Effects of Rhein on the Kidney

Subjects: Pharmacology & Pharmacy

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Rhein is a monomeric component of anthraquinone isolated from rhubarb, a traditional Chinese medicine. It has anti-inflflammation, anti-oxidation, anti-apoptosis, anti-bacterial and other pharmacological activities, as well as a renal protective effects. Rhein exerts its nephroprotective effects mainly through decreasing hypoglycemic and hypolipidemic, playing anti-inflflammatory, antioxidant and anti-fifibrotic effects and regulating drug-transporters. However, the latest studies show that rhein also has potential kidney toxicity in case of large dosages and long use times.

traditional Chinese medicine rhein kidney protection nephrotoxicity

1. Introduction

Rhein (molecular formula $C_{15}H_8O_6$), a lipophilic anthraquinone, is the main component of *Senna alexandrina* Mill., *Rheum palmatum* L., *Aloe barbadensis* Miller, and *Polygonum multiflorum* Thunb [1]. It contains two hydroxyl groups and one carboxyl group and has strong polarity and electrochemical REDOX properties [2]. Rhein has a lot of pharmacological effects, such as anti-inflammation [3], anti-cancer [4], anti-fibrosis [5], antioxidation [6], hepatoprotective [7], nephroprotective [8], lipid-lowering [9], and antimicrobial activities [10]. In spite of this, its poor solubility and low bioavailability limit its clinical applications. The study of rhein and its derivatives has been enriched by advances in drug separation and synthesis. Diacerein is one of the most common and representative derivatives of rhein, and it is used for the treatment of arthritis owing to its ability in reducing osteoclast formation and inhibiting the synthesis of resorptive factors [11]. Additionally, nanodrug delivery systems have been designed to overcome the poor solubility of rhein [12]. The pharmacological effects of these compounds lay the groundwork for the treatment of liver disease, osteoarthritis, diabetes, atherosclerosis, and a variety of cancers [13][14][15][16][17]. However, it has recently been reported that rhein also causes hepatotoxicity and nephrotoxicity [6][18].

2. Nephroprotective Effect

The pharmacological benefits of rhein for human health are increasingly recognized. Rhein, and its derivatives, analogs, and compound preparations show the nephroprotective activities against various kidney disease, especially diabetic nephropathy (DN) and drug-induced acute kidney injury (AKI). One of the most common microvascular complications of diabetes is DN [19], which is a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [20]. The pathophysiology of DN is quite complex with two main initiating factors: abnormal metabolism and hemodynamic activity [21]. Pathogenesis of DN includes metabolic dysregulation, inflammation, oxidative stress, abnormal cytokines, etc., [22]. Considering multiple pathogenic

mechanisms, scholars therefore need to develop drugs with multiple targets to treat DN effectively. The pathogenesis of AKI may be related to the specific injury of renal vessels, glomeruli, renal tubules, or interstitial compartments [23]. AKI can cause apoptosis, autophagy, and regulated and genetically controlled cell death as a result of cell damage [24]. Rhein has a variety of therapeutic targets as a TCM ingredient (or element), and its protective effect on the above targets has been gradually examined. Rhein is highly effective for the treatment of kidney-related diseases among natural components. The schematic mechanism of the signal pathway of the nephroprotective effects of rhein is shown in **Figure 1**.

