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Phellinus igniarius ameliorates renal aging in a rat model of focal and segmental glomerulosclerosis

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ABSTRACT

Objective: To comparatively investigate the ameliorative effect of *Phellinus igniarius* (*P. igniarius*) on renal aging in a rat model of focal and segmental glomerulosclerosis (FSGS).

Methods: The FSGS model was established in rats by uninephrectomy combined with tail vein injection of doxorubicin. The FSGS rats were randomly divided into the model group, the *P. igniarius* decoction group, the *P. igniarius* polysaccharides group, and the *P. igniarius* polyphenols group. Molecular indicators of cell senescence, renal function indexes, and podocyte injury markers were tested after ten weeks of intragastric administration. Besides, the pathological renal lesions and the ultrastructural changes were observed.

Results: FSGS developed in the model group within ten weeks and showed segmental glomerular scarring and renal aging. Following the 10-week intervention, 24 h proteinuria, serum creatinine, blood urea nitrogen, P16$^{INK4\alpha}$, thrombospondin-1, and transforming growth factor-β1 were decreased in each treatment group, whereas albumin, erythropoietin, nephrin, and podocin were increased; the pathological renal injury was alleviated, and the number of senescent cells was reduced, especially in rats treated with *P. igniarius* decoction.

Conclusions: *P. igniarius* ameliorates renal aging and renal injury in the FSGS rat model. Compared with the effective constituents (polysaccharides and polyphenols), *P. igniarius* decoction has a better curative effect, which is expected to provide a new therapeutic idea for FSGS.

KEYWORDS: *Phellinus igniarius*; Polysaccharides; Polyphenols; FSGS; Renal aging; Rat; Renal injury

1. Introduction

The main histological features of renal aging are segmental glomerulosclerosis, glomerular basement membrane thickening, mesangial matrix hyperplasia, and tubulointerstitial fibrosis, which mainly occur in chronic kidney disease[1]. As a common disease of chronic kidney disease, focal segmental glomerulosclerosis (FSGS) is the leading cause of nephrotic syndrome with severe proteinuria, accompanied by glomerular sclerosis and foot process effacement pathologically[2]. Currently, corticosteroids and immunosuppressants are the most widely used agents in treating FSGS, but steroid dependence or steroid resistance is frequently reported[3]. Thus, more and more scholars are focusing on traditional Chinese medicine

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Significance

Considering *Phellinus igniarius*'s biological effects, we speculated that it had positive effects on kidney disease. This study demonstrates that *Phellinus igniarius* ameliorated renal aging and renal injury in a rat model of focal and segmental glomerulosclerosis, which is expected to provide a safe and effective method to alleviate the progression of chronic kidney diseases.

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to reduce toxic side effects and find better treatments[4,5].

Phellinus igniarius (*P. igniarius*) is a valuable edible and medicinal fungus in China and other countries. As a preventive measure or a health care product for adjunct therapies, *P. igniarius* has been used to treat stomach aches, bloody stranguria, tumors, arthritis, and peptic ulcers in folk herbal formulas for thousands of years[6]. In addition, effective constituents of *P. igniarius*, such as polysaccharides and polyphenols, were reported to have various biological effects, including anti-tumor, anti-inflammatory, antioxidative, anti-diabetic, and anti-angiogenic activity[7]. Our preliminary studies showed that *P. igniarius* improved renal function in FSGS rats, but unknowns still need further investigation[8]. The current study aims to investigate the ameliorative effect of *P. igniarius* on renal aging in a rat model of FSGS.

2. Materials and methods

2.1. Preparation of drugs

Professor Li provided *P. igniarius* powder, *P. igniarius* polysaccharides (PIPS) powder, and *P. igniarius* polyphenols (PIP) powder. The preparation of *P. igniarius* decoction (PID) was referred to in the previous paper[8]. The appropriate dosage of *P. igniarius* powder is 4 g per day for an adult, and the dosage for a rat was calculated based on body surface area. PIPS powder and PIP powder were dissolved in distilled water, and the appropriate dosages for a rat were calculated based on body surface area. Intragastric administration was used in this study, similar to clinical administration.

2.2. Animals and treatment

Fifty clean-grade male SD rats (5-6 weeks, 160-200 g) were randomly divided into five groups: sham, model, FSGS+PID, FSGS+PIPS, and FSGS+PIP (*n*=10 per group). All rats were housed in clean cages and maintained at (23 ± 1) ℃ with 55% relative humidity, a 12-h light/dark cycle and *ad libitum* access to pure water and standard rat pellets. The feeding conditions of all rats were provided by Zhejiang Animal Experimental Research Center. The experimental practice was done in accordance with the international guidelines for laboratory animal use and care.

In this study, FSGS rats were anesthetized with isoflurane and a small animal anesthesia machine; then, rats were firstly subjected to right nephrectomy on day 1, followed by the injection of doxorubicin *via* tail vein on day 7 (5 mg/kg) and 28 (3 mg/kg). For different agents (PID, PIPS, and PIP) administration, FSGS rats were given oral gavage once per day for ten weeks from day 2; the sham and model groups were given oral gavage with the same volume

of saline. After 10-week treatment, all FSGS rats were placed in a closable box and euthanized by CO_2 inhalation. After CO_2 inhalation, FSGS rats were subjected to cervical dislocation with heart failure, eyeball whitening, and respiratory arrest, which resulted in death. After the experiments, we collected the whole left kidney, serums, and 24-hour urine samples.

2.3. Ethical statement

The animal procedures were approved by the Institute Animal Care and Use Committee (IACUC) with ethics approval ID: ZJCLA-IACUC-20030027. The date of approval by the ethics committee is April 30, 2021.

2.4. Biochemical assay

24 h Proteinuria, serum creatinine (Scr), blood urea nitrogen (BUN), and albumin (ALB) were measured by Hitachi 7180 automatic biochemical analyzer (Hitachi High-Tech Co., Tokyo, Japan).

2.5. Histopathological examination

A portion of the kidneys was fixed with acetone and frozen into sections; then frozen sections were stained with senescence β-galactosidase (SA-β-gal) kit (Beyotime, C0602, Jiangsu, China); another portion of the kidneys was fixed with 4% paraformaldehyde, embedded in paraffin, cut into 3 μm-thick sections, and stained with H&E and Masson's trichrome stain kit. The degree of lesions was described by ImageJ software. The other parts of the kidneys were fixed with 5% glutaraldehyde overnight, and ultrathin sections (50-100 nm) were cut and stained with 2% uranyl acetate and lead citrate. They were observed with JEM-1400 transmission electron microscopy (Nihon Kohden Kogyo Co., Japan).

