Buyuan decoction inhibits autophagy in a rat model of chronic obstructive pulmonary disease

Chunyan Huang[1], Shaofeng Li[2], Chao Xu[2], Wenlong Song[3], Lei Xu[2], Zhihui Lan[2],* and Liangji Liu[2],#

[1]*Jiangxi University of Chinese Medicine,* Nanchang 330004, Jiangxi Province, P.R. China

[2]*Department of Respiratory and Critical Medicine, The Affiliated Hospital of Jiangxi University of Chinese Medicine,* Nanchang 330006, Jiangxi Province, P.R. China

[3]*Internal Medicine, Jiande City Integrated Traditional Chinese and Western Medicine Hospital,* Jiande, Hangzhou 311612, Zhejiang Province, P.R. China

Corresponding authors: *13970989231@163.com; #liuliangjiLLJ@163.com

Received: November 4, 2021; **Revised**: November 25, 2021; **Accepted**: November 29, 2021; **Published online**: December 14, 2021

Abstract: Efforts have been made to find a better therapeutic approach with fewer side effects in treating chronic obstructive pulmonary disease (COPD). This study investigated the effect of Buyuan decoction (BYD) on autophagy in COPD rats. An experimental model with Sprague-Dawley rats was established by lipopolysaccharide (LPS) injection and cigarette smoke exposure. Rats were randomly allocated into blank control (normal control), experimental model, low-dose BYD (8.0 g/kg/day), medium-dose BYD (16.0 g/kg/day), high-dose BYD (32.0 g/kg/day) and 3-MA (methyladenine) groups (6 rats/group). Cell and tissue morphology were observed using hematoxylin and eosin staining. Autophagic vesicles were examined with a transmission electron microscope. Protein expression of LC3-II/I, BNIP-1, ATG7, p62, PI3K and p-PI3K in lung tissue was detected by Western blotting. Compared with the experimental model group, the inflammatory infiltrate in lung tissue was reduced, the nuclei of the pulmonary epithelial cells were restored to normal, and the expression of LC3, BNIP1, ATG7 and p-PI3K was significantly downregulated, while p62 expression was significantly upregulated after treatment with the BYD. The effect was most significant in the low-dose BYD group ($P<0.05$, all groups). These findings suggest that the BYD inhibits the occurrence of autophagy in the pathogenesis of COPD and that it can be a potential treatment.

Keywords: Buyuan decoction; chronic obstructive pulmonary disease (COPD); autophagy; PI3K pathway; lipopolysaccharide (LPS)

Abbreviations: chronic obstructive pulmonary disease (COPD); Buyuan decoction (BYD); microtubule-associated protein 1 light chain 3 (LC3); BCL2 interacting protein 1 (BNIP1); autophagy related 7 (ATG7); phosphoinositide 3-kinase (PI3K); traditional Chinese medicine (TCM); phosphorylated-phosphoinositide 3-kinase (p-PI3K); protein kinase B (Akt); glyceraldehyde 3-phosphate dehydrogenase (GAPDH); Bcl-2 homology 3 (BH3); Bcl-2 homologous antagonist killer (Bak)

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by persistent and partial reversibility of airflow restriction. The main symptoms of COPD include chronic cough, expectoration, shortness of breath, dyspnea, chest distress, all of which are easily aggravated. COPD is related to seasonal changes and tends to occur in autumn and winter when the weather is colder [1]. When the disease worsens, it can seriously affect the working ability and quality of life of patients, and it may even develop into respiratory failure and pulmonary heart disease, which can be life-threatening [2,3]. COPD is a disease that can be prevented and treated. Since smoking is a major risk factor and the underlying cause of the inflammatory response and oxidative stress in COPD, quitting smoking is important, as well as the most economical

and effective approach to its prevention [4]. At the same time, reducing occupational exposure to harmful dust gases, improving indoor and outdoor air quality and avoiding air pollution are also effective measures to prevent COPD [5].

