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Research Article

Peperomia pellucida Extract Ameliorates Secondhand Smoke Exposure-Induced Lung Fibrogenesis via Regulation of Matrix Metalloproteinase, Inflammatory, and Fibrotic Cytokines: A Pre-Clinical Study

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ABSTRACT

Secondhand smoke exposure (SHSE) induces pulmonary fibrogenesis. This process involves dysregulation of matrix metalloproteinase, inflammatory, and fibrotic cytokines. *Peperomia pellucida*, an Indonesian herbal plant, has been reported to possess anti-inflammatory activity. In this study, we aimed to evaluate the effect of *P. pellucida* extract on SHSE-induced pulmonary fibrogenesis. We utilized a post-test-only control group design and randomly divided 20 male Wistar rats into three groups (CON, SHS, and SHS+PP). Group CON was exposed to smoke-free room air. Group SHS and SHS+PP received daily SHSE (1 cigarette/rat/day) for four weeks. After cessation of SHSE, group SHS received normal saline, while group SHS+PP received daily doses of *P. pellucida* extract (400 mg/kg body weight [BW]/day, per oral) for four weeks. Finally, after eight weeks of interventions, the animals were euthanized, and the lung tissues were taken out. Matrix metalloproteinase-8 (MMP-8), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta1 (TGF-β1), and collagen-1 expression in the lung tissues were assessed using immunohistochemical techniques. The degree of SHSE-induced lung injury was evaluated using hematoxylin-eosin staining. Statistical analysis was carried out according to the data obtained, and a significant level of 0.05 was determined. Increased MMP-8, TNF-α, TGF-β1, and collagen-1 expression in the group receiving SHSE were evidence of pulmonary fibrogenesis. Daily administration of *P. pellucida* extract at 400 mg/kg BW for four weeks led to a marked reduction in the expression of MMP-8, TNF-α, and TGF-β1 compared with the SHSE-treated group ($p < 0.05$). Histomorphological analysis of the SHSE-received group showed a considerable lung injury with alveolar emphysema and wall thickening as well as infiltration in the alveoli, bronchioles, and vasculature. These alterations were alleviated with *P. pellucida* extract. Therefore, this pre-clinical study showed that *P. pellucida* extract ameliorates SHSE-induced pulmonary fibrogenesis by regulating MMP-8, TNF-α, TGF-β1, and collagen-1.

Keywords:
Secondhand smoke; Pulmonary fibrosis; *Peperomia pellucida*

1. INTRODUCTION

Exposure to cigarette smoke or environmental tobacco smoke has been linked to several illnesses[1]. Passive smoke exposure-induced changes have the greatest impact on the lungs [2]. According to the Global Adults Tobacco Survey, the prevalence of adult secondhand smoke exposure in Indonesia in 2021 was

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74.2% in restaurants, 59.3% at home, and 44.8% at work[3]. Exposure to cigarette smoke increases the likelihood of fibrous tissue growth in the lungs, a process known as fibrogenesis, which ultimately results in pulmonary fibrosis[4,5]. Despite the cessation of exposure, the process of fibrogenesis continues to progress, resulting in a decline in lung capacity and quality of life [6,7]. This ultimately leads to respiratory failure and subsequent mortality [6,8]. It is worth noting that approximately half of the individuals diagnosed with pulmonary fibrosis succumb to the disease within a period of 2 to 5 years [8]. The incidence of pulmonary fibrosis in Indonesia is estimated to be between 6.26 and 7.73 cases per one million individuals, and this figure is expected to rise [9].

Pirfenidone and nintedanib are pharmacological agents used for the treatment of pulmonary fibrosis [10]. Nevertheless, their utilization is limited due to the occurrence of adverse effects and their elevated cost [11]. Hence, it is imperative to investigate novel chemicals in order to regulate and manage this illness. The inhalation of cigarette smoke by the respiratory epithelium is a major risk to lung cells [2]. This is because it leads to the production of various proinflammatory cytokines, interferes with the wound healing process, and disrupts the balance of the extracellular matrix (ECM) [12–14]. Pulmonary fibrosis can occur as a result of these mechanisms. Therefore, focusing on these processes helps mitigate the damage to the lungs caused by passive exposure to cigarette smoke.

