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Myricetin protects airway epithelial cells against cigarette smoke extract-induced inflammation and oxidative stress by suppressing autophagy

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Abstract. Chronic obstructive pulmonary disease (COPD) is a common respiratory disease characterized by inflammation and oxidative stress, which is mainly caused by cigarette smoke (CS). The flavonoid myricetin was reported to exert protective effects in different diseases. This study aimed to explore the function of myricetin in COPD progression. Airway epithelial A549 cells were treated with CS extract (CSE) to establish an *in vitro* model, followed by the detection of inflammation, oxidative stress, and autophagy markers. Sprague-Dawley male rats were exposed to CS for 12 weeks to establish an *in vivo* model, followed by the evaluation of lung function parameters and lung histopathological changes. We found that myricetin relieved inflammation and oxidative stress in CSE-induced A549 cells, as demonstrated by the reduced MCP-1, IL-6, and IL-8 expression, ROS production, and MDA content and elevated SOD activity. Myricetin treatment reduced LC3B-II/LC3B-I ratio and Beclin-1 protein levels and elevated p62 protein level after CSE stimulation in A549 cells. I*n vivo* results revealed that myricetin restored pulmonary function and ameliorated pulmonary inflammation and emphysema in CS-induced COPD rats. Collectively, the anti-inflammatory and antioxidant effects of mMyricetin in COPD may be attributed to its suppressive effects on autophagy.

Key words: Chronic obstructive pulmonary disease — Myricetin — Autophagy — Oxidative stress — Inflammation

Abbreviations: COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CSE, CS extract; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.

Introduction

Chronic obstructive pulmonary disease (COPD) has been recognized as a common respiratory disease (Rabe and Watz 2017). COPD is characterized by airway progressive obstruction and inflammation, which results in emphysema (Barnes et al. 2014). According to WHO estimates, COPD will become the third most common cause of death in the world by 2030 (Hillas et al. 2015). More and more studies indicate that cigarette smoke (CS) is the main risk for COPD (Tuder and Petrache 2012). Cigarette smoke extract (CSE) contains many different types of components, which can induce lasting oxidative stress and inflammatory reactions in airway epithelial cells and eventually destroy these cells (Wu et al. 2020; Zhang MY et al. 2021). However, the mechanisms underlying the development of COPD remain largely unknown.

Autophagy is a self-phagocytosis process of cellular components and a mighty promoter of metabolic homeostasis. It

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is activated by oxidative stress or accumulation of damaged proteins (Glick et al. 2010). Importantly, a growing body of research suggests that autophagy can mediate cellular damage, contributing to disease development (Levine and Kroemer 2008, 2019). Autophagy is stronger in epithelial cells of patients with COPD compared to healthy individuals (Morse and Rosas 2014). Previous studies have demonstrated that enhanced autophagy results in inflammation and emphysema in COPD (Li et al. 2017). CS-induced activation of autophagy can exacerbate lung damage (Wang Y et al. 2018). Furthermore, it has been reported that silymarin attenuates CSE-induced inflammation *via* inhibition of autophagy in human bronchial epithelial cells (Li et al. 2016). Thus, more detailed studies are needed to explore the abnormal activation of autophagy in the pathogenesis of COPD.

Myricetin (3,5,7,3′,4′,5′-hexahydroxyflavonol) is a flavonoid compound widely found in vegetables, fruits, berries, and tea (Song et al. 2021). Myricetin possesses assorted biological activities such as anti-inflammatory, antioxidant, antitumor, and antiviral effects (Gupta et al. 2020). Importantly, the protective effects of myricetin against lung disease have been well-established. For example, myricetin ameliorates lung histological changes, alleviates lung barrier dysfunction and edema, and inhibits inflammation cell activation in a mouse model of lipopolysaccharide-induced acute lung injury (Hou et al. 2018). Myricetin suppresses fibroblast proliferation and differentiation and mitigates bleomycin-induced pulmonary fibrosis in mice by inactivating TGF-β signaling (Li et al. 2020). Myricetin prevents fatal lung infections caused by methicillin-resistant *Staphylococcus aureus* in mice by blocking the activity of ClpP (Jing et al. 2021). Myricetin inhibits cancer cell invasion and migration in lung adenocarcinoma (Shih et al. 2009). Importantly, it was reported that myricetin represses TNF-α-induced inflammatory response in A549 cells by regulating SIRT1/NF-κB signaling, thereby inhibiting COPD progression (Chen et al. 2020). However, whether myricetin can regulate autophagy in COPD remains unclear.

Herein, this study aimed to investigate the specific function of myricetin in the progression of COPD. Airway epithelial A549 cells were treated with CSE to establish the COPD cell model *in vitro*. We hypothesized that myricetin could protect A549 cells against CSE-induced inflammation and oxidative stress by suppressing autophagy. In addition, CS-exposed rats were used as COPD animal models to confirm the therapeutic effects of myricetin *in vivo*.

Table 1. The sequences of primers

Primer		
MCP-1	F:	5'-CCAGATGCAATCAATGCCC-3'
	R:	5'-TGGTCTTGAAGATCACAGCT-3'
IL-6	F:	5'-GATTCAATGAGGAGACTTGCC-3'
	R:	5'-TGTTCTGGAGGTACTCTAGGT-3'
IL-8	F:	5'-CTTGGCAGCCTTCCTGA-3'
	R:	5'-TTCTTTAGCACTCCTTGGCAAAA-3'
GAPDH	F:	5'-TCAAGATCATCAGCAATGCC-3'
	R:	5'-CGATACCAAAGTTGTCATGGA-3'

F, forward; R, reverse.

