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Taxifolin improves disorders of glucose metabolism and water-salt metabolism in kidney via PI3K/AKT signaling pathway in metabolic syndrome rats

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ABSTRACT

Aims: Our study was designed to explore the function and mechanism of taxifolin on glucose metabolism and water-salt metabolism in kidney with metabolic syndrome (MS) rats.

Main methods: Spontaneous hypertensive rats were induced by fructose to establish MS model. Systolic blood pressure (SBP) and homeostasis model assessment of insulin resistance (HOMA-IR) were measured after 7 weeks of continuous administration with taxifolin. Kidney injury indices and histopathological evaluation were done. The apoptosis rate of primary kidney cells was detected by flow cytometry. Insulin signaling pathway related proteins and renal glucose transport-related proteins were detected by western blotting. We assessed the effects of taxifolin on sodium water retention and renin-angiotensin-aldosterone system (RAAS) in MS rats. We examined not only changes in urine volume, osmotic pressure, urinary sodium and urinary chloride excretion, but also the effects on NA^+/K^+-ATPase and RAAS indicators. We also detected changes in inflammatory factors by immunohistochemical staining and immunofluorescence. In vitro experiment, high glucose and salt stimulated NRK-52E cells. By adding the PI3K inhibitor (wortmannin) to inhibit the PI3K, the effects of inhibiting the PI3K/AKT signaling pathway on glucose metabolism, water-sodium retention and inflammatory response were discussed.

Key findings: Taxifolin effectively reversed SBP, HOMA-IR, the kidney indices and abnormal histopathological changes induced by MS. Besides, taxifolin called back the protein associated with the downstream glucose metabolism pathway of PI3K/AKT. It also inhibited overactivation of RAAS and inflammatory response. In vitro experiments have demonstrated that the PI3K/AKT signaling pathway plays an important role in this process.

Significance: Taxifolin can improve homeostasis of glucose, inhibit overactivation of RAAS and reduce inflammatory response by PI3K/AKT signaling pathway.

1. Introduction

Metabolic syndrome (MS) is a pathological condition in which a variety of metabolic substances such as carbohydrate, fat and protein are disturbed [1,2]. With the improvement of modern living standard, great changes have taken place in people's diet structure. The imbalance between nutrient intake and consumption is caused by high-energy diet, which leads to the increasing incidence of MS year by year, and the resulting complications rise sharply [3]. Related studies have found that most MS patients suffer from serious harm to the body due to metabolic disorders of various substances, resulting in disorders of glucose metabolism, hypertension and hyperlipidemia [4,5]. Insulin resistance (IR), as the central link of MS, will reduce the sensitivity and reactivity of insulin target organs to insulin in damaged, which is reported as a major risk factor for the progression of chronic kidney disease [6–8].

Insulin is the key hormone to maintain glucose homeostasis and plays an important role in glucose homeostasis. When insulin resistance occurs, it will lead to a decrease in the uptake and utilization of glucose

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by insulin, and the body will compensatory secrete excessive insulin to maintain the stability of blood glucose. Glucose metabolism can be disrupted when impaired glucose tolerance occurs, resulting in sustained high glucose levels (glucose toxicity), which can cause kidney damage [9]. Several lines of evidence suggested that insulin resistance and the resultant hyperinsulinemia were causal related to hypertension [10–13]. Kidney is an important organ affecting hypertension, which can promote the reabsorption of sodium and the regulation of water and salt metabolism in kidney tubules [14,15]. The large amount of insulin produced by insulin resistance can excite sympathetic nerve regulation center, activate kidney β receptor, and make renin secrete increase, and thus activate RASS and increase blood pressure. In addition, the activation of RASS can enhance the activity of Na^+/K^+-ATPase and promote the reabsorption of sodium in renal tubule. The inflammatory signaling pathway in the cell to release a large number of inflammatory cytokines when RAAS is overactivated [16–19]. The imbalance of water and salt metabolism and the release of inflammatory factors will promote the injury and remodeling of renal tubular epithelial cells, the proliferation and hypertrophy of mesangial glomeruli, which will lead to kidney injury and further increase of blood pressure, thus forming a vicious circle [20–22]. Therefore, long-term insulin resistance will lead to the disorder of renal glucose metabolism and water-salt metabolism, further aggravating the degree of renal damage. However, as a key protein molecule in the insulin signaling system, phosphatidylinositol 3-kinase (PI3K) is also an important target for the prevention and treatment of MS.

Taxifolin is a natural flavonoid found in nature, modern pharmacological studies have shown that taxifolin has the biological activity of improving microcirculation, regulating immunity, antioxidant, antibacterial, anti-inflammatory and antiviral [23–27]. In recent years, because of its medicinal value, taxifolin has been widely used in the treatment of atherosclerosis, dyslipidemia, cardiovascular diseases and other chronic diseases. Does it improve metabolic disorders and hypertension caused by metabolic syndrome? Therefore, we studied taxifolin and discussed its mechanism of action.

2. Materials and methods

2.1. Chemicals and reagents

Fructose (A100226-0005) was purchased from Diamond (China). Taxifolin (480-18-2) was purchased from Hangzhou Shangjie Chemical Co. LTD (Zhejiang, China). Valsartan (H200505508) was purchased from Huanglong pharmaceutical co., Ltd. (Hainan, China). Wortmannin (19545-26-7) was purchased from the MCE Company (USA).

2.2. Animal experiments

Male spontaneously hypertensive (SHR) rats and Wistar-Kyoto (WKY) rats (180–200 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Rats were caged under a 12-h light-dark cycle in a specific pathogen-free (SPF) facilities and allowed free access to feeding food and drinking water. Rats were acclimated for at least a week before experimentation.

WKY rats fed with a normal chow diet were the control group (WKY). SHR rats were fed by 10% of fructose (SHR (F)) to induce metabolic syndrome [28–31]. BP-600A automatic non-invasive blood pressure measurement system was used to measure SBP of SHR rats. SHR (F) rats were divided into 4 groups (n = 10) according to the principle of equal blood pressure and weight, including: (1) the model group SHR(F) (1 mL/100 g distilled water, ig); (2) the low-dose taxifolin group SHR(F) + TA-L (25 mg/kg taxifolin, ig); (3) the high-dose taxifolin group SHR(F) + TA-H (50 mg/kg taxifolin, ig); and (4) the positive group SHR(F) + Va (30 mg/kg valsartan, ig). The study was conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

2.3. Cell culture and treatments

Rat kidney tubular ductal epithelial cell line NRK-52E was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM/LOW GLUCOSE 1× (PE2190108, Proteineasy, China) and 10% fetal bovine serum (11011-8611, Tianhang, China) in a water-saturated 5.0% CO_2 incubator (Thermo Scientific, MA, USA). The medium was added with triple antibodies containing 10kU/mL penicillin, 10 mg/mL streptomycin and 5 mg/mL gentamicin. The experiment was conducted when the cells grew to 70%–80% in logarithmic growth period.

NRK-52E Cells were randomly divided into 6 groups, including: (1) Normal control group (NC); (2) Normal control + Wortmannin group (NC + W); (3) Model group (M); (4) Model + Wortmannin group (M + W); (5) Taxifolin group (TA); (6) Taxifolin + Wortmannin group (TA + W). For establishment of high glucose and hypertonic cell model, NRK-52E cells were transferred to DMEM medium, containing 30 mM glucose and 30 mM NaCl for 24 h [32–35]. After 2 h of intervention with 1 μm wortmannin, added 10 mM taxifolin for 24 h.

2.4. Measurement of systolic blood pressure

All the rats were trained to adapt themselves to the restraining cages and tail-cuff apparatus for the standard non-invasive tail-cuff method before SBP measurement. Rats were put into the automatic non-invasive blood pressure measuring instrument (Techman, China) for 20 min to adapt, during which a certain amount of blocking pressure was appropriate. After the heart rate stabilized, each measurement was repeated sextic, and the average value was the final measurement result.

2.5. Insulin resistance index

Blood was taken from the rat orbital vein , the serum obtained by centrifugation was determined by radioimmunoassay. The blood samples were centrifuged at 4 °C, 3000 rcf for 20 min and the supernatant was taken. The fasting insulin level was measured by radioimmunoassay. The blood was collected from orbital venous plexus of the rats once every two weeks, and fasting blood glucose was measured by glucometer (On·Call EZII, China). Insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: HOMA IR = fasting plasma insulin × fasting plasma glucose / 22.5 [36].

2.6. Apoptosis assay

Flow cytometry was used to determine the apoptosis of kidney primary cells. It was measured by PE AnnexinV Apoptosis Detection Kit (Becton, Dickinson Company). Fresh kidney tissue was cut into pieces in 1.5 mL phosphate buffer solution (PBS) washing solution, centrifuged (4 °C, 1000 rpm) for 1 min, and washed twice. 1 mL 0.5% trypsin was added and digested for 5 min. The digestion was observed under an inverted microscope. When the cells became slightly round, the digestion was stopped and the supernatant was centrifuged. 1 mL erythrocyte lysate was added to lyse for 5 min, and the fragments were removed by centrifugation. 10 mL PBS was added and resuspended, filtered with a 75-mesh filter. The filtrate was transferred and centrifuged to collect kidney cells. After PBS was resuspended, cells were then incubated in 100 μL labeling solution (5 μL of PE, 5 μL of 7-AAD and 90 μL of 10× binding buffer) in darkness at room temperature for 15 min. After that, 400 μL of 10× binding buffer was added to stop the staining reaction. Flow cytometric analyses were performed on a FACS AriaIII (BD Biosciences, USA).

2.7. Histopathological examination

Kidney tissue samples were fixed and preserved in 10% neutral buffered formalin solution. The kidney samples isolated from rats were fixed in 4% paraformaldehyde, cleared in xylene, embedded in paraffin, and cut into 4 μm thickness by using a microtome. The kidney tissue sections were stained with haematoxylin-eosin (H&E), periodic acid schiff (PAS) and Masson's trichrome (Masson) for the analysis of histologic lesions. Images were viewed and acquired by microscope (Nikon, Shinagawa, Tokyo, Japan).

