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# Rhoifolin improves bleomycin-induced fibrosis in vivo and cell damage in vitro both related to NRF2/HO-1 pathway

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## Abstract

**Background** Pulmonary fibrosis (PF) is a chronic interstitial lung illness characterized by its high worldwide prevalence, unknown etiology, and dismal prognosis. *Lonicerae Japonicae Flos*, a commonly used traditional Chinese medicine for treating PF, is rich in Rhoifolin (ROF). Although numerous studies have demonstrated the anti-inflammatory properties of ROF, its potential anti-fibrotic effects remain uncertain.

**Methods** In this study, we established a PF model in Sprague-Dawley (SD) rats utilizing Bleomycin (BLM). We then assessed the impact of ROF on lung histology and appearance, measured the content level of Superoxide dismutase (SOD) in rat serum, and analyzed changes in α-SMA, TGF-β relative mRNA expression using PCR, measured SMAD Family Member 7 (Smad7), and Heme Oxygenase-1 (HO-1) protein expression in rat lung tissue by Western Blot. Additionally, we induced a cell injury model in A549 cells with BLM. Then after ROF administration, we detected the cell viability by MTT assay, measured N-cadherin, α-SMA, and Vimentin mRNA levels via real-time PCR, and analyzed the expression changes of N-cadherin, Nuclear factor erythroid 2-related factor 2(Nrf2), HO-1, Smad7 proteins by Western Blot.

**Results** The results indicated that ROF mitigated lung tissue damage and reduced the degree of PF in the lung tissue of rats with PF. Furthermore, In vivo, ROF reduced the expression of N-cadherin protein while increasing the expression of Smad7, and HO-1 proteins and decreasing the relative mRNA expression of α-SMA and TGF-β, and increased the expresson of SOD in rat serum In vitro, cell injury was induced in A549 cells using BLM. After ROF administration, the relative mRNA expression of α-SMA, N-cadherin, and Vimentin decreased significantly, and the protein expression of N-cadherin decreased, while the protein expression of Nrf2, HO-1, and Smad7 increased significantly.

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**Conclusion** This study demonstrates that ROF can mitigate the symptoms of PF to a certain degree, and its mechanism of action is intimately linked to the Nrf2/HO-1 signaling pathway. Therefore, this study indicates that ROF may serve as a potential therapeutic agent for treating PF.

**Clinical trial number** Not applicable.

**Keywords** Pulmonary fibrosis, Rhoifolin, Bleomycin

## Introduction

Pulmonary fibrosis (PF) is a chronic, progressive, fibrotic, and fatal respiratory disease of largely unknown etiology, typically resulting from a range of lung injuries such as autoimmune conditions, drug-induced damage, bacterial infections, or traumatic injuries [1–4]. Its pathogenic characteristics include inflammation, abnormal extracellular matrix (ECM) deposition, increased interstitial collagen, and excessive fibroblast activity, all of which deteriorate lung structure and impair respiratory function [5, 6]. The primary clinical symptom is progressive dyspnea, accompanied by coughing, sputum production, exhaustion, appetite loss, and weight loss. The condition can progress to heart and lung failure [7, 8]. Since there is currently no proven cure for PF, it is beneficial to search for medications to alleviate the condition aggressively.

Due to its beneficial healing effects and lack of adverse consequences, Chinese herbal therapy is gaining popularity. ROF, a flavonoid found in many plants including *Lonicerae Japonicae Flos*, *Chrysanthemi Flos*, and *Citrus Reticulata*, possesses anti-inflammatory, antiviral, antilipid, antitumor, antibacterial, and antiallergic activities [9]. Yan et al. [10] discovered that ROF can treat osteoarthritis by modulating autophagy. Zheng et al. [11] demonstrated that ROF inhibits pancreatic cancer via the AKT/JNK signaling pathway.

*Lonicerae Japonicae Flos* is frequently used to treat PF in traditional Chinese medicine [12], with its primary components being flavonoids, chlorogenic acid, and isochlorogenic acid. ROF is one of the active components found in *Lonicerae Japonicae Flos* [13–15].However, it has not been investigated whether ROF can alleviate PF. In this study, we induced fibrosis in SD rats using bleomycin (BLM) and established an A549 cells injury model to investigate the effects of ROF on PF both in vivo and in vitro.

## Materials and methods

### Chemicals

ROF (DY0053, purity > 99%) was purchased from Chengdu Lemeitian Medicine Technology Company (Chengdu, China). BLM was obtained from CSNpharm (CSN10472). Pifenidone (PFD) was purchased from Dalian Meilun Biotechnology Co., Ltd. (MB1603). Masson tricolor dye solution was purchased from Beijing Solaibao Technology Co., Ltd. (G1340).

