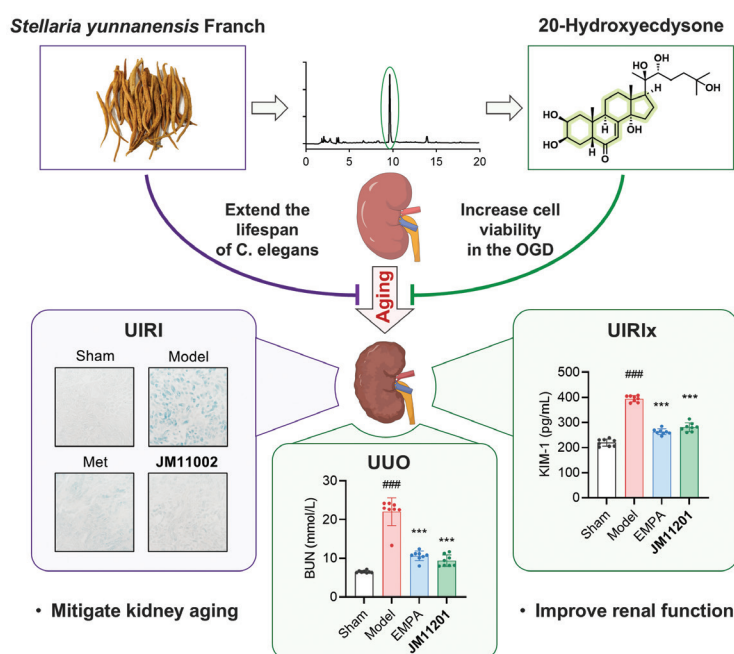


Stellaria yunnanensis Franch and its active constituent 20-hydroxyecdysone ameliorate renal failure

Graphical abstract



Highlights

- The 75% ethanol extract of *Stellaria yunnanensis* Franch exhibited anti-aging activity in *C. elegans* and mouse models.
- The compound 20-hydroxyecdysone was identified as a key bioactive constituent of the extract and showed efficacy in both chronic and acute renal failure models.
- The compound 20-hydroxyecdysone was found to exert renoprotective effects by regulating the TGF- β 1/Smad3 signaling pathway.

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In Brief

The 75% ethanol extract of *Stellaria yunnanensis* Franch and its active component 20-hydroxyecdysone exhibit anti-aging and renoprotective effects in *C. elegans*, cellular, and mouse CRF models. Mechanistically, 20-hydroxyecdysone suppresses TGF- β 1/Smad3 signaling and decreases senescence-associated markers (p21/p53), thus highlighting its therapeutic potential for CRF.

Research Article

Stellaria yunnanensis Franch and its active constituent 20-hydroxyecdysone ameliorate renal failure

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ABSTRACT

Chronic renal failure (CRF) is a progressive disease characterized by a sustained loss of kidney function, for which current therapies remain insufficient. Growing evidence closely links the CRF pathogenesis to renal aging; therefore, targeting aging processes might have therapeutic promise. Herein, we implemented a novel strategy to identify potential anti-CRF agents from anti-aging Chinese herbal medicines. Phenotypic screening indicated that the 75% ethanol extract of *Stellaria yunnanensis* Franch (**JM11002**) exhibited marked anti-aging activity, by prolonging the lifespan of *Caenorhabditis elegans* and decreasing the proportion of SA- β -gal-positive senescent MRC-5 cells. Importantly, **JM11002** also ameliorated renal dysfunction, inflammation, and fibrosis in both unilateral ischemia-reperfusion injury (UIRI) and unilateral ureteral obstruction (UUO) mouse models. Phytochemical investigation identified 20-hydroxyecdysone (**JM11201**), a major active component of **JM11002**, which recapitulated the renoprotective effects in both the UUO model and unilateral ischemia-reperfusion injury with contralateral nephrectomy (UIRIx) model. Mechanistically, 20-hydroxyecdysone suppressed the TGF- β 1/Smad3 signaling pathway and decreased the expression of fibrosis-related proteins in UUO kidneys. In summary, through an aging-intervention strategy, we discovered the renoprotective effect of *Stellaria yunnanensis* Franch extract and its active component, 20-hydroxyecdysone, two promising candidates for the development of novel CRF therapies.

Keywords: *Stellaria yunnanensis* Franch, 20-hydroxyecdysone, chronic renal failure, anti-aging

1. INTRODUCTION

Chronic renal failure (CRF) represents the end stage of progressive kidney dysfunction, typically classified as stage 4 or 5 chronic kidney disease (CKD) [1]. Its progression is driven by factors such as acute kidney injury (AKI), diabetes, hypertension, and age-related decline in renal function [2]. The fundamental pathological driver of CRF is fibrosis, which is characterized by tubular injury,

interstitial fibrosis, tubular atrophy, and glomerulosclerosis, and ultimately leads to irreversible loss of renal function [3]. CKD poses a major global health burden and was responsible for 1.48 million deaths worldwide in 2023 [4]. Current CRF treatment strategies rely primarily on angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and sodium-glucose cotransporter 2 inhibitors [5, 6]. Although these therapies usually target pathological causes underlying

kidney failure, they generally do not reverse established pathological changes within the kidneys [7, 8]. In clinical practice, dialysis, despite being resource intensive, remains the primary treatment option for most patients with advanced CRF [9]. Therefore, an urgent unmet need exists for novel therapeutic agents that effectively and safely halt or reverse disease progression in CRF.

Aging is a major risk factor for CRF development [10]. As people age, kidney function gradually declines, and the prevalence of CRF markedly increases in older populations [11]. According to epidemiological studies, CKD affects approximately 40% of individuals 65 years or older in the United States [12]. The hallmarks of kidney aging typically include impaired tubular function, tubulointerstitial fibrosis, glomerulosclerosis, and decreased renal blood flow [13, 14]. Aging kidneys are vulnerable to stressors such as hyperglycemia and hypertension, which exacerbate pathological fibrosis and inflammatory responses, and further accelerate the progression of renal failure [15, 16]. In recent years, anti-aging interventions have emerged as a promising therapeutic strategy for CRF [17]. For example, the flavonoid fisetin has been demonstrated to selectively eliminate senescent cells, decrease oxidative stress-induced senescence, and alleviate renal fibrosis in aging mice [18]. It also decreases senescence markers and myofibroblasts in lupus nephritis mouse models, thus further supporting the potential of senotherapeutic approaches in CRF treatment [19]. We have previously observed that the kidneys in naturally aging mice exhibit features of accelerated aging characterized by elevated senescence-associated β -galactosidase (SA- β -gal) activity [20]. Consequently, we developed an SA- β -gal-targeted senolytic prodrug that selectively eliminates senescent cells and effectively attenuates renal injury in mouse models. Collectively, these findings largely support aging intervention as a novel therapeutic strategy and theoretical framework for CRF treatment [21].

Traditional Chinese medicine theory and modern pharmacological studies have suggested that many herbal medicines have multiple activities, including antifibrotic effects and promotion of cellular regeneration [22, 23]. *Stellaria yunnanensis* Franch (*S. yunnanensis*), a medicinal herb endemic to Yunnan Province, China, was first documented in the Ming Dynasty text *Dian Nan Ben Cao*, which describes it as sweet in flavor and slightly warm in nature, with functions of nourishing the liver, spleen, and kidney. Traditionally, it has been used in folk medicine to treat fatigue and weakness associated with kidney deficiency in women, and it has also been described to promote spleen and kidney health while invigorating the liver and blood. These historical applications suggest its potential to modulate renal function. Moreover, a recent study has identified the cyclic nonapeptide Stellarin I from *S. yunnanensis* roots, which has shown potential anti-renal fibrosis activity *in vitro*, although its pharmacological effects have not been systematically evaluated [24]. Systematic pharmacological

studies on *S. yunnanensis* remain scarce, and direct experimental evidence supporting its anti-aging activity or efficacy against CRF remains largely lacking. Moreover, insufficient characterization of its bioactive constituents has hindered its development and integration into modern medical practice.