2.8. Urine biochemistry assay

The urine samples were collected in a metabolic cage the night before dissection and the urine volume were recorded. Freezing point osmotometer was used to detect the urine osmotic pressure of rats in each group. The concentration of sodium and chloride in urine were detected by colorimetry kit. *N*-acetyl-β-D-glucosaminidase (NAG) is one of the markers of kidney injury, and we detected the content of NAG (E-EL-R0647c, Elabscience, China) by Enzyme linked immunosorbent assay ELISA kit.

2.9. Detection of blood biochemical index measurement

After 7 weeks of administration, the rats fasted for 12 h, injected 10% chloral hydrate into the abdominal cavity for anesthesia (3 mL/kg), took blood from the abdominal aorta, centrifuged at 4 °C at 3000 rcf for 10 min, and collected serum. Creatinine (Cre) and urea nitrogen (BUN) are considered to be sensitive indicators of kidney tubular or glomerular epithelial injury and are used to evaluate the degree of kidney injury in rats. Cre was detected by picric acid method and BUN was detected by urease method. The contents of TNF-α (E-EL-R2856c, Elabscience, China) and IL-6 (E-EL-R0015c, Elabscience, China) were detected by ELISA according to the kit instructions. In addition, the indexes of renin-angiotensin-aldosterone system in plasma were detected. Concentrations of Renin (REN) (E-EL-R0030c, Elabscience, China), angiotensin converting enzyme (ACE) (E-EL-R2401c, Elabscience, China) and angiotensin II (AngII) (E-EL-R1430c, Elabscience, China) and aldosterone (ALD) (E-EL-R0070c, Elabscience, China) were examined by ELISA kits, according to the manufacturer's instructions.

2.10. Immunohistochemical staining

The kidney tissue samples were embedded in paraffinize and sliced into 4 μm thick sections. After deparaffinized and rehydrated, antigen retrieval was performed with 10 μM sodium citrate, and treated with hydrogen peroxide for 20 min to inactivate endogenous peroxidase. Sections were blocked with 5% goat serum for 1 h, incubated overnight at 4 °C with the primary antibody anti-CD45 (proteintech, 20103-1-AP). The dilution ratio of anti-CD45 was 1:200. After washing with PBS, the secondary antibody were sequentially incubated at 37 °C for 30 min washed with PBS, and stained with diaminobenzidine (DAB) (K5007, DAKO, Germany). The total and positively stained cells in 6 random fields of each section were counted at ×400 magnification. The optical density (OD) of positive cells was analyzed by the Image-pro plus 6.0 (IPP) software. The average integral OD (AIOD) was calculated as follows, AIOD = positive area × OD / total area.

2.11. Western blotting analysis

The total proteins of kidney were extracted from the pancreas or cells by using RIPA lysis buffer. After protein quantitation using bicinchoninic acid (BCA) method, 50 μg proteins in each lane were separated by 10% SDS-PAGE gels. The proteins were transferred to polyvinylidene fluoride (PVDF) film (ISEQ00010, Millipore, USA). The membranes were blocked with 5% BSA (9048-46-8, Genview, USA) for 1 h at room temperature. Subsequently, the membranes were then incubated with Ang-II (abcam, ab8452, diluted 1:500 with 5% BSA) antibody, AKT (cell signaling, #2920), p-AKT (cell signaling, #4060), GS (abcam, ab40867), p-GS (cell signaling, #47043), p-GSK-3β (abcam, ab131097), SGLT-2 (abcam, ab37296), GLUT-2 (abcam, ab54460), Na^+/K^+-ATPase (cell signaling, #23565) antibodies (diluted 1:1000 with 5% BSA), PI3K (cell signaling, #4292), GSK-3β (abcam, ab93926), β-actin (abclonal, AC004), β-Tubulin (abclonal, AC010) antibodies (diluted 1:5000 with 5% BSA) overnight at 4 °C. After washes with 0.2% Tween20 phosphate buffer solution (PBST) for four times, the membranes were incubated with secondary antibodies (diluted 1:5000 with 5% BSA) for 1 h at 37 °C. The membranes were washed four times with PBST, and the imaging was performed using an Odyssey Infrared Imaging System (LICOR, USA). The intensity of the western blot signals was quantitated using Image J software.

2.12. Mitochondrial membrane potential detection

NRK-52E cells were inoculated at a density of 10×10^4/mL on a 6-well plate, and the cells were randomly divided into 5 groups: (1) normal control group (NC); (2) model group (M); (3) 1 mM taxifolin group (TA-L); (4) 5 mM taxifolin group (TA-M); (5) 10 mM taxifolin group (TA-H). After being treated with different concentrations of drugs for 24 h, with JC-1 Kit (C2006 Beyotime, China) to dyeing of groups of cells, and then used flow cytometry instrument FACS AriaIII (BD Biosciences, USA) to analyze the level of mitochondrial membrane potential.

2.13. Cellular immunofluorescence

NRK-52E cells were uniformly inoculated at a density of 3×10^4/mL in 96-well plates (E190236X, PerkinElmer, USA). After the treatment of taxifolin, the cells were washed with PBS, fixed with 4% paraformaldehyde for 20 min, then permeated with 0.1% Triton for 20 min, and washed with PBS. Treated cells were blocked with 5% goat serum for 1 h at room temperature , and incubated at 4 °C overnight with the primary antibody (IL-6, ABclonal, A0286). Then incubated with secondary antibody (Cy3 Goat Anti-Rabbit IgG, abclonal, AS007) for 1 h at room temperature. Nuclei were counterstained with DAPI for 5 min. A High-content imaging system (Opera Phenix, PerkinElmer, USA) was used to scan. The relative protein expression level was normalized against the Harmony 4.8.

2.14. In-cell-western of NRK-52E cells

NRK-52E cells were inoculated in 96-well plates (1247971, Thermo, USA) with a density of 3×10^4/mL. After the drug intervention, the medium was discarded and 4% formaldehyde was added to fix the cells for 20 min. 0.1% Triton was permeated for 20 min, 5% BSA was closed for 1.5 h at room temperature, and incubated at 4 °C overnight with the primary antibody. Then incubated with secondary antibody (goat anti-rabbit 925-68071, goat-mouse 925-32210, Li-COR, MO, USA) for 1 h at room temperature, and the intensity of the proteins was quantified using Odyssey (Clx, Li-COR Biosciences, USA).

2.15. Statistical analysis

SPSS software program (version 20.0; SPSS Inc., Chicago, IL.USA) was used to perform the statistical analysis. Data were presented using single-factor ANOVA followed by *t*-test. All data are presented as mean ± S.D. Significant differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Taxifolin mitigates MS-related metabolic disorder in MS rats

At the end of the 7-week fructose feeding, the weight of SHR(F) rats were significantly increased compared with WKY rats (Fig. 1A). The systolic blood pressure of SHR(F) rats were significantly elevated compared with WKY group, and taxifolin administration to MS rats significantly reduced SBP compared with SHR(F) rats (Fig. 1B). As shown in Fig. 1C, fructose induced insulin resistance index were significantly reduced when taxifolin were administrated in MS rats. Furthermore,

MS rats exhibited both elevated triglyceride (TG) and low density lipoprotein cholesterin (LDL-c) compared with WKY group, and taxifolin significantly lowered MS-induced elevated TG and LDL-c (Fig. 1D, E). In addition, SHR(F) rats showed significantly decreased high density lipoprotein cholesterin (HDL-c) and taxifolin significantly increased HDL-c (Fig. 1F). These results suggest that the metabolic syndrome model was successfully established in SHR(F) rats and taxifolin mitigates MS-related metabolic disorder. All these changes suggest that taxifolin might contribute to ameliorate MS-related metabolic disorder in MS rats.

3.2. Taxifolin protects against kidney damage in MS rats

Renal dysfunction induced by metabolic syndrome was confirmed by the disturbance of biochemical parameters and histopathologic evaluation. As shown in Fig. 2A, B, the significant enhancement in blood urea nitrogen (BUN) and creatinine (Cre) levels, suggesting an intense protein catabolism and a decrease in glomerular filtration rate. Moreover, *N*-acetyl-β-D-glucosaminidase (NAG) levels were also increased (Fig. 2C), indicating renal dysfunction. Taxifolin treatment ameliorated renal dysfunction, since three parameters (BUN, Cre and NAG) were significantly decreased ($p < 0.01$). In addition, compared with WKY rats, kidneys in SHR(F) rats had remarkable morphological changes. Inflammatory cells infiltration, tubular necrosis, expansion of renal capsules and glomerular fibrosis were shown by H&E staining (Fig. 2D). But taxifolin lightened the pathological changes. In the glomeruli, mesangial matrix expansion of kidneys, increased glomerular tuft area and widening of Bowman's space were observed in SHR(F) group by PAS staining. But taxifolin treatment improved the phenomenon. We also performed Masson staining on the tissue sections to assess the level of collagen expression. Collagen deposition was profound in SHR(F) group, but taxifolin treatment reduced significantly the amount of collagen in renal interstitium. In contrast, taxifolin treatment ameliorated the injury of renal to mild, indicating that taxifolin significantly reduced MS-induced renal injury. All these changes suggest that taxifolin might contribute to ameliorate the renal dysfunction in SHR(F) rats.

3.3. Taxifolin inhibits renal cell apoptosis in MS rats

Apoptosis can interact with many vital processes such as inflammation, programmed cell death, and adaptive immunity; therefore, we measured the rate of kidney cell apoptosis. Flow cytometry shows that the rate of kidney cell apoptosis was significantly higher in SHR(F) rats than that in WKY rats, and taxifolin significantly reduced this level (Fig. 3A). The decrease of mitochondrial membrane potential is also a marker of early apoptosis. To explore the molecular mechanism of taxifolin, the levels of mitochondrial membrane potential in NRK-52E cells were detected by flow cytometry. As shown in Fig. 3B, taxifolin significantly elevated the levels of mitochondrial membrane potential in NRK-52E cells in a dose-dependent manner.