Peperomia pellucida, sometimes known as Chinese betel, is a highly diverse medicinal plant belonging to the *Peperomia* genus within the *Piperaceae* family [15]. This medicinal plant is commonly utilized in traditional medicine due to its analgesic, anti-inflammatory, antipyretic, and antioxidant properties [16]. This species predominantly thrives in wet environments in Indonesia and is seldom subjected to direct sunlight[17]. Nevertheless, the utilization of *P. pellucida* necessitates further enhancement owing to the need for more scientific investigation on this botanical specimen.

Thus far, there has been a limited amount of experimental study conducted on the effects of administering *P. pellucida* extract on the occurrence of lung fibrosis caused by exposure to cigarette smoke. This research addresses the abovementioned issues by investigating the efficacy of a cost-effective *P. pellucida* extract as a lung fibrogenesis inhibitor in Wistar rats (*Rattus norvegicus*) following exposure to cigarette smoke.

2. MATERIALS AND METHODS

2.1. Ethical considerations and research design

The Ethics Committee of the Faculty of Medicine, Surabaya Wijaya Kusuma University, had granted ethical approval for all experiments and procedures (approval no. 80/SLE/FK/UWKS/2023). In addition, the procedures complied with the National Institute of Health guidelines for the care and utilization of laboratory animals.

This study utilized 20 adult male Wistar rats (*Rattus norvegicus*) aged 8-12 weeks, weighing 180-220 grams. The rats were obtained from the Experimental Animal Laboratory, Faculty of Medicine, Surabaya Wijaya Kusuma University, Indonesia. The research design employed was a true experimental design with a post-test-only control group design.

2.2. *Peperomia pellucida* extraction

The leaves and stems of *P. pellucida* were determined by the East Java Provincial Government, Health Service, Technical Implementation Unit (UPT) Herbal Materia Medica Laboratory (No. 000.9.3/2684/102.20/2023). Wet sorting was carried out to separate impurities and weeds, followed by running water washing. The wet sorting results were cut and dried using an oven until the water content was less than 10% and ground into powder. The resulting powder was extracted using the maceration method for 3 × 24 hours using 96% ethanol as a solvent [18]. The filtrate of *P. pellucida* leaves and stems was evaporated using a rotary evaporator until a thick extract was obtained and stored in a refrigerator at a temperature of 5°C. Additionally, the powder had received the Production and Quality Testing Certificate (400.7.21.4/2382/102.20/2023).

2.3. Intervention, grouping, and organ collection

The rats were housed in cages with dimensions of 33 cm × 27.5 cm × 13 cm, accommodating a density of 3 rats per cage. Unlimited access to food and drink was granted. The feed utilized was chicken feed code 511, which was then called standard feed (SF). It has 14% water content, 20% crude protein, 5% crude fat, 5% crude fiber, 8% ash, 1.1% calcium, 0.5% phosphorus, 50 ppb aflatoxin, 1.2% lysine, 0.45% methionine, 0.8% methionine + cystine, 0.19% tryptophan, and 0.75% threonine. Cigarette smoke exposure was conducted within a smoke chamber apparatus. Each cigarette contained 1.99 mg of nicotine and 38.93 mg of tar.

Twenty rats were divided into 3 groups (CON, n = 6; SHS, n = 7; SHS+PP, n = 7). Group CON was administered SF for 9 weeks. Group SHS was exposed to cigarette smoke at a rate of 1 cigarette per rat per day, 7 days per week, for a duration of 4 weeks. Subsequently, the inhalation of cigarette smoke was ceased, and the rats in group SHS were administered with normal saline orally for a duration of 4 weeks. Group SHS+PP was exposed to cigarette smoke at a rate of 1 cigarette per rat

per day, 7 days per week, over a period of 4 weeks. Subsequently, the inhalation of cigarette smoke was halted, and rats in group SHS+PP were administered *P. pellucida* extract (400 mg/kg body weight [BW], oral gavage), along with SF, without any further exposure to cigarette smoke, for a duration of 4 weeks.

After the last *P. pellucida* extract treatment, the rats underwent a 12-hour fasting period and were subsequently euthanized using deep anaesthesia (Ketamine-Xylazine, intramuscular injection). Subsequently, the lung tissue was collected. The left lung was ligatured with nylon wire, extracted, measured, rinsed with 0.9% NaCl, and preserved in an organ container filled with 10% neutral-buffered formalin (NBF) for histological analysis using hematoxylin-eosin and immunohistochemistry (IHC) staining [19]. Meanwhile, the right lung tissue was perfused with phosphate-buffered saline (PBS) and stored at -80°C for further out of this study analysis.

