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## EDITED BY

Sirajudheen Anwar,  
University of Hail, Saudi Arabia

## REVIEWED BY

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University of Hail, Saudi Arabia  
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Ain Sham University, Egypt  
Mohammed break,  
University of Hail, Saudi Arabia  
Kareem M. Younes,  
University of Hail, Saudi Arabia

## \*CORRESPONDENCE

Wenqing Jia,  
✉ wenqingjia0312@163.com  
Yukui Ma,  
✉ yaoliduli@163.com

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# Efficacy of Oroxylin A in ameliorating renal fibrosis with emphasis on Sirt1 activation and TGF- $\beta$ /Smad3 pathway modulation

Guangzhuang Li<sup>1</sup>, Sentao Xian<sup>2</sup>, Xianchao Cheng<sup>3</sup>, Yunhua Hou<sup>1</sup>,  
Wenqing Jia<sup>2\*</sup> and Yukui Ma<sup>2\*</sup>

<sup>1</sup>School of Bioengineering, Shandong Academy of Sciences, Qilu University of Technology, Jinan, China,

<sup>2</sup>School of Chemistry and Chemical Engineering, Qilu Normal University, Jinan, China, <sup>3</sup>Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin, China

**Introduction:** Renal fibrosis poses a serious threat to human health. At present, there are few types of traditional Chinese medicine used to treat this disease, and Oroxylin A (OA), as a natural product with multiple biological activities, is expected to be used for the treatment of renal fibrosis.

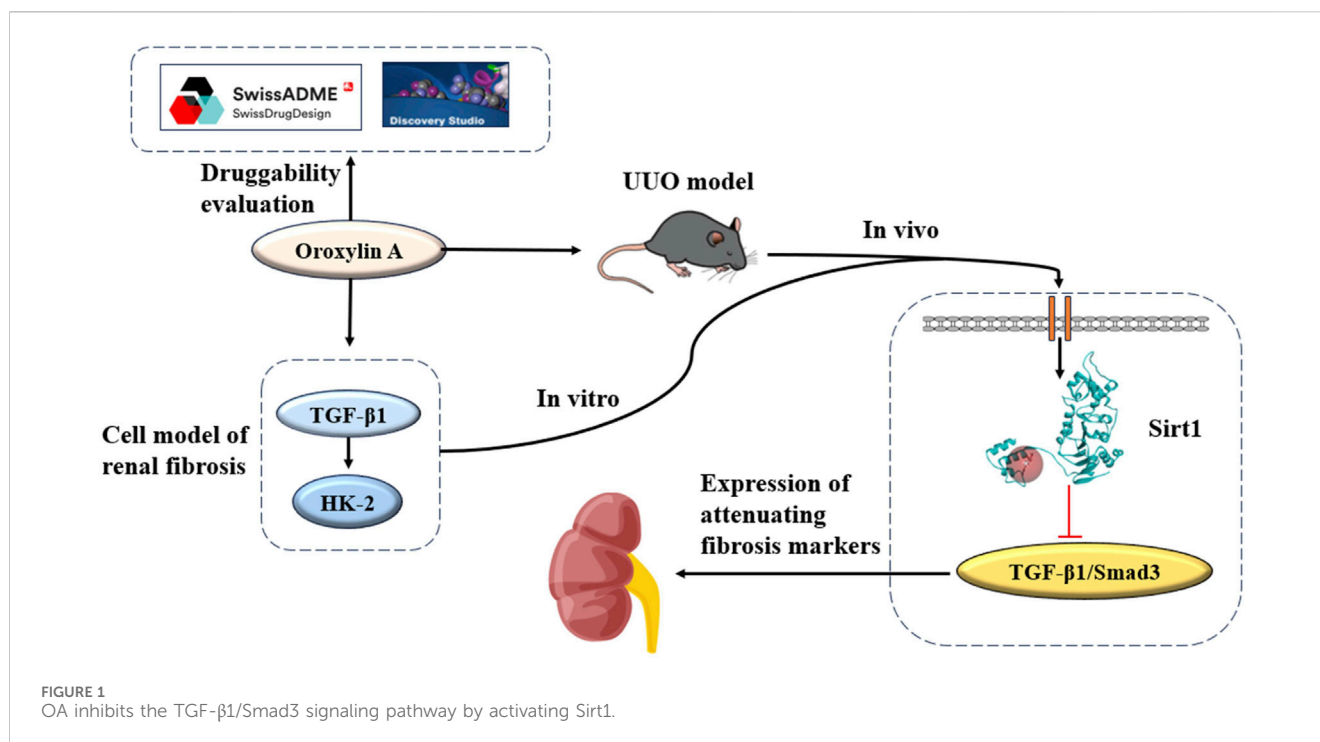
**Methods:** The tolerance of osteoarthritis and its impact on renal fibrosis were studied through ADMET, Lipinski's filter, establishment of a unilateral ureteral obstruction (UUO) model, and molecular docking.

**Results:** OA has good drug tolerance. Compared with the sham group, UUO mice that did not receive OA treatment showed severe tubular dilation and atrophy, extracellular matrix (ECM) deposition, and inflammatory cell infiltration in their kidneys, while OA-treated mice showed significant improvement in these symptoms. OA treatment remarkably restrained the accumulation of fibronectin and  $\alpha$ -SMA. Moreover, OA treatment remarkably decreased the abnormal upregulation of inflammatory factors (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) in the obstructed kidney of UUO mice. Sirtuin1 (Sirt1) expression was markedly diminished in the kidneys of UUO mice and TGF- $\beta$ 1-induced HK-2 cells, whereas this reduction was largely reversed after OA treatment. The results support that OA exerts antifibrotic effects partly through the promotion of the activity of Sirt1. In *in vitro* results, OA treatment markedly inhibited the activation of Smad3 in UUO mice, thereby ameliorating renal fibrosis. OA could form hydrogen bonds with key the amino acid ASN226 in Sirt1, thereby activating Sirt1, which might also be the reason why OA could resist renal fibrosis.

**Discussion:** Our study indicated that OA might exert anti-renal fibrosis effects through the activation of Sirt1 and the suppression of the TGF- $\beta$ /Smad3 signaling pathway.

## KEYWORDS

Oroxylin A, chronic kidney disease, renal fibrosis, Sirt1, molecular docking



## 1 Introduction

Chronic kidney disease (CKD) poses a serious threat to health. The incidence rate is increasing each year, and the main feature is renal fibrosis (Ledo et al., 2024; Speer et al., 2022; Ceccotti et al., 2024; Ruiz-Ortega et al., 2020; Miguel et al., 2021). In fibrotic kidneys, interstitial fibroblasts are the principal effector cells that produce the extracellular matrix (ECM), and their activation is an important pathogenetic event of renal fibrosis. The kidney's interstitial fibroblasts differentiate into myofibroblasts during obstruction, resulting in excessive ECM synthesis and renal fibrosis. Despite numerous attempts, the underlying mechanisms of renal fibrosis remain unclear. Therefore, it is necessary to identify and develop effective anti-renal fibrosis drugs to prevent the progression of CKD.