2.6. Immunohistochemistry (IHC)

Paraffin sections in 3 μm-thick were deparaffinized, hydrated, incubated with 3% hydrogen peroxide solution for 15 min, and blocked with normal goat serum for 1 h after antigen retrieval. Slides were incubated at 37℃ with primary antibodies against EPO (Beyotime, Jiangsu, China, AF6801, 1:180), P16$^{INK4\alpha}$ (Beyotime, Jiangsu, China, AF1672, 1:100), TSP-1 (Cell Signaling, 37879, 1:1 000, Boston, USA), and TGF-β1 (ImmunoWay, YT4632, 1:200, USA) for 1 h, followed by incubation in biotin-conjugated secondary antibody at room temperature for 30 min. DAB horseradish peroxidase substrate kit was used for color development, and hematoxylin was used for re-staining. Images were analyzed by ImageJ software.

2.7. Real–time PCR (RT–PCR)

Total RNA was isolated from individual rats' kidney tissues using the TRIzol reagent and then reverse-transcribed into cDNA. Afterward, target and reference genes were detected using RT-PCR (ABI Prism 7500, USA): 95 ℃ for 35 s, then 40 cycles of denaturation at 95 ℃ for 5 s, annealing at 60 ℃ for 30 s, extension at 60 ℃ for 60 s, and 95 ℃ for 1 s. TRIZOL, reverse transcription kit, and SYBR® Premix Ex Taq™ were purchased from Takara Company, Japan; primer design and synthesis were from Shanghai Sangon Company, China. Relative mRNA expression levels were normalized to *GAPDH* and compared by calculating $2^{-\Delta\Delta CT}$. The primer base sequence is shown in Supplementary Table 1.

2.8. Western blotting

The kidney tissues were incubated with RIPA lysis buffer containing a proteinase inhibitor cocktail (Beyotime, Jiangsu, China) for 30 min at 4 ℃. Cell lysates were centrifuged at 10 000 *g* for 10 min at 4 ℃ . 10% SDS-PAGE gels separated the protein extracts (60 μg) under reducing conditions. The proteins were combined with the primary antibodies after being transferred onto membranes and blocked by 5% skimmed milk. Antibodies used were as follows: anti-nephrin (ImmunoWay, YT3036, 1:1 500, USA); anti-podocin (ImmunoWay, YN2909, 1:1 000, USA); and anti-GAPDH (ImmunoWay, YM3029, 1:20 000, USA). After hybridization, the blots were washed and incubated with IRDye 800CW Goat anti-Rabbit IgG (LI-COR 926-32211, 1:15 000, USA) and IRDye 680RD Goat anti-Mouse IgG (LI-COR 926-68070, 1:15 000, USA). Finally, the signal was tested using LI-COR Odyssey CLx (LI-COR, USA). The relative band intensity was quantified by ImageJ software.

2.9. Statistical analysis

GraphPad Prism V8.3.0 and ImageJ software were used for the statistical analysis and data visualization. All values presented were expressed as mean ± standard deviation (SD). Comparisons between multiple groups were analyzed by one-way ANOVA, and comparisons between two groups were analyzed using Tukey test. Statistical significance was defined as *P*<0.05.

3. Results

3.1. PID and effective constituents improved renal function in FSGS rats

None of the rats died before being sacrificed at the end of the experiment. Compared with the sham group, the levels of 24 h proteinuria (Figure 1A), Scr (Figure 1B), and BUN (Figure 1C) in FSGS rats were significantly increased. In contrast, ALB (Figure 1D) was reduced, indicating that the FSGS model had been successfully established (*P*<0.01). After different administrations, 24 h proteinuria and Scr in each treatment group were significantly lower than those in the model group (*P*<0.05) (Figure 1A-B). Moreover, BUN was markedly reduced in the PID and PIPS groups. However, there was no significant difference in BUN level between the PIP and model groups (Figure 1C). Additionally, ALB in the PID and PIP groups was much higher, but no significant difference was observed between the PIPS and model groups (Figure 1D). These results indicated that *P. igniarius* and its effective constituents decreased urinary protein excretion and improved renal function in the FSGS rats.
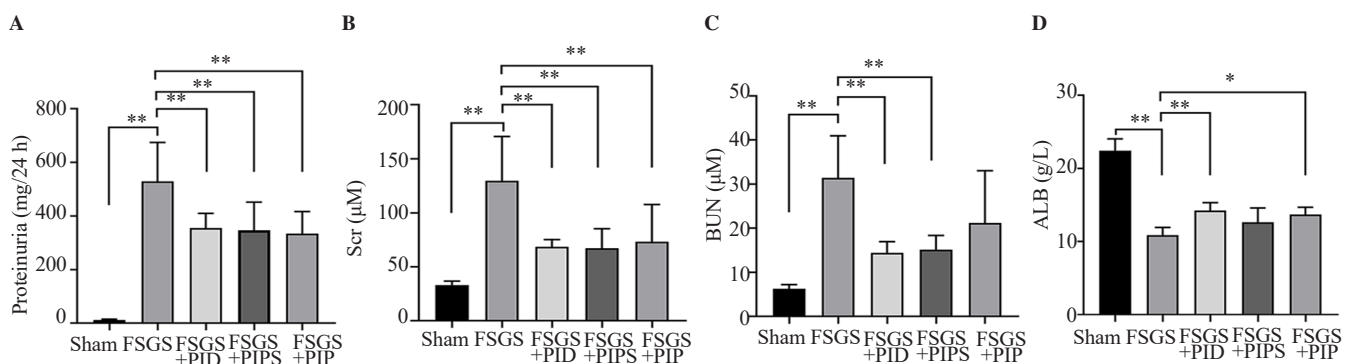

Figure 1. Levels of 24 h proteinuria (A), serum creatinine (Scr) (B), blood urea nitrogen (BUN) (C), and albumin (ALB) (D). The data are presented as mean ± SD (*n*=10). *P<0.05, **P<0.01. FSGS: focal and segmental glomerulosclerosis; PID: *Phellinus igniarius* decoction; PIPS: *Phellinus igniarius* polysaccharides; PIP: *Phellinus igniarius* polyphenols.

3.2. PID and effective constituents alleviated renal histopathological damage in FSGS rats

The glomerular pathomorphology was further detected by H&E staining and Masson's staining.

As seen in Figure 2A, the kidneys in model rats showed significant pathological changes compared to the sham group kidneys with normal renal structures. After different interventions, pathological damages (compensatory enlargement of glomeruli, focal segmental sclerosis, mesangial matrix hyperplasia, tubule dilation, vacuolar degeneration, and inflammatory cell infiltration) were alleviated in each treatment group (Figure 2A).

As seen in Figure 2B, Masson's staining showed that a large number of blue-green collagen fibers were deposited in the model group. After treatment, each intervention group's blue-green collagen fiber deposition significantly shrank (Figure 2B). As shown in Figure 2C, the positive area of the model group was found to be significantly larger than that of the sham group, and the positive area of the treatment groups was markedly smaller than that of the model group, suggesting that the interstitial damage was ameliorated to varying degrees after intervention (Figure 2C).