Materials and Methods

CSE preparation

Two cigarettes (Double Happiness, Chinese; 14 mg of carbon monoxide, 1.1 mg of nicotine, and 12 mg of tar *per* cigarette) were combusted with a reformative syringe-driven apparatus. The resultant smoke was bubbled through 30 ml serum-free medium. For quality control, the absorbance (OD value) of the solution was measured at 320 nm (A320) and 540 nm (A540) using a spectrophotometer, and CSE solution with an $OD_{(A320–A540)}$ of 0.9 to 1.2 was considered acceptable. Next, the solution was adjusted to pH 7.2 and filtered through an aseptic 0.22-μm filter. The obtained solution was gathered as "100% CSE" solution. Then, 100% CSE was diluted with PBS to obtain the desired concentration of CSE (10%).

Cell culture and treatment

A549 cells purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) were incubated in DMEM (Gibco, Carlsbad, CA, USA) added with 10% FBS (Gibco) at 37°C with 5% CO_2. Myricetin (Myr, purity: 98.42%; MedChemExpress, Monmouth Junction, NJ, USA) was dissolved in DMSO to make a stock solution and diluted to different concentrations for cell treatment. To assess the cytotoxicity of myricetin, A549 cells were treated with myricetin (7.8, 15.6, 31.25, 62.5, 125, 250, 500 or 1000 μM) for 24 h. To determine the effects of myricetin on CSE-induced cell injury, A549 cells were treated without or with 10% CSE in the absence or presence of myricetin (20, 40, and 60 μM) for 24 h.

RT-qPCR

After indicated treatments, A549 cells were seeded (5×10^5 cells/well) onto 6-well plates, and total cellular RNA was extracted using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA). After analyzing RNA concentration and quality by a Nano-Drop spectrophotometer, 1 μg of total RNA was reverse transcribed using the Reverse Transcription System Kit (Takara, Kusatsu, Japan) to synthesize cDNA. Then, qPCR analysis was performed on an Applied Biosystems 7500 Real Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) utilizing SYBR Green PCR Master Mix (Takara). The relative fold changes were estimated by the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. The sequences of primers are shown in Table 1.

Western blot

Total proteins were extracted from cells using RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, USA). Next, protein concentration was estimated *via* Bicinchoninic Acid Protein Assay Kit (Pierce Biotechnology, Inc., USA) in accordance with user guides. Next, 20 μg proteins were isolated *via* 12% SDS-PAGE gel and transferred to PVDF membranes (Millipore, Billerica, MA, USA), followed by blocking with 5% skimmed milk and incubation with primary antibodies against LC3B (Abcam, UK, ab192890, 1:2000), Beclin-1 (Abcam, ab207612, 1:2000), p62 (Abcam, ab207305, 1:1000), and the loading control β-actin (Abcam, ab8226, 1:1000) at 4°C for one night. The next day, the membranes were rinsed with Tris-buffered saline solution and then cultured with the secondary antibody (Abcam, ab7090, 1:1000) for 2 h. In the end, the protein bands were visualized utilizing the ECL detection kit (Millipore) and analyzed by ImageJ software (National Institutes of Health, USA).

CCK-8 assay

The cytotoxicity of myricetin was estimated *via* Cell Counting Kit-8 (CCK-8; Dojindo, Tokyo, Japan) in accordance with user guides. Cells were put in 96-well plates (5×10^3 cells/well) for 24 h. Myricetin at the concentrations of 7.8, 15.6, 31.25, 62.5, 125, 250, 500 or 1000 μM was separately supplemented into cells. After 24 h of incubation, cells were cultured with 10 μl of CCK-8 solution for 2 h at room temperature. The absorbance at 450 nm was tested by a microplate reader (Thermo Fisher Scientific).

Animal assay

Thirty-two Sprague-Dawley male rats (8 weeks, 250 ± 20 g) purchased from Vital River Co. Ltd. (Beijing, China) were raised in a room at controlled temperature of 25°C with a 12/12 h light/dark cycle. Rats were allowed free access to diet and water *ad libitum*. Animal experimental protocols were approved by the Ethics Committee of Yichang Central People's Hospital. Rats were stochastically divided into four groups (8 rats/group) as follows: Control, CS, CS+Myr 25, and CS+Myr 50. COPD animal models were established as previously described and some modifications were made (Wang et al. 2018a). Rats were exposed to CS from four cigarettes burning together (Double Happiness, Shanghai). Each smoke exposure lasted two hours, twice a day, five days a week for a total of 12 weeks by a custom-designed and purpose-built nose-only, directed flow inhalation and smoke-exposure system (handmade) housed in a fume and laminar flow hood. Rats in CS+Myr groups were treated with myricetin (25 or 50 mg/kg) by intraperitoneal injection 1 h before the first CS exposure of each day. The dose of myricetin was selected based on the previous studies (Rehman and Rather 2019; Li et al. 2020). Rats in the Control group were subjected to room air exposure and treated with normal saline. Rats in CS group were subjected to CS exposure and treated with normal saline. After 12 weeks, rats were anesthetized with 40 mg/kg intraperitoneal sodium pentobarbital for pulmonary function tests, followed by the collection of lung tissues.

Pulmonary function test

Pulmonary function was estimated by employing Buxco's Research System. Rats were anaesthetized with 40 mg/kg sodium pentobarbital before tracheotomy. A specific spirometer for rats was utilized for observing lung function, including the ratio of forced expiratory volume in the first 0.1 s and forced vital capacity (FEV0.1/FVC%), maximal mid-expiratory flow (MMEF), functional residual capacity (FRC), and peak expiratory flow (PEF).

