Effect of Renshen Pingfei Decoction, a traditional Chinese prescription, on IPF induced by Bleomycin in rats and regulation of TGF-β1/Smad3

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A B S T R A C T

Aim of the study: Idiopathic pulmonary fibrosis (IPF), one of the clinical common diseases, shares similar pathogenesis with ancient disease “Feibi” in Chinese medicine, Renshen pingfei decoction (RPFS), a classical prescription, was commonly used in treating Feibi. In the current study, the protective role of RPFS in rats model of IPF and the mechanism via regulation of TGF-β1/Smad3, were evaluated and explored.

Methods: The chemicals of RPFS were analyzed by UPLC-QTOF-MS. Under the optimized chromatographic and MS condition, the major components in RPFS were well separated and detected. An IPF model was established in rats which were induced with Bleomycin (BLM). After treated with corresponding medicine for 7 days, 14 days, 21 days and 28 days respectively, lung function of rats were measured; peripheral blood and bronchoalveolar lavage fluid (BALF) were assessed; histopathological changes and homogenate of lung tissue were detected; TGF-β1 and Smad3 mRNA and protein expressions in lung tissue were examined as well.

Results: 43 signal peaks of chemical components in RPFS were identified by UPLC-QTOF-MS method. Compared with model group, RPFS group exerted significant effects on IPF model rats in improving lung function and decreasing HYP content of lung tissue (P < 0.01), reducing the level of TGF-β1 and NFκB in BALF (P < 0.05), decreasing SOD and MDA level in serum (P < 0.01), as well as down-regulating TGF-β1 and Smad3 mRNA and protein expressions of lung tissue (P < 0.01).

Conclusion: RPFS could reduce the lung injury and fibrosis degree and improve lung function of IPF model rats. The protective role might mediated by down-regulating TGF-β1/Smad3 signaling pathway.

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1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a special type of chronic progressive fibrosis interstitial pneumonia with unknown etiology. Due to its irreversible damage to lung function (Raghu et al., 2011; Bando et al., 2015), patients with IPF have an estimated median survival of 2–5 years (Marks, 2013; Sun et al., 2015; Morais et al., 2015). Therefore, it is important to search for the effective medicine to treat IPF in medical field. Feibi, an ancient disease in TCM, as described in (Huangdi Neijing Suwen) of Han Dynasty was characterized by dry cough, progressive dyspnea and unfavorable prognosis, which shared similar symptoms with IPF. According to the earlier studies, it was considered that IPF shared similar pathogenesis with Feibi in TCM (Rui and Peng, 2005; Weilong, 2014). Renshen Pingfei Decoction (RPFS) from (Zhengyinmaizhi · volume 3) (1706) was normally used to treat Feibi for hundreds of years (Manyi and Yuqian, 2015) and commonly applied in treating modern clinical disease IPF. It consists of Radix and Rhizoma of Panax ginseng C.A.Mey., Radix of Asparagus cochinchinensis (Lour.) Merr., Cortex of Morus alba L., Cortex of Lycium chinense Mill., Radix and Rhizoma of Glycyrrhiza uralensis Fisch., Rhizoma of Anemarrhena asphodeloides Bunge and Pericarpium of Citrus reticulata Blanco. However, the mechanism of RPFS, a traditional Chinese prescription, in treating IPF remains unknown.

At present, Bleomycin (BLM) through intratracheal instillation is the most important and widely used method for inducing IPF in various animal species to cause lung injury and fibrotic lesions (Hay et al., 1991; Veronica et al., 2015) and this study created IPF model in rats by BLM. Then the effect and mechanism of RPFS...
interfering the BLM-induced IPF model of rats were determined through the changes of pulmonary pathology and function, TGF-β1/Smad3, and NFκB level.

2. Materials and methods

2.1. Drugs and reagents

The ratio of Radix and Rhizoma of P. ginseng C.A.Mey., Radix of A. cochinchinensis (Lour.) Merr., Cortex of M. alba L., Cortex of L. chinense Mill., Radix and Rhizoma of G. uralensis Fisch., Rhizoma of A. asphodeloides Bunge and Pericarpium of C. reticulata Blanco in RPFS was 1:1:1:1:1:1. All crude dried herbs were purchased from Jiangsu Province Hospital of TCM. The botanical identities of the herbs were well identified by Prof. Dekang Wu of Medicinal Plant Department, Nanjing University of Chinese Medicine. The herbs were decocted and concentrated, with each millilitre containing 0.65 g of crude drugs.