3.3. PID and effective constituents improved the renal ultrastructure changes in FSGS rats

TEM was used to observe the degree of damage in the podocyte foot process. The results showed normal structures on the glomerular basement membrane and podocytes in the sham group (Figure 3).
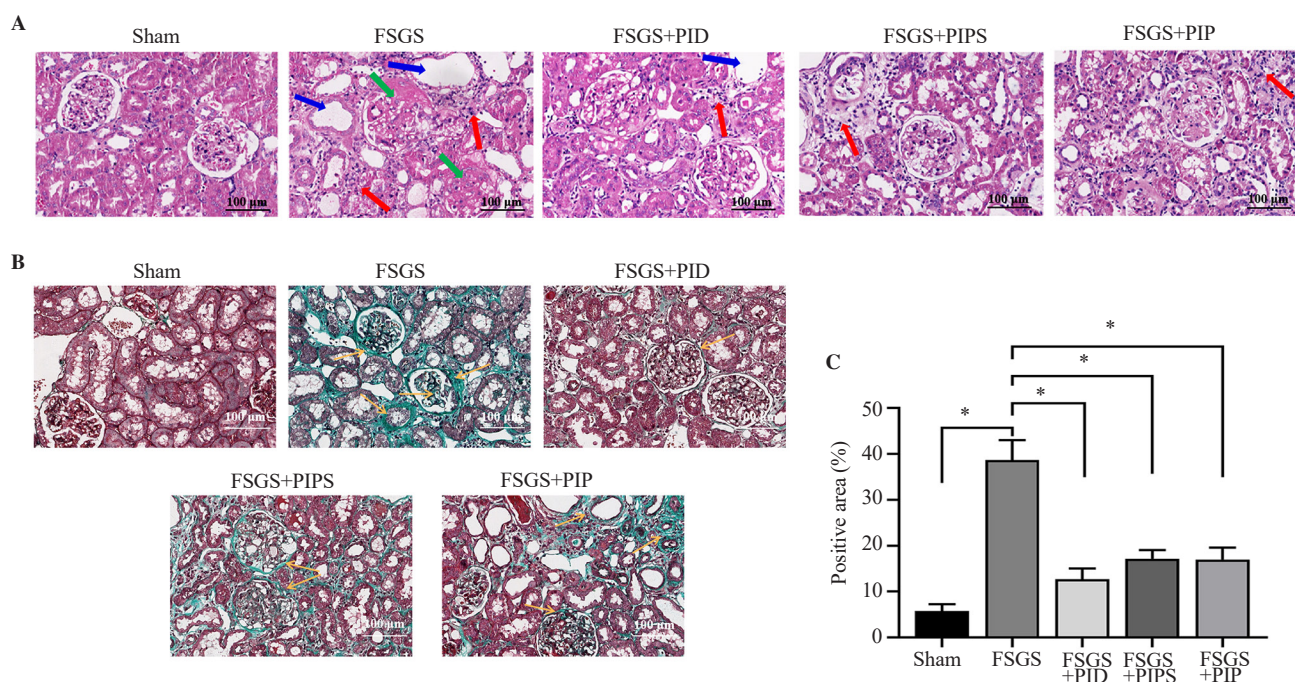

Figure 2. Renal histopathological damage in FSGS rats. Representative images of H&E staining (magnification: ×200) (A) and images of Masson's staining (magnification: ×200) (B). C: The positive area of Masson staining. Data are presented as mean ± SD, $^*P<0.01$. Arrows indicate focal segmental sclerosis (green), inflammatory cell infiltration (red), tubular vacuolar degeneration (blue), and blue-green collagen fibers (yellow).
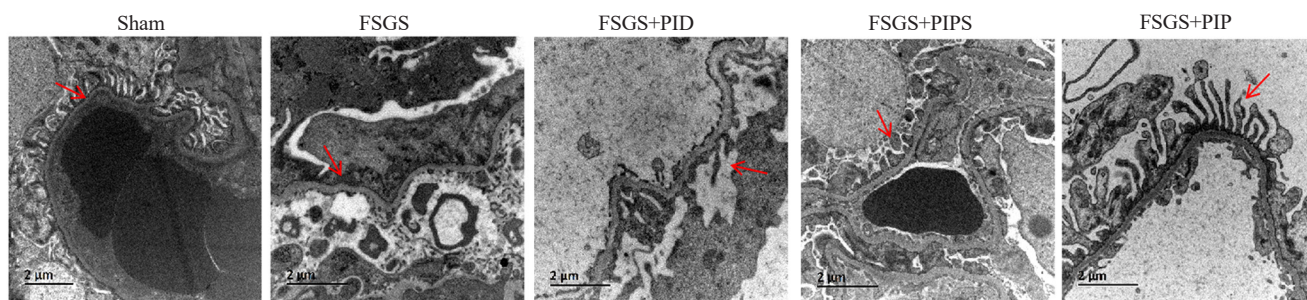

Figure 3. Renal ultrastructure changes (magnification: ×10 000). Arrows indicate the podocyte foot process. The degree of foot process damage in each treatment group was alleviated compared with the model group, and the lesions in the PIP group were slighter than those in the PID and PIPS groups.

However, the foot process diffusely effaced, became flat and fused, and lost its normal shape in the model group (Figure 3). The degree of foot process damage in each treatment group was alleviated compared with the model group, and the lesions in the PIP group were slighter than those in the PID and PIPS groups (Figure 3). As expected, these results indicated that *P. igniarius* improved the renal ultrastructure changes in FSGS rats.

3.4. PID and effective constituents improved podocyte injury in FSGS rats

The podocyte proteins nephrin and podocin were evaluated to further assess the effect of PID and effective constituents on FSGS progression. RT-PCR results showed that mRNA expression levels of *nephrin* (Figure 4A) and *podocin* (Figure 4B) were markedly decreased in the model group. In the PID and PIP groups, the mRNA expression level of *nephrin* was significantly increased compared with that in the model group; however, there was no significant difference between the model and PIPS groups (Figure 4A). *Podocin* mRNA expression was significantly increased in different intervention groups compared with the model group (Figure 4B).

The protein expressions of nephrin and podocin were observed by Western blotting. The nephrin and podocin expressions in the model group were all lower than those in the control group (Figure 4C-D). After different administrations, the protein levels of nephrin in the PID and PIPS groups were significantly higher than those in the model group (Figures 4C). Moreover, podocin protein expression was markedly increased in the PID and PIP groups (Figure 4D). These results suggested that PID protected the podocyte slit diaphragm proteins, nephrin, and podocin.