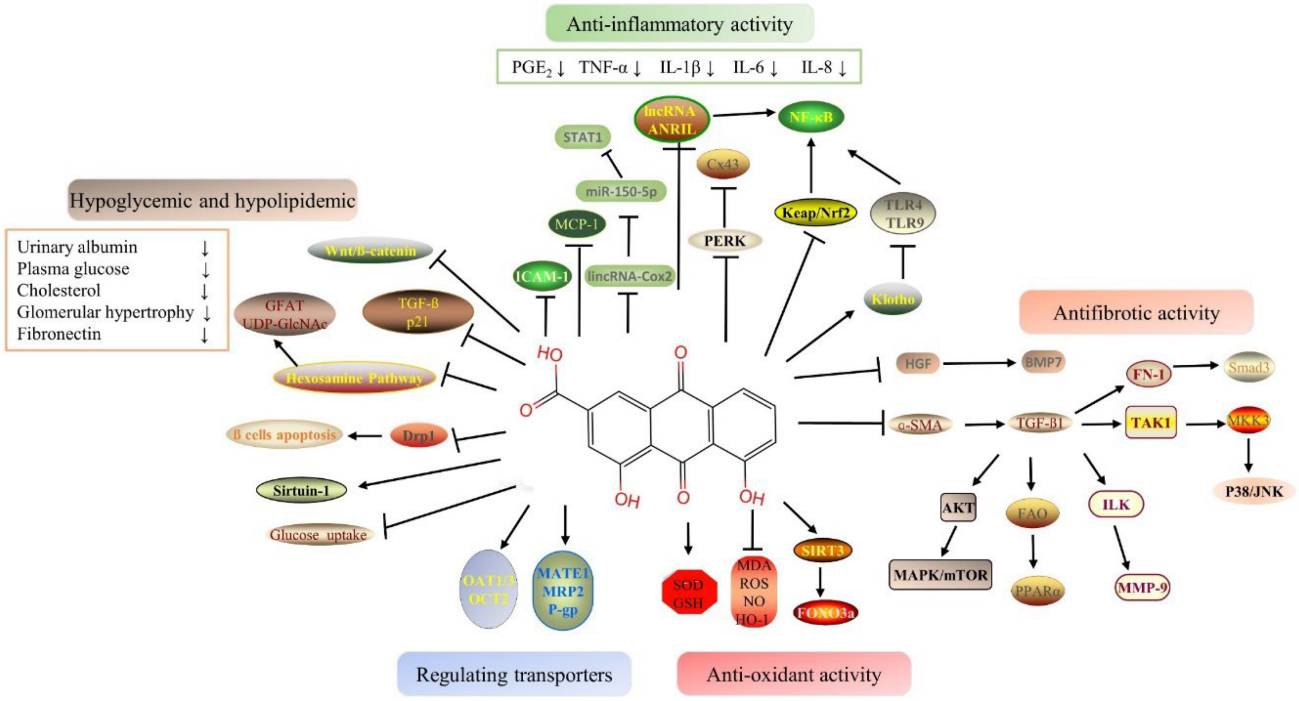

Figure 1. Signal pathway of the nephropropective effects of rhein.

2.1. Hypoglycemic and Hypolipidemic Proprieties of Rhein

Some studies have shown that the mitochondrial event chain caused by hyperglycemia may be one of the pathogeneses of diabetic nephropathy [25]. Rhein treatment showed significant improvements in glucose-dependent and independent insulin secretion in db/db mice by preserving β-cell mass and inhibiting apoptosis [26]. Another study [27] showed that rhein protects pancreatic cells from apoptosis by inhibiting the expression of dynamin-related protein 1 (Drp1). It may be possible to prevent or treat diabetes by rhein in the near future. Rhein has been shown to improve insulin resistance and renal injury in rats and db/db mice [28]. Furthermore, rhein and benazepril were evaluated individually and in combination in diabetic mice. Benazepril and rhein showed similar kidney protection [29]. In db/db mice, a combined application had a better kidney protection effect [29]. This reduced urinary albumin excretion, decreased plasma glucose, cholesterol, relieved glomerular hypertrophy, mesangial expansion and proliferation, and inhibited the expression of fibronectin (FN) and transforming growth factor- β1 (TGF-β1) [29]. In order to elucidate the therapeutic mechanism of rhein on DN, Zheng J et al. (2008) examined the effect of rhein on the hexosamine pathway [30]. Due to its role as a nutrient sensor, the hexosamine pathway has

been associated with metabolic disorders and cellular hypertrophy when glucose levels are high [31]. The hexosamine pathway is one of the mechanisms responsible for renal damage in diabetes. To mimic mesangial cells under diabetic conditions, transgenic mesangial cell lines (MCGT1) overexpressing glucose transporter 1 (GLUT1) were used. GLUT1, an integral membrane protein, transports glucose into mesangial cells via glucose gradients [32].