We previously established a library of 836 herbal extracts from Yunnan Province [25]. Herein, using *Caenorhabditis elegans* (*C. elegans*) lifespan assays as an aging-intervention-oriented screening strategy, we identified the 75% ethanol extract of *S. yunnanensis* (JM11002) as a candidate with anti-aging potential. Subsequent studies demonstrated that JM11002 exerted significant renoprotective effects in UIRI and UUO models. Phytochemical investigation led to the isolation of four constituents, among which 20-hydroxyecdysone (JM11201) had the strongest protective activity in an oxygen-glucose deprivation (OGD)-induced HK-2 cell injury model. Although 20-hydroxyecdysone has been reported to have beneficial effects against kidney damage in gerbils with streptozotocin-induced diabetes, and to alleviate TGF- β 1-induced renal tubular cell fibrosis *in vitro*, systematic pharmacological research on its role in CRF remains lacking [26, 27]. Herein, 20-hydroxyecdysone was found to exhibit marked renoprotective effects in the UUO and UIRI models, characterized by attenuated renal fibrosis and senescence-associated markers. Given the central role of the TGF- β 1/Smad3 pathway in renal fibrogenesis and CRF progression, and findings from our cellular experiments, we performed further mechanistic analysis, which demonstrated that 20-hydroxyecdysone suppresses TGF- β 1/Smad3 pathway activation and decreases the expression of downstream fibrosis-related proteins. Overall, this study identified 20-hydroxyecdysone as an anti-aging and renoprotective agent in *C. elegans*, cellular, and mouse models of renal failure, and established 20-hydroxyecdysone as a major active constituent that ameliorates both chronic and acute renal injury by suppressing the TGF- β 1/Smad3 pathway.

2. METHODS

2.1 Plant material and extraction

The roots of *S. yunnanensis* collected from the Bai Autonomous Prefecture of Dali (Yunnan, China) were used as the raw material. After air-drying in the shade, dried roots (3 kg) were pulverized and passed through a 30-mesh sieve. The powdered material was then subjected to reflux extraction three times with 75% ethanol at 95°C for 1 h each. The combined extracts were concentrated under reduced pressure to obtain a viscous residue, which was subsequently freeze-dried, thus yielding 1,057 g crude extract powder, corresponding to an extraction yield of 35.23%. This extract was designated JM11002.

2.2 *C. elegans* culture and maintenance

Wild-type *C. elegans* (strain N2) worms were obtained from the *Caenorhabditis* Genetics Center and maintained

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at 20°C on solid nematode growth medium (NGM) plates seeded with *E. coli* OP50 as the food source.

2.3 *C. elegans* lifespan assays

Synchronized populations of *C. elegans* were prepared by collection of worms from NGM agar plates in M9 buffer (1 L H₂O, 5 g NaCl, 6 g Na₂HPO₄, 3 g KH₂PO₄, and 1 mL 1 mol/L MgSO₄). Eggs were isolated with a bleaching solution (2.5 mL NaClO with 13,600–18,400 mg/L available chlorine, 0.5 mL 10 mol/L NaOH, and 7 mL H₂O). Eggs were cultured on NGM plates at 20°C until the L4 larval stage. Synchronized L4 worms were then transferred to NGM plates containing the test compound or vehicle. A 0.5% (v/v) dimethyl sulfoxide (DMSO)-treated group served as the vehicle control. To prevent progeny development, we added 5-fluoro-2'-deoxyuridine (50 µg/mL) daily from day 0. Survival was recorded daily, and worms were transferred to fresh plates every other day.

2.4 Bacterial growth assays

According to previously published protocols [28], *E. coli* OP50 was diluted in liquid LB medium to an OD₆₀₀ of 0.4, and 30 µL was seeded onto NGM plates, which were incubated at 20°C. At each time point (12, 24, 36, 48, 60, and 72 h), bacteria were washed off and resuspended in 1 mL M9 buffer, and the OD₆₀₀ was measured with a Hitachi U-2910 spectrophotometer. M9 buffer served as a blank control.

2.5 Pharyngeal pumping and body bending assays

Pharyngeal pumping was measured according to established methods [29]. A total of 20 worms were placed on NGM plates seeded with OP50. On days 3 and 8 of adulthood, the number of pharyngeal contractions was recorded over 30 seconds for 15–20 worms per group. For assessment of body bending, a drop of M9 buffer was placed on an NGM plate without bacteria, and each worm was allowed to acclimate for 30 seconds. Body bends (defined as complete lateral movement of the midsection) were counted for 30 seconds.

2.6 Thermotolerance assays

According to established methods [30], 20 worms per group were placed on OP50-seeded NGM plates on day 4 of treatment and incubated at 35°C. Viability was assessed every 3 h through gentle prodding with a platinum wire, and nonresponsive worms were scored as dead. Survival curves were generated in GraphPad Prism, and statistical significance was evaluated with the log-rank (Mantel-Cox) test.

2.7 Osmotic stress resistance assays

According to established methods [31], 20 worms were transferred on day 4 of treatment to NGM plates containing 500 mM NaCl. Crawling ability was recorded every 2 minutes from 3 minutes after exposure until all

worms became immobile. Immobility under platinum wire stimulation was scored as death. GraphPad Prism was used to generate and compare survival curves with the log-rank test.

2.8 Cell culture and treatment

MRC-5 cells were obtained from the Stem Cell Bank of the Chinese Academy of Sciences and cultured in MEM (Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% GlutaMAX (Gibco), 1% non-essential amino acids (Gibco), 1% sodium pyruvate (Gibco), and 1% penicillin/streptomycin (Yeasten). HK-2 cells were purchased from Wuhan PriCells Biotechnology and maintained under the same conditions in 0.1% gelatin-coated dishes. NRK-49F cells were obtained from Shanghai Jinyuan Biotechnology and cultured in DMEM (Gibco) containing 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂.

2.9 *In vitro* anti-fibrotic assays

NRK-49F cells in logarithmic growth phase were seeded into six-well plates at a density of 2 × 10⁵ cells per well and cultured for 24 h. The control group was maintained under normal culture conditions. The model group was treated with TGF-β1 (10 ng/mL) for 36 h. In the treatment groups, cells were co-treated with TGF-β1 (10 ng/mL) and the indicated compounds for 36 h. JM11201 and empagliflozin (EMPA) were tested at concentrations of 30 and 60 µM. After treatment, cells were collected for subsequent analyses.

2.10 CCK-8 assays

When HK-2 cells reached >90% confluency, they were seeded into 96-well plates at a density of 40,000 cells per well in 100 µL MEM. After 24 h, test compounds were added, and the cells were incubated for an additional 12 h. The medium was then replaced with PBS (with or without compounds), and the cells were subjected to hypoxic conditions (5% CO₂, 1% O₂, and 94% N₂) for 6 h. After hypoxic exposure, 10% (v/v) CCK-8 reagent (Targetmol) was added in fresh MEM and incubated for 2 h before absorbance measurement at 450 nm with a microplate reader (Bio-Tek Synergy H1).

2.11 SA-β-gal staining

SA-β-gal staining was performed with a commercial kit (Beyotime) according to the manufacturer's instructions to evaluate SA-β-gal positivity in MRC-5 cells and mouse kidneys. MRC-5 cells were seeded in 24-well plates at a density of 10,000 cells per well in 1,000 µL MEM, then treated with test compounds for 72 h. After treatment, the cells were washed three times with PBS and fixed at room temperature for 15 minutes. Fresh staining solution was applied, and the cells were incubated overnight at 37°C. On the following day, the cells were washed again and counterstained with DAPI for quantification.

Mouse kidney tissues were embedded in optimal cutting temperature compound (Leica), cryosectioned at 20 μm thickness (CRYOSTAR NX 50, Thermo), and mounted on slides (Servicebio). Kidney staining was performed as described for cultured cells. Images were captured with a Nikon inverted microscope.

2.12 Animals

C57BL/6J mice were purchased from Charles River (Beijing, China). The animals were housed under specific-pathogen-free conditions at a controlled temperature of 25°C and a relative humidity of 55%, under a 12-h light/dark cycle. Food and water were provided *ad libitum*.

2.13 UIRI model

The UIRI model was established according to previously described procedures [21]. Mice were randomly assigned into five groups ($n = 8$): (1) sham-operated with vehicle; (2) UIRI with vehicle; (3) UIRI treated with 200 mg/kg metformin; (4) UIRI treated with 1 g/kg **JM11002**; and (5) UIRI treated with 2 g/kg **JM11002**. Under anesthesia, the left renal pedicle was clamped for 35 minutes with a non-traumatic microvascular clamp, whereas the right kidney was left untouched. In the sham group, the same surgical steps were performed without vascular clamping. Met and **JM11002** were dissolved in DMSO and formulated in a vehicle consisting of 65% PBS, 30% polyethylene glycol (PEG)-200, and 5% DMSO. Met served as the positive control. From day 7 post-surgery, treatments were administered orally every other day for 30 days. The mice were euthanized on day 37 post-surgery.

2.14 UUO model

The UUO model was established according to standard protocols [32]. In one experiment, mice were randomized to three groups ($n = 4$): (1) sham-operated with vehicle; (2) UUO with vehicle; and (3) UUO treated with 2 g/kg **JM11002**. In another experiment, four groups ($n = 8$) were examined: (1) sham-operated with vehicle; (2) UUO; (3) UUO treated with 20 mg/kg EMPA; and (4) UUO treated with 10.88 mg/kg **JM11201**. Under anesthesia, the left ureter was exposed via a flank incision and ligated twice with 5-0 surgical sutures, with a cut made between the ligatures. Sham-operated mice underwent identical procedures without ureter ligation. EMPA and **JM11201** were dissolved in DMSO and formulated in a vehicle consisting of 65% PBS, 30% PEG-200, and 5% DMSO. EMPA served as the positive control. Treatments were administered once daily by oral gavage from day 7 to day 13 post-surgery, and mice were euthanized on day 14 post-surgery.