BLM was purchased from Japanese chemical drug kabushiki kaisha (Batch no. 730342); Prednissone acetate tablets were purchased from Zhejiang Xianju Pharmaceutical Co., Ltd. (Batch no. 140138); Chloral hydrate (Batch no. 20121204) was purchased from Shanghai BioSun Sci&Tech Co., Ltd.; Formalin solution (Batch no. 130706) was purchased from Xilong Chemical Co., Ltd.; Normal saline (NS) was purchased from Shanghai Y-Y Biotech Co., Ltd.; TGF-β1 ELISA kit (CK-E30636R), NFκB ELISA kit (CK-E91672R), HYP kit (Batch no. 20140625), MDA assay (TBA) kit (Batch no. 20140627) and SOD assay kit (WST-1) (Batch no. 20140618) were purchased from Nanjing KeyGEN Biotech Co., Ltd.; Trizol (No. 15596-026) was purchased from Life Technologies Invitrogen Company, USA; 1st-Strand cDNA Synthesis Kit (No. K1622) and Taq DNA Polymerase (No. EP0405) were purchased from Thermo Fisher Scientific Inc., USA.

2.2. UPLC and mass spectrometric conditions

UPLC was performed on a Waters ACQUITY UPLC system (Milford MA), consisting of a binary solvent delivery system, an autosampler and PDA detector system. An ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm i.d., 1.7 μm; Waters) was used for all analyses. The mobile phase was composed of 0.1% formic acid (v/v) (A) and acetonitrile (B), with 30 min of gradient elution: 0–1 min, 5–5% B; 1–10 min, 5–20% B; 10–25 min, 20–50% B; 25–27 min, 50–95% B; 27–29 min, 95% B; 29–30 min, 95–5% B. The flow rate of the mobile phase was 0.4 ml/min and the temperatures of the column and autosampler were maintained at 30 and 10 °C, respectively.

The MS analysis was performed on a Waters ACQUITY Synapt Q-TOF mass spectrometer connected to the Waters ACQUITY UPLC system via an electrospray ionization (ESI) interface. High purity nitrogen was used as the nebulizer and auxiliary gas and argon as the collision gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas. The Q-TOF MS was operated in negative ion mode with spray voltage of 3 kV, a sampling cone voltage of 35 V, a desolvation gas.

Mass accuracy was maintained by using LockSpray. The [M–H]− and [M+H]+ ions of leucine-enkephalin at m/z 554.2615 and 556.2771 were used as the lock mass in negative and positive ESI modes, respectively. The concentration of leucine-enkephalin was 50 pg/ml and the infusion flow rate was 10 μL/min.

2.3. Apparatus

Enzyme-linked immune detector was purchased from Bio-Tek Instruments Inc., USA; BUXCO PFT was purchased from BUXCO Inc., USA; Gel documentation system was purchased from Bio-Rad Laboratories Inc., USA; Veriti PCR (96-well Thermal cycler) was purchased from Applied Biosystems Inc., USA; Nucleic acid electrophoresis apparatus (DYV-6B) was purchased from Liuyi Instruments Inc., Beijing, China.

2.4. Animals

A total of 192 male SD rats (weighting 180 ± 20 g) were used in this study. All animals were purchased from Vital River Laboratory Animal Technology Co., Ltd., Beijing, China, with the certification No. SCXK (Jin) 2012-0001. The rats were housed in microisolator cages (each cage 5 rats) with free access to tap water and pellet feed provided by Xietong Medical Bioengineering Co., Ltd., Jiangsu, China. The laboratory temperature was under 22–24 °C, and relative humidity was between 50% and 70%. The rats were housed for 2–3 days to help them adapt to the environment before experimentation. All experiments were performed in accordance with the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Ethical Committee for the Experimental Use of Animals at Nanjing University of Chinese Medicine (Nanjing, Jiangsu, China).