Oroxylin A (OA) is a natural product derived from *Scutellaria baicalensis* (Chen et al., 2024). Reports show that OA has anti-inflammatory, anti-tumor, and vascular protective effects (Wang et al., 2024; Liu et al., 2020; Bai et al., 2021). Previous studies have found that OA markedly mitigates carbon tetrachloride-triggered liver fibrosis by activating autophagy (Chen et al., 2018). OA also exerts anti-inflammatory effects in liver fibrosis (Zhang et al., 2018; Shen et al., 2020). Moreover, OA attenuated the pyroptosis of hepatocytes by blocking mitochondrial ROS to promote PGC-1 $\alpha$ /Mfn2 signaling. Oxidative stress and inflammation are the key factors in renal fibrosis (Oyama et al., 2021), and it has been found that OA may have anti-renal fibrosis functions.

Transforming growth factor-beta-1 (TGF- $\beta$ 1) emerges as a primary initiator of renal fibrosis. Once fibrogenesis is initiated, the secretion of TGF- $\beta$ 1 triggers the activation of receptors. The initiation of this process triggers a series of events, of which the most significant was the phosphorylation of Smad2/3, which leads to the transfer of activated p-Smad3 to the nucleus. This sequence of

actions enhances the transcription of genes related to the ECM, thereby increasing matrix production (Sureshbabu et al., 2016). Based on these findings, therapeutic approaches targeting the TGF- $\beta$ /Smad3 signaling pathway may provide a new avenue for treating CKD (Yu et al., 2022). Sirtuin1 (Sirt1) plays an important role in regulating the cell cycle, differentiation, apoptosis, and metabolism (Wang et al., 2023). Sirt1 is a key regulatory factor in various cellular processes, and it participates in the regulation of renal fibrosis. Studies indicate that activating Sirt1 may mitigate renal fibrosis, as evidenced by its capacity to attenuate fibrosis by repressing HIF-2 $\alpha$  (Li et al., 2021). Conversely, in liver-specific contexts, the absence of Sirt1 exacerbates liver fibrosis, especially post-injury (Liu et al., 2017). Consequently, targeting Sirt1 through pharmacological modulators holds promise as a therapeutic approach for renal fibrosis. It was observed that OA exhibits renoprotective properties and possesses anti-renal fibrosis effects, primarily attributed to its activation of Sirt1. Conversely, the inhibition of Sirt1 may compromise the renoprotection of OA. We studied the durability of OA and the influence of OA on renal fibrosis, which mainly included ADMET, Lipinski's filter, establishing a unilateral ureteral obstruction (UUO) model, and molecular docking (Figure 1).

## 2 Material and methods

### 2.1 ADMET prediction and Lipinski's filter

The ADMET module in Discovery Studio 3.5 was used to predict the pharmacokinetics and toxicity of OA (Zhu et al., 2021). In ADMET prediction, the pharmacokinetics (water solubility, blood-brain barrier [BBB], cytochrome P450 2D6 [CYP2D6], liver toxicity, human intestinal absorption

TABLE 1 Primer sequences used in the study.

Gene	Forward	Reverse
m <i>Fibronectin</i>	CAGCCAGGCACTGACTACAA	AGGGGATCCAGGCTTCTCAT
m $\alpha$ -SMA	GTACCCAGGCATTGCTGACA	GCTGGAAGGTAGACAGCGAA
m <i>Il-1<math>\beta</math></i>	TGCCACCTTTTGACAGTGATG	AAGGTCCACGGGAAAGACAC
m <i>Il-6</i>	CAACGATGATGCACCTTGACAGA	TGTGACTCCAGCTTATCTCTTGG
m <i>TNF-<math>\alpha</math></i>	ATGGCCTCCCTCTCATCAGT	TTGCTACGACGTGGGCTAC
m <i>Sirt1</i>	CGGCTACCGAGGTCCATATAC	ACAATCTGCCACAGCGTCAT
m $\beta$ -Actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC
h <i>Sirt1</i>	CCTACTGGCCTGAGTTGA	GGACGGAGGAAAAGAGCGAAT
h <i>ACTIN</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

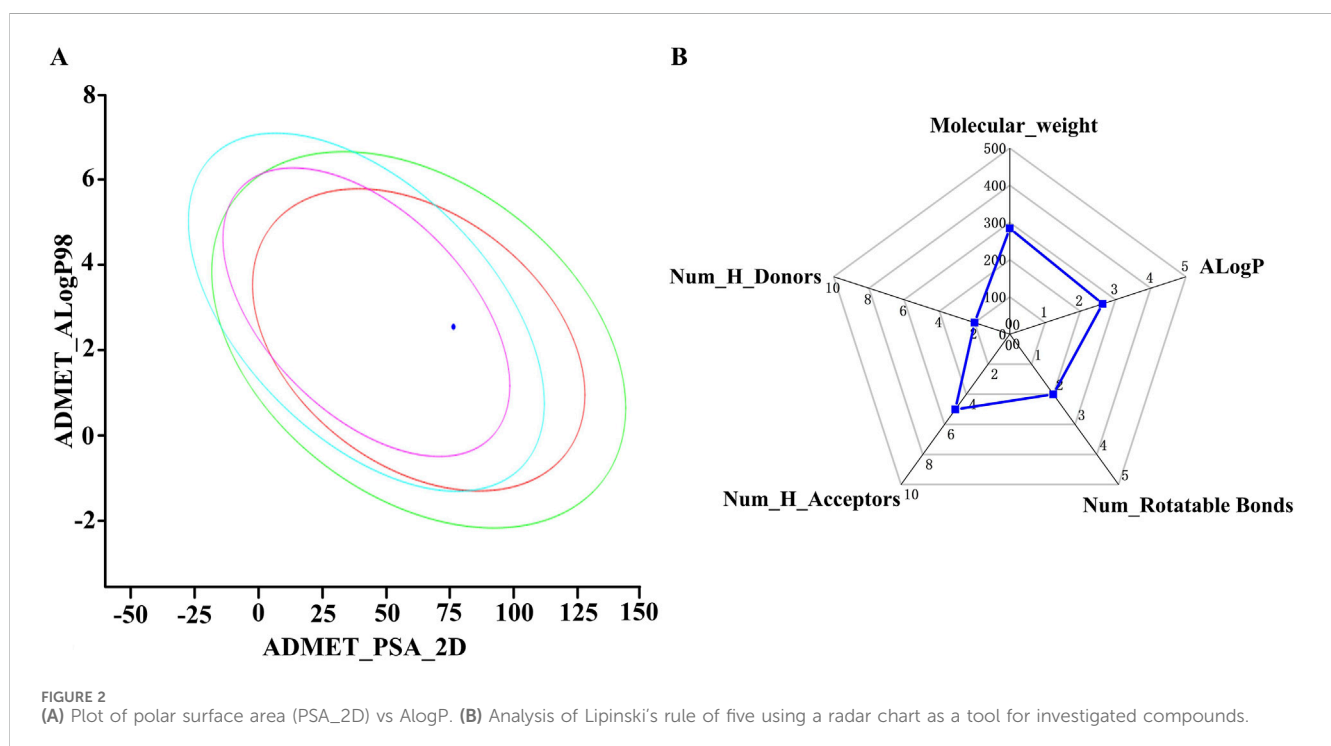


TABLE 2 ADMET prediction for OA.

