

Effect of baicalin on renal function in patients with diabetic nephropathy and its therapeutic mechanism

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Abstract. The present study observed the effect of baicalin on blood glucose and renal function in patients with diabetic nephropathy and explored its mechanism of action. A total of 95 patients diagnosed with diabetic nephropathy by clinical and laboratory examinations were selected and randomly divided into a control and treatment group. The control group included 45 patients who were treated with routine symptomatic treatment. The remaining 50 patients in the treatment group received baicalin, in addition to routine symptomatic treatment. The treatment course was 6 months. Following this, the changes of indicators such as fasting plasma glucose (FPG), glycosylated hemoglobin (HBA1c), aldose reductase (AR) activity, 24-h urinary microalbumin, urinary β 2-microglobulin (β 2-MG) and urinary albumin excretion rate (UAER) were compared before and after treatment; at the same time, the variations of indexes, including superoxide dismutase (SOD), glutathione peroxidase (GSH-px), nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B), transforming growth factor- β 1 (TGF- β 1) and vascular endothelial growth factor (VEGF) were detected. Compared with those in the control group, baicalin had little effect on the treatment group's FPG and HBA1c, but it clearly reduced the AR activity and the difference was significant ($P < 0.05$). Baicalin visibly decreased the 24-h urinary microalbumin, urinary β 2-MG and UAER ($P < 0.05$) and had notable effect on improving renal function. Following treatment, compared with those in the control group, baicalin distinctly increased the levels of SOD and GSH-px ($P < 0.05$) and decreased the content of NF- κ B and VEGF ($P < 0.05$), however, its impact on the expression of TGF- β 1 was not statistically significant ($P > 0.05$). The results showed that baicalin may improve the renal function in patients with diabetic nephropathy and delay

the progression of diabetic nephropathy through various ways, including anti-inflammation and anti-oxidation.

Introduction

Diabetic nephropathy is currently the most common chronic complication of patients with diabetes, and its incidence accounts for about 20-40% of that in the entire population with diabetes. Diabetic nephropathy can increase the risk of the occurrence of cardiovascular-related events. It is an important cause for easy occurrence of end-stage renal diseases. Also, it can affect the prognosis of patients and improve the quality of life of patients (1). In view of the universality and harmfulness of its occurrence, scholars around the world began to focus on this disease, and there are increasing research directed to its pathogenesis. Microalbuminuria is the first clinical symptom of patients with diabetic nephropathy. When the patient's condition is gradually aggravated, the renal function of the patient will be significantly decreased, represented by visible increases in the massive proteinuria, urinary β 2-microglobulin (β 2-MG) and urinary albumin excretion rate (UAER), and ultimately kidney replacement therapy will be used (2). At present, the routine treatment of diabetic nephropathy is mainly to reduce proteinuria, prevent glomerular sclerosis and interstitial fibrosis, but the curative effect is not satisfactory and it is difficult to prevent the progression of renal failure. In recent years, the treatment of diabetic nephropathy using traditional Chinese medicine has attracted increasing attention. Baicalin as an important compound extracted from skullcap and is the most recognized traditional Chinese medicine in the treatment of diabetic nephropathy. Baicalin has the pharmacological effects of detoxification, anti-allergy, immune regulation and anti-tumor activity. Besides, baicalin has also been proven to have anti-inflammatory, anti-oxidation, aldose reductase (AR) inhibition and other effects. Baicalin can inhibit the progress of diabetic nephropathy through a variety of ways (3).

A previous study found that inflammation reaction is closely related to the occurrence of diabetes, and strict control of blood glucose can significantly reduce the incidence of diabetic microvascular disease (4). Peripheral blood mononuclear cells can invade the intima and form foam cells. As an important inflammatory factor, nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) may play an important role in the pathogenesis of diabetic nephropathy by mediating