2.5. Sample preparation

For the traditional decoction, samples of Radix and Rhizoma of P. ginseng C.A.Mey. (10 g), Radix of A. cochinchinensis (Lour.) Merr. (10 g), Cortex of M. alba L. (10 g), Cortex of L. chinense Mill. (10 g), Radix and Rhizoma of G. uralensis Fisch. (10 g), Rhizoma of A. asphodeloides Bunge (10 g) and Pericarpium of C. reticulata Blanco (10 g) were accurately weighed and mixed. The above mixed dry herbs were soaked into 10 times of water for 1 h and boiled 30 min to get the extracted solution, then boiled another 30 min with 8 times of water to get the extracted solution. The two times extracted solutions were mixed together and centrifuged at 3000 rpm for 5 min, and the supernatant was concentrated to each millilitre containing 0.65 g of crude drugs, which was regarded as the sample of a traditional decoction.

2.6. Model establishment and drug treatment

192 rats were randomly divided into normal group (7d, 14d, 21d, 28d), model group (7d, 14d, 21d, 28d), prednisone acetate positive group (7d, 14d, 21d, 28d) and RPFS group (7d, 14d, 21d, 28d) totally 16 groups with 12 rats each. Rats in the above groups other than normal group were given 10% chloral hydrate (0.3 ml/100 g) intraperitoneally, then as the rats were inhaling, quickly insert the dedicated needle into rats bronchial bifurcation and slowly inject 2.5 mg/ml BLM (0.2 ml/100 g) intragastrically administrated with RPFS (0.65 g/100 g d) one time per day, while rats from normal group were given 10% chloral hydrate (0.3 ml/100 g) intraperitoneally, then as the rats were inhaling, quickly insert the dedicated needle into rats bronchial bifurcation and slowly inject 2.5 mg/ml BLM (0.2 ml/100 g) e to establish the model. After the injection, the rats were kept erectly and rotated for several minutes to make the medicine well distributed in lung. 24 h after the model establishment, rats from RPFS group were intragastrically administrated with RPFS (0.65 g/100 g d) and rats from positive group were treated with prednisone acetate (0.54 mg/100 g d) one time per day, while rats from normal group and model group were given normal saline (NS) of equal volume.

2.7. Lung function measurements

On day 7, 14, 21 and 28, rats of each group were anaesthetized by 10% chloral hydrate (0.3 ml/100 g). PFT Pulmonary Maneuvers was used for lung function measurements of rats. The recorded variables included forced vital capacity (FVC), resistance (RI), total lung capacity (TLC) and Chord compliance (Cchord).
2.8. MDA and SOD test in serum

After the last challenge, the blood was withdrawn from the orbit of rats and centrifuged (3500 r min⁻¹) for 5 min to get the serum, which were subjected to the detection of malondialdehyde (MDA) and superoxide dismutase (SOD). In the study, MDA was measured by TBA colorimetric assay and SOD was assayed with a water-soluble tetrazolium salt (WST-1).

2.9. TGF-β1 and NFκB test in BALF

Samples were obtained after the blood collection. The trachea was cannulated by using a blunt needle attached to a syringe. BALF was performed 3 times with 3 ml of phosphate buffer saline (PBS). Each BALF sample was centrifuged and the supernatants were stored at −80 °C for further cytokine (TGF-β1, NFκB) analyses with ELISA kits.

2.10. HYP test in lung tissue

30 to 100mg of lung tissue samples were hydrolyzed with 1ml of hydrolysate and boiled for 20min followed the instructions of HYP kit. Samples were centrifuged at 3000 r min⁻¹ of hydrolysate and boiled for 20min followed the instructions of 2.10. HYP test in lung tissue

2.11. Lung histopathology

Rat lung tissues were fixed in 10% formalin and processed for routine paraffin embedding. Serial sections were cut into 5 μM each. Hematoxylin and Eosin (HE) stain and Masson’s trichrome stain were performed as Tanaka et al. (2013) previously described. Histopathological changes in the lung tissues were observed under a light microscope. The severity of alveolitis and fibrosis was blindly assessed semi-quantitatively, according to the criteria previously described by Szapiel et al. (1979). The degree of fibrosis was also shown through the areas of blue-stained collagen on the sections stained with Masson.

