RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.072

ScienceAsia 50 (4): 2024: ID 2024072: **1–7**

Pyrroloquinoline quinone mitigates glomerular filtration barrier damages in diabetic mice

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Received 8 Oct 2023, Accepted 11 May 2024
Available online 20 Aug 2024

ABSTRACT: Diabetic nephropathy (DN) is a common complication of diabetes and can lead to kidney failure if left untreated. Pyrroloquinoline quinone (PQQ) is a natural compound that functions as both a redox cofactor and an antioxidant. Accumulating evidence denotes that oxidative stress plays a central role in the development of renal injury in diabetes, and the use of antioxidant agents is an effective approach to combat DN. In this study, we evaluated whether PQQ has potential protective effects against glomerular damage due to DN by establishing type 1 diabetes in mice via streptozotocin. We found that PQQ decreased renal malondialdehyde (MDA) levels and increased renal glutathione peroxidase (GPX) activities. It also reduced renal tumor necrosis factor alpha (TNF-α) levels. PQQ significantly decreased urinary excretions of albumin as well as specific podocyte damage markers, i.e. nephrin, synaptopodin, and podocin. Additionally, it improved creatinine clearance (Ccr) and, at the same time, improved renal histoarchitecture. Overall, these findings demonstrate the protective properties of PQQ against glomerular filtration barrier in diabetic mice.

KEYWORDS: diabetic nephropathy, glomerular filtration barrier, pyrroloquinoline quinone, podocyte

INTRODUCTION

Diabetic nephropathy (DN) is a progressive kidney complication of diabetes mellitus, affecting a significant percentage of individuals with type 1 and type 2 diabetes [1, 2]. The condition is characterized by damages to the glomeruli in the kidneys, leading to impaired kidney function over time. Ultimately, DN may progress to end-stage renal disease (ESRD), the final stage of chronic kidney disease (CKD) where the kidneys have lost almost all of their function. In ESRD, the kidneys are no longer able to function well enough to meet the needs of daily life [3]. This stage is characterized by a significant decline in kidney function, with a glomerular filtration rate (GFR) less than 15 milliliters per minute per 1.73 square meters [4].

Early detection and effective management of DN are of utmost importance to prevent or delay the progression to ESRD [5]. The glomerular filtration barrier, a specialized structure in the kidneys, plays a crucial role in filtering blood to form urine while retaining essential components in the bloodstream. DN is associated with disturbances in this filtration barrier, particularly in the podocytes, specialized cells within the barrier [6].

Hyperglycemia, a prolonged exposure to high blood glucose levels, and other metabolic changes in diabetes lead to detrimental effects on podocytes, compromising the structural integrity and functional selectivity of the glomerular filtration barrier [7]. As a result, there is an increased filtration of proteins, particularly albumin, into the urine; and albuminuria becomes a hallmark feature of DN [8].

Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, contributes to podocyte injury and apoptosis [9, 10]. Efforts to reduce oxidative stress and enhance antioxidant defense mechanisms have shown promise in preserving podocyte function and mitigating kidney damage in DN [11, 12].

Pyrroloquinoline quinone (PQQ) is a naturally occurring compound known for its antioxidant properties and potential health benefits [13]. It acts as a redox cofactor in cellular energy metabolism, interacting with mitochondria to generate adenosine triphosphate (ATP) and supporting overall mitochondrial functions [14]. Additionally, PQQ has been reported to have neuroprotective properties, promoting nerve cell growth and cognitive function [15].

Given the antioxidant properties of PQQ, we hypothesized that it could be effective in treating DN by protecting the glomerular filtration barrier. To test this hypothesis, we conducted a study on diabetic mice, evaluating tissue and urinary markers of kidney and podocyte damage.

MATERIALS AND METHODS

Materials

PQQ and streptozotocin (STZ) were obtained from the Shaanxi Iknow Biotechnology (Xi'an, Shaanxi, China)

and Santa Cruz Biotechnology (Dallas, Texas, USA), respectively.

