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Protocatechuic acid alleviates chronic obstructive pulmonary disease model by reducing inflammation and oxidative stress *via* the AHR/CYP1A1/CYP1A2 pathway

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Abstract. This study aimed to investigate the protective effects of protocatechuic acid (PCA), a natural phenolic acid, on a chronic obstructive pulmonary disease (COPD) model. The COPD mouse model was induced through cigarette smoke (CS) exposure, followed by administration of dexamethasone or PCA during modeling. Human bronchial epithelial (HBE) cells were stimulated with CS extract and treated with or without PCA (40 μM) and/or the aryl hydrocarbon receptor (AHR) activator FICZ (20 nM). Model mice exhibited significant weight loss, impaired lung function, and severe lung injury. Moreover, CS exposure promoted inflammation and oxidative stress and activated the AHR/CYP1A1/CYP1A2 pathway in mice. PCA significantly alleviated COPD symptoms by inhibiting inflammation, oxidative stress, and the AHR/CYP1A1/CYP1A2 pathway. CS extract-stimulated HBE cells exhibited decreased cell viability, increased inflammatory response and oxidative stress, and upregulation of AHR/CYP1A1/CYP1A2 pathway. These effects were reversed by PCA, but FICZ inhibited the protective effects of PCA on HBE cells. In conclusion, PCA improves COPD model by reducing inflammation and oxidative stress through inhibition of the AHR/CYP1A1/CYP1A2 pathway.

Key words: Chronic obstructive pulmonary disease — Protocatechuic acid — Inflammation — AHR/CYP1A1/CYP1A2 pathway — Oxidative stress

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous lung disease that affects a large portion of the global population, bringing a substantial medical and economic burden on society (Liao et al. 2024). The pathogenesis of COPD involves genetic susceptibility, respiratory viruses, tobacco use, and ambient fine particulate matter (Lahmar et al. 2022). Inhalation of harmful substances leads to oxidative stress and inflammation in the lungs, with chemokines recruiting and activating immune cells (Pang and Liu 2024). Persistent inflammation damages tissues and contributes to emphysema, which impairs the defense and repair functions of the respiratory system, eventually resulting in fibrosis of small airways and airflow obstruction (Xu J et al. 2024). Commonly used drugs for COPD include corticosteroids and muscarinic antagonists, which focus on symptom relief (Vogelmeier et al. 2020). However, safe and effective therapies for COPD are still limited.

Aryl hydrocarbon receptor (AHR) is a member of the basic helix-loop-helix superfamily and exists in the cyto-

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sol in an inactivated state (Sládeková et al. 2023). When ligands bind to AHR, it translocates to the nucleus, where it combines with nuclear transporters and interacts with xenobiotic-responsive elements to regulate numerous Cytochrome P450 (CYP) family genes, such as CYP1A1 and CYP1A2 (Bahman et al. 2024). Both CYP1A1 and CYP1A2 can metabolize diverse compounds and drugs, showing overlapping substrate specificity (Kapelyukh et al. 2019). Additionally, AHR collaborates with other transcription factors and signaling molecules in a ligand-, cell-, and microenvironment-dependent manner to regulate physiological and pathological functions, including cell fate determination, immune response regulation, and adaptation to oxidative stress (Sondermann et al. 2023). Studies have shown that AHR and its downstream gene (CYP1A1 and CYP1A2) levels are increased in the kidneys of patients with idiopathic membranous nephropathy, and inhibition of AHR signaling can reduce oxidative stress and inflammation, thereby improving kidney injury (Wang YN et al. 2023). Moreover, AHR is widely expressed in lung epithelial cells and contributes to barrier defense (Xu L et al. 2024). Particulate matter 2.5 exposure activates AHR translocation to upregulate Transmembrane Protein Synthesis Suppressor 2 and interleukin (IL)-18 expression, which promotes lung cancer progression (Wang TH et al. 2023). The environmental pollutant 1-nitropyrene induces oxidative stress and increases CYP1A1 and CYP1A2 levels in rats (Li et al. 2017). However, the role of the AHR/CYP1A1/CYP1A2 pathway in COPD remains unclear.

Protocatechuic acid (PCA) is a benzoic acid polyphenol found in grapes, star anise, and rosemary (Kelidari et al. 2024). PCA has multiple properties, such as antioxidant, anti-inflammatory, and anti-aging effects (Song et al. 2020). PCA regulates oxidative stress, inflammation, and drug-metabolizing enzyme activities to protect liver (Asejeje et al. 2023). Additionally, PCA has been proven to have a significant binding affinity for CYP1A2 (Zhuang et al. 2021). Research has shown that PCA may suppress oxidative stress and inflammation to ameliorate septic lung injury (Alsharif et al. 2021). Furthermore, PCAME (the methylated form of PCA) reduces fluoride accumulation and oxidative stress, and improves fluoride-induced lung changes in rats (Ameeramja et al. 2018). However, the role and mechanisms of PCA in COPD have not been explored.

Based on these findings, this study used cigarette smoke (CS)-induced mice and CS extract-stimulated human bronchial epithelial (HBE) cells to investigate the protective effects of PCA on COPD models, focusing on changes in inflammation, oxidative stress, and the AHR/CYP1A1/CYP1A2 pathway. The role of the AHR/CYP1A1/CYP1A2 pathway in PCA's prevention of COPD models was confirmed using the AHR activator FICZ. The workflow of this study is shown in Figure 1.

Materials and Methods

Animals and experimental protocol

BALB/c male mice (6–8 weeks, 20 ± 3 g, Yangzhou University) were kept in a room at 24 ± 2°C with a 12-h light/dark cycle. PCA was dissolved in normal saline. The Experimental Animal Ethics Committee of Yangzhou University approved these animal experiments (No.202408035).

