Renal fibrosis is considered as the pathway of almost all kinds of chronic kidney diseases (CKD) to the end stage of renal diseases (ESRD). Ganoderic acid (GA) is a group of lanostane triterpenes isolated from Ganoderma lucidum, which has shown a variety of pharmacological activities. In this study we investigated whether GA exerted antirenal fibrosis effect in a unilateral ureteral obstruction (UUO) mouse model. After UUO surgery, the mice were treated with GA (3.125, 12.5, and 50 mg·kg⁻¹·d⁻¹, ip) for 7 or 14 days. Then the mice were sacrificed for collecting blood and kidneys. We showed that GA treatment dose-dependently attenuated UUO-induced tubular injury and renal fibrosis; GA (50 mg·kg⁻¹·d⁻¹) significantly ameliorated renal dysfunction during fibrosis progression. We further revealed that GA treatment inhibited the extracellular matrix (ECM) deposition in the kidney by suppressing the expression of fibronectin, mainly through hindering the over activation of TGF-β/Smad signaling. On the other hand, GA treatment significantly decreased the expression of mesenchymal cell markers alpha-smooth muscle actin (α-SMA) and vimentin, and upregulated E-cadherin expression in the kidney, suggesting the suppression of tubular epithelial-mesenchymal transition (EMT) partially via inhibiting both TGF-β/Smad and MAPK (ERK, JNK, p38) signaling pathways. The inhibitory effects of GA on TGF-β/Smad and MAPK signaling pathways were confirmed in TGF-β1-stimulated HK-2 cell model. GA-A, a GA monomer, was identified as a potent inhibitor on renal fibrosis in vitro. These data demonstrate that GA or GA-A might be developed as a potential therapeutic agent in the treatment of renal fibrosis.

**Keywords:** chronic kidney disease; renal fibrosis; ganoderic acid; epithelial-mesenchymal transition; TGF-β; UUO mice; HK-2 cells

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**INTRODUCTION**

Renal fibrosis is considered to be the pathway involved in almost all types of chronic kidney disease (CKD) [1–3]. The prevalence of CKD in the general population is high, reaching 13% in some countries and is increasingly becoming a public health problem worldwide [4–6]. For most CKD patients that reach end stage of renal disease (ESRD), dialysis and kidney transplantation are the only therapeutic options. The 5-year survival rate of people with ESRD on dialysis is 13%–60% lower than that of people of similar ages in the general population [7]. Importantly, only 25% of patients with ESRD undergo kidney transplantation in a timely manner, whereas 6% of patients die each year while waiting for a transplant [8]. In CKD, fibrosis is an active biosynthetic healing response initiated to protect tissue from injury. However, fibrosis can lead to serious organ damage when it becomes independent from the initial stimulus. However, approaches to attenuate or reverse renal fibrogenesis are limited [8, 9]. Recently, natural products have been studied in the search for novel and promising alternative drugs for preventing and treating fibrosis [10].

Ganoderma lucidum (G. lucidum), a well-known Chinese traditional medicine [11], has been used as a dietary supplement or drug to increase energy, improve immunity, and promote health and longevity for over 2000 years in Asian countries. Numerous bioactive constituents of G. lucidum, including terpenes, proteins, polysaccharides, amino acids, flavonoids, alkaloids, steroids, mannitol, and other compounds, have been revealed [12]. Pharmacological studies have determined that G. lucidum polysaccharides and triterpenes have multiple pharmacological activities [13–16].

We recently studied the therapeutic effect of Ganoderma triterpenoids (GTs) on autosomal dominant polycystic kidney disease (ADPKD) [15] and hyperhomocysteinemia (HHcy)-induced endothelial injury [14]. GTs exerts a fascinating inhibitory effect on renal cyst development in an ADPKD mouse model via down-regulating Ras/mitogen-activated protein kinase (MAPK) signaling and promoting cell differentiation [15]. It was determined that, in a bovine aortic endothelial cell model, GT protects against HHcy-induced EMT via inhibiting canonical TGF-β/Smad- and non-Smad-dependent signaling pathways [14]. Considering the common
pathogenesis and signaling pathways, these data strongly suggest that GTs and their components can be developed as novel agents for preventing and treating CKD.