2.15 UIRx model

The UIRx model was established according to a previously published method [33]. Mice were randomly divided into four groups ($n = 8$): (1) sham-operated with

vehicle; (2) UIRx with vehicle; (3) UIRx treated with 20 mg/kg EMPA; and (4) UIRx treated with 10.88 mg/kg **JM11201**. Under anesthesia, a right flank incision was made to expose and remove the right kidney by ligating the renal pedicle with surgical thread. Subsequently, the left renal pedicle was clamped for 30 minutes with a 3.7 cm curved microvascular clamp. Sham-operated mice underwent right nephrectomy but no ischemia-reperfusion procedure on the left kidney. EMPA and **JM11201** were dissolved in DMSO and formulated in a vehicle consisting of 65% PBS, 30% PEG-200, and 5% DMSO. EMPA served as a positive control. Oral administration of the test compounds was conducted once daily for three consecutive days before surgery. The mice were euthanized 24 h after surgery.

2.16 Ki67 immunohistochemistry

Mouse kidney tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated with a graded ethanol series, cleared in xylene, and embedded in paraffin. Tissue sections were cut into 5 μm sections. After deparaffinization and rehydration, antigen retrieval was performed with sodium citrate buffer (pH 6.0) at 95°C for 20 minutes, followed by natural cooling and washing with PBS three times. Sections were blocked with 5% BSA at room temperature for 1 hour, then incubated overnight at 4°C with rabbit anti-Ki67 primary antibody (CST, 1:200). After being washed, sections were incubated with Alexa Fluor-conjugated secondary antibody (1:500) for 1 hour in the dark at room temperature, counterstained with DAPI for 5 minutes, washed in PBS, and mounted. Images were captured with a Nikon inverted fluorescence microscope.

2.17 Sirius Red staining

Kidney tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4 μm sections. After deparaffinization, sections were stained with Sirius Red to visualize collagen deposition. Images were acquired with a Nikon Eclipse Ts2R inverted microscope. The positive staining area was quantified in ImageJ software.

2.18 Western blotting

Cells and mouse kidney tissues were lysed with RIPA buffer (Yeasen, China). Protein concentration was determined with a BCA assay kit. Equal amounts of protein were separated with SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies to fibronectin (Proteintech, 15613-1-AP), collagen I (Proteintech, 14695-1-AP), p21 (Santa Cruz, sc-6246), p53 (Santa Cruz, sc-126), Smad3 (Abclonal, A19115), p-Smad3 (S423/S425) (Abclonal, AP0727), TGF- β 1 (HUABIO, HA721143), and GAPDH (Proteintech, 60004-1-Ig), and subsequently incubated with secondary antibodies. Signals were developed with enhanced chemiluminescence (ECL, Yeasen, China) and visualized.

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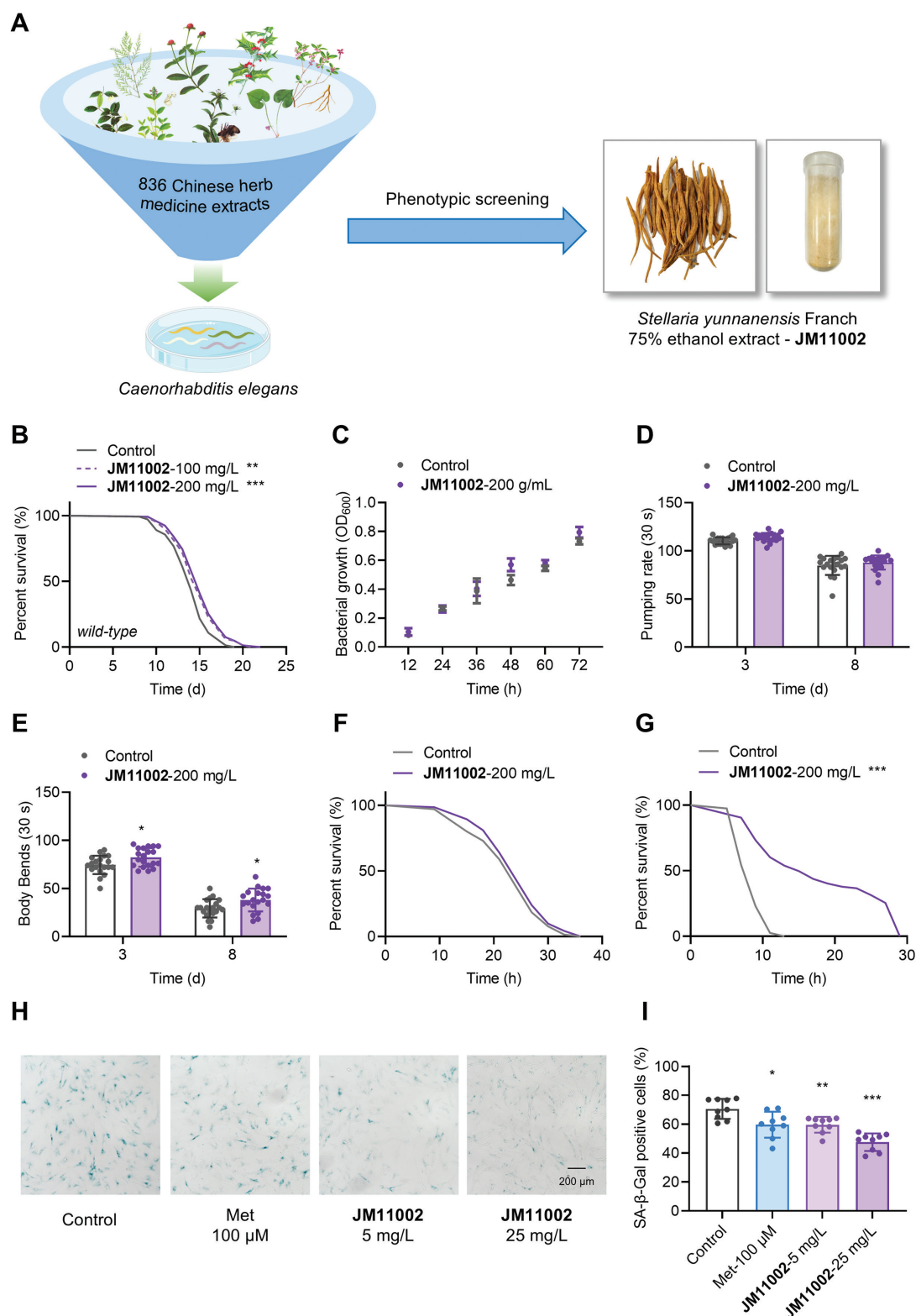


Figure 1 | JM11002 anti-aging effects in *C. elegans* and MRC-5 cells.

(A) Screening of a crude extract library of traditional Chinese medicines from western Yunnan with lifespan assays in wild-type *C. elegans*. (B) Lifespan assay in *C. elegans*. n = 150. (C) Growth assay of *E. coli* OP50. n = 3. (D) Pharyngeal pumping frequency in *C. elegans*. n = 20.

(E) Body bend counts in *C. elegans*. n = 20. (F) Heat stress assay. n = 150. (G) Osmotic stress assay. n = 150. (H) Representative images of SA- β -gal staining in MRC-5 cells. n = 3, Scale bars, 200 μ m. (I) Quantification of SA- β -gal staining. Data are mean $\pm$ SD; * P < 0.05, ** P < 0.01, *** P < 0.001 compared with the control group.

2.19 Data analysis

Image data were processed in ImageJ (version 1.54). Statistical analysis was conducted in GraphPad Prism software. All results are presented as mean $\pm$ standard deviation (SD). For two-group comparisons, an unpaired Student's t-test was applied. For multiple-group comparisons, one-way ANOVA was used. *In vivo* experiments were conducted in animals randomly assigned to groups. No animals or data were excluded, and the study was not blinded.

3. RESULTS

3.1 JM11002 enhances lifespan and healthspan in *C. elegans*

Through lifespan assays in wild-type *C. elegans*, we screened a library of crude plant medicine extracts from western Yunnan. The 75% ethanol extract of *S. yunnanensis* (JM11002) was found to significantly extend *C. elegans* lifespan (Figure 1A). Treatment with JM11002 at concentrations of 100 mg/L and 200 mg/L increased the mean lifespan by 5.99% and 7.76%, respectively (Figure 1B). Notably, the 200 mg/L dose exhibited the most pronounced lifespan-extending effect and was therefore selected for subsequent studies. To confirm that the observed lifespan extension was not attributable to dietary restriction, we examined the proliferation of *E. coli* OP50, the food source of *C. elegans*, and observed no significant effect of JM11002 at a dose of 200 mg/L, thereby ruling out dietary restriction as a confounding factor (Figure 1C).

