Quercetin Treatment Improves Renal Function and Protects the Kidney in a Rat Model of Adenine-Induced Chronic Kidney Disease

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Hu Yang**
BCDE 2 **Yan Song**
BDE 1 **Ya-nan Liang**
ACDEFG 1 **Rong Li**

1 Department of Nephrology, The Second Hospital of Tianjin Medical University, Tianjin, P.R. China
2 Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital, Tianjin, P.R. China

Corresponding Author: Rong Li, e-mail: rongli1@yahoo.com
Source of support: Departmental sources

Background: The aim of this study was to examine the effects of the natural flavonoid, quercetin, in a rat model of adenine-induced chronic kidney disease.

Material/Methods: Forty male Wister rats were divided into four groups: normal (no adenine or quercetin) (n=10); untreated model (treated withadenine but not quercetin) (n=10); quercetin-treated model (5 mg/kg/day for 21 days) (n=10); quercetin-treated model (10 mg/kg/day for 21 days) (n=10). Urine and blood samples were collected and rat kidneys were examined histologically.

Results: Comparison of the findings of the model rats treated with quercetin (n=20) with non-treated model rats (n=10) showed reduced levels of fibroblast growth factor 23 (FGF23): normal group, 19.6 pg/ml; untreated group, 73.6 pg/ml; quercetin-treated group (5 mg/kg), 34.25 pg/ml; and quercetin-treated group (10 mg/kg), 21.3 pg/ml. Quercetin-treated model rats had reduced serum levels of parathyroid hormone (PTH), inorganic phosphate, increased urine protein-to-creatinine ratio, increased urine antioxidants, serum lactate dehydrogenase (LDH), and interleukin (IL)-8 when compared with the untreated model group and the control group. Quercetin treatment 10 mg/kg (n=10) reduced the levels of creatinine, blood urea nitrogen (BUN), and urinary uric acid. Renal histopathology in model rats treated with quercetin (n=20) showed reduced inflammation compared with the untreated model rats (n=10).

Conclusions: In a rat model of adenine-induced chronic kidney disease, treatment with quercetin improved renal function, reduced oxidative stress factors, serum levels of FGF23, and kidney inflammation.

MeSH Keywords: **Anti-Inflammatory Agents • Monocytes • Renal Insufficiency, Chronic**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909259
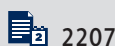
📄 2207 ⊞ — 📊 5 📖 24

Background

Worldwide, chronic kidney disease is one of the most commonly diagnosed health problems and is a major cause of morbidity and mortality in patients with diabetes and hypertension [1]. Recent studies have shown that increased levels of fibroblast growth factor 23 (FGF23) in the serum and an increased urine protein-to-creatinine ratio are associated with the development of end-stage renal disease [1–4]. FGF23 is a hormone that is a member of the fibroblast growth factor family that inhibits the formation of vitamin D, and is mainly secreted by osteoblasts and osteocytes in bone tissue and is involved in the excretion of inorganic phosphate by the kidneys [5,6]. This activity of FGF23 is responsible for the maintenance of the inorganic phosphate levels and calcium ions in the body [5,6]. Previously published studies have shown that the presence of FGF23, parathyroid hormone (PTH), and inorganic phosphate have a role in the development of chronic kidney disease, and with hypercalcemia, and hyperparathyroidism [7–9]. Increased urinary protein and reduced urinary creatinine are the characteristic features of chronic kidney and heart diseases [10]. Previously published studies have shown that inhibition of proteinuria can prevent renal damage, and a variety of approaches have been studied to inhibit proteinuria and improve renal functions in patients with chronic kidney disease [8–10].

Several naturally derived agents have been used in Chinese herbal medicine, including flavonoids (or bioflavonoids), which form an important group of secondary plant metabolites. Quercetin is an important flavonoid that has been reported to exhibit several biological activities which include, but are not limited to, antioxidant, anti-diabetic and anti-inflammatory activities. Given the strong antioxidant activity of quercetin, the present study was designed to investigate the effect of quercetin in an established rat model of chronic kidney disease. The aim of this study was to examine the effects of the natural flavonoid, quercetin, in a rat model of adenine-induced chronic kidney disease.