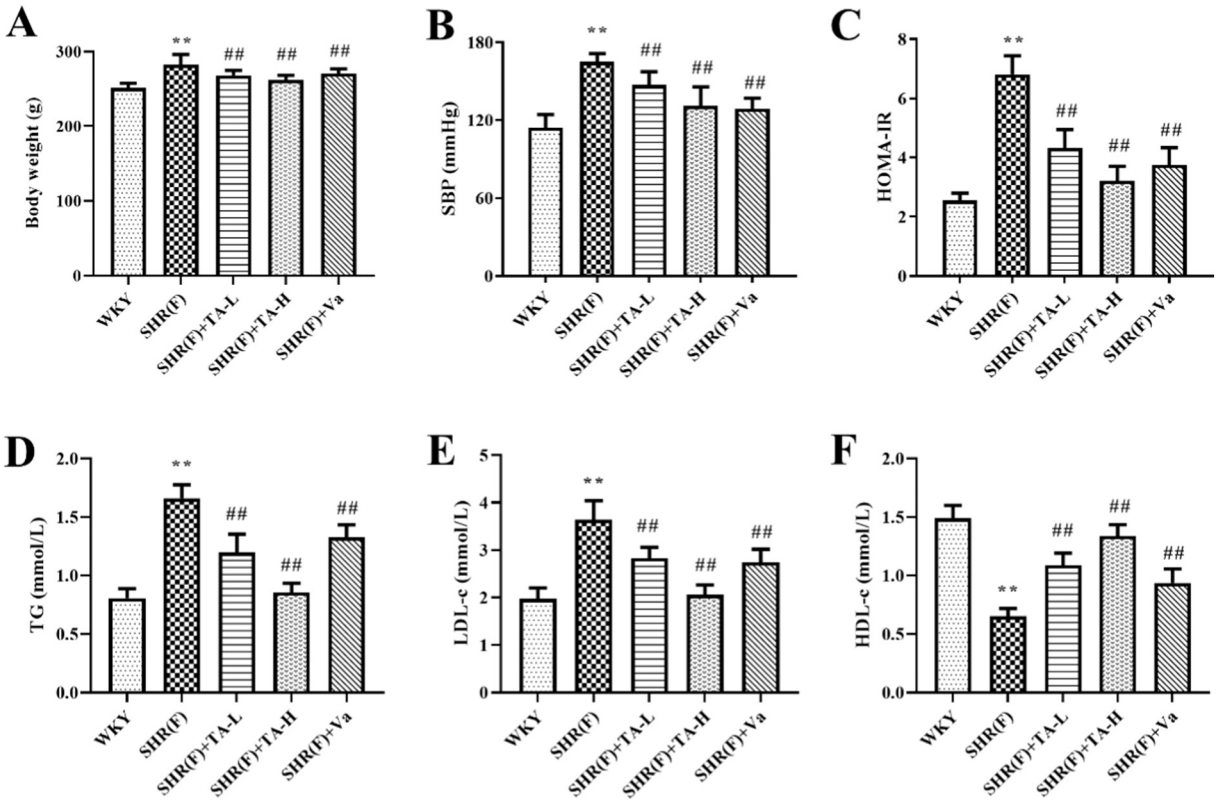

Fig. 1. Taxifolin mitigates MS-related metabolic disorder in MS rats. Rats were treated as described in "Materials and methods". (A) The effect of taxifolin on body weight. (B) The effect of taxifolin on systolic blood pressure. (C) The effect of taxifolin on insulin resistance index. (D) The effect of taxifolin on serum triglyceride. (E) The effect of taxifolin on serum low density lipoprotein cholesterin. (F) The effect of taxifolin on serum high density lipoprotein cholesterin. $^{**}p < 0.01$ compared with WKY group; $^{\#\#}p < 0.01$ compared with SHR(F) group.

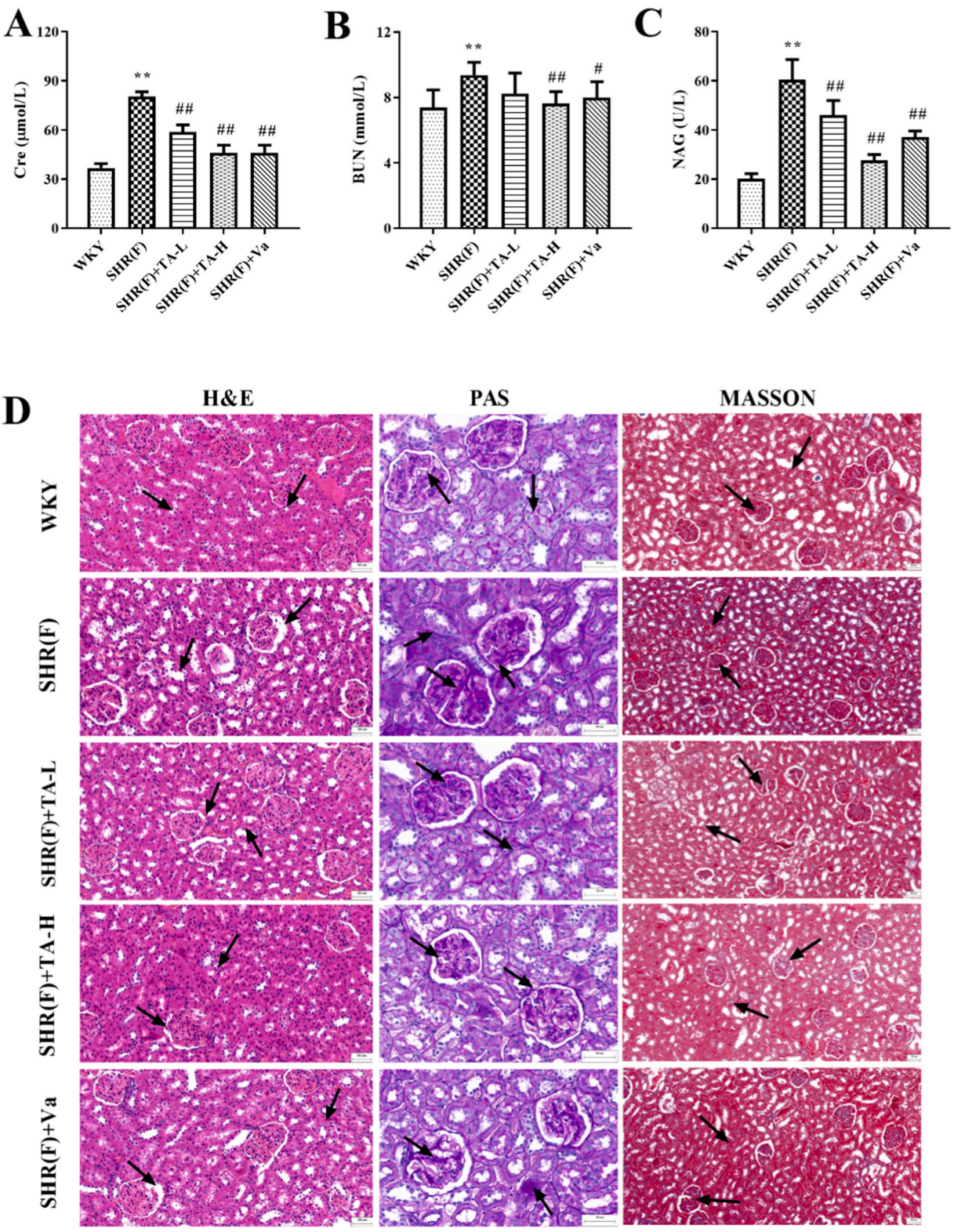

Fig. 2. Taxifolin protects against kidney damage in MS rats. The levels of Cre, BUN and NAG were determined. Rats were treated as described in "Materials and methods". (A) The effect of taxifolin on Cre. (B) The effect of taxifolin on BUN. (C) The effects of taxifolin on NAG. The effect of taxifolin on BUN. Kidney tissue sections from each rat were stained with H&E, PAS or Masson's trichrome. (D) Representative histological images are shown for H&E, PAS and Masson staining of formalin-fixed kidney tissues in each group. H&E and Masson staining (20× magnification). PAS staining (40× magnification). The magnification of H&E and Masson staining results were 200×. $^{**}p < 0.01$ compared with WKY group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with SHR(F) group.