Pharyngeal pumping rate and body bend frequency are widely used indicators of nematode health status. To comprehensively evaluate the anti-aging effects of JM11002, we measured these parameters on days 3 and 8 of adulthood (Figure 1D). Treatment with JM11002 at 200 mg/L did not decrease the pharyngeal pumping frequency at either time point, thus indicating no adverse effects on basic physiological function, whereas locomotor performance showed marked improvements over the control group (Figure 1E). Because prolonged organismal lifespan is often associated with enhanced stress resistance [34], we assessed the ability of JM11002 to increase *C. elegans* stress resistance in stress tolerance assays. In heat stress assays, the average survival time of *C. elegans* in the 200 mg/L JM11002-treated group at 35°C was extended by 1.22 h, although this increase did not reach statistical significance (Figure 1F). In hyperosmotic stress assays under 500 mM NaCl, the survival time of *C. elegans* in the 200 mg/L JM11002-treated group was extended by 108.9%, thereby indicating a significant enhancement in *C. elegans* resistance to osmotic stress (Figure 1G).

3.2 JM11002 decreases SA- β -gal positivity in MRC-5 cells

In agreement with the observations in *C. elegans*, SA- β -gal staining of human embryonic lung fibroblasts (MRC-5 cells) further confirmed the anti-aging effect of JM11002 (Figure 1H, I). At concentrations of 5 mg/L and 25 mg/L, JM11002 significantly decreased the proportion of SA- β -gal-positive cells, by 10.98% and 23.02%, respectively, thus outperforming the Met positive control (20 mg/L, 10.95%). Because JM11002 not only extended the lifespan and healthspan in *C. elegans* but also decreased the proportion of senescent cells in mammalian cells, it might potentially serve as an effective anti-aging agent.

3.3 JM11002 ameliorates renal injury and renal aging in a UIRI-induced chronic renal injury model and UUO-induced renal interstitial fibrosis model

Renal aging is closely associated with CRF progression and accelerates functional decline. Therefore, targeting aging pathways is a promising therapeutic strategy for CRF. Having demonstrated the anti-aging effects of JM11002 *in vitro*, we next evaluated its renal protective effects *in vivo*. Notably, Dian Nan Ben Cao described *S. yunnanensis* as capable of strengthening the spleen and tonifying the kidney, thus implying potential renal protective properties (Figure 2A). Accordingly, we evaluated JM11002 in the UIRI model, which recapitulates the progression from AKI to CRF. Beginning on day 7 after surgery, mice were orally administered JM11002 at 1 g/kg or 2 g/kg every other day for 30 days (Figure 2B). Met served as a positive control. The UIRI group exhibited typical features of renal damage, including elevations in serum BUN and Cr, injury markers, and histopathological alterations. In contrast, JM11002 treatment notably decreased serum BUN and Cr (Figure 2C, D), thereby indicating improved kidney function, and decreased the injury markers KIM-1 and NGAL (Figure 2E, F), thereby suggesting notable protective effects on renal tubular cells. Furthermore, JM11002 showed anti-inflammatory properties, by significantly decreasing the pro-inflammatory cytokines TNF- α and IL-6 (Figure 2G, H). Histological analysis corroborated these findings. Hematoxylin and eosin (H&E) and picrosirius red (PSR) staining showed decreased brush border loss, tubular dilation, and fibrosis (Figure 2I, J). Immunofluorescence staining revealed diminished Ki67 and SA- β -gal-positive cells in the JM11002-treated groups (Figure 2I, K, L); therefore, the renoprotective effects of this compound might involve modulation of aging-related processes.

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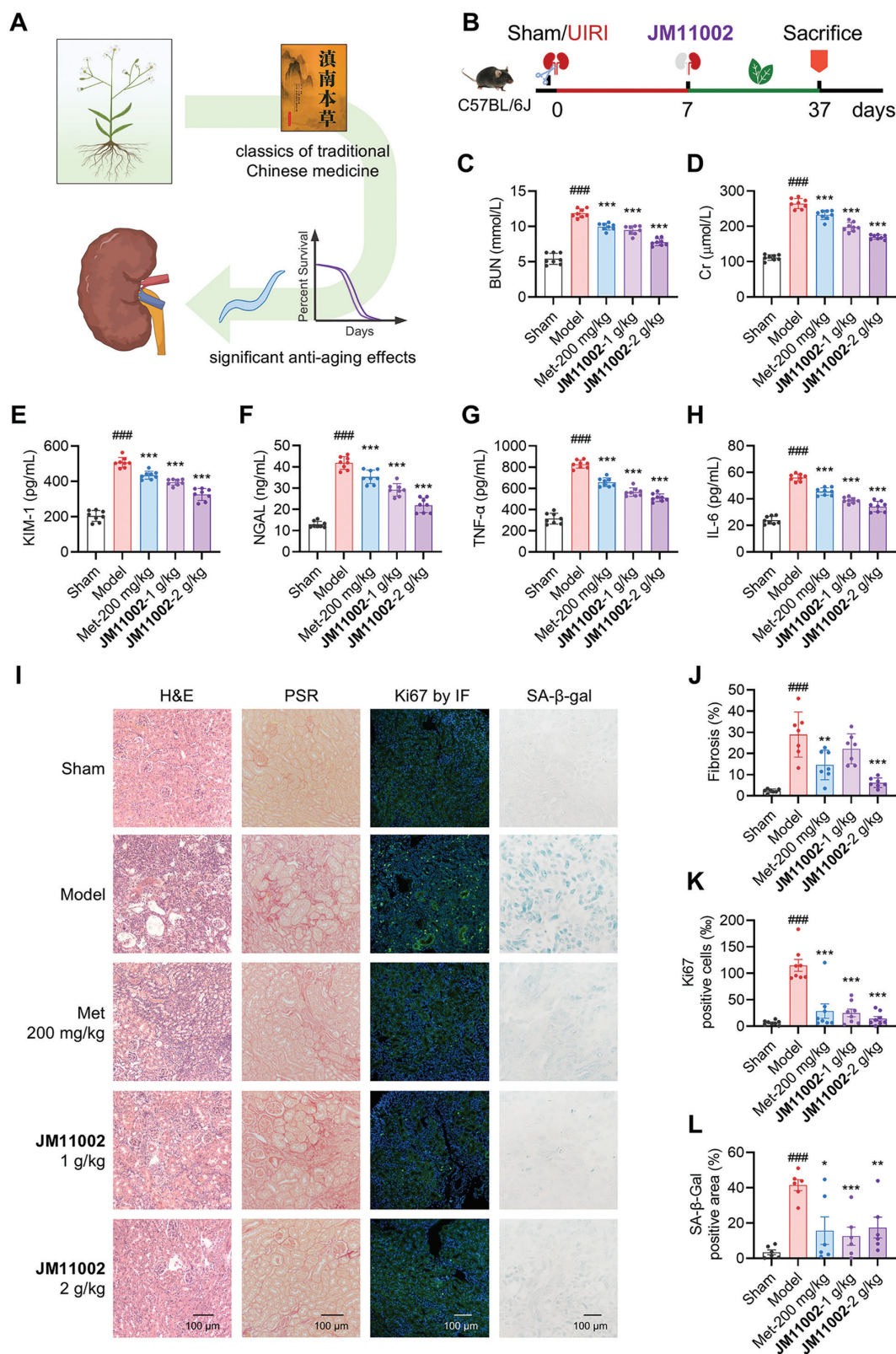


Figure 2 | JM11002 alleviates renal injury and aging in the UIRI model.

(A) Application of *S. yunnanensis* in renal failure treatment. (B) Schematic representation of the UIRI experiment. (C) Serum BUN. (D) Serum Cr. (E) Serum KIM-1. (F) Serum NGAL. (G) Serum TNF- α . (H) Serum IL-6. (I) Representative kidney tissue images with H&E staining, PSR staining, Ki67 immunohistochemistry, and SA- β -gal staining. Scale bars, 100 μ m. (J) Quantification of Sirius Red staining. (K) Quantification of Ki67 immunofluorescence. (L) Quantification of SA- β -gal staining. n = 6–8. Data are mean $\pm$ SD; ###P < 0.001 compared with the sham group, *P < 0.05, **P < 0.01, ***P < 0.001 compared with the model group. Mice received vehicle (sham and model), **JM11002** (1 g/kg or 2 g/kg, i.g., every other day), or Met (positive control) from day 7 post-surgery for 30 consecutive days.