### BLM-induced PF rat model

SD rats (250–280 g, lot: 00314136) were purchased from Beijing Wei Tong Li Hua. The experimental animals were randomized into five groups, each consisting of eight rats: control (CON), Model (MOD), high dose of ROF (HROF), low dose of ROF (LROF), and Pirfenidone (PFD) groups. To induce PF in rats, all groups except the CON group received intratracheal BLM (4.5 mg/kg). The CON and MOD groups received physiological saline daily. The HROF and LROF groups were administered 20 mg/kg and 10 mg/kg of ROF, respectively, dissolved in 0.25% carboxymethylcellulose sodium (i.g.). The PFD group received 5 mg/kg of PFD. Each rat was intragastrically administered once daily for 28 days.

### Lung coefficient

After modeling the SD rats and 28 days of drug administration, the entire lung from each group was removed. The water from the lung tissue was filtered using filter paper, and the lungs were then photographed and weighed. The lung coefficient (wet weight/body weight×100%) was calculated for each group.

### Histology

Rat lung tissue was fixed in 4% paraformaldehyde (Boster, China) for 48 h, followed by dehydration and embedding. Sections were cut at a thickness of 4 μm and stained with hematoxylin-eosin (HE). The tissue sections were then examined under light microscopy.

Grading was performed using the modified Ashcroft method (grades 0–8). Under the same magnification (20 × 10), three fields of view were randomly selected in the H&E-stained sections of lung tissue from each group (avoiding bronchi and large and medium-sized vessels) to assess the degree of PF. The grading scoring criteria are shown in Tables 1, 2.

### Masson staining

Masson's trichrome staining kits were purchased from Solarbio Technology Co., Ltd., Beijing, China (Catalog No. G1340), and staining was performed according to the instructions. PF was assessed based on the collagen volume fraction (CVF), and the area of collagen fibers was measured. After imaging, ImageJ software was used for analysis, with blue collagen serving as the color selection standard, to calculate the integral of the pixel area of blue

**Table 1** Ashcroft score

| Fibrosis grade | Improved Ashcroft method scoring scale |
|---|---|
| 0 | Normal lung |
| 1 | Minimal fibrous, thickening of alveolar or bronchiolar walls |
| 2–3 | Moderate thickening of walls without obvious damage to lung architecture |
| 4–5 | Increased fibrosis with definite damage to lung architecture and formation of fibrous bands or small fibrous one-mass |
| 6–7 | Severe distortion of structure and large fibrous areas |
| 8 | Total fibrotic obliteration |

**Table 2** Primer sequence of genes of rats

| Genes | Primer sense(5′-3′) | Primer antisense(5′-3′) |
|---|---|---|
| GAPDH | AGTGCCAGCCTCGTCTCATA | GATGGTGATGGGTTTCCCGT |
| TGF-β | GAGCCTGAGGCCGACTACTA | CGGAGCTCTGATGTGTTGAA |
| α-SMA | GCTGTGCTATGTCGCTCTGG | TCCTTCTGCATCCTGTCAGC |

collagen fibers in the target area, thereby determining the CVF.

### ELISA

We assessed the levels of Superoxide Dismutase (SOD) in the serum of rats from each group. The ELISA kits, with kit numbers BC0175-100T/48S, were acquired from Solarbio, Beijing, China.

### Cell culture

A549 cells were obtained from Guangzhou Saiku Biology Technology Company (Guangzhou, China) and cultured in 1640 medium (Gibco BRL, Grand Island) in a humidified incubator with 95% air and 5% CO2, supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island).

### Cell viability assay

Logarithmic-phase A549 cells were plated into 96-well plates (Thermo, China), with each well containing 5000 cells. The ROF groups were treated with different concentrations of ROF (0, 3.125, 6.25, 12.5, 25, 50, 100, 200μM) dissolved in 1640 medium (Gibco BRL, Grand Island). After 24 and 48 h of incubation, the medium was discarded, and 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, USA) was added to each well for 4 h. Subsequently, 20μL of the MTT solution was added to each well and incubated continuously at 37 °C for 4 h. Finally, MTT was discarded, 150μL of DMSO was added, and the wells were shaken for 10 min before detection of the absorbance at 570 nm using an enzyme marker.

Meanwhile, we also explored the viability of A549 cells during the modeling and ROF intervention. Each plate was divided into five groups: control, BLM, LROF, MROF, and HROF. Except for the control group, each group received BLM (20 μg/mL). The LROF, MROF, and HROF groups received 25μM, 50μM, and 100μM of ROF, respectively. Subsequent procedures were the same as those described above.

**Table 3** Primer sequence of genes of A549 cell

| Genes | Primer sense(5′-3′) | Primer antisense(5′-3′) |
|---|---|---|
| GAPDH | AAGAAGGTGGTGAAGCAGG | GTCAAAGGTGGAGGAGTGG |
| N-cadherin | ATCAAGCCTGTGGGAATCCG | AGCTGTGGGGTCATTGTCAG |
| α-SMA | TAGCACCCAGCACCATGAAG | CTGCTGGAAGGTGGACAGAG |
| Vimentin | GGACTCTGATTAAGACGGTT | AGAAAGGCATTGAAAGCTG |
| IL-6 | GAGGGCTCTTCGGCAAATGT | GCCCAGTGGACAGGTTTCTG |
| IL-1β | CACCTCTCAAGCAGAGCACAG | GGGTTCCATGGTGAAGTCAAC |
| TNF-α | GCCCTGGTATGAGCCCATCT | AGTAGACCTGCCCAGACTCG |

### Cell handling

A549 cells were plated in six-well culture plates (Thermo, China). The trial included six groups: control, BLM (20 μg/mL), PFD (0.5 mg/mL), and LROF (25 μM), MROF (50 μM), and HROF (100 μM). BLM was administered to all groups except the control group. Following 24 h of stimulation, the cells were collected.

