Kidney protection effects of dihydroquercetin on diabetic nephropathy through suppressing ROS and NLRP3 inflammasome

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A B S T R A C T

Background: Diabetic nephropathy (DN), the leading cause of end-stage renal disease, is acknowledged as an independent risk factor for cardiovascular disease, which underlines the urgent need for new medications to DN. Dihydroquercetin (DHQ), an important natural dihydroflavone, exerts significant antioxidant, anti-inflammatory, and antithrombotic properties, but its effects on DN have not been investigated yet.

Purpose: We aimed to explore the kidney protection effects of DHQ on DN rats induced by high-fat diet/streptozotocin in vivo and the underlying mechanisms of DHQ on renal cells including HBZY-1 and HK2 exposed to high glucose in vitro.

Methods: Major biochemical indexes were measured including urine microalbumin, fasting serum glucose, serum levels of creatinine, total cholesterol and low density lipoprotein cholesterol. Renal histologic sections were stained with hematoxylin-eosin, periodic acid-Schiff and Masson. The cell proliferation was assessed by MTT assay. Reactive oxygen species (ROS) generation was detected by DCFH-DA assay and laser scanning confocal microscope. Expression of all proteins was examined by western-blot.

Results: In high-fat diet/streptozotocin-induced DN rats, DHQ at the dose of 100 mg/kg/day significantly attenuated the increasing urine microalbumin excretion, hyperglycemia and lipid metabolism disorders, and mitigated renal histopathological lesions. In in vitro studies, DHQ significantly suppressed cell proliferation and the excessive ROS generation, and alleviated the activation of nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome and the expression of renal fibrosis-associated proteins in renal cells exposed to high glucose.

Conclusion: The results revealed that DHQ possesses kidney protection effects including attenuating urine microalbumin excretion, hyperglycemia and lipid metabolism disorders, and mitigating renal histopathological lesions on DN, and one of the possible renal-protective mechanisms is suppressing ROS and NLRP3 inflammasome.

Introduction

As one of the most important complications of diabetes mellitus, diabetic nephropathy (DN) is the major cause of end-stage renal disease (ESRD) with gradually increased urine microalbumin excretion and glomerulosclerosis, and is acknowledged as an independent risk factor for cardiovascular disease (Si et al., 2014). Despite the exact mechanisms have not been well elucidated, it’s known that a variety of factors are involved in the onset and progression of DN including genetic factors, representative glomerular hypertension, hyperfiltration and hyperperfusion of abnormal renal hemodynamics in early stage, and hyperglycemia-induced metabolic disorders (Zheng et al., 2016). Lots of data show that the oxidative stress and chronic low-grade inflammation play fundamental key roles in DN (Elmarakby and Sullivan, 2012). Although with intensive control of glucose, lipids and blood pressure, DN is lack of effective treatments except for kidney replacement...
therapy when progresses to ESRD with the average incidence rate ranging from 30% to 40% globally in diabetic patients (Lopes, 2009; Vega-Díaz et al., 2015). Therefore, exploring new drugs or treatments is urgent for DN.

Dihydroquercetin (DHQ), also known as taxifolin, is an important dihydroflavone compound commonly found in Larix sibirica Ledeb. (Pinaceae) and Pseudotsuga taxifolia Lamb. & Britton (Pinaceae) (Yang et al., 2016). It exerts lots of biological effects, including anti-oxidant, anti-inflammatory, anti-tumor, antiviral and prevention of Alzheimer's disease, among others (Weidmann, 2012). It attenuated cerebral ischemia-reperfusion injury by inhibiting the production of reactive oxygen species (ROS) (Maksimovich et al., 2014). Studies also proved that DHQ improved capillary microrculcation and antiplatelet aggregation, and reduced the production of lipid-free radicals in a dose-dependent manner (Vladimirov et al., 2009). A recent study of mouse model established by transverse aortic constriction confirmed that DHQ attenuated left ventricular fibrosis and collagen synthesis through abrogating the phosphorylation of Smad2 and Smad2/3 nuclear translocation, and inhibiting excess production of ROS, ERK1/2, JNK1/2 after pressure overload (Guo et al., 2015). DHQ also reduced the cholesterol esterification of hepatocytes, triacylglycerol and phospholipid synthesis, decreased apolipoprotein B secretion and inhibited the microsomal triglyceride synthesis (Casaschi et al., 2004). Recent studies revealed that DHQ showed cardio-protective effects against diabetic cardiomyopathy by inhibiting NADPH oxidase, activating JAK2/STAT3 cascade activation and decreasing angiotensin II production (Sun et al., 2014). However, the effect of DHQ against DN hasn't been explored before and whether DHQ is effective to treat DN is unclear.