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inflammation. Therefore, detection of NF- κ B may facilitate investigation of the mechanism of the function of baicalin. A previous study has shown that (5), the pathogenesis of early diabetic nephropathy is similar to vascular proliferation. Vascular endothelial growth factor (VEGF) has the effect of promoting vascular endothelial cell migration and increasing vascular permeability. VEGF can induce neovascularization, and participate in the pathogenesis of diabetic nephropathy. A previous study has shown that baicalin can reduce the level of VEGF (6), but its role in the treatment of diabetic nephropathy has not been reported. It has been reported that TGF- β is closely related to diabetic nephropathy. However, baicalin can reduce the deposition of immune complex on renal tubular and delay the process of diabetic nephropathy by down-regulating the expression of TGF- β (7). Therefore, the detection of TGF- β is also important. Free radicals can severely damage the body. As an antioxidant enzyme, superoxide dismutase (SOD) can scavenge superoxide anion, antagonize lipid peroxidation and repair damaged injury. Glutathione peroxidase (GSH-px) can remove harmful peroxide metabolites and catalyze harmful peroxides to become harmless, so as to protect the integrity of the cell membrane structure and function (8). In this study, patients with diabetic nephropathy were studied to observe the effect of baicalin on blood glucose and renal function. NF- κ B, SOD, GSH-px, TGF β 1, VEGF and other factors were detected to explore the mechanism of the functions of baicalin.

Patients and methods

General information. A total of 95 patients who were diagnosed with diabetes in Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University (Shanghai, China) from January 2014 to December 2015 were selected and the diagnoses conformed to World Health Organization (WHO) diagnostic criteria (9). The patients were diagnosed with early diabetic nephropathy and the diagnoses were in line with Mogensen early diabetic nephropathy diagnostic criteria (10). None of the patients had medical history of primary glomerular diseases, acute and chronic nephritises, urinary tract infections and connective tissue diseases. Patients with the following conditions were excluded: Primary kidney diseases, cardiac, respiratory, hepatic and renal insufficiencies caused by other reasons, those who could not have regular medication or had insufficient medication time and incomplete clinical data. Among them, 45 patients (21 males and 24 females) were in the control group, with an average age of (54.8 \pm 8.4) years, a mean body mass index (BMI) of (24.6 \pm 1.5) kg/m², and a medial disease course of (9.3 \pm 4.2) years; the treatment group had 50 patients (26 males and 24 females), the average age was (55.7 \pm 9.2) years, the mean BMI was (24.9 \pm 1.2) kg/m², and the medial disease course was (8.9 \pm 4.2) years. The statistical analysis showed that there were no statistically significant differences in age, sex, BMI and disease course between the two groups ($P > 0.05$). The study was approved by the Ethics Committee of Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University and informed consents were signed by the patients or the guardians.

Inclusion and exclusion criteria. Inclusion criteria: i) Patients with type II diabetes who met the WHO diagnostic criteria for

diabetes (9); ii) patients in the early stage of diabetic nephropathy and who met the Mogensen diagnostic criteria (10); and iii) patients with 24 h UAER of 20-200 μ g/min. Exclusion criteria: i) Patients with primary renal disease, urinary tract infections, or connective tissue disease; ii) patients with dysfunction of heart, lung, liver and kidney caused by other factors; and iii) patients that could not regularly take medication or patients with incomplete clinical data.

Treatment. All patients were asked to maintain their previous exercise and lifestyle during treatment. Both groups were given low-sugar and low-protein diet. According to the patient's blood glucose level and standard body weight, total daily calories intake was 104.6 kJ/kg, and daily protein intake was 0.6-0.8 g/kg. Metformin hydrochloride tablet (0.5-0.85 g) was taken with meals three times a day. Treatment with insulin was performed if necessary (NovoRapid, Copenhagen, Denmark). Atorvastatin calcium [Pfizer (China) Pharmaceutical Co., Ltd.; Shanghai, China] was also used to reduce lipid with a dose of 0-40 mg/day. Benazepril hydrochloride was used to control blood pressure if necessary. Besides the treatments, patients in observation group were also asked to take baicalin at a dose of 800 mg, three times a day. Meanwhile, control group was given placebo. Treatment was performed for 6 months.

Observation items and experimental methods. Fasting plasma glucose (FPG), erythrocyte AR and glycosylated hemoglobin (HBA1c) values of all patients with diabetic nephropathy in the two groups were measured before treatment and after the 6-month treatment. Moreover, the changes in the 24-h urinary albumin, urinary β 2-MG and UAER were monitored and the levels of SOD, GSH-px, NF- κ B, transforming growth factor- β 1 (TGF- β 1) and VEGF were measured. Immunofluorescence assay was utilized to measure blood glucose. Fluorescence was used to determine the activity of AR. The levels of HBA1c, 24 h urinary albumin, urinary β 2-MG and UAER were measured by using radioimmunoassay. SOD was detected via the xanthine oxidase method.