Material and Methods

Chemicals and reagents

Quercetin and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solution.

Animal groups

Forty male Wistar rats were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). The animals were kept in the animal facility under specific pathogen-free (SPF) conditions, with free access to food and water. All the experimental procedures involving the animals were performed with the approval of the Committee of Experimental Animal Administration of the Second Military Medical University Laboratory (Shanghai, China) (approval number: MUL067A/2017) and the all the animal procedures were carried out according to published guidelines [11].

Development of animal model and treatment strategy

Forty male Wister rats were included in the study, 30 of which were given a 200 mg/kg dose of adenine through the intragastric route to produce rat model of adenine-induced chronic kidney disease. The 40 rats were divided into four groups: normal (untreated with adenine or quercetin) (n=10); untreated model (untreated with quercetin) (n=10); quercetin-treated model (5 mg/kg) (n=10); quercetin-treated model (10 mg/kg) (n=10). The rats in the 5 mg/kg and 10 mg/kg quercetin treatment groups were given daily doses of 5 mg/kg or 10 mg/kg quercetin for 21 days. Rats in the normal and untreated groups received the same volume of normal saline. On day 22 urine and blood samples were collected as described previously [12], and the animals were euthanized using anesthesia with chloral, and the kidneys were removed for histological analysis.

Laboratory testing of renal function

The levels of creatinine and blood urea nitrogen (BUN) in the rat blood samples were examined using an automatic biochemistry analyzer, the Hitachi 7600-020/7170A (Hitachi High-Technologies Corp., Tokyo, Japan).

Biochemical examination

The blood samples obtained from the rats on day 22 of the study, and following treatment, underwent centrifugation for 15 min at 11,000×g. In the serum, levels of uric acid, calcium, and phosphorus ions, and parathyroid hormone (PTH) were determined using an auto-analyzer (Abbott, Chicago, IL, USA). Urine antioxidant capacity was evaluated using the Trolox equivalent antioxidant capacity (TEAC) assay, a method for measuring total antioxidant capacity (TAC). Commercial spectrophotometer analysis kits were used to measure lactate dehydrogenase (LDH) levels (bioMérieux, Marcy-l'Étoile, France) and superoxide dismutase (SOD) levels using the RANSOD method (RANDOX Laboratories Ltd., Co. Antrim, UK) according to the manufacturers' protocols. The urine samples of the rats were collected over 24 hours on day 22, and the samples underwent centrifugation, and then analyzed for the urine protein (mg/dL)-to-creatinine (mg/dL) ratios, obtained by dividing urine protein by urine creatinine at 24 hours, using standard protocols [13]. All analyses were performed in triplicate.
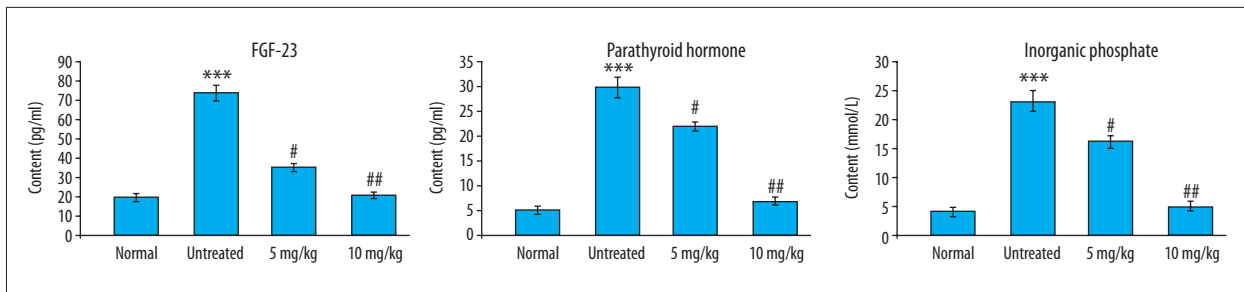
Figure 1. Quercetin treatment for 21 days inhibited the expression of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and inorganic phosphate in the adenine-induced chronic kidney disease rat model. The rats were treated with 5 mg/kg (n=10) and 10 mg/kg (n=10) doses of quercetin for 21 days, whereas the normal (n=10) and untreated model rats (n=10) were given equal volume of normal saline. Values of *** $P<0.0001$ represent significant differences between the untreated group compared with the normal group. Values of # $P<0.01$, and ## $P<0.001$ represent significant differences between the untreated group compared with the treated group.