After one week of adaptation, twenty-five mice were randomly assigned to five groups (n = 5): Control, CS, Dex (dexamethasone 2 mg/kg; MCE®, Cat. No. HY-14648), L-PCA (low-dose PCA, 5 mg/kg; MCE®, Cat. No. HY-N0294), and H-PCA (high-dose PCA, 10 mg/kg). The dosage of PCA was determined based on previous research (Yang X et al. 2023a). Control mice were placed in an indoor environment, while the other mice were exposed to 3R4F standard cigarettes for 60 min daily using a whole-body exposure setup, 6 times a week, over 15 weeks, to establish COPD mouse model. Animals in the Dex, L-PCA, and H-PCA groups were orally administered the corresponding drug starting on day 9 of modeling, 1 h before CS exposure, once a day until day 105 (Huang et al. 2022). In the CS group, mice were given equal normal saline. Mice were weighed on days 0 and 105 after modeling. All animals were euthanized on day 105 with 5% isoflurane inhalation.

Detection of lung function

The mice were anesthetized by intraperitoneal injection of pentobarbital sodium, followed by tracheotomy. Functional residual capacity (FRC), resistance index (RI), dynamic compliance (Cdyn), and volume *per* minute (MV) were detected *in situ* using a Forced Maneuvers apparatus (DSI, Buxco PFT) to assess mouse lung function (Wang et al. 2022). FRC was calculated by allowing the mice to dilute the nitrogen in the alveoli by repeatedly inhaling pure oxygen, and by measuring the nitrogen concentration in the lungs and the total amount of breathing gas before and after pure oxygen irrigation. The principle of RI measurement is based on the relationship between the airway pressure and the airflow velocity. Cdyn refers to the dynamic response of lung volume to changes in airway pressure during breathing, measured by applying a certain pressure change. Tidal volume and respiratory rate were measured, and the volume per minute (MV) was calculated as the product of the two. These parameters are closely related to COPD progression, and lung function tests based on these indicators are often used in clinical practice to assess lung hyperinflation and airway resistance in COPD patients (Chen Y et al. 2024a, Li et al. 2024).

Collection of tissue samples

The whole blood of mice was left for 1–2 h and centrifuged to obtain serum. After lung function measurements were

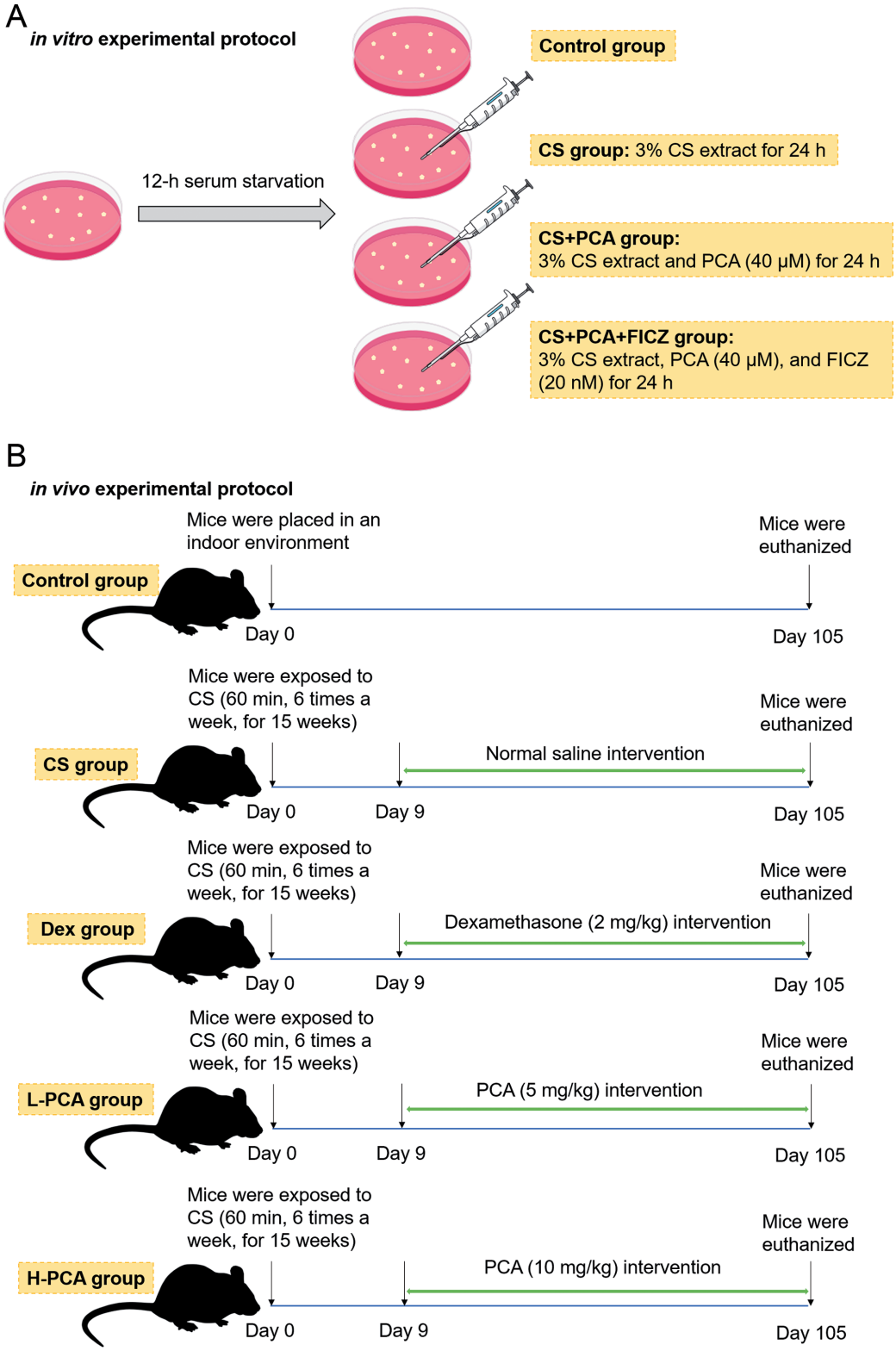


Figure 1. The workflow of this study. **A.** *In vitro* experimental protocol. HBE cells were subjected to 12-h serum starvation and were divided into the Control group (normal treatment), CS group; CS+PCA group, and CS+PCA+FICZ group. **B.** *In vivo* experimental protocol. Mice were randomly divided into the Control group, CS group, Dex group, L-PCA group, and H-PCA group. On day 105, the mice were euthanized. CS, cigarette smoke; Dex, dexamethasone; PCA, protocatechuic acid.