2.4. Hematoxylin-eosin staining

The left lung was fixated in a 10% NBF solution for 24 hours, and then the tissue was sliced into cross-sections and placed in a cassette. The tissue underwent processing utilizing a Thermo Scientific STP 120 for a duration of 16 hours. The tool involved a dehydration procedure with varying amounts of ethanol and subsequent cleaning with xylene. Subsequently, the tissue was embedded using liquid paraffin to create a paraffin block. The paraffin blocks were sectioned to a size of 4 microns using a microtome. The sections were then placed on slides in a water bath and stained with hematoxylin-eosin using established techniques. The slides were assessed using an Olympus BX43 microscope and Image Processor DP21 software. Briefly, at a magnification of 400x, 10 randomly selected nonoverlapping fields from each slide were quantified to determine the extent of lung injury and fibrogenesis process using the Klopfleisch score, as shown in Table 1 [20]. The evaluation was conducted blindly by a pathologist.

2.5. Immunohistochemistry staining of MMP-8, collagen-1, TNF-α, and TGF-β1

In order to conduct an IHC evaluation, a paraffin block was prepared and subsequently subjected to deparaffinization, dehydration, and incubation with 3% hydrogen peroxide to suppress the activity of endogenous peroxidase, subsequently, the sample was given a blocking solution to prevent undesired staining. The sample was incubated overnight at a temperature of 4°C with antibodies specific to MMP-8 (1 mg/mL; 1:50; catalog no. ab53017; Abcam, MA, USA), collagen-1 (1 mg/mL; 1:50; catalog no. ab34710; Abcam, MA, USA), TNF-α (1 mg/mL; 1:100; catalog no. ab6671; Abcam, MA, USA), and TGF-β1 (0.5 mg/mL; 1:500; catalog no. ab215715; Abcam, MA, USA). The incubation process was followed by exposing the sample to a secondary antibody at 37°C for 1 hour. This was then followed by another incubation step, including 0.05% solution of diaminobenzidine (DAB). Subsequently, counterstain and rinse with PBS to terminate the reaction. Coverslips were employed to safeguard the slides prior to analysis. The slide results were assessed using an Olympus BX43 microscope and Image Processor DP21 software.

MMP-8, collagen-1, TNF-α, and TGF-β1 expressions were measured at 5 randomly selected nonoverlapping fields from each slide (1000x magnification) using the Immunoreactive Score (IRS) technique [21]. The color intensity of positive IHC reaction scoring was conducted blindly by a pathologist.

2.6. Statistical analysis

All data analysis was carried out using Statistical Package for the Social Sciences (SPSS) software, Version 27.0, and the results were visualized using GraphPad Prism software, Version 9.0. The two-

Table 1. Klopfleisch scoring system for histopathological analysis of the lung.

	Score per field of view (FOV)					
Parameter	0	1	2	3	4	5
Alveolar Hemorrhage	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV
Alveolar Emphysema	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV
Alveolar Wall Thickening	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV
Alveolitis	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV
Bronchiolitis	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV
Vasculitis	none	<10% FOV	11-25% FOV	26-50% FOV	51-75% FOV	>75% FOV