Enzyme-linked immunosorbent assay (ELISA)

The blood samples of the rats were collected after the completion of the treatment and centrifuged at 1200×g. The fibroblast growth factor 23 (FGF23) enzyme-linked immunosorbent assay (ELISA) kit (Immutopics, San Clemente, CA, USA) was used for the determination of the concentration of FGF23, according to the manufacturer's protocol, as previously described [14].

Histological examination of the rat kidneys

The rat kidneys were removed on day 22 of the treatment. The kidneys were washed with phosphate-buffered saline (PBS), formalin-fixed, embedded in paraffin wax, and sectioned at 2 µm onto glass slides. Sections were de-waxed and stained with hematoxylin and eosin (H&E) for histological examination by light microscopy for the analysis of the renal damage.

Statistical analysis

Data were analyzed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA). All the assays were performed in triplicate, and the values were expressed as the mean ± standard deviation (SD). The differences between the different groups were analyzed by using one-way analysis of variance (ANOVA) with a post hoc Tukey's test for multiple comparisons. The values at $P<0.01$, $P<0.001$, and $P<0.0001$ were taken to indicate statistically significant differences.

Results

Quercetin reduced the levels of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and inorganic phosphate in the adenine-induced chronic kidney disease rat model

Analysis of fibroblast growth factor 23 (FGF23) in the serum of rats in the model of adenine-induced chronic kidney disease showed significantly reduced levels of FGF23 when compared with normal rats, and the inhibitory effect on the levels of FGF23 in the serum of the rats was dose-dependent. Comparison of the findings of the model rats treated with quercetin (n=20) with non-treated model rats (n=10) showed reduced levels of FGF23 as follows: normal group (n=10), 19.6 pg/ml; untreated group (n=10), 73.6 pg/ml; quercetin-treated group (5 mg/kg) (n=10), 34.25 pg/ml; and quercetin-treated group (10 mg/kg) (n=10), 21.3 pg/ml. The inhibition of FGF23 was maximum at the higher dose of quercetin, of 10 mg/kg (Figure 1).

Rats in the model of adenine-induced chronic kidney disease showed significantly increased levels of parathyroid hormone (PTH) and inorganic phosphate compared with normal group, but treatment with quercetin reduced the concentration of both parathyroid hormone and inorganic phosphate in a concentration-dependent manner, with the concentration levels of parathyroid hormone and inorganic phosphate reduced significantly at a dose of 10 mg/kg of quercetin in the rat model of adenine-induced chronic kidney disease (Figure 1).

Quercetin reduced urine protein-to-creatinine ratios and urinary uric acid in the adenine-induced chronic kidney disease rat model