completed, intubation was performed on the mice. The right lung was ligated, while the left lung was rinsed three times with 0.3 ml of pre-cooled saline to collect bronchoalveolar lavage fluid (BALF) (Zhang et al. 2024). The lung tissue of mice was mixed with normal saline and grinding beads, and then homogenized with a tissue grinder to acquire lung tissue homogenate.

Pulmonary pathological staining

The mouse lungs were placed in 4% paraformaldehyde (Beyotime, P0099), embedded in paraffin wax (Sinopharm, 69019061), cut into sections with 5 μm thickness, and stained with an HE staining kit (Beyotime, C0105S). The sections were photographed using a microscope (Leica, DM3000). Three regions were randomly selected from the images of each section for mean linear intercept (MLI) analysis. MLI is a quantitative parameter to determine the enlargement of the pulmonary air cavity, and it is calculated by dividing the length of the line drawn on the lung section by the total number of alveolar intercepts counted within that line (Kettawan et al. 2024).

Immunohistochemistry

Lung sections were repaired with a citric acid repair solution (Beyotime, P0083). Following treatment with 3% H_2O_2 solution (Sinopharm, 10011208), the sections were incubated with normal goat serum solution (Solarbio, SL038) for 10–15 min. CYP1A1 (1:100, Abcam, ab235185) and CYP1A2 (1:200, Abcam, ab151728) antibodies were applied to sections overnight. Following incubation with the secondary antibody (Abcam, ab6721) for 10–15 min, sections were treated with 3,3'-Diaminobenzidine (Beyotime, P0202) and stained with hematoxylin. The results were observed under an optical microscope (Olympus, CKX53) after the sections were dehydrated and sealed. CYP1A1 and CYP1A2-positive cells in mouse lung tissue were analyzed by Olympus Image Analyzer.

Preparation of CS extract

A 3R4F standard cigarette was smoked for 5 min, and the smoke was extracted using 10 ml RPMI-1640 medium (Gibco, C11875500BT). After filtration using a 0.22 μm filter, 100% CS extract solution was obtained.

Cell culture

HBE cells (Shanghai Chuanqiu Biotechnology Co., LTD) were inoculated into RPMI-1640 medium containing 10% fetal bovine serum at 37°C in 5% CO_2. HBE cells were serum starved for 12 h before the experiment to synchronize the cell cycle. Cells were divided into Control, CS, CS+PCA, and CS+PCA+FICZ (FICZ is an AHR activator, MCE®, Cat. No. HY-12451) groups. For Control group, HBE cells were cultured normally, while the other groups were exposed to 3% CS extract for 24 h, with or without PCA (40 μM) and/or FICZ (20 nM).

Cell counting kit-8 (CCK-8) assay

PCA at 0, 20, 40, and 80 μM concentrations was added to HBE cells for 24 h. Cells were then incubated with 10 μl CCK-8 (Beyotime, C0037) for 2 h, and absorbance was detected at 450 nm using a microplate reader.

Assessment of inflammation and oxidative stress levels

IL-6, IL-1β, tumor necrosis factor-alpha (TNF-α), CXCL1, CXCL2, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels in serum, BALF, lung tissue homogenate, and supernatant were measured by kits following manufacturer's instructions. Mouse IL-6, mouse IL-1β, mouse TNF-α, mouse CXCL1, mouse CXCL2, human TNF-α, human CXCL1, human CXCL2 kits were purchased from Mlbio, while human IL-6 and human IL-1β were obtained from Beyotime. The type of these kits is Sandwich ELISA. MDA kits were purchased from Nanjing Jiancheng, CAT kits were acquired from Solarbio, and SOD kits were obtained from Mlbio. These kits use colorimetric measurements. Absorbance of the samples was detected by a microplate reader (Wuxi Hiwell Diatek, DR-200Bs).

Western blot

RIPA lysis buffer (Beyotime, P0013B) was used to prepare the lysates of lung tissue and HBE cells. The total protein concentrations of the samples were detected using a bicinchoninic acid assay kit (Beyotime, P0010S). The proteins were isolated by electrophoresis and transferred to polyvinylidene fluoride membranes (Beyotime, FFP24). Membranes were treated with 5% skim milk (Beyotime, P0216) for 1 h before incubation with anti-AHR (1:500, Abcam, ab308215), anti-CYP1A1 (1:1000), and anti-CYP1A2 (1:1000) antibodies overnight. Subsequently, membranes were treated with secondary antibodies for 1 h and observed by enhanced chemiluminescence (Applygen, P1000). The blot density was quantified by iBright® CL750 Imaging system (Applied Biosystems, NY, USA).