2.12. Reverse transcription PCR

Total RNA was extracted from 30 mg of lung tissue following the Trizol reagent instructions as Kang et al. (2011) previously described. Following the instructions of Revert Aid First Strand cDNA Synthesis kit, first-strand cDNA synthesis was performed from 20 μl of reaction mixture including 2 μg of total RNA and 2 μl of oligo (dT) as a primer and reverse transcriptase. 50 μl of reaction mixture including 0.5U Taq DNA polymerase, polymerase chain reaction was performed by starting denaturation at 95 °C for 5 min, followed by 28 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min.

2.13. Western blot analysis

The Total Protein was drawn from the lung tissue, got protein quantification using Bradford method, and diluted by denaturing Lysis Buffer. The equivalent samples were put into tubes under 95–100 °C for 5 min, and contain 50 μg proteins each. Then, the protein samples were subjected to SDS-PAGE (Nanjing Keygen Biotech Co., Ltd.). After electrophoresis, proteins were transferred, blocked 2 h in 5% skim milk powder block buffer and washed with PBST 4times. Appropriate amount of rat anti-Smad3 and TGF-β1 (294 bp) antibodies were diluted by the dilution and incubated by the developing solution were laid aside under room temperature for 5 min, and exposed with G:BOX chemiXR5. The results were analyzed with Gel-Pro32 software.

2.14. Statistical analyses

All data were presented as the mean ± standard deviation. The statistics and data evaluation were respectively performed by SPSS18.0 and one-way analysis of variance (ANOVA). P < 0.05 was considered to be significant.
3. Results

3.1. Identified chemical compounds of RPFS

RPFS consists of seven herbs, forming a very complex matrix. The major components that could be detected in RPFS are mainly saponin. UPLC–QTOF-MS was applied to analyze chemicals in RPFS, and the major components were well separated and detected under the optimized chromatographic and MS condition. Components in RPFS were identified by UPLC–QTOF-MS method and listed in Table 1. Taking retention time (tR), mass fragment ions (m/z), UV absorption wavelength, and relevant references into consideration, 43 peak signals of chemical components were identified. Total ion currents were shown in Fig. 1.

3.2. Histopathologic changes of lung induced by BLM

Through HE stain, it was found that on day 7 and 14, rats from all groups except normal group had significant increase in the thickness of alveolar interval. Also exudates were found in lumen. Bronchial submucosa and muscular layer were infiltrated by large amount of inflammatory cells, including eosinocytes, neutrophils, lymphocytes, macrophages and some plasma cells. Fibrous connective tissue had moderate hyperplasia, bronchial ciliated epithelium partly fell off, cilia were found adhesion, lodging, degeneration and necrosis, airway epithelium stratified, and also part of the alveolar had moderate emphysema. On day 21 and 28, the amount of hyperplasia of blue-stained collagen in positive group and RPFS group was obviously less than that in model group (Fig. 3).

3.3. HYP content of lung

At each stage, compared with normal group, HYP content in model group was obviously increased (P < 0.01). Compared with model group, HYP content in RPFS group was decreased, especially on day 21 and 28 (P < 0.01) (Fig. 4).

3.4. Lung function changes

Lung function changes of rats, forced vital capacity (FVC), total lung capacity (TLC), resistance (RI) and Chord compliance (Cchord), were detected through PFT Pulmonary Maneuvers. The results were shown in Figs. 5–8.

3.5. Genetic and protein expressions of Smad3 and TGF-β1 in lung tissue

RT-PCR and western blotting results showed that both genetic and protein expressions of Smad3 and TGF-β1 in rats lung tissues were highly expressed in model group (P < 0.01). After interfered by RPFS and prednisone acetate, the genetic and protein expressions of Smad3 and TGF-β1 were obviously lower than that of model group (P < 0.01). (Tables 3 and 4, Figs. 9 and 10).
3.6. TGF-β1 and NFκB level in BALF

Levels of TGF-β1 and NFκB in BALF were detected with ELISA. The results showed that.

(1) TGF-β1 level changes in BALF: compared with normal group, TGF-β1 level increased in model group at each stage (P < 0.05); compared with model group, the level decreased in both RPFS group (day 21 and 28) and positive group (day 28) (P < 0.05). (Fig. 11).