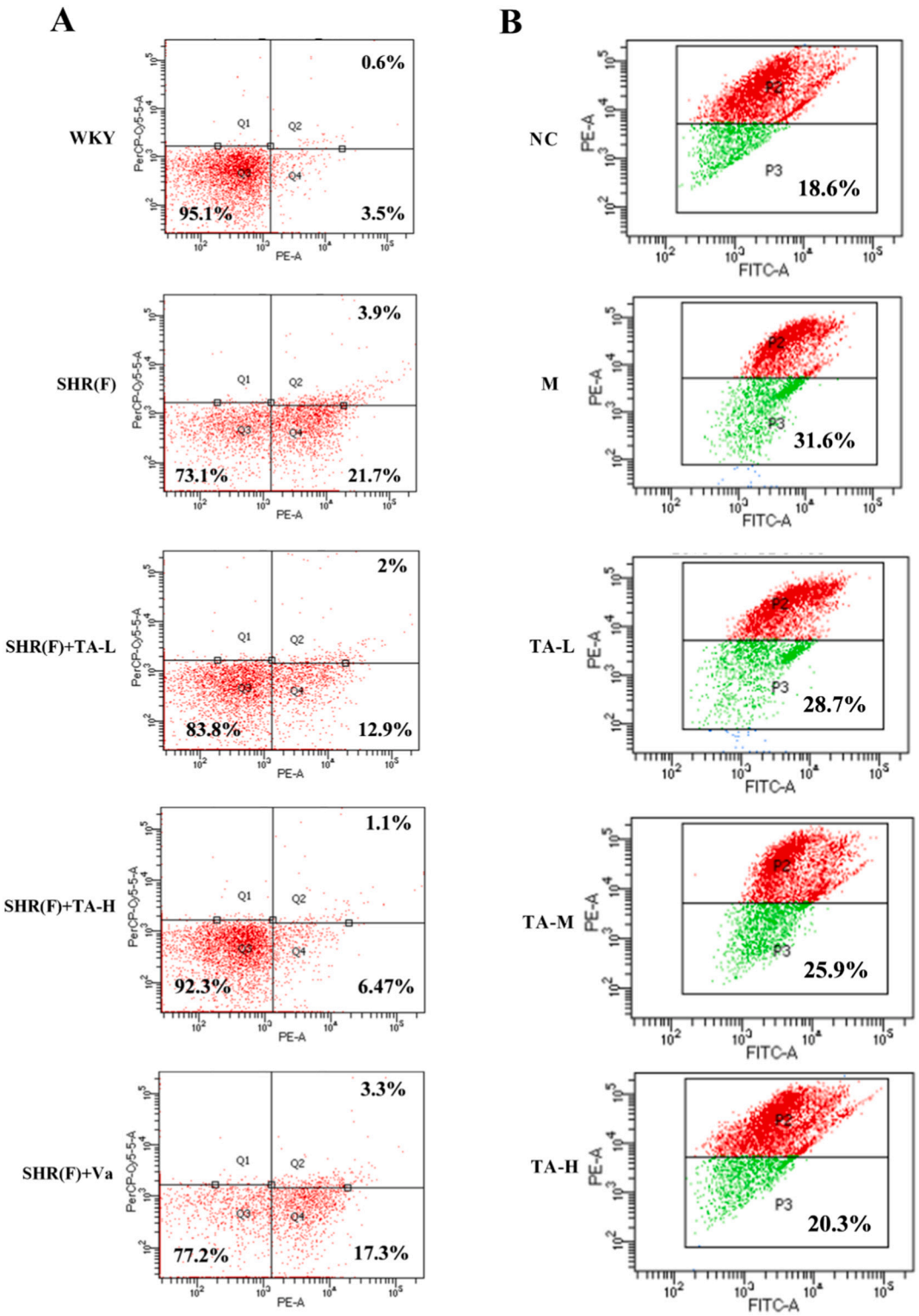

Fig. 3. Taxifolin inhibits renal cell apoptosis in MS rats. Rats and cells were treated as described in "Materials and methods" respectively. (A) The effect of taxifolin on the rate of kidney cell apoptosis in rats. The lower right quadrant represents the rate of early apoptosis. (B) The effect of taxifolin on the levels of mitochondrial membrane potential. Increased membrane potential is represented in red and decreased membrane potential is represented in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Taxifolin improves glucose homeostasis and insulin signaling via PI3K/AKT pathway in MS rats

Insulin signaling pathway in the kidney is thought to contribute to the maintenance of the renal glucose homeostasis. Therefore, it was studied whether taxifolin modulates the insulin signaling in the renal, and total and phosphorylated levels of main proteins of this pathway were assayed by Western blot. PI3K is associated to an earlier response to insulin stimulation, was diminished in SHR(F) rats in comparison with WKY rats. But taxifolin could upregulation of PI3K (Fig. 4A). As

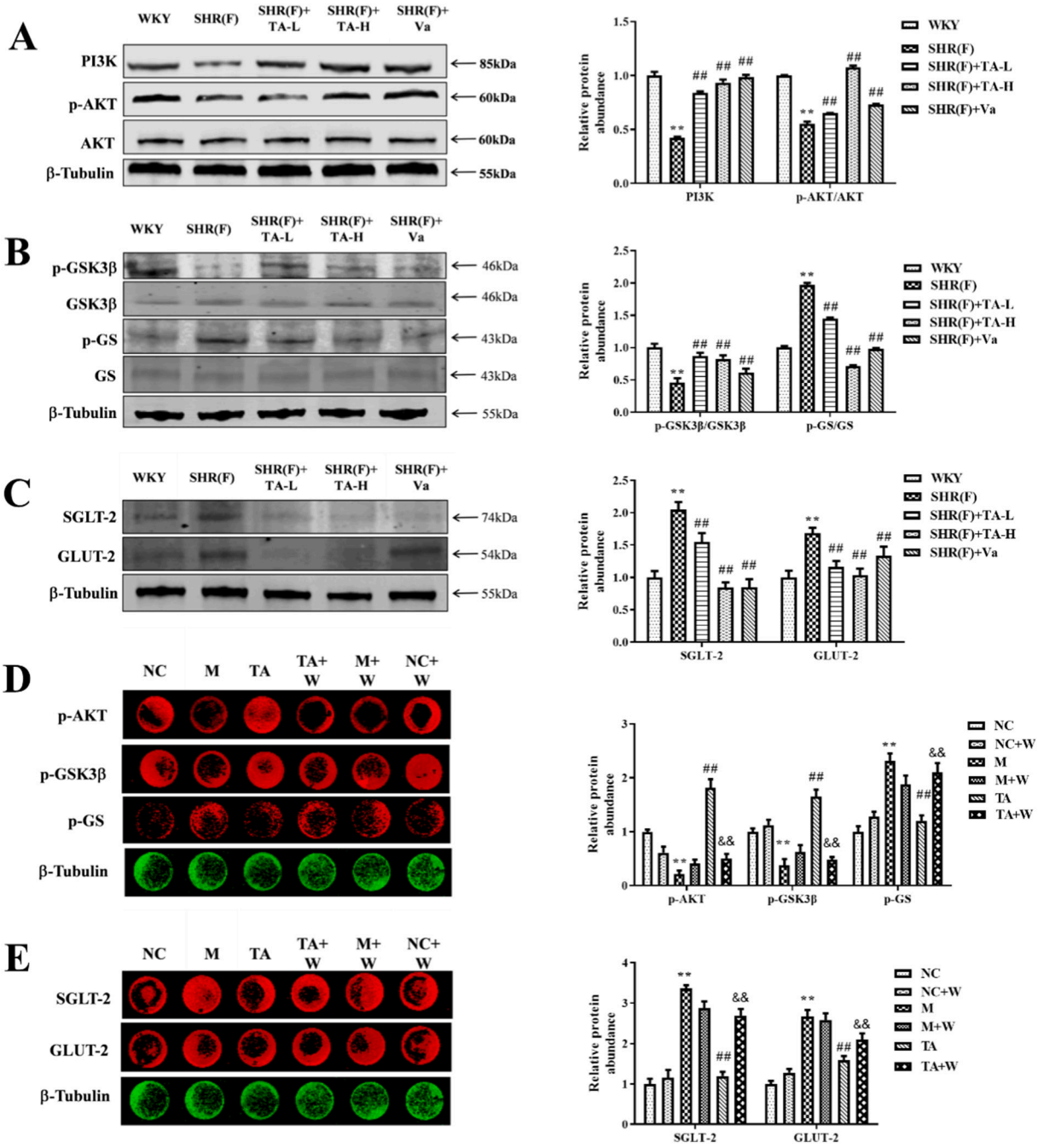

Fig. 4. Taxifolin improves glucose homeostasis and insulin signaling via PI3K/AKT pathway in MS rats. Rats and cells were treated as described in "Materials and methods" respectively. (A) The effects of taxifolin on levels of PI3K and p-AKT/AKT. (B) The effects of taxifolin on levels of p-GSK3β/GSK-3β and p-GS/GS. (C) The effects of taxifolin on levels of SGLT-2 and GLUT-2. (D) The effects of wortmannin on levels of p-AKT, p-GSK3β and p-GS in. NRK-52E cells. (E) The effects of wortmannin on levels of SGLT-2 and GLUT-2 in NRK-52E cells. In A, B and C, *$p < 0.05$, **$p < 0.01$ compared with WKY group; $^{\#\#}p < 0.01$ compared with SHR(F) group. In D and E, * *$p < 0.01$ compared with NC group; $^{\#\#}p < 0.01$ compared with M group. $^{\&\&}p < 0.01$ compared with TA group.

shown in Fig. 4B, p-GSK3β decreased in SHR(F) rats when compared to WKY rats. Moreover, p-GS increased in SHR(F) rats. These results indicated that taxifolin prevents the blockage of the insulin signaling cascade observed in SHR(F) rats by modulating main proteins of the insulin pathway, contributing to the glucose homeostasis. In addition, to continue with the study of the glucose homeostasis, key proteins involved in renal glucose transport, namely SGLT-2 and GLUT-2, were evaluated by Western blot. SGLT-2 and GLUT-2 levels significantly increased in the renal of SHR(F) rats when compared to WKY rats, and these effects were partly reverted in SHR(F) rats receiving taxifolin (Fig. 4C). To evaluate the potential protective effect of taxifolin against metabolic syndrome, NRK-52E cells were pre-treated with PI3K antagonist (wortmannin). We found SGLT-2 and GLUT-2 levels significantly increased and the blockage of the insulin signaling cascade after wortmannin was added (Fig. 4D and E). All these suggest that taxifolin might modulate the glucose homeostasis and insulin signaling via PI3K/AKT pathway in MS rats.