Data analysis

Statistical analysis was performed on GraphPad 7.0 software. One-way analysis of variance was utilized to compare the

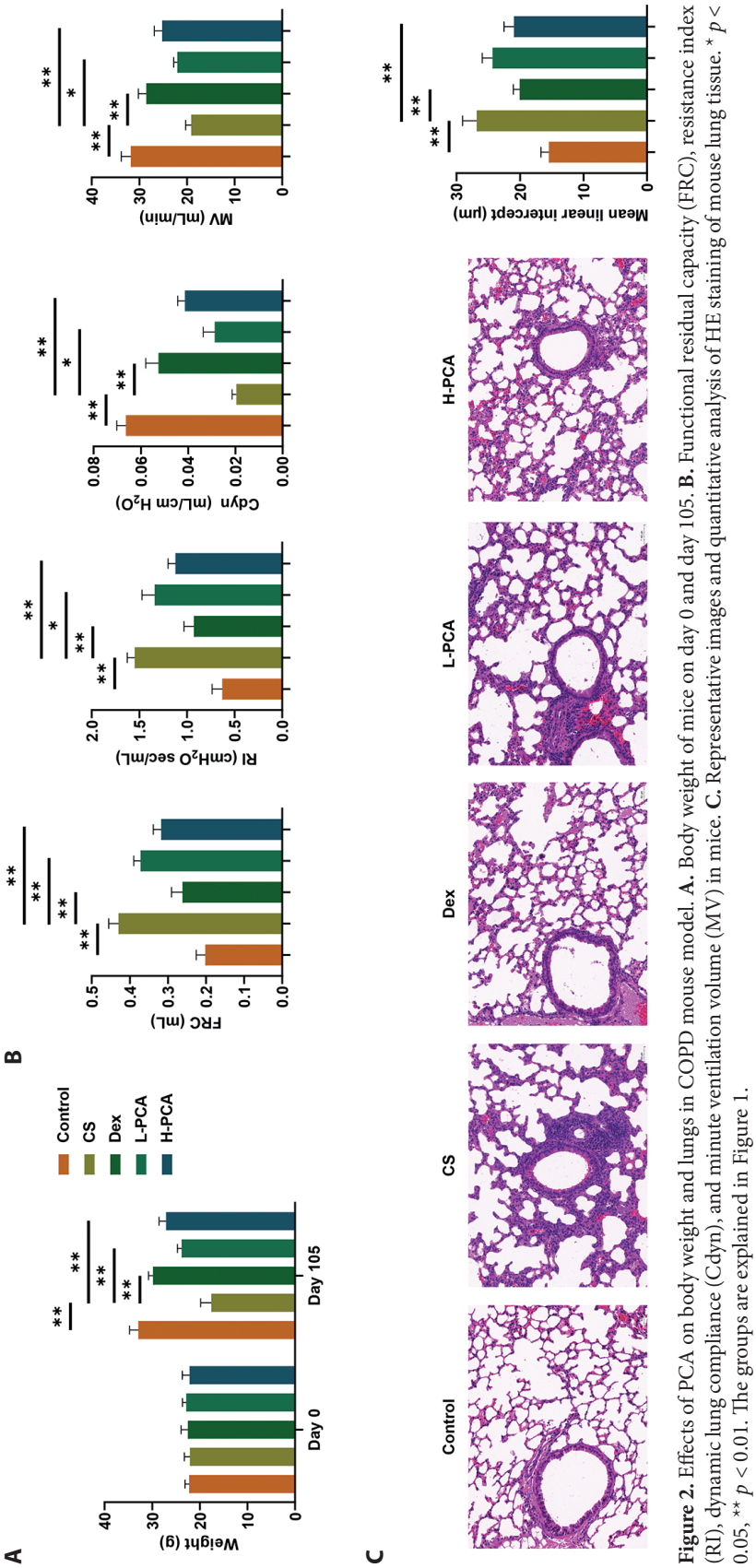
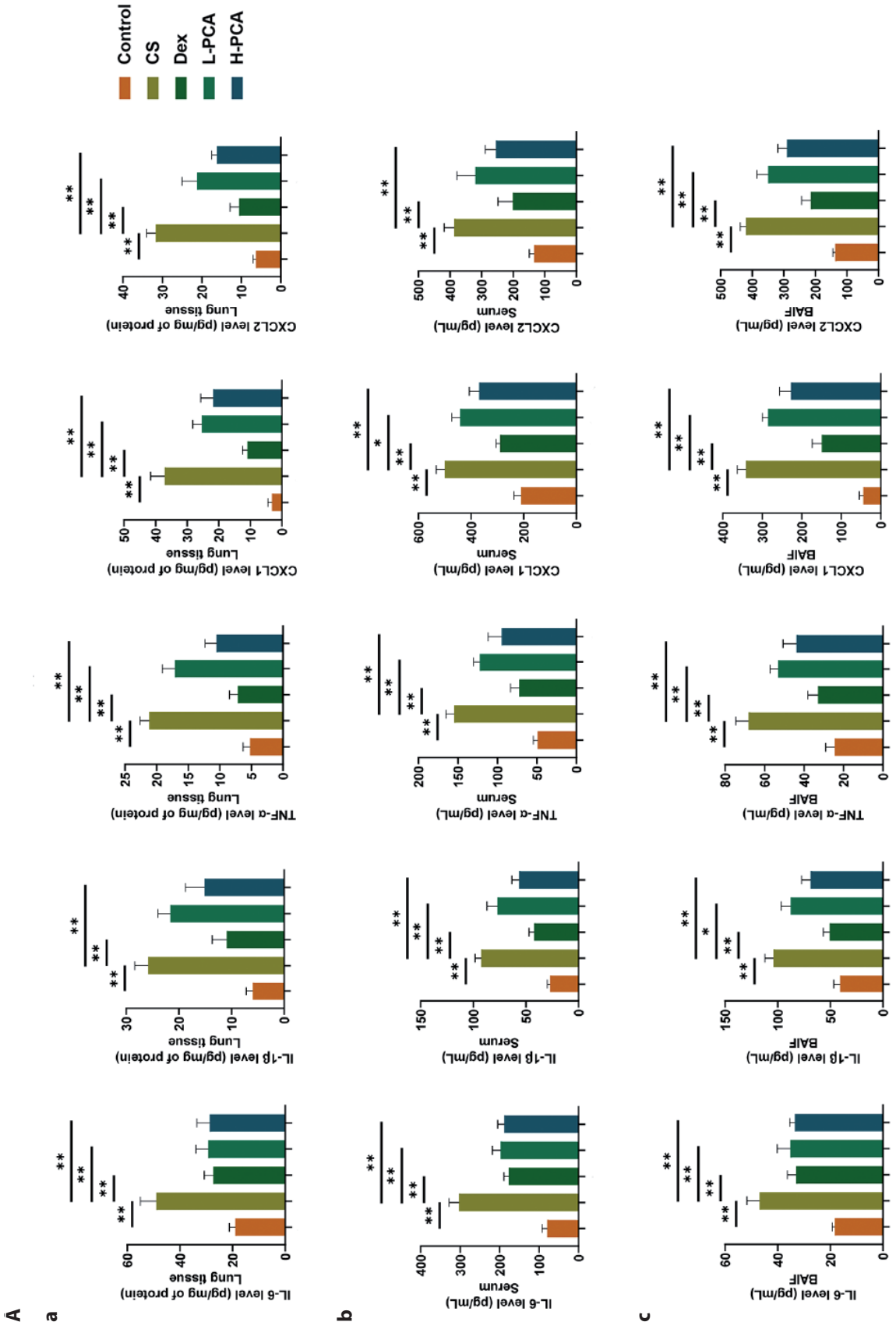