ID number	Solubility level <sup>a</sup>	CYP450 2D6 <sup>b</sup>	Absorption level <sup>c</sup>	PPB <sup>d</sup>
Oroxylin A	3	-2.79684	0	1.79429

<sup>a</sup>Solubility\_level: 1, very low; 2, yes, low; 3, yes, good; and 4, optimal.

<sup>b</sup>CYP450 2D6: <0.161, non-inhibitor; >0.161, inhibitor.

<sup>c</sup>Absorption\_level: 0, good; 1, moderate; 2 low; and 3, very poor.

<sup>d</sup>PPB: < -2.209,  $\geq$ 90%; > -2.209,  $\leq$ 90%.

[HIA], and plasma protein binding [PPB]) of OA were studied (Ju et al., 2016). SwissADME (Daina et al., 2017) was used (<http://www.swissadme.ch/index.php>) to evaluate the physicochemical properties of OA molecular weight (MW), number of hydrogen bond acceptors (nHAs), number of hydrogen bond donors (nHBs), lipid water partition coefficient (ALogP), and number of rotatable bonds (nRot).

## 2.2 Animal model

The UUO model was established as described in a previous study (Zhong et al., 2024), and male C57BL/6 mice aged 10–12 weeks were selected. After anesthesia, the abdominal cavity was accessed through a midline incision, and double ligation was performed on the left ureter. The mice were randomly divided into

TABLE 3 TOPKAT prediction for OA.

Mouse female FDA		Male mouse FDA		Rat female FDA		Rat male FDA		WOE prediction		Ames prediction		Skin irritancy	
Probability	Effect	Probability	Effect	Probability	Effect	Probability	Effect	Probability	Effect	Probability	Effect	Probability	Effect
0.216984	NC	0.246201	NC	0.22312	NC	0.294397	NC	0.472273	NC	0.039966	NM		None

NC, non-carcinogen; NM, non-mutagen.

three groups, with six animals in each group: sham operation (sham), where sham mice underwent ureteral exposure surgery without ligation of the ureter; UUO; and UUO + OA (Cat No. HY-N0560, purity = 99.74%, MedChemExpress, United States). After 14 days of induction with UUO, the mice were fasted overnight. The blood samples were collected from the eyeballs and centrifuged (3,000 r/min, 15 min), and serum was collected and stored. Then, the mice were euthanized by cervical dislocation. The animal experiments were approved by the Animal Ethics Committee (YSY-DWLL-2024438).

## 2.3 Cell culture

HK-2 cells were provided by Dr. Yong Liu (Chongqing University of Traditional Chinese Medicine, China). The HK-2 cells were cultured in DMEM/F12 medium (MeilunBio, China) and stored in a humidified chamber (37°C, 5%, CO<sub>2</sub>). To evaluate the anti-fibrotic properties of OA, OA was directly added to subcloned HK-2 cells and incubated. The HK-2 cells were subjected to a 24-h starvation period using DMEM containing 0.5% FBS and then exposed to TGF-β1 (10 ng/mL, PeproTech, United States) or Ex-527 (Shyuanye, China) for 24 h.

## 2.4 Real-time quantitative PCR

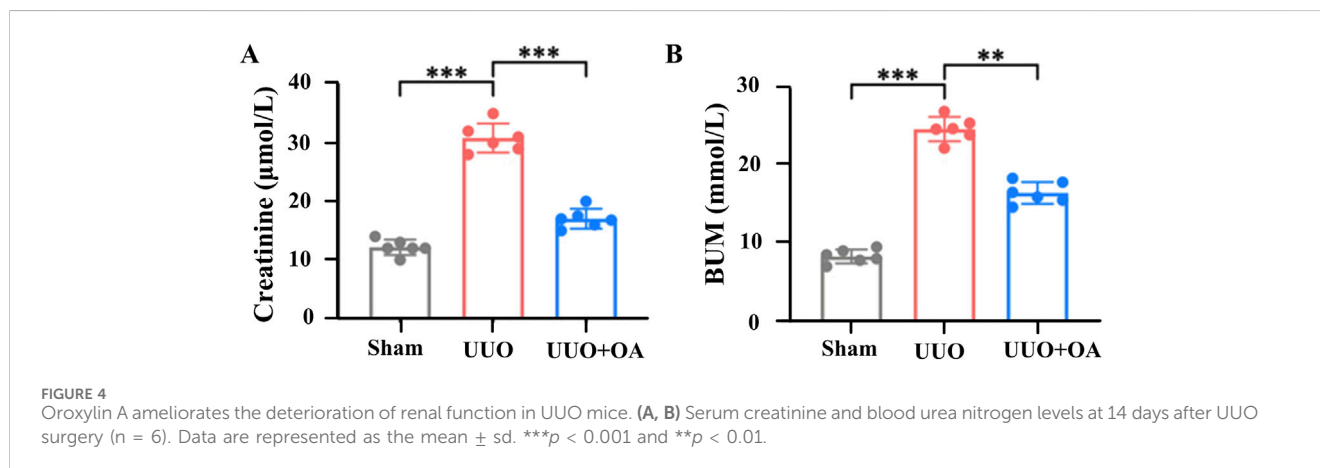
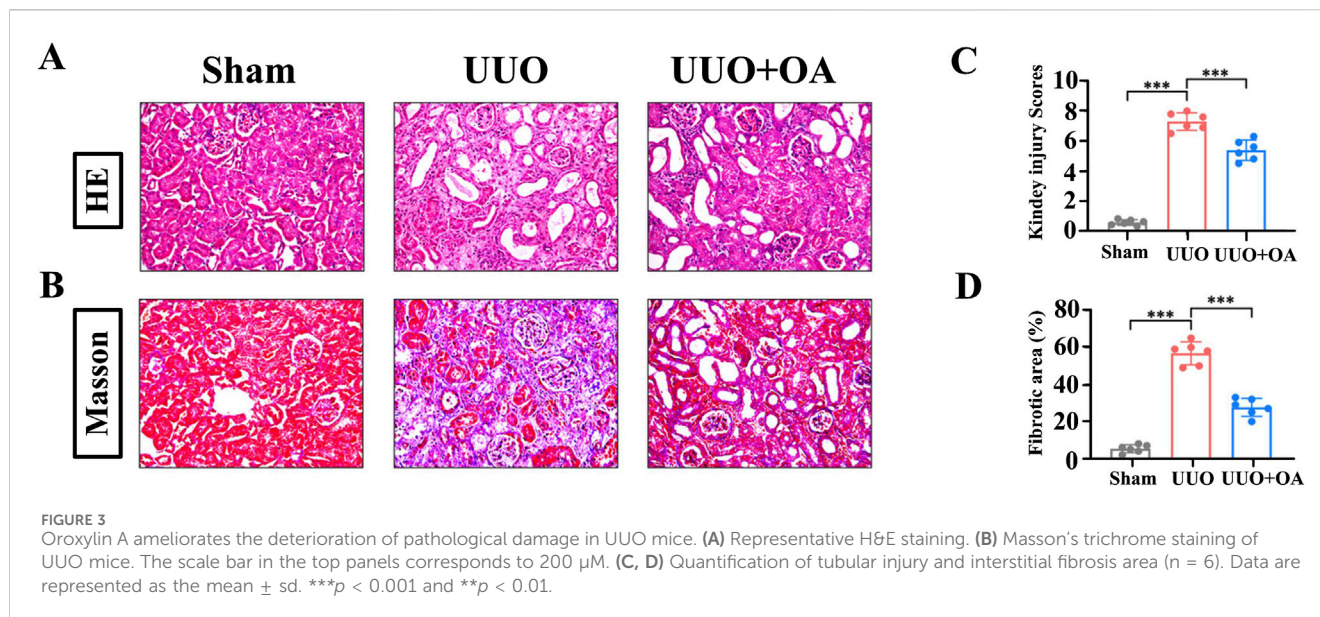
The RNAiso Plus reagent was used to extract RNA from kidneys and HK-2 cells. The cDNA was synthesized using the PrimeScript™ RT Kit (Takara, Japan). A measure of 2 μg RNA extracted from tissues and cells was used as a template for RT-PCR. RT-PCR was performed on cDNA using the SYBR Green method and QuantStudio. The amplification protocol was as follows: 30 s for the pre-read stage at 60°C, 10 min for denaturation at 95°C, 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min, and then 30 s for post-read stage at 65°C. The Ct values for each sample were used in the analysis of 2<sup>-ΔΔCt</sup> data. Table 1 lists the primers used.