3.5. *Taxifolin ameliorates water and sodium retention via PI3K pathway in MS rats*

To assess the effects of taxifolin on renal excretory function, we

measured urine volume, osmotic pressure, urinary sodium and chloride excretion. As shown in Fig. 5A, urine volume and osmotic pressure were significantly reduced in SHR(F) rats compared to WKY rats. We also observed significant decrease in urinary sodium and chloride excretion. Fig. 5B showed Na^+/K^+-ATPase activity increased in SHR(F) rats. The changes of these indexes indicated that renal excretory function in SHR (F) rats were weakened and water and sodium retention occurred. However, taxifolin treatment significantly ameliorated these indexes. In addition, the effects of taxifolin on RAAS related indexes and Na^+/K^+-ATPase were also detected. The results showed that these indicators were significantly increased, suggesting RAAS activation in MS rats (Fig. 5C). Taxifolin treatment inhibited the activation of RAAS. As a positive control, valsartan also inhibited the activation of RAAS. In cell experiments, Na^+/K^+-ATPase and AngII activity were increased in the cell model induced by high glucose and high salt, and their activity were significantly reduced after treatment with taxifolin. After the addition of PI3K antagonist (wortmannin), the activity of Na^+/K^+-ATPase and AngII increased, suggesting that taxifolin improved the water and sodium retention, inhibited the activation of RAAS through PI3K, and thus control the increase of blood pressure (Fig. 5D).

3.6. *Taxifolin decreases the production of inflammatory cytokines via PI3K pathway in MS rats*

To assess the effect of taxifolin on the proinflammatory cytokine production, the levels of TNF-α, IL-6, CD45 were evaluated. As shown in Fig. 6A, B and C, the levels of inflammatory cytokines TNFα, IL-6, CD45 significantly elevated while taxifolin attenuated MS-induced changes in

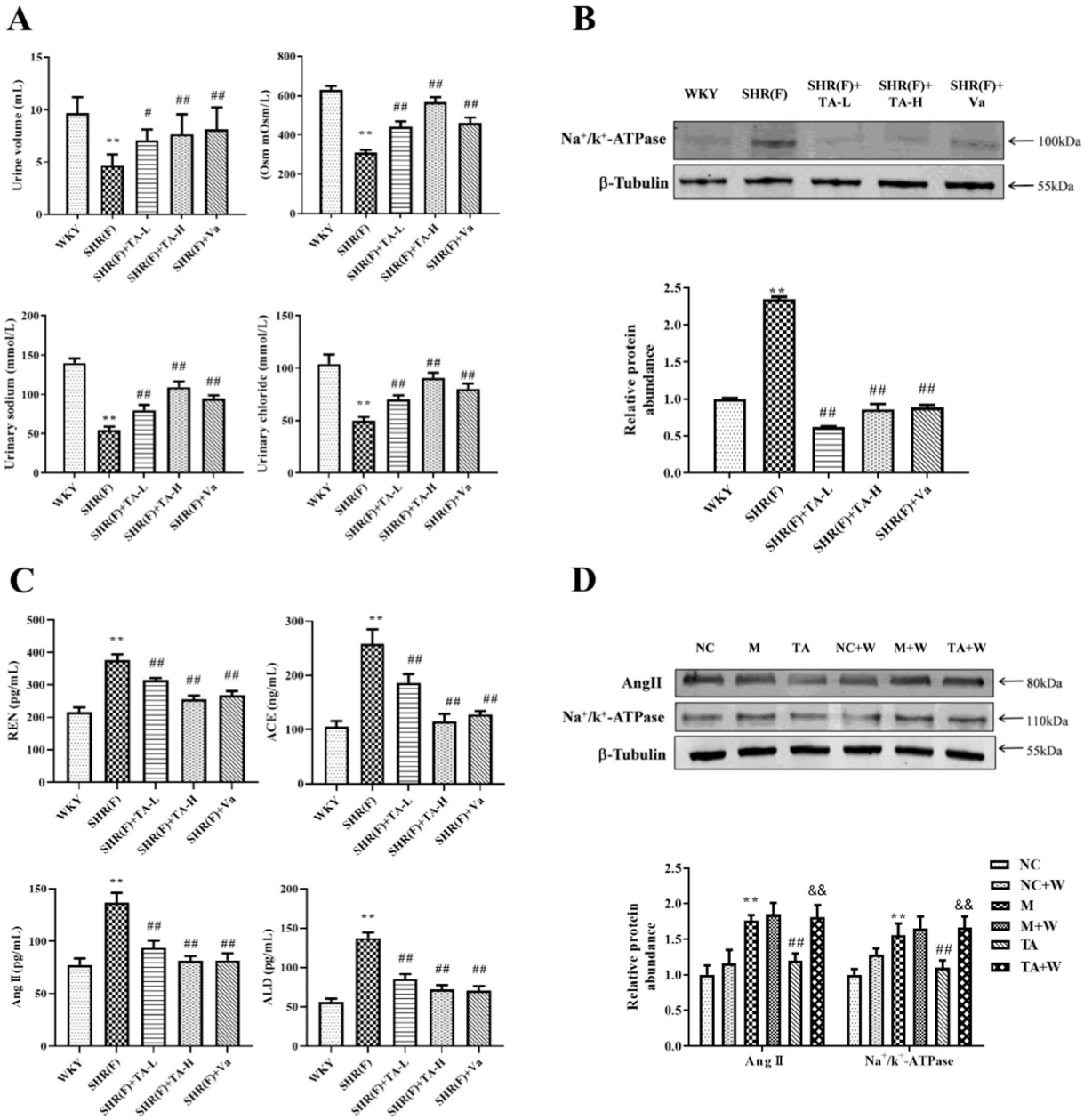

Fig. 5. Taxifolin ameliorates water and sodium retention via PI3K pathway in MS rats. Rats and cells were treated as described in "Materials and methods" respectively. (A) The effects of taxifolin on urine volume, osmotic pressure, urinary sodium and chloride excretion. (B) The effect of taxifolin on levels of Na^+/K^+-ATPase in MS rats. (C) The effect of taxifolin on levels of RAAS. (D) The effects of wortmannin on levels of Na^+/K^+-ATPase and AngII. In A, B and C, $^{**}p < 0.01$ compared with WKY group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with SHR(F) group. In D, $^{**}p < 0.01$ compared with NC group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with M group. $^{\&\&}p < 0.01$ compared with TA group.

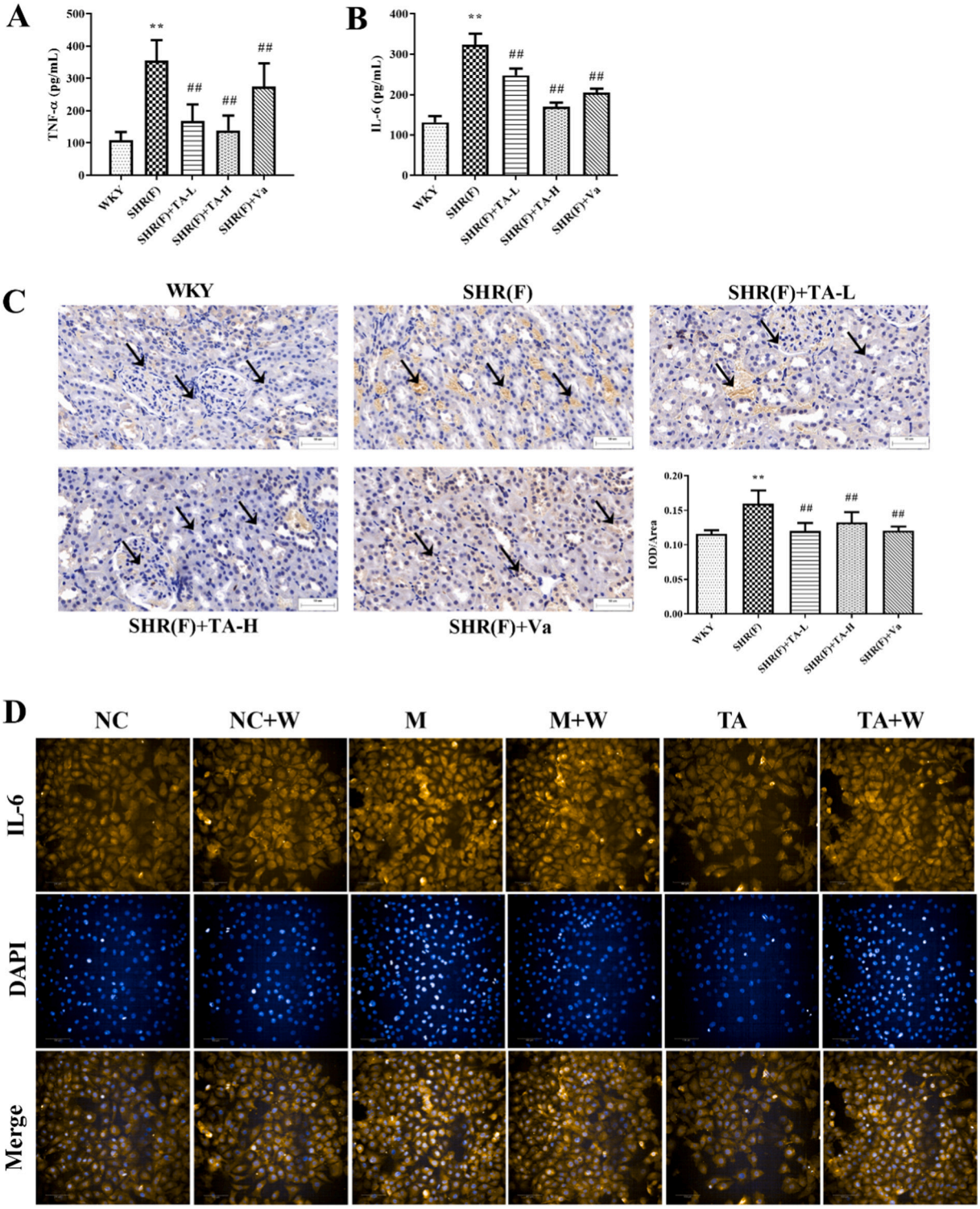

Fig. 6. Taxifolin decreases the production of inflammatory cytokines via PI3K pathway in MS rats. Rats and cells were treated as described in "Materials and methods" respectively. (A) The effect of taxifolin on TNF-α. (B) The effect of taxifolin on IL-6. (C) The effect of taxifolin on CD45 (40× magnification). (D) The effect of wortmannin on IL-6 (20× magnification). **p < 0.01 compared with WKY group; ##p < 0.01 compared with SHR(F) group.

the levels of inflammatory cytokines to different degrees. In the vitro experiment, we evaluated the levels of IL-6. The levels of IL-6 significantly elevated after the addition of PI3K antagonist (wortmannin). This indicated that the inflammatory response of cells was enhanced after the inhibition of PI3K, and taxifolin might decrease the production of inflammatory cytokines via PI3K pathway in MS rats (Fig. 6D).

