Extract of Corallodiscus Flabellata Attenuates Renal Fibrosis in Aging Mice via the Wnt/β-catenin/RAS Signaling Pathway

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Research

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Abstract

Background: Renal fibrosis is a common pathological feature of chronic kidney disease (CKD). Aging accelerates renal fibrosis and further aggravates the process of CKD. *Corallodiscus flabellata* B. L. Burtt (*C. flabellata*) is a commonly used botanical drug in the Chinese population. However, few studies have reported its physiological effects. This study aimed to explore the effect of *C. flabellata* extract on renal fibrosis in aging mice and identify potentially active compounds.

Methods: Using Senescence-accelerated mouse-prone 8 (SAMP8) mice and β-galactosidase (β-gal)-induced normal rat kidney epithelial cells (NRK-52E cells) as a model, to explore the mechanism of *C. flabellata* extract on senescence-related renal fibrosis, and initially clarified the material basis of *C. flabellata*.

Results: The *C. flabellata* extract reduced the senescence and fibrosis of the kidneys in SAMP8 mice by activating nuclear factor erythroid 2–related factor 2 (Nrf2) to balance oxidative stress and inflammation, and interrupting the fibrinogen signaling in the Wnt/β-catenin/renin–angiotensin system. Moreover, 3,4-dihydroxyphenylethanol (SDC-0-14, 16) and (3,4-dihydroxyphenylethanol-8-O-[4-O-trans-caffeoyl-β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl (1→6)]-β-D-glucopyranoside (SDC-1-8) were isolated compounds from *C. flabellata*, which reduced the senescence of NRK-52E cells, and maybe the material basis for the function of *C. flabellata*.

Conclusion: Our experiments illuminated that the extract of *C. flabellata* may improve the aging-related renal fibrosis through the Wnt/β-catenin/RAS pathway, and the material basis of its function may be SDC-0-14, 16 and SDC-1- 8.

Background

Chronic kidney disease (CKD) is a syndrome that continuously changes the structure and function of the kidneys [1]. Epidemiological studies showed that CKD caused 1.19 million deaths worldwide in 2016 and was the 11th leading cause of death worldwide in the same year [2]. Aging is one of the risk factors that induce CKD [3]. The most common pathological manifestation of CKD is some form of renal fibrosis [4]. Aging is closely related to the progression of renal fibrosis. Studies have shown that mitochondrial dysfunction and kidney fibrosis are related to the activation of the Wnt/β-catenin signaling pathway and the renin-angiotensin system (RAS) during aging [5]. Severe CKD needs to be treated by hemodialysis and peritoneal dialysis. Delaying the development of CKD is critical. Studies have found that using RAS inhibitors and controlling salt levels, blood pressure, and blood lipid levels can effectively delay the development of CKD, providing the direction for the development of specific therapeutic drugs [1, 6]. In recent years, the replacement therapy of CKD fibrosis by natural products has attracted the attention of many scholars [7]. This study also explored the effect of a natural plant extract on renal fibrosis.

Corallodiscus flabellata B. L. Burtt (*C. flabellata*) is a medicinal plant in China. It was first recorded in the “Dian Nan Ben Cao.” The whole plant is commonly used for treating dysentery, premature ejaculation,

seminal vesicle disease, and kidney disease in ethnic minority areas of China [8]. The chemical constituents of *C. flabellata* were studied, revealing that the main chemical constituents were phenylethanoid glycosides and flavonoids [9, 10]. Few pharmacological studies have been conducted on *C. flabellata*. The *C. flabellata* extract was found to have a diuretic effect [11]. This study aimed to explore the effect of *C. flabellata* extract on renal fibrosis in the elderly patients and elucidate its possible mechanism so as to investigate whether *C. flabellata* could be used to treat kidney diseases as described in ancient literature.

Materials And Methods

Collection and extraction of the plant material

The *C. flabellata* plants were collected in September in the Xixia County, Henan province, China, and authenticated by Prof. Sui-qing Chen of the Henan University of Chinese Medicine. The plants (1 kg) were refluxed with 50% ethanol (3 × 12 L, each 1 h), and the mixture was filtered. The combined filtrates were dried by rotary evaporation using a freeze drier. Finally, the percentage yield of 50% ethanol crude extract of *C. flabellata* was 15.5%. The dried extract was kept in a fridge until further use.

Another separation process previously reported was used in this study to obtain the water elution fraction, 20% ethanol elution fraction, 30% ethanol elution fraction, and 40% ethanol elution fraction from *C. flabellata* [11]. The 40% ethanol fraction was separated using Sephadex LH-20 and silica gel column and purified by semi-preparative high-performance liquid chromatography (HPLC) to finally obtain compounds such as SDC-0-14,16, SDC-1-8, SDC-0-60 (p-hydroxybenzyl alcohol).

Cell culture and *in vitro* study

Normal rat kidney epithelial cells (NRK-52E cells) purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were cultured to investigate the effect of *C. flabellata* extract on cell aging caused by D-galactose (S11050, Yuanye, Shanghai, China). The NRK-52E cells were grown in Dulbecco's modification of Eagle's medium Dulbecco (DMEM) supplied with 10% fetal bovine serum in an incubator at 37 °C and in the presence of 5% CO_2. The NRK-52E cells were plated in 96-well or 6-well plates for 24 h. Then, the cells were treated with D-galactose (20 mg/mL), *C. flabellata* extract (75 μg/mL), and its monomeric compound (10 μM) for 48 h. Subsequently, the methyl thiazolyl tetrazolium assay was used to detect cell viability, and β-galactosidase staining was used to observe cell senescence.

Animals and administration

Six-month-old male senescence-accelerated mouse-prone 8 (SAMP8) and SAMR1 mice from the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China) were used in this study. The animals were housed under controlled light (12-h light/dark cycle), temperature (23 °C–25 °C),

and humidity (45–55%) conditions and received a standard diet and water ad libitum. All animal experiments were approved by the ethics committee of Henan University of Traditional Chinese Medicine and performed under the institutional guidelines. A total of 40 SAMP8 mice were divided into four experimental groups (*n* = 10/group): SAMP8 model mice, low-dose CF-treated SAMP8 mice (CF-L, 387.5 mg kg^{-1}, intragastrically), medium-dose CF-treated SAMP8 mice (CF-M, 775 mg kg^{-1}, intragastrically), and high-dose CF-treated SAMP8 mice (CF-H, 1550 mg kg^{-1}, intragastrically). The mice in the SAMR1 control and SAMP8 model groups were treated with physiological saline (0.9%). All mice were treated orally for 1 month.