2.2. Anti-Inflammatory Proprieties of Rhein

As a result of hyperglycemia, advanced glycation end products (AGEs) damage the vascular endothelium, glomeruli, and tubules, which facilitates the emergence of DN eventually [33]. Diabetes is an entity of inflammation because its pathogenesis involves multiple inflammatory/proinflammatory factors, and it is characterized by chronic low-grade inflammatory disease [34]. The model of early diabetic nephropathy was established and showed increases in serum microalbuminuria, NADPH oxidase expression, and PKR-like eukaryotic initiation factor 2α kinase (PERK) level, and a decrease in connexin 43 (Cx43) in renal tissue. Upregulation of nuclear transcription factors, such as PERK, reveals the presence of endoplasmic reticulum stress (ER stress) [35]. Cx43, a family of connexins, may play a key role in exchanging small molecules within glomeruli and tubules in the kidney, which is essential for normal renal function [36]. Argirein, a derivative of rhein, can be produced by combining rhein with L-arginine by forming a hydrogen bond. It has anti-inflammatory activity derived from rhein. Argirein (200, 100, and 50 mg/kg) is more effective than aminoguanidine (AMG) (100 mg/kg), which has anti-inflammatory activity as a positive reference agent in reversing the above changes [36].

The mortality rate of AKI is about 70–80 % [37][38]. Currently, renal replacement therapy (RRT) is the only effective treatment for AKI [39]. Inflammatory response inherent to sepsis is thought to be the direct mechanism of AKI [40]. Based on rhein's anti-inflammatory pharmacological activity, Yu C et al. (2015) explored the effects of rhein on sepsis-induced AKI by using lipopolysaccharide (LPS) and cecal ligation and puncture (CLP) models [41]. The potential mechanism of rhein may be related to its anti-inflammatory effects. Rhein inhibited the activation of NF-κB via restraining the expression and phosphorylation of the relevant proteins in the NF-κB signal pathway and hindering the transcription of NF-κB p65 [41]. It brings a new research direction for solving AKI related to endotoxemia. At the same time, the research of Liu M et al. (2021) also confirms this point [42]. The 5/6 nephrectomy model (5/6 Nx) in rats and LPS-induced HK-2 cells were used, and the results indicated that rhein inhibits inflammatory signaling pathways via decreasing the production of TNF-α, IL-6, and monocyte chemotactic protein (MCP-1) [42]. In addition, by inhibiting NF-κB phosphorylation, rhein diminished LPS-induced NF-κB activation [42]. The above results clearly indicate that rhein can be a promising therapeutic agent for renal disease by inhibiting multiple inflammatory mediators [41][42].

2.3. Antioxidant Proprieties of Rhein

Rhein attenuated APAP-induced hepatotoxicity and nephrotoxicity in a dose-dependent manner [7]. The levels of serum glutamate-pyruvate transaminase (GPT), glutamate-oxaloacetic transaminase (GOT), urea nitrogen (UREA), creatinine (Crea), and reactive oxygen species (ROS) production were significantly decreased, and the

contents of nitric oxide (NO), MDA, and GSH were recovered in the rhein treatment group [7]. Rhein relieved APAP-induced liver and kidney injury by ameliorating oxidative stress [7]. Diacerein (DIA) is used to treat osteoarthritis. DIA enters the body and is rapidly converted into its active metabolite rhein [43]. Furthermore, studies have been conducted one after another on whether DIA can alleviate AKI. The antioxidant effects of DIA were reported to protect renal function against doxorubicin-induced AKI [43].

2.4. Antifibrotic Proprieties of Rhein

CKD and chronic kidney failure (CRF) develop as a result of renal fibrosis. The pathological mechanism of renal fibrosis is relatively complex. There is a variety of stimulating factors or mediators, such as growth factors, cytokines and toxins, which induce the occurrence of fibrosis through a variety of mechanisms and signal pathways [44][45]. TGF-β has been proven to be a major pathogenic factor for the progressive development of renal fibrosis [46]. TGF-β can induce renal tubular epithelial cells to transform into renal mesenchymal fibroblasts through the epithelial–mesenchymal transition (EMT) process [47]. In addition to the TGF-β pathway, the Notch, Wnt, and Hedgehog signaling pathways can also be activated in response to renal injury, thereby promoting renal fibrosis [48].

TGF-β regulates renal fibrosis progression through classic and non-classic pathways. The classical TGF-β pathway includes two signaling pathways, namely TGF-β/Smad and bone morphogenetic protein (BMP) [46]. However, they have opposite effects although they have similar downstream Smad signaling pathways. Smad3 is a key downstream mediator of TGF-β signal transduction [46]. BMP7 is a member of the TGF-β superfamily that antagonizes the effects of TGF-β [49]. Like liver growth factor (HGF), they both have anti-fibrotic effects [49]. Combined therapy of rhein and Danshensu (DSS) had a certain renal protective effect on chronic kidney damage. The mechanism may be related to anti-inflammatory and anti-fibrosis by downregulating the NF-κB-related pathway and inhibiting the TGF-β/Smad3 pathway, respectively [50]. Other studies have shown that rhein improved renal function and reduced renal fibrosis and interstitial inflammation by inducing HGF and BMP7 production [51].