Figure 2. Effects of PCA on body weight and lungs in COPD mouse model. **A.** Body weight of mice on day 0 and day 105. **B.** Functional residual capacity (FRC), resistance index (RI), dynamic lung compliance (Cdyn), and minute ventilation volume (MV) in mice. **C.** Representative images and quantitative analysis of HE staining of mouse lung tissue. * $p < 0.05$, ** $p < 0.01$. The groups are explained in Figure 1.


A
a
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Control
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IL-6 level (pg/mg of protein) Lung tissue
IL-6 level (pg/mL) Serum
IL-6 level (pg/mL) BALF
IL-1β level (pg/mg of protein) Lung tissue
IL-1β level (pg/mL) Serum
IL-1β level (pg/mL) BALF
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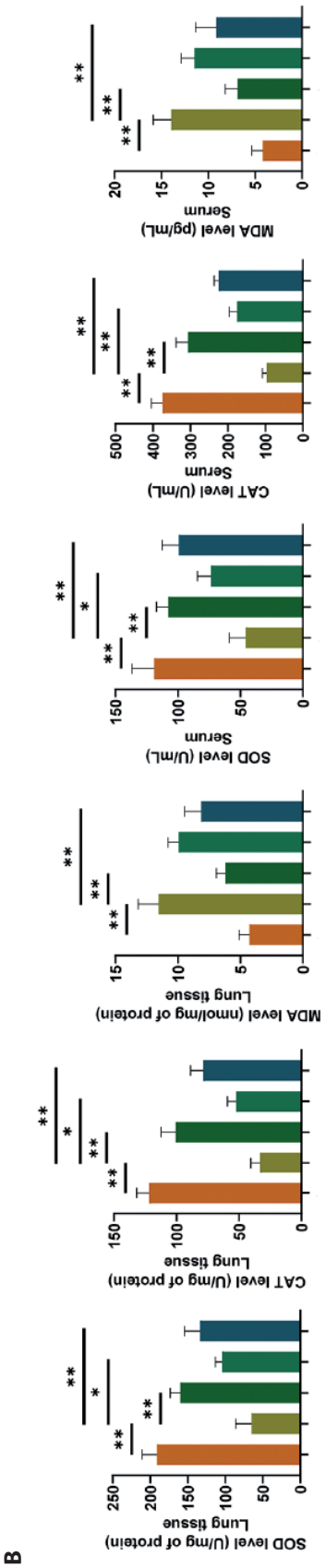

Figure 3. Effects of PCA on inflammation and oxidative stress in COPD model. **A.** The levels of pro-inflammatory factors (IL-6, IL-1β, and TNF-α) and chemokines (CXCL1 and CXCL2) in mouse lung tissue (a), serum (b), and BALF (c). **B.** The levels of oxidative stress markers (SOD, CAT, and MDA) in mouse lung tissue and serum. * $p < 0.05$, ** $p < 0.01$. IL, interleukin; TNF, tumor necrosis factor; BALF, bronchoalveolar lavage fluid; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. The groups are explained in Figure 1.

differences between groups. All data are expressed as mean ± standard deviation. $p < 0.05$ was considered statistically different.

Results

PCA improved COPD symptoms in CS-induced mice

Typical symptoms of COPD are systemic, one of them is weight changes. Mice were weighed on days 0 and 105, and results showed no significant differences among all groups at the beginning (Fig. 2A). However, CS induction markedly decreased the mice's body weight on day 105. A forced motor device was used to assess lung function in mice. FRC and RI were significantly elevated, while Cdyn and MV were decreased after CS induction (Fig. 2B). The lung tissue structure of control mice was intact, with normal alveolar shape, while in CS-induced mice, the alveolar structure was disordered, alveolar septa were disrupted, and pulmonary bronchus exhibited abnormal shape with obvious inflammatory infiltration (Fig. 2C). For quantitative morphological changes of mouse lungs, MLI was significantly increased after CS induction (Fig. 2C). These changes were consistent with the typical pathological features of COPD, indicating the successful establishment of COPD mouse model. In COPD model, both low and high doses of PCA significantly increased body weight, Cdyn, and MV levels, reduced FRC and RI levels and alleviated lung tissue injury. PCA improved COPD symptoms. Dexamethasone is an effective drug for treating COPD (Zeng et al. 2023), therefore, it was used as a positive drug to evaluate the efficacy of PCA in this study. Dexamethasone also significantly improved COPD model, and was more effective than PCA.

PCA alleviated inflammation and oxidative stress in COPD model

The levels of inflammation- and oxidative stress-associated markers were measured. The results showed that IL-6, IL-1β, TNF-α, CXCL1, and CXCL2 levels in lung tissue, serum, and BALF of COPD model were markedly higher than those in controls, while both dexamethasone and high-dose PCA markedly inhibited the expression of these factors (Fig. 3A). Moreover, SOD and CAT expression in both lungs and serum of CS-induced mice was significantly reduced compared to controls, while both PCA and dexamethasone partially restored their levels in COPD model (Fig. 3B). Additionally, MDA levels significantly increased after CS induction, but were decreased in both the Dex and H-PCA groups compared to the CS group. PCA reduced the levels of markers of inflammation and oxidative stress in COPD model.