3.5. PID and effective constituents improved SA−β−gal staining in FSGS rats

SA-β-gal staining shows the blue-green nucleus of senescent cells, essential in detecting cell senescence. As shown in Figure 5A, the
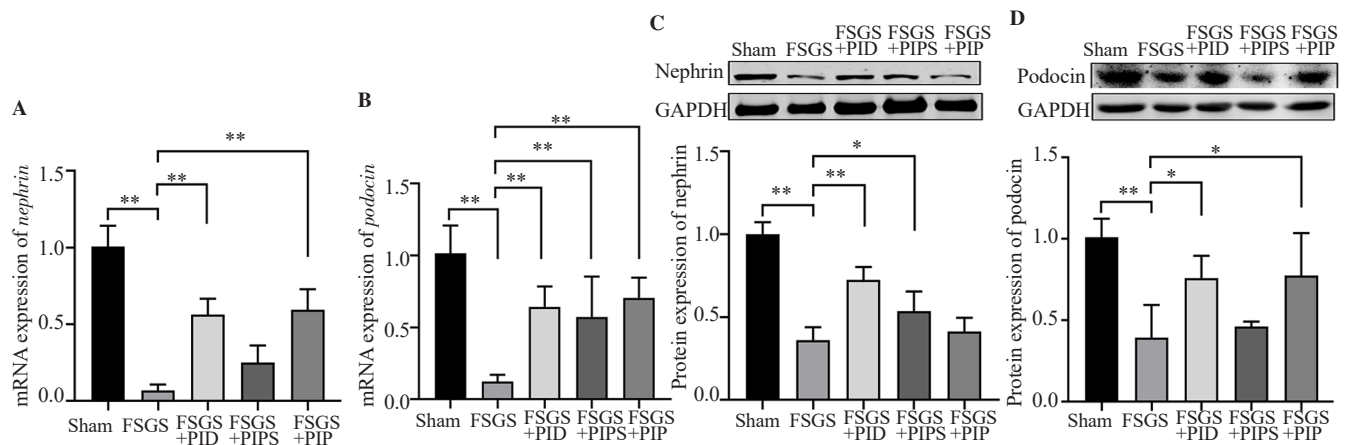

Figure 4. mRNA and protein expressions of nephrin (A, C) and podocin (B, D). Data are presented as mean ± SD, $^{*}P<0.05$, $^{**}P<0.01$.
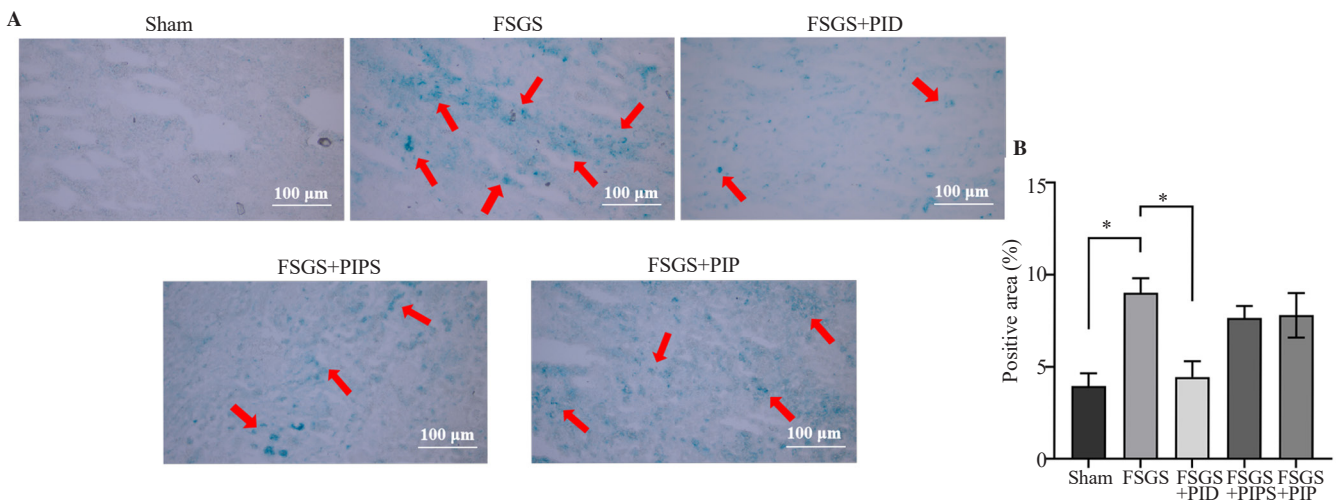

Figure 5. Analysis of renal aging by SA-β-gal staining (A) and positive area (B) (magnification: ×200). Data are provided as mean ± SD, $^{*}P<0.01$. Arrows indicate a large number of senescent cells.

senescent cells were more strongly positive in the model group than in the sham group. After treatment, the blue-green area was smaller, the color was lighter, and senescent cells were weakly positive in each group than in the model group. Figure 5B indicates that the number of senescent cells decreased significantly in the PID group than in the model group.

3.6. PID and effective constituents upregulated the expression of EPO in FSGS rats

EPO is a critical regulatory protein for renal aging. RT-PCR results showed that the expression of *EPO* mRNA was significantly reduced in the model group compared with that in the sham group (Figure 6A). The expressions of *EPO* mRNA in the treatment groups were markedly increased compared with that in the model group (Figure 6A). Meanwhile, Figure 6B indicates that EPO was significantly upregulated in each treatment group, especially in the PID group. As shown in Figure 6C, EPO was narrowly distributed as brownish-yellow granules in the model group; unlike the model group, positive cells were darker in color and more prominent in the area after different treatments.

3.7. PID and effective constituents reduced the expression of P16$^{INK4\alpha}$ in FSGS rats

P16$^{INK4\alpha}$ is a main senescence marker in kidney biopsies. RT-PCR results showed that the expression of *P16$^{INK4\alpha}$* mRNA was markedly increased in the model group (Figure 7A). Compared with the model group, the expression of *P16$^{INK4\alpha}$* mRNA in the PIPS and PIP groups was significantly reduced (Figure 7A). Meanwhile, Figure 7B indicates that the expression of P16$^{INK4\alpha}$ was significantly reduced in all treatment groups. IHC staining showed that P16$^{INK4\alpha}$ was rarely seen in the glomerulus and tubulointerstitium of the sham group (Figure 7C). Positive cells were darker and more prominent in the model group than in the sham group. In contrast, positive cells were lighter in color and smaller in the area in each treatment group than in the model group (Figure 7C).