Studies have found that autophagy participates in the regulation of COPD [6]. Autophagy is a lysosomal-dependent degradation pathway that is ubiquitous in cells and is very important for the maintenance of their homeostasis [7]. Autophagy is manifested as autophagosome formation resulting in the encapsulation of endogenous substances in single- or double-layer membranes, fusion with lysosomes to form autophagolysosomes, and degradation of their contents. Studies have shown that autophagy plays an important role in intracellular homeostasis, tumors, heart failure, neurodegenerative diseases, aging-related diseases and other life processes [7,8]. Exposure to first- and second-hand cigarette smoke is the leading cause of COPD initiation and progression [9], which may cause elevated inflammatory-oxidative stress-mediated cellular apoptosis [10], and increased expression of autophagy markers [11].

It has been suggested that novel therapeutics can be developed based on autophagy in COPD pathophysiology, to provide a target-specific treatment for COPD [7]. Increased autophagy was observed in lung tissue of COPD patients, and induction of autophagy at early stages of COPD progression points to novel therapeutic targets for the treatment of cigarette smoke-induced lung injury [12].

There are many drugs used in COPD treatment, and although they are effective in disease treatment, their side effects are widely observed, such as increased airway wall thickness caused by bronchodilators [13] and skin bruising caused by corticosteroids [14]. Thus, alternative therapeutic strategies with fewer or no side effects have been explored for a better clinical approach, and herbal medicines are among the substitutive therapies studied. Xiaoqinglong decoction, for example, a classic herbal formula in traditional Chinese medicine (TCM), which is composed of eight herbal materials, including *Ephedrae Herba*, Radix Paeoniae Alba, *Asarum sieboldii* Miq., Rhizoma Zingiberis, honey-fried licorice root (*Glycyrrhiza uralensis*), cassia twig (*Ramulus Cinnamomi*), *Schisandra chinensis* and *Pinellia ternata*, and was recorded in Shang Han Lun in the Han Dynasty of China, was shown to attenuate COPD in rats via inhibition of autophagy [15]. Chinese herbs are often used in formulae to obtain synergistic effects or diminish possible adverse reactions [16], In TCM, the pathogenesis of chronic pulmonary disease is believed to be closely related to the deficiency and excess in lung and spleen functions, respectively. In the present study, we evaluated the effect of the Buyuan decoction (BYD), an effective herbal formula developed by Professor Hong Guangxiang, a famous TCM physician from the Jiangxi University of Chinese Medicine, through his experience in treating patients with COPD. The decoction has the effect of "reinforcing deficiency and purging excess", which is the principle of treating COPD in TCM [17], as well as "replenishing qi" to strengthen defense.

Our previous studies found that this formula produces a certain effect on COPD rats [18-21]. It can significantly improve clinical symptoms and dyspnea, walking distance in 6 min, lung function, reduce the frequency and severity of the disease compared to western medicine, delay or even block further development of COPD, improve the quality of life of patients, and it is safe to use [22].

The BYD is composed of *Codonopsis pilosula*, *Atractylodes macrocephala*, Radix Astragali (*Astragalus mongholicus*), Radix Angelicae Sinensis (*Angelica sinensis*), Rhizoma Cimicifugae (*Cimicifuga foetida*), Radix Bupleuri (*Bupleurum chinense*), Herba Cynomorii (*Cynomorium songaricum*), Fructus Corni (*Cornus officinalis*), Pericarpium Citri Reticulatae (*Citrus reticulata*), and Radix Glycyrrhizae Preparata (*Glycyrrhiza uralensis*). The main effects and activities of these herbal materials are presented in Supplementary Table S1.

In this study, we explored the mechanism of the BYD on autophagy in COPD rats by observing changes in autophagy-related proteins after treatment. We hypothesized that the BYD can suppress the occurrence of autophagy via regulation of the expression of the autophagy-related proteins and PI3K pathway activation. The findings may help to develop a novel therapeutic approach for COPD.

MATERIALS AND METHODS

Ethics statement

The research protocol was approved by the Animal Care and Use Committee of the Jiangxi University of Chinese Medicine and conducted in compliance with institutional guidelines.