H&E staining

Lungs of rats were fixed by 10% buffered formalin for 48 h and embedded in paraffin. Next, lung tissues were cut into 4 μm thick slices. The slices were dyed using hematoxylin and eosin (H&E) staining kit (Solarbio, Beijing, China), and then analyzed by microscopic (Carl Zeiss, Oberkochen, Germany) observation. The inflammatory response was quantified *via* the inflammation scores. The destructive index (DI) was utilized to determine the alveolar wall injury.

Immunofluorescence staining

Cells were gathered and seeded on glass slides in 12-well plates for 48 h. Then, cells were fixed with 4% paraformaldehyde for 10 min and treated with blocking solution including 0.1% Triton X-100 (Beyotime, China) and 3% BSA (Beyotime, China) in PBS. Next, cells were cultured with anti-LC3B (ab192890, 1:2000, Abcam) at 4°C for one night. Next, sections were rinsed and then incubated with secondary antibodies (1:200, A-11012, Goat anti-rabbit Alexa Fluor 594, Thermo Fisher Scientific) for 1 h. DAPI was applied for nucleus staining. In the end, a fluorescence microscope (Olympus, BX53, Japan) was employed to capture the image.

Detection of ROS production

Cells were loaded with 10 μM of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) solution at 37°C for half an hour. After rinsing with PBS, the ROS level was tested *via* detecting the fluorescent intensity by microscopy at excitation wavelength 485 nm and emission wavelength 530 nm and then analyzed by Image-Pro Plus software (version 5.0).

Detection of MDA and SOD activities

Cells were lysed in PBS *via* ultrasonic pyrolysis and subjected to centrifugation at 3000×*g* for 10 min at 4°C. Then, 100 µl supernatant was mixed with MDA and SOD assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) in accordance with user guides. Next, a microplate reader (BioTek Instruments) was applied for measuring absorbance value at 532 nm (for MDA) and at 520 nm (for SOD).

ELISA

Cell cultures were gathered and centrifuged at 3000×*g* for 10 min at 4°C. IL6 and IL8 concentrations in the supernatant were estimated *via* their corresponding ELISA kits (R&D Systems, Minneapolis, MN, USA) in line with user guides. The absorbance value at 450 nm was measured by a microplate reader (BioTek Instruments).

Statistical analysis

GraphPad Prism software (version 7.0, USA) was utilized to analyze the data. The measurement data were expressed as means ± SD from three individual repeats. Student's *t*-test was applied for comparison between two groups. The comparison among multiple groups was analyzed by one-way ANOVA followed by Tukey's *post hoc* analysis. $p < 0.05$ indicated statistically significant difference.

Results

Cytotoxicity of myricetin in A549 cells

The chemical structure of myricetin is displayed in Figure 1A. For the sake of choosing the appropriate concentration of myricetin for later assays, we first detected the cytotoxicity of A549 cells. A549 cells were treated with 0–1000 µM of myricetin for 24 h and then subjected to CCK-8 assay. We discovered that there were no notable alterations in cell viability after treatment with myricetin (0–250 µM). We further observed that 500 or 1000 µM myricetin treatment markedly decreased cell viability (Fig. 1B). Therefore, 20, 40, and 60 µM of myricetin was chosen for assays.

Myricetin relieves CSE-induced inflammatory response in A549 cells

To construct the cellular model of COPD, A549 cells were exposed to 10% CSE. Through ELISA, we discovered that the contents of MCP-1, IL-6, and IL-8 were notably elevated in CSE-induced cells, while their concentrations were then gradually decreased after treatment with 20, 40, or 60 µM of myricetin in a dose-dependent manner (Fig. 2A–C). Additionally, RT-qPCR further illustrated that the elevation in MCP-1, IL-6, and IL-8 mRNA levels caused by CSE exposure was markedly repressed by myricetin treatment (Fig. 2D–F). Thus, we confirm that myricetin alleviates inflammatory response in CSE-induced A549 cells.

Myricetin represses CSE-induced oxidative stress in A549 cells

To assess the influence of myricetin on oxidative stress in CSE-exposed A549 cells, DCFH-DA staining was applied to determine the intracellular ROS production. We discovered that ROS production in cells exposed to CSE was notably higher than that in control cells. However, ROS production was markedly reduced by myricetin treatment (Fig. 3A,B). Moreover, the content of MDA and the activity of SOD were also measured using commercial kits. The results showed that, compared with the Control group, MDA content in