Experimental design

Sixteen male C57BL/6 mice of 8–10 weeks' old, weighing 20–22 grams, were purchased. The experiment was performed with adherence to the principles of laboratory animal care (NIH publication no. 85-23, revised 1985), and the study protocol was approved by the ethical committee of the Xinzhou District People's Hospital, Integrated Traditional Chinese and Western Medicine, Wuhan, Hubei Province, China under the ethical code 20220412. The mice were allocated to three groups, eight per group. The first group was the healthy control group. The other two were received a single 50 mg/kg dose of STZ dissolved in citrate buffer to induce diabetes [16]. The confirmation of diabetes induction involved checking the glucose levels in the blood collected from the tails of the mice 48 h after the STZ injection. Animals with glucose levels exceeding 250 mg/dl were selected to be part of the study [16]. Diabetic kidney damage typically becomes apparent after approximately 8 weeks of diabetes induction by STZ [17]. Furthermore, in order to ascertain the optimal time point for observing kidney damage in our specific experimental conditions, we conducted a pilot study. This pilot study involved monitoring the mice for varying durations after STZ injection to determine when diabetic kidney damage was consistently observable. Our findings from the pre-study indicated that a duration of 12 weeks post-STZ injection was optimal for reliably observing the emergence of diabetic kidney damage in our experimental model. Pyrroloquinoline quinone (PQQ) was prepared in a solution containing 1% v/v dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS). The treatment group was administered a daily intraperitoneal dose of PQQ at 10 mg/kg. At the end of a 12-week treatment period, the mice were placed in metabolic cages to collect urine samples over a 24-hour period. Following that, tissue and blood samples were obtained from the mice after administering the appropriate anesthesia, consisting of a combination of 50 mg/kg ketamine and 1 mg/kg midazolam. It should be mentioned that in accordance with the latest guidelines on animal ethics, research articles involving animals should employ the minimal number of animals necessary [18]. To address this concern, we omitted the application of three treatment groups and opted against the conventional method of dose-dependent study. Instead, we adopted a more refined approach by utilizing a single PQQ treatment group. The decision to employ a singular treatment group was based on our meticulous evaluation of previously ascertained studies in the field [19]. This strategic selection ensured not only the scientific rigor of our study but also aligns with the ethical imperative of minimizing the number of animals used in our investigation.

Blood pressure assessment

The measurement of systolic blood pressure (SBP) was conducted using a non-invasive tail-cuff method (AD Instrument PowerLab Data Acquisition System, Dunedin, Australia) a day prior to placing the mice in the metabolic cages. The mice were placed in a heated restrainer set at a temperature of 37 ± 1 °C for a duration of 10 min during the blood pressure measurements. Three readings of blood pressure were taken for each mouse, and the average of these readings was considered as the SBP.

Measurement of biochemical markers

To determine creatinine clearance (Ccr), we measured the levels of creatinine in both the serum and the urine using commercially accessible kits (Cayman Chemical, Ann Arbor, Michigan, USA). The levels of malondialdehyde (MDA) as well as the activities of glutathione peroxidase (GPX) in the kidney tissue samples were assessed by means of commercial tests (Sigma Aldrich, St. Louis, Missouri, USA). Commercial enzyme-linked immunosorbent assay (ELISA) kits were utilized to measure urinary levels of albumin, nephrin, synaptopodin, and podocin (BioValley, Strasbourg, France).

Assessment of renal histopathology

The samples of kidney tissue were preserved in a solution of neutral buffered formalin. We utilized hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and Masson's trichrome stains to stain the paraffin sections and assessed the sections qualitatively using a light microscope.

Real time quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was extracted from kidney tissues using TRIzol reagent (Invitrogen Waltham, Massachusetts, USA), and cDNA was synthesized using a reverse transcription kit (InterLabServices, Moscow, Russia) as per the provided instructions. Real-time PCR was conducted on Mic thermocycler (BioMolecular Systems, Upper Coomera, Australia) using SYBR Premix Ex Taq II, with GAPDH serving as the internal control. The relative expression of each target gene was calculated by the $2^{-\Delta\Delta CT}$ method [20]. The primers employed were as stated (Table S1).

Statistical analysis

The data were presented as mean ± standard deviation (SD). Group differences were evaluated using one-way analysis of variance (ANOVA) with Tukey's post hoc test, conducted through SPSS statistical software. Statistical significance was determined at a p-value of less than 0.05.

RESULTS

PQQ had no effect on serum glucose and systolic blood pressure

As shown in Table 1 the serum glucose levels of sham mice were 78 ± 6 mg/dl. This value was increased to 351 ± 32 mg/dl for DN mice ($p < 0.05$). The serum glucose levels in PQQ + DN mice was 365 ± 37 mg/dl that had no statistically significant difference with DN mice. Moreover, systolic blood pressure values for the studied groups were 119 ± 7, 122 ± 10, and 117 ± 12 mmHg for sham, DN, and DN + PQQ mice, respectively. No statistically significant difference was noted for the values of systolic blood pressure among the studied groups.