4. Discussion

Metabolic syndrome (MS) centers on insulin resistance and is characterized by elevated blood pressure, abnormal blood glucose and dyslipidemia. When insulin resistance occurs, glucose tolerance decreases, resulting in imbalance of glucose homeostasis in the body. Persistent high glucose levels (glucose toxicity) can cause kidney damage

[14,34,37–39]. All these metabolic alterations were observed after 7 weeks of fructose induction in SHR(F) rats, which demonstrate that metabolic syndrome occurred in rats. However, all these parameters were highly reverted to normal value after taxifolin treatment, indicating that taxifolin improves insulin resistance, lowers blood pressure and dyslipidemia. In this experiment, we also found that the kidney function of rats with metabolic syndrome was seriously damaged by detecting the indexes of kidney function: Cre, BUN and NAG. The pathological changes of the kidney were observed by H&E, Masson and PAS staining, in addition to the development of glomerular cyst dilatation, renal tubular epithelial cell shedding and vacuolization, which indicated that long-term metabolic disturbance would cause obvious kidney injury. While taxifolin significantly protected against kidney damage in MS rats.

The kidney plays a crucial role in the maintenance of glucose homeostasis. The PI3K/AKT signaling pathway is a classic pathway for insulin signaling, which can regulate glucose transport, cell differentiation, proliferation and apoptosis by activating insulin [2]. Under normal circumstances, insulin activates the downstream substrate through receptors on the cell membrane, binding it to PI3K, and activating AKT [40,41]. As a downstream protein of AKT, GSK3β is a rate-limiting enzyme with phosphorylation regulation of glycogen synthesis [42]. GSK3β reduces glycogen synthesis by inhibiting glycogen synthase GS. In the metabolic syndrome, glucose uptake is increased in the kidney, and also the insulin signaling pathway seems to be altered, contributing all these pathological changes to sustain the high levels of glucose. As shown in the results, compared with WKY rats, the levels of p-GSK3β decreased and the levels of p-GS increased in the kidney cortex in SHR(F) rats, suggesting that the glycogen synthesis content increased. However, taxifolin prevented the blocking of insulin signaling cascade observed in SHR(F) rats, reduced glycogen synthesis and promoted glucose homeostasis by calling back these changes.

At the same time, sustained high levels of glucose (glucose toxicity) in the body can cause damage to tubular cells in the kidney due to increased glycogen synthesis [39]. This may be related to glucose transporters SGLT-2 and GLUT-2 in the kidney [43–45]. In general, insulin promotes glucose uptake via a signaling cascade involving many enzymes. Insulin activates the PI3K/AKT signaling pathway by binding to the IRS-1, then phosphorylates protein p-AKT to promote GLUT2 transportation of glucose into kidney. Moreover , SGLT-2 is a transporter with low affinity and high transport capacity, mainly distributed in the proximal convoluted tubule of the kidney. Its main physiological function is to complete the reabsorption of 90% glucose in the glomerular filtration fluid. The filtration of glucose in the glomeruli and the reabsorption of glucose in the proximal convoluted tubules increased under high glucose conditions. Therefore, the activity of SGLT-2 is enhanced. Our experimental results also confirmed this hypothesis. The expression levels of SGLT-2 and GLUT-2 in SHR(F) rats were significantly increased, and the levels of these two glucose transporters were significantly decreased after treatment with taxifolin, suggesting that taxifolin can inhibit the uptake of glucose by cells, reduce the reabsorption of glucose by kidney tubules, promote the excretion of glucose, and thereby improve the homeostasis of glucose. In addition, due to diminished the level of SGLT-2 and decreased proximal glucose reabsorption, normal tubule-glomerular feedback mechanisms may be restored, contributing to reduced glomerular filtration. To further explore the pharmacologic activities of taxifolin, we established a high glucose and salt cell model to evaluate its efficacy and investigate their mechanism. In vitro experiments, we added the PI3K antagonist (wortmannin) to further verify whether taxifolin can improve glucose metabolism disorder via PI3K/AKT pathway. Our findings demonstrated that taxifolin no longer exerted its pharmacological action and had no improvement on the disorder of glucose metabolism after the addition of wortmannin. The above research showed that taxifolin can improve glycaemic metabolism and insulin sensitivity of MS rats via PI3K/AKT pathway.

The important role of kidney in blood pressure regulation is manifested in the regulation of sodium reabsorption and water and salt metabolism in kidney tubules [46]. Metabolic syndrome is often associated with elevated blood pressure. Studies have shown that the pathogenesis of hypertension is related to water and sodium retention. Moreover, the highest content of Na^+/K^+-ATPase in kidney tissue can promote the reabsorption of sodium in glomerular filtrate, and the water can also be reabsorbed under the effect of osmotic pressure. The change of its activity will cause the abnormal reabsorption of sodium by the kidney, resulting in the disorder of the internal environment of the body and playing an important role in the occurrence and development of hypertension [47]. In addition, long-term insulin resistance can lead to overactivation of renin-angiotensin-aldosterone system. An inappropriately activated intrarenal RAAS promotes angiotensin II formation, causing sodium retention and increased arterial pressure [48–50]. In this study, we assessed the effects of taxifolin on sodium water retention and RAAS in MS rats. The results showed that urine volume, urine osmotic pressure, urine sodium and chlorine excreted decreased, and the activity of Na^+/K^+-ATPase increased, which suggested that water and sodium retention occurred in SHR(F) rats. The renin-angiotensin-aldosterone system was activated, aldosterone secretion increased and blood pressure increased. After taxifolin treatment, it could significantly increase urine volume and urinary sodium excretion, reduce the reabsorption of sodium by renal tubules, increase urine osmotic pressure and inhibit the activity of Na^+/K^+-ATPase, which suggested that taxifolin can improve water and sodium retention caused by metabolic syndrome and regulate blood pressure. While in vitro experiments, our findings demonstrated that the activity of Na^+/K^+-ATPase increased and the overexpression of Ang II (a key factor of RAAS) in M group cells. Taxifolin can reduce the activity of Na^+/K^+-ATPase and the levels of AngII. However, the activity of Na^+/K^+-ATPase and the RAAS were still in an overactivated state after the addition of wortmannin. These results suggested that taxifolin may affect sodium water retention and the activation of renin-angiotensin-aldosterone system by inhibiting PI3K.

Apoptosis can interact with many vital processes such as inflammation, programmed cell death, and adaptive immunity. Apoptosis has been previously shown to play a key role in MS progression [51,52]. PI3K/AKT signaling pathways plays an important regulatory role in the release process of inflammatory mediators and the proliferation process of inflammatory cells. After activation of the PI3K/Akt signaling pathway, the activation of AKT (P-AKT) by enhancing the activity of NF-κB increases the expression of a variety of inflammatory factors, such as: TNF-α and IL-6. Flow cytometry results show that in comparison with WKY rats, the apoptosis rate of primary renal cells was increased in SHR (F) rats, and taxifolin significantly reduced this rate. The decrease of mitochondrial membrane potential is one of the markers of early apoptosis. In vitro experiments, flow cytometry results showed that taxifolin increased the mitochondrial membrane potential in a dose dependent manner. The excessive release of inflammatory mediators also plays an important role in the development of metabolic syndrome. Kidney damage induced by metabolic syndrome will cause inflammatory reaction in the body and release a large number of inflammatory cytokines [53]. In the present study, the proinflammatory factors, TNF-α and IL-6 were measured by ELISA method, and CD45 was measured by immunohistochemical staining. The results showed that SHR(F) rats released a large number of inflammatory factors, of which TNF-α, IL-6, and CD45 play important roles in the pathologic process of renal injury. But taxifolin can alleviate kidney damage by inhibiting these inflammatory factors. However, this protective effect was blocked by the inhibition of PI3K gene, indicating taxifolin might decrease the production of inflammatory cytokines via PI3K pathway in MS rats.

The kidney is the main organ regulating glucose homeostasis and blood pressure and plays an important role in metabolic syndrome rats. Insulin resistance is a central component of the metabolic syndrome, and the PI3K/AKT insulin signaling pathway also plays an important role. This study explored the regulation mechanism of taxifolin on kidney

glucose and blood pressure by selecting this signaling pathway. Through the PI3K/AKT signaling pathway, taxifolin can improve the downstream GSK3β/GS signaling pathway to reduce glycogen synthesis, inhibiting glucose uptake by SGLT-2 and GLUT-2. At the same time, it can also reduce blood pressure through inhibiting RAAS and the level of Na^+/K^+-ATPase to improve water and sodium retention and reduce the inflammatory response.

5. Conclusion

Taxifolin can regulate glucose homeostasis, inhibit excessive activation of RAAS system, improve water and sodium retention, reduce inflammatory reaction, and thereby improve disorders of glucose metabolism and water-salt metabolism in kidney through PI3K/AKT signaling pathway in metabolic syndrome rats.

CRediT authorship contribution statement

Xiaoke Zheng and Liyuan Gao designed the experiments. Liyuan Gao performed the experiments, analyzed the raw data, and wrote the manuscript. Peipei Yuan assisted with the experiments and modified the manuscript. Qi Zhang assisted in feeding the rats and provided suggestions. Yang Fu and Ying Hou also provided suggestions. Yaxin Wei assisted with ELISA and assays. Weisheng Feng supervised the project.