In this work, we attempted to explore the kidney protection effects of DHQ on DN rats induced by high-fat diet (HFD)/streptozotocin (STZ) in vivo and on renal cells including HBZY-1 and HK2 exposed to high glucose in vitro. We particularly investigated the protective effects of DHQ on the urine microalbumin excretion, glucose and lipid metabolisms and renal lesions, and explored the underlying mechanisms of these renal protective effects.

Materials and methods

Materials and reagents

High-fat diet food (40 kJ/g, 20% fat) was purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Streptozotocin (STZ) was acquired from Sigma (St Louis, MO, USA). Cozaar (Losartan Potassium Tablets) and DHQ (purity >99%) were obtained from Merck Sharp & Dohme (Hangzhou, China) and the Yuanye company (Shanghai, China) respectively. Labrasol was purchased from Gattefosse (Gennevilliers, France). Urine microalbumin enzyme-linked immunosorbent assay (ELISA) kits were obtained from Jiancheng Bioengineering Institute (Nanjing, China) for measurement of glucose, creatinine, total cholesterol and low density lipoprotein cholesterol. The primary antibodies used in the in vitro study for Western-blot were as follows: anti-Fibronectin and anti-Collagen IV (Abcam, Cambridge, MA, USA); anti-NLRP3 (Epitomics, Burlingame, CA, USA); anti-Cleaved Caspase-1, anti-IL-1β and anti-β-actin (Cell Signaling Technology, Danvers, MA, USA). The secondary antibodies including peroxidase-conjugated goat anti-rabbit and anti-mouse immunoglobulin G (IgG) were obtained from Jackson ImmunoResearch Laboratory (West Grove, PA, USA).

Animals and model establishment

Male SD rats (4–5 weeks old, 110–150 g) were obtained from the Sipper-BK Laboratory Animal Co. Ltd. (Shanghai, China), and three rats were housed per cage in a specific-pathogen-free-conditioned room (at 25 ± 1 °C) with 55–60% relative humidity under a 12-h light/dark cycle and provided with food and water ad libitum. All animals-involved procedures were conducted in strict accordance with the guidelines and protocols approved by the Animal Ethics Committee of School of Pharmacy at Fudan University. To minimize suffering of rats, surgery was performed under anesthesia with intraperitoneal injection of 10% chloral hydrate. Rats were randomly divided into 2 groups after a week adaption period: (1) control group: 10 rats; (2) High-fat diet group: 63 rats. Rats were fed with high fat diet (40 kJ/kg, 20% fat) in the HFD group and were fed with normal pellet diet (20 kJ/kg, 5% fat) in the control group for 4 weeks, then rats in the HFD group were given a single intraperitoneal dose of 30 mg/kg streptozotocin (STZ) dissolved in ice-cold sodium citrate buffer (0.1 M, pH 4.4) and rats in control group were injected with same volume of sodium citrate buffer. Three days after STZ injection, rats with tail fasting blood glucose level beyond 16.7 mmol/l measured using ACCU-CHEK (Roche, Switzerland) were considered as diabetic and randomly divided into 5 groups with treatment for 12 weeks: (1) DN group; (2) DN + DHQ 25 mg/kg/day group; (3) DN + DHQ 50 mg/kg/day group; (4) DN + DHQ 100 mg/kg/day group; (5) DN + Losartan 20 mg/kg/day group, each group consisted of 10 rats. DHQ was dissolved in Labrasol at 35 °C in ultrasound bath for 30 min in vivo study. Body weight and fasting serum glucose were determined every two weeks. At the end of the experimental protocol, urine of 24 h was collected to determine the urine microalbumin, then all rats were weighted and sacrificed under anesthesia, and samples were soon collected including blood and kidney. The renal index (mg/g) was calculated as: renal index = kidney weight (mg)/body weight (g).

Serum and urine biochemical parameters analysis

The levels of urine microalbumin were measured by ELISA according to the manufacturer's instructions. The relevant assay kits were obtained for determining levels of fasting serum glucose by glucose-oxidase/peroxidase method, determining serum levels of creatinine through sarcosine oxidase method, determining serum levels of total cholesterol by GPO-PAP enzymatic method and measuring the serum level of low density lipoprotein cholesterol through two direct methods (Nankar and Doble, 2017).