PQQ alleviated renal inflammation and oxidative stress

To evaluate inflammation, we measured the levels of TNF-α in kidney tissue homogenates. TNF-α levels in kidneys of sham mice were 0.20 ± 0.042 pg/mg protein. Its levels raised significantly to 0.62 ± 0.048 pg/mg protein in DN mice ($p < 0.05$). PQQ treatment mice significantly decreased TNF-α levels to 0.37 ± 0.051 pg/mg protein ($p < 0.05$) (Table 1). Additionally, for assessing the status of oxidative stress, we measured the levels of MDA and activities of GPX in the kidney tissue homogenates. Our findings revealed that MDA level in sham mice was 4.13 ± 0.77 nmol/mg protein, and the value was increased to 16.25 ± 1.13 nmol/mg protein in DN mice ($p < 0.05$). PQQ treatment effectively decreased MDA level in DN + PQQ mice to 10.55 ± 1.05 nmol/mg protein ($p < 0.05$). In line with this, the GPX activity in the renal tissues of sham mice was 6.42 ± 1.17 U/mg protein. It was noted that, in DN mice, the activity was elevated to 14.82 ± 1.33 U/mg protein ($p < 0.05$), and DN + PQQ mice significantly increased GPX activities to 18.32 ± 1.24 U/mg protein ($p < 0.05$) (Table 1).

PQQ improved renal function and alleviated podocyte injury

In this study we measured Ccr values to assess renal function. We found that Ccr in sham mice was 2.18 ± 0.16 ml/min/kg. It was significantly reduced to 1.01 ± 0.14 ml/min/kg in DN mice ($p < 0.05$). Ccr significantly increased to 1.39 ± 0.21 in DN + PQQ mice ($p < 0.05$) (Table 1). Moreover, we measured albumin levels in the urine to evaluate glomerular injury. According to our findings, urine albumin level in sham mice was 28.58 ± 3.77 µg/day. This value was significantly higher in DN mice (85.26 ± 14.25 µg/day) ($p < 0.05$); and compared with the DN mice, the value was significantly reduced to 54.71 ± 9.15 µg/day ($p < 0.05$) in DN + PQQ mice (Table 1). Additionally, we measured urinary levels of nephrin, synaptopodin, and podocin to assess podocyte injury. The gene expressions of these markers were also measured in the kidney tissue homogenates (Fig. 1). While the urinary levels of nephrin, synaptopodin, and podocin were all increased in the urine of DN mice ($p < 0.05$), their respective gene expressions were decreased in the renal tissues of DN mice ($p < 0.05$). On the other hand, DN + PQQ mice reduced urinary levels of nephrin, synaptopodin, and podocin ($p < 0.05$) and increased gene expressions of nephrin, synaptopodin, and podocin in the kidneys ($p < 0.05$).

PQQ improved kidney histoarchitecture

We subjectively evaluated the H&E, PAS, Masson's trichrome stained renal sections under light microscope and found obvious shrinkage in the glomeruli of DN mice. Moreover, evident vacuolations were observed in the renal tubuli of the DN mice. While these derangements were also observable in the renal sections of DN + PQQ mice, their intensity were observably milder. PQQ enhanced the structural integrity of the kidneys and mitigated cell vacuolization, along with a decrease in the count of condensed nuclei observed in H&E stained renal sections. Additionally, in Masson's Trichrome stained sections, PQQ led to a reduction in blue-stained fibrillar deposits. Furthermore, in PAS-stained sections, PQQ diminished polysaccharide deposits and contributed to a decrease in overall glomerular volumes (Fig. 2).

DISCUSSION

MDA and GPX are two main indicators of oxidative stress [21]. Here, we showed that PQQ effectively reduced MDA levels and, at the same time, increased GPX activities in the kidney tissues of diabetic mice. Therefore, it can be concluded that PQQ relieves oxidative stress in the kidneys. The antioxidant properties were previously shown to be protective in several conditions including Parkinson's disease [22], skin aging [23], and heat failure [24]. This effect could be attributed to the induction of nuclear factor erythroid 2-related factor 2 (Nrf2) by the PQQ, which is the master regulator of antioxidant enzymes. For example, it has been reported that PQQ induces the expressions of GPX, heme oxygenase (HO-1), and superoxide dismutase (SOD) by stimulating nrf2 in IPEC-J2 intestinal epithelial cell lines [25].