Ganoderic acid (GA), as one of the most abundant GTs, has been reported to have multiple therapeutic activities, such as antibacterial, antiviral, antitumor, hepatoprotective, antioxidative, antihypertensive, and antiaggregation activities [11]. GA-A, GA-B, and GA-C2 are representative active components and the most abundant components of crude GA. In addition, GA-A is viewed as a marker for evaluating G. lucidum quality [17] and has been reported to exhibit antioxidative, cytotoxic, hepatoprotective, and anticancer activities [18–20].

In this study, we used a unilateral ureteral obstruction (UUO) mouse model to identify the antirenal fibrotic effect of GA. A TGF-β1-stimulated HK-2 cell model was used to identify the antifibrotic effects of active compounds of GA and reveal the related mechanisms. The experimental results showed that GA significantly inhibited the progression of renal fibrosis partially via suppressing the TGF-β1/Smad and MAPK signaling pathways. The monomer GA-A, which was isolated from GA, had a potent antirenal fibrotic effect.

MATERIALS AND METHODS
Ganoderic acid preparation
The purified natural product GA and three monomers (GA-A, B, and C2) were extracted and isolated from G. lucidum. In the in vivo experiment, GA was dissolved in saline with 5% Tween 80. GA and these three monomers were dissolved in DMSO for the in vitro experiment. According to the results of HPLC, the concentrations of GA-A, GA-B, and GA-C2 were 16.101, 10.586, and 5.404 μg/mL, respectively. These three monomers accounted for 16.1% (GA-A), 10.6% (GA-B), and 5.4% (GA-C2) of crude GA. The purity of these three monomers was >98%, as determined by HPLC [21].

Cell culture
The HK-2 cells (human proximal tubular epithelial cells) were gifted by Dr Hui-wen Ren at Tianjin Medical University, Tianjin, China. Briefly, the HK-2 cells were cultured in DMEM/F12 containing 8% fetal bovine serum (FBS; Gibco, Australia), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO2 at 37 °C. The cells reached 40%–50% confluence, they were incubated with 10 ng/mL TGF-β1 with or without test compounds, all of which were dissolved in DMSO (0.1%) for 1 h. Specifically, DMSO was adopted as the solvent for the control group.

Cytotoxicity assay
The CCK-8 assay kit (Dojindo, Kumamoto, Kyushu, Japan) was used to test cytotoxicity in vitro. HK-2 cells were plated in 96-well plates at a density of 5000 cells/well. HK-2 cells were incubated with GA (mix, A, B, and C2) for 24 h. Then, the CCK-8 solution diluted 1:10 with 8% FBS DMEM/F12, was added to each well, and the cells were incubated for 2 h at 37 °C. The absorbance at 450 nm was measured with a microplate reader (Biotek, MQX200, Winooski, VT, USA). Cell viability was calculated as follows:

\[
\text{Cell viability} (\%) = \frac{(OD_{\text{treatment}} - OD_{\text{blank}})}{(OD_{\text{control}} - OD_{\text{blank}})} \times 100
\]

Mouse UUO model
The UUO model was established in male C57BL/6J mice (8 weeks of age, 25–30 g body weight, purchased from the Animal Center of Peking University Health Science Center, Beijing, China), which were housed with a 12-h:12-h light/dark cycle with food and water available ad libitum. The mice were randomly divided into experimental groups, including the sham plus vehicle control (5% Tween 80 dissolved in saline) group, the sham plus 50 mg/kg GA group, the UUO plus vehicle control group, and the UUO plus 3.125, 12.5, and 50 mg kg−1 day−1 GA groups. UUO was performed as follows: the abdominal cavity was exposed by a midline incision, and the left ureter was isolated and ligated. The sham group only received incision as a control [22, 23]. To examine the efficacy of GA in renal fibrosis after UUO injury, GA was intraperitoneally administered at a concentration of 3.125, 12.5, or 50 mg kg−1 day−1 for 7 or 14 days beginning immediately after UUO. Seven or 14 days after surgery, the animals were sacrificed, and the blood and kidneys were collected for biochemical and protein analysis and histologic examination.

BUN and blood creatinine measurement
The urea concentration in the blood was measured using a QuantiChrom Urea Assay kit (BioAssay Systems, Hayward, CA, USA). The creatinine concentration in the blood was measured with commercial kits (NJUC Bio, Nanjing, Jiangsu, China) according to the manufacturer’s instructions.

