# Yifei Sanjie Formula Treats Chronic Obstructive Pulmonary Disease by Regulating Lung Microbiota Dysbiosis

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**Research**

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# Abstract

Background

Chronic obstructive pulmonary disease (COPD) is one of the most common pulmonary diseases. There is evidence to suggest that dysbiosis of pulmonary microbiota participates in COPD development. Yifei Sanjie Formula (YS) is widely used to treat diseases in respiratory systems, yet its mechanisms are little known.

Methods

In the present study, the efficacy of YS was evaluated by analyzing its effects on the severity of pulmonary pathological damage, pulmonary function, pro-inflammation cytokines, the activation of NLRP3/caspase-1/IL-1β signaling pathway, and changes of lung microbiota.

Results

YS improved animal behaviors, prevented declines in pulmonary ventilatory function and lung injury in a rat model of COPD. Administration of YS significantly suppressed the release of proinflammatory cytokines and collagen deposition and downregulated NLRP3/caspase-1/IL-1β signaling in vivo. YS changed the relative abundance of specific pulmonary microbiota and modulated bacterial flora in the rat model.

Conclusions

These results suggest that the effects of YS involved lung microbes and anti-inflammatory mechanisms.


# 1. Introduction

Respiratory symptoms and airflow limitation characterize chronic obstructive pulmonary disease (COPD); it has become the third most common life-threatening disease worldwide[1]. The pathogenesis of COPD includes inflammation, oxidative stress, and protease/antiprotease imbalance. Regulation of the pulmonary inflammatory microenvironment to remove the pathological products produced by the inflammatory response might reduce the level of inflammation, restoring the balance of the lung microenvironment and delaying the development of COPD[2]. Nod-like receptor protein 3(NLRP3) is a potent inducer of inflammation that, when overactive, may be targeted therapeutically in inflammatory lung diseases[3]. Studies[4] showed that acute exacerbations of COPD are related to NLRP3 activation, and its activation may be involved in initiating the inflammatory response by binding to cytoplasmic pathogen-associated molecular patterns or damage-associated molecular patterns.

Bacteria in the respiratory tract resist colonization by foreign pathogens. Cigarette smoking alters the composition of the lung microbiota[5]. Lung microbiota dysbiosis is related to inflammation, pathological airway alterations, immune responses, and the aggravation of clinical symptoms in patients with

COPD[6]. Pathogens stimulate inflammatory cells to produce inflammatory media that often destroy the immune function of the airway and mucosa, leading to chronic inflammation and lung microbiota dysbiosis, further aggravating COPD[7]. Our understanding of pulmonary microorganisms is limited, and many questions remain. How does the lung microbiota dysbiosis in COPD patients, and how do these changes affect disease development? How does the NLRP3 signal pathway interact with lung microbiota dysbiosis?

A great deal of attention has been paid to the development of Chinese herbal medicines to treat COPD[8]. Yifei Sanjie Formula (YS), a traditional Chinese medicine, comprises eight medicinal herbs and has been shown to possess extensive pharmacological effects against COPD, including reduction of lung injury, inflammatory responses, and pulmonary fibrosis in a COPD animal model. Nevertheless, the pharmacological mechanisms of action of YS remain poorly understood and warrant further investigation[9-10]. Therefore, we determined whether the regulation of lung microbiota by YS would alleviate lung injury, ventilatory function, inflammatory reactions, and collagen deposition in a rat model of COPD. We aimed to provide theoretical support for the efficacy of YS in correcting lung microbiota dysbiosis (Fig.

# 2. Material And Methods

### 2.1 Groups and treatments

According to the “Guidelines for the Care and Use of Laboratory Animals” published by the National Institutes of Health, all rats experiments were approved by the Animal Ethics Experiment Committee of Yunnan University of Traditional Chinese Medicine and were conducted by the guidelines of the committee. SPF Wistar rats (n=18; 200±20g; male) were provided by Chengdu Dashuo Laboratory Animal Co., Ltd.(Chengdu, China; license no. SCXK(Chuan)-2015-030). The rats were randomly divided into Control (CT), COPD, COPD+YS. Except for CT group, the rat of the COPD model was replicated by using cigarette smoke exposure (Hongyun cigarette, Tar11mg, Nicotine1.1mg, CO12mg) combined with airway instillation of lipopolysaccharide(LPS, Sigma)[11]. 11.6g·kg$^{-1}$·d$^{-1}$ of YS was administered daily by intragastric administration from day 57 to day 84(The same dosage as is used clinically). Animal weight and behaviorism were monitored regularly and recorded. The composition of YS is shown in Table 1.

Table 1 Prescription of YS

| NO. | Chinese Pinyin name | Latin name |
|---|---|---|
| 1 | Huangqi | *Hedysarum Multijugum Maxim.* |
| 2 | Baizhu | *Atractylodes Macrocephala Koidz.* |
| 3 | Fangfeng | *Saposhnikoviae Radix* |
| 4 | Jiezi | *Sinapis Semen* |
| 5 | Zhebeimu | *Fritillariae Thunbrgii Bulbus* |
| 6 | Sangbaipi | *Mori Cortex* |
| 7 | Ezhu | *Curcumae Rhizoma* |
| 8 | Sanqi | *Panax Notoginseng (Burk.) F. H. Chen Ex C. Chow* |

### 2.2 Pulmonary function testing

Pulmonary function testing was performed with Whole-body Plethysmograph（EMMS，ALton，Hants， UK） after the experiment is over(12 weeks). According to the Whole-body Plethysmograph, data for EF50(Expiratory Flow at 50%), Tidal Volume (TV), and Minute Volume (MV) were collected.

### 2.3 Histological evaluation

The upper lobe of the right lung of the rat was fixed with 4% formalin for 48 hours and then embedded in paraffin, stained with hematoxylin and eosin (H&E) and Masson, and finally evaluated histopathologically under an optical microscope (OLYMPUS VS200, magnification×200).