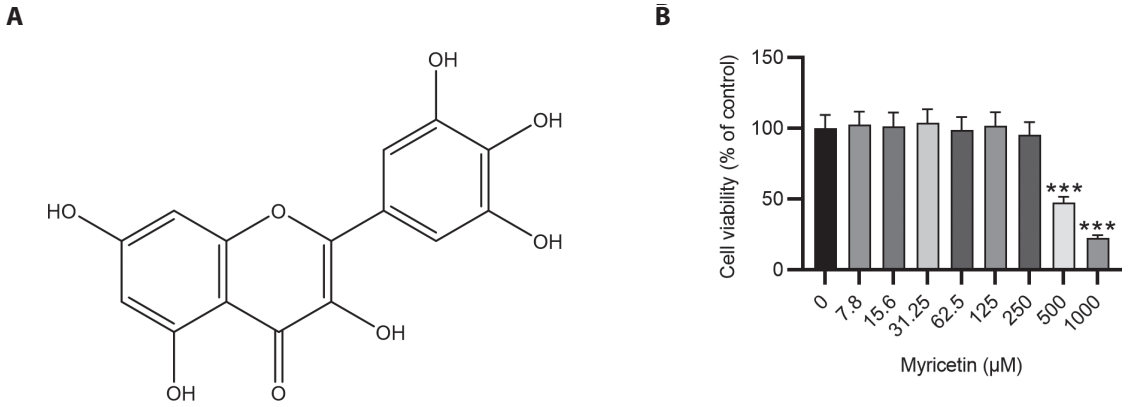

Figure 1. Cytotoxicity of myricetin in A549 cells. **A.** The chemical structure of myricetin. **B.** CCK-8 assay was utilized to test the viability of A549 cells treated with different concentrations of myricetin. *** $p < 0.001$.

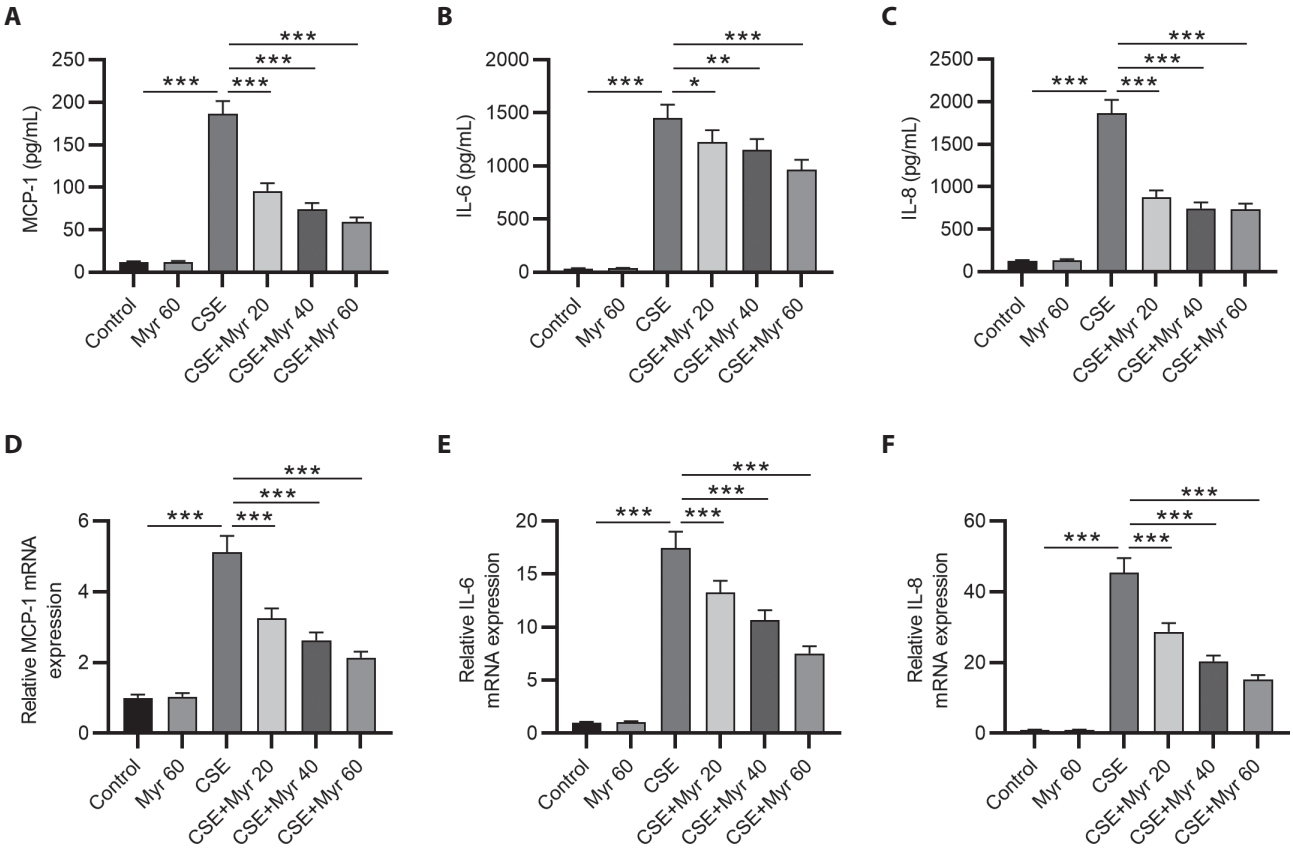

Figure 2. Myricetin alleviates CSE-induced inflammatory response in A549 cells. ELISA results of MCP-1 (**A**), IL-6 (**B**), and IL-8 (**C**) contents in A549 cells of different groups. RT-qPCR was utilized to test the mRNA levels of MCP-1 (**D**), IL-6 (**E**), and IL-8 (**F**) in different groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Control, control group; Myr 60, cells treated with 60 μM myricetin; CSE group, cells treated with 10% CSE in the absence of myricetin; CSE+Myr 20, cells with 10% CSE in the presence of 20 μM myricetin; CSE+Myr 40, cells with 10% CSE in the presence of 40 μM myricetin; CSE+Myr 60, cells with 10% CSE in the presence of 60 μM myricetin.

the CSE group was prominently increased, while myricetin treatment significantly reduced MDA content (Fig. 3C). In contrast, SOD activity was reduced by CSE stimulation, while myricetin treatment counteracted this effect (Fig. 3D). Thus, we prove that myricetin represses oxidative stress in CSE-induced A549 cells.