Renal histologic staining and ultrastructural examination
Kidneys were collected and fixed in 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS) overnight, dehydrated, embedded in paraffin, and cut into 5-μm sections. After deparaffinization, the kidney tissue sections were rehydrated and stained with hematoxylin and eosin and Masson’s trichrome. Tissue damage was assessed based on the tubular damage score, as previously described [24]. Briefly, injury was scored in a blinded manner according to the percentage of damage, which included the loss of the brush border, tubular dilation and intertubular hemorrhage (0 = normal; 1 ≤ 20%; 2 = 20%–40%; 3 = 40%–60%; 4 = 60%–80%; and 5 ≥ 80%). Representative fields were captured.

For ultrastructural evaluation, kidneys that were cut into 1 mm3 pieces were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. The specimens were thinly sectioned and examined under a transmission electron microscope. Electron microscopy images were randomly taken for each group.

Western blot analysis
Renal tissues or cells were homogenized in tissue protein extraction reagent (MeiSi, MF188-01, Beijing, China) containing aprotinin, leupeptin, and PMSF. The lysates were electrophoresed on polyacrylamide gels and electrophoresed to polyvinylidene difluoride membranes (Amershams Biosciences, Boston, MA, USA). After blocking, the membranes were incubated with antibodies against GAPDH (Proteintech, 60004-1-lg, 1:5000 dilution, Rosemont, PA, USA), fibronectin (Proteintech, 15613-1-AP, 1:1000 dilution), α-SMA (ACTA2, Abclonal, A1011, 1:1000 dilution, Wuhan, Hubei, China), TGF-β (CST, 3711, 1:1000 dilution, Danvers, MA, USA), Smad2 (CST, 5339, 1:1000 dilution), p-Smad2/3 (abcam, ab63399, 1:1000 dilution, San Francisco, CA, USA), Smad7 (Abclonal, A12343, 1:1000 dilution, E-cadherin (CST, 24E10, 1:1000 dilution), vimentin (CST, 5741, 1:1000 dilution), CTNNB1 (β-catenin, Abclonal, A11512, 1:1000 dilution), p-JNK (CST, 9255, 1:1000 dilution), p-ERK1 (CST, 4370, 1:2000 dilution), ERK2 (Abclonal, A11186, 1:1000 dilution), p-p38 (CST, 4511, 1:1000 dilution), and p38 (CST, 8690, 1:1000 dilution). Goat anti-rabbit IgG or goat anti-mouse IgG (EASYBIO, Beijing, China) was added, and the blots were developed with an ECL plus kit (Biodragon, Beijing, China) and visualized with a chemiluminescence detection system (Syngene, GeneGnome XQR, Cambridge, Cambridgeshire, UK). Quantitation was performed by scanning and analyzing the intensity of the hybridization bands.
Immunohistochemical staining and laser confocal microscopy
The expression of the epithelial-to-mesenchymal transition (EMT) marker α-SMA in the kidneys was evaluated by immunohistochemistry and laser confocal microscopy. Briefly, the kidneys were fixed with 4% paraformaldehyde in PBS for 24 h and incubated with 30% sucrose overnight at 4 °C. The sections were embedded in optimal cutting temperature medium and cut into 5-μm-thick tissue sections. The sections were dewaxed and hydrated in graded ethanol solutions and then microwaved in sodium citrate buffer. Endogenous peroxidase activity was blocked using 3% H2O2 for 10 min at room temperature. The samples were blocked with 5% goat serum for 30 min and then treated with a primary antibody against α-SMA (1:200) at 4 °C overnight. The next day, the sections were incubated with the goat anti-rabbit IgG secondary antibody for 30 min at 37 °C. Then, the sections were stained with 3,3′-diaminobenzidine and counterstained with hematoxylin. After dehydrating and drying, the sections were mounted with neutral gum and observed under a microscope. Laser confocal microscopy was performed as previously described [25, 26].

Statistical analysis
Statistical analyses were performed using GraphPad Prism software (San Diego, CA, USA). All results are expressed as the means ± SEM. Each experiment was performed at least three times. A two-tailed t test, one-way ANOVA analysis of variance followed by post hoc Bonferroni test, or a general linear model with repeated
measures followed by post hoc Bonferroni test was then performed to assess the differences between the groups. \( P \) values <0.05 were considered statistically significant.