Declaration of competing interest

The authors declare no conflict of interest.

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References

[1] D.T. de Oliveira, I.D. Fernandes, G.G. de Sousa, T.A.P. dos Santos, N.C.N. de Paiva, C.M. Carneiro, E.A. Evangelista, N.R. Barboza, R. Guerra-Sa, High-sugar diet leads to obesity and metabolic diseases in ad libitum-fed rats irrespective of caloric intake, Arch. Endocrinol. Metab. 64 (1) (2020) 71–81, https://doi.org/10.20945/2359-3997000000199.

[2] G. Sadi, G. Sahin, A. Bostanci, Modulation of renal insulin signaling pathway and antioxidant enzymes with streptozotocin-induced diabetes: effects of resveratrol, Medicina-Lithuania 55 (1) (2019), https://doi.org/10.3390/medicina55010003. UNSP 3.

[3] J. Dupas, A. Feray, C. Goanvec, A. Guernec, N. Samson, P. Bougaran, F. Guerrero, J. Mansourati, Metabolic syndrome and hypertension resulting from fructose enriched diet in Wistar rats, Biomed. Res. Int. 2017 (2017), 2494067, https://doi.org/10.1155/2017/2494067.

[4] S.G. Zhu, Y.L. Zhu, Z.F. Huang, D.Y. Zhai, T.T. Zhang, Q. Zhou, Y.F. Mu, D.Z. Liu, W.H. Liu, G.L. Xu, X.F. Liu, Effects of metabolic syndrome on multi-vessel lesions of symptomatic intracranial atherosclerosis, Zhonghua Yi Xue Za Zhi 93 (21) (2013) 1622–1625.

[5] I.M. Wahba, R.H. Mak, Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease, Clin. J. Am. Soc. Nephrol. 2 (3) (2007) 550–562, https://doi.org/10.2215/Cjn.04071206.

[6] V. Jha, G. Garcia-Garcia, K. Iseki, Chronic kidney disease: global dimension and perspectives (vol 382, pg 260, 2013), Lancet 382 (9888) (2013) 208.

[7] J. Baynes, D.B. Murray, Cardiac and renal function are progressively impaired with aging in Zucker diabetic fatty type II diabetic rats, Oxidative Med. Cell. Longev. 2 (5) (2009) 328–334, https://doi.org/10.4161/oxim.2.5.9831.

[8] A. Mitrakou, Kidney: its impact on glucose homeostasis and hormonal regulation, Diabetes Res. Clin. Pr. 93 (2011) S66–S72, https://doi.org/10.1016/S0168-8227(11)70016-X.

[9] D. Alvarez-Cilleros, E. Lopez-Oliva, L. Goya, M.A. Martin, S. Ramos, Cocoa intake attenuates renal injury in Zucker Diabetic fatty rats by improving glucose homeostasis, Food Chem. Toxicol. 127 (2019) 101–109, https://doi.org/10.1016/j.fct.2019.03.002.

[10] W. Cao, M. Shi, L.L. Wu, Z.C. Yang, X.B. Yang, H.F. Liu, X. Xu, Y.H. Liu, C. S. Wilcox, F.F. Hou, A renal-cerebral-peripheral sympathetic reflex mediates insulin resistance in chronic kidney disease, Ebiomedicine 37 (2018) 281–293, https://doi.org/10.1016/j.ebiom.2018.10.054.

[11] O.D. John, P. Mouatt, M.E. Majzoub, T. Thomas, S.K. Panchal, L. Brown, Physiological and metabolic effects of yellow mangosteen (Garcinia dulcis) rind in rats with diet-induced metabolic syndrome, Int. J. Mol. Sci. 21 (1) (2020), https://doi.org/10.3390/ijms21010272. ARTN 272.

[12] C. Sierra-Ramos, S. Velazquez-Garcia, A. Vastola-Mascolo, G. Hernandez, N. Faresse, D.A. de la Rosa, SGK1 activation exacerbates diet-induced obesity, metabolic syndrome and hypertension, J. Endocrinol. 244 (1) (2020) 149–162, https://doi.org/10.1530/Joe-19-0275.

[13] V. Sukumaran, H. Tsuchimochi, T. Sonobe, M.T. Waddingham, M. Shirai, J. T. Pearson, Liraglutide treatment improves the coronary microcirculation in insulin resistant Zucker obese rats on a high salt diet, Cardiovasc. Diabetol. 19 (1) (2020), https://doi.org/10.1186/s12933-020-01000-z. ARTN 24.

[14] F. Artunc, E. Schleicher, C. Weigert, A. Fritsche, N. Stefan, H.U. Haring, The impact of insulin resistance on the kidney and vasculature, Nat. Rev. Nephrol. 12 (12) (2016) 721–737, https://doi.org/10.1038/nrneph.2016.145.

[15] B. Brouwers, V.P.E.G. Pruniau, E.J.G. Cauwelier, F. Schuit, E. Lerut, N. Ectors, J. Declercq, J.W.M. Creemers, Phlorizin pretreatment reduces acute renal toxicity in a mouse model for diabetic nephropathy, J. Biol. Chem. 288 (38) (2013) 27200–27207, https://doi.org/10.1074/jbc.M113.469486.

[16] A. Siani, P. Russo, F.P. Cappuccio, R. Iacone, A. Venezia, O. Russo, G. Barba, L. Iacoviello, P. Strazzullo, Combination of renin-angiotensin system polymorphisms is associated with altered renal sodium handling and hypertension, Hypertension 43 (3) (2004) 598–602, https://doi.org/10.1161/01.HYP.0000117985.57001.b3.

[17] X. Xiao, H.J. Du, W.J. Hu, P.X. Shaw, The influence of long term hydrochlorothiazide administration on the relationship between renin-angiotensin-aldosterone system activity and plasma glucose in patients with hypertension, Oxidative Med. Cell. Longev. 2013 (2013), https://doi.org/10.1155/2013/434618. Unsp 434618.

[18] E. Faloia, C. Gatti, M.A. Camilloni, B. Mariniello, C. Sardu, G.G. Garrapa, F. Mantero, G. Giacchetti, Comparison of circulating and local adipose tissue renin-angiotensin system in normotensive and hypertensive obese subjects, J. Endocrinol. Investig. 25 (4) (2002) 309–314, https://doi.org/10.1007/BF03344010.

[19] Q.L. Fang, L.P. Zhao, Y. Wang, Y.L. Zhang, Z.Y. Li, Y. Pan, K. Kanchana, J.Y. Wang, C. Tong, D. Li, G. Liang, A novel chalcone derivative attenuates the diabetes-induced renal injury via inhibition of high glucose-mediated inflammatory response and macrophage infiltration, Toxicol. Appl. Pharm. 282 (2) (2015) 129–138, https://doi.org/10.1016/j.taap.2014.10.021.

[20] Z. Ming, D.D. Smyth, W.W. Lautt, Decreases in portal flow trigger a hepatorenal reflex to inhibit renal sodium and water excretion in rats: role of adenosine, Hepatology 35 (1) (2002) 167–175, https://doi.org/10.1053/jhep.2002.30425.

[21] R.A. Gonzalez-Villalobos, T. Janjoulia, N.K. Fletcher, J.F. Giani, M.T. Nguyen, A. D. Riquier-Brison, D.M. Seth, S. Fuchs, D. Eladari, N. Picard, S. Bachmann, E. Delpire, J. Peti-Peterdi, L.G. Navar, K.E. Bernstein, A.A. McDonough, The absence of intrarenal ACE protects against hypertension, J. Clin. Invest. 123 (5) (2013) 2011–2023, https://doi.org/10.1172/JCI65460.

[22] L.A. Calo, V. Ravarotto, F. Simioni, E. Naso, F. Marchini, L. Bonfante, L. Furian, P. Rigotti, Pathophysiology of post transplant hypertension in kidney transplant: focus on calcineurin inhibitors induced oxidative stress and renal sodium retention and implications with RhoA/Rho kinase pathway, Kidney Blood Press. Res. 42 (4) (2017) 676–685, https://doi.org/10.1159/000483023.

[23] A.V. Galochkina, V.V. Zarubaev, O.I. Kiselev, V.A. Babkin, L.A. Ostroukhova, Antiviral activity of the dihydroquercetin during the coxsackievirus B4 replication in vitro, Vopr. Virusol. 61 (1) (2016) 27–31.

[24] E. Awad, A.S. Awaad, M.A. Esteban, Effects of dihydroquercetin obtained from deodar (Cedrus deodara) on immune status of gilthead seabream (Sparus aurata L.), Fish Shellfish Immunol. 43 (1) (2015) 43–50, https://doi.org/10.1016/j.fsi.2014.12.009.

[25] V.S. Rogovskii, A.I. Matiushin, N.L. Shimanovskii, A.V. Semeikin, T.S. Kukhareva, A.M. Koroteev, M.P. Koroteev, E.E. Nifant'ev, Antiproliferative and antioxidant activity of new dihydroquercetin derivatives, Eksp. Klin. Farmakol. 73 (9) (2010) 39–42.

[26] M.B. Plotnikov, O.I. Aliev, A.V. Sidekhmenova, A.Y. Shamanaev, A. M. Anishchenko, A.V. Nosarev, E.A. Pushkina, Modes of hypotensive action of dihydroquercetin in arterial hypertension, Bull. Exp. Biol. Med. 162 (3) (2017) 353–356, https://doi.org/10.1007/s10517-017-3614-4.

[27] Y. Zhang, J. Yu, X.D. Dong, H.Y. Ji, Research on characteristics, antioxidant and antitumor activities of dihydroquercetin and its complexes, Molecules 23 (1) (2018), https://doi.org/10.3390/molecules23010020. ARTN 20.

[28] M. Oron-Herman, Y. Kamari, E. Grossman, G. Yeger, E. Peleg, Z. Shabtay, A. Shamiss, Y. Sharabi, Metabolic syndrome: comparison of the two commonly used animal models, Am. J. Hypertens. 21 (9) (2008) 1018–1022, https://doi.org/10.1038/ajh.2008.218.