Experimental animal

Thirty-six 4-week-old specific-pathogen-free grade male Sprague-Dawley rats (130-150 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd, Hunan, P.R. China (license SCXK (Hunan) (2016-0002)). The rats were raised in non-toxic plastic boxes and metal cages with a stainless-steel wire cover and movable cage frame, which could be sterilized by various disinfection methods. The rats were kept in a constant 12 h light/dark cycle and allowed food and water *ad libitum*.

Preparation of the BYD

The BYD is composed of 30 g *Codonopsis pilosula*, 15 g *Atractylodes macrocephala* stir-fried with bran, 30 g Radix Astragali, 10 g Radix Angelicae Sinensis, 10 g Rhizoma cimicifugae, 10 g Radix Bupleuri, 15 g Herba Cynomorii, 15 g Fructus Corni, 10 g Pericarpium Citri Reticulatae, and 10 g Radix Glycyrrhizae Preparata. All the medicinal materials used in this experiment were TCM formula granules purchased from Guangdong Yifang Pharmaceutical Co., Ltd., Guangdong, P.R. China, that passed the inspection of the Guangdong Food and Drug Administration. The formula granules were diluted with physiological saline before use.

Reagents

Jinsheng cigarettes were purchased from Nanchang Cigarette Factory of China Tobacco Jiangxi Industrial LLC, Jiangxi, P.R. China; 3-methyladenine was obtained from MedChemExpress LLC, NJ, USA. The following reagents were used: radioimmunoprecipitation (RIPA) lysis buffer (Applygen Technologies Inc., Beijing, P.R. China); polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Burlington, MA, USA); enhanced chemiluminescence (ECL) (Thermo Fisher Scientific, Waltham, MA, USA); mouse monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000), goat anti-mouse immunoglobulin G (IgG) high and light chains (H+L) horseradish peroxidase (HRP) conjugate (1:2000), goat anti-rabbit IgG (H+L) HRP conjugate (1:2000) (ZSGB-BIO; OriGene Technologies, Inc., Beijing, China); rabbit polyclonal anti-microtubule-associated protein 1 light chain 3 (LC3)-II/I (1:2000), rabbit polyclonal anti-BCL2 interacting protein 1 (BNIP1) (1:5000), rabbit polyclonal anti-autophagy related 7 (ATG7) (1:30000), rabbit polyclonal anti-p62 (1:30000), rabbit polyclonal anti-phosphoinositide 3-kinase (PI3K) (1:1000), rabbit polyclonal anti-phosphoinositide 3-kinase (p-PI3K) (1:500) (Abcam PLC, Cambridge, UK); lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA); hematoxylin solution, eosin (BosterBio, Pleasanton, CA, USA); Scott's Bluing reagent (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China); uranium acetate and lead citrate (Nanjing SenBeiJia Biological Technology Co., Ltd., Jiangsu, P.R. China); bicinchoninic acid (B CA) assay kit (Beijing Cowinbioscience Co. Ltd., Beijing, P.R. China); sodium pentobarbital (Wuhan HeChang Chemical Co., Ltd., Hubei, P.R. China).

Instrumentation

The instrumentation was as follows: fluorescence microscope (CKX53, Olympus Corp., Shinjuku, Tokyo, Japan); transmission electron microscope (TEM) (JEM-1230 (80 KV), JEOL Inc., Akishima, Tokyo, Japan); vertical protein electrophoresis apparatus (DYY-6C, Beijing Liuyi Biotechnology Co., Ltd., Beijing, P.R. China); ultra-high sensitivity chemiluminescence imaging system (Chemi Doc™ XRS+, Bio-Rad Laboratories, Inc., Shanghai, P.R. China); ultramicrotome (EM UC7, Leica Microsystems, Wetzlar, Germany); Quantity One software (v4.6.6, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Experimental grouping

The rats were randomly allocated into 6 groups: 1) blank control (normal control), 2) COPD model + saline (experimental model), 3) COPD model + low-dose BYD (8.0 g/kg/day) (low-dose BYD), 4) COPD model + medium-dose BYD (16.0 g/kg/day) (medium-dose BYD); 5) COPD model + high-dose BYD

(32.0 g/kg/day) (high-dose BYD); 6) COPD model + 3-methyladenine group (3-MA) (n=6 per group).