### 2.4 Western blotting

Protein was extracted from lung tissue, protein quantification was performed using the BCA protein quantification kit (P0012, Beyotime, Jiangsu, China) to calculate loading capacity, samples were added to a precast SDS polyacrylamide gel for electrophoretic separation, and proteins in the gel were electrotransferred to a PVDF membrane using water bath electroblotting. the primary antibody was added, which was anti-TGF-β1 (rabbit polyclonal antibody, Abcam, ab179695), anti-NLRP3 (rabbit polyclonal antibody, Bioss, bs-10021R)，overnight at 4℃; after washing the membrane added secondary antibody, shaker at 4℃, incubated for 1h; PVDF was dropped ECL luminescence liquid, developed, photographed (GeneGnome). Using gene tools software, the grayscale values of individual bands were analyzed.

### 2.5 Enzyme-linked immunosorbent assay

ELISA kit (Jiangsu Enzyme-Linked Biotechnology Co., Ltd.) was used to detect the concentration of TNF-α, IL-6 in serum as well as the concentration of IL-1β, IL-18 and SIgA in the lung. The method and procedure were completed according to the kit operating instructions. Finally, read the absorbance at 450nm after adding stop solution and within 15min.

### 2.6 16S rRNA gene high-throughput sequencing to detect the lung microbiota

Total genome DNA from samples was extracted using CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water.16S rRNA genes of distinct regions(16S V4/16S V3/16S V3-V4) were amplified used a specific primer(16S V4:515F-806R) with the barcode. PCR products were mixed in equidensity ratios. Then, mixture PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and AgilentBioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

### 2.7 Statistical analysis

Data were expressed as means ± standard deviation(`x ± s). The normal distribution of the data was assessed with the Shapiro-Wilk test. Significant differences in the variance of parameters were evaluated either with ANOVA or Kruskal-Wallis test, depending on the data normality distribution. All statistical tests were two-sided, and a *P* value of <0.05, or FDR adjusted *P*adj value < 0.05 was considered as statistically significant.

# 3. Results

### 1. YS improves pulmonary function in COPD rats

Pulmonary function is an important index in evaluating COPD, including TV, MV and EF50, reflecting small airway obstruction. Studies[12] found that COPD patients showed decreased TV and MV. We found that EF50, TV and MV were significantly lower in COPD rats ($P < 0.05$), while EF50 ($P < 0.05$), TV and MV ($P > 0.05$) were higher after YS intervention (Fig. 1C-1E). COPD rats displayed hair withering, slow action, and shortness of breath. There were significant improvements in animal behaviors after the YS intervention. The body weights of COPD rats significantly decreased ($P < 0.05$). Weights in the YS+COPD group increased ($P > 0.05$) (Fig. 1B). These findings suggest that YS improves animal behaviors and pulmonary ventilatory function in COPD rats.

### 2. YS improved immune function in COPD rats

The spleen and thymus are critical immune organs, and their organ indexes reflect the strength of immune function to a certain extent [13]. Results show that thymus index and spleen index were decreased significantly in COPD rats ($P > 0.05$); after YS intervention, thymus index and spleen index were increased ($P > 0.05$) (Fig. 2A-2B). SIgA is a major effector molecule of the mucosal immune defense system against the colonization and adhesion of pathogenic microorganisms on mucosal surfaces[14]. The amount of SIgA in the sputum and alveolar lavage fluid of patients with COPD was significantly lower than in the control group. In the present study, we have found that SIgA was decreased in COPD rats ($P > 0.05$), while the SIgA of the COPD+YS group was significantly increased ($P > 0.05$) (Fig. 2C). All

these suggested that YS could enhance the local mucosal immunity and improve immune function in COPD rats.

### 3.YS relieves inflammation in COPD rats

The inflammatory response of COPD primarily involves neutrophil infiltration. TNF-α activates neutrophils, and IL-6 inhibits apoptosis [15]. HE staining showed that substantial amounts of neutrophil infiltration plugged the bronchi. The lumens were significantly narrowed, the alveolar walls become thinner and fused in COPD rats. There were fewer of these pathological changes in the YS intervention group (Fig. 3A). Levels of TNF-α and IL-6 were significantly higher in the serum of COPD rats ($P > 0.05$) with YS intervention (Fig. 3C-3D). TGF-β1 is a powerful profibrotic cytokine that participates in inflammatory repair [16]. Masson staining showed that much collagen fiber deposition occurred around the trachea and pulmonary interstitium in COPD rats. Collagen deposition was less severe in YS rats (Fig. 3B). TGF-β1 protein expression was significantly elevated in lung tissue of COPD rats ($P > 0.05$). TGF-β1 levels in the COPD+YS group were significantly lower ($P > 0.05$) (Fig. 3F). These findings suggest that YS ameliorates lung tissue damage, attenuates inflammatory responses, and reduces collagen deposition in COPD rats.

### 4.YS reduces NLRP3/caspase-1/IL-1β signaling expression in COPD rats

NLRP3 promotes the release of downstream inflammatory cytokines, inducing acute and chronic inflammatory responses. When NLRP3 is overactivated, ASC acts as an adaptor protein to recruit the precursor caspase-1, promoting IL-1β and IL-18 secreted outside the cell, exerting proinflammatory effects[17]. Compared with Control, NLRP3 protein expression was significantly greater in lung tissues of COPD rats ($P > 0.05$). Caspase-1 and ASC were significantly increased in lung tissues ($P > 0.05$), and IL-1β and IL-18 levels were significantly higher in serum ($P > 0.05$). In the YS group, NLRP3 protein expression was significantly lower in lung tissue ($P > 0.05$), caspase-1 and ASC levels were significantly lower in lung tissue ($P > 0.05$), and serum IL-18 levels were significantly lower ($P > 0.05$). These findings suggest that YS inhibits the NLRP3/caspase-1/IL-1β signaling pathway (Fig. 4A-4E), alleviating the inflammatory response.