To evaluate the effects of **JM11002** on renal fibrosis and chronic kidney injury relevant to CRF, we used the well-established UO model, which involves obstructive nephropathy and tubulointerstitial fibrosis, key features of CKD progression and renal aging. Mice were treated with **JM11002** (2 g/kg) once daily from day 7 to day 13 post-surgery (**Figure S1A**). The UO group exhibited typical features of renal injury, including marked renal enlargement; upregulation of fibrosis-related markers; and notable histological changes, such as tubular atrophy and collagen deposition. **JM11002** treatment significantly attenuated the elevations in serum Cr and Urea levels (**Figure S1B, C**), improved tissue morphology, and reduced renal interstitial fibrosis, as indicated by H&E and PSR staining (**Figure S1D**). Additionally, the findings that **JM11002** decreased the expression of fibronectin (FN) and collagen I (Col I) confirmed its potential to alleviate renal fibrosis and chronic renal failure (**Figure S1E, F**). Therefore, **JM11002** has potential in anti-aging and improving renal function, particularly in alleviating chronic renal decline; enhancing kidney structure and function after acute kidney injury; and effectively ameliorating renal interstitial fibrosis.

3.4 *In vivo* safety profile of JM11002

To systematically assess the safety of **JM11002**, we conducted a 14-day high-dose toxicity study in male and female C57BL/6J mice (8 weeks old). Mice were orally administered **JM11002** at 10 g/kg/day, corresponding to ten times the effective dose. Throughout the study period, the mice showed stable body weight (**Figure 3A**) indicative of an absence of overt toxicity. Post-treatment examination of the major organs (heart, liver, spleen, lungs, and kidneys) showed no abnormalities in gross morphology or organ/body weight ratios (**Figure 3B–D**). Histological evaluation with H&E staining showed no evidence of necrosis, edema, or inflammatory infiltration in major organs (**Figure 3E**) in the treatment group with respect to the control group. Serum biochemistry analysis revealed no significant changes in ALT, AST, urea, and Cr levels (**Figure 3F–I**), thus indicating no impairments in liver and kidney function. Therefore, **JM11002**'s excellent *in vivo* tolerability and biosafety, even at high doses, provide a sound foundation for subsequent pharmacological investigations.

3.5 Isolation, identification, and bioactivity evaluation of constituents from *S. yunnanensis*

To investigate the bioactive constituents responsible for the pharmacological effects of *S. yunnanensis*, we

systematically isolated, structurally characterized, and evaluated the bioactivity of its chemical constituents (**Figure 4A**). Using preparative liquid chromatography, we isolated two steroidal compounds, **JM11201** and **JM11203**, from the 75% ethanol extract of *S. yunnanensis* (**JM11002**). Comparative spectral analysis identified **JM11201** as 20-hydroxyecdysone (**Figure 4B**) and **JM11203** as taxisterone (**Figure 4C**), both of which are known plant-derived ecdysteroids. Subsequent purification of a more polar fraction yielded an indole alkaloid **JM11205** and a flavonoid glycoside **JM11206**, which were characterized as lycoperodine-1 (**Figure 4D**) and schaftoside (**Figure 4E**), respectively. The structures of all compounds were confirmed with high-resolution mass spectrometry-electrospray ionization, proton and carbon-13 nuclear magnetic resonance, and ultraviolet-visible spectroscopy, in accordance with published data [35–37] (**Supporting Information Figures S3–S6**).

To evaluate potential anti-CRF activity, we tested the isolated compounds in an OGD-induced HK-2 cell injury model. **JM11201** exhibited the most pronounced cytoprotective effects, by restoring cell viability to a level comparable to that observed with the parent extract **JM11002** and surpassing that of all other tested constituents (**Figure 4F**). In addition, in a TGF- β 1-induced NRK-49F cell model, 60 μ M **JM11201** significantly reversed the upregulation of FN and Col I, thus indicating effective suppression of cellular fibrosis (**Supporting Information Figure S2**). We therefore selected **JM11201** for further pharmacological studies.

3.6 JM11201 alleviates renal fibrosis in a UO-induced renal interstitial fibrosis model

Given that **JM11201** exhibited cytoprotective activity comparable to that of **JM11002** *in vitro*, we further evaluated its *in vivo* efficacy in a UO model to assess its potential against chronic renal injury. Mice were treated with **JM11201** (10.88 mg/kg) once daily from day 7 to day 13 post-surgery. This dose was derived from its relative peak area in **JM11002** (0.54%), according to the 2 g/kg extract dose used in previous experiments (**Figure 5A**). EMPA (20 mg/kg), a well-established clinical treatment for CRF, was chosen as the positive control [38]. **JM11201** ameliorated UO-induced renal dysfunction, as demonstrated by decreased serum levels of Cr and BUN (**Figure 5B, C**). Notably, **JM11201** achieved greater effects than EMPA in decreasing plasma Cr and therefore might potentially have stronger renoprotective efficacy. Histopathological analysis confirmed these

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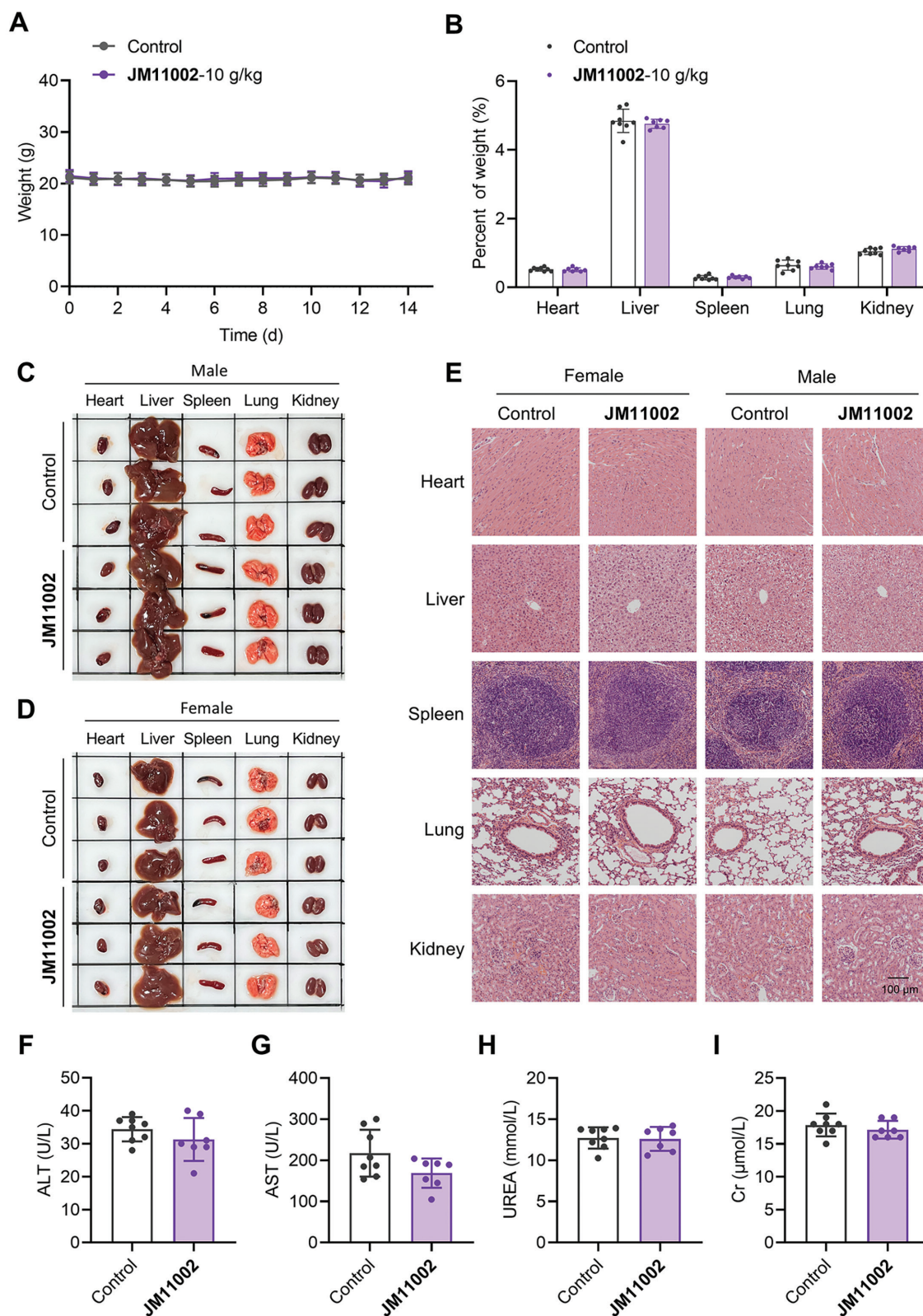


Figure 3 | *In vivo* safety evaluation of JM11002.