Myricetin inhibits autophagy of CSE-induced A549 cells

To further determine the regulatory role of myricetin on autophagy of CSE-induced A549 cells, LC3 immunofluorescence assay was performed. We found that the fluorescence intensity of LC3B was enhanced in the CSE group compared with the Control group. Then, with the increase of myricetin concentration, the fluorescence intensity of LC3B gradually weakened, suggesting that the autophagy of CSE-induced A549 cells was repressed by myricetin treatment (Fig. 4A). Furthermore, Western blot demonstrated that the ratio of LC3B-II/LC3B-I and the protein levels of Beclin-1 were increased while the protein levels of p62 was decreased by CSE stimulation and then decreased by 20, 40, or 60 μM of myricetin treatment (Fig. 4B). Overall, these data suggest that myricetin inhibits autophagy of CSE-induced A549 cells.

Myricetin restores pulmonary functions of COPD rats

To confirm whether myricetin represses CS-induced pulmonary injury in COPD rats, the pulmonary function parameters were measured *via* Buxco's Research System. The results revealed that FEV0.1/FVC, mean mid-expiratory flow, and peak expiratory flow were reduced whereas functional residual capacity was elevated in CS-treated rats compared with the control rats. Nevertheless, myricetin (25 mg/kg or 50 mg/kg) treatment significantly reversed CS-induced changes in these parameters (Fig. 5A–D). Overall, these data suggest that myricetin improves pulmonary functions of CS-treated rats.

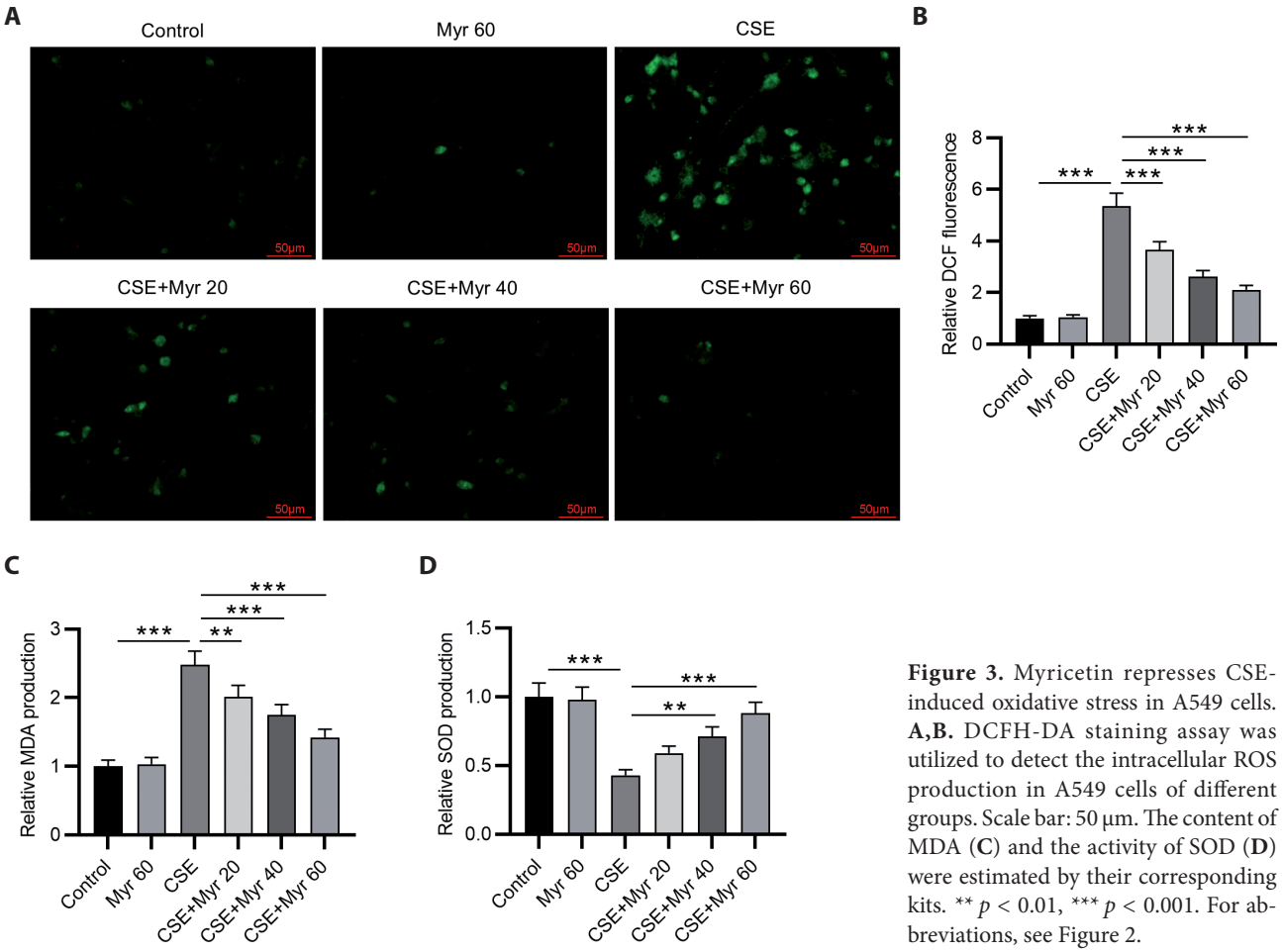

Figure 3. Myricetin represses CSE-induced oxidative stress in A549 cells. **A,B.** DCFH-DA staining assay was utilized to detect the intracellular ROS production in A549 cells of different groups. Scale bar: 50 μm. The content of MDA (**C**) and the activity of SOD (**D**) were estimated by their corresponding kits. ** $p < 0.01$, *** $p < 0.001$. For abbreviations, see Figure 2.