(2) NFκB level changes in BALF: compared with normal group, NFκB level in model group increased, obviously increasing from day 14 to 28 (P < 0.01, P < 0.05); compared with model group, the level in RPFS group all decreased at each stage and obviously decreased on the 28th day (P < 0.01); compared with model group, the SOD level in RPFS group all increased on day 14, 21 and 28, and obviously increased on the 28th day (P < 0.01), while positive group increased on the 21th day (P < 0.05) and obviously increased on the 28th day (P < 0.01). (Figs. 13 and 14).

3.7. MDA and SOD level in serum

The results showed: (1) Compared with normal group, the MDA level in model group increased on day 14, 21 and 28 (P < 0.01, P < 0.05); compared with model group, the MDA level in RPFS group and positive group all decreased on day 14, 21 and 28 (P < 0.05). (2) Compared with normal group, the SOD level in model group all decreased at each stage and obviously decreased on the 28th day (P < 0.01); compared with model group, the SOD level in RPFS group all increased on day 14, 21 and 28, and obviously increased on day 28 (P < 0.01), while positive group increased on the 21th day (P < 0.05) and obviously increased on the 28th day (P < 0.01). (Figs. 13 and 14).

4. Discussion

It is considered in TCM that Feibi is caused by the deficiency of Qi and Yin, and the stasis of phlegm and heat. Chinese medicine has a rich experience in treating feibi as early studied (Hui et al., 2010; Xia et al., 2012). The herbs in RPFS are all recorded in Pharmacopoeia of the People’s Republic of China (2015 Edition). These herbs were commonly used in treating lung disease and many studies did a lot on their effects (Xiuli et al., 2011; Qun, 2013; Wanghui et al., 2014). Radix and Rhizoma of *P. ginseng* C.A.Mey. strongly nourishes Qi; Radix of *A. cochinchinensis* (Lour.) Merr. and Rhizoma of *A. asphodeloides* Bunge nourish Yin; Yin Cortex of *M. alba* L. and Cortex of *L. chinense* Mill. clear the heat in lung; Pericarpium of *C. reticulata* Blanco regulates Qi; Radix and Rhizoma of *G. uralensis* Fisch. harmonizes the herbs in the prescription. Therefore, RPFS was chosen to treat rats model of IPF induced by...
The histopathological results in the study showed that in early stages experiments, compared with model group, the alveolar inflammation and fibrosis degree in RPFS group and positive group had no big difference until day 21 and 28 experiment, which showed that the effect of RPFS was taken from the 21th day experiment (Bei et al., 2013). On clinic, lung function is considered as a mature measurement to assess pulmonary fibrosis and an important indicator to diagnose pulmonary fibrosis (Shiyuan and Wei, 2011). In this study, FVC, TLC and Chord level were all decreased in model group at each stage, while lung resistance increased. After interfered by RPFS, the situation of all indicators improved and the effect was taken from day 21 of the experiment.

Being the major part of collagen fibre in connective tissue, collagen contains HYP most in human protein. HYP is an efficient index of collagen deposition, and HYP content can reflect the collagen metabolism of desmosis and fibrosis degree of the tissue (Zhou et al., 2013; Cao et al., 2014). HYP content increased obviously in all stages of the experiment, which showed the model was successfully established. And compared with model group, HYP content in rats decreased after interfered by RPFS, indicating the improvement of fibrosis degree.

TGF-β1 is one of the important cytokines in pulmonary fibrosis pathogenesis, which is considered to act as mediators of fibroblast activation, accumulation of fibroblast and/or myofibroblasts in damaged tissue (Chapman, 2011; Todd et al., 2012), to promote the progression of the disease. Smad3 is related to the signal transduction of TGF, especially TGF-β1, of animals or human beings, that is why the TGF-β1/Smad3 signaling pathway is considered to have close relation with deposits of extracellular matrix and tissue.