Establishment of the COPD rat model

Except for the blank control group, the rats in the other groups were given intratracheal administration of lipopolysaccharide (LPS) (0.01 mg/kg, formulated as a 1 mg/mL solution) on day 1 and day 14, and regularly exposed to cigarette smoke for 30 min each morning, 1 cigarette per exposure, once daily, on days 2-13 and days 15-28. To reduce the effect of water vapor produced by the process of cigarette burning on rats, activated charcoal was placed at the bottom of the smoke box. The rats were fed regularly after smoke exposure.

The treatments were started on day 15. The BYD groups received 20-mL/kg twice-daily intragastric administration of low-dose BYD (8.0 g crude drug/kg/day), medium-dose BYD (16.0 g crude drug/kg/day) or high-dose BYD (32.0 g crude drug/kg/day). The experimental model group received intragastric administration of equal amounts of physiological saline. The 3-MA group received intragastric administration of 3-methyladenine 15 mg/kg once daily. The blank control group was not given any medication. All animals were maintained for 42 days. Their weights were recorded at the same time every Monday. Thereafter, the rats were anesthetized with an intraperitoneal injection of 45 mg/kg sodium pentobarbital and euthanized by cervical dislocation. The lungs were removed by thoracotomy and the lung tissue was collected. The successful establishment of the experimental rat model was followed by histopathological staining.

Histopathological examination of rat lung tissue by hematoxylin-eosin staining (H&E)

The lung tissue was rinsed with water for several hours and subjected to gradient dehydration in 70%, 80% and 90% ethanol solutions. The specimen was placed in a mixed solution containing equal amounts of pure alcohol and xylene for 15 min, followed by xylene I and II for about 15 min each (until clear). The specimen was then placed in a mixed solution containing equal amounts of xylene and paraffin for 15 min, and in paraffin I and II, for 50-60 min each, and paraffin embedded. The paraffin section was baked, de-waxed and hydrated. The sections that were placed in distilled water and stained with a hematoxylin aqueous solution for 3 min, followed by an ethanol hydrochloride solution for 15 s, rinsed and placed in a bluing reagent for 15 s. The sections were washed with running water, stained with eosin for 3 min, washed with running water, dehydrated, cleared, mounted and examined under the microscope. The histopathological assessment was performed blind on randomized sections. The severity of the inflammatory cell infiltration in the airway was estimated with a 4-point scoring system [41] as follows: 0 – normal; 1 – few cells; 2 – a ring of inflammatory cells one cell layer deep; 3 – a ring of inflammatory cells, two to four cells deep; 4 – a ring of inflammatory cells more than four cells deep.

Examination of rat lung tissue under a transmission electron microscope (TEM)

Specimens were fixed in 2.5% glutaraldehyde and phosphate buffer solution for 2 h or more and rinsed three times with 0.1 M phosphoric acid cleaner for 15 min. The specimens were fixed in 1% osmic acid fixative for 2-3 h, rinsed three times with 0.1 M phosphoric acid cleaner for 15 min, dehydrated, embedded and solidified. The specimen was then sliced with an ultramicrotome into 70 nm thickness, stained with uranium acetate and lead citrate, and observed under a TEM.

Protein expression of LC3-II/I, BNIP-1, ATG7, p62, PI3K and p-PI3K in rat lung tissue detected by Western blotting

The corresponding lysis buffer was added to the cells in each group, lysed at 4°C for 30 min, and centrifuged at 13500*x*g for 30 min. The supernatant was carefully aspirated to obtain the total protein. The protein concentration was determined by the bicinchoninic acid protein assay, followed by protein denaturation and sample loading. Electrophoresis was conducted for 1-2 h, followed by wet transfer for 30-50 min. Primary antibody incubation was performed at 4°C overnight. The secondary antibody incubation was performed at room temperature for 1-2 h and enhanced chemiluminescence (ECL) was completed. The gray value of each antibody band was analyzed using "Quantity one" software.