Myricetin ameliorates pulmonary inflammation and emphysema in COPD rats

Through H&E staining, we observed that the alveolar structure of the control rats was complete and infiltration of inflammatory cells was few. However, the number of alveoli was decreased and the pulmonary bulla was formed in the lungs of CS-stimulated COPD rats. Additionally, notable inflammatory changes including hemorrhage, edema, and neutrophil infiltration into the parenchyma and alveolar spaces of the lung were observed in CS-stimulated rats. Nevertheless, these histopathological alterations were ameliorated by myricetin administration (Fig. 6A). Inflammation score and destructive index are crucial parameters that are utilized to quantify the degree of pulmonary inflammation and the injury of alveolar walls. Compared to the Control group, the values of inflammation score and destructive index were markedly elevated in the CS group. However, myricetin administration antagonized CS-induced increment in inflammation score and destructive index (Fig. 6B,C). Thus, myricetin mitigates pulmonary inflammation and emphysema in COPD rats.

Discussion

COPD is a chronic inflammatory disease with high mortality (Bagdonas et al. 2015). Under normal circumstances, the respiratory system is in a balanced state in the body, which is maintained by the interplay between airway epithelium and immune cells (Strzelak et al. 2018). However, in the case of long-term inhalation of harmful gases such as tobacco smoke, this balance can be broken, resulting in inflammatory response and tissue remodeling (Strzelak et al. 2018). Thus, airway epithelial dysfunction may initiate the pathogenesis of COPD. In this study, we used CSE-stimulated bronchial epithelial A549 cells to establish the COPD cell model. Currently, many drugs are clinically used for treating COPD, which, however, have some ineluctable side effects (Rennard and Drummond 2015). Thus, it is urgent to develop safer and more effective therapeutic drugs. Myricetin is a flavonoid

compound and has been confirmed to exhibit beneficial effects in curbing the development of various diseases (Song et al. 2021). Inflammation is one of the main pathological manifestations of COPD (Brightling and Greening 2019). CS can stimulate the infiltration of inflammatory cells in the respiratory tract, releasing inflammatory mediators and chemokines, such as IL-6, IL-8, TNF-α, and MCP-1, which is conducive to emphysema formation (Lu et al. 2021;