SOD kit (cat. no. AOO1-2) and GSH-px kit (cat. no. A005) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Colorimetric assay was applied to detect GSH-px. The treatment was performed at 37°C for 60 min. Double antibody sandwich enzyme-linked immunosorbent assay was used to detect the VEGF level (Genesis RMP200 VEGF; Tecan Group, Ltd., Mannedorf, Switzerland). The kit was purchased from Jingmei Biotech Co., Ltd. (Shenzhen, China), and the mouse anti-human VEGF monoclonal antibody (cat. no. KL03672) was purchased from Shanghai Kanglang Biological Technology Co., Ltd. (Shanghai, China). After the 96-well polyacrylonitrile plate was coated with the mouse anti-human VEGF monoclonal antibody, 100 μ l of sample dilution was added to each well, then 100 μ l of standard and 100 μ l of test serum were added. The dilution was used as the blank control. Double-well detection was used for all specimens. The color was developed for 15 min in the dark at room temperature, and the action was terminated by adding 50 μ l of sulfuric acid (2 mol/l). The optical density value of each well was measured by a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at a wavelength of 450 nm. The concentration of the VEGF standard was plotted

Table I. Baseline data of patients in the treatment group and the control group.

Influence factor	No. of people (n)	Age (years)	Sex (male/female)	BMI (kg/m ²)	Disease course (years)
Treatment group	50	55.7±9.2	26/24	24.9±1.2	8.9±4.2
Control group	45	54.8±8.4	21/24	24.6±1.5	9.3±4.2
χ^2/t		-0.468	0.270	-1.197	0.484
P-value		0.641	0.604	0.234	0.630

Comparison of age, BMI index, and disease duration of treatment and control groups was performed using two independent samples t-test. Sex comparison between two groups was performed using χ^2 test. BMI, body mass index.

Table II. Changes in blood glucose and other related indicators before and after baicalin treatment.

Group	FPG (mmol/l)	HBA1c (%)	AR activity (U/g Hb)
Treatment group			
Before treatment	11.4±2.4	9.5±1.3	4.5±1.2
After treatment	10.2±2.0 ^a	8.9±1.6 ^a	2.1±1.2 ^a
F/P-value	7.017/0.009	4.942/0.029	97.740/<0.001
Control group			
Before treatment	10.8±1.6	9.6±1.1	4.4±1.3
After treatment	9.6±1.8	9.0±1.5	3.8±1.3
F/P-value	10.632/0.002	5.107/0.026	5.102/0.026

ANOVA analysis and post hoc test was used to compare blood glucose and other related indicators before and after the treatment of baicalin. F, F-value in ANOVA. ^aP<0.05, compared with control group after treatment. FPG, fasting plasma glucose; HBA1c, glycosylated hemoglobin; AR, glycosylated hemoglobin.

on the abscissa, and the corresponding optical density value was plotted on the ordinate. The corresponding VEGF content was detected on a straight line according to the optical density value of the specimen to be tested. The concentration of TGF- β 1 in the sample was detected by double antibody sandwich ELISA. The biotinylated anti-human TGF- β 1 antibody (cat. no. hz-H0109c) was purchased from Shanghai Huzhen Biological Technology Co., Ltd. (Shanghai, China). A 96-well microplate was coated with TGF- β 1 antibody to prepare a solid phase carrier, and samples were added to the micro wells. TGF- β 1 was ligated to the solid phase carrier, then biotinylated TGF- β 1 antibody was added. The unbound biotinylated antibody was washed, and HRP-labeled avidin was added, washed again thoroughly, then TMB substrate was added for color development. TMB was converted to blue under the catalysis of peroxidase and converted to yellow by the action of an acid. There was a positive correlation between how light the color was and TGF- β 1 in the sample. The absorbance (O.D. value) was measured at a wavelength of 450 nm using a microplate reader to calculate the sample concentration. The NF- κ B content of peripheral blood mononuclear cells was detected by immunohistochemical two-step method. The kit including the rabbit anti-human NF- κ B polyclonal antibody (cat. no. 7834) was purchased from Cell Signaling Technology, Inc., (Danvers, MA, USA). All of the above experiments have clear negative controls and were repeated three times.