Statistical analysis

All data was analyzed by SPSS 19.0 (IBM Corp., Armonk, NY, USA). The results were presented as mean±standard deviations ($\bar{}$ ± s). Significant difference between groups were determined using analysis of variance (ANOVA) with $P<0.05$ as the threshold value for statistical significance.

RESULTS

Establishment of the COPD rat model

The alveoli in the normal control group were clearly visible and the amount of collagen fibers was not obviously increased. The experimental model group had a large number of inflammatory infiltrates (indicated by arrows), the alveoli were significantly enlarged, and emphysema was observed (Supplementary Fig. S1A). The lung injury score for the experimental model group was significantly higher than that of the control group ($P<0.05$) (Supplementary Fig. S1B). These findings indicated the successful establishment of the COPD rat model.

BYD reduced inflammatory cell infiltration in rat lung tissue observed by H&E staining

In the normal control group, the alveoli were clearly visible, and the amount of collagen fibers was not obviously increased. The experimental model group showed a large number of inflammatory infiltrates (indicated by arrows), the alveolar cavity was significantly enlarged, and emphysema was obvious. Compared with the experimental model group, the inflammatory infiltrates in the lung tissue were reduced in the treatment groups, and this was most obvious in the low-dose BYD group (Fig. 1A).

BYD improved karyopyknosis in rat lung tissue as observed under TEM

As shown in Fig. 1B, for the normal control group the structure of the pulmonary epithelial organelles was normal and the air-blood barrier was intact. In the experimental model group, the endoplasmic reticulum was expanded and karyopyknosis was observed (indicated by the arrow). Compared with the experimental model group, the nuclei of the pulmonary epithelial cells in the BYD-treatment groups were restored to normal, and this was most obvious in the low-dose BYD group.