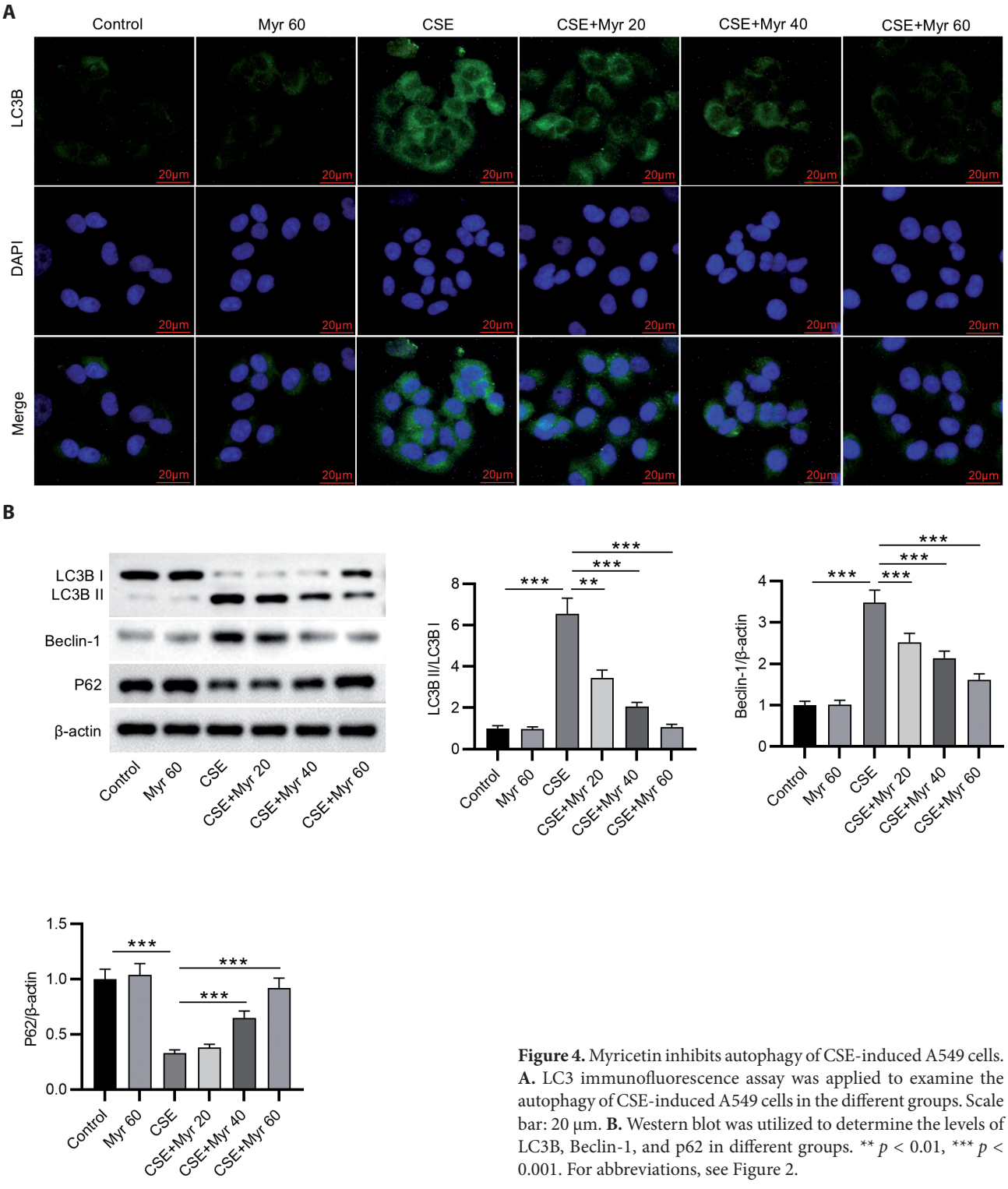

Figure 4. Myricetin inhibits autophagy of CSE-induced A549 cells. **A.** LC3 immunofluorescence assay was applied to examine the autophagy of CSE-induced A549 cells in the different groups. Scale bar: 20 μm. **B.** Western blot was utilized to determine the levels of LC3B, Beclin-1, and p62 in different groups. ** $p < 0.01$, *** $p < 0.001$. For abbreviations, see Figure 2.

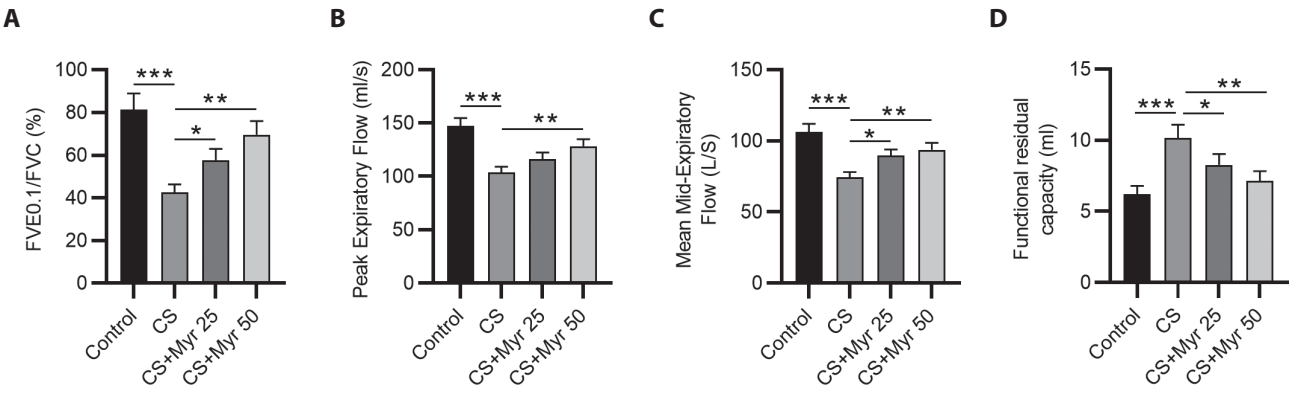

Figure 5. Myricetin restores pulmonary functions of COPD rats. Detection of pulmonary function parameters including FEV0.1/FVC (**A**), peak expiratory flow (**B**), mean mid-expiratory flow (**C**), and functional residual capacity (**D**) in the different groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CS, rats exposed to cigarette smoke. Rats in CS+Myr groups were treated with myricetin (25 or 50 mg/kg) by intraperitoneal injection.

Racanelli et al. 2018). In this study, we discovered that CSE stimulation elevated IL-6, IL-8, and MCP-1 levels in A549 cells. However, myricetin treatment reduced their concentrations in a dose-dependent manner. Previously, myricetin was reported to relieve mastitis by repressing inflammation and restoring the blood-milk barrier (Kan et al. 2019). Myricetin effectively attenuates eosinophil infiltration in the lungs and airway inflammation in asthmatic mice (Huang et al. 2024). Furthermore, myricetin represses TNF-α-induced inflammatory reactions in A549 cells by regulating the SIRT1/NF-κB pathway (Zhang et al. 2018). Thus, we further confirm that myricetin exerts an anti-inflammatory effect in COPD.

CS-induced oxidative stress of the airway epithelium is involved in initiating the pathogenesis of COPD (Sun et al. 2019). Oxidative stress is defined as the unbalance between ROS production and the antioxidant defense mechanism (Sies 2015). The continuous oxidative stress can increase the release of inflammatory mediators, thus aggravating COPD development (Kirkham and Barnes 2013). MDA is the lipid peroxidation product, which reflects the severity of oxidative stress (Tsikas 2017). SOD is a vital antioxidant for scavenging oxygen free radicals, and its activity represents the total antioxidant capacity (Inoue et al. 1991). In this study, we observed that CSE-induced elevation in ROS production and MDA content as well as reduction in SOD activity were reversed by myricetin treatment. Accordingly, we validate that myricetin impedes COPD progression by inhibiting oxidative stress injury. Myricetin protects NK cells from