In the adenine-induced chronic kidney disease rat model, the urinary protein content was significantly increased, and creatinine was decreased when compared with the normal group of
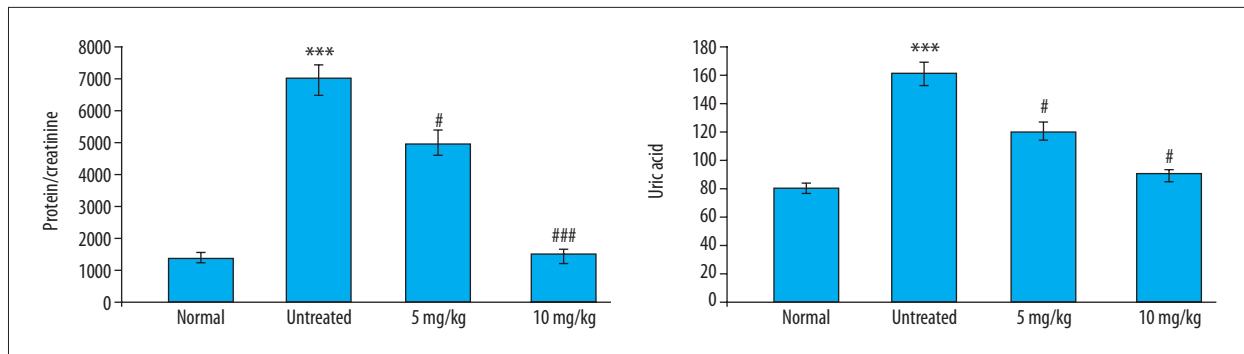
Figure 2. Quercetin treatment on day 22 reduced the values of the urine protein-to-creatinine ratio and urine uric acid in the adenine-induced chronic kidney disease rat model. The urine samples of the rats were collected over 24 hours on day 22 of the treatment and the values of rhe urine protein-to-creatinine ratio and uric acid were determined. Values of *** $P<0.0001$ represent significant differences between the untreated group compared with the normal group. Values of # $P<0.01$ and, ### $P<0.0001$ represent significant differences between the untreated group compared with the treated group.
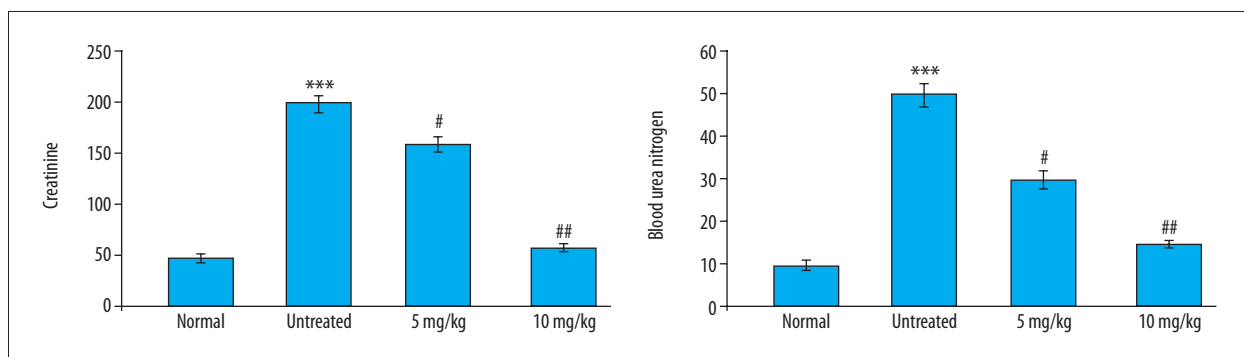

Figure 3. Quercetin treatment reduced the levels of creatinine and blood urea nitrogen (BUN) in the serum of the adenine-induced chronic kidney disease rat model. The blood samples from the rats were collected after the completion of treatment, centrifuged and then analyzed to measure the levels of creatinine and blood urea nitrogen (BUN). Values of *** $P<0.0001$ represent significant differences between the untreated group compared with the normal group. Values of # $P<0.01$ and ## $P<0.001$ represent significant differences between the untreated group compared with the treated group.

rats (n=10) ($P<0.01$). Quercetin treatment resulted in a significant reduction in the urine protein-to-creatinine ratios in adenine-induced chronic kidney disease rats (Figure 2).

Comparison of the findings of the model rats treated with quercetin (n = 20) with non-treated model rats (n=10) showed urine protein (mg/dL)-to-creatinine (mg/dL) ratios as follows: normal group (n=10), 1,454±421; untreated group (n=10), 7,053±643; quercetin-treated group (5 mg/kg) (n=10), 4,953±542; and quercetin-treated group (10 mg/kg) (n=10), 1,534±376. The reduction in the urine protein-to-creatinine ratio was maximum at the higher dose of quercetin, of 10 mg/kg. The level of uric acid was also reduced significantly in the chronic kidney disease rat model following treatment with a 10 mg/kg dose of quercetin (Figure 2).