[29] K.L.H. Wu, C.W. Wu, Y.L. Tain, Y.M. Chao, C.Y. Hung, P.C. Tsai, W.S. Wang, C. D. Shih, Effects of high fructose intake on the development of hypertension in the spontaneously hypertensive rats: the role of AT(1)R/gp91 (PHOX) signaling in the

rostral ventrolateral medulla, J. Nutr. Biochem. 41 (2017) 73–83, https://doi.org/10.1016/j.jnutbio.2016.11.013.
[30] L. Zhou, Y.Q. Pan, R. Chonan, R. Batey, X.L. Rong, J. Yamahara, J.W. Wang, Y. H. Li, Mitigation of insulin resistance by mangiferin in a rat model of fructose-induced metabolic syndrome is associated with modulation of CD36 redistribution in the skeletal muscle, J. Pharmacol. Exp. Ther. 356 (1) (2016) 74–84, https://doi.org/10.1124/jpet.115.229005.
[31] P.W. Cheng, Y.T. Lin, W.Y. Ho, P.J. Lu, H.H. Chen, C.C. Lai, G.C. Sun, T.C. Yeh, M. Hsiao, C.J. Tseng, C.P. Liu, Fructose induced neurogenic hypertension mediated by overactivation of p38 MAPK to impair insulin signaling transduction caused central insulin resistance, Free Radical Biol. Med. 112 (2017) 298–307, https://doi.org/10.1016/j.freeradbiomed.2017.07.022.
[32] Y.N. Tong, J.L. Chuan, L. Bai, J.Y. Shi, L. Zhong, X.M. Duan, Y.X. Zhu, The protective effect of shikonin on renal tubular epithelial cell injury induced by high glucose, Biomed. Pharmacother. 98 (2018) 701–708, https://doi.org/10.1016/j.biopha.2017.12.112.
[33] D. Alvarez-Cilleros, M.A. Martin, S. Ramos, (−)-Epicatechin and the colonic 2,3-dihydroxybenzoic acid metabolite regulate glucose uptake, glucose production, and improve insulin signaling in renal NRK-52E cells, Mol. Nutr. Food Res. 62 (4) (2018), https://doi.org/10.1002/mnfr.201700470 (doi:ARTN 1700470).
[34] D. Alvarez-Cilleros, M.A. Martin, S. Ramos, Protective effects of (−)-epicatechin and the colonic metabolite 3,4-dihydroxyphenylacetic acid against glucotoxicity-induced insulin signalling blockade and altered glucose uptake and production in renal tubular NRK-52E cells, Food Chem. Toxicol. 120 (2018) 119–128, https://doi.org/10.1016/j.fct.2018.07.003.
[35] P. Yuan, X. Zheng, M. Li, Y. Ke, Y. Fu, Q. Zhang, X. Wang, W. Feng, Two sulfur glycoside compounds isolated from Lepidium apetalum Willd protect NRK52e cells against Hyp ertonic-induced adhesion and inflammation by suppressing the MAPK signaling pathway and RAAS, Molecules 22 (11) (2017).
[36] J. Dong, H. Xu, H. Xu, P.F. Wang, G.J. Cai, H.F. Song, C.C. Wang, Z.T. Dong, Y. J. Ju, Z.Y. Jiang, Nesfatin-1 stimulates fatty-acid oxidation by activating AMP-activated protein kinase in STZ-induced type 2 diabetic mice, PLoS One 8 (12) (2013), e83397, https://doi.org/10.1371/journal.pone.0083397.
[37] I. Cordero-Herrera, M.A. Martin, L. Goya, S. Ramos, Cocoa flavonoids attenuate high glucose-induced insulin signalling blockade and modulate glucose uptake and production in human HepG2 cells, Food Chem. Toxicol. 64 (2014) 10–19, https://doi.org/10.1016/j.fct.2013.11.014.
[38] S. Tiwari, V.K.M. Halagappa, S. Riazi, X.Q. Hu, C.A. Ecelbarger, Reduced expression of insulin receptors in the kidneys of insulin-resistant rats, J. Am. Soc. Nephrol. 18 (10) (2007) 2661–2671, https://doi.org/10.1681/Asn.2006121410.
[39] S. Song, M. Dang, M. Kumar, Anti-inflammatory and renal protective effect of gingerol in high-fat diet/streptozotocin-induced diabetic rats via inflammatory mechanism, Inflammopharmacology 27 (6) (2019) 1243–1254, https://doi.org/10.1007/s10787-019-00569-6.
[40] I. Cordero-Herrera, M.A. Martin, L. Bravo, L. Goya, S. Ramos, Cocoa flavonoids improve insulin signalling and modulate glucose production via AKT and AMPK in HepG2 cells, Mol. Nutr. Food Res. 57 (6) (2013) 974–985, https://doi.org/10.1002/mnfr.201200500.
[41] Y.J. Lee, H.J. Han, Troglitazone ameliorates high glucose-induced EMT and dysfunction of SGLTs through PI3K/Akt, GSK-3 beta, Snail1, and beta-catenin in renal proximal tubule cells, Am. J. Physiol. Renal. 298 (5) (2010) F1263–F1275, https://doi.org/10.1152/ajprenal.00475.2009.
[42] M.M. Mariappan, S. Prasad, K. D'Silva, E. Cedillo, K. Sataranatarajan, J.L. Barnes, G.G. Choudhury, B.S. Kasinath, Activation of glycogen synthase kinase 3beta ameliorates diabetes-induced kidney injury, J. Biol. Chem. 289 (51) (2014) 35363–35375, https://doi.org/10.1074/jbc.M114.587840.
[43] K. Jaikumkao, A. Pongchaidecha, V. Chatsudthipong, S.C. Chattipakorn, N. Chattipakorn, A. Lungkaphin, The roles of sodium-glucose cotransporter 2 inhibitors in preventing kidney injury in diabetes, Biomed. Pharmacother. 94 (2017) 176–187, https://doi.org/10.1016/j.biopha.2017.07.095.
[44] A. Mima, Renal protection by sodium-glucose cotransporter 2 inhibitors and its underlying mechanisms in diabetic kidney disease, J. Diabetes Complicat. 32 (7) (2018) 720–725, https://doi.org/10.1016/j.jdiacomp.2018.04.011.
[45] L. Norton, C.E. Shannon, M. Fourcaudot, C. Hu, N.S. Wang, W. Ren, J. Song, M. Abdul-Ghani, R.A. DeFronzo, J. Ren, W.P. Jia, Sodium-glucose co-transporter (SGLT) and glucose transporter (GLUT) expression in the kidney of type 2 diabetic subjects, Diabetes Obes. Metab. 19 (9) (2017) 1322–1326, https://doi.org/10.1111/dom.13003.
[46] L. te Riet, J.H.M. van Esch, A.J.M. Roks, A.H. van den Meiracker, A.H.J. Danser, Hypertension renin-angiotensin-aldosterone system alterations, Circ. Res. 116 (6) (2015) 960–975, https://doi.org/10.1161/Circresaha.116.303587.
[47] Y. Kimura, A. Kuno, M. Tanno, T. Sato, K. Ohno, S. Shibata, K. Nakata, H. Sugawara, K. Abe, Y. Igaki, T. Yano, T. Miki, T. Miura, Canagliflozin, a sodium-glucose cotransporter 2 inhibitor, normalizes renal susceptibility to type 1 cardiorenal syndrome through reduction of renal oxidative stress in diabetic rats, J. Diabetes Invest. 10 (4) (2019) 933–946, https://doi.org/10.1111/jdi.13009.
[48] S. Crestani, A. Gasparotto, M.C.A. Marques, J.C. Sullivan, R.C. Webb, J.E. da Silva-Santos, Enhanced angiotensin-converting enzyme activity and systemic reactivity to angiotensin II in normotensive rats exposed to a high-sodium diet, Vasc. Pharmacol. 60 (2) (2014) 67–74, https://doi.org/10.1016/j.vph.2013.12.001.
[49] W.W. Batenburg, X.F. Lu, F. Leijten, U. Maschke, D.N. Muller, A.H.J. Danser, Renin- and prorenin-induced effects in rat vascular smooth muscle cells overexpressing the human (pro)renin receptor does (pro)renin-(pro)renin receptor interaction actually occur? Hypertension 58 (6) (2011) 1111–1119, https://doi.org/10.1161/Hypertensionaha.111.180737.
[50] S. Yoshida, K. Ishizawa, N. Ayuzawa, K. Ueda, M. Takeuchi, W. Kawarazaki, T. Fujita, M. Nagase, Renin inhibition ameliorates renal damage through prominent suppression of both angiotensin I and II in human renin angiotensinogen transgenic mice with high salt loading, Clin. Exp. Nephrol. 18 (4) (2014) 593–599, https://doi.org/10.1007/s10157-013-0893-6.
[51] V. Malek, A.B. Gaikwad, Telmisartan and thiorphan combination treatment attenuates fibrosis and apoptosis in preventing diabetic cardiomyopathy, Cardiovasc. Res. 115 (2) (2019) 373–384, https://doi.org/10.1093/cvr/cvy226.
[52] X. Chen, L. Ren, X. Liu, X. Sun, C. Dong, Y. Jiang, Y. Qin, H. Qu, J. Jiao, S. Wang, Y. Bai, B. Yang, Ranolazine protects against diabetic cardiomyopathy by activating the NOTCH1/NRG1 pathway, Life Sci. 261 (2020), 118306, https://doi.org/10.1016/j.lfs.2020.118306.
[53] L. Sun, Y.S. Kanwar, Relevance of TNF-alpha in the context of other inflammatory cytokines in the progression of diabetic nephropathy, Kidney Int. 88 (4) (2015) 662–665, https://doi.org/10.1038/ki.2015.250.