PCA inhibited the AHR/CYP1A1/CYP1A2 pathway in COPD model

As shown in Figure 4A, AHR protein expression was observably increased after CS induction, while both PCA and dexamethasone markedly reduced AHR expression in CS-induced mice. Moreover, the results revealed that CYP1A1 and CYP1A2 levels were markedly elevated in COPD model compared to controls, and decreased after PCA or dexamethasone intervention (Fig. 4B). PCA inhibited the AHR/CYP1A1/CYP1A2 signaling pathway in CS-induced COPD model.

PCA reduced inflammation and oxidative stress via the AHR/CYP1A1/CYP1A2 pathway in CS extract-induced HBE cells

CCK-8 results showed that PCA at 20, 40, and 80 μM all had no significant toxicity to HBE cells (Fig. 5A), and 40 μM PCA was selected for subsequent experiments. The protein levels of the AHR/CYP1A1/CYP1A2 pathway were detected by Western blot. The expression of AHR, CYP1A1, and CYP1A2 was markedly elevated following CS stimulation but decreased after PCA intervention (Fig. 5B,C). Notably, the AHR activator FICZ markedly increased AHR expression

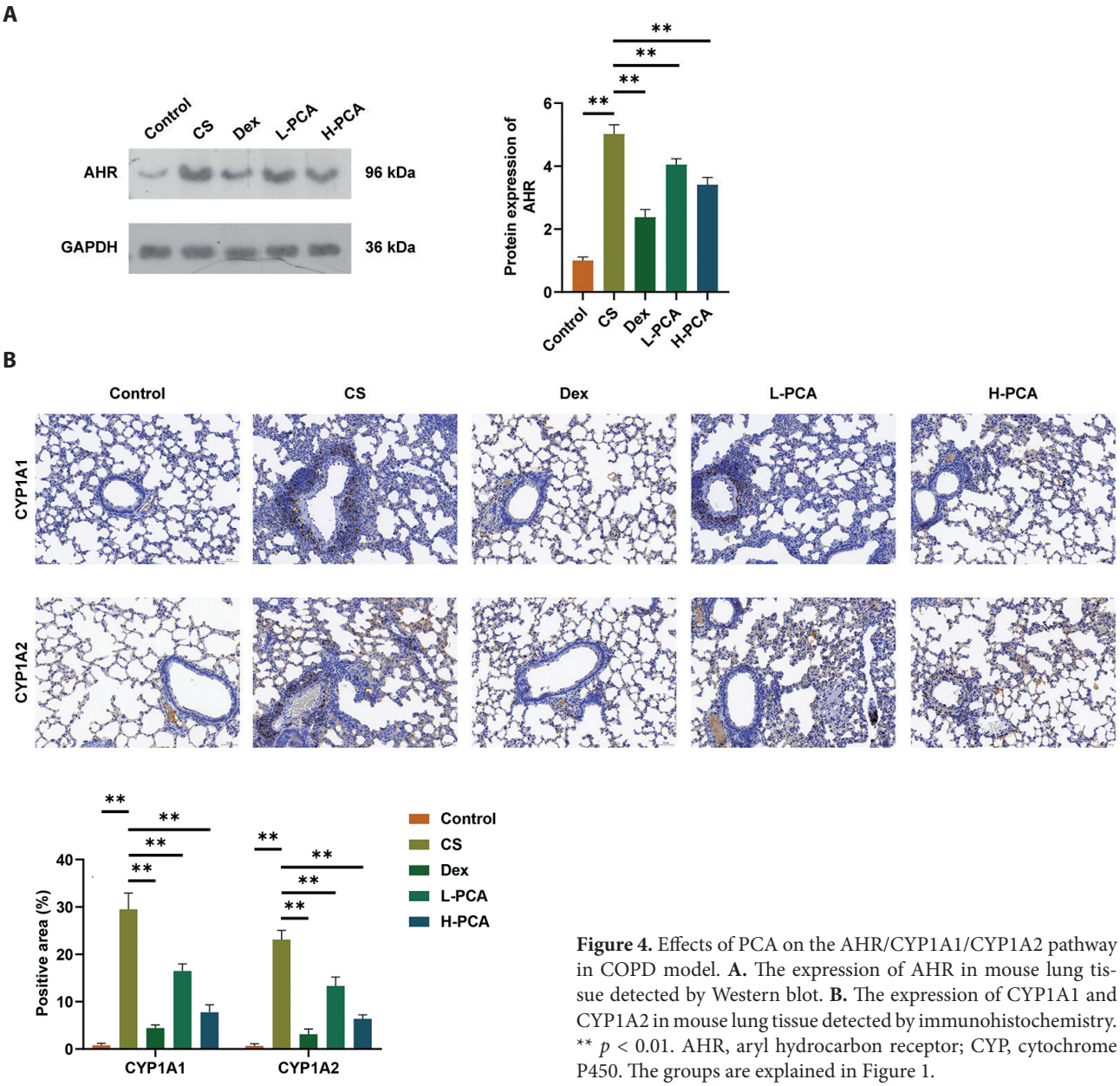

Figure 4. Effects of PCA on the AHR/CYP1A1/CYP1A2 pathway in COPD model. **A.** The expression of AHR in mouse lung tissue detected by Western blot. **B.** The expression of CYP1A1 and CYP1A2 in mouse lung tissue detected by immunohistochemistry. ** $p < 0.01$. AHR, aryl hydrocarbon receptor; CYP, cytochrome P450. The groups are explained in Figure 1.

