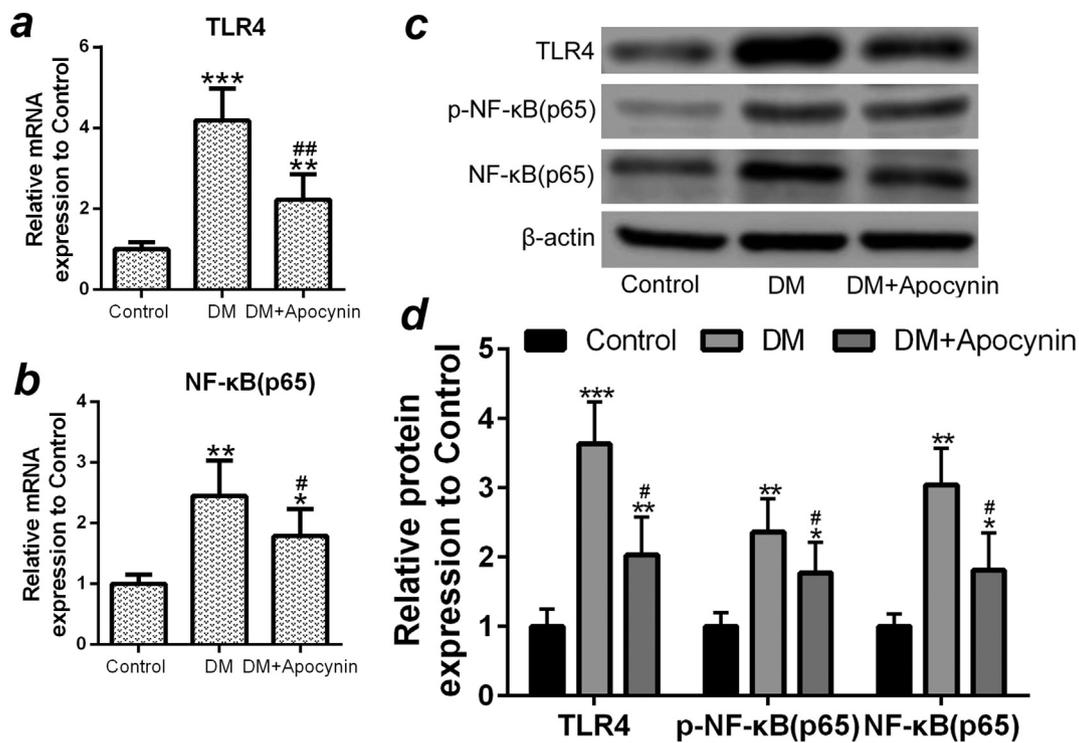


Fig. 7. Apocynin inhibited TLR4/NF-κB signaling pathway in retinas of diabetic rats after 12 weeks treatment.

a, b. qRT-PCR analysis of TLR4 and NF-κB(p65) mRNA expressions in retinas of rats among different groups. Relative expression pattern was analyzed by comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to control group. c. Representative immunoblots of TLR4, p-NF-κB(p65) and NF-κB(p65) in retinas of rats among different groups. β-actin used as loading control. d. Quantification of immunoblots of TLR4, p-NF-κB(p65) and NF-κB(p65). Expressions were normalized to control group. Data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control group, # $p < 0.05$ and ## $p < 0.01$ compared to DM group.

both down-regulated by apocynin treatment in retinas of diabetic rats. It is possible that the inhibition of TLR4/NF-κB pathway by apocynin plays an important role in the amelioration of diabetic retinopathy in rats.

As a serious-threatening complication of diabetes, diabetic retinopathy is one of the leading causes of blind and demanded to be treated urgently. Several different therapeutic strategies were performed in the treatment of diabetic retinopathy, such as steroids treatment, vitrectomy, laser, and anti-VEGF drug treatment [27,28]. As a consequence of the existence of side effects and limitations in these therapeutic strategies, new treatment method based on the further understanding of molecular mechanisms in the pathology of diabetic retinopathy is needed. In our study, we demonstrated that apocynin inhibits the diabetic retinopathy development in diabetes induced rat model through the inhibition of TLR4/NF-κB signaling pathway activity. This result indicated us that the treatment of apocynin may be a potential therapeutic strategy in diabetic retinopathy therapy.

Of note, there are several limitations in the current study. First, there is no positive control to make the comparisons of therapeutic efficiency between apocynin and some known drug impossible. Second, although the observed dose was chosen based on the published paper and pilot study, it would be more interesting to see the dose-related effects.

5. Conclusion

In conclusion, the treatment of apocynin ameliorates diabetic retinopathy in rat model and the inhibition of TLR4/NF-κB signaling pathway activity by apocynin is involved in this process.

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

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