Tissue collection and renal histopathology

After rats sacrificed, kidneys harvested were sectioned longitudinally and fixed in 4% paraformaldehyde solution for 24 h, then were paraffin-embedded and sectioned at 4 μm. At least 10 randomly selected renal histologic sections in each group were stained with hematoxylin-eosin (H&E) for general morphological analysis, periodic acid-Schiff (PAS) for glomerulosclerosis evaluation, and Masson for collagen deposition and interstitial lesions assessment, respectively. 20 glomeruli randomly selected from each group were quantified in PAS-stained sections using ImageJ software 1.42q (National Institute of Health, Bethesda, MD, USA) to assess the percentage of PAS-positive area in glomerulus which was denoted as mesangial index.

Cell culture

Rat kidney mesangial cells (HBZY-1) and human proximal renal tubular epithelial cells (HK2) were purchased from the Chinese Type Culture Collection (CTCC, Shanghai, China), and were maintained in RPMI-1640 medium (Thermo Scientific HyClone, Beijing, China) supplemented with 100 U/ml of a penicillin/streptomycin mixture (Beyotime, Shanghai, China) and 10% fetal bovine serum (Capricorn Scientific GmbH, Ebsdorfergrund, Germany). The cells were routinely grown in 25-cm² cell culture plates (Corning Inc., Corning, NY, USA) at 37 °C in a humidified atmosphere with 5% carbon dioxide. DHQ was freshly dissolved in dimethyl sulphoxide (DMSO) as a stock solution and diluted with RPMI 1640 medium when used (The concentration of
DMSO was equal in all groups of 0.1%.

Cell proliferation assay

Cell proliferation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells in monolayer culture growing to subconfluence were washed twice with phosphate-buffered saline (PBS), and were then re-suspended in culture medium, counted, and seeded in 100 µl media at 4000 cells/well in 96-well microliter plates with 6 parallel wells in each group, and then exposed to normal glucose (5 mmol/l) or high glucose (30 mmol/l) with DHQ added to each well at a final concentration of 10 µM, 20 µM, 40 µM and 80 µM, respectively. The plates were further incubated at 37 °C for 48 h and 72 h in a CO₂ incubator, respectively. The cells were incubated with MTT solution (0.5 mg/ml) for 4 h at 37 °C. Then, 100 µl DMSO was added to each well following the MTT solution discarded. Evenly shaking the 96-well plates resulted in complete dissolution of formazan, and the optical density (OD) was measured at an absorbance wavelength of 570 nm.

Detection of intracellular ROS generation and mitochondrial ROS production

Cells were seeded in 100 µl media at 4000 cells/well in 96-well black plates with 6 parallel wells in each group, and then exposed to normal glucose (5 mmol/l) or high glucose (30 mmol/l) with DHQ added to each well at a final concentration of 5 µM, 10 µM, 20 µM, respectively. The plates were further incubated at 37 °C for 24 h. Cells then were loaded with DCFH-DA, a fluorescent probe for ROS (Beyotime, China) at 10 µM in all wells. After further culture for 20 min
Fig. 2. Effect of DHQ on the proliferation of HBZY-1 and HK2 cells induced by high glucose. (A) The proliferation of rat kidney mesangial cells (HBZY-1) induced by high glucose (30 mM) at 48 h and 72 h. (B) The proliferation of human proximal renal tubular epithelial cells (HK2) induced by high glucose (30 mM) at 48 h and 72 h. N = 6 per group (*P < 0.05, **P < 0.01 versus the NG group).

in dark, the cells were detected by fluorescence spectrophotometer. To investigate the mitochondrial ROS generation, cells were plated on glasscover-slips in 35 mm diameter dishes at a density of 1 × 10^6 cells/ml. After adhesion, cells then exposed to normal glucose (5 mmol/l) or high glucose (30 mmol/l) and were treated simultaneously with or without 20 µM DHQ for 24 h. Meanwhile, the mannitol group without DHQ was set to exclude osmotic impact (5 mmol/l glucose + 25 mmol/l mannitol). Then, cells were stained with MitoSOX dye (red) and Hoechst 33342 (blue) at 37 °C for 30 min following the manufacturer's instruction. All the procedures were done in the dark and the samples were observed by a laser scanning confocal microscope.