### Real-time polymerase chain reaction (RT-PCR)

RNA was extracted from lung tissues or A549 cells using TRIzol (AG, China), followed by reverse transcription using the PrimeScript™ RT reagent kit (AG11718, USA). Finally, real-time PCR detection was performed using SYBR Premix Ex Taq™ (AG11701, USA) on a ViiA7 real-time PCR instrument (Thermo Fisher Scientific).

The primer pairs detailed in Table 3 were employed, with GAPDH utilized as the internal reference gene. All primers used in this study were synthesized by Sangon Biotech.

### Western blotting assay

Proteins from lung tissue or A549 cells were extracted using RIPA buffer (CST, 9806 S) supplemented with 1% phosphatase and protease inhibitors, and the protein concentration was determined by the BCA kit (Thermo Fisher Scientific, USA). Firstly, the total protein was separated by SDS polyacrylamide gel electrophoresis and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. Thereafter, the membranes were blocked with 5%BSA in TBST for 1 h. Following this, the membranes were incubated overnight at 4 °C with the primary antibody. On the following day, the blots were incubated with the secondary antibody at room temperature for 1 h. Finally, the blots were developed using a Bio-rad imaging system (Bio-rad Biosciences, Hercules, CA, USA) to visualize the bands. The relative protein expression levels were quantified using ImageJ software in this study.

### Statistical analysis

Experimental data were analyzed using SPSS version 26.0, with results presented as the mean ± standard deviation ( $\bar{x} \pm s$), and the statistical analysis results were plotted using GraphPad Prism 5.0. When the data were normally distributed and exhibited homogeneity of variances, one-way analysis of variance (ANOVA) was employed. In the event of significant differences among groups, multiple comparisons were conducted using Tukey's test. Conversely, when the data were normally distributed but displayed heterogeneity of variances, one-way ANOVA was utilized, and multiple comparisons were performed using Scheffe's test in the presence of significant intergroup differences. Non-parametric tests were applied when the data did not conform to a normal distribution.

$P < 0.05$ was considered statistically significant.

## Result

### Effect of ROF on lung tissue damage in PF rats

BLM, a family of compounds produced by *Streptomyces verticillis*, is a commonly used inducer for the preparation of experimental PF [16]. A 28-day BLM intratracheal instillation regimen is currently accepted as a standard PF model [17]. As a result, we employed this model to explore the beneficial effect of oral ROF treatment on an experimental model of lung fibrosis. The weights of the rats were recorded every two days (Fig. 1a). In Fig. 1b, the rats in the CON group exhibited smooth, well-touchable lung surfaces with no visible lesions. In contrast, in the MOD group, the softness of the lung tissue had decreased, and there were localized hemorrhagic areas with congestion and increased lung stiffness. The appearance of the lung tissue in the ROF/PFD-treated group was significantly improved. Additionally, the lung coefficient of the rats in the MOD group significantly increased compared to that of the CON group (Fig. 1c). Conversely, when compared to the MOD group, the lung coefficient of the HROF group and the PFD group decreased.

In the HE staining (Fig. 1d), we found that lung tissue sections from the CON group showed no evidence of pulmonary fibrosis (PF), inflammatory cell infiltration in the alveolar septum, or inflammatory exudate in the bronchial and alveolar cavities. By contrast, the MOD group exhibited significant lung tissue consolidation, alveolar wall rupture and collapse, massive fibroblast proliferation, and evident infiltration of large inflammatory cells. In the HROF and PFD groups, the alveolar system was essentially unharmed, with only a small percentage of alveoli exhibiting hemorrhage and rupture, and minimal inflammatory cell infiltration. To further assess the degree of fibrosis, the modified Ashcroft scoring criteria were applied to HE-stained rat sections. As shown in Fig. 1e, the Ashcroft score of the MOD group increased significantly compared to the CON group. In comparison, the Ashcroft scores of the LROF and HROF groups decreased, and the Ashcroft score of the PFD group also decreased significantly.

### Effect of ROF on fibrosis severity in PF rats

The light blue region in Fig. 2a indicates the presence of collagen. Figure 2b illustrates the calculated area of collagen fibers. The CVF in the lung tissue of rats in the MOD group was significantly higher than that in the CON group. Rats in the LROF and HROF groups, as well as the PFD group, showed a significant decrease in the areas of collagen fibers compared to the MOD group.

In this study, we utilized RT-PCR to assess the expression levels of TGF-β and α-SMA mRNA in the lung tissues of rats from all groups. Compared to the CON group, the mRNA levels of TGF-β were elevated in the MOD group, and significantly decreased following LROF and HROF interventions (Fig. 2c). Similarly, compared to the CON group, the MOD group exhibited elevated mRNA levels of α-SMA, which significantly decreased following PFD and HROF interventions (Fig. 2d).