TNF-α is a prominent marker of inflammation [26]. Here, we showed that PQQ significantly reduced renal TNF-α levels in diabetic mice. So, it can be deduced that PQQ alleviated renal inflammation. Oxidative stress and inflammation are two closely related entities, as the induction of oxidative stress leads to inflammation in the kidney [27, 28]. Therefore, it can be concluded that PQQ alleviated inflammation by opposing oxidative stress. Moreover, PQQ is an effective repressor of nuclear factor kappa B (NF-kB), the principal effector of inflammation. It has been

Table 1 List of measured markers.

	Sham (N=6)	DN (N=6)	DN + PQQ (N=6)
Serum glucose (mg/dl)	78 ± 6	351 ± 32[a]	365 ± 37[a]
SBP (mmHg)	119 ± 7	122 ± 10	117 ± 12
TNF-α (pg/mg protein)	0.20 ± 0.042	0.62 ± 0.048[a]	0.37 ± 0.051[a,b]
MDA (nmol/mg protein)	4.13 ± 0.77	16.25 ± 1.13[a]	10.55 ± 1.05[a,b]
GPX (U/mg protein)	6.42 ± 1.17	14.82 ± 1.33[a]	18.32 ± 1.24[a,b]
Nephrin (pg/mg protein)	0.95 ± 0.22	4.67 ± 0.53[a]	3.04 ± 0.41[a,b]
Synaptopodin (pg/mg protein)	5.12 ± 0.71	24.38 ± 2.02[a]	17.45 ± 1.89[a,b]
Podocin (pg/mg protein)	8.28 ± 1.55	36.92 ± 3.62[a]	23.84 ± 2.95[a,b]
Albumin (μg/day)	28.58 ± 3.77	85.26 ± 14.25[a]	54.71 ± 9.15[a,b]
Ccr (ml/min/kg)	2.18 ± 0.16	1.01 ± 0.14[a]	1.39 ± 0.21[a,b]

Sham, healthy control mice; DN, diabetic control mice; and DN + PQQ, diabetic mice treated with 10 mg/kg PQQ. Values are expressed as mean ± SD. Ccr, creatinine clearance; GPX, glutathione peroxidase; MCL, micheliolide; MDA, malondialdehyde; NF-kB, nuclear factor kappa beta; Nrf2, nuclear factor erythroid 2 (NFE2)-related factor 2; SBP, systolic blood pressure; and TNF-α, tumor necrosis factor alpha. [a] $p < 0.05$ vs. Control; [b] $p < 0.05$ vs. DN.

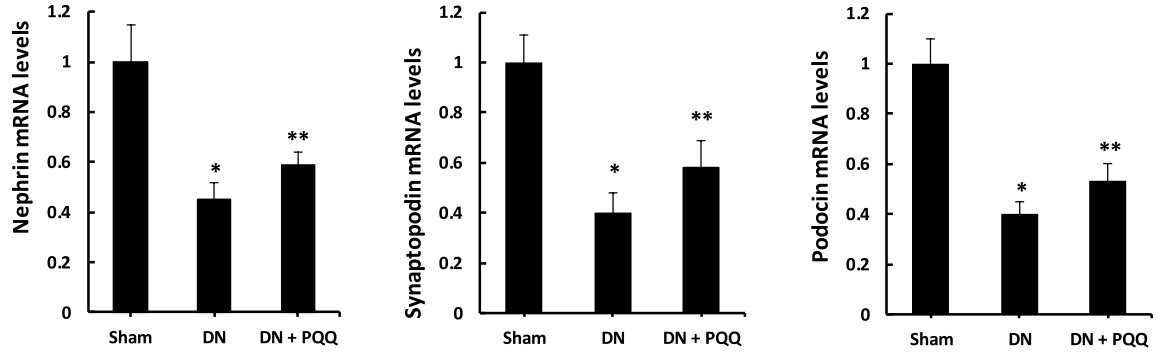

Fig. 1 PQQ effects on gene expressions of nephrin, synaptopodin, and podocin in kidney tissue homogenates. Sham, healthy control mice; DN, diabetic control mice; DN + PQQ, diabetic mice treated with 10 mg/kg PQQ. * $p < 0.05$ vs. Control; ** $p < 0.05$ vs. DOX.

shown in a previous study that PQQ reduced TNF-α, interleukin 1 (IL-1), and IL-6 levels of mice with cardiac hypertrophy by inhibiting NF-kB [29].