In addition to the classical Smad signaling pathway, TGF-β can also regulate the downstream cellular response through other non-classical pathways and then adjust the pathological process of renal fibrosis [46]. p38 mitogen-activated protein kinase (MAPK) is one of the atypical signaling pathways of TGF-β1. TGF-β1 activates the downstream signaling pathway MKK3-p38 MAPK cascade through the activation of TAK1, ultimately leading to cell fibrosis [52]. Rhubarb and Astragalus capsules (RAC) that contain 2.25 mg/g rhein have been used in a clinical treatment for chronic kidney disease [53].

2.5. Benefits of Rhein Via Drug-Transporter

Renal transporters transport endogenous substances, poisons, and drugs from the blood to the urine. As a result of renal injury, uptake transporters and efflux transporters are altered, which affects toxic excretion and aggravates renal injury [54]. Previous research has shown that the expressions of organic anion transporter 1 (OAT1), OAT3, and multidrug resistance related protein 2 (MRP2) were significantly decreased after cisplatin-induced AKI, which

reduced the excretion of endotoxin and aggravated renal injury [54]. There are few studies on the relationship between the various pharmacological effects of rhein and transporters. Zhu Y et al. (2022) [8] showed that the gene levels and protein expressions of the renal transporters including Oat1, Oat3, Organic cation transporter 2 (OCT2), mammal multidrug, and toxin extrusion proteins 1 (Mate 1), Mrp2, and P-glycoprotein (P-gp) in vancomycin-induced nephrotoxicity (VIN) were significantly decreased. Plasma creatinine, BUN, and plasma indoxyl sulfate were not excreted efficiently. Rhein reversed the expressions of the above transporters, and thereby promoted the excretion of endotoxins and finally alleviated renal injury [8]. The discovery of VIN's pathogenesis expands the field of study on the kidney protection effects of VIN.

3. Toxicological Effects in Kidney

Total rhubarb anthraquinones (TRAs) include emodin, rhein, chrysophanol, aloe emodin, and other substances [55]. However, the clinical cases of liver injury and the progression of kidney disease caused by TCM contained TRAs are increasing. Rhein is an important ingredient in TRAs, which affects the kidneys in a positive and negative way. Studies on the nephrotoxicity effects of rhein and the related mechanisms are shown in **Figure 2**.