### 5. Effect of YS on lung microbiota in COPD rats

The lung microbiota is strongly associated with COPD. To explore the mechanisms, 16s rRNA high-throughput sequencing was employed to analyze the lung microbiota. The chao1 index reflects the relative abundance of flora, and the Shannon index reflects the diversity of flora. The results are represented in Figure 5A. Compared with the CT group, the relative abundance of flora in the COPD group decreased. After YS intervention, the relative abundance of the flora showed no significant change. Compared with the CT group, the diversity of the flora in the COPD group was significantly decreased ($P > 0.05$). The diversity of the flora was significantly greater after YS intervention ($P > 0.05$).

On the phylum level, the lung flora of rats in each group was dominated by *Proteobacteria*, *Firmicutes*, and *Bacteroidota*. Compared with the CT group, the COPD group showed an increased relative abundance

of *Proteobacteria* and *Firmicutes,* with a decreased relative abundance of *Bacteroidota*; after YS intervention, the relative abundance of *Bacteroidota* increased those of *Proteobacteria* and *Firmicutes* decreased (Fig. 5B). At the genus level, the lung flora of rats in each group was dominated by *Ralstonia, Mycoplasma, Halomonas, Lactobacillus, Dizetzia,* and *Bacteroides.* Compared with the CT group, the COPD group showed an increased relative abundance of *Ralstonia*, *Mycoplasma*, *Halomonas*, and *Dizetzia,* while there was a decreased relative abundance of *Lactobacillus* and *Bacteroides*. The relative abundance of *Halomonas, Lactobacillus, Dizetzia*, and *Bacteroides* were increased, and there was a lower relative abundance of *Ralstonia* and *Mycoplasma* after YS intervention (Fig. 5C).

Linear discriminant analysis of effect size shows the iconic microorganisms in each group that contributed significantly to differences in microbial structure. As shown in Figure 5D, *Mycoplasma* was more abundant in the gut microbiota of the COPD group. *Mycoplasma* was significantly increased in COPD patients, while *Halomonas*, *Dietzia*, and *Nesterenkonia* were enriched after YS intervention. These results suggest that YS changes the relative abundance of specific bacteria and modulates the bacterial flora in COPD rats.

### 6. Environmental factor correlation analysis

Correlation heatmap plots were used to assess the top 20 species at the genus level and correlations with EF50, MV, TV, SIgA, TNF-α, IL-6, TGF-β1, NLRP3, caspase1, ASC, IL-1β, and IL-18.

As shown in the Figure 6, *Mycoplasma* positively correlated with IL-1β ($P < 0.05$) and TNF-α ($P < 0.05$), and negatively correlated with MV ($P < 0.001$), TV ($P < 0.001$) and EF50 ($P < 0.001$). *Bacteroides* positively correlated with EF50 ($P < 0.001$) and negatively correlated with TNF-α ($P < 0.001$), IL-6 ($P < 0.001$), TGF-β1 ($P < 0.05$), caspase1 ($P < 0.001$), ASC ($P < 0.05$) and IL-18 ($P < 0.001$). *Halomonas* positively correlated with SigA ($P < 0.001$), and negatively correlated with IL-6 ($P < 0.05$) and TGF-β1 ($P < 0.001$). *Nesterenkonia* positively correlated with IL-6 ($P < 0.001$), caspase 1 ($P < 0.001$), and ASC ($P < 0.001$) and negatively correlated with TV ($P < 0.05$). *Parabacteroides*negatively correlated with IL-6 ($P < 0.001$), caspase1 ($P < 0.001$), and ASC ($P < 0.001$). In summary, we presumed that inflammatory and immune indicators were the most closely related to this study.

# Discussion

Airway inflammation and remodeling are the primary pathological features of COPD and are causes of airflow limitation and remodeling[18]. Smoking cessation, bronchodilators, and hormones have not been effective in halting the progressive deterioration of pulmonary function and the progression of the disease in patients with COPD, although they may improve symptoms to some extent[19]. Traditional Chinese medicine is characterized by the effects of multiple components, targets, and pathways, and these are used to treat COPD[20]. YS preserves the pathological morphology of lung tissue and reduces collagen deposition. It also promotes the expression of SIgA to strengthen local mucosal immunity and regulate the inflammatory microenvironment in COPD rats. Pharmacological studies[21] suggested that

astragalus polysaccharide regulates the inflammatory response and exerts anti-inflammatory effects by inhibiting the TLR4/NF-κB pathway. Huangqi-Fangfeng protected against allergic airway remodeling by inhibiting the epithelial-mesenchymal transition process in mice via regulating epithelial-derived TGF-β1[22]. Total flavonoids of cortex mori reduced airway inflammation and had a significant effect on asthma in mice[23]. Sinapine acted as an antiasthmatic by dilating airway smooth muscle and increasing lung and tracheal volumes[24]. Quercetin-3-O-β-D-glucopyranosyl-7-O-β-D-gentiobioside reduced apoptosis in lung tissues, repaired damaged tissue, and maintained the integrity of the organ[25]. In summary, the components of YS relieve COPD by improving autoimmune function, alleviating inflammatory response, inhibiting apoptosis, and reducing collagen deposition. In the present study, the COPD group was reproduced by two intratracheal injections of LPS combined with cigarette smoke. A substantial amount of neutrophil infiltration in the lung tissue of COPD rats plugged the bronchi. The alveolar walls were thinned and fused. The lung interstitium displayed substantial inflammatory cell infiltration and collagen fiber deposition around the trachea and pulmonary interstitium.

TGF-β1 is a profibrotic cytokine that stimulates collagen deposition and aggravates local inflammatory responses[26]. Its expression is elevated in acute and chronic lung diseases. Our previous study[27] found that the Yuping Fengsan Jiawei formula downregulated TGF-β1 levels, suggesting that this is an intervention that prevents airway remodeling. In the present study, expression levels of TGF-β1, TNF-α, and IL-6 were reduced in COPD rats after YS intervention. These findings suggest that YS ameliorates the pathological changes of lung tissue, reduces collagen deposition, and attenuates inflammatory responses in COPD rats.