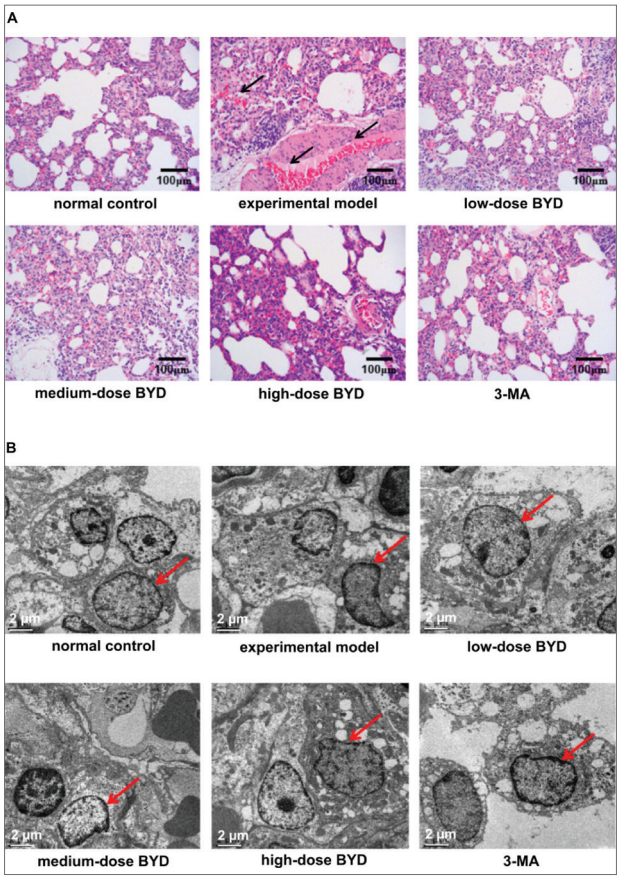

Fig. 1. A – H&E staining of lung tissue in each experimental group of rats. In the normal control group, the alveoli were clearly visible, and the amount of collagen fibers were not obviously increased. The experimental model group showed inflammatory infiltrates (indicated by arrows), the alveolar cavity was significantly enlarged, and emphysema was observed. Compared to the experimental model group, the inflammatory infiltrates in the lung tissue were reduced in the treatment groups, and this was most obvious in the low-dose BYD group. n=6 per group, designated as the hematoxylin-eosin (HE), Buyuan decoction (BYD) and methyladenine (MA) groups. **B** – Lung tissue of rats in each group under TEM. In the normal control group, the structure of the pulmonary epithelial organelles was normal, and the air-blood barrier was intact. In the experimental model group, the endoplasmic reticulum was expanded and karyopyknosis was detected (indicated by arrow). Compared with the experimental model group, the nuclei of the pulmonary epithelial cells in the BYD treatment groups were restored to normal, and this was most obvious in the low-dose BYD group (indicated by arrows). n=6 per group.

BYD downregulated LC3, BNIP1, ATG7 and p-PI3K and upregulated p62 protein expressions in rat lung tissue

The expressions of proteins that promote autophagic vesicle formation in the experimental rat model (LC3, BNIP1, ATG7 and p-PI3K) were significantly upregulated, while p62 was significantly downregulated (P<0.05, all). Compared with the experimental model group, protein expression of LC3, BNIP1, ATG7 and p-PI3K in the treatment groups was significantly downregulated to varying degrees, while p62 was significantly upregulated (P<0.05, all); the effect was most obvious in the low-dose BYD group (Fig. 2).

DISCUSSION

COPD is a disease that is characterized by partial reversibility of airflow restriction, which can be prevented and treated. Its pathogenesis mainly includes inflammatory response, oxidative stress imbalance, and protease and antiprotease imbalance; additionally, environmental and genetic factors may also play a role in its pathogenesis [42].

The COPD model is an essential platform for COPD research. There are many types of animals that can be used to establish the model. Rats are an often-selected animal model due to their docile character, strong reproductive ability, low breeding cost and short life cycle; the genome of the rat is very close to the human genome and is widely studied; the histopathological changes and immune response of rats exhibit a high degree of similarity to that of humans [43]. Since cigarettes are recognized as a high-risk factor for COPD, combined with pathogenesis to establish the COPD model, the modeling time can be shortened, and the established model is closer to the COPD observed in patients in many aspects, such as changes in pathophysiology, tissue structural changes and general status [44]. Autophagy can be dysregulated by different COPD risk factors, leading to cell death or senescence and COPD progression. When autophagy is dysregulated by factors such as cigarette smoking, different environmental insults and ageing, it can lead to aggresome-body formation and enhanced production of reactive oxygen species (ROS), which contribute to the pathogenesis of COPD [7].