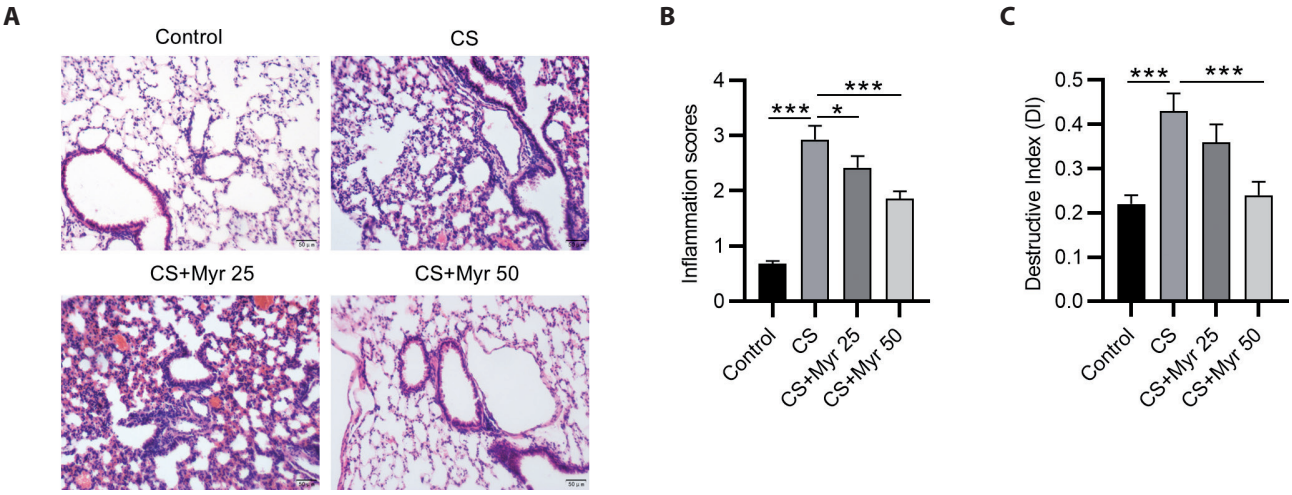

Figure 6. Myricetin ameliorates pulmonary inflammation and emphysema in COPD rats. **A.** H&E staining assay was utilized to reveal the histopathological changes of rat lungs in the different groups. **B.** The lung inflammation is measured by the inflammation scores. **C.** The destruction of alveolar walls is defined as DI. * $p < 0.05$, *** $p < 0.001$. For abbreviations, see Figure 5.

DNA injury by repressing oxidative stress (Ma et al. 2021). Myricetin suppresses tert-butyl hydroperoxide-induced upregulation in the levels of oxidative stress parameters in diabetic erythrocytes (Pandey et al. 2009). Myricetin represses acute liver injury by inhibiting oxidative stress and inflammatory response (Berköz et al. 2021). These studies further support our findings.

Autophagy dysregulation is closely associated with the pathogenesis of COPD (Tan et al. 2019). Exposure to CS-induced oxidative stress and inflammation leads to autophagy, which promotes emphysema formation and accelerates COPD deterioration (Zhang Q et al. 2021). The upregulation of autophagic protein levels is observed in the lung tissues from COPD patients (Chen et al. 2008). LC3B is the main autophagy-related protein, and it represents autophagosome formation by conversing from LC3B-I to LC3B-II (Schaaf et al. 2016). It was reported that LC3B plays avital pathogenic function in CS-induced emphysema (Chen et al. 2010). Beclin-1 is an essential molecule during autophagosome formation, and it can mediate the localization of other autophagy proteins to phagocytic vesicles, thereby regulating autophagosome formation and maturation (Pabón et al. 2018). p62 is a ubiquitin-binding protein that forms a complex with LC3-II protein localized in the inner membrane of autophagosomes during autophagy and is degraded in autophagic lysosomes (Vishnupriya et al. 2020). The level of p62 indirectly reflects the clearance level of autophagosomes, and its expression decreases when autophagy occurs in the cell (Vishnupriya et al. 2020). In this study, we observed that the ratio of LC3B-II/LC3B-I and the protein levels of Beclin-1 were elevated and the protein level of p62 was reduced in A549 cells stimulated by CSE, while CSE-induced changes in the levels of these autophagy markers were reversed by myricetin treatment. It suggested that myricetin could repress autophagy in COPD. Myricetin was reported to induce apoptosis through the MAPK pathway and regulate JNK-mediated autophagy in SK-BR-3 cells (Han et al. 2022). Myricetin slows the liquid-liquid phase separation of Tau and activates ATG5-dependent autophagy to suppress Tau toxicity (Dai et al. 2021). Our study confirms for the first time that myricetin is involved in the development of COPD by mediating autophagy.

CS-induced COPD animal model has been studied extensively (Vij et al. 2018). In this study, we established a CS-induced COPD rat model. We found that myricetin treatment effectively restored lung functions of COPD rats. Furthermore, myricetin treatment repressed the inflammatory response and the destruction of alveolar walls in the lungs of COPD rats. These data suggest that myricetin treatment inhibited pulmonary inflammation and emphysema *in vivo*.

Taken together, this study confirms that myricetin protects A549 cells against CSE-induced inflammation and oxidative stress by suppressing autophagy, which can provide a novel therapeutic agent for COPD. The limitation of this study is that we did not explore the detailed molecular mechanism through which myricetin regulates autophagy in COPD, which will be the focus of future research.

Conflicts of interests. No conflicts of financial interest are enclosed in this study.

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Ethical approval. All the experiments were approved by the Ethics Committee of Yichang Central People's Hospital and were performed in line with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals".

Authors' contributions. Mi Zhang conceived and designed the experiments. Mi Zhang and Ming Zhan carried out the experiments. Mi Zhang and Ming Zhan analyzed the data. Mi Zhang and Ming Zhan drafted the manuscript. Xinyu Son revised the manuscript. All authors agreed to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

Data availability statement. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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