Quercetin reduced the creatinine and blood urea nitrogen (BUN) levels in the adenine-induced chronic kidney disease rat model

The creatinine and blood urea nitrogen (BUN) were significantly increased in the adenine-induced chronic kidney disease rat model. However, treatment of the rats in the model with quercetin at a dose of 5 mg/kg and 10 mg/kg resulted in a reduction in the levels of creatinine and blood urea nitrogen (BUN) (Figure 3).

Quercetin treatment increased the expression of serum lactate dehydrogenase (LDH), superoxide dismutase (SOD), and total antioxidant activity in the adenine-induced chronic kidney disease rat model

As shown in Figure 4A and 4B, the rats in the adenine-induced chronic kidney disease rat model had inhibited total antioxidant activity. However, this effect was considerably, but not completely, restored by quercetin treatment at the two test
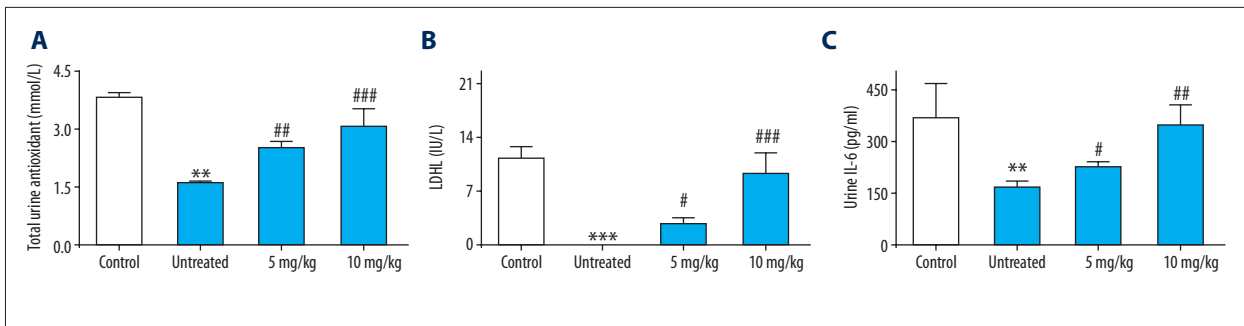
Figure 4. Effect of quercetin on (**A**) urinary antioxidants, (**B**) lactate dehydrogenase (LDH) activity, and (**C**) urine interleukin (IL)-6 concentrations, in the adenine-induced chronic kidney disease rat model. All experiments were performed in triplicate and presented as the mean ± standard deviation (SD). Values of ** P<0.001, and *** P<0.0001 represent significant differences between the untreated group compared with the normal group. Values of # P<0.01, ## P<0.001, and ### P<0.0001 represent significant differences between the untreated group compared with the treated group.
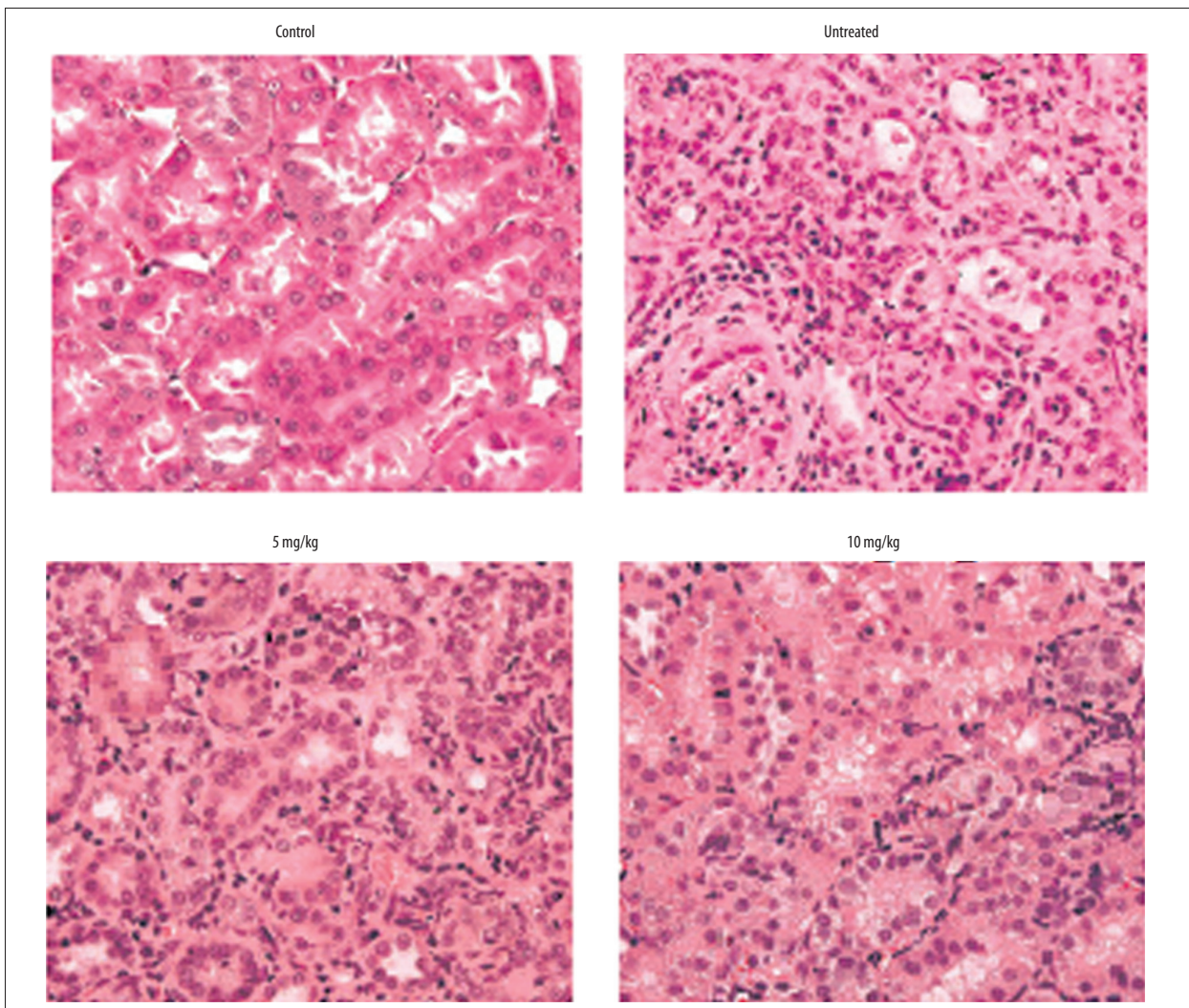