In this study, we found that PQQ improved renal histoarchitecture and renal function by increasing Ccr. Moreover, it alleviated glomerular injury as demonstrated by decreased urinary excretions of albumin. Finally, PQQ protected glomerular podocytes as confirmed by decreased urinary excretions of nephrin, synaptopodin, and podocin. It should be emphasized that PQQ promoted the gene expressions of nephrin, synaptopodin, and podocin in the kidney. All these findings denote that PQQ is protective against diabetes-induced kidney injury in mice. In line with our findings, it has been reported that PQQ ameliorates renal fibrosis in diabetic mice via suppressing pyroptosis [30]. PQQ attenuates oxidative stress in the kidneys of rats and improves renal function as evidenced by the reductions in blood urea nitrogen (BUN) and serum creatinine levels [31]. The protective effects of PQQ is not restricted to diabetes related kidney injury. It has been found that PQQ not only protects the kidneys of rats against ischemia-reperfusion injury [32], but also improves renal histoarchitecture and function via promoting nrf2 activities and inhibiting the LRR- and pyrin domain-containing 3 (NLRP3) pathway [33].

The noted reduction in gene expressions of nephrin, synaptopodin, and podocin within the kidneys, coupled with an increase in urinary excretions, indeed presents an intriguing aspect of our study. One potential explanation could be the presence of increased renal turnover or cellular shedding, leading to the release of these podocyte-specific molecules into the urine. This phenomenon might be indicative of podocyte injury or dysfunction, prompting the release of nephrin, synaptopodin, and podocin into the urinary space.

PQQ has been associated with the activation of signaling pathways related to mitochondrial biogenesis. It might interact with peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial biogenesis [34]. Enhanced mitochondrial function contributes to increased energy production, providing