Sample collection

At the end of the experiment, the mice were housed individually in metabolic cages for 12-h urinary collection. All blood samples were collected by retro-orbital bleeding. The kidneys from each mouse were then surgically removed and kept at − 80 °C until the analyses.

Biochemistry measurements

The serum and kidney homogenate samples were thawed to room temperature, and the enzyme-linked immunosorbent assay kit method was employed according to the manufacturer's protocols to measure serum superoxide dismutase (SOD, Wuhan Huamei Biological Technology, Wuhan, China) levels and activities of interleukin-1β (IL-1β, ABclonal Technology, Wuhan, China) and tumor necrosis factor-α (TNF-α, ABclonal Technology), and the expression levels of collagen type Ⅰ (Col-Ⅰ, Bio-Swamp Life Science Lab, Wuhan, China), α-smooth muscle actin (α-SMA, Bio-Swamp Life Science Lab), and fibronectin (FN, Bio-Swamp Life Science Lab) in kidney tissue. Serum creatinine (Cr), blood urea nitrogen, glutathione peroxidase (GSH-Px), and urinary total protein levels were measured using detection kits (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China).

Senescence-associated β-galactosidase staining

Frozen kidneys from mice sliced into 10-μm-thick sections and NRK-52E cells were stained with senescence-associated β-galactosidase (SA-β-gal) (C0602; Beyotime Biotechnology, Shanghai, China) following the manufacturer's protocols.

Histological analysis

The kidney sections were fixed with 4% buffered paraformaldehyde, and 10-μm-thick paraffin-embedded sections were stained with Masson's trichrome (G1006; Servicebio, Wuhan, China) and observed microscopically. The blue-colored areas in Masson's trichrome–stained sections were measured quantitatively from six randomly selected fields and analyzed by Image-Pro Plus 6.0 software.

Immunohistochemical analysis

The immunohistochemical analysis was performed using the routine method [12]. The antibodies used included the following: Wnt4 (14371-1-AP; Proteintech, IL, USA), β-catenin (17565-1-AP; Proteintech), renin (14291-1-AP; Proteintech), type 1 angiotensin II receptors (AGTR1) (25343-1-AP; Proteintech), p-nuclear factor erythroid 2–related factor 2 (*p*-Nrf2) (ab76026; Abcam, Cambridge, MA, USA), *p*-c-Fos (ab27793; Abcam), connective tissue growth factor (CTGF) (GB11078; Servicebio), and Kelch-like Ech-associated protein-1 (Keap1) (GB11847; Servicebio). The sections were observed under a microscope (Olympus, Tokyo, Japan), and the mean of density value was measured using the Image-Pro Plus analysis software 6.0.

Western blot analysis

Western blot assay was conducted as described in a previous study [13]. Briefly, the kidney tissues were homogenized in lysis buffer and quantified using a Bradford Protein Assay Kit (Wuhan Boster Biological Technology, Ltd., China). The homogenates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membrane, and blocked in blocking buffer (4% nonfat dry milk) for 90 min. They were then incubated with primary antibodies (Wnt4; renin; AGTR1; *p*-Nrf2; *p*-c-Fos; Keap1; β-actin; AC026; Abclonal, Wuhan, China; and glyceraldehyde-3-phosphate dehydrogenase, AC033, Abclonal) overnight at 4℃, followed by incubation with an appropriate fluorescence-conjugated secondary antibody for 1 h at room temperature. The proteins of interest were scanned with an Odyssey IR scanner (LI-COR Biosciences, CO, USA), and the signal intensities were quantified using Image Studio software.

UPLC-Q-TOF-MS analysis for kidney samples

The renal tissues were weighed and homogenized in ice-cold physiological saline (w/v = 1:1). Then, 1 mL of acetonitrile was added to 200 μL of tissue homogenate samples, followed by ultrasonic extraction for 30 min. The extract was centrifuged at 12,000 *g* and 4℃ for 10 min. The supernatant was taken into the vial for analysis. Chromatographic separation was carried out on ultraperformance liquid chromatography (UPLC) (Dionex UltiMate 3000 System, Thermo Scientific, MA, USA), with the LC system comprising an Acclaim RSLC 120 C_{18} column (2.2 μm, 2.1 × 100 mm; Thermo Scientific). The mobile phase consisted of solvent A (acetonitrile) and water with 0.1% formic acid (B). The separation was performed by gradient elution as follows: 10–70% A from 0 to 3 min, 70–78% A from 4 to 13 min, 78–90% A from 14 to 15 min, 90–10% A from 15 to 16 min, and 10% A from 16 to 20 min. The injection volume of the test sample was 2 μL. The optimal conditions for mass spectrometry (MS) were as follows: capillary voltage, 3.0 kV; cone voltage, 40 V; desolvation gas temperature, 500 °C; source temperature, 110 °C; desolvation gas flow, 800 L/h; and cone gas flow, 50 L/h. The scan range was from 50 to

1200 *m/z*. Leucine–enkephalin was used for accurate mass acquisition. Waters MassLynx v4.1 was used for the acquisition and analysis of data in both positive and negative ion modes.

Statistical analysis

The acquired raw data from ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) analysis were first preprocessed using profile analysis (version 2.1, Bruker, Germany). The “bucket table” was obtained and imported into the SIMCA-P software (version13.0 Umetrics AB, Sweden) for principal component analysis (PCA). Other results were presented as mean ± standard deviation (SD). Comparisons between groups were conducted using one-way analysis of variance. A *P* value less than 0.05 was considered statistically significant.

Results

Effect of C. flabellata **crude extract on renal aging and histopathological alterations**

Studies found that SAMP8 mice had age-related renal fibrosis [5]. First, the SA-β-gal activity in the kidney was observed to investigate whether *C. flabellata* could improve senescence-related renal fibrosis. As shown in Fig. 1a, the SA-β-gal activity in kidney tissues significantly increased in the M group; low and moderate doses of *C. flabellata* crude extract caused effective inhibition. Also, a significant decrease in the kidney coefficient was observed in the model group compared with the control group (Fig. 1b, $P < 0.05$). After treatment with the moderate dose of *C. flabellata* crude extract, the organ coefficients for the kidney improved ($P < 0.01$). Next, the Masson’s trichrome staining results revealed that the mice in the model group had obvious fibrosis in the glomeruli and renal tubules (Fig. 1c, $P < 0.01$). Meanwhile, the expression levels of Col-Ⅰ, α-SMA, and FN in the kidney tissue of mice in the model group mice were found to be significantly increased (Fig. 1e-g, $P < 0.01$), indicating that SAMP8 mice indeed developed renal fibrosis. After treatment with *C. flabellata*, renal fibrosis was reduced.