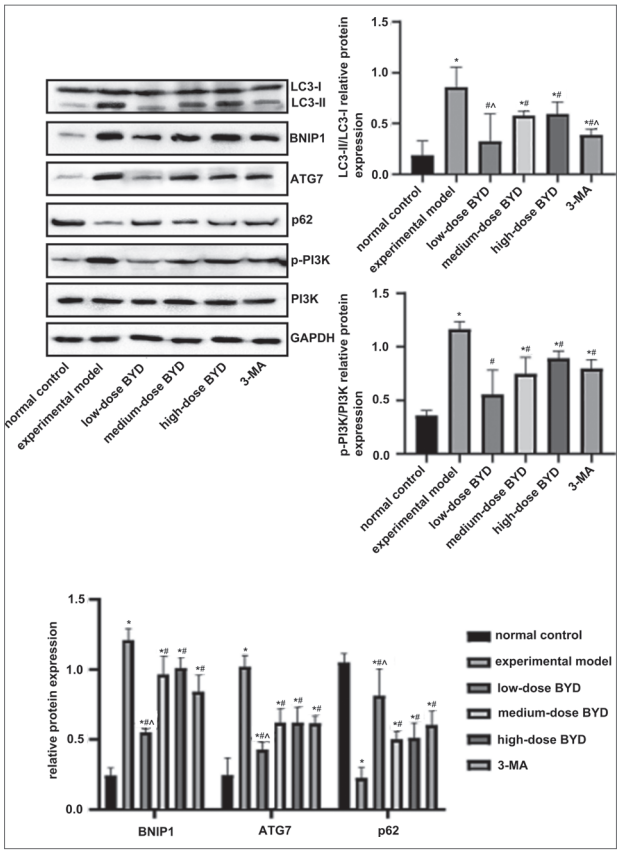

Fig. 2. The LC3-II/I, BNIP1, ATG7, p62, PI3K and p-PI3K protein expression detected by Western blotting in lung tissue of rats in each group. Compared with the normal control group, *P<0.05; compared with the experimental model group, #P<0.05; compared with the medium dose BYD group, ^P<0.05. The expression of proteins that promote autophagic vesicle formation in the rat model (LC3, BNIP1, ATG7 and p-PI3K) were significantly upregulated, while the expression of p62 was significantly downregulated (P<0.05, all). Compared to the model group, the protein expression of LC3, BNIP1, ATG7 and p-PI3K was significantly downregulated in varying degrees, while p62 was significantly upregulated in the treatment groups (P<0.05, all); the effect was most obvious in the low-dose BYD group. Data are presented as the mean±standard deviation. n = 6 per group. Microtubule-associated protein 1 light chain 3 (LC3); BCL2 interacting protein 1 (BNIP1); autophagy related 7 (ATG7); phosphoinositide 3-kinase (PI3K); phosphorylated-phosphoinositide 3-kinase (p-PI3K); Buyuan decoction (BYD); methyladenine (MA).

In this experiment, the COPD model was established by intratracheal administration of LPS and exposure to cigarette smoke for 8 weeks. The histopathological findings in lungs demonstrated a large number of inflammatory infiltrates, significant expansion of the alveoli and obvious pulmonary emphysema in the experimental model group, indicating the successful establishment of the COPD rat model. Previous studies

showed that the BYD can enhance immunity, and that it acts as an effective regulator of the inflammatory response and signaling pathway [45]. 3-Methyladenine is a cell autophagy inhibitor that was used as a positive control in this study [46]. The histopathological and TEM results showed decreased inflammatory infiltrates in rat lung tissue and epithelial cell nuclei were restored to normal, particularly in the low-dose BYD group, suggesting that the BYD exerts a beneficial effect in alleviating COPD in the rat experimental model.

LC3 is a marker protein on the autophagosome membrane and a specific index reflecting autophagy. When autophagy is established, LC3I decreases while LC3II increases. Autophagic activity can be determined by LC3 expression and changes in the LC3II/LC3I ratio [15]. Previous studies showed that autophagosome formation increased in patients with COPD and that the conversion of LC3B-I to LC3B-II increased, with the mRNA and protein levels of some autophagy-related genes increasing significantly [47]. The results of this study established that LC3II/LC3I in the COPD model group increased significantly, indicating the occurrence of autophagy, with LC3II/LC3I decreasing after treatment with different doses of BYD, suggesting that the decoction inhibits the occurrence of autophagy. The findings were consistent with the results of a previous study with Xiaoqinglong decoction [15].

BNIP belongs to the BCL-2 family, which includes BNIP1, BNIP2 and BNIP3. BNIP1 has the same Bcl-2 homology 3 (BH3) domain as the Bcl-2 homologous antagonist/killer (Bak) that promotes apoptosis. This protein is located in the endoplasmic reticulum, and studies have shown that BNIP1 protein can serve as an important junction molecule of the endoplasmic reticulum signaling network. It can also cause mitochondrial fragmentation and has the function of inducing apoptosis [48]. In recent years, there have been many studies on the relationship between BNIP1 protein and autophagy. It has been reported that polyubiquitination of BNIP1 protein can recruit autophagy receptor p62, which can simultaneously bind ubiquitin and LC3 proteins, and link ubiquitination and autophagy together [49]. The results of this study found that BNIP1 was significantly increased in the COPD model group, and decreased after treatment with different doses of BYD, indicating that the decoction inhibits BNIP1 protein expression and thus inhibits the occurrence of autophagy.