3.8. PID and effective constituents reduced the expressions of TSP–1 and TGF–β1 in FSGS rats

TSP-1 and TGF-β1 are aging-related phenotype indicators, and the abnormal increases in their levels indicate that the kidney loses its normal function and accelerates renal aging. RT-PCR results showed
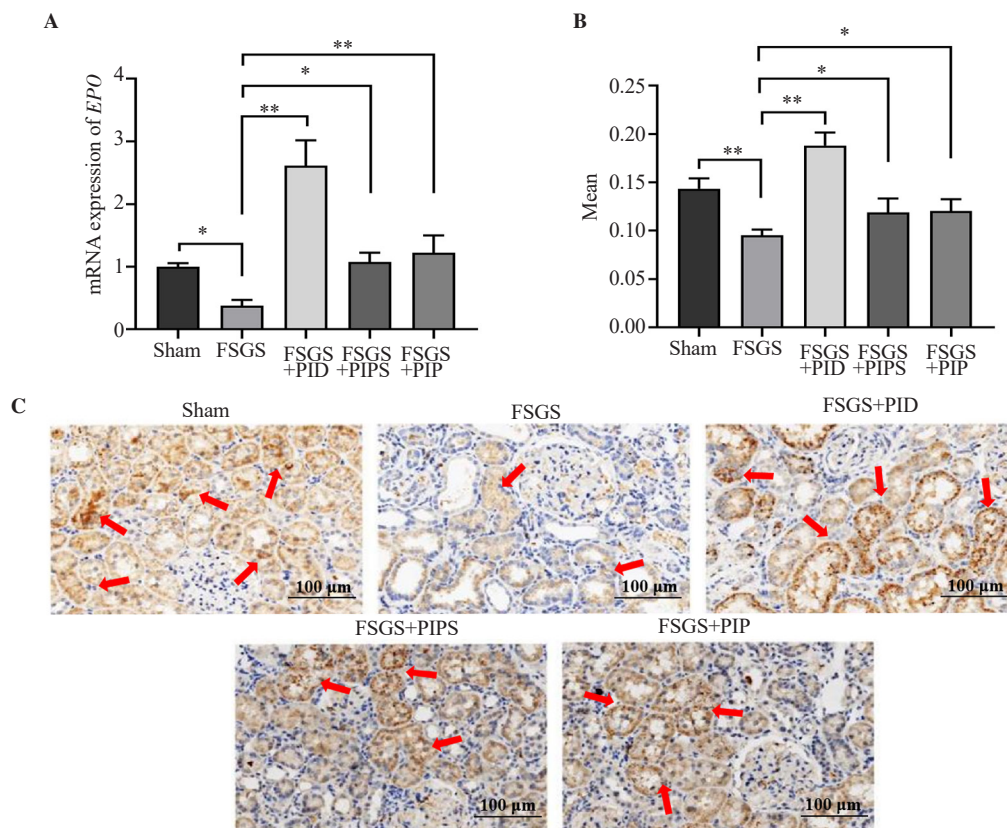

Figure 6. mRNA expression of *EPO* (A) and analysis of renal aging by IHC (B&C) (magnification: ×200). Data are presented as mean ± SD, $^*P<0.05$, $^{**}P<0.01$. Arrows indicate where EPO is mainly deposited in the kidney tissue.

Figure 7. mRNA expression of $P16^{INK4a}$ (A) and analysis of renal aging by IHC (B&C) (magnification: ×200). Data are presented as mean ± SD, *$P<0.01$. Arrows indicate where P16^{INK4a} is mainly deposited in the kidney tissue.

Figure 8. mRNA expression and IHC analysis of TSP-1 (A, C, and E) and TGF-β1 (B, D, and F) (magnification: ×200). Data are presented as mean ± SD, *$P<0.05$, **$P<0.01$. Arrows indicate where TSP-1 and TGF-β1 are mainly deposited in the kidney tissue.
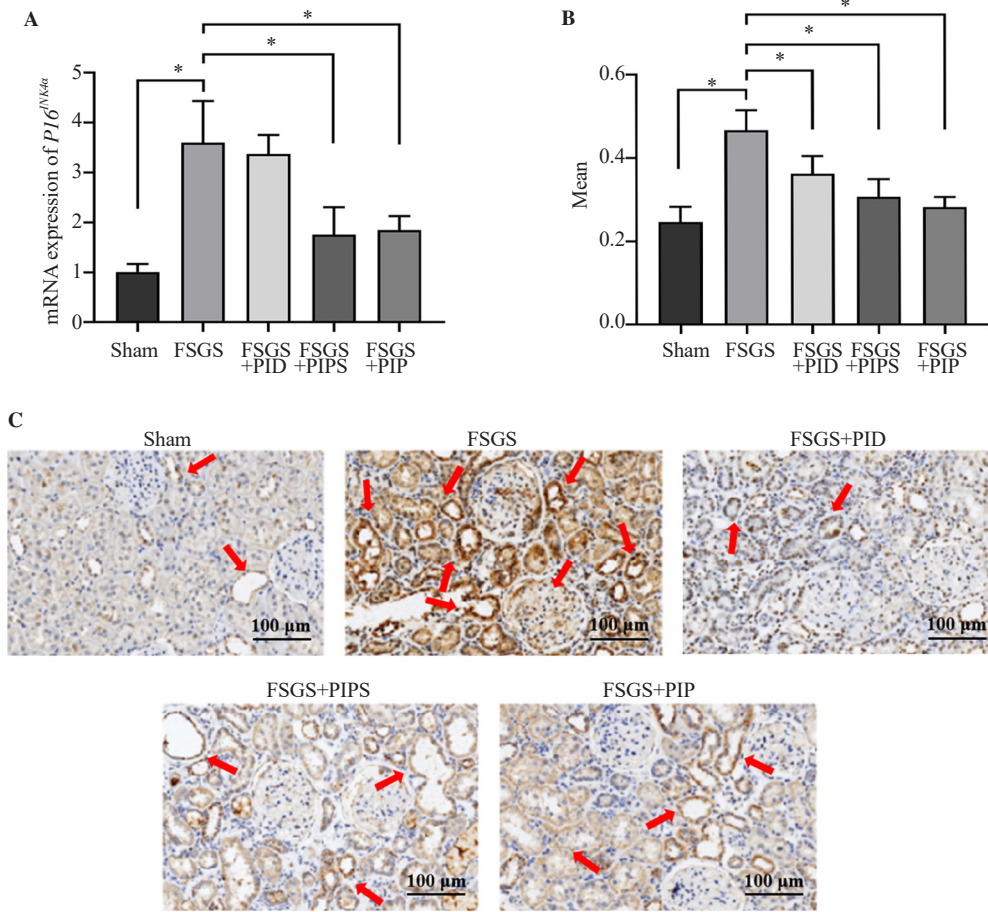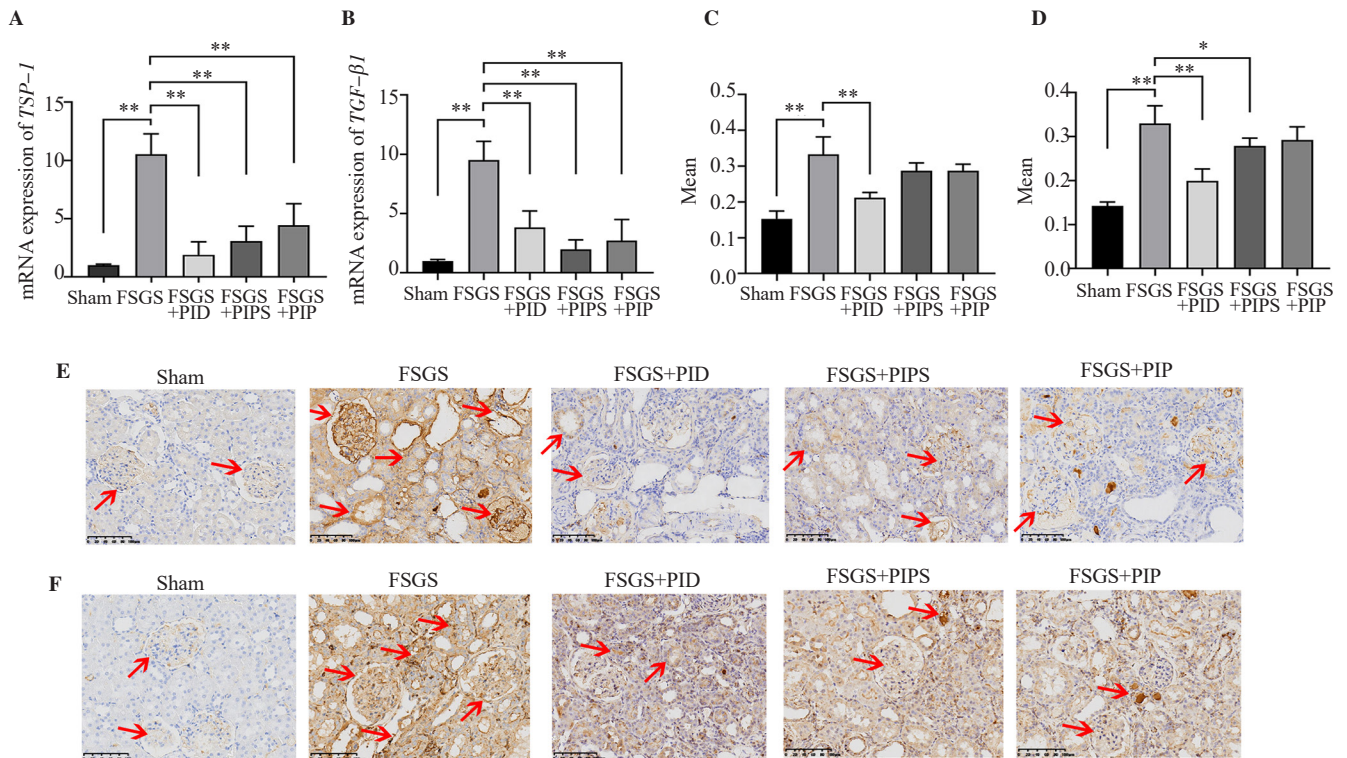
that the expressions of *TSP-1* (Figure 8A) and *TGF-β1* (Figure 8B) mRNA were markedly increased in the model group. After administration, *TSP-1* and *TGF-β1* mRNA expressions in each treatment group were significantly reduced compared with those in the model group (Figure 8A&B).