Effect of C. flabellata **crude extract on biochemical indicators in rapidly aging mice**

In addition, the indicators of renal function in responding mice were examined, which included urine volume in mice for 12 h, Cr and urea nitrogen levels in the serum, and urine protein levels. As shown in Fig. 2a–d, the urine protein, serum Cr, and serum urea nitrogen levels increased, whereas the urine volume of the mice decreased (Fig. 2a, $P < 0.01$) in SAMP8 mice compared with SAMR1 mice (Fig. 2b–d, $P < 0.01$). *C. flabellata* supplementation reduced the urine protein, serum Cr, and serum urea nitrogen levels (Fig. 2b-d, $P < 0.05$ or $P < 0.01$), while it increased the urine volume in SAMP8 mice (Fig. 2a, $P < 0.01$). Collectively, the results indicated that SAMP8 mice had aging-related renal damage, and *C. flabellata* treatment effectively ameliorated aging-induced injury in kidneys. Next, whether *C. flabellata* beneficially modulated oxidative stress and inflammation in the kidneys of rapidly aging mice was explored. As shown in Fig. 2e, f, C. *flabellata* significantly ameliorated the reduced enzyme activity of total SOD and GSH-Px in the kidneys of aging mice. Besides, *C. flabellata* treatment significantly reduced aging-induced TNF-α and IL-1β levels (Fig. 2g, h).

Renal expression of Nrf2 in aging mice and effects of C. flabellata crude extract

Nrf2 is a master transcriptional regulator for genes related to redox status and antioxidant effects [14]. Studies have shown that phosphorylation is required for Nrf2 activation and target gene induction [15]. The content of *p*-Nrf2 was measured in kidney tissues to investigate whether the Nrf2 pathway was involved in the protective effect of *C. flabellata* (Fig. 3a, c, and f) in the present study. The expression level of *p*-Nrf2 significantly decreased in the SAMP8 group alone ($P < 0.01$, Fig. 3c), and the downregulation was reversed to some extent by the treatment of *C. flabellata* crude extract. Keap1 is known as the major regulator of antioxidant response; no significant difference in the expression levels was found between the groups (Fig. 3d, f). Further, the expression of *p*-c-Fos was detected and analyzed by immunohistochemical and Western blot analyses. The *C. flabellata* crude extract significantly attenuated the increase in *p*-c-Fos levels in the kidneys of SAMP8 mice (Fig. 3b, e, and f).

C. flabellata crude extract might regulate renal fibrosis in senescence-accelerated mice through the Wnt/β-catenin/RAS pathway

The effect of the extract on CTGF, Wnt, and RAS signaling was examined in senescence-accelerated mice to explore the mechanism underlying the antifibrotic effect of the *C. flabellata* crude extract. First, the localization of Wnt4, β-catenin, renin, AGTR1, and CTGF was performed using immunohistochemistry. As shown in Fig. 4a, b, the expression of Wnt4 and β-catenin was induced predominantly in renal tubular cells. Similar results were observed when renin and AGTR1, the downstream pathway targets of Wnt signaling, were assessed (Fig. 4c, d). Next, the activation of Wnt4, β-catenin, renin, and AGTR1 was analyzed. The results showed that Wnt4, β-catenin, renin, and AGTR1 proteins accumulated in the kidney tissue of mice in the SAMP8 group alone, whereas *C. flabellata* crude extract treatment significantly inhibited the alterations (Fig. 4f–i and k). Furthermore, many studies indicated that CTGF played an important role in renal fibrosis [16]. Hence, the localization of CTGF was also detected. Consistent with the aforementioned results, *C. flabellata* treatment significantly enhanced the expression of CTGF (Fig. 4e and 4j).

Effects of chemical compounds in C. flabellata extract in D-galactose-treated NRK-52E cells

D-galactose-induced NRK-52E cells were used to screen some active ingredients in *C. flabellata* extract so as to identify the material basis of the effect of *C. flabellata* extract on the aging kidney. The results revealed that 75 μg/mL of *C. flabellata* extract, SDC-0-14,16, and SDC-1-8 had a better effect on cell viability. Also, they reduced the expression of β-galactosidase compared with that in the model group (Fig. 5).

Metabolic profiling

The PCA analysis of the kidney tissue extracts of each group of mice showed that the normal and model groups were clustered into one group, and the separation trend was obvious (Fig. 6a), indicating that the renal metabolites in SAMP8 mice differed from those in SAMR1 mice. Besides, the treated groups were

also different from the control and model groups, but the treated groups tended to be close to the control group, indicating that the regulation of metabolite levels by *C. flabellata* crude extract tended to improve in SAMP8 mice.

Discussion

Aging plays an important role in the progression of CKD [17]. As CKD fibrosis progresses, senescent cells express and secrete pro-fibrotic factors (TGF-β, CTGF, and so forth) and pro-inflammatory factors (IL-1β, IL-6, TNF-α, and so forth), which are senescence-associated secretory phenotype factors, thereby accelerating renal fibrosis [18, 19]. At present, traditional Chinese medicine has achieved good results in treating renal fibrosis with fewer side effects. This study explored the interventional effects of the extract of *C. flabellata* on renal fibrosis in aging mice.

The animal species selected for this study was SAMP8, which was developed based on the lifespan, senescence, and pathological phenotypic grading scores of AKR/J mice and was an accelerated aging model solely of genetic origin [20, 21]. Studies have shown that the changes in the renal pathology of SAMP8 mice (9 months) include tubulointerstitial fibrosis and focal segmental glomerulosclerosis [22]. SAMP8 mice were used in the present study to investigate the protective effect of *C. flabellata* crude extract on kidney damage caused by aging. The renal coefficient reduced, and aging (using SA-β-gal staining) and fibrosis (using Masson staining, and levels of Col-Ⅰ, α-SMA, and FN) were observed in SAMP8 mice compared with SAMR1 mice. The pathological characteristics of the kidneys improved with *C. flabellata* crude extract treatment, especially at low and moderate doses (Fig. 1). Consistent with previously published studies indicating that the extracts of *C. flabellata* obtained by two different processes had diuretic effects [11], the extract significantly increased the urine volume of SAMP8 mice. Also, the present study showed that supplementation with the *C. flabellata* crude extract improved the renal function, the activity of antioxidant enzymes, and levels of inflammatory factors in SAMP8 mice (Fig. 2).