RESULTS
GA hindered tubular injury and renal fibrosis induced by UUO
Based on research by Pillai et al., who found that treatment with 50 mg/kg \( G. \) lucidum terpenes exerts a renoprotective effect against cisplatin-induced nephrotoxicity \[27\], we chose 3.125–50 mg/kg GA for use in our in vivo study. GA was administered at concentrations of 3.125, 12.5, or 50 mg/kg via intraperitoneal injection once a day for 14 days beginning immediately after UUO surgery. Treatment with GA significantly reduced the tubular injury score (Fig. 1a, b). Masson-positive area (Fig. 1a, c) and fiber accumulation (Fig. 1a) in a dose-dependent manner. Compared with the low and medium doses, the high dose of GA (50 mg/kg) significantly lowered the BUN and blood creatinine concentrations (Fig. 1d, e) and thus ameliorated renal dysfunction during fibrotic progression. Moreover, the high dose of GA significantly decreased the expression of fibronectin and \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA) in kidney tissue (Fig. 1f). These results indicate that GA exhibits potent antirenal fibrotic bioactivity at high doses. Therefore, 50 mg/kg GA was used for the subsequent experiments to explore the underlying mechanisms.

GA hindered renal tubular EMT progression in the UUO model
As expected, UUO mice developed typical renal EMT characterized by elevated expression of the mesenchymal cell marker \( \alpha \)-SMA and vimentin and decreased expression of the epithelial cell marker E-cadherin compared with that in sham mice (Fig. 2a). Interestingly, Western blotting showed that GA markedly reversed the elevation of \( \alpha \)-SMA and vimentin levels and the reduction in E-cadherin protein levels (Fig. 2a). Furthermore, laser confocal microscopy and immunohistochemical staining for \( \alpha \)-SMA verified that GA downregulated the \( \alpha \)-SMA protein levels in UUO mice (Fig. 2b). These results demonstrate that GA inhibits renal EMT in fibrotic kidneys.

GA downregulated the TGF-\( \beta \)/Smad and MAPK signaling pathways in vivo
To further explore the underlying mechanism, we examined whether GA affects the TGF-\( \beta \)-induced activation of Smad and non-Smad pathways in UUO mice. The protein levels of TGF-\( \beta \), fibronectin, phosphorylated Smad2/3 and total Smad (represented by Smad2) were notably increased in UUO mice compared with sham group mice (Fig. 3a, c). GA significantly attenuated TGF-\( \beta \) expression, TGF-\( \beta \)-induced Smad2/3 phosphorylation, and total Smad levels. As the inhibitory component, Smad7 is able to inhibit renal fibrosis in a number of experimental models of CKD mainly by blocking Smad2/3 activation and anti-inflammation \[28\]. We found obviously decreased expression of Smad7 in fibrotic kidneys, which was significantly rescued by GA (Fig. 3a, c).

In addition to the TGF-\( \beta \)/Smad pathway, non-Smad signaling pathways, such as the MAPK signaling pathway, have been demonstrated to play a major role in EMT progression and in many pathophysiological processes associated with renal fibrosis \[29–31\]. Therefore, we used Western blotting to detect the activation of the MAPK signaling pathway in kidney tissue. The results showed that the phosphorylation levels of three key MAPK signaling proteins (ERK, JNK, and p38) were significantly increased in obstructed kidneys. GA significantly suppressed the phosphorylation of these proteins (Fig. 3b, c). Moreover, a previous study suggested that \( \beta \)-catenin levels are elevated in the UUO model and that Wnt/\( \beta \)-catenin signaling activation contributes to EMT in UUO nephropathy \[32\]. Many studies have also identified a link between \( \beta \)-catenin and EMT in many other diseases \[33, 34\]. Our results showed a remarkable reduction in \( \beta \)-catenin after 14 days of GA treatment in UUO mice (Fig. 3a, c). In addition, GA inhibited the activation of p38 (Fig. 3b, c). Taken together, these data suggest that GA potently partially downregulates both Smad and non-Smad signaling, thereby hindering renal fibrotic progression.