IHC analysis showed that the protein expressions of TSP-1 and TGF-β1 were increased in the model group compared with the sham group (Figure 8E&F). Figures 8C&D indicate that the protein expressions of TSP-1 and TGF-β1 in the PID group were significantly reduced compared with that in the model group. These results suggested that *P. igniarius* reduced the gene and protein expressions of TSP-1 and TGF-β1 in FSGS rats.

4. Discussion

In kidney diseases, including acute and chronic, senescent cells accumulate in renal tissues, where their inflammatory phenotype disrupts standard structure and function, causing renal aging[9]. Renal aging is an inevitable process and a result of functional degeneration, mainly manifesting as segmental glomerulosclerosis, basement membrane thickening, mesangial matrix hyperplasia, renal tubule atrophy, tubulointerstitial fibrosis, arteriosclerosis, and compensatory hypertrophy of remnant nephrons[10,11]. In a recent study, Verzola *et al.* demonstrated that senescence, as expressed by p16^{INK4α} overexpression in the tubular compartment, contributed to accelerated loss of eGFR in FSGS, suggesting that cellular senescence is associated with faster progression of FSGS[12]. Thus, as the disease progresses, FSGS causes renal aging and chronic renal failure, eventually leading to end-stage renal disease and threatening the lives of patients[13–15]. Reducing proteinuria levels and improving kidney function is generally believed to be important in treating FSGS at different stages[16]. Moreover, therapeutic interventions targeting senescent cells also attenuate FSGS-related renal dysfunction and improve disease outcomes[17].

Tradition Chinese Medicine has advantages for kidney diseases and has been widely applied in China. *P. igniarius* plays a significant regulatory role in anti-hepatic fibrosis, and considering its biological effects, we speculated that *P. igniarius* had positive effects on kidney disease[18,19]. Our preliminary studies found that *P. igniarius* improved renal function and alleviated pathological renal injury in FSGS rats. However, it has not been studied in the field of renal aging[8]. Thus, our study aimed to explore the ameliorative effect of *P. igniarius* decoction and its active ingredients on renal aging in a rat model of FSGS. Research results showed that *P. igniarius* ameliorated renal aging in a rat model of FSGS, which is expected to provide a safe and effective method to alleviate the progression of chronic kidney diseases.

FSGS typically presents with abrupt-onset proteinuria, severe nephrotic syndrome, and decreased renal function[20]. 24 h Proteinuria is associated with the occurrence and development of FSGS. In addition to 24 h proteinuria, Scr, BUN, ALB, and other biochemical indicators reflect kidney function[21]. This study showed that after treating PID and effective constituents, the biochemical indicators significantly improved, indicating that *P. igniarius* improved renal function in FSGS rats. Meanwhile, H&E staining and Masson's staining are the most common methods for assessing pathological damage in FSGS, reflecting the degree of glomerulosclerosis and interstitial fibrosis[22,23]. This study showed that *P. igniarius* protected against pathological renal injury in FSGS rats. Besides, the essence of FSGS is podocyte disease. Podocyte injury is an essential feature of FSGS, which leads to foot process fusion and cell detachment from the glomerular basement membrane, resulting in glomerulosclerosis[24]. Nephrin and podocin are the signature proteins in the slit diaphragm region of podocytes, and the decrease in their levels indicates podocyte injury[25–27]. Furthermore, TEM is also a vital method for observing podocyte lesions. The degree of podocyte injury can be determined by evaluating the degree of foot process fusion[28]. This study showed that after treating with *P. igniarius*, the expressions of nephrin and podocin were markedly increased, and foot process fusion was significantly reduced, suggesting that *P. igniarius* protected against podocyte injury in FSGS rats. Since *P. igniarius* improved renal function, reduced pathological damage, and decelerated podocyte injury, we speculated that the compounds of *P. igniarius*, such as polysaccharides and polyphenols, might have antioxidant, anti-inflammatory, and anti-fibrosis activity, which alleviated renal injury.