**Fig. 3.** Lung tissue sections with Masson staining (Masson, ×200).

**Fig. 4.** Comparison of HYP content of all groups.

**Fig. 5.** FVC changes in all groups.
fibrosis (Wang et al., 2013; Soumyakrishnan et al., 2014). In this study, protein and genetic expressions of Smad3 and TGF-β1 after interfered by RPFS were obviously lower than that in model group. RPFS could adjust the protein and genetic expressions of Smad3 and TGF-β1 in lung tissue and decrease the TGF-β1 level in the BALF, to reduce the severity of pulmonary fibrosis. NFκB is the cytokine which participates in the early time of immunoreaction and every period of inflammatory reaction (Li et al., 2013; Kandhare et al., 2015). The result of our study showed that positive group was better than RPFS group in decreasing NFκB level in BALF, indicating that RPFS had no obvious statistical significance on the alveolitis of rats induced by BLM on day 14. Recently many researches have found that one of the pathogenesis of pulmonary fibrosis is the imbalance of oxygen free radical and anti-oxygen free radical system. Being one of the decomposition product of lipid hydroperoxide, MDA level could in a way reflect the injury degree of cells attacked by oxygen free radical (Chitra et al., 2013). MDA is usually measured together with SOD, which is an antioxidase and can protect the tissue from injury by catalyzing the dismutation of superoxide into oxygen and hydroperoxides (Soumyakrishnan et al., 2011). Our study showed that at each stage MDA level increased and SOD level decreased in model group, indicating there was oxidative damage in the process and development of pulmonary fibrosis. After interfered by RPFS, MDA level decreased and SOD level increased, indicating the oxidative damage situation improved as compared with model group.

Table 3
Genetic expressions of Smad3 and TGF-β1 in lung tissue (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Smad3</th>
<th>TGF-β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.235 ± 0.008</td>
<td>0.216 ± 0.004</td>
</tr>
<tr>
<td>Model</td>
<td>0.851 ± 0.026**</td>
<td>0.843 ± 0.018***</td>
</tr>
<tr>
<td>Positive</td>
<td>0.627 ± 0.063**</td>
<td>0.576 ± 0.021**</td>
</tr>
<tr>
<td>RPFS</td>
<td>0.491 ± 0.015**</td>
<td>0.445 ± 0.008**</td>
</tr>
</tbody>
</table>

Notes: *P < 0.05 vs normal group. **P < 0.01 vs normal group.

Table 4
Protein expressions of Smad3 and TGF-β1 in lung tissue (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Smad3</th>
<th>TGF-β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.144 ± 0.034</td>
<td>0.127 ± 0.012</td>
</tr>
<tr>
<td>Model</td>
<td>0.583 ± 0.046**</td>
<td>0.566 ± 0.061**</td>
</tr>
<tr>
<td>Positive</td>
<td>0.353 ± 0.064**</td>
<td>0.286 ± 0.022**</td>
</tr>
<tr>
<td>RPFS</td>
<td>0.284 ± 0.029**</td>
<td>0.257 ± 0.032**</td>
</tr>
</tbody>
</table>

Notes: *P < 0.05 vs normal group. **P < 0.01 vs model group. **P < 0.01 vs model group.
The result also showed the trend in RPS group was even better than that in positive group on day 14, indicating that anti-oxidative damage would also be one of the effects and mechanisms of RPS.

5. Conclusion

In summary, this study demonstrated that RPSF had a significant effect on IPF model rats in improving lung function and histopathology, decreasing HVP content of lung tissue. And most of the effect indicators improved on day 21. Furthermore, RPSF could lower gene and protein expressions of TGF-β1 and Smad3 in lung tissue, reduce TGF-β1 and NFκB level in BALF of rats, as well as regulate the level of SOD and MDA in serum of rats. The protective role of RPSF in rat model of IPF might be operated by down-regulating TGF-β1/Smad3 mediated intracellular signal transduction pathway. Also, the role of the chemical components identified in RPSF is worthy of further study in IPF treatment.

Conflict of interest

The authors declare no conflict of interest.

Authors’ contributions

Xinsheng Fan (shshfan@njutcm.edu.cn), Jinghua Yu (18351897316@126.com) and Yue Zhu (fanxsh126@126.com) conceived and designed the experiments; Fei Chen (disillus163.com), Jinghua Yu and Zhenhua Zhu (4040416@163.com) performed the experiments; Fei Chen, Zhenhua Zhu and Pengli Wang (wangpengli2012@163.com) analyzed and collected the data; Zhenhua Zhu contributed analysis tools; Fei Chen, Pengli Wang, Yue Zhu and Xinsheng Fan wrote the original manuscript and Pengli Wang translated it into English version. All authors have read and approved the final manuscript.

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