Statistical analysis. SPSS v19.0 (IBM Corp., Armonk, NY, USA) software was used for statistical analysis. For comparison of baseline data, quantitative data were analyzed by t-test, and qualitative data were analyzed by χ^2 test. Comparison of age, BMI index, and disease course were performed by independent samples Student t-tests. Sex comparison between two groups was performed using χ^2 test. Comparison of indicators between treatment group and control group was performed by one-way analysis of variance and the LSD test as a post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline data of patients in the treatment group and the control group. There were 95 patients with diabetic nephropathy in total. Comparing the baseline data of patients in the two groups the age was (55.7±9.2) years, the BMI was (24.9±1.2) kg/m², the disease course was (8.9±4.2) years, and the male to female ratio was 26:24 in the treatment group; the age, BMI, disease course and male to female ratio in the control group was (54.8±8.4) years, (24.6±1.5) kg/m², (9.3±4.2) years and 21:24, respectively. The statistical analysis results revealed that comparisons of age, gender, weight index and disease course of the patient between the two groups had no statistical difference (P>0.05; Table I).

Table III. Changes in renal function before and after baicalin treatment.

Group	24 h urinary microalbumin (mg)	Urine β 2-MG (mg/l)	UAER (μ g/min)
Treatment group			
Before treatment	256.6 \pm 25.2	3.5 \pm 1.5	129.1 \pm 40.8
After treatment	121.5 \pm 66.2 ^a	2.5 \pm 0.9 ^a	110.7 \pm 3.9 ^a
F/P-value	181.823/<0.001	17.095/<0.001	7.890/0.006
Control group			
Before treatment	260.1 \pm 22.8	3.6 \pm 1.4	130.2 \pm 34.3
After treatment	232.0 \pm 51.3	3.0 \pm 0.9	125.1 \pm 29.5
F/P-value	11.258/0.001	5.420/0.022	0.577/0.449

ANOVA analysis and post hoc test was used to compare renal function indicators before and after the treatment of baicalin. F, F value in ANOVA. ^aP<0.05, compared with control group after treatment. MG, macroglobulin; UAER, urinary albumin excretion rate.

Table IV. Changes in mechanism-related indexes of baicalin in treatment of diabetic nephropathy.

Group	SOD (U/ml)	GSH-px (U/l)	NF- κ B (%)	TGF- β 1 (pg/ml)	VEGF
Treatment group					
Before treatment	75.7 \pm 12.9	107.2 \pm 13.8	27.5 \pm 4.7	76.0 \pm 13.7	222.1 \pm 20.7
After treatment	81.8 \pm 9.0 ^a	113.1 \pm 12.4 ^a	23.6 \pm 6.0 ^a	69.3 \pm 15.8 ^a	210.4 \pm 19.2 ^a
F/P-value	7.539/0.007	5.196/0.025	12.949/0.001	5.092/0.026	8.507/0.004
Control group					
Before treatment	76.1 \pm 12.8	106.5 \pm 12.2	28.4 \pm 4.0	79.2 \pm 12.0	223.7 \pm 13.7
After treatment	77.1 \pm 12.9	108.3 \pm 9.9	26.2 \pm 5.3	72.4 \pm 13.3	218.2 \pm 17.9
F/P-value	0.142/0.707	0.582/0.447	4.729/0.032	6.381/0.013	2.676/0.105

ANOVA analysis and post hoc test was used to compare nephropathy mechanism-related indicators before and after the treatment of baicalin. F, F-value in ANOVA. ^aP<0.05, compared with control group after treatment. SOD, superoxide dismutase; GSH-px, glutathione peroxidase; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; TGF- β 1, transforming growth factor- β 1; VEGF, vascular endothelial growth factor.

Levels of blood glucose and other related indicators before and after baicalin treatment. Before treatment, there was no significant difference in FPG, HBA1c and AR activity between the groups (P>0.05). After treatment, FPG, HBA1c and AR activity in both groups decreased significantly (P<0.05), but no significant differences in FPG and HBA1c were found between the groups, while AR activity was significantly lower in treatment group than in control group (P<0.05; Table II).