Figure 5. Photomicrographs showing the histological changes in the kidney, including the renal tubules, in the adenine-induced chronic kidney disease rat model. The kidneys of the euthanized rats in the study were removed after the completion of treatment. Renal tissue sections were prepared and histochemically stained with hematoxylin and eosin (H&E). Magnification, ×200.

doses of 5 mg/kg and 10 mg/kg. In the model rats treated with quercetin at the two doses, serum lactate dehydrogenase (LDH) and superoxide dismutase (SOD) activity in the urine were significantly increased. Concomitant administration with quercetin and adenine partially restored this effect (Figure 4A, 4B). Also, quercetin treatment increased the levels of interleukin (IL)-8 in the urine (Figure 4C).

Histologic changes in the kidneys of the normal rats and the adenine-induced chronic kidney disease rat model with and without treatment with quercetin

Histopathological examination of the rat kidney tissue showed changes in the renal tubules of the rats in the adenine-induced chronic kidney disease rat model, which showed swelling, and cystic renal tubule dilatation, with interstitial chronic inflammation inflammatory cells (Figure 5). In the adenine-induced chronic kidney disease rat model, abnormal renal tubular and interstitial changes, including chronic inflammation, were apparent when compared with normal rat kidney. Treatment with quercetin of the rats in the chronic kidney model reduced the abnormal histopathological renal changes, including the chronic interstital inflammation (Figure 5).

Discussion

The aim of this study was to examine the effects of the natural flavonoid, quercetin, in a rat model of adenine-induced chronic kidney disease. The findings showed that quercetin treatment in the rat model resulted in the prevention of proteinuria and improvement of the renal function, including dose-dependent inhibition of fibroblast growth factor 23 (FGF23) and parathyroid hormone, both of which play a vital role in the regulation of the levels of calcium and phosphorus in the kidneys [15].

Previously published studies have shown that increased expression of FGF23 and parathyroid hormone levels in rat serum leads to the development and progression of chronic kidney disease [3,16,17]. In the present study, the chronic kidney disease rat model was developed by adenine administration to the rats through an intragastric route. The levels of FGF23, parathyroid hormone, and inorganic phosphate in the rat serum, following adenine administration, were found to be markedly increased. In this study, the administration of quercetin to the rats with chronic kidney disease caused a significant reduction in the expression of FGF23, parathyroid hormone and inorganic phosphate in the serum.

Quercetin treatment improved kidney function of the model rats by regulating the expression of FGF23, parathyroid hormone and inorganic phosphate. The chemotherapeutic agents used for treatment of renal disease and heart diseases exhibit their effects through the regulation of the protein-to-creatinine ratio [18–21]. In patients with chronic kidney disease, the values of the protein-to-creatinine ratio have previously been shown to be markedly increased [21]. The findings of the present study showed a marked increase in the protein-to-creatinine ratio in the urine of the rat model of adenine-induced chronic kidney disease. However, quercetin treatment caused a significant reduction in the protein-to-creatinine ratio in the urine of the rat model, and the treatment of the rats with quercetin reduced the level of protein and increased the content of creatinine in the urine.

In this study, the adenine-induced chronic kidney disease associated increase in the levels of creatinine, blood urea nitrogen (BUN), and uric acid in the rat serum were reduced significantly on treatment with 5 mg/kg and 10 mg/kg doses of quercetin. Also, quercetin administration improved the level of total urine antioxidants, when compared with the untreated control group. Histopathological examination of the rat kidney tissues showed that the renal tubules of rats with chronic kidney disease showed both damage to the renal tubules and inflammation in the renal interstitium. However, treatment of the model rats with quercetin prevented renal tubular damage and chronic interstitial inflammation, findings that could be due to the antioxidant activity of quercetin, which has been previously reported [22].

The results of the present study are supported by the findings from previously published studies, where quercetin has been reported to show some protective effects on renal injury in rats [23]. Also, quercetin has previously been reported to alleviate the cisplatin-induced oxidative stress in the rat kidneys [24]. These previously published studies, together with the findings of the present stud, indicate the protective effects of quercetin in chronic kidney disease rat models.

Conclusions

In a rat model of adenine-induced chronic kidney disease, treatment with quercetin improved renal function, reduced oxidative stress factors, reduced serum levels of fibroblast growth factor 23 (FGF23), and reduced renal inflammation and renal tubular damage. The findings of this preliminary study indicate that further studies are required to confirm these findings and to investigate the potential for the use of the natural flavonoid, quercetin, in the prevention and treatment of chronic kidney disease.