SOD is a marker of lipid peroxidation, and increased oxidative stress plays a crucial role in the development of PF. As shown in Fig. 2e, SOD levels were significantly reduced in the MOD group compared to the CON group. While the PFD and LROF groups exhibited an increasing trend, these changes did not reach statistical significance when compared to the MOD group. Conversely, the HROF group demonstrated substantial variations.

### Effect of ROF on the expression of N-cadherin, Smad7, and HO-1 in rats with PF

As shown in Fig. 3, the expression of Smad7 and HO-1 was found to decrease in lung tissue after BLM modeling. The expression of N-cadherin significantly increased in the MOD group. On the other hand, in the LROF, HROF and PFD groups, the expression of N-cadherin decreased, while Smad7 expression increased. Furthermore, compared with the MOD group, the PFD and HROF groups showed an increase in the expression of HO-1, although this increase was not statistically significant.

### Effect of ROF on BLM-induced A549 cells

Lung conditions were studied using highly proliferative A549 cells, also referred to as human alveolar basal epithelial cells from lung cancer. In this experiment, we established an A549 cell injury model induced by BLM. Initially, the cytotoxicity of ROF was assessed in A549 cells using the MTT assay (Fig. 4a). We found that ROF at concentrations ranging from 3.125 to 200 μM had no significant impact on A549 cell viability at 24 and 48 h. Therefore, we selected BLM concentrations of 25,50 and 100μM for subsequent interventions.