Renal aging plays an important role during the progression of kidney disease, which coincides with the pharmacological effects of *P. igniarius*[29]. Renal aging manifests as cell cycle stagnation in the G$_1$ phase, and P16^{INK4α}/EPO is the key regulatory protein[30,31]. As an aging mediator in the glomeruli, tubules, and interstitium, P16^{INK4α}, which positively correlates with the degree of interstitial fibrosis, can predict the occurrence and development of cell senescence[32,33]. EPO is a glycoprotein hormone, naturally produced by the peritubular cells of the kidney, that stimulates red blood cell production[34]. Besides, EPO promotes the repair of endothelial function and alleviates renal pathological changes[35]. In addition, SA-β-gal staining is the gold standard of cell senescence and predicts renal aging progression[36]. This study showed that FSGS induced renal aging in rats, but after treatment, the P16^{INK4α} protein and the number of senescent cells were significantly decreased. In contrast, EPO protein was markedly increased, suggesting that both *P. igniarius* and its effective constituents improved the expression of aging markers. TSP-1 and TGF-β1 are aging-related phenotype indicators, and the abnormal increases in their levels indicate that the kidney loses its normal function and accelerates renal aging[37,38]. Theoretical and empirical research results have shown that the level

of TSP-1 in the renal tissues of FSGS rats is significantly increased, which is positively correlated with the apoptosis of podocytes[39]. As an important physiological activator of TGF-β1, overexpression of TSP-1 will also continuously stimulate the activation of TGF-β1. Activated TGF-β1 is involved in cell apoptosis by blocking the cell cycle, resulting in glomerulosclerosis gradually. In this study, FSGS rats showed high levels of TSP-1 and TGF-β1, and treatment groups generally had different degrees of reduction. The above results showed that *P. igniarius* had a protective effect on renal injury and aging in FSGS rats. The protective effect was related to suppressing the expression of TSP-1 and TGF-β1.

In the current study, however, we did not have more in-depth investigations on the mechanism of action of *P. igniarius*. In the future, we will study whether *P. igniarius* plays a protective role in kidney disease through the TSP-1-TGF-β1 pathway, exploring the potential effect of *P. igniarius* on anti-aging.

In conclusion, the present findings confirmed that *P. igniarius* down-regulated the levels of TSP-1 and TGF-β1, improved kidney function, and ameliorated renal aging and podocyte injury in FSGS rats. Compared with the effective constituents, polysaccharides and polyphenols, *P. igniarius* decoction had a more prominent curative effect. It is anticipated that this study will provide a theoretical basis for the application of *P. igniarius* in treating kidney diseases.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Funding

Authors' contributions

ZTW designed the study, conducted the animal experiments and molecular indicators, and drafted the manuscript. YWT and FW conducted animal experiments, detected renal function indexes and immunostaining. FW and RCY were responsible for financial support of the study. RCY conceived and designed the study, and provided final supervision. YG performed TEM, and modified the manuscript. JZ provided efforts in data analysis. YGL provided the *Phellinus igniarius*. WMH modified the manuscript. All authors have approved the final version of the manuscript.

References

[1] Ortiz A, Mattace-Raso F, Soler MJ, Fouque D. Ageing meets kidney disease. *Nephrol Dial Transplant* 2023; **38**(3): 523-526.

[2] Bose B, Cattran D. Toronto Glomerulonephritis Registry. Glomerular diseases: FSGS. *Clin J Am Soc Nephrol* 2014; **9**(3): 626-632.

[3] De Vriese An S, Wetzels Jack F, Glassock Richard J, Sethi S, Fervenza FC. Therapeutic trials in adult FSGS: Lessons learned and the road forward. *Nat Rev Nephrol* 2021; **17**(9): 619-630.

[4] Shabaka A, Tato RA, Fernández-Juárez G. Focal segmental glomerulosclerosis: State-of-the-art and clinical perspective. *Nephron* 2020; **144**(9): 413-427.

[5] Sun QF, Chen SX, Tang ZF, Song XY, Jing F, Wu HT, et al. *Origanum vulgare* L. leaf extract alleviates finasteride-induced oxidative stress in mouse liver and kidney. *Asian Pac J Trop Biomed* 2021; **11**(5): 194-204.

[6] Li HX, Zhang XY, Gu LL, Li Q, Ju Y, Zhou XB, et al. Anti-gout effects of the medicinal fungus *Phellinus igniarius* in hyperuricaemia and acute gouty arthritis rat models. *Front Pharmacol* 2022; **12**: 801910.

[7] Bai XX, Feng ZA, Peng S, Zhu TY, Jiao LN, Mao NN, et al. Chitosan-modified *Phellinus igniarius* polysaccharide PLGA nanoparticles ameliorated inflammatory bowel disease. *Biomater Adv* 2022; **139**: 213002.

[8] Wan F, Yang RC, Shi YP, Tang YW, Tang XL, Zhu XL et al. The protective effect of *Phellinus linteus* decoction on podocyte injury in the kidney of FSGS rats. *BMC Complement Altern Med* 2019; **19**(1): 272.

[9] Kitai Y, Nangaku M, Yanagita M. Aging-related kidney diseases. *Contrib Nephrol* 2021; **199**: 266-273.

[10] Fang Y, Chen B, Liu Z, Gong AY, Gunning WT, Ge Y, et al. Age-related GSK3β overexpression drives podocyte senescence and glomerular aging. *J Clin Invest* 2022; **132**(4): e141848.

[11] Docherty MH, O'Sullivan ED, Bonventre JV, Ferenbach DA. Cellular senescence in the kidney. *J Am Soc Nephrol* 2019; **30**(5): 726-736.

[12] Verzola D, Saio M, Picciotto D, Ferenbach DA. Cellular senescence is associated with faster progression of focal segmental glomerulosclerosis. *Am J Nephrol* 2020; **51**(12): 950-958.

[13] Ossareh S, Yahyaei M, Asgari M, Bagherzadegan H, Afghahi H. Kidney outcome in primary focal segmental glomerulosclerosis (FSGS) by using a predictive model. *Iran J Kidney Dis* 2021; **15**(6): 408-418.

[14] Ahn W, Bomback AS. Approach to diagnosis and management of primary glomerular diseases due to podocytopathies in adults. *Am J Kidney Dis* 2020; **75**(6): 955-964.

[15] Müller-Deile J, Schenk H, Schiffer M. Minimal change disease and focal segmental glomerulosclerosis. *Internist (Berl)* 2019; **60**(5): 450-457.

[16] de Cos M, Meliambro K, Campbell KN. Novel treatment paradigms: Focal segmental glomerulosclerosis. *Kidney Int Rep* 2022; **8**(1): 30-35.

[17] Yu SM, He JC. Aged glomeruli: A link between PD-1 and podocytes. *J Clin Invest* 2022; **132**(16): e162330.

[18] Dong Y, Qiu P, Zhao LS, Zhang PH, Huang XW, Li CY, et al. Metabolomics study of the hepatoprotective effect of *Phellinus igniarius* in chronic ethanol-induced liver injury mice using UPLC-Q/TOF-MS

combined with ingenuity pathway analysis. *Phytomedicine* 2020; **74**: 152697.

[19]Contato AG, de Araújo CAV, Zanzarin DM, Guilherme MA, Priscila AS, Eduardo JP, et al. Biological characterization and antimicrobial bioactives of mycelium extracts from medicinal mushrooms *Phellinus linteus* and *Pleurotus albidus* (Agaricomycetes). *Int J Med Mushrooms* 2022; **24**(6): 47-55. .

[20]Musiała A, Donizy P, Augustyniak-Bartosik H, Jakuszko K, Banasik M, Kościelska-Kasprzak K, et al. Biomarkers in primary focal segmental glomerulosclerosis in optimal diagnostic-therapeutic strategy. *J Clin Med* 2022; **11**(12): 3292.

[21]Gipson DS, Troost JP, Spino C, Attalla S, Tarnoff J, Massengill S, et al. Comparing kidney health outcomes in children, adolescents, and adults with focal segmental glomerulosclerosis. *JAMA Netw Open* 2022; **5**(8): e2228701.

[22]Zhong J, Whitman JB, Yang HC, Fogo AB. Mechanisms of scarring in focal segmental glomerulosclerosis. *J Histochem Cytochem* 2019; **67**(9): 623-632.

[23]Nagano C, Hara S, Yoshikawa N, Takeda A, Gotoh Y, Hamada R, et al. Clinical, pathological, and genetic characteristics in patients with focal segmental glomerulosclerosis. *Kidney360* 2022; **3**(8): 1384-1393.

[24]Dou Y, Shang Y, Shen Y, Qu JT, Liu CL, Cao JS. Baicalin alleviates adriamycin-induced focal segmental glomerulosclerosis and proteinuria by inhibiting the Notch1-Snail axis mediated podocyte EMT. *Life Sci* 2020; **257**: 118010.

[25]Pereira LHM, da Silva CA, Monteiro MLGDR, Araújo LS, Rocha LP, da Rocha Reis MB, et al. Podocin and uPAR are good biomarkers in cases of focal and segmental glomerulosclerosis in pediatric renal biopsies. *PLoS One* 2019; **14**(6): e0217569.

[26]Surya M, Rajappa M, Vadivelan M. Utility of urinary nephrin in patients with and without diabetic nephropathy and its correlation with albuminuria. *Cureus* 2021; **13**(12): e20102.

[27]Ashraf A, Akhtar T, Shabbir A, Aftab U, Shahzad M. Sitagliptin ameliorates diabetic nephropathy by upregulating renal nephrin and podocin expression through modulation of adipokines levels. *Fundam Clin Pharmacol* 2023; **37**(3): 549-555.

[28]Chen Y, Lin L, Tao X, Song Y, Cui J, Wan J. The role of podocyte damage in the etiology of ischemia-reperfusion acute kidney injury and post-injury fibrosis. *BMC Nephrol* 2019; **20**(1): 106.

[29]Buchanan S, Combet E, Stenvinkel P, Shiels PG. Klotho, aging, and the failing kidney. *Front Endocrinol (Lausanne)* 2020; **11**: 560.

[30]Fu B, Yang J, Chen J, Lin L, Chen K, Zhang W, et al. Preventive effect of Shenkang injection against high glucose-induced senescence of renal tubular cells. *Front Med* 2019; **13**(2): 267-276.

[31]Felaco P, Felaco M, Franceschelli S, Ferrone A, Gatta DMP, Speranza L, et al. Erythropoietin induces miRNA-210 by JAK2/STAT5 signaling in PBMCs of end-stage renal disease patients. *FEBS J* 2020; **287**(23): 5167-5182.

[32]Watts D, Gaete D, Rodriguez D, Hoogewijs D, Rauner M, Sormendi S, et al. Hypoxia pathway proteins are master regulators of erythropoiesis. *Int J Mol Sci* 2020; **21**(21): 8131.

[33]Sha JY, Li JH, Zhou YD, Yang JY, Liu W, Jiang S, et al. The p53/p21/p16 and PI3K/Akt signaling pathways are involved in the ameliorative effects of maltol on *D*-galactose-induced liver and kidney aging and injury. *Phytother Res* 2021; **35**(8): 4411-4424.

[34]Chen L, Yang R, Qiao W, Zhang W, Chen J, Mao L, et al. 1,25-Dihydroxyvitamin D exerts an antiaging role by activation of Nrf2-antioxidant signaling and inactivation of p16/p53-senescence signaling. *Aging Cell* 2019; **18**(3): e12951.

[35]Kobayashi H, Davidoff O, Pujari-Palmer S, Drevin M, Haase VH. EPO synthesis induced by HIF-PHD inhibition is dependent on myofibroblast transdifferentiation and colocalizes with non-injured nephron segments in murine kidney fibrosis. *Acta Physiol (Oxf)* 2022; **235**(4): e13826.

[36]Martínez-Zamudio RI, Dewald HK, Vasilopoulos T, Gittens-Williams L, Fitzgerald-Bocarsly P, Herbig U. Senescence-associated β-galactosidase reveals the abundance of senescent CD8^{+} T cells in aging humans. *Aging Cell* 2021; **20**(5): e13344.

[37]Peng J, Ren XF, Yang C, Lan TB, Shao ZY, Chen Y. Effects of inflammatory response on renal function and TGF-β1 pathway of rats with aging-related kidney damage by upregulating the expression of CD36. *Eur Rev Med Pharmacol Sci* 2020; **24**(17): 8957-8967.

[38]Ma G, Chen F, Liu Y, Zheng L, Jiang X, Tian H, et al. Nur77 ameliorates age-related renal tubulointerstitial fibrosis by suppressing the TGF-β/Smads signaling pathway. *FASEB J* 2022; **36**(2): e22124.

[39]Song S, Shi C, Bian Y, Yang Z, Mu L, Wu H, et al. Sestrin2 remedies podocyte injury *via* orchestrating TSP-1/ TGF-β1/Smad3 axis in diabetic kidney disease. *Cell Death Dis* 2022; **13**(7): 663.

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