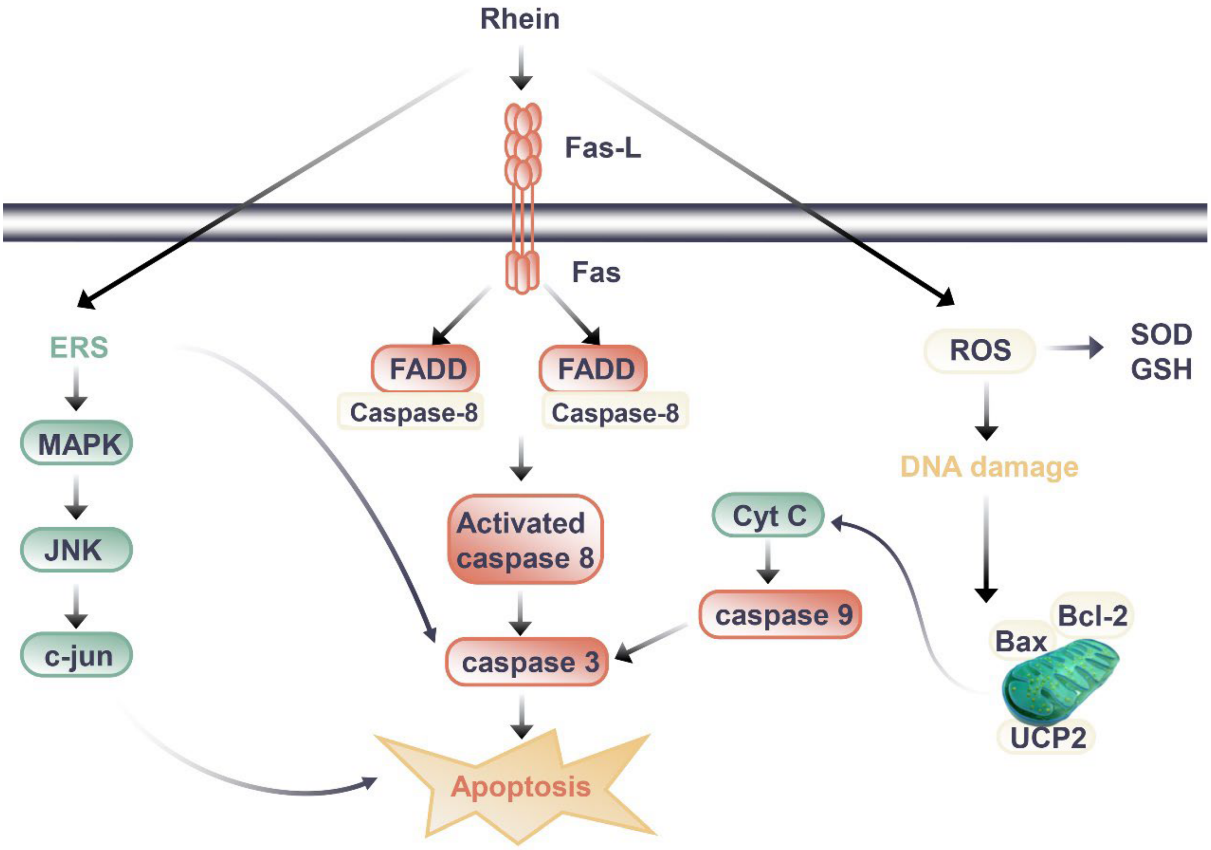

Figure 2. Signal pathway of nephrotoxic effect of rhein.

3.1. Rhein Nephrotoxicity: Mechanisms of Action and Possible Causes

Through the literature review, the difference between the kidney protection and nephrotoxicity effects of rhein may be related to the dosage and duration of rhein. When rhein exerted kidney protection effects, the dosage of rhein in animal experiments was mostly 20 to 150 mg/kg/day, and the duration of administration was mostly less than 14 days, with the longest time being 8 weeks when the rhein was at a slightly lower dosage. In mice, HU Y et al. (2019) observed renal toxicity after long-term administration of rhein [56]. Mice were randomly divided into three groups: blank group, low-dose rhein group (0.175g/kg) and high-dose rhein group (0.35g/kg). The drug was administered by gavage for 60 days [56]. Compared to the blank group of the same sex, BUN and SCr of mice in the administration group were increased, and the body weight of mice in the rhein high-dose group decreased [56]. The renal index of male mice in the administration group decreased significantly, and the content of GSH-Px decreased and the expression of TGF-β1 increased in male mice in the rhein high-dose group [56]. Its potential toxic mechanism may be caused by the imbalance of glutathione antioxidant system that can induce excessive oxidation, inflammatory reaction, and the apoptosis induced by the activation of caspase-3 [56].