The Keap1–Nrf2 system gained the attention of many scholars in recent years due to its antioxidant and anti-inflammatory properties. Its pharmacological potential in treating kidney diseases has been extensively studied in nonclinical and clinical studies [23]. Activating Nrf2 might improve CKD progression by preventing oxidative stress and maintaining cellular redox homeostasis [24]. As a key transcription factor, Nrf2 plays a crucial role in defense against oxidative stress by regulating its downstream antioxidants and detoxification enzymes [25]. Kim et al. reported that resveratrol, as a potent Nrf2 activator, could ameliorate aging-related progressive renal injury [26]. In the present study, the crude extract of *C. flabellata* improved the attenuation of Nrf2 in the kidney of SAMP8 mice without affecting the expression level of Keap1, suggesting that the crude extract might improve oxidative damage in the kidneys by activating the Nrf2 pathway (Fig. 3).

Renal fibrosis is characterized by excessive extracellular matrix (ECM) deposition leading to the formation of scars in the renal parenchyma [27]. Transforming growth factor-β (TGF-β) is thought to be a

key cytokine in fibroblast overactivation [28, 29]. Of course, targeting only the TGF-β signaling pathway is insufficient to reduce renal fibrosis. Some studies indicated that CTGF, Wnt/β-catenin, renin–angiotensin system, oxidative stress, and so forth, were implicated in renal fibrosis [16, 30, 31]. Wnt/β-catenin is an evolutionarily conserved signaling pathway involved in the regulation of tissue homeostasis, organ development, and injury repair [32]. Cisternas et al. discussed the pro-fibrotic effect of Wnt signaling in both skeletal muscle and kidney [33]. Mounting evidence established that the Wnt/β-catenin signaling pathway plays a crucial role in regulating the development and progression of renal fibrotic lesions following injury [34–36]. The Wnt/β-catenin signal is relatively silent in the kidneys of healthy adults and is activated once the kidneys are subjected to various kinds of damages [37]. In mammals, the Wnt family has at least 19 family members critical for kidney development. In senescent kidneys, at least 15 of these family members are upregulated to varying degrees, including Wnt4 [5]. The results of the present study showed that the expression level of Wnt4 protein in the kidneys of SAMP8 mice was significantly higher than that in SAMR1 mice, which was verified by both Western blot and immunohistochemical analyses (Fig. 4c, h, k). Coincidentally, the expression of β-catenin protein was also upregulated. Also, both Wnt4 and β-catenin were expressed in renal tubular epithelial cells, as revealed by the immunohistochemical analysis (Fig. 4c, d, i). Wnt/β-catenin elicited renal fibrosis by inducing multiple fibrogenic genes such as RAS components, matrix metalloproteinase-7 (MMP-7), plasminogen activator inhibitor 1 (PAI-1), and Snail1 [31]. Zhou et al. described Wnt/β-catenin as the major upstream regulator, which controls the expression of all tested RAS components in the kidneys [38]. Zhou et al. used a 5/6 nephrectomy (5/6 NX) rat model to show that the expression of major components of RAS in the brain and kidneys, such as angiotensinogen, angiotensin-converting enzyme, and angiotensin II AT1-receptor, was significantly upregulated. The upregulated expression was inhibited by a central blocker of Wnt, which was an adeno-associated virus vector overexpressing the *DKK1* gene [39]. Similarly, the present study found that renin and AGTR1 were still expressed in the renal tubular epithelium, and the expression levels of both proteins in the kidney tissues of SAMP8 mice were significantly greater than those in SAMR1 mice (Fig. 4a, b, f, g). These results indicated that the Wnt/β-catenin/RAS signaling pathway was activated in the kidneys of SAMP8 mice, and the crude extract of *C. flabellata* could effectively inhibit the activation of this pathway.

CTGF exerts multiple biological functions, including promoting mitosis of chemotactic cells, inducing adhesion, and promoting cell proliferation and ECM synthesis [16, 40]. CCN2 (CTGF) modulated Wnt signaling by binding to low density lipoprotein receptor-related protein 5/6 (LRP5/6) to further mediate fibrosis [33, 41]. Other studies used gene silencing to discover and confirm that Nrf2 regulated the Wnt pathway by regulating the expression of CTGF, affecting renal interstitial disease. Also, Nrf2 regulated the CTGF transcription level mainly via CTGF transcriptional regulator c-Fos [42]. The immunohistochemical analysis revealed that CTGF and *p*-c-Fos were expressed mainly in renal tubular epithelial cells, and *p*-c-Fos was also distributed in glomeruli. The quantitative analysis showed that the expression of both proteins was inhibited by *C. flabellata* crude extract (Fig. 3b, e, 4e, j). Finally, the PCA analysis was used to further verify that the *C. flabellata* extract improved kidney damage in SAMP8 mice (Fig. 6). The present study provided evidence that the *C. flabellata* crude extract not only activated Nrf2 signaling but also

relied on Nrf2 to balance oxidative stress and inflammation, inhibited Wnt/β-catenin/RAS signaling, and improved kidney aging and renal fibrogenesis.

As a reducing monosaccharide, D-galactose is widely used in various age-related diseases *in vivo* and *in vitro*. Studies found that D-galactose could cause senescence and damage to NRK-52E cells [43] induce senescence of human kidney proximal tubular epithelial cells (HKC-8 cells), and increase the expression of two renal fibrosis marker proteins FN and α-SMA [5]. In this study, D-galactose was used to induce NRK-52E cells *in vitro*. The study found that SDC-0-14,16 and SDC-1-8 might be one of the active components of *C. flabellate* against kidney aging (Fig. 5). However, whether these compounds work through the Wnt/β-catenin/RAS signaling pathway still needs further experimental investigation.

Conclusion

In conclusion, *in vivo* studies showed that *C. flabellata* extract could reduce renal fibrosis in elderly mice. Some potential active ingredients were found in *in vitro* experiments. These findings might provide pharmacological support for treating kidney disease using *C. flabellata* and a direction for further research on the active ingredients of *C. flabellata*.