![Fig. 2](image-url)  
**Fig. 2** GA hindered renal tubular EMT progression in the UUO mouse model. **a** Representative Western blot (left) and the relative protein levels of \( \alpha \)-SMA, E-cadherin, and vimentin in the kidneys (right panel). The data were normalized to the intensity of GAPDH and are expressed relative to the value of the Sham group. **b** Representative micrographs of immunofluorescence (top panel) and immunohistochemical staining (bottom panel) with an \( \alpha \)-SMA antibody (typical \( \alpha \)-SMA positive cells are indicated by yellow or black arrows, magnification of \( \times200 \)). The data are presented as the mean ± SEM \(( n = 6–8 \) ). \(* P < 0.05, ** P < 0.01, \) versus the Sham group; \( ^{\#} P < 0.05 \) versus the UUO group.
GA downregulated the TGF-β/Smad and MAPK signaling pathways in HK-2 cells. To confirm the suggested mechanisms, a TGF-β1-stimulated HK-2 cell model was adopted. As shown, the addition of TGF-β1 for 1 h significantly increased the phosphorylation levels of Smad2/3 and ERK/JNK/p38 in HK-2 cells (Fig. 4). According to our previous research experience, the terpenes isolated from *Ganoderma* exert their pharmacological activities often at doses between 6.25 and 100 μg/mL in vitro [14, 15, 35, 36]. Therefore, we chose 6.25, 25, and 100 μg/mL GA for the in vitro study. GA treatment obviously reduced the expression levels of phosphorylated Smad2/3 and phosphorylated ERK/JNK/p38 in a dose-dependent manner (Fig. 4). These data suggest that GA exerts its antirenal fibrotic activity partially by downregulating the TGF-β/Smad and MAPK signaling pathways both in vitro and in vivo.

The GA monomer GA-A was shown to be a potent inhibitor of renal fibrosis. To determine the compound with the strongest antifibrotic bioactivity, the monomers GA-A, GA-B, and GA-C2 were isolated and identified by HPLC (Fig. 5a); the concentrations of GA-A, GA-B, GA-C2 were 161.013, 105.864, and 54.041 μg/mL, respectively. A CCK-8 assay for cell viability revealed that GA mix, GA-A, GA-B, and GA-C2 at doses under 200 μg/mL had no cytotoxic effect on HK-2 cells (data not shown).

Cell morphology showed that HK-2 cells lost their epithelial appearance and presented elongated and spindle-shaped morphology after being stimulated with 10 ng/mL TGF-β1 for 48 h (Fig. 5b). GA-A (100 μg/mL) significantly reduced spindle-like morphology induced by TGF-β1 stimulation and decreased the expression of fibronectin. However, GA-B and GA-C2 at equivalent doses did not affect the morphological changes or the expression of fibronectin (Fig. 5b, c). It was also found that GA-A dose-dependently reversed the levels of fibrotic markers upregulated by TGF-β1 (Fig. 5d). These results indicate that GA-A potently inhibits renal fibrosis.

**DISCUSSION**

The UUO mouse model is widely accepted and has been widely adopted to investigate renal fibrotic mechanisms and to develop therapeutic strategies [22]. Ureteral obstruction results in marked renal dysfunction and morphological changes with excess deposition of ECM and renal fibrosis. Various researchers have used various obstruction durations (from 7 to 14 days) in the UUO mouse model [37, 38]. We found that, both 7 (data not shown) and 14 days after ureteral ligation, the obstructed kidneys showed typical features of tubular injury and interstitial fibrosis. Renal dysfunction, characterized by elevated BUN and serum creatinine levels, was more significant in the 14-day UUO model than in the 7-day UUO model. Consistently, the morphological changes, including tubular injury and ECM deposition, were more severe in the 14-day UUO kidneys. Western blot analysis also showed that fibronectin, collagen IV and α-SMA expression was increased more...
obviously in the 14-day UUO kidneys. Therefore, we adopted the 14-day UUO model to determine the therapeutic effect of GA.

Using the UUO mouse model, we first demonstrated that GA significantly hindered renal fibrosis. GA inhibited morphologic changes and the expression of profibrotic and ECM proteins in a dose-dependent manner. In vivo, GA reduced the renal expression of fibronectin and collagen, and this effect was accompanied by the inhibition of EMT progression via decreasing α-SMA, vimentin levels and increasing E-cadherin levels, which indicates that GA prevented renal fibrosis mainly by inhibiting Smad and non-Smad signaling. Specifically, GA suppressed Smad2/3 phosphorylation and increased the expression of Smad7 in fibrotic kidneys, directly affecting the production and accumulation of ECM.

Renal tubular EMT is an important mechanism in the development of renal fibrosis [39–41]. Epithelial cells, through EMT, which is characterized by the reduced or absent expression of the epithelial intercellular adhesion E-cadherin and the increased expression of α-SMA, translocate from the epithelial compartment to the interstitium and gain a full mesenchymal phenotype, through which fibrotic matrix proteins, such as fibronectin and collagens, are produced [39, 42]. Many lines of research have demonstrated that the deletion of major transcriptional regulators of EMT, such as Twist and Snail, rescue renal fibrosis [43]. Therefore, developing a novel approach that inhibits EMT progression and ECM deposition would be an effective therapeutic option for inhibiting renal fibrosis and preserving renal function in CKD.