The NLRP3 inflammasome is involved in developing diseases by promoting the release of downstream inflammatory cytokines, inducing acute and chronic inflammatory responses[28]. It comprises NLRP3, apoptosis-associated speck-like protein containing a CARDI(ASC), and cysteine-requiring aspartate protease-1 (caspase-1)[29]. In response to endogenous or exogenous stimuli, NLRP3 is activated, and ASC acts as an adaptor protein to recruit the precursor caspase-1, which has an enzymatic activity after cleaving pro-interleukin-1β and IL-18 precursor, producing mature IL-1β and IL-18, exerting proinflammatory effects[30]. In the present study, we found that levels of NLRP3, caspase-1, ASC, IL-18, and IL-1β were decreased.

Respiratory mucosal surfaces are colonized by a community of commensal bacteria and are also the primary site of entry for pathogenic agents[31]. Mucosal B cells release large amounts of immunoglobulin (Ig) molecules via both follicular and extrafollicular routes to prevent microbial invasion[32]. IgA and IgG are the most abundant antibodies in mucosal secretions, and SIgA is the predominant antibody secreted by the respiratory mucosa. When SIgA levels secreted in the respiratory mucosa are reduced, the organism has a greater probability of developing an upper respiratory tract infection, and SIgA levels directly correlate with the organism's ability to fight an upper respiratory tract infection. We found that SIgA levels were significantly decreased in lung. These findings suggest YS decreases NLRP3/caspase-1/

IL-1β signaling pathway expression, alleviates inflammation, and enhances local mucosal immune function in the lungs of COPD rats.

Bacterial colonization has been found in the lower respiratory tract of patients with stable COPD[33]. Analysis of bronchoalveolar lavage fluid from patients with COPD revealed that airway bacterial load is associated with inflammatory factors. Studies found that cigarette smoke and inhalation of contaminated air can affect the composition of the lung microbiota[34]. When the mucosal immune system is defective, bacteria can cross the epithelial barrier and stimulate inflammatory responses. Typically, this response is a feature of the protective immune defense response. Persistent airway inflammatory response damages normal lung tissue and defense functions. Reduced defense function allows other infections and inflammatory reactions, creating an infection-inflammation-injury vicious cycle. We observed lower microbial alpha diversity in the COPD group than in the Control group. Lung microbial alpha diversity of mice in the COPD+YS groups differed from that of the COPD group. Analysis of flora diversity showed that YS prevented lung microbiota dysbiosis in COPD rats. Consistent with this finding, linear discriminant analysis of effect size showed different lung microbial compositions in these groups. *Mycoplasma* belongs to *Firmicutes*, which accelerates disease processes by promoting chronic inflammation[35]. *Halomonas* are gram-positive bacteria. Various oligotypes of *Christensenella* and *Clostridium* were detected in lung tissue and a few oligotypes of *Halomonas* in both airway fluid and lung tissue from pulmonary fibrosis and lung cancer patients, suggesting the involvement of these microbial communities in fibrotic diseases[36]. *Nesterenkonia* belongs to *Actinobacteria* and *Lactobacillaceae*. The microbiome might play a significant role in maintaining normal lung function, including structural and immune barriers[38]. These findings suggest that, in COPD treatment, the relative abundance of *Halomonas* and *Nesterenkonia* are increased by decreasing the relative abundance of *Mycoplasma*, which might inhibit the inflammatory factors IL-1β, TNF-α, IL-6, IL-1β, and TGF-β1, increasing SIgA expression, enhancing immunity, alleviating collagen deposition, and improving lung function.

### Summary and prospects

What role does the lung microbiota dysbiosis play in COPD formation? We suggest that lung microbiota dysbiosis promotes the progression of COPD. The colonization of pathogens in the lower respiratory tract due to mucociliary clearance dysfunction caused by inhalation of smoke particles impairs mucociliary clearance, which leads to increased mucus production, airway epithelial damage, downregulation of IgA levels, and disrupts phagocytic function. Bacteria-related products and bacterial-induced epithelial damage impair host immunity, further allowing microbes to enter the lower respiratory tract and ultimately leading to persistent chronic inflammation and microbial colonization of the lungs. Ultimately, this generates a vicious cycle. In conclusion, we found altered lung microbiota in COPD rats, and the imbalance of lung microbiota was associated with lung inflammation. The mechanism of action by which YS prevents this process is not fully elucidated and should be studied in the future.

## Conclusion

These results suggest that the effects of YS involved lung microbes and anti-inflammatory mechanisms.

# Abbreviations

COPD: Chronic obstructive pulmonary disease; YS: Yifei Sanjie Formula; SIgA: secretory immunoglobulin A; IL-1β: Interleukin-1β; TNF-α: tumor necrosis factor-α; IL-6: Interleukin-6; IL-8：Interleukin-8; TGF-β1： transforming growth factor-β1; ASC: Apoptosis-Associated Speck-Like Protein Containing A CARD; NLRP3: Nod-like receptor protein 3; TV: Tidal Volume; MV: Minute Volume; EF50: Expiratory Flow at 50%.

# Declarations

## Ethics approval and consent to participate

The experimental protocol was approved by the Experimental Animal Care and Ethics Committees of Yunnan University of Chinese Medicine (Approval No.: R-06202023). Consent for participation was not applicable to this study because it does not involve the use of any human data.


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## Author contributions

Author contributions were as follows: study design (Zhongshan Yang and Jiali Yuan), data collection (Yueying Wu), statistical analysis (Yueying Wu and Bo Qiao), data interpretation (Yueying Wu and Bo Qiao), manuscript preparation (Qiang Zhang, Wenqing Jia, Hui Meng, Haijing Xing, Yuqing Li), and funds collection (Zhongshan Yang, Jiali Yuan and Yueying Wu).

## Availability of data and materials

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

## Competing interests

The authors declare that there is no confict of interests regarding the publication of this paper.