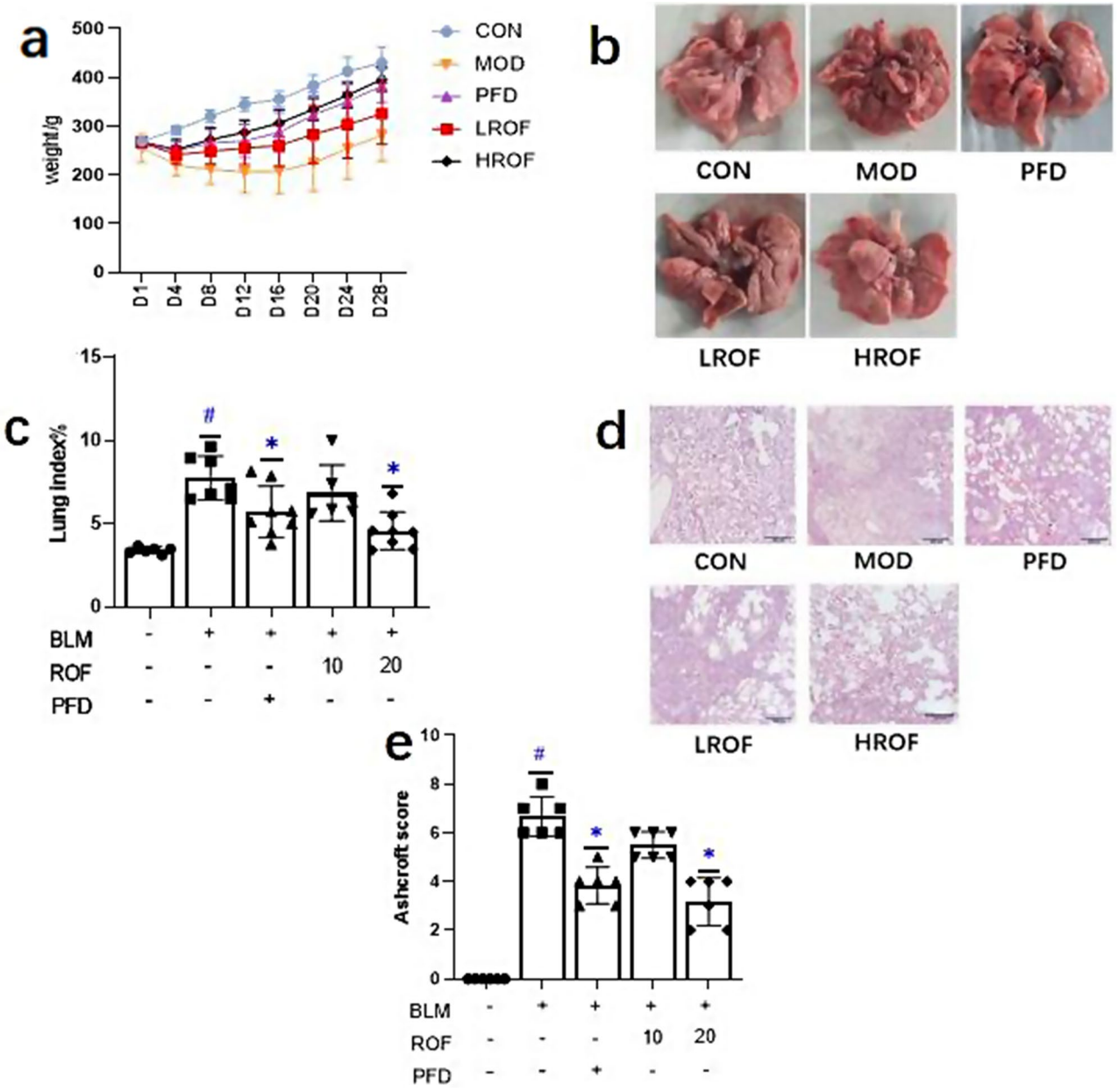


**Fig. 1** The lung damage brought on by BLM in rats can be improved with ROF: Rats were treated with BLM (4.5 mg/kg), ROF (20 and 10 mg/kg), and PFD (5 mg/kg). (**a**) Trends of body weight gain in rats were observed. (**b**) showed the appearance of rat pulmonary. (**c**) showed the rat lung coefficient. (**d**) showed the PF damage of the lung (x200). (**e**) Shows the Ashcroft scoring scale for evaluating the degree of fibrosis in H&E stained sections of rats in each group; Data are expressed as mean ± SEM ($n = 6–8$)

We found that the BLM group showed a significant decrease in cell viability compared to the normal group after 24 h. Additionally, we found that the viability of the cells in the BLM + ROF group was not substantially impacted relative to the BLM group. In addition, we found that when BLM was added for 48 h, the cell viability was significantly lower than when BLM was added for 24 h. However, when BLM intervened for 48 h, compared with the BLM group, the cell viability of the BLM + ROF group was not significantly affected (Fig. 4b).

To further examine the anti-fibrotic effects of ROF in vitro, we measured the mRNA levels of α-SMA, N-cadherin, and Vimentin. After BLM treatment, α-SMA, N-cadherin, and Vimentin mRNA expression were significantly increased. Compared with the BLM group, the mRNA expression levels of α-SMA and N-cadherin were reduced considerably in the medium-dose and HROF groups (Fig. 4c and d)). In the PFD group, the Vimentin mRNA expression levels were decreased in the low, middle and high ROF group (Fig. 4e). After BLM treatment,

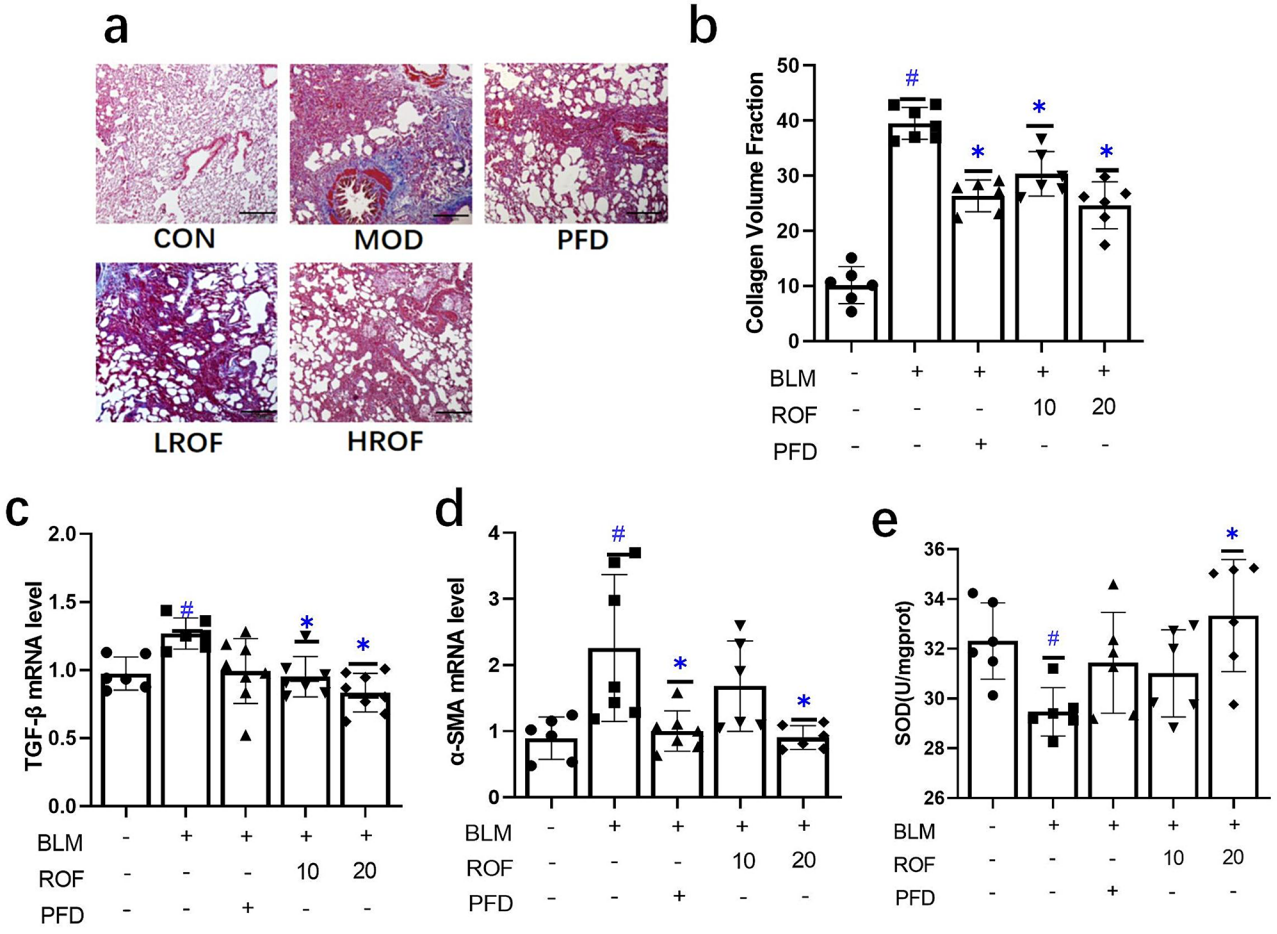


**Fig. 2** ROF can lessen the PF that BLM causes in rats: (**a**) showed the lung histopathology of rats (Masson, ×200); (**b**) The collagen volume fraction of each group was evaluated. (**c**) (**d**) Effect of ROF on the rats and production of TGF-β (**c**), α-SMA (**d**). (**e**) The vitality of SOD was determined. Data are expressed as mean ± SEM ($n$=6-8). $^{\#}P<0.05$ compared with the control group; $^{*}P<0.05$ and compared with the BLM model group

the mRNA expression levels of α-SMA, N-cadherin, and Vimentin were significantly increased. Compared to the BLM group, the mRNA expression levels of α-SMA and N-cadherin were considerably reduced in the MROF and HROF groups (Fig. 4c and d). In the PFD group, the mRNA expression levels of Vimentin were decreased in the LROF, MROF, and HROF groups (Fig. 4e).

We investigated the effects of ROF on the expression of PF-related proteins, including N-cadherin and Smad7, as well as proteins in the Nrf2/HO-1 pathway. Compared to the control group, the BLM group showed a significant increase in the protein expression of N-cadherin, while the protein expressions of Nrf2, HO-1, and Smad7 were significantly decreased. In comparison, the ROF-treated group exhibited significant trends in the protein expressions of N-cadherin, HO-1, and Smad7, and a significant difference in the protein expression of Nrf2 was observed only in the HROF group (Fig. 4).

## Discussion

Pulmonary fibrosis is closely related to inflammation. In previous studies, we found that ROF can alleviate inflammation in LPS induced A549 inflammation model, reduce the relative mRNA expression levels of IL-1β, TNF-α, IL-6, and decrease P-JAK1/JAK1, The protein expression levels of P-P65/P65 and P-IKKβ/IKKβ (see supplementary materials), in this study, we mainly explore whether ROF can improve PF.

Numerous clinical contexts can give rise to PF. Excessive accumulation of the extracellular matrix and the activation of fibroblasts and myofibroblasts are hallmarks of lung fibrosis and remodeling. The disease progresses and ultimately results in cardiopulmonary failure. The primary clinical sign is progressive dyspnea [18]. Inflammatory immunological responses, extracellular matrix deposition, epithelial-mesenchymal transition, and oxidative stress are thought to be associated with the development of PF. Currently, two medicines for PF, PFD and nidanib, have been approved for global sale, while more than a dozen others are in clinical trials. However, these

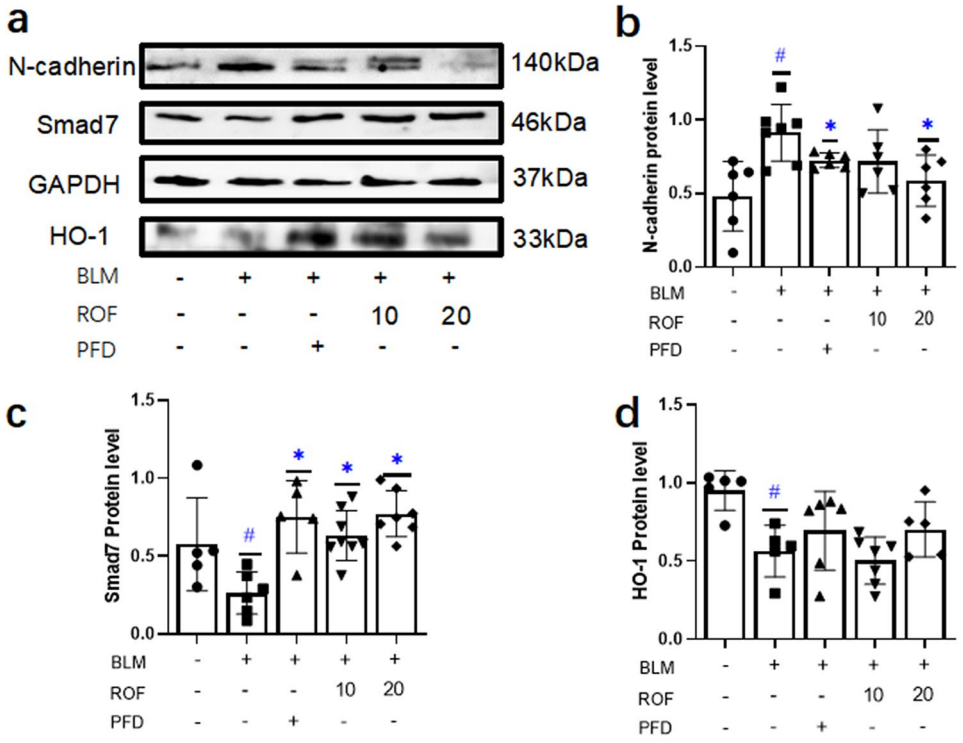


**Fig. 3** Rats with fibrosis' lung tissue have N-cadherin, Smad7, and HO-1 expression levels that ROF can control: The rats were divided into the control group, BLM (4.5 mg/Kg) model group, ROF group (10,20 mg/Kg), and PFD (5 mg/Kg) group. The expression levels of N-cadherin, Smad7, and HO-1 proteins were assessed via Western blot (**a**–**d**). Data are expressed as mean ± SEM ($n = 6$–8). $^{\#}P < 0.05$ compared with the control group; $^{*}P < 0.05$ and compared with the BLM model group

treatments have several drawbacks, including significant costs and suboptimal efficacy. Nevertheless, pharmaceutical treatments still have many side effects and are rather expensive. Because Chinese herbal therapy has low side effects, people are increasingly accepting it.

ROF, a flavonoid that is abundant in *Lonicerae Japonicae Flos*. This plant has been highly regarded in traditional Chinese medicine (TCM), receiving the highest rating in historical texts such as "A Record of Famous Doctors." *Lonicerae Japonicae Flos* contains numerous chemical components, exhibits wide-ranging pharmacological effects, and possesses great medical value. It is widely utilized in TCM for anti-PF treatment, and is among the various TCMs and their constituents used in the clinical treatment of PF [19]. For instance, in rats with BLM-induced PF, early treatment with Qingjin Bufei Decoction, which contains *Lonicerae Japonicae Flos*, can lower serum TNF-α levels and slow down the progression of the disease [20].

One of *Lonicerae Japonicae Flos* key active ingredients is ROF. To further explore the effect of ROF on PF, BLM was used to simulate the pathogenesis of PF. BLM was utilized as both an in vitro and in vivo model to study the effect of ROF on PF. A rat PF model was established in vivo, and LROF and HROF were administered. Based on behavioral observations, morphological changes in lung tissue, and rat body weight data, the anti-fibrotic effect of LROF was found to be negligible; however, HROF demonstrated a significant anti-PF effect.

The impact of ROF on cells following BLM modeling was initially investigated in vitro using A549 cells. Epithelial-mesenchymal transition (EMT)is a major contributor to lung fibrosis [21]. In vitro research has shown that ROF can reduce fibrosis by significantly reducing the relative mRNA expressions of α-SMA, N-cadherin, and Vimentin, which are key fibrosis factors. Nrf2 is a key regulator of the oxidative stress response and plays a significant role in lung fibrosis [22]. In addition, HO-1, one of the most abundant antioxidant enzymes, protects against lung fibrosis in rats [23]. This study found that ROF has a therapeutic effect on PF rats in vivo and can mitigate the degree of damage in A549 cells in vitro. Zhao et al. [24] suggests that anti-fibrosis medication could be a potential technique for slowing the progression of fibrosis in the early stages and preventing the disease from worsening in the middle and late stages. As a possible medication to treat fibrosis, ROF has some research value.

To summarize, ROF can improve the pulmonary fibrosis in rats and the A549 cell damage in vitro caused by BLM.