Abbreviations

CKD: Chronic kidney disease; *C. flabellate*: *Corallodiscus flabellata* B. L. Burtt; RAS: Renin-angiotensin system; SDC-0-14, 16: 3,4-dihydroxyphenylethanol; SDC-1-8: (3,4-dihydroxyphenylethanol-8-O-[4-O-trans-caffeoyl-β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl (1→6)]-β-D-glucopyranoside; SDC-0-60: p-hydroxybenzyl alcohol; HPLC: High-performance liquid chromatography; NRK-52E cells: normal rat kidney epithelial cells; DMEM: Dulbecco's modification of Eagle's medium Dulbecco; D-gal: D-galactose; SAMP8: Senescence-accelerated mouse-prone 8; SAMR1: Senescence-resistant mouse-prone 1; SOD: superoxide dismutase; IL: interleukin; TNF-α: tumor necrosis factor-α; Col-Ⅰ: Collagen type Ⅰ; α-SMA: α-smooth muscle actin; FN: fibronectin; Cr: creatinine; GSH-Px: glutathione peroxidase; AGTR1: type 1 angiotensin II receptors; CTGF: connective tissue growth factor; p-Nrf2: p-nuclear factor erythroid 2–related factor 2; Keap1: Kelch-like Ech-associated protein-1; UPLC-Q/TOF-MS: ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry; PCA: principal component analysis; SD :standard deviation; SA-β-gal: senescence-associated β-galactosidase; ECM: excessive extracellular matrix; TGF-β: transforming growth factor-β; HKC-8 cells: Human kidney proximal tubular epithelial cells

Declarations

Acknowledgements

Not applicable

Authors' contributions

MZ, BC, XZ, and WF conceived and designed the experiments in the manuscript. BC, YS and YW performed the experiments. BC, BZ and RX analyzed data, plotted the graphs for figures. BC and MZ drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures were approved by the Research Ethics Committee of Henan University of Chinese Medicine.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

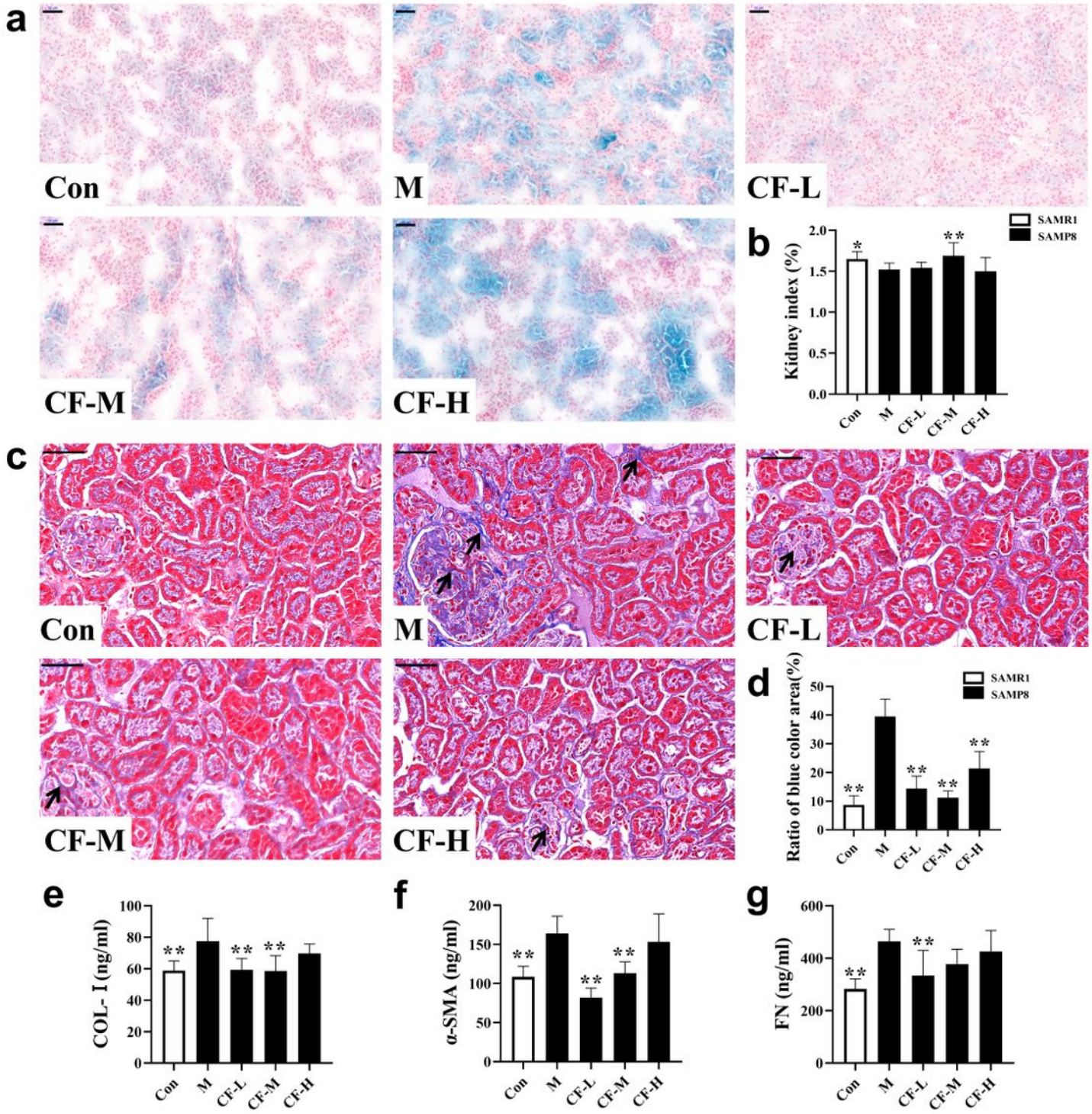

Figure 1

Effect of crude extract of C. flabellata on the kidney of SAMP8 mice. a The activity of SA-β-gal in frozen sections of kidneys. Scale bars: 50 μm, magnification 200×. b Changes in the mouse kidney index. c Masson's trichrome staining of kidneys. Renal fibrosis was expressed by collagen deposition (blue color area) in Masson's trichrome staining. Scale bars: 50 μm, magnification 400×. d Semiquantification of renal interstitial fibrosis. The ratio of the blue color area to the field area from Masson's trichrome–

stained sections. e–g Expression levels of Col-Ⅰ, α-SMA, and FN in kidney tissue. Values expressed as mean ± SD; *P < 0.05 and **P < 0.01 vs M group (n = 6).