(A) Body weight changes over 14 days. (B) Organ-to-body weight ratios of major organs. (C) Representative gross images of the heart, liver, spleen, lung, and kidney in male mice. (D) Representative gross images of the heart, liver, spleen, lung, and kidney in female mice. (E) Representative H&E staining images of the heart, liver, spleen, lung, and kidney. Scale bars, 100 μ m. n = 8. (F) Serum ALT. (G) Serum AST. (H) Serum urea. (I) Serum Cr. Mice received vehicle (control) or **JM11002** (10 g/kg, i.g., daily) for 14 consecutive days.

results: H&E staining indicated that **JM11201** markedly attenuated structural damage in UUO kidneys (**Figure 5D, E**). We subsequently evaluated the anti-aging effects of **JM11201**. UUO induced a pronounced increase in renal p53 and p21 protein expression, a finding reflecting the association of renal aging with interstitial fibrosis. Treatment with **JM11201** significantly decreased these elevations to a greater extent than EMPA (**Figure 5F, G**). In summary, **JM11201** not only improved renal function and decreased pathological injury in the UUO model but also effectively suppressed renal fibrogenesis, thus supporting its therapeutic potential against CRF.

3.7 JM11201 alleviates acute kidney injury in a UIRIx-induced acute kidney injury model

AKI, characterized by a sudden loss of renal function, is critical in CRF onset and progression. To evaluate the therapeutic potential of **JM11201** in AKI, we established the UIRIx model, which closely mimics the pathophysiological features of acute renal failure and is relevant to clinical renal transplantation scenarios. Mice were pre-treated with **JM11201** (10.88 mg/kg) via oral gavage for three consecutive days before surgery, and EMPA (20 mg/kg) served as the positive control. Mice were sacrificed 24 h post-surgery, and tissue and blood samples were collected for analysis (**Figure 6A**). The UIRIx group exhibited distinct renal injury characteristics, as evidenced by elevated expression of renal injury biomarkers and significant inflammatory cell infiltration. However, **JM11201** treatment significantly decreased the kidney hypertrophy induced by UIRIx, to levels comparable to those in the EMPA-treated group (**Figure 6B, C**). Serum analysis revealed that **JM11201** significantly suppressed the elevation of the acute kidney injury biomarkers KIM-1 and NGAL (**Figure 6D, E**), thereby suggesting effective mitigation of acute tubular epithelial damage. Histopathological analysis further supported these findings: H&E staining indicated that **JM11201** markedly alleviated typical AKI lesions, such as brush border loss, nuclear pyknosis, and tubular epithelial swelling (**Figure 6F**). The overall renal architecture in the **JM11201**-treated group, in contrast to the untreated UIRIx group, was substantially preserved. In conclusion, **JM11201** demonstrated nephroprotective effects in the UIRIx model, by alleviating structural damage and inhibiting the rise of early injury markers, with efficacy comparable to that of EMPA. Therefore, **JM11201** was determined to be the primary active component responsible for the anti-CRF pharmacologic effects of **JM11002**.

3.8 JM11201 exerts anti-fibrotic effects by regulating the TGF- β 1/Smad3 signaling pathway in the UUO model

Given the anti-fibrotic activity of **JM11201** observed in the TGF- β 1-induced cellular fibrosis model, together with previous *in vitro* evidence that 20-hydroxyecdysone attenuates TGF- β 1-induced renal cellular fibrosis and modulates TGF- β 1/Smad-related signaling in tubular cells [27], we examined whether this pathway might be active *in vivo*. Given the prominent tubulointerstitial fibrosis and extracellular matrix accumulation in UUO kidneys, together with the clear anti-fibrotic efficacy of **JM11201** in this setting, we selected UUO kidneys for mechanistic analysis. Western blot analysis indicated that UUO, compared with the sham control, resulted in a markedly greater p-Smad3/Smad3 ratio and greater expression of TGF- β 1, FN, and Col I. **JM11201** treatment effectively reversed these changes in UUO kidneys (**Figure 7**). Therefore, **JM11201** suppresses TGF- β 1/Smad3 pathway activation and decreases downstream profibrotic protein expression *in vivo*, and this pathway might be involved in its anti-fibrotic effect in the UUO model.

4. DISCUSSION

CRF is a progressive and age-associated disorder for which existing therapeutic options have limited efficacy in halting renal fibrosis progression and functional decline. Given that aging is a major pathomechanism underlying the development and progression of CRF, interventions targeting renal aging are promising therapeutic strategies. Natural products with both anti-aging and renoprotective properties offer potential alternative CRF treatments. Herein, we investigated the potential of the 75% ethanol extract of *S. yunnanensis* (**JM11002**) and its active constituent 20-hydroxyecdysone (**JM11201**). **JM11002** exhibited marked anti-aging effects in both *C. elegans* and MRC-5 cells, by decreasing senescence markers and increasing stress tolerance. These effects align with growing interest in anti-aging therapies as a novel approach for managing age-related diseases, including CRF. *In vivo*, **JM11002** demonstrated significant renoprotective effects in both acute (UIRI) and chronic (UUO) kidney injury models, by improving kidney function and decreasing fibrosis, as evidenced by decreased serum biomarkers and preserved histoarchitecture. Therefore, **JM11002** not only alleviates age-related deterioration but also confers marked protection against renal injury and fibrosis. These dual capabilities highlight the potential of **JM11002** as a therapeutic strategy for addressing renal decline and aging.

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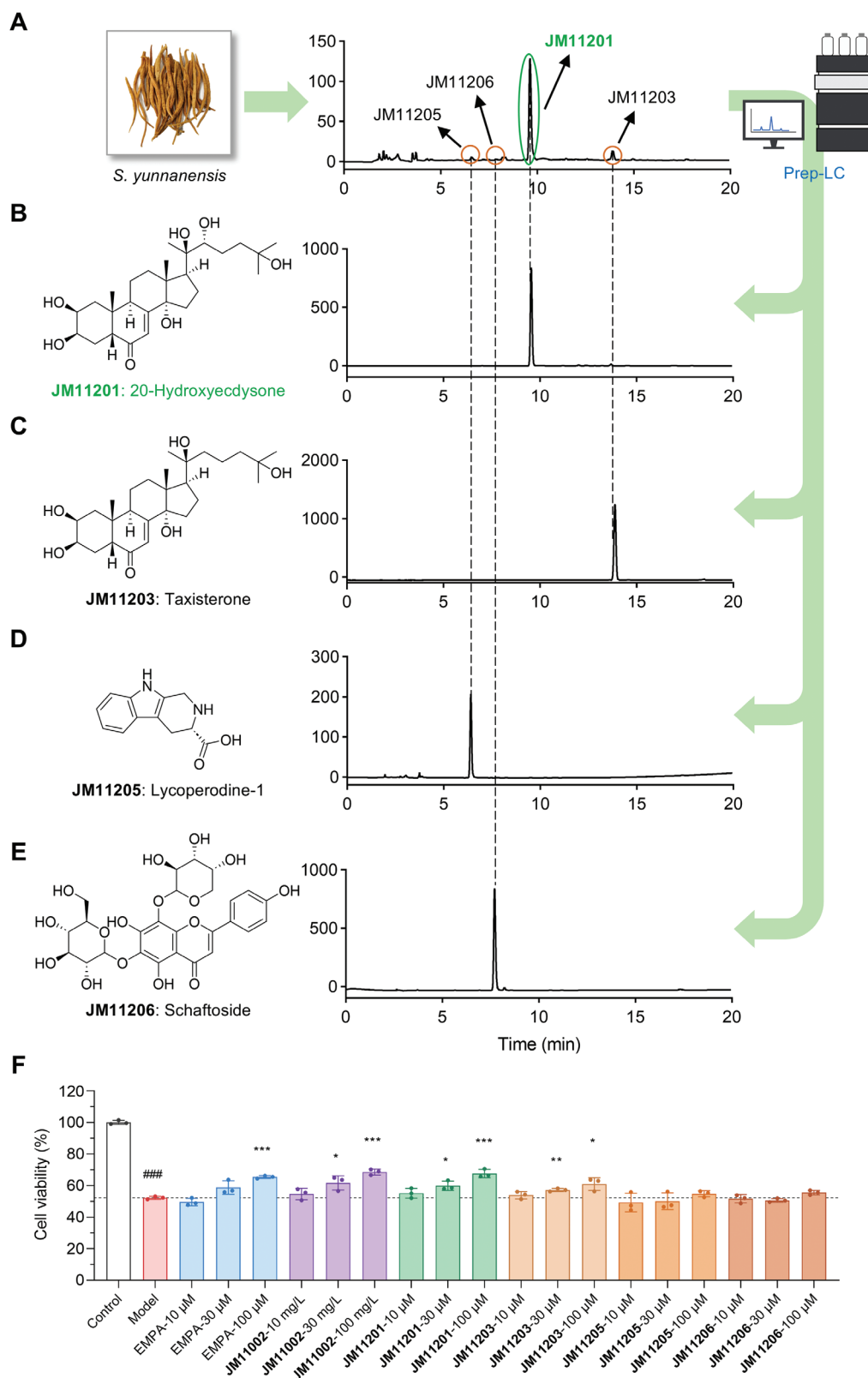


Figure 4 | Phytochemical investigation of constituents from *S. yunnanensis*.