Western blotting

Cells were seeded in 2 ml media at 8 × 10^4 cells well in 6-well plates with 3 parallel wells in each group. After adhesion, cells then exposed to normal glucose (5 mmol/l) or high glucose (30 mmol/l) with DHQ added to each well at a final concentration of 5 µM, 10 µM, 20 µM respectively for 72 h. Meanwhile, the mannitol group without DHQ was set to exclude osmotic impact (5 mmol/l glucose + 25 mmol/l mannitol). Then cells were harvested using Radio Immunoprecipitation Assay (RIPA) Lysis Buffer (Beyotime, China). Cell lysates (20 µg) were electrophoresed on polyacrylamide gels and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with TBS containing 3% bovine serum albumin and 0.1% Tween-20 (Sigma-Aldrich), and afterward incubated with the primary antibody and secondary antibodies and then analyzed using an enhanced chemiluminescent detection kit (Pierce, Rockford, IL, USA). Semi-quantitative Western-blot analysis was conducted through measuring the Optical Densitometry in three independent experiments using ImageJ software.

Statistical analysis

Statistics analysis was carried out with GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). The results from at least three independent experiments were expressed as mean ± standard deviations (S.D). Comparisons between different groups were performed using Student's t test (two-tailed) or one-way ANOVA. A p value < 0.05 was considered statistically significant.

Results

Effect of DHQ treatment on the urine microalbumin excretion, serum level of creatinine, hyperglycemia and lipid metabolism disorders, and renal histopathological lesions in HFD/STZ -induced DN rats.

The urine microalbumin excretion of 24 h reflecting the renal injuries and serum levels of creatinine reflecting the renal functions were measured at the end of DHQ administration for 12 weeks in HFD/STZ-induced DN rats. Urine microalbumin and serum creatinine distinctly increased in DN group compared with the control group, and decreased in the groups administrated with DHQ in a dose-dependent manner. Especially, the levels of urine microalbumin in the group with DHQ 100 mg/kg/day was significantly lower than those in the DN group (P < 0.05) (Fig. 1A). The serum levels of creatinine were significantly lower in the groups with DHQ 50 mg/kg/day and DHQ 100 mg/kg/day than those in the DN group (P < 0.05, P < 0.01 respectively) (Fig. 1B). Fasting serum glucose was determined to assess the effect of DHQ on abnormal glucose metabolism. The increased levels of fasting serum glucose in the DN group compared with the control group (P < 0.05) were significantly decreased in the DN rats treated with DHQ 25 mg/kg/day and DHQ 50 mg/kg/day (P < 0.05, P < 0.05 respectively), but the glucose levels were not altered in the groups with DHQ 100 mg/kg/day compared with the DN group (Fig. 1C). The elevated levels of low density lipoprotein cholesterol and total cholesterol in the DN group compared with the control group decreased with administration with DHQ. Especially, levels of low density lipoprotein cholesterol of the group with DHQ 50 mg/kg/day and DHQ 100 mg/kg/day were significantly lower than those of the DN group (P < 0.05, P < 0.05) (Fig. 1D), and the levels of total cholesterol of the group with DHQ 100 mg/kg/day were significantly lower than the DN group (P < 0.05), and there was no difference between the groups with DHQ less than 100 mg/kg/day and the DN group (Fig. 1E). The renal index increased significantly in the DN group compared with the control group and overtly decreased in the DN + DHQ 100 mg/kg/day group compared with the DN group (P < 0.01) (Fig. 1F).

Renal histologic sections were stained with H&E, PAS and Masson respectively. Without DHQ, rats in the DN group experienced renal pathological alterations compared with the control group including increased mesangial matrix indicated by hematoxylin and eosin (H&E) staining and obvious segmental glomerulosclerosis indicated by PAS staining. Since the limitations of DN animal models, no interstitial lesions were observed in all DN rats from Masson staining. Overtly, DHQ treatment alleviated the increased renal lesions especially in the groups with DHQ 100 mg/kg/day (Fig. 1G and H). DHQ attenuated renal fibrosis through inhibition of extracellular matrix(ECM) accumulation and mesangial matrix expansion in DN. Furthermore, immunohistochemical staining was conducted to evaluate the expression of IL-1β in each group and found that IL-1β was expressed in normal glomerular tissues and was significantly up-regulated in diabetic nephropathy rats. With the DHQ treatment at the dose of 50 mg/kg/day and 100 mg/kg/day, IL-1β was significantly down-regulated compared to DN group (Fig. S1A and S1B).

Collectively, DHQ exerted kidney protection effects on HFD/STZ -induced DN rats by attenuating the increasing urine microalbumin...
excretion, hyperglycemia and levels of low density lipoprotein cholesterol and total cholesterol, and mitigating renal histopathological lesions.