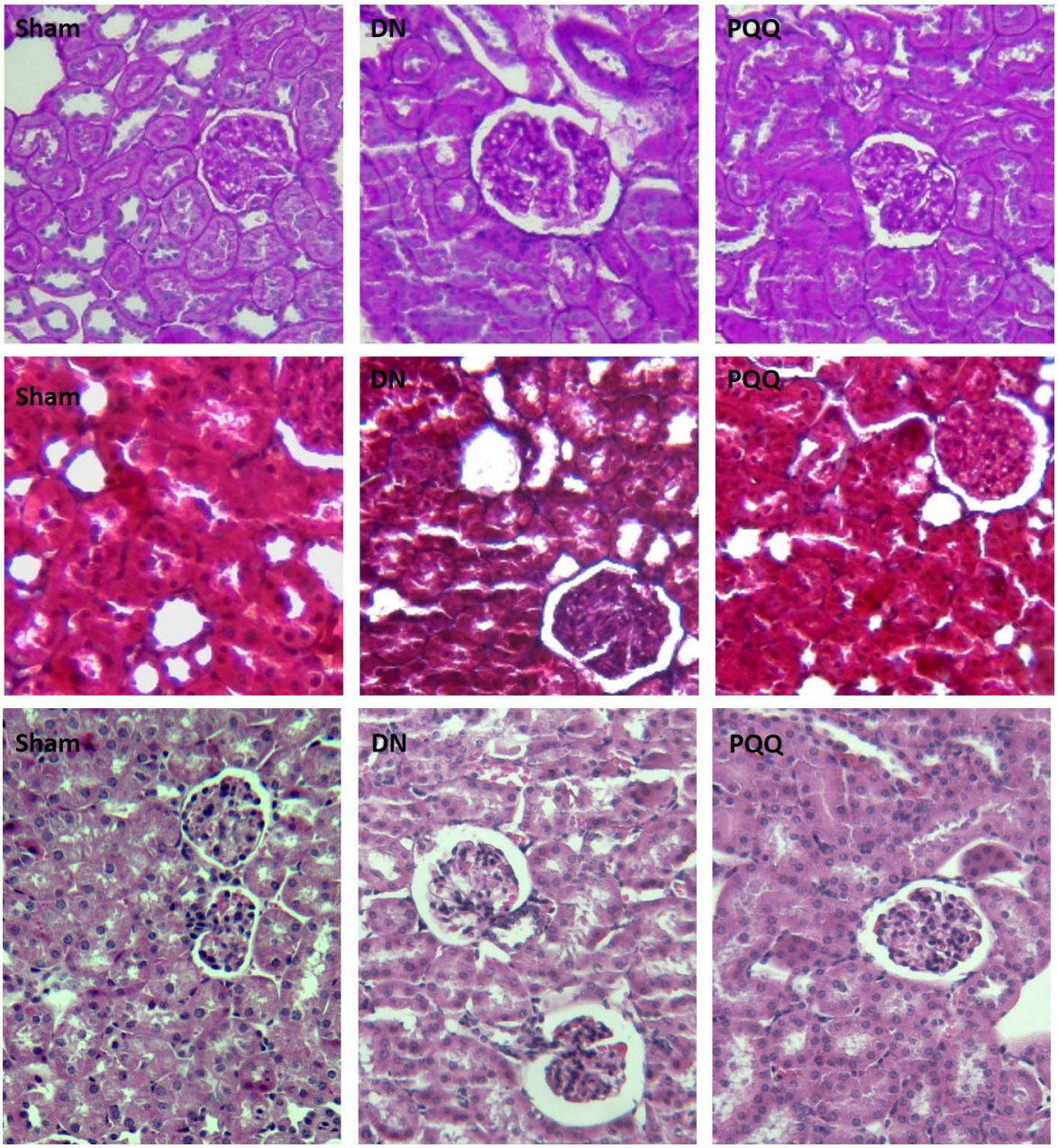

Fig. 2 PQQ effects on kidney histoarchitecture (400 ×). Micrographs illustrate the periodic acid Schiff (upper row), Mason's trichrome (middle row), and H&E stain sections (lower row) of renal tissue. Sham, healthy control mice; DN, diabetic control mice; DN + PQQ, diabetic mice treated with 10 mg/kg PQQ.

podocytes with the necessary energy for maintaining their structural integrity and supporting the slit diaphragm (14). Moreover, PQQ might activate prosurvival and anti-apoptotic pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt pathway. Activation of these pathways would promote cell survival, preventing apoptosis and maintaining the overall health of podocytes [35]. Additionally, PQQ might influence cell adhesion molecules and cytoskeletal proteins. It could impact the organization of actin filaments and other cytoskeletal components, contributing to the maintenance of podocyte structure and slit diaphragm integrity [36, 37].

Antioxidants represent the oldest category of medications employed to mitigate the generation of ROS and address issues related to dysfunction in podocytes.

In this context, substances akin to PQQ, such as coenzyme Q10, resveratrol, curcumin, and alpha-lipoic acid (ALA), have demonstrated efficacy in ameliorating podocyte damage in experimental models of diabetic nephropathy. These compounds achieve this effect by mitigating mitochondrial dysfunction and enhancing the integrity of the podocyte slit diaphragm [38–41].

Our study had two potential limitations. While it was evident that PQQ protected podocytes in diabetic kidneys of the mice, its mechanism of actions remained elusive. Additional *in vitro* investigations should be conducted in the future to reveal the associated signaling pathways in the kidney cell lines. Moreover, this study investigated only the 10 mg/kg dose of PQQ. Therefore, it was not evident whether this is the most effective dose of the compound. The decision to conduct a single treatment group study was primarily driven by ethical considerations, aiming to minimize the number of animals used while still obtaining significantly meaningful results. Moreover, previous studies demonstrated that the selected dose of PQQ (10 mg/kg) had effective therapeutic effects [19]. While we acknowledge that a dose-response study could provide additional insights, it was beyond the scope of this initial investigation.

CONCLUSION

The current study unveils that PQQ is protective against diabetes related kidney injury in mice via relieving glomerular and podocyte damages. This is indicated by decreased urinary excretions of albumin as well as nephrin, synaptopodin, and podocin. Moreover, PQQ improves both kidney tissue derangements and improves kidney function by promoting creatinine clearance rate.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.072.

Acknowledgements: The authors are thankful to Xinzhou District People's Hospital, Integrated Traditional Chinese and Western Medicine, Wuhan, Hubei Province, China. The study protocol has been approved by the ethical committee of mentioned institution under the ethical code 20220412.

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Appendix A. Supplementary data

Table S1 List of primers used in the RT-qPCR analysis.

Gene name	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Nephrin	CGTCAGCATCAGCAGCAACC	AGCCACATCTTCCAGCCTCTC
Synaptopodin	AGCCTAAGGTGACGCCGAATC	TCTCTGCCTCCGCTTCTCATC
Podocin	TGAGGATGGCGGCTGAGAT	GGTTTGGAGGAACTTGGGT
GAPDH	CGGAGTCAACGGATTTGGTCGTA	AGCCTTCTCCATGGTGGTGAAGA