References:

1. Bello AK, Hemmelgarn B, Lloyd A et al: Associations among estimated glomerular filtration rate, proteinuria, and adverse cardiovascular outcomes. Clin J Am Soc Nephrol, 2011; l6: 1418–26

2. Sarnak MJ, Astor BC: Implications of proteinuria: CKD progression and cardiovascular outcomes. Adv Chronic Kidney, Dis 2011; 18: 258–66

3. Faul C: Fibroblast growth factor 23 and the heart. Curr Opin Nephrol Hypertens, 2012; 21: 369–75

4. Nakano C, Hamano T, Fujii N et al: Intact fibroblast growth factor 23 levels predict incident cardiovascular event before but not after the start of dialysis. Bone, 2012; 50: 1266–74

5. Razzaque MS, Lanske B: The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. J Endocrinol, 2009; 194: 1–10

6. Shimada T, Kakitani M, Yamazaki Y et al: Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest, 2004; 113: 561–68

7. Seiler S, Heine GH, Fliser D: Clinical relevance of FGF23 in chronic kidney disease. Kidney Int Suppl, 2009; 76: S34–42

8. El-Abbadi MM, Pai AS, Leaf EM et al: Phosphate feeding induces arterial medial calcification in uremic mice: Role of serum phosphorus, fibroblast growth factor-23, and osteopontin. Kidney Int, 2009: 75; 1297–307

9. Gutiérrez OM, Mannstadt M, Isakova T et al: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med, 2008: 359: 584–92

10. Sharma SK, Zou H, Togtokh A et al: Burden of CKD, proteinuria, and cardiovascular risk among Chinese, Mongolian, and Nepalese participants in the International Society of Nephrology screening programs. Am J Kidney Dis, 2010; 56: 915–27

11. Percie du Sert N, Alfieri A, Allan SM et al: The IMPROVE guidelines (ischaemia models: Procedural refinements of *in vivo* experiments). J Cerebral Blood Flow Metabol, 2017; 37: 3488–517

12. Deda O, Gika HG, Taitzoglou I et al: Impact of exercise and aging on rat urine and blood metabolome. An LC-MS based metabolomics longitudinal study. Metabolites, 2017; 7: 10–13

13. Farombi EO, Ekor M: Curcumin attenuates gentamicin-induced renal oxidative damage in rats. Food Chem Toxicol, 2006; 44: 1443–48

14. Cliquet F, McElhinney LM, Servat A et al: Development of a qualitative indirect ELISA for the measurement of rabies virus-specific antibodies from vaccinated dogs and cats. J Virological Meth, 2004; 117: 1–8

15. Bergwitz C, Jüppner H: Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu Rev Med, 2010; 61: 91–104

16. Pontoriero G, Cozzolino M, Locatelli F, Brancaccio D: CKD patients: The dilemma of serum PTH levels. Nephron Clin Pract, 2010; 116: c263–68

17. Levin A, Djurdjev O, Beaulieu M, Er L: Variability and risk factors for kidney disease progression and death following attainment of stage 4 CKD in a referred cohort. Am J Kidney Dis, 2008; 52: 661–71

18. Setti G, Hayward A, Dessapt C et al: Peroxisome proliferator-activated receptor-{g agonist rosiglitazone prevents albuminuria but not glomerulosclerosis in experimental diabetes. Am J Nephrol, 2010; 32: 393–402

19. Hu W, Yu Q, Zhang J, Liu D: Rosiglitazone ameliorates diabetic nephropathy by reducing the expression of Chemerin and ChemR23 in the kidney of streptozotocin-induced diabetic rats. Inflammation, 2012; 35: 1287–93

20. Kincaid-Smith P, Fairley KF, Farish S, Best JD, Proietto J: Reduction of proteinuria by rosiglitazone in non-diabetic renal disease. Nephrology (Carlton), 2008; 13: 58–62

21. Betz B, Schneider R, Kress T et al: Rosiglitazone affects nitric oxide synthases and improves renal outcome in a rat model of severe ischemia/reperfusion injury. PPAR Res, 2012; 2: 193–99

22. Anjaneyulu M, Chopra K: Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol, 2004; 31: 244–48

23. Kahraman A, Erkasap N, Serteser M, Köken T: Protective effect of quercetin on renal ischemia/reperfusion injury in rats. J Nephrol, 2003; 16: 219–24

24. Behling EB, Sendão MC, Francescato HD et al: Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol Rep, 2006; 20: 526–32