Effect of DHQ on the proliferation of HBZY-1 and HK2 cells induced by high glucose

MTT assay was employed to investigate the impact of DHQ on the proliferation of HBZY-1 and HK2 cells. As demonstrated in Fig. 2A, cell proliferation of HBZY-1 increased after 48 h ($P < 0.05$) and 72 h ($P < 0.05$) compared with the normal glucose group when cells exposed to high glucose, and gradually decreased after supplement with DHQ at different concentrations. Specially, cell proliferation of groups with DHQ at the concentration more than 20 µM was significantly lower than those of the HG group after 48 h ($P < 0.05$) and 72 h ($P < 0.01$). As shown in Fig. 2B, cell proliferation of HK2 increased after 48 h ($P < 0.01$) and 72 h ($P < 0.05$) exposure to high glucose compared with the normal glucose group, and decreased significantly in the groups with DHQ at a concentration higher than 20 µM after 48 h ($P < 0.01$) and 72 h ($P < 0.01$) compared with the HG group without DHQ. These results suggested that DHQ significantly suppressed the proliferation of HBZY-1 and HK2 cells induced by high glucose.
Effect of DHQ on the intracellular ROS generation and mitochondrial ROS production induced by high glucose in HBZY-1 and HK2 cells

Intracellular and mitochondrial ROS of HBZY-1 and HK2 cells were measured by using DCFH-DA assay and fluorescent probe MitoSOX, respectively. A significant rise in ROS levels was observed for these cells when exposed to the high glucose compared with the normal glucose group, and ROS levels decreased after supplement with DHQ in a concentration-dependent manner. Specially, DHQ at the concentration of 10 µM and 20 µM significantly inhibited the ROS generation in HBZY-1 (**P < 0.01, ***P < 0.01 versus the NG group) (Fig. 3A) and HK2 cells (**P < 0.05, ***P < 0.01) (Fig. 3B). As shown in Fig. 3C and D, the increased mitochondrial ROS generation observed in the HG group, represented by the red fluorescence, was inhibited by DHQ at the concentration of 10 µM and 20 µM (**P < 0.01, ***P < 0.01 versus the HG group).
20 µM. Moreover, there was no significant difference between the normal glucose group and the mannitol group in the mitochondrial ROS generation. The two ROS detection experiments thus confirmed the antioxidant ability of DHQ in a concentration-dependent manner on the renal cells induced by high glucose. Taken together, DHQ suppressed the intracellular ROS generation and mitochondrial ROS production induced by high glucose in HBZY-1 and HK2 cells.

**Effect of DHQ on the activation of NLRP3 inflammasome induced by high glucose in HBZY-1 and HK2 cells**

As shown in Fig. 4A, Western-blot of HBZY-1 cell lysates demonstrated that the elevated protein activations of NLRP3, Cleaved Caspase-1 and IL-1β in cells exposed to high glucose were decreased after supplementation with DHQ in HG groups. Especially, semi-quantitative analysis proved that levels of these proteins in the group with DHQ 20 µM were significantly lower than those in the HG group (NLRP3: *P* < 0.05; Cleaved Caspase-1: *P* < 0.01; IL-1β: *P* < 0.01) (Fig. 4B–D). Furthermore, the changes of NLRP3-related proteins appeared in the HK2 cells were similar to the HBZY-1 cells (Fig. 4E), and the semi-quantitative analysis showed that the NLRP3-related proteins were normalized in the group of DHQ 20 µM compared to the NG group and were overly lower than in the HG group without DHQ (NLRP3: *P* < 0.01; Cleaved Caspase-1: *P* < 0.05; IL-1β: *P* < 0.01) (Fig. 4F–H).

These results demonstrated that DHQ attenuated the activation of NLRP3 inflammasome as well as Cleaved Caspase-1 and IL-1β expression induced by high glucose in HBZY-1 and HK2 cells.

**Effect of DHQ on the expressions of renal fibrosis-associated proteins induced by high glucose in HBZY-1 and HK2 cells**