In cell experiments, the transition between kidney protection and nephrotoxicity is more closely related to dose and duration of administration. Da H et al., (2009) evaluated the cytotoxic effects of emodin and rhein in HK-2 cells [57]. The results showed that both emodin and rhein could inhibit the growth of HK-2 cells, but the inhibitory effect of rhein was weaker than that of emodin [57]. From the experimental results of rhein on the survival rate of HK-2 cells, we found that rhein had obvious inhibitory effect on cell proliferation when it was treated with 40 μM for 24 hours, and the inhibitory effect gradually increased with the extension of incubation time; while the cell proliferation could be significantly inhibited after 12 hours of administration when the dosage of rhein was 100 μM [135]. It was preliminarily elucidated that rhein caused renal injury by inducing apoptosis. Researchers have also carried out a series of studies to further clarify the nephrotoxicity mechanisms of rhein. Most studies have shown that it is related to the induction of apoptosis (**Figure 2**). In addition to death-receptor signaling, the mitochondrial death pathway, oxidative stress, and endoplasmic reticulum stress all contribute to apoptosis [58]. It was found that rhein directly inhibited HK-2 cell growth and increased apoptosis in a dose- and time-dependent manner according to the study by Yang J et al. (2015) [59]. Rhein (50 and 100 μM, 24 h after administration) increased the mRNA levels of amino terminal kinase (c-Jun), activated transcription factor-2 (ATF-2), and caspase-3, and upregulated the expression of p38 MAPK and cleaved caspase-3. These results suggest that rhein may induce apoptosis in HK-2 cells through the MAPK signaling pathway [59]. Hao S et al. (2015) [60] also found rhein could dose-dependently inhibit the viability of HK-2 cells, increase the release of lactate dehydrogenase (LDH) and apoptosis rate, and significantly upregulate the mRNA or protein expressions of Fas, FasL, FADD, caspase-3, caspase-8, and Cytochrome C (Cyt-c). Apoptosis induced by the Fas pathway may be the mechanism behind rhein's toxic effects on HK-2 cells in vitro. In another study [61], rhein reduced mitochondrial membrane potential and intracellular ATP level, released Cyt-c, and decreased Bcl-2 and Bax protein levels in HK-2 cells. Meanwhile, rhein increased the intracellular ROS level and inhibited mitochondrial uncoupling protein 2 (UCP2) expression, which regulates mitochondrial membrane potential, ROS generation, and ATP synthesis [61]. Rhein inhibited the expression of UCP2, significantly enhanced oxidative stress in cells, and thus promoted cell apoptosis, indicating the potential role of UCP2 in rhein nephrotoxicity [61].

3.2. Methods for Controlling Rhein Toxicity

In order to use rhein reasonably and safely, there may be some measures we should take. On the one hand, it is important to control the dosage and duration of rhein administration as described above; on the other hand, the compatibility of TCM can enhance its protective effects and reduce the toxicity. For instance, different doses of astragaloside IV (10, 20 and 40 μM) could reduce the occurrence of rhein-induced vacuolation, cell fusion, and the increase of necrotic cells in HK-2 cells [62]. After the combination of rhein and astragaloside IV in HK-2 cells for 48 hours, the cell inhibition rate and LDH leakage rate were significantly reduced [62]. The compatibility significantly increased the contents of SOD and GSH in cells and down-regulated the expression of MDA, which indicates that astragaloside IV could significantly inhibit the oxidative stress injury caused by rhein and then protect cells [62].

Abbreviations

AP allopurinol; APAP acetaminophen; APS astragalus polysaccharide; α-SMA α-smooth muscle actin; BMP-7 bone morphogenic protein 7; BSA bovine serum albumin; CAN chronic allograft nephropathy; CCl4 carbon tetrachloride; db/db diabetic obese; Cox2 cytochrome oxidase subunit 2; DFD Dahuang Fuzi Decoction; DNMTs DNA methyltransferases; Dox doxorubicin; EMT epithelial-mesenchymal transition; FN fibronectin; GFAT glutamine fructose 6-phosphate aminotransferase; GSK3b glycogen synthase kinase 3 beta; HEK human embryonic kidney; HGF hepatocyte growth factor; HK-2 human kidney-2; IgAN IgA nephropathy; IL-1 interleukin 1; ILK integrin-linked kinase; JNK c-JunNH2-terminal kinase; lncRNAs long noncoding RNAs; LPS lipopolysaccharide; MAPK mitogen-activated protein kinase; MMP-9 matrix metalloproteinase-9; MCGT1 mesangial cells transinfected with the human GLUT1 gene; MCLacZ mesangial cells transinfected with bacterial β-galactosidase; NF-κB nuclear factor kappa-B; Nrf2 the nuclear factor E2-related factor 2; NRK-49F normal rat kidney-49F; Nx nephrectomied; p38-MAPK p38 mitogen-activated protein kinase; PPARα peroxisome proliferator-activated receptor-α; RAC Rhubarb and astragalus capsule; RHL rhein lysinate; SAM senescence-accelerated mouse; SAMR1 senescence-resistant inbred strain 1; SAMP10 senescence-prone inbred strain 10; SD Sprague–Dawley; SIRT1 Sirtuin-1; STZ streptozotocin; TCMK-1 transformed C3H mouse kidney-1; TGF-β1 transforming growth factor- β1; TIMP1 TIMP metallopeptidase inhibitor 1; TLR4 toll-like receptor 4; TNF tumor necrosis factor; UUO unilateral ureteral obstruction; VCM vancomycin.

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