Changes in renal function before and after baicalin treatment. No significant differences in 24 h urinary albumin, urinary β 2-MG and UAER were found between the groups before treatment. After treatment, 24 h urinary albumin, urinary β 2-MG and UAER decreased in both groups, while the decreases in treatment group were more significant (P<0.05; Table III).

Study on the mechanism of Baicalin in treating diabetic nephropathy. Before treatment, the differences in the SOD and GSH-px levels between the groups were not manifest. After treatment, the SOD and GSH-px levels were ascended in the two groups, the increases were clearer in the treatment group and the differences were notable (P<0.05). The NF- κ B content

between the two groups had no significant difference before treatment. After treatment, the content of NF- κ B in the two groups were decreased overtly compared with those before treatment, but the decrease in the treatment group was more evident and the difference was significant (P<0.05). No significant differences were observed in the content of TGF- β 1 and VEGF between the two groups before treatment. After treatment, the content of TGF- β 1 in the two groups were declined, but the decreases were not remarkable (P>0.05). While the VEGF was different, its decline in the treatment group was more obvious (P<0.05; Table IV).

Discussion

The main risk of diabetes is the occurrence of chronic complications. Hyperglycemia can often induce retina, nerve, kidney and other locations damage; therefore, the prevention of the occurrence of complications is essential for patients with diabetes. Diabetic nephropathy is the most important chronic complication of diabetic patients, and its pathogenesis is very complex, including heredity, polyol pathway activation, inflammatory response and oxidative stress response. Patients

with diabetic nephropathy generally have raised urinary microalbumin, urinary β 2-MG and UAER. Among them, the increases in the urinary microalbumin and urinary β 2-MB indicate renal tubular injury, and the UAER rise manifests glomerular injury (11). Baicalein, 5-7-trihydroxyflavone, is the flavonoid in *Scutellaria baicalensis* with the highest content. Baicalein can present with baicalin at the same time. Baicalin is a natural extract of *Scutellaria baicalensis*. After oral intake, baicalin will enzymatically hydrolyze into baicalein, which can be absorbed into the blood. Then, baicalein in blood will quickly transform into baicalin to play its biological roles. At present, the therapeutic effects of baicalin were as follow (12): i) antibacterial antiviral effect; ii) scavenging oxygen free radicals and antioxidant; iii) Antipyretic, analgesic and anti-inflammatory effects; iv) antitumor effects; v) cardio-cerebral vascular protection; vi) neuroprotective effects; vii) hepatic protection and viii) treatment and prevention of diabetes and its complications; This study found that baicalin could significantly reduce the 24 h urinary microalbumin, urinary β 2-MG and UAER levels of the patient with diabetes, improve the patient's renal function, delay the progress of diabetic nephropathy, and improve the quality of life of the patient.

A previous study has shown that when patients have diabetes, the high concentration of blood glucose can activate AR, so that the activity is clearly increased, the polyol pathway is activated, and extensive sorbitol is produced. Sorbitol has very strong heteropolarity and it is difficult to traverse the cell membrane. Therefore, massive sorbitol is accumulated in the cell, resulting in osmotic damage and further causing glomerular and tubular structural damage, so that its function is gradually decreased and eventually kidney damage is induced (13). Therefore, the control of blood glucose can prevent and treat diabetic nephropathy to some extent and it is the basis of treatment. AR inhibitors can further control the above performances to improve renal function of patients. Currently, there are more synthetic AR inhibitors worldwide, and the vast majorities do not enter into the clinical application stage because of the great side effects. The results of this study disclosed that the AR activity in patients with diabetic nephropathy was visibly increased, but it was overtly dropped after taking baicalin, the discrepancy was significant ($P < 0.05$), indicating that baicalin could obviously inhibit the AR activity of patients with diabetic nephropathy. Furthermore, baicalin has wide sources and low cost, no obvious side effects have been found, suitable for patients with diabetic nephropathy.