in CS+PCA group, as well as CYP1A1 and CYP1A2. The results revealed that CS induction significantly decreased the cell viability of HBE cells, but PCA partially restored the cell viability inhibited by CS, and FICZ weakened the effect of PCA (Fig. 5D). Moreover, IL-6, IL-1β, TNF-α, CXCL1, CXCL2, and MDA levels were significantly increased after

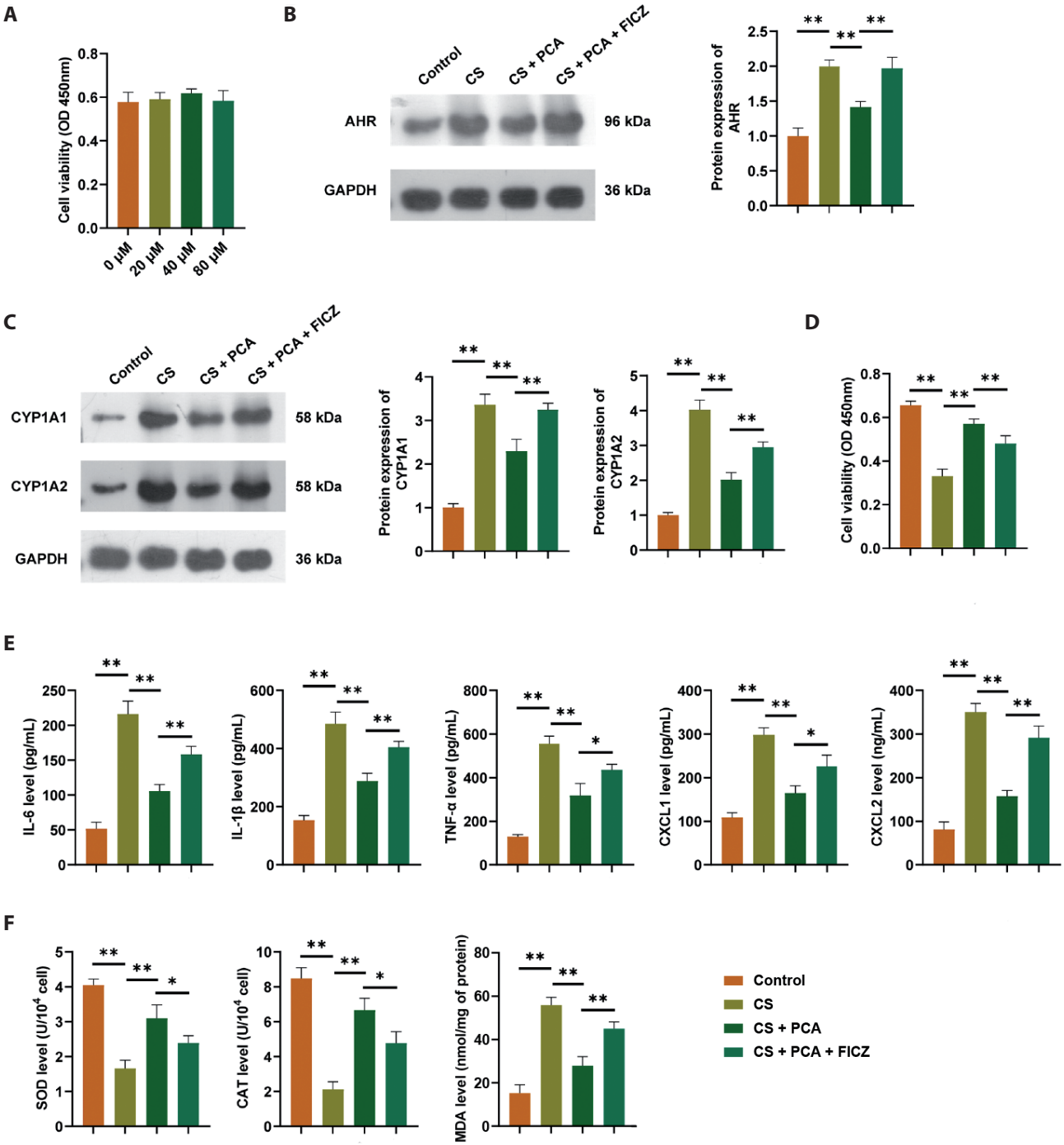

Figure 5. PCA improved inflammation and oxidative stress by inhibiting the AHR/CYP1A1/CYP1A2 pathway in CS extract-induced HBE cells. **A.** Effect of different concentrations of PCA on cell viability of HBE cells. **B.** The expression of AHR detected by Western blot. **C.** The expression of CYP1A1 and CYP1A2 detected by Western blot. **D.** Cell viability detected by cell counting kit-8 assay. **E.** The levels of pro-inflammatory factors (IL-6, IL-1β, and TNF-α) and chemokines (CXCL1 and CXCL2) in HBE cells. **F.** The levels of oxidative stress markers (SOD, CAT, and MDA) in HBE cells. * $p < 0.05$, ** $p < 0.01$. The groups are explained in Figure 1.

CS stimulation, while the levels of SOD and CAT were observably decreased. PCA altered the expression of these factors, but this effect was reversed by FICZ (Fig. 5E,F). PCA reduced inflammation and oxidative stress in COPD model, which may be related to the inhibition of the AHR/CYP1A1/CYP1A2 pathway.

Discussion

COPD is a fatal lung disease with no current cure, and treatments can only relieve symptoms and delay progression. This study explored PCA's effect on CS-induced COPD models, both *in vivo* and *in vitro*, and investigated its underlying mechanisms. The results showed that PCA reduced inflammation and oxidative stress, thereby improving COPD models through AHR/CYP1A1/CYP1A2 pathway.