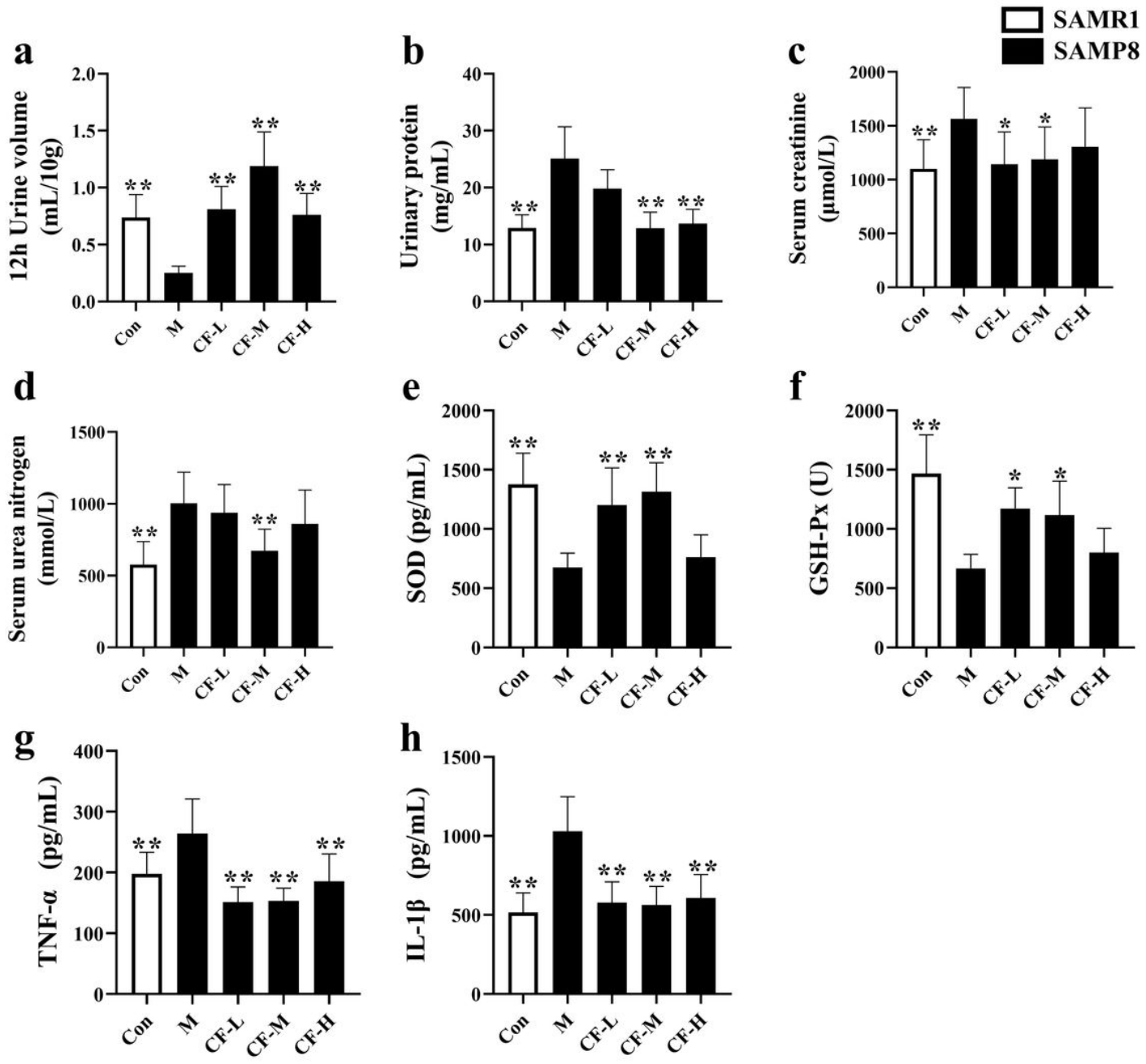

Figure 2

Effect of C. flabellata crude extract on biochemical indicators in rapidly aging mice. a-d Effect of C. flabellata on the urine volume, urine protein levels, and serum Cr and urea nitrogen levels, respectively. e-h The activities of SOD and GSH-Px, and the level of TNF-α and IL-1β in serum, respectively. Values expressed as mean ± SD; *P < 0.05 and **P < 0.01 vs M group (n = 8).