Free radicals cause great damage to the body, especially cardiovascular diseases and other chronic diseases. SOD, as an antioxidant enzyme, can antagonize lipid peroxidation, scavenge oxygen free radicals and repair oxygen free radical-caused damage to cells. The SOD level often reflects the body's abilities in self-repair, anti-oxidation and oxygen free radical elimination. GSH-px can catalyze the reduction of harmful peroxides into harmless compounds and protect the cell membrane structure and function as an antioxidant enzyme (14). A study has revealed that baicalin can increase the SOD content in rats with myocardial injury, reduce their malonyldialdehyde (MDA) content, and mitigate cell infiltrations and myocardial cell loss (14). The study of Cui *et al* (15), showed that baicalin can reduce H_2O_2 -induced oxidative stress

injury in rat nerve cells, increase the content of SOD in culture liquid and decrease the MDA content. The results of this study suggest that baicalin can significantly raise the levels of SOD and GSH-px ($P < 0.05$), protect the patients' renal function by anti-oxidative reaction and delay the progress of diabetic nephropathy. NF- κ B, as an important nuclear transcription factor, may be involved in the occurrence and development of diabetic nephropathy by mediating inflammation (16). Under normal conditions, NF- κ B is bound to I κ B protein to be in non-activated state. When a cell has internal and external stimulations, the I κ B kinase is activated and I κ B protein is degraded by phosphorylation, and thus the NF- κ B is released, transferred into the nucleus, and bound to specific target genes to initiate the transcription of the target gene and further induce acute inflammatory response (17). This study found that the NF- κ B activity in peripheral blood of patients with diabetic nephropathy was significantly increased, suggesting that the patients with diabetic nephropathy were in inflammatory state. After treatment, the decrease in NF- κ B of the baicalin treatment group was clearer ($P < 0.05$), indicating that baicalin had anti-inflammatory effect. Modern medicine believes that the key to the onset of diabetic nephropathy is a microvascular lesion. As a vascular endothelial cell mitogenic factor, VEGF can increase vascular permeability, promote vascular endothelial cell proliferation and neovascularization, produce substantial plasma proteins, and further stimulate vascular endothelium cells, promote epithelial hyperplasia, and thus cause glomerular damage and renal interstitial injury, and the patient's condition continues to worsen (18). The study of Li *et al* (19) showed that VEGF in serum of patients with diabetic nephropathy is raised, and serum VEGF can be used as an important indicator to determine the degree of kidney injury in diabetic patients. Another study has found that VEGF can induce the increase in glomerular basement membrane permeability, change renal hemodynamics and produce a large number of proteinuria by binding to the glomerular basement membrane (20). The results of this study showed that the levels of TGF- β 1 and VEGF between the treatment group and the control group had no clear difference before treatment. After treatment, the levels of TGF- β 1 and VEGF in the two groups were declined, but the decrease of TGF- β 1 was not remarkable while the decline in VEGF was obvious ($P < 0.05$). This indicates that baicalin can significantly reduce the level of VEGF and improve the renal function of patients. This study also has some limitations. No significant differences in age, gender, body mass index and course of disease was shown between the groups ($P > 0.05$), but exercise and patient's lifestyle may affect the experimental results. However, those factors cannot be easily quantified and statistically analyzed. Therefore, all patients were asked to maintain previous exercise and lifestyle during treatment. This study is also limited by the small sample size. Future studies with larger number of samples are needed to further confirm the conclusions in this study.

This study revealed that patients' 24 h urine albumin, urinary β 2-MG and UAER in the group receiving baicalin were overtly lower than those in the group undergone conventional treatment. This indicates that baicalin can reduce the level of proteinuria and distinctly improve renal function of the diabetic patients. After receiving baicalin treatment, the SOD and GSH-px levels of patients were obviously increased and the AR activity,

NF- κ B and VEGF content were decreased significantly, indicating that baicalin could reduce renal vascular permeability, improve renal function of patients with diabetic nephropathy and postpone the progress of diabetic nephropathy through anti-oxidative stress, polyol pathway, anti-inflammatory and other ways. Further research into whether baicalin protects renal function through other mechanisms is required.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MY conceived and designed the study. LK and LW were responsible for the collection and analysis of the data. MY, YZ and QW interpreted the data and drafted the manuscript. MY and LK revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University (Shanghai, China). Signed written informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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