## 2.5 Histology staining

Conventional methods were used to prepare the paraffin-embedded sections of mouse kidneys. In brief, the kidneys were immersed in 4% paraformaldehyde solution for an extended period, followed by dehydration using a series of increasing ethanol concentrations. The kidneys were embedded in paraffin and cut into sections with a thickness of 4 μM. Then, these sections were dewaxed and stained to evaluate the histopathological changes in kidney tissue. The analysis was conducted using ImageJ software (Bethesda, Maryland, United States).

## 2.6 Immunofluorescence assay

The mouse kidney sections were incubated overnight at 4°C with fibronectin and α-SMA antibodies (Servicebio, China). Then, the kidney sections were stained with the Cy3 conjugated secondary



antibody at 37°C for 1 h. DAPI was used to visualize the nuclei of cells. The slices were incubated with biotin-linked secondary antibodies at 37°C for 1 h. Antigen antibody reactions were visualized using a DAB substrate kit (ab64238, Abcam). The slides were counterstained with hematoxylin, dehydrated, and mounted.

## 2.7 Western blot analysis

Tissue and cell extracts were obtained using RIPA buffer. The BCA kit (Bio-Rad Lab, California, United States) was used to quantify the total protein concentration. The protein lysate was loaded onto 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membranes (Bio-Rad Lab, California, United States). The membranes were then sealed with 5% skim milk for 1 h. Then, they were incubated with primary antibodies (Servicebio, China) anti-fibronectin (1:400), anti- $\alpha$ -SMA (1:1,000), anti-Sirt1 (1:1,000), anti-Smad3 (1:1,000), anti-p-Smad3 (1:1,000), and anti- $\beta$ -Actin (1:1,000) at 4°C overnight. After being washed three times with TBST, the polyvinylidene fluoride membranes were exposed to a horseradish

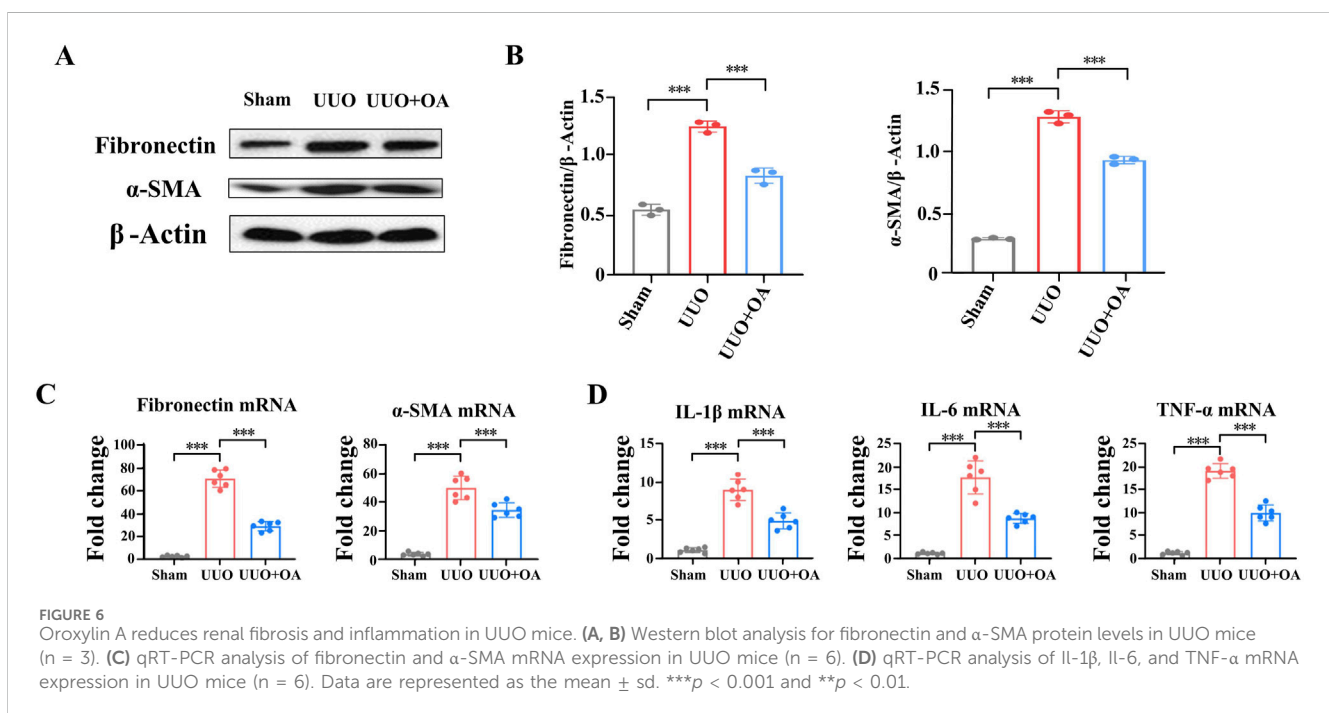
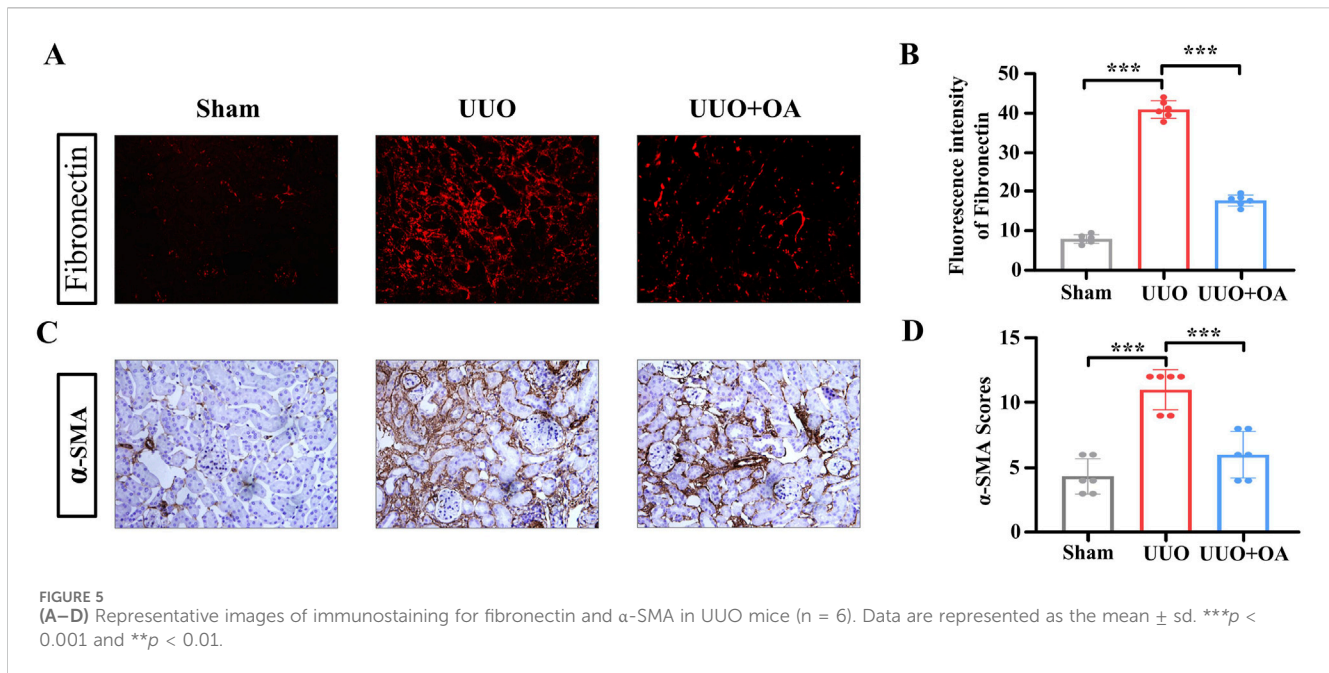
peroxidase-conjugated secondary antibody at a release degree of 1:2,000 (Servicebio, China) and incubated for 1 h at room temperature.

## 2.8 Statistical analyses

GraphPad Prism 9.0 (GraphPad Software, United States; <http://www.graphpad.com/>) was used to visualize and analyze data. The normality of the data was assessed using the Shapiro–Wilk test. One-way ANOVA was used to analyze the differences between groups, and Tukey's *post hoc* test was used to determine the level of significant difference between each group in *post hoc* comparisons. All data are expressed as the mean  $\pm$  sd. *p* < 0.05 was considered statistically significant (Yang et al., 2024).

## 2.9 Molecular docking

CDOCKER is a grid-based molecular docking method that uses a CHARMM force field (Jia et al., 2018). Sirt1 (ID: 4ZZH) was obtained from the PDB database (<https://www.rcsb.org/>). This study



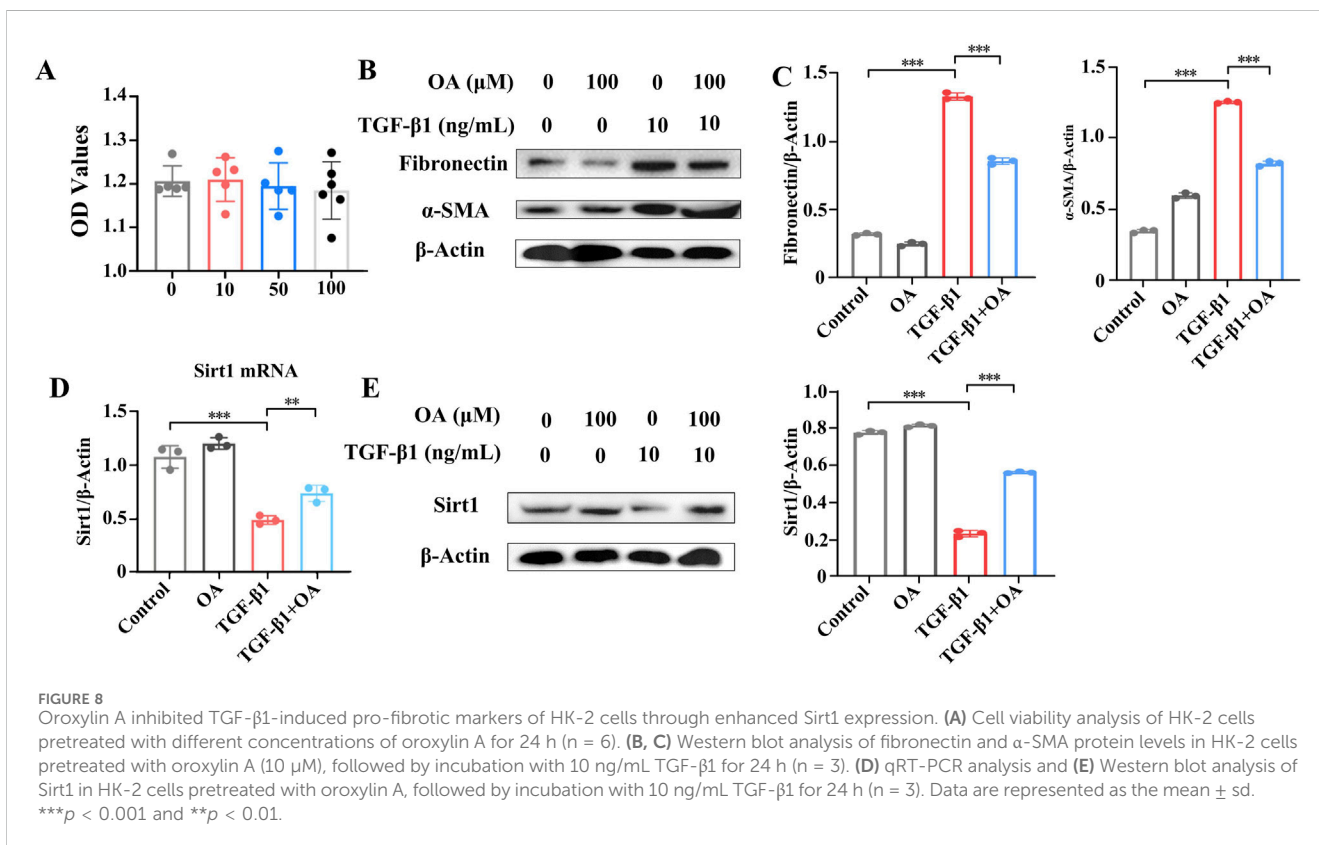
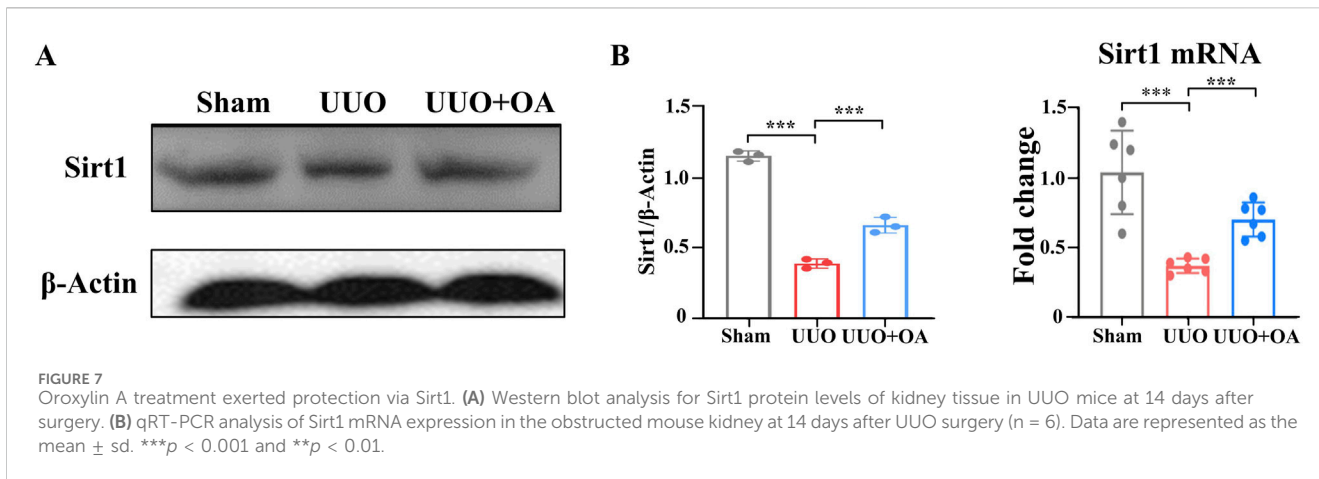
used Discovery Studio 3.5 software for molecular docking. Since the proteins downloaded from the PDB have missing residues, the “Prepare Proteins” module was used for protein repair, and the “From Current Selection” module was used to establish binding sites. Next, we prepared this protein, including dehydration, addition of hydrogen atoms, and addition of residue sequences. The binding site was a sphere with a radius of 9.27 Å and coordinates of X = 25.63, Y = -53.99, and Z = 2.23. SMILES of OA obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) used the “Prepare Ligands” module to obtain the isomers of OA. The CDocker method was used to study the combination of OA and

Sirt1. Finally, the results were visualized in the CDocker report and used for further analyses.

## 3 Results

### 3.1 ADMET prediction and Lipinski’s filter

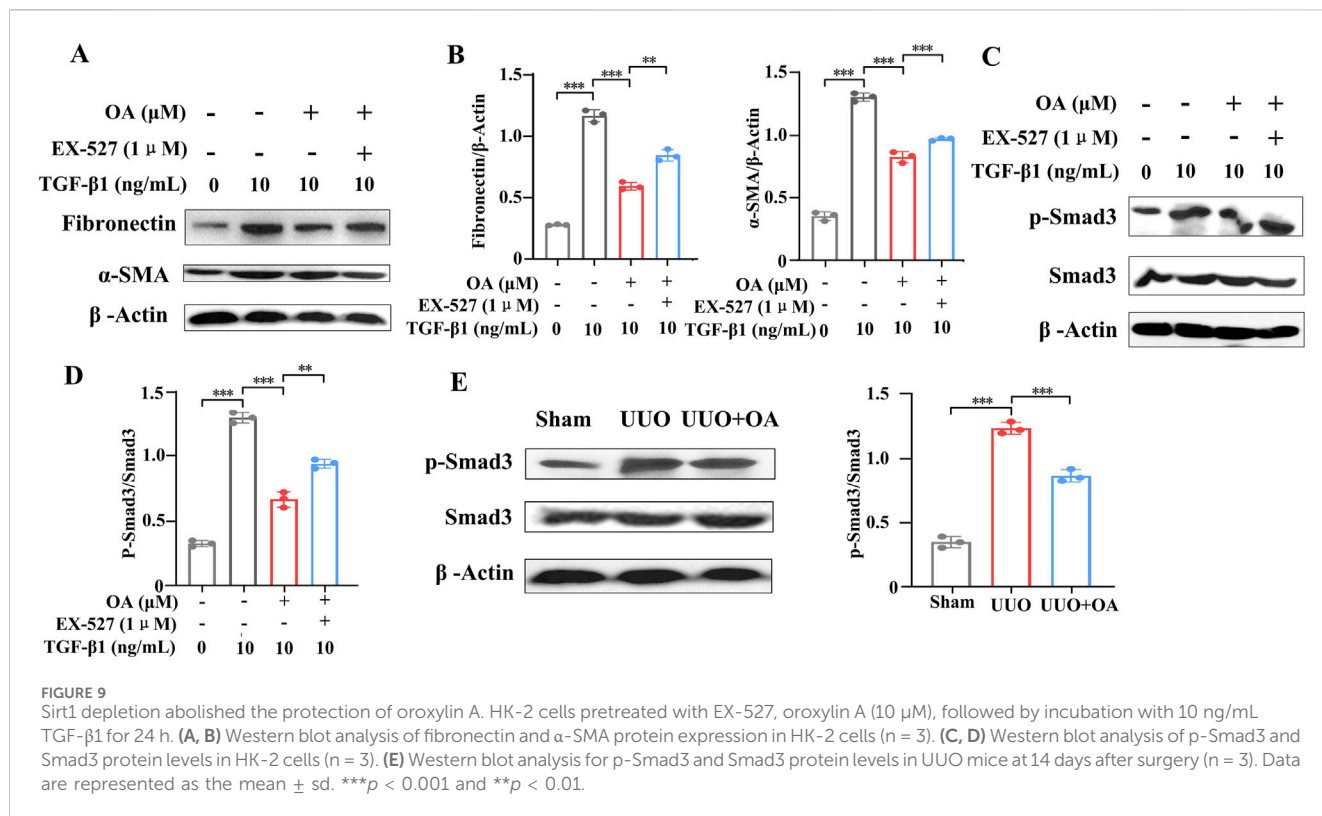
The druggability of OA was researched. Figure 2A shows that OA had good HIA and BBB. The MW, nHA, nHD, nRot, and ALogP followed Lipinski’s rule (Figure 2B).



Next, ADMET characteristics of OA were evaluated (Table 2). Human intestinal absorption and solubility are two key factors that affect oral bioavailability. The solubility level of OA was 3, which indicated relatively good solubility. The absorption level of OA was 0, indicating high intestinal absorption. The inhibition of CYP2D6 by drugs constitutes the majority of cases of drug-drug interaction. It could be found that OA might not inhibit CYP2D6. In addition, the prediction results of toxicity (mouse female FDA, mouse male FDA, rat female FDA, rat male FDA, WOE prediction, Ames prediction, and skin irritancy) showed that OA had no risk of carcinogenicity, mutagenicity, and skin irritation (Table 3). So, the physicochemical and ADMET properties of OA were within acceptable ranges.

### 3.2 OA alleviated renal fibrosis progression by improving kidney function in UUO mice

UUO mice were orally administered OA (5 mg/kg/day, po), referring to previous studies (Kim et al., 2007). As shown in Figures 3A–D, HE and Masson’s staining showed that the obstructed kidneys in UUO mice without OA treatment displayed severe tubular dilation and atrophy, ECM deposition, and inflammatory cell infiltration. In contrast, these conditions were significantly ameliorated in OA-treated UUO mice. Additionally, OA treatment significantly reduced BUN and Scr levels in UUO mice, indicating that OA treatment improved renal function in UUO mice (Figures 4A, B). Altogether, these results indicated that OA can alleviate renal fibrosis in UUO mice.



### 3.3 OA treatment reduced the expression levels of fibronectin and α-SMA in UVO mice

We analyzed the expression levels of proteins through immunostaining and Western blotting. Compared to the sham surgery group, the expression levels of fibronectin and α-SMA in the renal tissue of UVO mice were significantly increased. However, OA treatment remarkably restrained the accumulation of fibronectin and α-SMA (Figures 5A–D). Western blot and RT-PCR analyses showed that OA reduced the expression of fibrosis markers in obstructed kidneys (Figures 6A–C). Collectively, these results further confirmed that OA treatment reduced the expression levels of fibronectin and α-SMA in UVO mice.

### 3.4 OA restrained kidney inflammation in UVO mice

The process of renal fibrosis is accompanied by a large number of inflammatory reactions. According to reports, OA has anti-inflammatory effects (Liu et al., 2024). We then evaluated the influence of OA treatment on various inflammatory factors in renal tissue. The mRNA levels of IL-1β, IL-6, and TNF-α were significantly increased in the obstructed kidneys of UVO mice. However, OA treatment remarkably decreased the production of the abovementioned inflammatory factors (Figure 6D). These results further confirmed that OA treatment might suppress the inflammatory response in UVO mice.

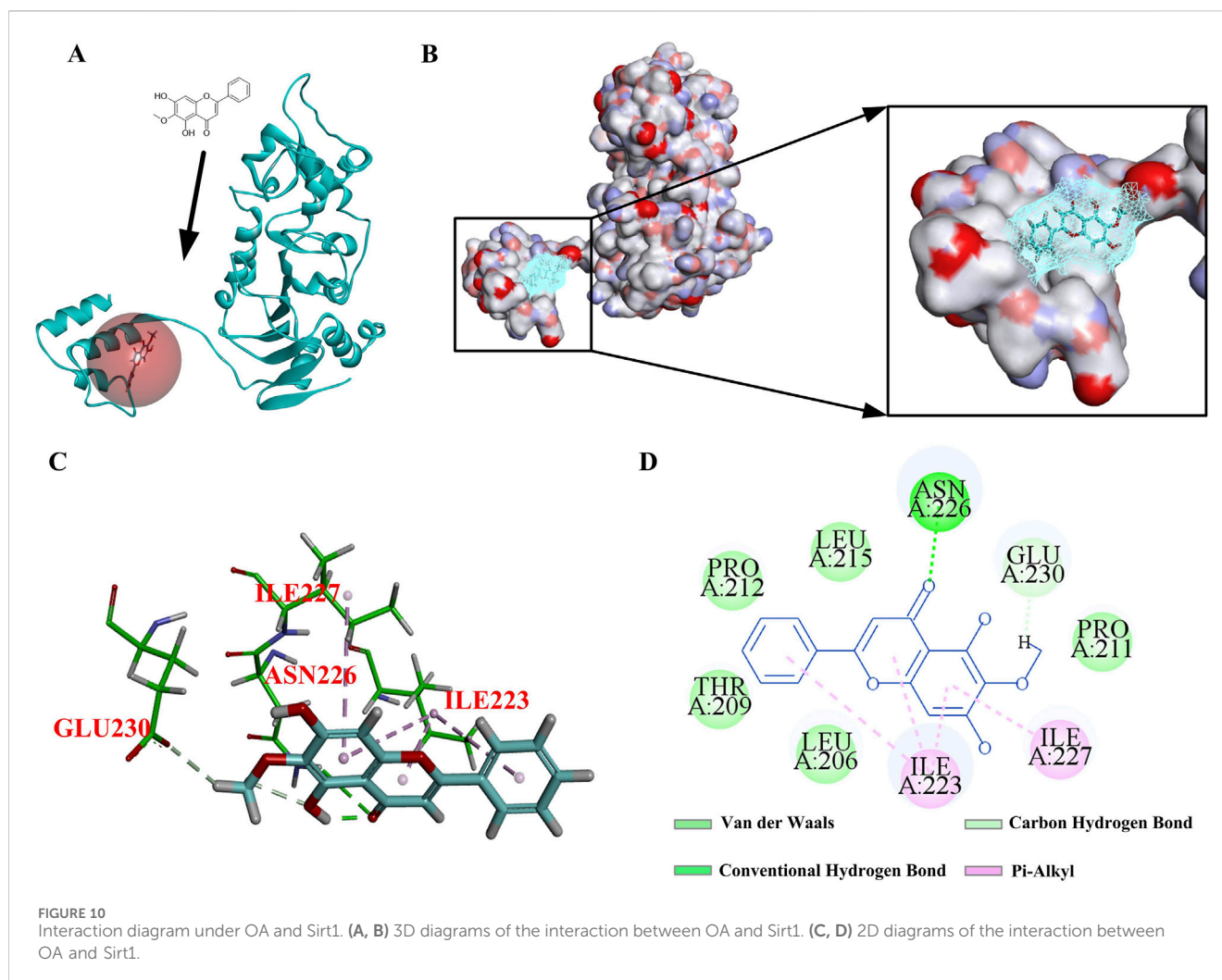
### 3.5 OA inhibited renal fibrosis progression by activating Sirt1

Previous studies have confirmed that OA protects mice from dox-induced acute cardiac injury by activating the Sirt1 signaling pathway (Zhang et al., 2021; Yao M. et al., 2022). We aimed to determine whether OA activated the Sirt1 signaling pathway. Therefore, we detected the expression level of Sirt1 in the kidney tissue of UVO mice. As expected, the expression level of Sirt1 in the kidneys of UVO mice was significantly reduced (Figures 7A, B), whereas these reductions were largely reversed by OA administration. The above data suggested that OA could activate the Sirt1 signaling pathway, which might be an essential reason for the anti-fibrotic effect of OA.

### 3.6 OA reversed TGF-β1-induced pro-fibrotic markers by activating the Sirt1 signaling pathway

We then investigated the protective effects of OA on HK-2 cells treated with TGF-β1. First, we assessed the cytotoxicity of OA in HK-2 cells. OA showed no obvious cytotoxicity toward HK-2 cells at different concentrations (10, 50, and 100 μM), as shown in Figure 8A. Therefore, a concentration of 10 μM of OA was used in subsequent experiments. In cultured HK-2 human kidney epithelial cells, TGF-β1 significantly increased the expression levels of fibronectin and α-SMA. *In vitro*, OA pretreatment significantly reduced the expression of fibronectin and α-SMA (Figures 8B, C). We then wondered whether OA plays a role in renal fibrosis by regulating Sirt1 levels in renal tubular epithelial cells. *In vitro*, Sirt1 levels were





significantly reduced, but they were upregulated after OA treatment (Figures 8D, E). The results further confirmed that OA also has a strong anti-fibrotic effect *in vitro* by increasing the expression of Sirt1.

### 3.7 OA inhibited fibrosis by activating Sirt1 and inhibiting the TGF- $\beta$ /Smad3 pathway

The cells were pretreated with a specific Sirt1 inhibitor (EX-527) following treatment with TGF- $\beta$ 1. The results showed that OA impeded TGF- $\beta$ 1-stimulated aberrant expression of fibronectin and  $\alpha$ -SMA, whereas EX-527 pretreatment eliminated the protective effect of OA (Figures 9A, B). Together, the above results support that OA exerts an anti-fibrotic effect partly through the promotion of the activity of Sirt1. Given the important role of TGF- $\beta$ /Smad3 signaling in renal fibrosis, the expression of Smad3 was examined. The results showed that OA inhibited TGF- $\beta$ -induced Smad3 activation, and EX-527 pretreatment abrogated the suppressive effect of OA on Smad3 activation (Figures 9C, D). Following the *in vitro* results, OA treatment markedly inhibited the activation of Smad3 in UUO mice, thereby ameliorating renal fibrosis (Figure 9E). Collectively,

these results indicated that OA repressed renal fibrosis by activating the expression of Sirt1 and blocking the TGF- $\beta$ /Smad3 pathway.

### 3.8 OA mediated the TGF- $\beta$ /Smad3 signaling pathway by activating Sirt1

In order to better analyze the rationality of the new molecular structure, molecular docking was selected to observe the interaction between the active site of the receptor and the compound. In general, interactions, including hydrogen bonding and hydrophobic and electrostatic interactions, play an important role in the stability of these receptor–ligand complexes in docking analysis (Jia et al., 2018). Based on the above research, we hypothesized that OA might regulate downstream signaling pathways by activating Sirt1, alleviate the degree of renal fibrosis, and have a protective effect on the kidneys. According to research reports, GLU230 was a key amino acid in the Sirt1 pocket (Dai et al., 2015). Resveratrol is a typical Sirt1 agonist that can target Sirt1 to form hydrogen bonds with the key amino acid GLU230. Next, molecular docking was used to investigate the binding of OA and Sirt1. The results showed that OA binds to the Sirt1 active site (Figures 10A, B). OA formed hydrogen bonds with the key amino acid GLU230, and it also formed hydrogen bonds with the amino acid ASN226 that

resveratrol did not have, which might enhance the binding ability of OA to Sirt1. In addition, OA could also form pi-alkyl interactions, which are a type of hydrophobic interactions with ILE223 and ILE227 in the active site (Figures 10C, D). Altogether, this might be the main reason why OA is more stable than resveratrol binding.

## 4 Discussion

Currently, the mechanism of renal fibrosis is still largely unknown, and there is no effective treatment. The major findings of this study showed that OA ameliorates renal fibrosis by activating the expression and activity of Sirt1 and inhibiting the TGF- $\beta$ /Smad3 signaling pathway. Thus, our current findings provide a new pathway and promising treatment for renal fibrosis and CKD.

OA is a natural compound and has a wide range of pharmacological effects (Yao J.Y. et al., 2022). Previous studies mainly focused on the antitumor effects of OA (Yu et al., 2023; Zhu Q. Q. et al., 2020), revealing that OA has a very strong inhibitory effect on tumor invasion and metastasis. As reported, OA can exert an anti-colon cancer effect by promoting the apoptosis of colon cancer cells (Qiao et al., 2015). Furthermore, OA can also prevent the invasion of non-small-cell lung cancer cells (Huo et al., 2022; Wei et al., 2015). An increasing number of studies have confirmed that OA plays a significant role in liver fibrosis, acute liver injury, osteoarthritis, and myocardial cell injury (Jin et al., 2018; Chen et al., 2021). Our study reports the role of OA in the treatment of renal fibrosis for the first time. We fully confirmed that OA had a significant anti-fibrotic effect. Subsequent results confirmed that OA inhibited renal fibrosis by regulating Sirt1 activity. Sirt1 is one of the most interesting members, with multiple functions, including cellular metabolism, immune response, and senescence (Huang et al., 2021; Shi et al., 2024; Cui et al., 2022; Kadono et al., 2022). Sirt1 has protective effects on various kidney diseases, such as acute renal injury, diabetes nephropathy, and CAD (Shu et al., 2024; Zhong and Zhang, 2024; Yan et al., 2022). Sirt1 ameliorated renal fibrosis by reducing endoplasmic reticulum stress (Chang et al., 2016). In our study, OA treatment remarkably mitigated renal fibrosis and conserved kidney function by activating Sirt1 and suppressing the TGF- $\beta$ /Smad3 pathway. The above results support that OA exerts an anti-fibrotic effect at least partly through promoting the activity of Sirt1.

Some effective drugs for the prevention or treatment of renal fibrosis have also been found in abundant natural resources. For example, quercetin alleviates renal fibrosis by inhibiting the TGF- $\beta$ /Smad3 signaling pathway. However, *in vitro*, it is administered orally twice as much as OA (Lu et al., 2018). Epigallocatechin gallate inhibits the TGF- $\beta$ /Smad3 signaling pathway and attenuates renal fibrosis (Zhu Y. et al., 2020). Pectin alleviates renal fibrosis by inhibiting Smad3 and TGF- $\beta$ /Smad3 signaling (Ren et al., 2022). The TGF- $\beta$ /Smad3 signaling pathway is one of the classic signaling pathways for the treatment of renal fibrosis. OA inhibits the TGF- $\beta$ /Smad3 signaling pathway through different targets and has great potential for the treatment of renal fibrosis.

In our study, through the UO mouse model, we observed that OA administration improved renal fibrosis and renal function in mice. It upregulated the expression of Sirt1 and inhibited the TGF- $\beta$ /Smad3 pathway, thus significantly and markedly improving renal fibrosis and renal function. OA mainly formed hydrogen bonds with

the key amino acid GLU230 in the active site. Among them, hydrogen bond interactions are more critical for evaluating the binding affinity and stable conformations. The results showed that OA had good binding ability to proteins. The above experimental results together proved that OA exerts an anti-fibrosis effect through the activation of Sirt1 activity. Furthermore, based on previous studies, we speculated that OA had potential and great benefits for the treatment of human diseases in clinical settings. First, OA is the main effective component of astragalus, which is the active ingredient of natural food sources. Second, OA has multiple biological effects and is safe without side effects and cytotoxicity. Third, together with previous reports, it collectively reveals that OA exerts an anti-fibrotic effect. In conclusion, our findings present strong evidence for OA in the clinical therapy of renal fibrosis. Next, we will explore the relationship between OA and Sirt1 activity in more depth.

## 5 Conclusion

We studied the druggability and anti-renal fibrosis effect of OA based on gene and protein levels. The results showed that physicochemical and ADMET properties of OA were within an acceptable range, and OA could significantly reduce the expression of fibrosis markers. The renal fibrosis-related mRNA expression and proteins levels of fibronectin and  $\alpha$ -SMA were significantly reduced compared to the model group. Inflammation is a hallmark of renal fibrosis. Through the treatment of OA, we found that the mRNA expression levels of inflammatory factors (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) decreased, while the mRNA expression level of Sirt1 increased. OA could promote the expression of Sirt1. Through protein and gene validation, we found that the anti-fibrosis renal protective effect of OA was tightly linked to Sirt1 and the TGF- $\beta$ /Smad3 signaling pathway. OA might serve as a treatment for CKD, considering its anti-fibrosis effect. These claims need further investigation.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

## Ethics statement

The animal study was approved by Tianjin Yishengyuan Biotechnology Co., Ltd. (YSY-DWLL-2024438). The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

GL: writing—original draft, conceptualization, data curation, investigation, and methodology. SX: investigation, methodology, and writing—original draft. XC: writing—original draft, investigation, methodology, software, and visualization. YH: conceptualization, investigation, methodology, and

writing–original draft. WJ: conceptualization, formal analysis, methodology, and writing–original draft. YM: writing–review and editing, conceptualization, and investigation.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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