(A) Image of *S. yunnanensis* and HPLC chromatogram of **JM11002**. (B) Chemical structure and HPLC chromatogram of **JM11201**. (C) Chemical structure and HPLC chromatogram of **JM11203**. (D) Chemical structure and HPLC chromatogram of **JM11205**. (E) Chemical structure and HPLC chromatogram of **JM11206**. (F) Cell viability in the OGD model, $n = 3$. Data are mean $\pm$ SD; ### $P < 0.001$ compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the model group.

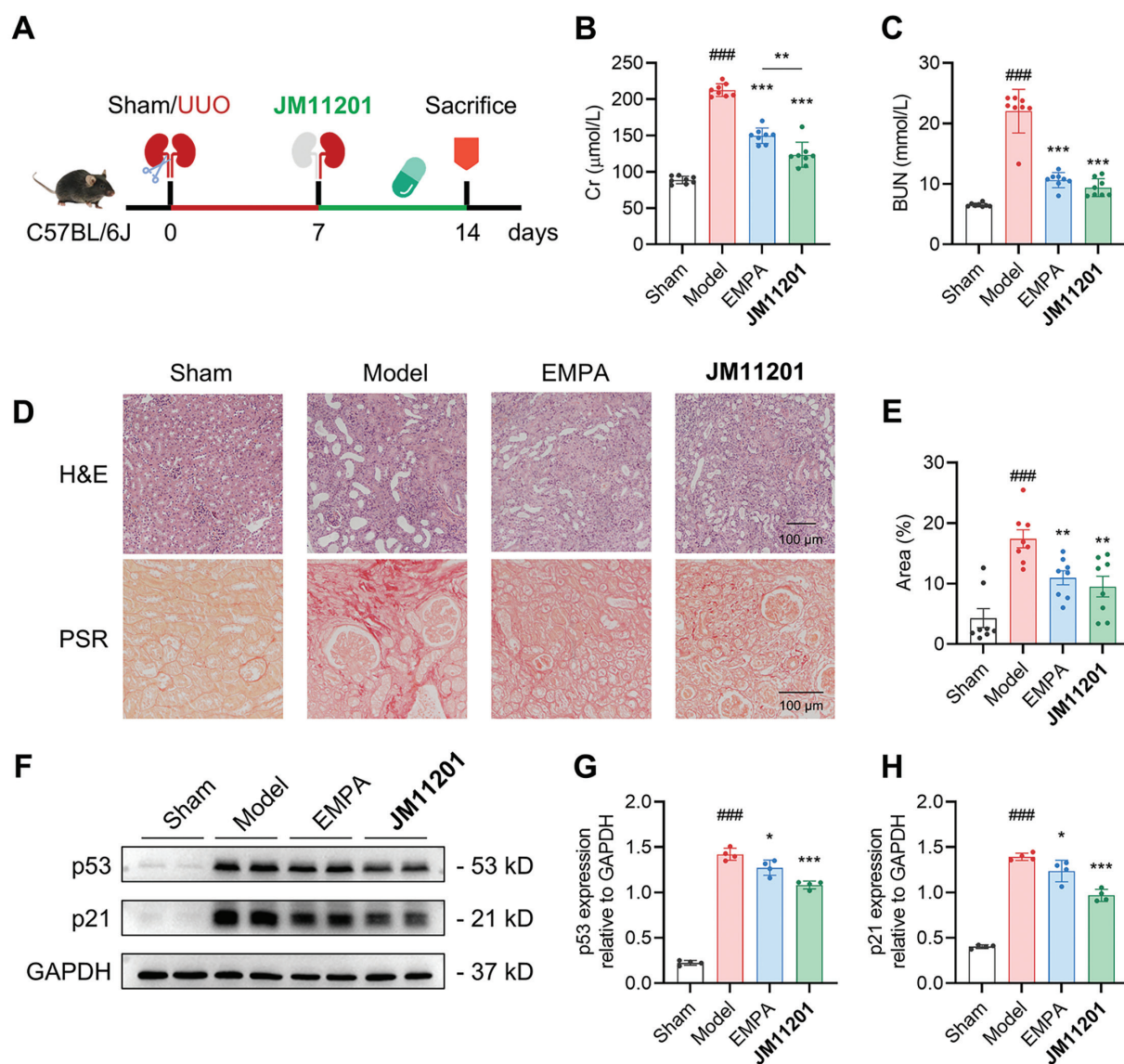


Figure 5 | JM11201 attenuates renal fibrosis in the UUO model.

(A) Schematic representation of the UUO experiment. (B) Serum BUN. (C) Serum Cr. (D) Representative kidney sections with H&E and Sirius Red staining. Scale bars, 100 μ m. (E) Quantification of Sirius Red staining. $n = 8$. (F) Representative western blot images of p53 and p21. (G) Relative expression of p53. (H) Relative expression of p21. $n = 4$. Mice received vehicle (sham and model), **JM11201** (10.88 mg/kg, i.g., daily), or EMPA (20 mg/kg, i.g., daily) from day 7 to day 13 post-UUO surgery. Data are mean $\pm$ SD; ### $P < 0.001$ compared with the sham group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the model group.

Phytochemical analysis identified 20-hydroxyecdysone (**JM11201**) as the major bioactive component of **JM11002**. Previous studies have reported beneficial effects of

JM11201 on kidney damage in diabetes-associated settings, and *in vitro* studies have shown that it attenuates TGF- β 1-induced renal cellular fibrosis in proximal

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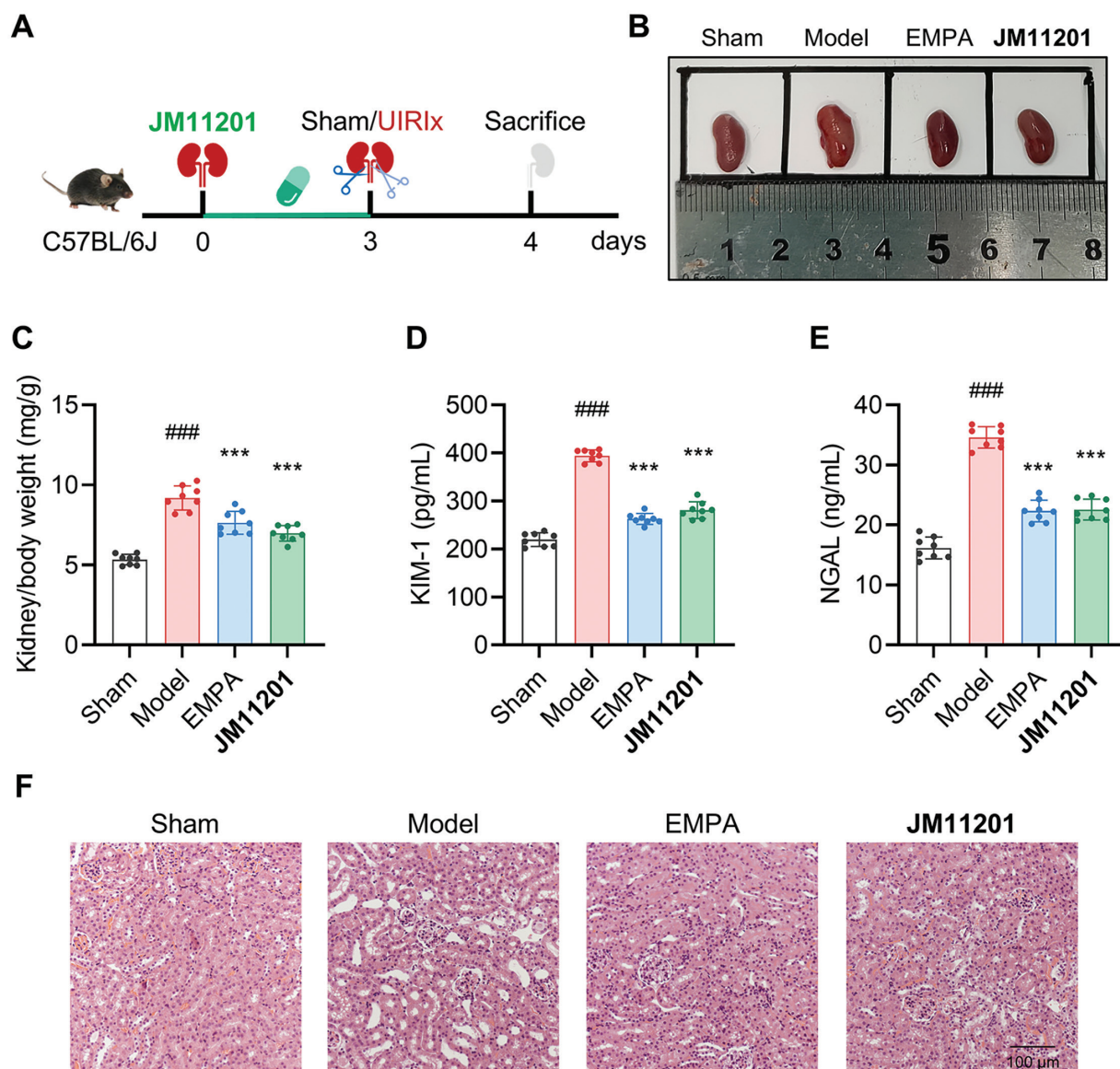


Figure 6 | JM11201 alleviates renal injury in the UIRIx model.