As demonstrated in Fig. 5A, the expressions of Fibronectin and Collagen IV in HBZY-1 cells, also known as ECM proteins, increased after exposure to high glucose and gradually decreased after supplement of DHQ. Semi-quantitative analysis of three independent experiments verified that the expressions of Fibronectin and Collagen IV increased significantly in the HG group compared with those in the NG group (Fibronectin: *P* < 0.01; Collagen IV: *P* < 0.05) and were significantly lower in groups with DHQ at the concentrations of 10 µM and 20 µM than those in the HG group (Fibronectin: *P* < 0.01; Collagen IV: *P* < 0.01) (Fig. 5B and C). As shown in Fig. 5D, the change in the expressions of Fibronectin and Collagen IV in HK2 cells was similar to the change in HBZY-1 cells. Semi-quantitative analysis demonstrated that the expressions of Fibronectin and Collagen IV in HK2 cells increased significantly in the HG group compared with those in the NG group (Fibronectin: *P* < 0.01; Collagen IV: *P* < 0.01) and decreased significantly in groups with DHQ at the concentration of 20 µM specially (Fibronectin: *P* < 0.01; Collagen IV: *P* < 0.01) (Fig. 5E and F).
Discussion

Diabetes results in inflammation, increased adiposity, and chronic hyperglycaemia, and progresses to DN characterized by clinical microalbuminuria and impaired glomerular filtration function. Microalbuminuria resultant from glomerular hyperfiltration in the early stage of DN is an early risk indicator of DN and a predictor of progression as well as a risk factor to cardiovascular disease, and remains the gold standard marker for early diagnosis of DN even though with some limitations (Uwaezuoke, 2017). The characteristic pathological lesion of DN is the accumulated ECM components in glomeruli, mostly resulting in ESRD with development of glomerulosclerosis and loss of renal function although with intensive control of glucose, lipids and blood pressure (Betz and Conway, 2016). We firstly investigated the kidney protection effects and potential mechanisms of DHQ on DN both in vivo and in vitro. Our results revealed that DHQ significantly attenuated the increasing urine microalbumin excretion, serum level of creatinine, hyperglycemia and lipid metabolism disorders, and mitigated renal histopathological lesions in DN rats through attenuating renal fibrosis via inhibition of ECM accumulation and mesangial matrix expansion in DN, which suggested that DHQ might be a new medicine to treat DN.

It has been generally known that oxidative stress and inflammatory response are interrelated in DN (Sharaf El Din et al., 2016). High glucose not only promotes generation of ROS and activation of NLRP3 inflammasome, but also stimulates the increased accumulation of ECM proteins in DN which can cause renal glomerulosclerosis. The activation of Cleaved Caspase-1 induced by NLRP3 inflammasome promotes IL-1β secretion followed by renal inflammatory cytokinesis. DHQ could attenuate the progression of DN through suppressing ROS and NLRP3 inflammasome as well as cell proliferation and expressions of ECM proteins.

Taken together, these results suggested that DHQ alleviated the expressions of renal fibrosis-associated proteins including Fibronectin and Collagen IV induced by high glucose in HBZY-1 and HK2 cells.

![Diagram](Image)

**Fig. 6.** Overview of potential mechanisms underlying kidney protection effects of DHQ on DN. High glucose state not only promotes generation of ROS and activation of NLRP3 inflammasome, but also stimulates the increased accumulation of ECM proteins in DN which can cause renal glomerulosclerosis. The activation of Cleaved Caspase-1 induced by NLRP3 inflammasome promotes IL-1β secretion followed by renal inflammatory cytokinesis. DHQ could attenuate the progression of DN through suppressing ROS and NLRP3 inflammasome as well as cell proliferation and expressions of ECM proteins.
intracellular and mitochondrial ROS generation induced by high glucose, and significantly decreased the elevated activation of NLRP3 inflammasome as well as the cleaved Caspase-1 and IL-1β expressions induced by high glucose in HBZY-1 and HK2 cells. Furthermore, DHQ attenuated the expression of renal fibrosis-associated proteins Fibronectin and Collagen IV, which reflected the underlying mechanism of the decreased ECM accumulation and mesangial matrix expansion.

As one of the most important microangiopathy of diabetes, DN brings about increasing fractional excretion of calcium, phosphorus, and uric acid when progresses to chronic kidney disease accompanied by a progressive reduction in glomerular filtration (Musslo et al., 2012). Recent researches demonstrated that DHQ improved micro-vascularization and microcirculation in the cerebral cortex of SHR rats during the formation of arterial hypertension (Plotnikov et al., 2017), and suppressed uric acid production in plasma and liver via inhibition of hepatic xanthine oxidase activity (Adachi et al., 2017). This work was supported by grants from the National Natural Science Foundation of China (81773620 and 81573332), National Key Basic Research Program of China (2015CB931800), the Scientific Research Projects of Shanghai Municipal Commission of Health and Family Planning (201740140), and the Research and Innovation Projects of Shanghai Municipal Education Commission (13ZZD03).

Conflict of interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2018.01.026.

References


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