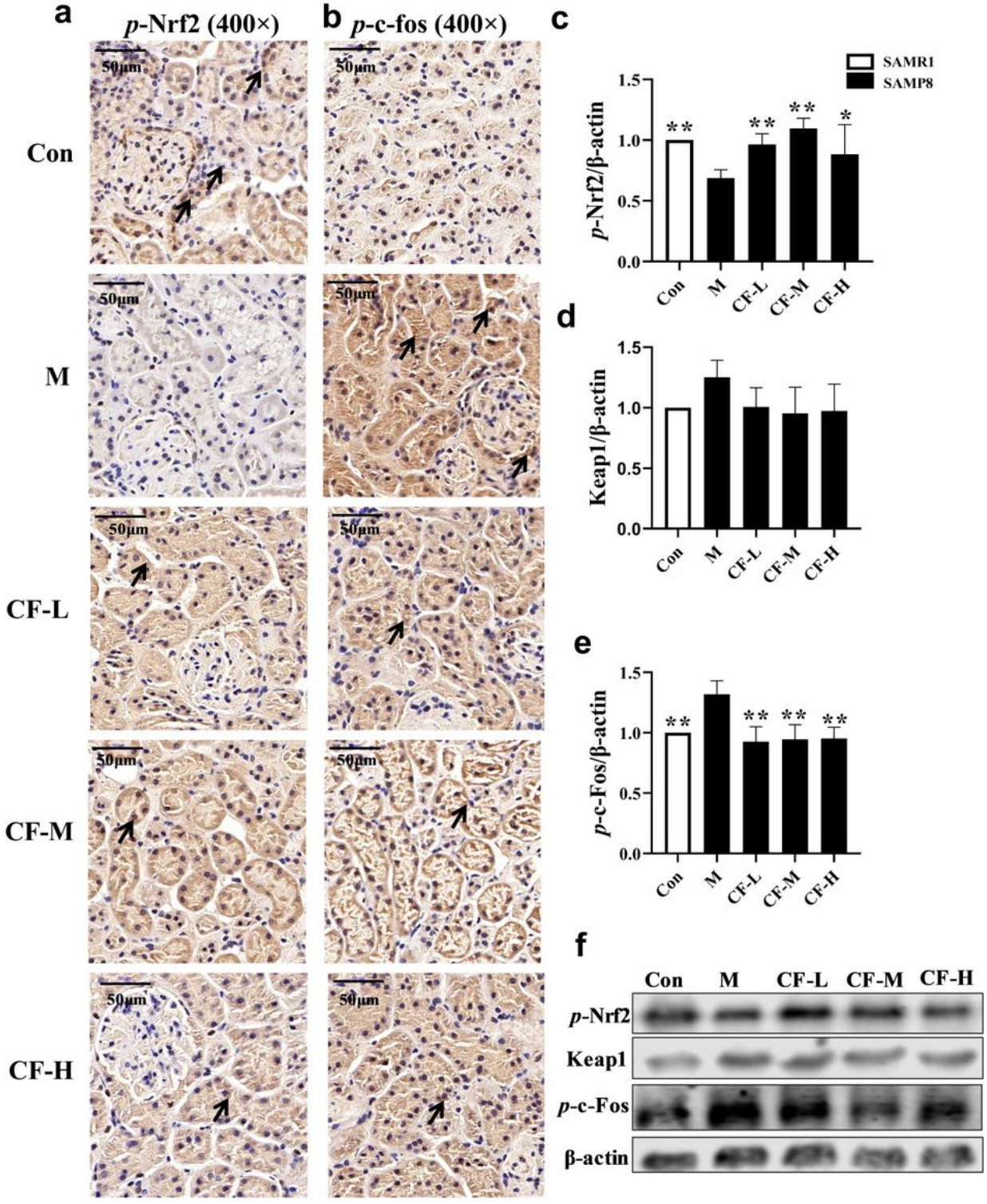

Figure 3

Effects of C. flabellata crude extract on the expression of Nrf2 in senescence-accelerated mice. Scale bars: 50 μm. a, b Immunohistochemical staining of kidney sections for a p-Nrf2 and b p-c-Fos. c-f Representative f Western blots and graphical representations of c p-Nrf2, d Keap1, and e p-c-Fos protein expression levels in kidneys. Arrows indicate positive expression. The data are presented as mean ± SD. *P < 0.05 and **P < 0.01 vs M group (n = 3 or n = 6).

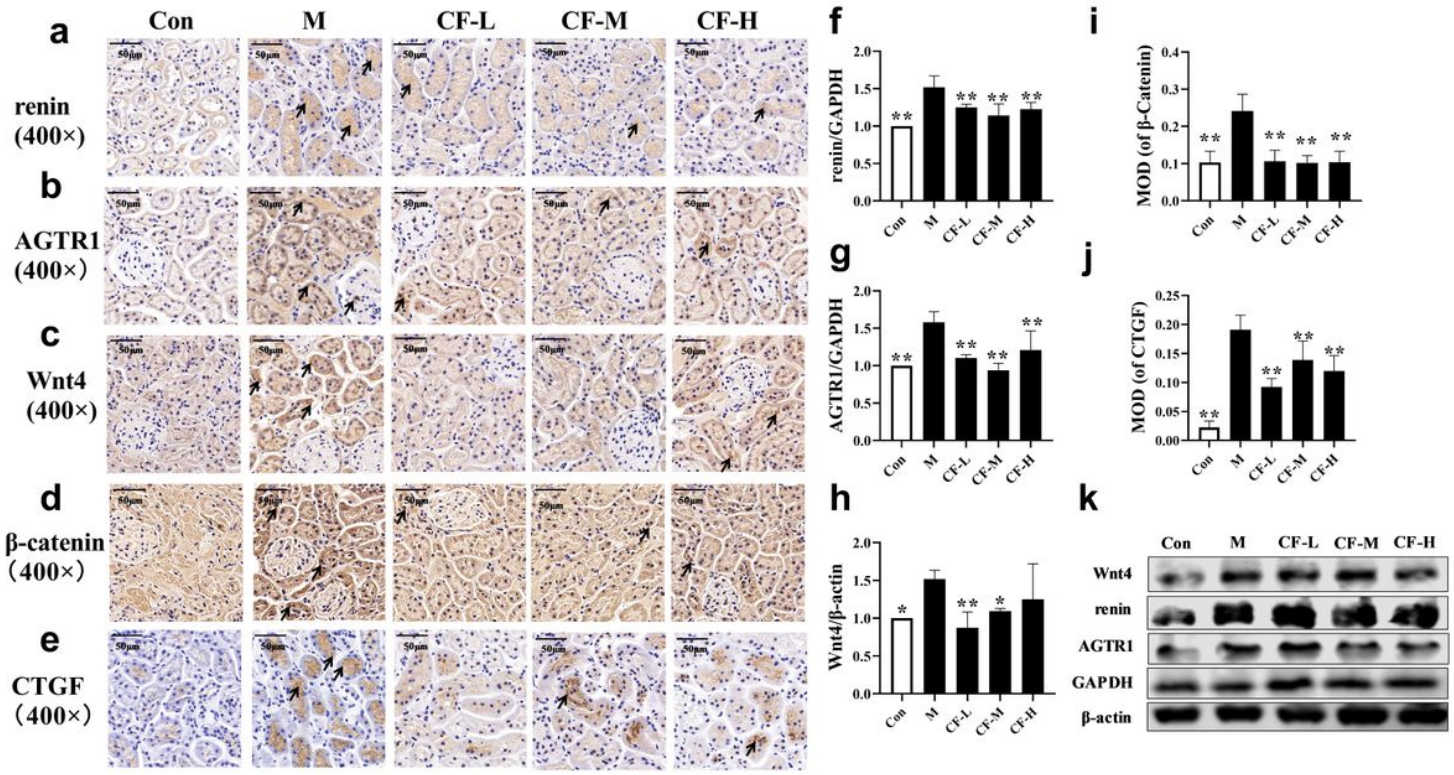

Figure 4

Effects of C. flabellata crude extract on the expression of the Wnt/β-catenin/RAS pathway in senescence-accelerated mice. Scale bars: 50 μm. a-e Immunohistochemical staining of kidney sections of a renin, b AGTR1, c Wnt4, d β-catenin, and e CTGF. f-h Representative k Western blots and graphical representations of the expression levels of f renin, g AGTR1, and h Wnt4 proteins in kidneys are presented. i and j Mean optical density (MOD) of β-catenin and CTGF. Arrows indicate positive expression. The data are presented as the mean ± SD. *P < 0.05 and **P < 0.01 vs the M group (n = 3 or n = 6).