(A) Schematic representation of the UIRIx experiment. (B) Representative gross images of the kidneys. (C) Kidney/body weight ratio. (D) Serum KIM-1. (E) Serum NGAL. (F) Representative kidney sections with H&E staining. $n = 8$. Mice received vehicle (sham and model), **JM11201** (10.88 mg/kg, i.g., daily), or EMPA (20 mg/kg, i.g., daily) for 3 consecutive days before surgery. Data are mean ± SD; ### $P < 0.001$ compared with the sham group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the model group.

tubular cells. In our study, **JM11201** exhibited renoprotective effects in the UUO and UIRIx models. On the basis of existing reports and our previous cellular experiments, we focused on exploring the effects of **JM11201** on the TGF- β 1/Smad3 pathway in UUO kidneys. Observations that **JM11201** decreased TGF- β 1 expression, Smad3 phosphorylation, and downstream profibrotic proteins *in vivo* supported that regulation of the TGF- β 1/Smad3 pathway contributes to this compound's anti-renal fibrotic effects. These findings, together with

the observed beneficial effects on renal function, histopathology, and senescence-associated markers, position **JM11201** as a promising antifibrotic and renoprotective candidate for chronic progressive renal disease.

In addition, **JM11002**'s favorable safety profile in high-dose toxicity studies reinforced its potential for further pharmacological development. Collectively, our findings support the potential of **JM11002** and its active constituent **JM11201** as promising candidates for CRF treatment, by offering a dual-action strategy with potential

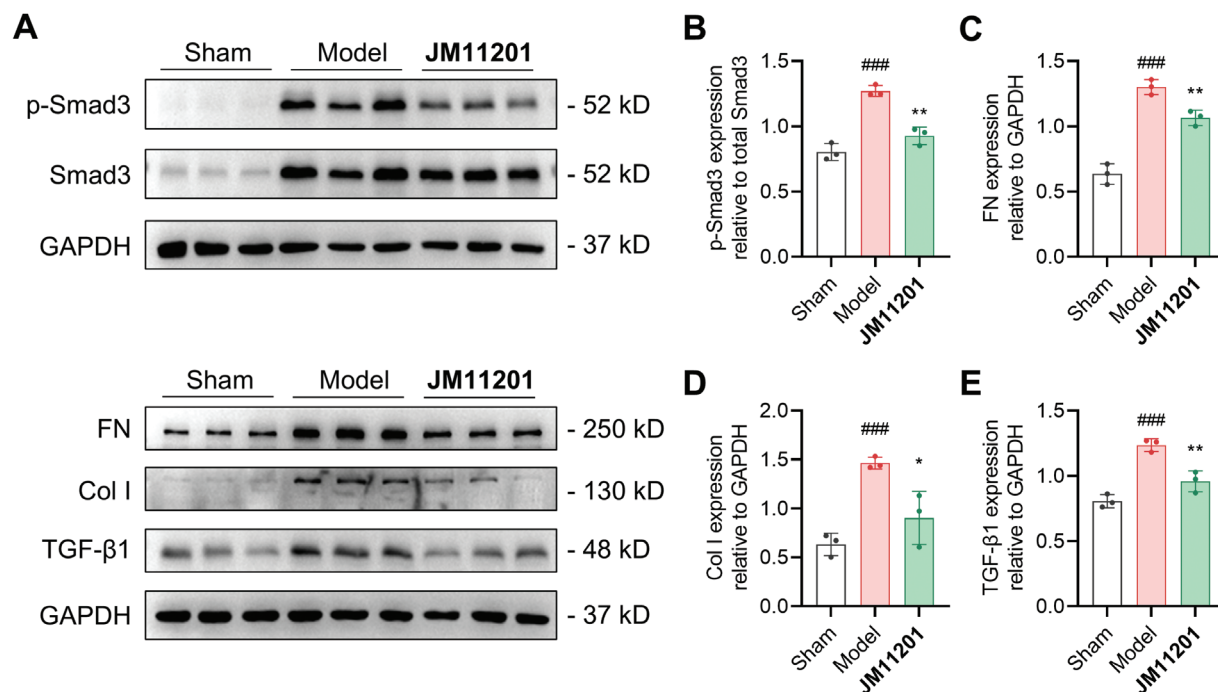


Figure 7 | JM11201 suppresses TGF-β1/Smad3 signaling in the UUO model.

(A) Representative western blot images of Smad3, p-Smad3, TGF-β1, Col I, and FN. (B) Relative expression level of p-Smad3. (C) Relative expression level of FN. (D) Relative expression level of Col I. (E) Relative expression level of TGF-β1. $n = 3$. Data are mean $\pm$ SD; ### $P < 0.001$ compared with the sham group, * $P < 0.05$, ** $P < 0.01$ compared with the model group.

to address both renal aging and injury-induced dysfunction. By integrating aging-intervention-oriented screening, phytochemical investigation, and validation in both chronic fibrotic and acute ischemic renal injury models, our study expands the pharmacological understanding of *S. yunnanensis* and provides further support for aging-guided discovery of candidate therapeutics for CRF. Nevertheless, the precise molecular targets and broader mechanism of JM11201 remain to be further clarified, and additional studies are needed to evaluate its pharmacokinetic properties and *in vivo* exposure characteristics.

5. CONCLUSION

This study demonstrated that *S. yunnanensis* extract (JM11002) and its active constituent, 20-hydroxyecdysone (JM11201), exert marked renoprotective effects in experimental models relevant to CRF. JM11002 showed anti-aging activity and ameliorated functional and structural renal injury in both acute and chronic models. JM11201 reproduced these beneficial effects in UUO and UIRx models, attenuated aging-related changes *in vivo*, and suppressed TGF-β1/Smad3 pathway activation and downstream profibrotic protein expression. Collectively, our findings support JM11002 and JM11201 as promising candidates for further development against CRF. Future studies should focus on elucidating

the molecular mechanism of JM11201 and evaluating its pharmacokinetic properties and translational potential.

ABBREVIATIONS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; Col I, collagen I; DMSO, dimethyl sulfoxide; EMPA, empagliflozin; FN, fibronectin; H&E, hematoxylin and eosin; HPLC, high-performance liquid chromatography; IL-6, interleukin-6; KIM-1, kidney injury molecule-1; MRC-5, human embryonic lung fibroblasts; NGM, nematode growth medium; NGAL, neutrophil gelatinase-associated lipocalin; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PSR, picrosirius red; SA-β-gal, senescence-associated β-galactosidase; TNF-α, tumor necrosis factor-α; UIRI, unilateral ischemia-reperfusion injury; UIRx, unilateral ischemia-reperfusion injury with contralateral nephrectomy; UUO, unilateral ureteral obstruction.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The animal assays were approved by the Institutional Animal Care and Use Committee of Hainan University, in

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compliance with Chinese laws for experimental animals, under approval number SYXK (Hainan) 2023-0031. All efforts were made to minimize animal suffering and the number of animals used.

AUTHOR CONTRIBUTIONS

X.L. and Y.X. conceived and designed the study, provided funding, and revised the manuscript. J.W. and C.G. performed the main experiments and analyzed the data. C.G. drafted the initial manuscript. Y.Z. conducted the cell experiments and provided additional NMR data. T.T., C.L., and D.Z. assisted with partial experiments and provided support during project implementation. J.L., W.L., M.W., C.X., and F.A. provided valuable guidance and suggestions for the study. All authors have read and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

Jian Li is Deputy Editor-in-Chief of Acta Materia Medica. He was not involved in the peer-review or handling of the manuscript. The other authors have no other competing interests to disclose.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://amm-journal.org/wp-content/uploads/2026/06/amm20260003_Suppl.pdf.

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