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### Consent for publication

Not applicable

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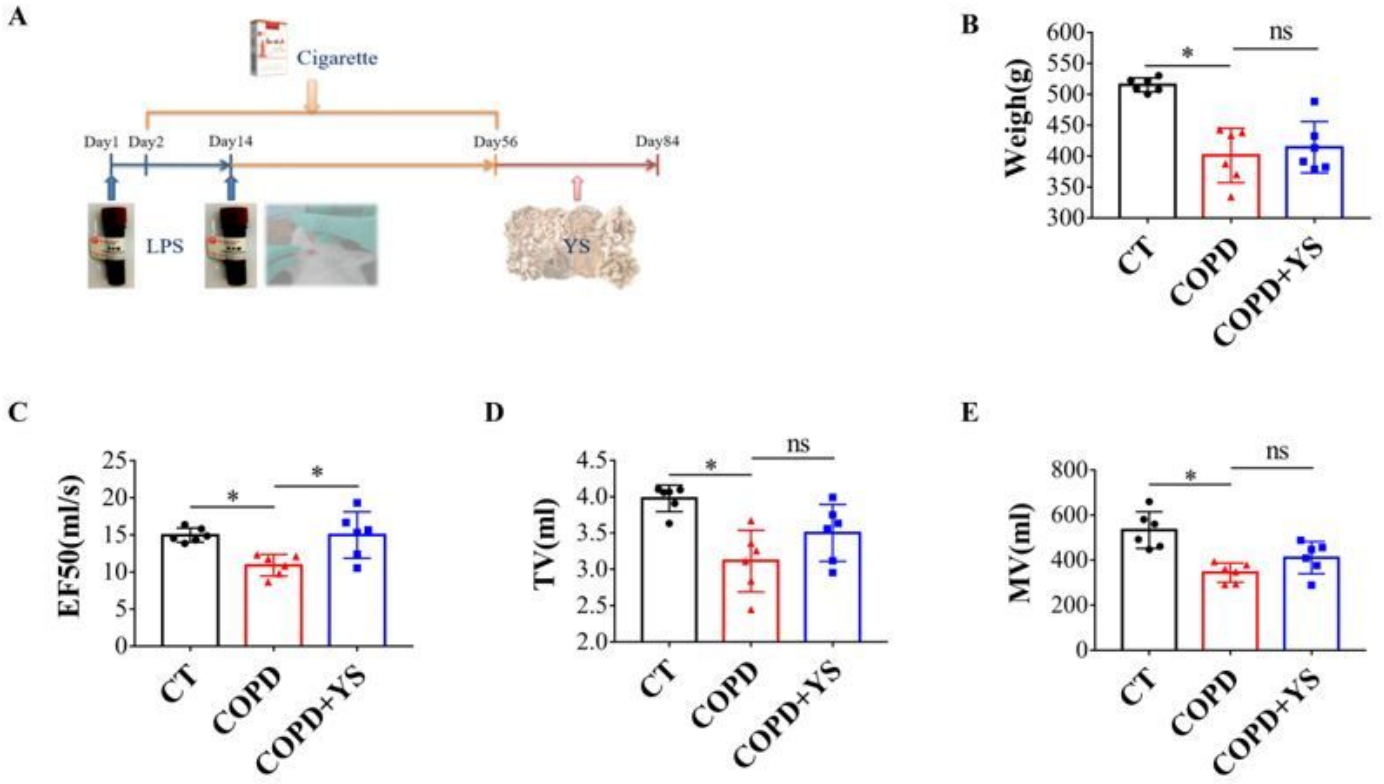
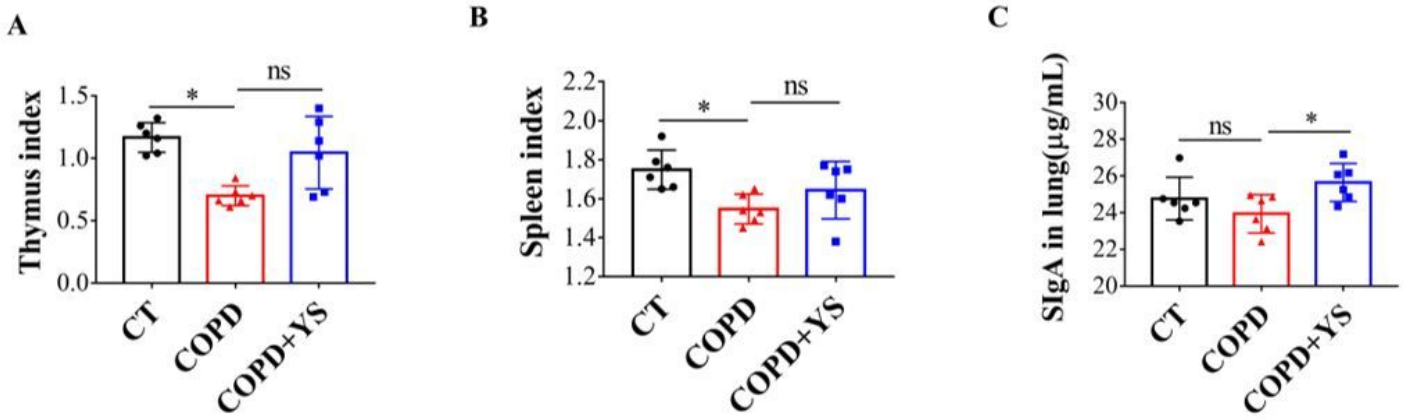
# Figures