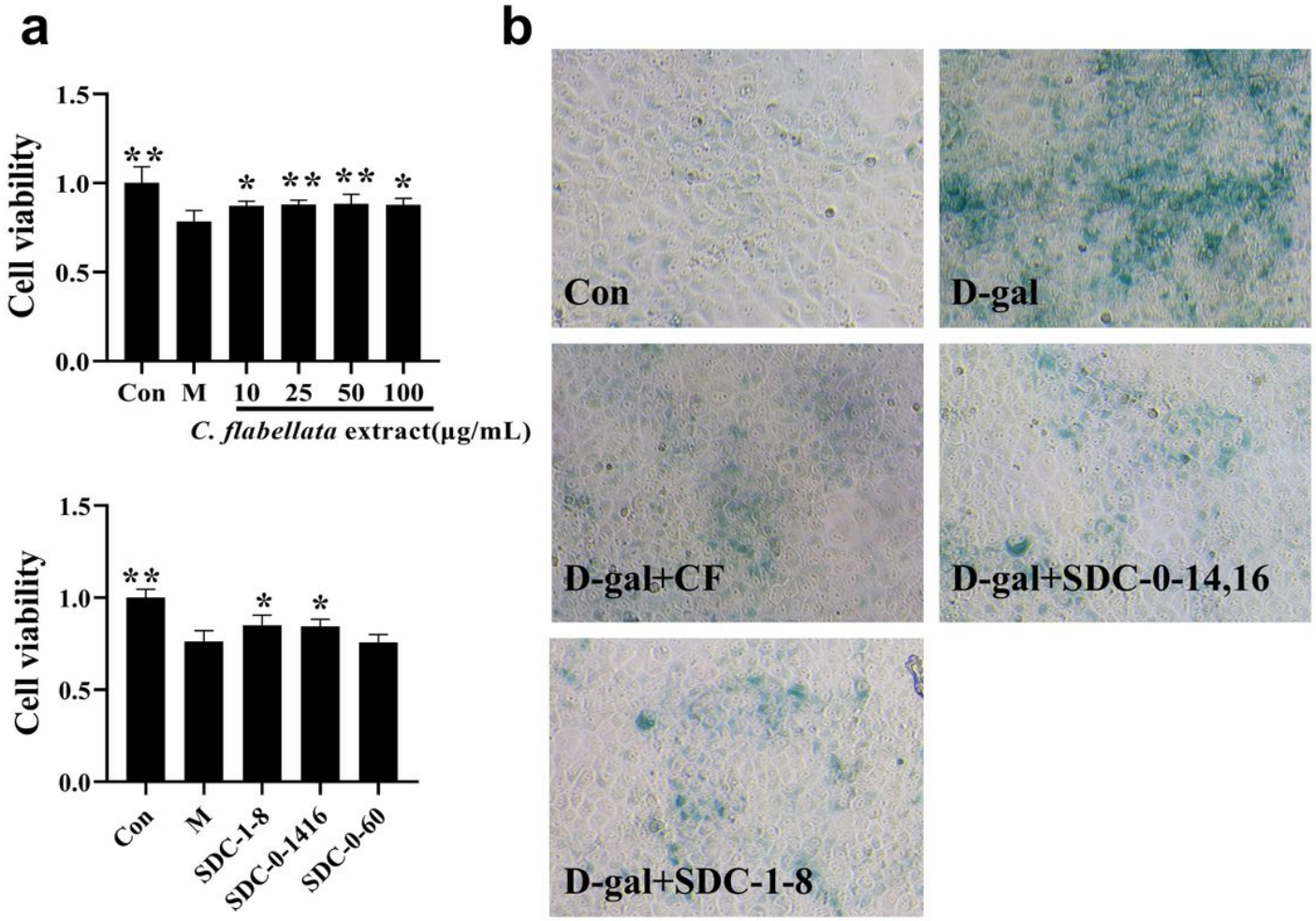

Figure 5

Effect of C. flabellata on D-gal-induced NRK-52E cells. a Detection of cell viability of C. flabellata extract and its chemical components. b β-galactosidase staining of NRK-52E cells. CF, C. flabellata extract; D-gal, D-galactose; SDC-0-14,16, 3,4-dihydroxyphenylethanol; SDC-1-8, (3,4-dihydroxyphenylethanol-8-O-[4-O-trans-caffeoyl-β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl (1→6)]-β-D-glucopyranoside; SDC-0-60, p-hydroxybenzyl alcohol. The data are presented as mean ± SD. *P < 0.05 and **P < 0.01 vs the D-gal group.

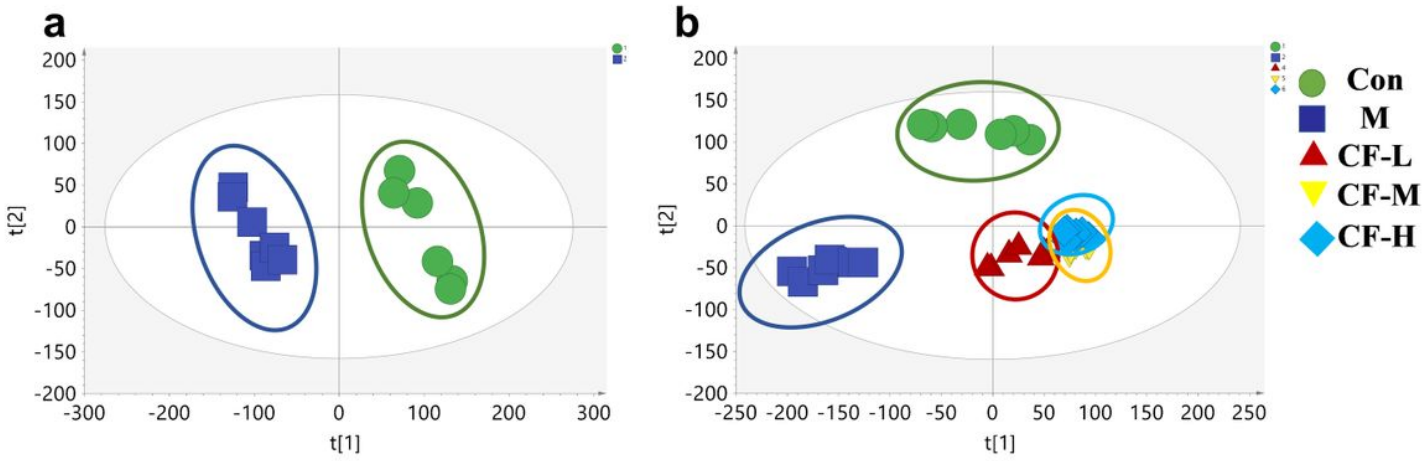

Figure 6

PCA score plot of kidney homogenate extract in positive modes. a PCA score plot for the control and M groups; and b PCA score plot for all groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx