Fluorofenidone alleviates liver fibrosis by inhibiting hepatic stellate cell autophagy via the TGF-β1/Smad pathway

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Article

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Abstract

Objectives
Liver fibrosis is a key stage in the progression of various chronic liver diseases to cirrhosis, but at present, there is no effective treatment. This study examined the therapeutic effect of the new antifibrotic drug Fluorofenidone (AKF-PD) on liver fibrosis and its related mechanism.

Materials and methods
The effects of AKF-PD on hepatic stellate cell (HSC) autophagy and extracellular matrix (ECM) expression were assessed in a carbon tetrachloride (CCl\textsubscript{4})-induced rat liver fibrosis model. In vitro, HSC-T6 cells were transfected with Smad2 and Smad3 overexpression plasmids and treated with AKF-PD. The viability and number of autophagosomes in HSC-T6 cells were examined. Beclin-1, LC3 and P62 protein expression were analysed by Western blotting.

Results
AKF-PD attenuated liver injury and ECM production in CCl\textsubscript{4}-induced liver fibrosis. In vitro, the viability and the number of autophagosomes in HSCs were decreased significantly by AKF-PD treatment. In addition, the protein expression of FN, α-SMA, collagen III, Beclin-1 and LC3 was increased, and P62 expression was decreased by the overexpression of Smad2 and Smad3; however, AKF-PD reversed these effects.

Conclusions
AKF-PD alleviates liver fibrosis by inhibiting HSC autophagy via the transforming growth factor (TGF)-β1/Smad pathway.

1. Introduction
Liver fibrosis is characterized by excessive extracellular matrix (ECM) deposition in the liver, which is triggered by various types of chronic liver injury, including viral infection, alcohol, toxic substances, metabolic diseases\textsuperscript{1,2}. Liver fibrosis can be reversed at the early stage, but without efficient treatment, persistent liver fibrosis will progress to liver cirrhosis and even hepatocellular carcinoma\textsuperscript{3}. According to statistics, approximately one million people die from liver cirrhosis each year worldwide, and the trend has been increasing in recent years\textsuperscript{4}. Currently, there is no effective drug for treating human liver fibrosis.

The activation of hepatic stellate cells (HSCs) is a pivotal link in the development of liver fibrosis\textsuperscript{2}. Under normal physiological conditions, the main function of HSCs is to store and metabolize vitamins\textsuperscript{5}, but
after liver damage, HSCs are activated and transform into myofibroblast cells, which markers include fibronectin (FN) and α-smooth muscle actin (α-SMA)\(^6\). Activated HSCs can release transforming growth factor (TGF)-β1, which is deemed to be the most effective factor to promote fibrocyte\(^7\). TGF-β1 can induce the activation of HSCs and the production of collagen through TGF-β1/Smad pathway, thereby promoting the development of liver fibrosis\(^8\).

Autophagy is a degradative process by which impaired organelles, long-lived proteins and invading microorganisms in the cytoplasm of eukaryotes are degraded to maintain cell homeostasis\(^9\). So far, there are many researches on autophagy mechanism. The formation of autophagosomes is a characteristic manifestation of autophagy, and many markers related to autophagy have been found, including Beclin-1 and Microtubule-associated protein light chain 3 (LC3)\(^10,11\). Meanwhile, several signal pathways related to autophagy were also found, such as AMPK-mTOR pathway\(^12\) and TGF-β1/Smad pathway\(^13\). Autophagy plays a regulatory role in multiple liver diseases, including liver fibrosis and liver cancer\(^14\). HSC autophagy promotes lipid droplet degradation and provides energy for inducing HSC activation\(^15\). Recently, it has been reported that some drugs play an anti-liver fibrosis role by inhibiting the autophagy of HSCs\(^16-18\).

Fufang Biejia Ruangan Tablets (FFBJ) was the first traditional Chinese medicine for treating liver fibrosis authorized by the China Food and Drug Administration (CFDA) in 1999\(^19\). FFBJ can attenuate liver fibrosis by inhibiting TGF-β1/Smad in carbon tetrachloride (CCl\(_4\))-induced rat liver fiber model\(^20\). FFBJ can also treat liver fibrosis by preventing HSC proliferation and activation\(^19\).

Fluorofenidone [1-(3-fluorophenyl)-5-methyl-2-(1H)-pyridone; AKF-PD] is an antifibrotic drug independently developed by Central South University, which has entered phase II clinical trials. Preceding researches have proved that AKF-PD has anti liver fibrosis effects in several animal models\(^8,21\), but the specific mechanism of its anti-liver fibrosis effect is not completely clear. Peng et al found that AKF-PD can alleviate liver fibrosis by restraining HSC activation and proliferation\(^21\), and it can regulate the TGF-β1/Smad and MAPK pathways to inhibit the activation of hepatic stellate cells\(^8,22\). However, the relationship between AKF-PD and autophagy is not completely clear. The purpose of this research was to study whether AKF-PD alleviates liver fibrosis by restraining HSC autophagy via the TGF-β1/Smad pathway.

### 2. Materials And Methods

#### 2.1 Liver fibrosis model and AKF-PD treatment

All animals used in this research were five- to six-week-old male Sprague–Dawley rats (Hunan SJA Laboratory Animal Co., Ltd. Changsha, China). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of University of South China (Hengyang, China). The rats accepted humane care in accordance with the university’s guidelines. The rats were separated into four
groups (6 rats/group): normal group; CCl₄ group; CCl₄ + AKF-PD (240 mg/kg/day) group and CCl₄ + FFBJ (550 mg/kg/day) group. In Groups I and II, the rats were subjected to intraperitoneal injection of CCl₄ (#40006861 Sinopharm Chemical Reagent Co. Ltd., China) (2 ml/kg body weight, twice weekly) for 6 weeks. The rats in Group I were received intraperitoneal injection of the same dose of olive oil. The rats in Group III were intragastrically administered AKF-PD (lot No. 20190810 Haikou, China) (240 mg/kg/d once a day) for 6 weeks. In Group I, the rats were intragastrically administered FFBJ (#C0121041, Inner Mongolia Furui Medical Science Co., Ltd., China) (550 mg/kg/d once daily) for 6 weeks. In Groups II and III, the rats were intragastrically administered 0.5% carboxymethyl cellulose sodium (CMCNa) once daily for 6 weeks. All rats were euthanized by CO₂ asphyxiation at the end of week 6. Serum and liver samples were acquired. Serum was applied to examine indicators of liver function. Liver tissues were harvested for histopathological staining and Western blotting.

2.2 ALT/AST/TBIL/ALB assays

The serum levels of alanine aminotransferase (ALT), albumin (ALB), aspartate aminotransferase (AST) and Total Bilirubin (TBIL) were detected by commercial kits (Jiancheng, Nanjing, China) following the manufacturer's instructions.

2.3 Histopathological staining

Haematoxylin and eosin (H&E), Sirius Red staining and Masson's trichrome staining were used for histological analyses. Sirius Red staining and Masson's trichrome staining were used to show fibrosis degree. The blue area of Masson's trichrome staining and the red area of Sirius Red staining in the image showed the distribution areas of collagen in the liver, which were quantified using Image-Pro Plus 8.0.

2.4 Cell Culture And Treatment

The rat HSC line (HSC-T6) was provided by ATCC (Rockville, MD, USA). HSC-T6 cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin and cultured to 60–70% confluence for 24 h at 37°C. The cells were followed by 2 mM AKF-PD exposure for 24 h after stimulation with recombinant human TGF-β1 (5 ng/ml, 24 h).

2.5 Cell viability assay

HSC-T6 cells were grown in 96-well plates, the cells were followed by 2 mM AKF-PD and incubated for 0 h, 24 h, 48 h, and 72 h. Cell viability were analysed by Cell Counting Kit-8 (CCK-8; APEXBio, Houston, TX, USA).

2.6 Acridine orange fluorescent staining
HSC-6 cells were grown in 6-well plates for 48 h, treated with 2 mM AKF-PD for 24 h, wash by filling each well with PBS, and stained with 0.01% acridine orange (Solarbio, CA1142, China) for 30 min, Protect from light. The cells were examined with a fluorescence microscope (magnification, ×400) (Olympus ABX50, Tokyo, Japan). The area of red fluorescence was measured using Image-Pro Plus 8.0.

2.7 Western blot analysis

Liver tissues and HSC-T6 cells were were lysed with RIPA buffer (#ab156034, Abcam, UK) containing a phosphatase inhibitor and PMSF (#ab141032, Abcam, UK). The protein content of each sample was then determined by the Bradford assay. A total of 20 mg of protein per sample was separated on 10–15% SDS-polyacrylamide gel, and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked and incubated with primary antibodies at 4°C overnight. then followed by HRP anti-rabbit IgG (1:2000; #ab288151, Abcam, UK) for 60min.The primary antibodies were as follows: Collagen I (1:1000; #ab270993, Abcam, UK), TGF-β1 (1:500; #sc-130348, Santa Cruz, CA), Collagen III (1:1000; #ab184993, Abcam, UK), Smad2 (1:500; #sc-393312, Santa Cruz, CA), Smad3 (1:500; #sc-101154, Santa Cruz, CA), p-Smad2 (1:1000; #ab280888, Abcam, UK), p-Smad3 (1:1000; #ab52903, Abcam, UK), LC3 (1:1000; #ab192890, Abcam, UK), Beclin-1 (1:1000; #ab207612, Abcam, UK), P62 (1:1000; #sc-28359, Santa Cruz, CA), α-SMA (1:1000; #55135-1-AP Proteintech, Rosemont, USA), fibronectin(FN) (1:500; #sc-8422, Santa Cruz, CA), and GAPDH(1:1000; #ab8245, Abcam, UK).Finally, the bands were visualized using the ECL- chemiluminescent kit (Proteintech, USA) and quantified using Image J software

2.8 Transmission electron microscopy (TEM)

HSC-T6 cells were maintained in 2.5% glutaraldehyde for 12 h and fixed with 1% osmic acid for 3 h at 4°C. Subsequently, the cells were dehydrated, embedded and stained with uranyl acetate and lead citrate. At last, Autophagosomes were observed by TEM (Philips, Holland).

2.9 Smad2 and Smad3 overexpression analysis

Plasmids containing smad2-pcDNA3.1(+) and smad3-pcDNA3.1(+) were designed and synthesized by General Bio (Anhui) Co., Ltd (China). HSC-T6 cells were prepared in dishes for 24 h, 1.0 µg of plasmid/10^6 cells as transfected into HSC-T6 cells using Lipofectamine 2000. The medium was changed to serum-free DMEM for 24 h, and 2 mM AKF-PD was added and incubated for 24 h. HSCs were detected by Western blotting and acridine orange fluorescent staining. Then, 2 mM AKF-PD was added and incubated for 0 h, 24 h, 48 h, and 72 h, HSC activity was observed by CCK-8 assays.

2.10 Statistical analysis

All experiments were repeated at least three times. GraphPad Prism 9 was used to analyse data. The data were presented as the mean ± SD, and calculated by Student-Newman-Keuls tests and one-way analysis of variance (ANOVA). P < 0.05 was considered statistically significant.

3. Results
3.1 AKF-PD attenuated CCl₄-induced liver fibrosis in rats

In this study, a classic rat liver fibrosis model was created by intraperitoneal injection of CCl₄ for 6 weeks. We observed the role of AKF-PD on liver injury by H&E staining, intraperitoneal injection of CCl₄ induced a large number of hepatocyte necrosis, inflammatory cell invasion and fiber formation compared to the normal liver structure. However, AKF-PD and FFBJ (as a positive control group) evidently decreased hepatocyte injury, reduced fibrous scar areas, and delayed liver fibrosis (Fig. 1A). Liver fibrosis is always along with collagen accumulation. Masson staining and Sirius Red staining were used to detect collagen content. Our results showed that in CCl₄ group, massive collagen was deposited in the fibrosis scar of liver, and collagen deposition was effectively reduced by AKF-PD and FFBJ treatment. But there were no remarkable differences between the CCl₄+AKF-PD group and CCl₄+FFBJ group. (P > 0.05) (Fig. 1A). Moreover, collagen and collagen, which are fibrotic markers, were measured in the rat liver. Western blotting showed that the expression of collagen and were observably increased by CCl₄ treatment. However, AKF-PD and FFBJ therapy observably reduced their expression, but there were no remarkable differences between the two groups (P > 0.05) (Fig. 1B). The serum levels of ALT, TBIL, AST, and ALB were measured to examine liver function. Our results demonstrated that compared to the normal group, ALT, TBIL and AST level were increased and ALB level was decreased in the CCl₄ group. However, AKF-PD treatment reversed these effects. (Fig. 1C). Overall, our results indicated that AKF-PD could attenuate CCl₄-induced liver fibrosis in rats.

3.2 AKF-PD inhibited TGF-β1/Smad pathway in CCl₄-induced liver fibrosis.

The TGF-β1/Smad pathway is an important pathway that promotes liver fibrosis. Western blot analysis revealed that TGF-β1 expression in the CCl₄-induced liver fibrosis model was remarkably increased, and reduced by AKF-PD and FFBJ treatment. In addition, p-Smad2 and p-Smad3 expression were upregulated in the CCl₄ group and reduced in the CCl₄+AKF-PD group and CCl₄+FFBJ group. However, total Smad2 and Smad3 expression were not affected. (Fig. 2A).

3.3 AKF-PD inhibited autophagy in CCl₄-induced liver fibrosis

To study the effect of AKF-PD on autophagy, we examined the protein levels of Beclin-1, P62 and LC3, which are markers of autophagy. Western blot analysis revealed that the protein expression of Beclin-1 and LC3- / was observably increased in the CCl₄ group; however, these levels were effectively reduced by AKF-PD and FFBJ treatment. In contrast, P62 protein expression was observably decreased in the CCl₄ group and increased by AKF-PD and FFBJ therapy (Fig. 2B).

3.4 AKF-PD alleviated liver fibrosis by inhibiting the TGF-β1/Smad pathway in cultured HSCs
We further studied the impact of AKF-PD on the TGF-β1/Smad pathway in cultured HSCs. HSC activation is a characteristic of liver fibrosis, The protein expression of α-SMA, FN and collagen, which are markers of activated HSCs, was measured by Western blotting. TGF-β1 stimulation increased α-SMA, FN and collagen protein expression in HSC-T6 cells, and AKF-PD treatment observably reduced their expression. Furthermore, the expression of α-SMA, FN and collagen in HSCs were observably increased by the overexpression of Smad2 and Smad3 and were reduced by treatment with AKF-PD (Fig. 3).

3.5 AKF-PD alleviated liver fibrosis by inhibiting HSC autophagy via the TGF-β1/Smad pathway in cultured HSCs

We investigated the effect of AKF-PD on HSC autophagy in vitro. HSC-T6 cells were treated by TGF-β1 for 0h, 24h, 48h and 72h. We analysed the viability of HSCs using a CCK-8 assay. The viability of TGF-β1-stimulated HSC-T6 cells at 48h and 72h was observably increased and was significantly reduced by AKF-PD. Furthermore, the viability of HSC-T6 cells increased dramatically in response to the overexpression of Smad2 and Smad3 and decreased significantly after treatment with AKF-PD (Fig. 4A). We researched the effect of AKF-PD on autophagy-lysosomes in HSCs by acridine orange fluorescent staining. The nucleus fluoresces green when acridine orange binds to double-stranded DNA, and red fluorescence occurs when it reacts with autophagy-lysosomes at low pH. We found that the area of red fluorescence was particularly clear, and the morphology of HSC-T6 cells was intact in the TGF-β1 group. The area of red fluorescence was reduced and HSC-T6 cells were deformed in the TGF-β1 + AKF-PD group. In addition, the area of red fluorescence was dramatically increased by the overexpression of Smad2 and Smad3 and decreased significantly after AKF-PD treatment. These results demonstrated that AKF-PD could decrease the number of autophagy-lysosomes in HSCs via the TGF-β1/Smad pathway (Fig. 4B). Electron microscopy was used to directly observe autophagy. the number of autophagosomes in HSC-T6 cells were observably increased by TGF-β1 stimulation and were reduced after AKF-PD treatment (Fig. 4C). In addition, Western blot analysis revealed that the protein expression of Beclin-1 and LC3-I / -II in HSC-T6 cells was dramatically increased by TGF-β1, and AKF-PD significantly reduced their expression. In contrast, P62 expression was significantly reduced by TGF-β1 stimulation, and increased after treatment with AKF-PD. Furthermore, LC3-I / -II and Beclin-1 protein expression were observably increased by the overexpression of Smad2 and Smad3 and decreased after treatment with AKF-PD. In contrast, P62 protein expression was observably reduced by the overexpression of Smad2 and Smad3 and was increased by treatment with AKF-PD. Thus, AKF-PD alleviated liver fibrosis by restraining HSC autophagy via the TGF-β1/Smad pathway (Fig. 4D).

4. Discussion

Liver fibrosis is a healing reaction of chronic liver injury, which is characterized by excessive deposition of ECM dominated by collagen in the liver. Hepatic inflammation activates HSC, which leads to the synthesis and deposition abundant ECM, and finally forms liver fibrosis\(^ {23,24} \). The generation of fibrous scar will lead to the destruction of normal liver and the loss of liver cell function, and eventually lead to liver failure\(^ {25} \). Except for the therapy of various causes of liver fibrosis, currently, there is no effective
treatment, but liver fibrosis is considered an invertible pathological process. HSCs, which play a leading part in liver fibrosis, are crucial targets for antifibrotic treatment. Inhibiting HSCs activation is an important therapeutic approach to relieve liver fibrosis.

AKF-PD is a broad-spectrum anti-fibrosis drug, which has significant effects on liver, kidney and lung fibrosis. In this study, AKF-PD was applied to treat CCl₄-induced liver fibrosis model, which is a classic animal model for researching the mechanism of liver fibrosis. AKF-PD down-regulated the levels of serum ALT, TBIL and AST, and up-regulated the levels of ALB, indicating that AKF-PD improved the liver damage caused by carbon tetrachloride, and played a protective role in liver function. Furthermore, AKF-PD reduced the damage of hepatocytes, fibroplasia induced by carbon tetrachloride. In addition, AKF-PD treatment dramatically lessened collagen and protein expression, which are the main ECM contents in liver cirrhosis. In cultured HSCs, this study indicated that AKF-PD also reduced α-SMA, FN and collagen protein expression, which are activated HSC markers. We found that AKF-PD not only improved liver function but also improved the CCl₄-induced fibrosis in the liver.

Autophagy can decompose and recycle organelles, proteins and macromolecules in the cytoplasm to maintain homeostasis. The characteristic manifestation of autophagy is the formation of autophagosomes. Autophagy is regulated by autophagy-related genes (ATGs). LC3 promotes the formation of autophagosomes. The Beclin1 gene is a specific autophagy gene, and its upregulation can stimulate the occurrence of autophagy. p62 is an important autophagy protein, and its content is inverse correlation with autophagy level. The activation of HSCs is related to autophagy, several studies have showed that regulating autophagy of HSCs can effectively inhibit their activation and improve liver fibrosis. We confirmed that AKF-PD could decrease the number of autophagosomes in HSCs. In addition, we detected the protein levels of LC3, P62 and Beclin-1 which are known autophagy markers in the CCl₄ fibrosis model and cultured HSC-T6, AKF-PD decreased the expression of Beclin-1 and LC3, and increased that of P62. These results suggested that AKF-PD could inhibit autophagy in HSCs.

TGF-β1 is an intermediate substance that mediates fibrosis. TGF-β1 can promote the activation of HSCs and the synthesis of ECM. TGF-β1 binds to the type II receptor (TβRII) on the HSC membrane, triggering the phosphorylation of the TGF-β1 receptor (TβR); subsequently, TβR binds Smad2 and Smad3. This complex becomes phosphorylated and forms hetero-oligomers with Smad4, finally, they enter the nucleus to manage the transcription of target genes. These target genes include fibrosis related genes, such as collagen I, α-SMA, MMPs, and TIMPs, and autophagy-related genes, such as Beclin-1. The TGF-1/Smad pathway can promote ECM synthesis and regulate HSC autophagy during liver fibrosis. In our study, we discovered that AKF-PD decreased the protein expression of TGF-β1, p-Smad2 and p-Smad3 in the CCl₄-induced rat liver fibrosis model. These indicated that AKF-PD can inhibit TGF-β1/Smad pathway. Furthermore, the viability and the number of autophagosomes in cultured HSCs increased significantly in response to the overexpression of Smad2 and Smad3 and decreased significantly after AKF-PD treatment. Moreover, the expression of Beclin-1 and LC3 were observably increased and that of
P62 was reduced by overexpression of Smad2 and Smad3, and AKF-PD reversed these effects. These indicated that AKF-PD inhibited TGF-β1/Smad pathway to suppress HSC autophagy. In addition, FN, α-SMA and collagen III expression were observably increased by overexpression of Smad2 and Smad3, and were reduced by AKF-PD treatment. This study confirmed that AKF-PD reduced TGF-β1 secretion and inhibited TGF-β1/Smad signalling pathway to inhibit HSC autophagy and reduce ECM synthesis, ultimately alleviating liver fibrosis (Fig. 5).

**Conclusion**

In summary, our findings prove that the inhibition of HSC autophagy plays an important part in the antifibrotic effects of AKF-PD. AKF-PD inhibits HSC autophagy, at least partly, via the TGF-β1/Smad signalling pathway.

**Declarations**

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All animal procedures performed in this study were reviewed and approved by the Ethics Review Committee for Animal Experimentation of University of South China.

**Author Contributions**

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**Disclosure**

The authors have declared that no competing interest exists

**Statement**
The experimental protocol was approved by the Animal Care and Conformed to the NIH Guide for the Care and Use of Laboratory Animals (No. 2013-99). All the animal experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All methods were carried out in accordance with ARRIVE guidelines.

**Data availability statement**

All data generated or analyzed during this study are included in this article.

**References**


Figures
Figure 1

AKF-PD attenuated CCl4-induced liver fibrosis induced in rats. A rat liver fibrosis model was established by intraperitoneal injection of CCl4 for 6 weeks. AKF-PD and FFBJ were intragastrically administered to the rats. (A) Liver sections were stained with H&E, Masson's trichrome and Sirius Red (scale bar: 50 μm). (B) Western blot analysis of collagen I and collagen III protein expression in the livers. (C) The levels of...
ALT, AST, ALB and TBIL in rat serum were calculated. Value represent mean ± SD (n=6). *p < 0.05, **p < 0.01. ***p < 0.001, N.S. not significant.

Figure 2

AKF-PD inhibited TGF-β1/Smad signalling and autophagy in CCl₄-induced liver fibrosis.
(A) Western blot analysis of TGF-β1, p-Smad2, Smad2, p-Smad3 and Smad3 protein expression in the liver. (B) Western blot analysis of Beclin-1, LC3- / and P62 protein expression in the livers. Value represent mean ± SD(n=6). *p < 0.05, **p < 0.01. ***p < 0.001, N.S. not significant.

Fig.3

Figure 3

AKF-PD alleviated liver fibrosis by inhibiting the TGF-β1/Smad pathway in cultured HSCs.

Western blot analysis of α-SMA, FN and collagen III protein expression in HSCs. Values represent mean ± SD of 3 independent experiments. *p < 0.05, **p < 0.01. ***p < 0.001, N.S. not significant.
Figure 4

AKF-PD alleviated liver fibrosis by inhibiting HSC autophagy via the TGF-β1/Smad pathway in cultured HSCs.

(A) The viability of HSC-T6 cells was examined by CCK-8 assays. (B) Effect of AKF-PD on HSC autophagy, as determined by fluorescence microscopy after staining with acridine orange (scale bar: 50
µm). (C) Autophagosome structures (denoted by white triangles) in HSCs were obtained by TEM (scale bar: 0.5 µm). (D) Western blot analysis of Beclin-1, LC3- / and P62 protein expression in HSCs. Values represent mean ± SD of 3 independent experiments. *p < 0.05, **p < 0.01. ***p < 0.001, N.S. not significant.

**Fig.5**

![Diagram of liver fibrosis mechanism](image)

**Figure 5**

**Mechanism of AKF-PD treatment of liver fibrosis.**

After liver injury caused by carbon tetrachloride, HSCs are activated and secrete TGF-β1. TGF-β1 binds to the type II receptor (TβRII) on the HSC membrane, triggering the phosphorylation of the TGF-β1 receptor (TβR); subsequently, TβR binds Smad2 and Smad3. This complex becomes phosphorylated and forms hetero-oligomers with Smad4, finally, they enter the nucleus to manage the transcription of target genes. These target genes include fibrosis related genes, such as collagen I, α-SMA, MMPs, and TIMPs, and autophagy-related genes, such as Beclin-1. In this study, our findings demonstrated that AKF-PD reduced TGF-β1 secretion and inhibited TGF-β1/Smad signalling to inhibit HSC autophagy and reduce ECM synthesis, ultimately alleviating liver fibrosis.

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- originaldata.zip