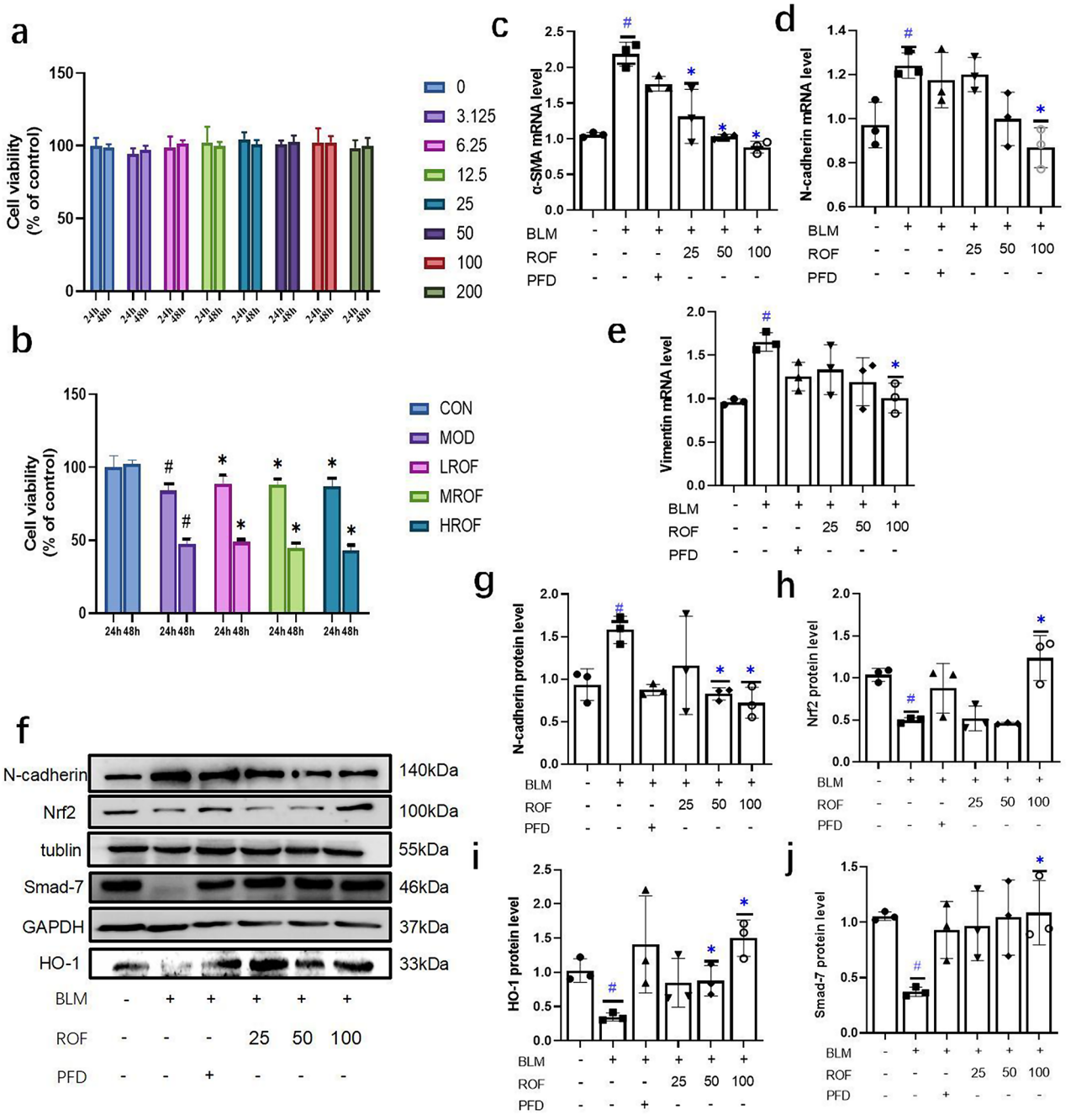


**Fig. 4** The effect of ROF on the survival of A549 cells and the expression of mRNA and protein associated with lung fibrosis following BLM modeling: Cell viability was determined by MTT (**a**, **b**) after 24–48 h of treatment with BLM (20 μg/mL). The A549 cells were divided into the group:0,3.125, 6.25,12.5,25, 50, 100,200 μM. Then, explore the expression of α-SMA (**c**), N-cadherin(**d**), and Vimentin(**e**) of BLM-induced A549 cells. Furthermore, the cells were split into control groups: BLM (20 μg/mL) model group, ROF group (25, 50, 100 μM), and PFD group. N-cadherin, Nrf2, HO-1, and Smad7 protein expression were assessed by Western blot (**f**-**j**). Data are expressed as mean ± SEM ($n = 3$). # $P < 0.05$ compared with the control group; *$P < 0.05$ and compared with the BLM model group

## Conclusion

In conclusion, ROF possesses anti-fibrosis properties and may be developed into an effective drug for treating PF. This paper suggests that the amelioration of PF by ROF may be related to the influence of the Nrf2/HO-1 signaling pathway. In the follow-up study, we will further clarify the signaling mechanism of ROF by using related inhibitors.

### Abbreviations

| | |
|---|---|
| PF | Pulmonary fibrosis |
| ROF | Rhoifolin |
| BLM | Bleomycin |

| | |
|---|---|
| SD | Sprague-Dawley |
| SOD | Superoxide dismutase |
| Smad7 | SMAD Family Member 7 |
| HO | 1-Heme Oxygenase-1 |
| Nrf2 | Nuclear factor erythroid 2-related factor 2 |
| ECM | Extracellular matrix |
| PFD | Pirfenidone |
| HROF | High dose of ROF |
| LROF | Low dose of ROF |
| HE | Hematoxylin-eosin |
| CVF | Collagen volume fraction |
| EMT | Epithelial-mesenchymal transition |
| TBST | Tris-Buffered Saline |
| BSA | Bovine Serum Albumin |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-025-03526-y .

Supplementary Material 1

Supplementary Material 2

### Author contributions

XW, QW, and PZ are in charge of the experiment. JZ, HS, and FL are in order of the data analysis, and J W is in order of the experimental technology support. FX, LL, and LH gave guidance on the practical direction.

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### Data availability

The datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

This animal experiment was reviewed and approved by the Animal Care and Use Committee of Guangzhou University of Traditional Chinese Medicine (2020058).

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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