COPD is the leading cause of death worldwide, contributing to progressive airflow restriction, chronic inflammation, lung damage, and reduced lung function (Vlahos et al. 2023). Both host factors (genes, sensitivity of lung cells to stimuli, and incomplete lung development at an early age) and external factors (smoking and air pollutants) are risk factors for the development of COPD (Tanner and Single 2020). Tobacco smoke exposure is a commonly used method for inducing a COPD model (Upadhyay et al. 2023). Research has demonstrated that CS-induced mice exhibit impaired lung function and significant lung injury, while CS extract treatment increases apoptosis in BEAS-2B cells (Li et al. 2024). Similarly, we found that the COPD mouse model displayed weight loss, decreased lung function, and lung tissue damage. Additionally, CS extract significantly reduced cell viability in HBE cells compared to controls.

The potential for immunomodulation in treating COPD is gradually being uncovered, as various cells, such as epithelial cells, dendritic cells, neutrophils, and macrophages, are all involved in COPD progression (Bu et al. 2020). Moreover, both structural and immune cells in lung tissue express chemokines and their receptors, and targeting these can regulate chronic inflammation and bronchial remodeling (Henrot et al. 2019). Therefore, inhibiting inflammatory response and chemotaxis may be an effective way to delay airway remodeling in COPD. Moreover, maintaining redox homeostasis in the respiratory system is crucial for the host immune system. Excessive free radical production or a decrease in endogenous antioxidant defense can lead to oxidative stress, resulting in lung injury in COPD (Thomson 2018). Studies have shown that hochuekkito treatment reduced neutrophils and lymphocytes, as well as Cxcl1 and Cxcl2 levels, in mice with acute exacerbation of COPD (Fukuda et al. 2024). In mice with a COPD model induced by CS extract combined with lipopolysaccharide, the levels of IL-8, TNF-α, and MDA increased, while SOD decreased. However, Codonopsis Radix inhibited inflammation and oxidative stress, enhanced lung function, and reduced lung tissue damage in this COPD model (Chen Z et al. 2024). Consistent with the above evidence, we found that CS-induced COPD models exhibited increased expression of pro-inflammatory factors, chemokines, and peroxidation markers, along with decreased expression of antioxidant markers, suggesting the occurrence of inflammation and oxidative stress in the COPD models.

PCA, a phenolic acid with various biological activities, is found in many natural plants and herbs, such as green tea and *Salvia miltiorrhiza* (Chen Y et al. 2024b). As a plant-derived chemical, the adverse effects of PCA are minimal, and its dietary intake is at least 100–500 times lower than the intake required to cause negative impacts (Riaz et al. 2024). PCA has demonstrated therapeutic effects on various diseases such as obesity, tumors, and atherosclerosis (Ding et al. 2024, Inacio et al. 2024, Xiang et al. 2024). Research has shown that PCA administration enhances cellular antioxidant ability by modulating Nrf2/HO-1 pathway and inhibiting inflammation in myocardial infarction (Li et al. 2023). Additionally, PCA can reduce cyclophosphamide-induced alveolar emphysema and bronchiolar epithelial hyperplasia, which is linked to its antioxidant and anti-inflammatory activities (Salama et al. 2023). Consistent with this, we revealed that PCA reduced the expression of inflammatory factors and markers of oxidative stress, decreased cell death, and improved lung function and injury in COPD models.

AHR is a multifunctional environmental sensor that is highly expressed in the lungs (Pariano et al. 2023). The expression of both lung and systemic AHR is reduced in COPD patients, with the mRNA level of systemic AHR positively correlated with lung function, and AHR deficiency promotes COPD development by controlling multiple pathogenic mechanisms (Guerrina et al. 2021). Mice exposed to Indeno[1,2,3-cd]pyrene (IP, an air pollutant) developed antigen-induced allergic inflammation, which was not observed in mice with dendritic cell-specific AHR deficiency, suggesting that IP-induced lung inflammation may be caused by AHR-dependent regulation of dendritic cell function (Wong et al. 2018). Furthermore, CYP1 family members, such as CYP1A1 and CYP1A2, are regulated by AHR, and it has been confirmed that co-exposure to methylmercury and TCDD increases CYP1A1 and CYP1A2 expression in lungs of mice (Alqahtani et al. 2024). Previous studies have shown that AHR can regulate the production of excessive reactive oxygen species and inflammation in hydroquinone-induced JHP cells (Yang X et al. 2023b). Inhibiting the AHR/CYP1A1 pathway can improve oxidative stress, reduce inflammation, and delay the development of COPD model (Zhu et al. 2023). Similarly, we revealed that the AHR/CYP1A1/CYP1A2 pathway was activated in CS-induced mice and HBE cells, and PCA inhibited this pathway, reducing inflammation and

oxidative stress. However, the AHR activator FICZ reversed the protective effects of PCA in the COPD model, suggesting that PCA alleviates COPD model by reducing inflammation and oxidative stress through inhibition of the AHR/CYP1A1/CYP1A2 pathway.

Several limitations of this study should be acknowledged. First, further experiments are necessary to verify the role of the AHR/CYP1A1/CYP1A2 pathway in COPD mouse model. Moreover, the COPD models used in this study cannot fully capture the symptoms experienced by patients. More studies are needed to evaluate the clinical efficacy and safety of PCA, focusing on its pharmacokinetics, optimal dosage, and effects for long-term use. Additionally, developing PCA in combination with other drugs for future clinical applications may maximize its role.

Conclusion

Overall, this study revealed that PCA improved COPD model by inhibiting inflammation and oxidative stress *via* the AHR/CYP1A1/CYP1A2 pathway. PCA may serve as a promising strategy for COPD prevention.

Author contributions. Xingyu Pan: conceptualization, resources, supervision, writing – original draft preparation. Xiaowei Zhang: methodology, validation, investigation. Ruizhi Li: software, data curation. Sheng Zhang: validation. Chengda Zhang: formal analysis, data curation. Yongjia Zhu: conceptualization, resources, visualization, project administration, writing – review and editing.

Statement of ethics. The Experimental Animal Ethics Committee of Yangzhou University approved these animal experiments (No.202408035).

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Conflicts of interest. The authors declare no conflict of interest.

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