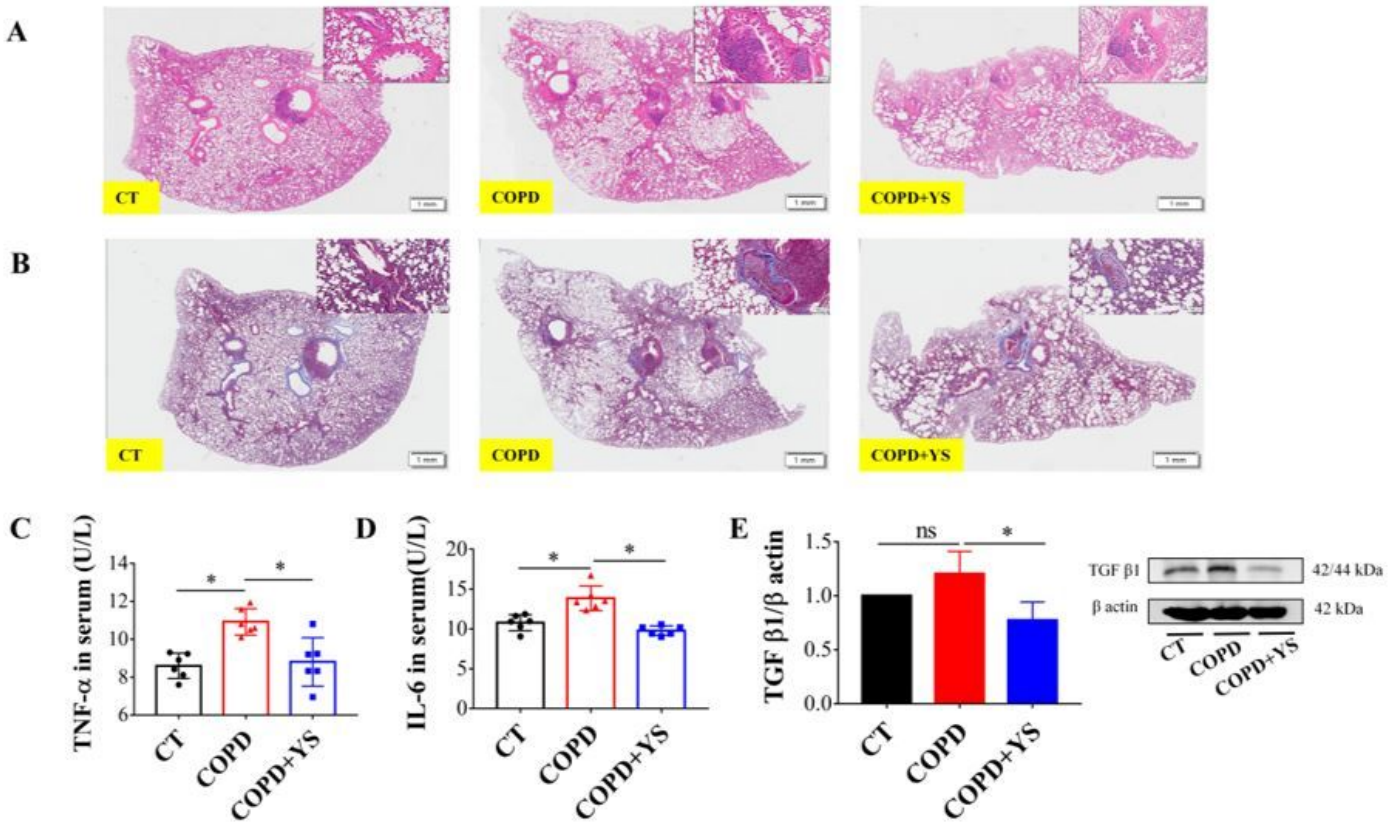

## Figure 1

(A) Experimental design and general conditions of the animals. (B) Weights in the three groups. (C) EF50 of the three groups. (D) TVs of the three groups. (E) MVs of the three groups. Datas are expressed as means ± SEM of three independent experiments (ns non-significant, *P < 0.05).