ATG7 is an autophagy-related genes. ATG7 is an E1-like activating enzyme, which is essential for the autophagy coupling system and autophagosome formation. At the same time, ATG7 can control the number and size of autophagosomes [50]. It plays an important role in the formation of ATG5-ATG12-ATG16L and LC3 complexes and is involved in the regulation of the autophagy pathway [51]. Studies have shown that high expression of ATG7 is associated with autophagy [52], and that inhibition of ATG7 expression can inhibit autophagic death of cardiomyocytes [53]. The results of this study show that ATG7 was significantly increased in the COPD model group, and that it decreased after treatment with different doses of the BYD. The findings suggest that high levels of ATG7 may participate in the occurrence of autophagy, and that the BYD may inhibit the occurrence of autophagy by inhibiting ATG7 expression.

p62 is involved in different physiological and pathological processes, including autophagy. It is one of the marker proteins reflecting autophagic activity [54]. p62 is an adaptor protein in autophagy that also regulates the process of autophagy, and when autophagic activity is lowered, p62 protein accumulates in the cytoplasm. High expression of p62 will activate the mTORC1 signaling pathway, leading to the inhibition of autophagy and abnormal accumulation of p62 protein [55]. Studies showed that inhibition of p62 can increase ROS level and decrease LC3II expression and the LC3II/LC3I ratio [56]. The findings of this study showed that p62 regulates autophagy. p62 was significantly decreased in the COPD model group and increased after treatment with different doses of the BYD, which indicates that p62 expression decreased when autophagic activity was robust, and that the BYD increased p62 expression by diminishing autophagic activity. These findings were consistent with the results of the previous study with Xiaoqinglong decoction [15].

PI3K, as an important component connecting extracellular signals and multiple key cellular pathways, can influence a series of downstream signaling molecules participating in the process of apoptosis and autophagy [57]. Studies have shown that PI3K/Akt/FoxO3a can regulate ubiquitin-proteasome and autophagy-lysosome systems. It was also found that when autophagy occurs, PI3K is activated, significantly increasing the expression of p-PI3K [58]. The results of this study showed that p-PI3K in the COPD

experimental model group increased significantly and decreased after treatment with different doses of the BYD, indicating that autophagy is related to the activation of PI3K and that the BYD can inhibit autophagy through inhibition of PI3K activation.

Although activation of autophagy exhibits a protective effect in many diseases, in COPD the continuous activation of autophagy due to prolonged exposure to smoke exceeds the beneficial aspect of autophagy-mediated cell death. Thus, the protective effect of herbal medicines on COPD may be due to their inhibition of autophagy, while the protective effect on other tissue damage is due to the activation of autophagy [15].

The findings of this study show that the BYD possesses a protective effect in attenuating COPD in rats. There is likely a cooperative effect among the active components involved in the therapeutic mechanism [59]. The BYD can be a potential treatment for COPD, acting via the combination of these active components, which are responsible for its pharmacological effect. Further studies should explore the active components in the BYD and the mechanism of its effects. This study adds to the growing literature on the use of herbal medicines to attenuate COPD and contributes towards the development of a novel therapeutic approach based on the context of autophagy in COPD pathophysiology.

Funding: This study was supported by the National Natural Science Foundation of China (NSFC) (Grant 81760859); the Jiangxi Provincial Department of Science and Technology Project devoted to research on lung disease diagnosis and treatment technology by the National Medical Master Hong Guangxiang (Grant 20192BBG70044); the Jiangxi Provincial Health and Family Planning Commission of Traditional Chinese Medicine research project (Grant 2019A035); the National Research and Training Program for Excellent Talents of Traditional Chinese Medicine (Human Resources and Education Department of State Administration of Traditional Chinese Medicine (Grant 2017/24).

Author contributions: Conceptualization, C. Huang, S. Li, Z. Lan and L. Liu. Methodology, C. Huang, S. Li, Z. Lan and L. Liu. Investigation, C. Huang, S. Li, C. Xu, W. Song and L. Xu. Intellectual input, Z. Lan and L. Liu. Writing – original draft, C. Huang. Reviewing and editing, Z. Lan. Supervision, Z. Lan and L. Liu.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: Data will be made available on request.

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Supplementary Material

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