In renal fibrosis, TGF-β1 is considered a master regulator of EMT and ECM accumulation and consequently a potential key driver of renal fibrosis [44, 45]. TGF-β signals are transduced mainly by TGF-β receptor (TβR)-mediated Smad and non-Smad signaling. Upon TGF-β stimulation, regulatory Smads (Smad2 and Smad3, R-Smads) are recruited to TβRs and activated by phosphorylation. Subsequently, activated R-Smads heterologomerize with Smad4 and translocate to the nucleus, where they jointly transactivate downstream profibrogenic protein expression [46], which can be inhibited by Smad7 [28] (Fig. 6). In the present study, the overexpression of TGF-β1 significantly induced the phosphorylation of Smad2/3, which directly activated the accumulation of fibronectin and collagen and EMT progression (Fig. 6).
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Fig. 6 Schematic diagram of the proposed underlying mechanisms involved in the antirenal fibrotic activity of GA. Please see the text for explanations.

It has been suggested that, as additional downstream pathways of TGF-β, the MAPK (ERK, JNK, and p38) signaling pathways are overactivated and promote the EMT process in obstructed kidneys and in TGF-β1-treated renal tubular epithelial cells [30, 47]. In 2008, it was reported that p38 MAPK leads to the accumulation of β-catenin via GSK3β signaling [48], which has been indicated to play an essential role in renal tubular EMT [42]. In addition, many studies have demonstrated that the MAPK pathways, as non-Smad signaling pathways, regulate EMT. The suppression of MAPK signaling pathways reverses EMT progression and renal fibrosis [30, 49–52]. Our present data showed that GA obviously partially downregulated the phosphorylation of ERK, JNK, and p38, indicating that the downregulation of the Smad-independent pathway also ameliorated renal fibrosis.

Above all, GA exhibited potent inhibitory bioactivity against TGF-β1 signaling in a mouse renal fibrosis model and in an HK-2 cell model via inhibiting the same signaling pathways. Considering that the TGF-β1 signaling pathway is closely associated with a number of fibrotic diseases, including renal fibrosis, lung fibrosis, hepatic fibrosis, atrial fibrosis, and cardiac fibrosis as well as the progression and metastasis of various cancers, connective tissue and skeletal diseases [53–55], GA might be developed as a potent drug to treat TGF-β1 signaling-related diseases more extensively.

GA-A, one of the representative active components and the most abundant component of crude GA, has been reported to exhibit various pharmacological activities. Jiang et al. reported that GA-A inhibits the growth and invasive behaviors of human breast cancer cells by modulating AP-1 and NF-κB signaling [18]. Radwan et al. proved that GA-A has anti-B-cell lymphoma potential, mainly through inducing apoptosis and mediating immune responses [56]. Das et al. found that GA-A plays a potent antitumorigenic role in meningioma by suppressing the Wnt/β-catenin signaling pathway [57].

We subsequently elucidated that GA-A is the most effective antirenal fibrotic component of GA. Because of the limited production of GA-A/B/C2, we only identified the effective monomer of GA through in vitro research. Further in vivo studies are necessary to investigate the relevance of our in vitro data using the UUO model. In our study, the treatment of fibrictic kidneys with GA significantly downregulated various downstream signals of TGF-β, suggesting that GA may target novel factors of renal fibrosis. However, the specific target of GA is another key question and should be further studied.

In conclusion, our study identified that GA possesses renoprotective activity, especially in hindering renal fibrosis, in a UUO mouse model. Based on our experimental data, we suggest a mechanism by which GA protects renal fibrosis. On the one hand, GA hinders the EMT process, partially by downregulating TGF-β1 signaling via downstream Smad and non-Smad pathways. In addition, GA also directly suppresses the deposition of ECM proteins, such as fibronectin, by inhibiting the activation of TGF-β1/Smad2/3 signaling. Specifically, GA-A was identified as the monomer with the strongest protective effect against TGF-β1-stimulated fibrosis in HK-2 cells. Therefore, our study suggests that GA can be developed as a promising therapeutic agent to suppress the progression of renal fibrosis, providing a potential effective treatment for CKD.

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AUTHOR CONTRIBUTIONS

XQG and BXY designed the research. XQG, AM, JZH, YLJ, GYS, ML, and JHR performed the research. XQG, JHR, and SQL analyzed the data. XQG and BXY wrote the manuscript. HZ and BXY revised the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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