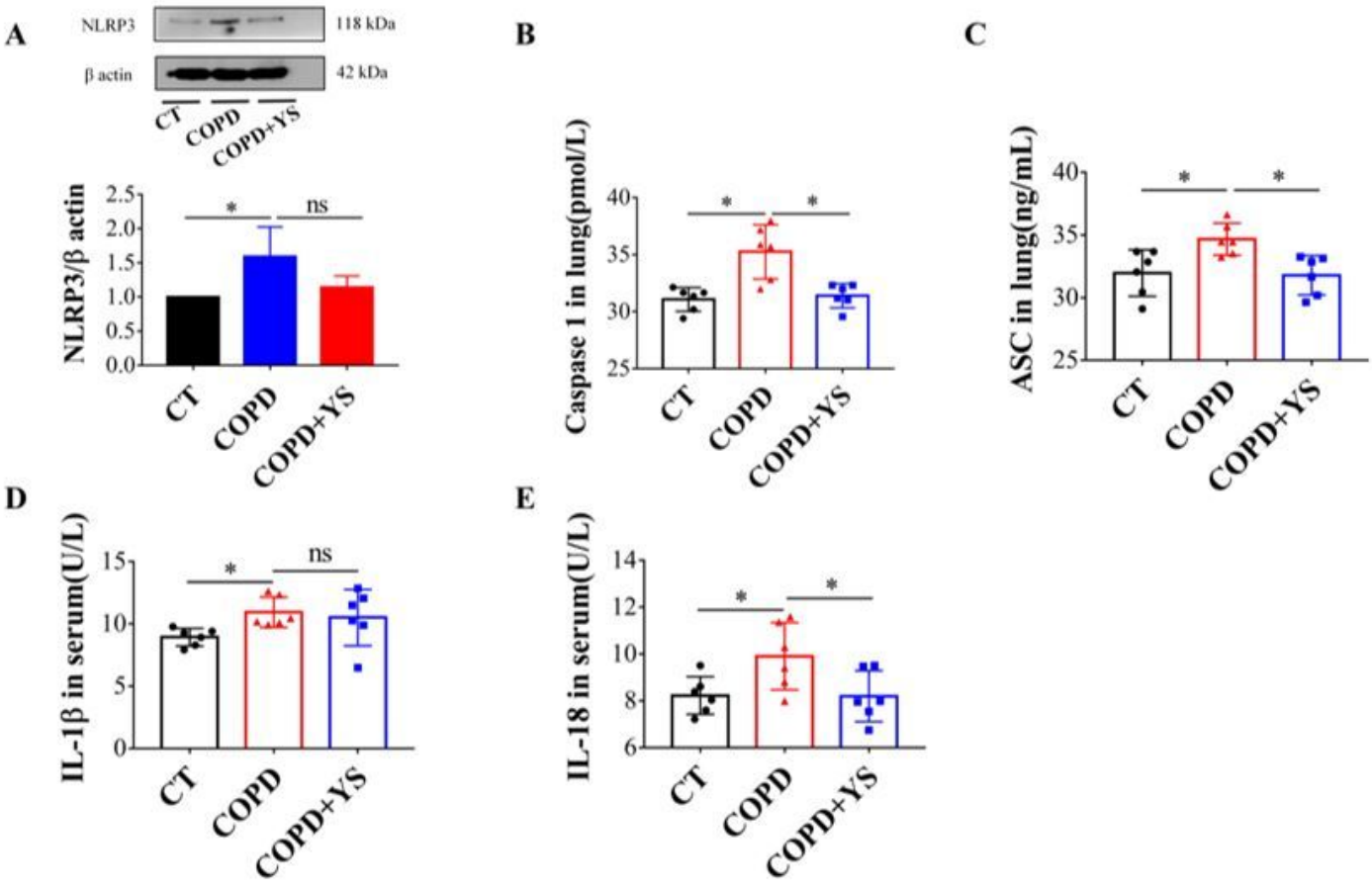

## Figure 2

(A) Thymus index of three groups. (B) Spleen index of three groups. (C) The expression of SIgA in lung. Dates are expressed as means ± SEM of three independent experiments (ns non-significant, *P < 0.05).



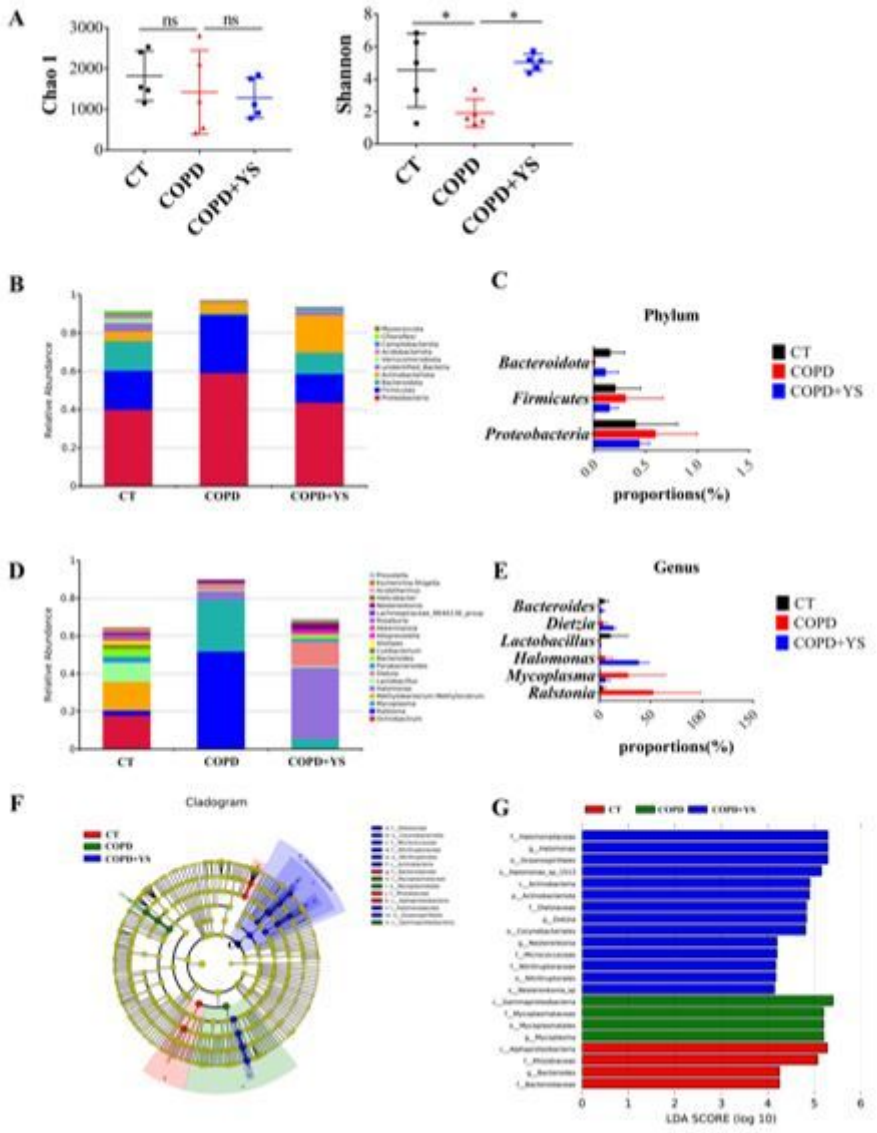

## Figure 3

(A) HE staining of lung tissues from each group (×200). (B) Masson staining of lung tissues from each group (×200). (C) TNF-α in serum. (D) IL-6 in serum. (E) The relative expression of TGF-β1 in lung. Dates are expressed as means ± SEM of three independent experiments (ns non-significant, *P < 0.05).



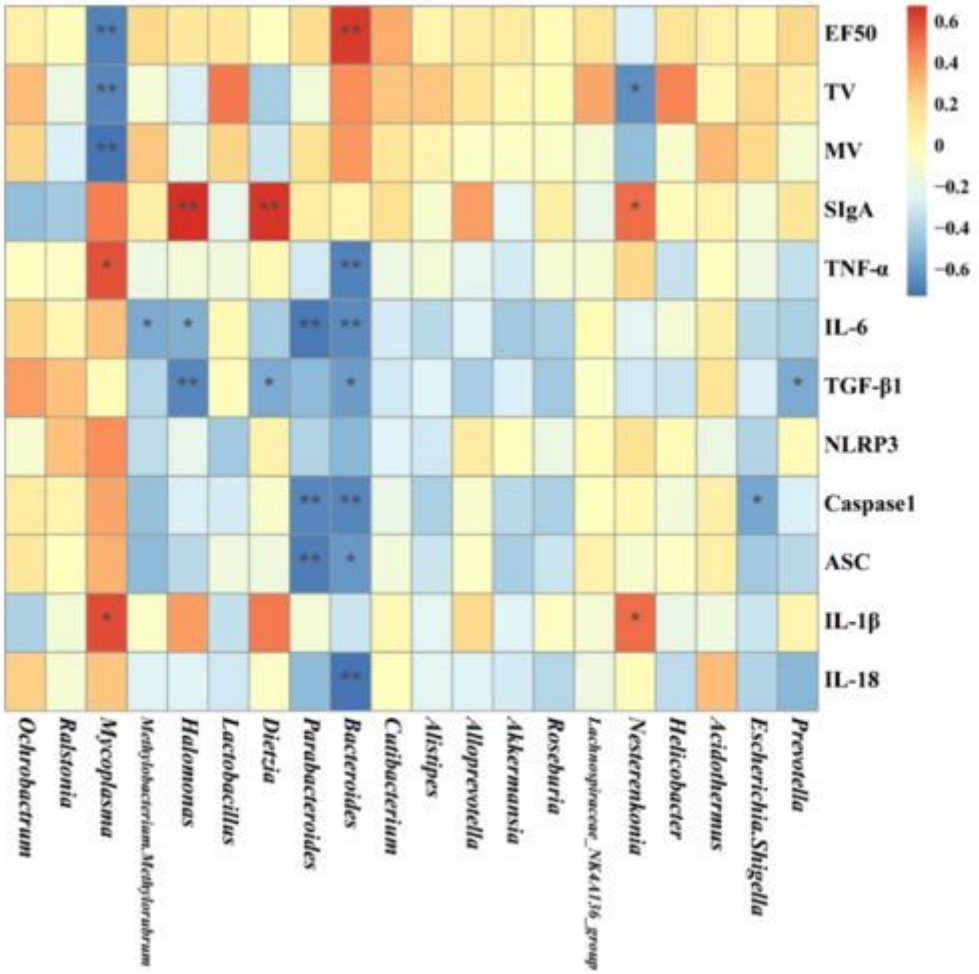

## Figure 4

(A) The relative expression of NLRP3 in lung. (B) Caspase1 in lung. (C) ASC in lung. (D) IL-1β in serum. (E) IL-18 in serum. Dates are expressed as means ± SEM of three independent experiments (ns non-significant, *P < 0.05).




## Figure 5

(A) Analysis of alpha diversity (Chao1, Shannon). (B)The relative abundance of pulmonary microbiota in the phylum. (C) The relative abundance of pulmonary microbiota in the genus. (D) Linear discriminant analysis score of each group of flora samples. Data shown are means ± SEM of three independent experiments (ns non-significant, *P < 0.05.




## Figure 6

Heatmap of the correlation between the lung microbiota of the rats and environmental factors (*P < 0.05, **P < 0.01; blue represents negative correlation; red represents positive correlation).

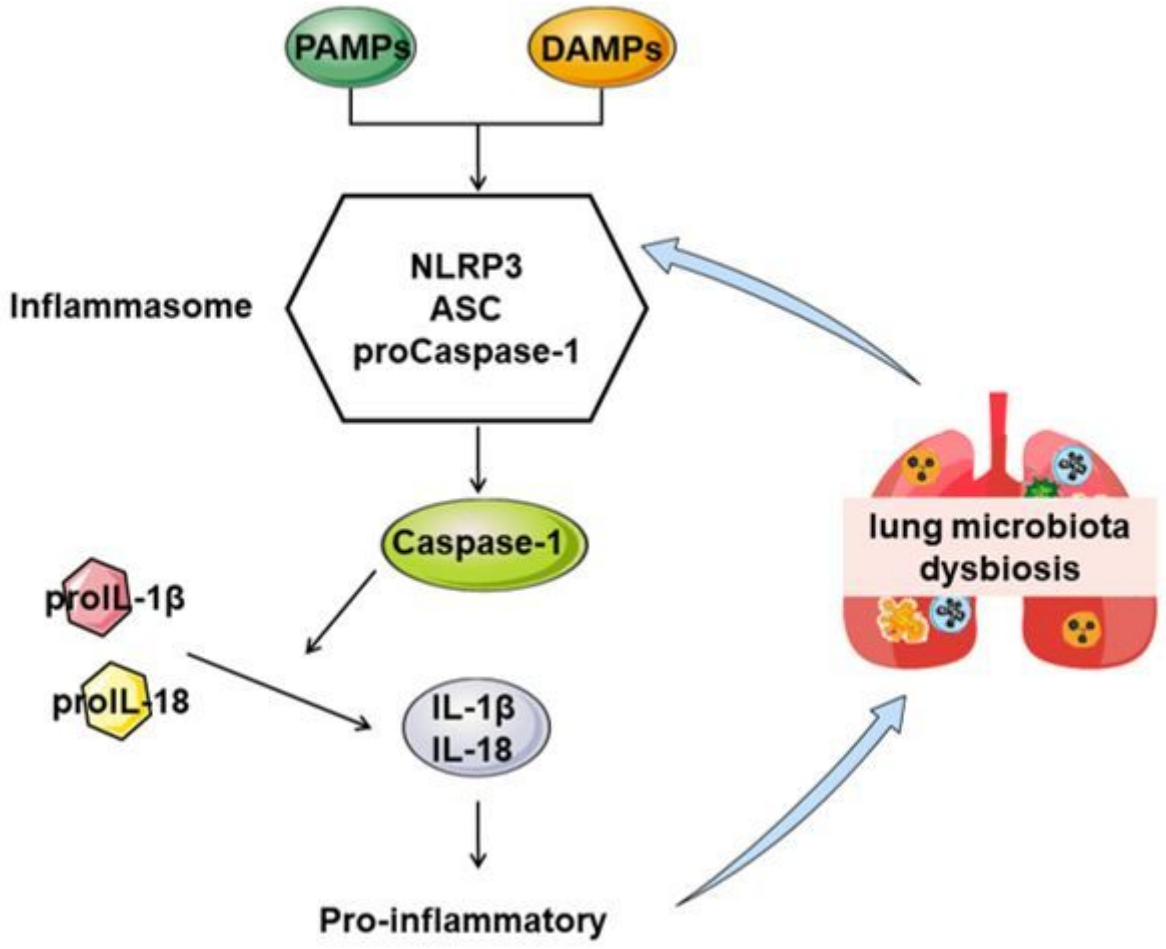



**Figure 7**

a The proposed mechanism by which YS treats COPD.

## Supplementary Files

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