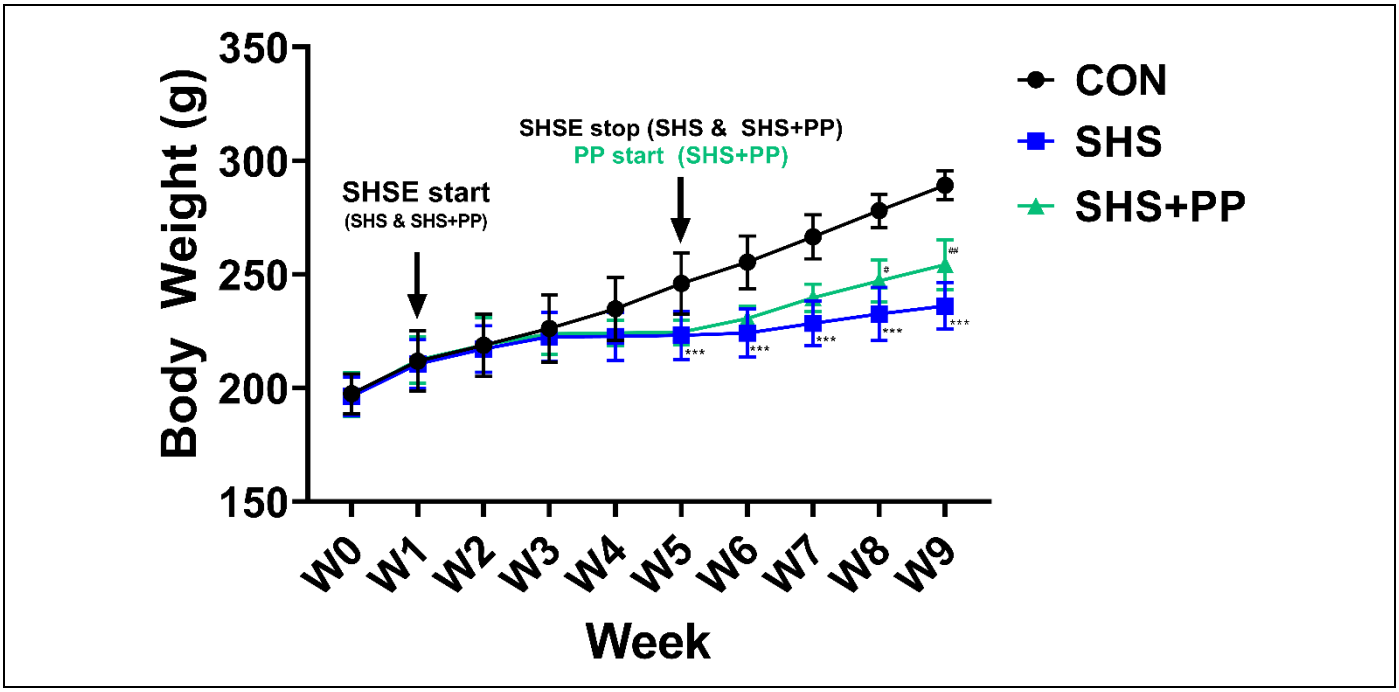

Figure 1. Effects of *Peperomia pellucida* extract on body weight. CON group = 6 rats, SHS group = 7 rats, SHS+PP group = 7 rats. Data are presented as mean ± SD. ***p<0.001 indicates significant differences between the CON group and the SHS group. #p<0.05 and ##p<0.01 indicates significant differences between the SHS group and the SHS+PP group. CON: Control group; SHS: Secondhand smoke-exposed group; SHS+PP: Secondhand smoke-exposed group treated with *P. pellucida* extract; PP: *P. pellucida* extract.

way analysis of variance (ANOVA) followed by Bonferroni's test was used to compare BW among groups. Meanwhile, the one-way ANOVA followed by the Fisher's Least Significant Difference (LSD) post-hoc test was utilized to compare left lung weight among each group. Furthermore, the histopathological score and the immunoreactivity score were analyzed using the Mood's median test. Then, the result of the ANOVA test was expressed as mean ± standard deviation (SD), whereas the result of the non-parametric test was expressed as the median and interquartile range (IQR). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. *Peperomia pellucida* extract maintain rats' body weight

Four weeks of SHS exposure significantly decreased the BW of rats ($p < 0.001$). Meanwhile, four weeks of treatment with *P. pellucida* extract at 400 mg/kg significantly reduced SHS exposure-induced BW loss ($p < 0.05 \sim 0.01$) (Figure 1). All experimental animals in group CON experienced an increase in BW.

3.2. Lung weight is increased in the secondhand smoke exposed group

The exposure to cigarette smoke resulted in a notable elevation in the weight of the left lung ($p < 0.05$) when compared to group CON, as seen in Figure 2. The administration of *P. pellucida* extract at a dose of 400 mg/kg effectively suppressed the rise in organ weight, as compared to the group exposed alone to cigarette smoke ($p < 0.05$).

3.3. *Peperomia pellucida* extract alleviates cigarette-smoke induced-ECM disruption and lung inflammation

The group exposed alone to cigarette smoke exhibited significantly elevated levels of MMP-8 and collagen-1 expression, suggesting a disturbance in the ECM. In addition, there was an increase in proinflammatory and profibrotic cytokines, specifically TNF-α and TGF-β1, respectively. This rise exhibited statistical significance ($p < 0.05$) when compared to the control group (Figure 3). Administering *P. pellucida* extracts at a dosage of 400 mg/kg effectively maintains the balance of the ECM by reducing the production of MMP-8. However, there was no significant decrease in collagen-1 expression between the SHS and SHS+PP groups. Furthermore, this extract demonstrates the ability to inhibit the processes of inflammation and fibrogenesis, as evidenced by a reduction in the expression of TNF-α and TGF-β1. This outcome exhibited statistical significance ($p < 0.05$) when compared to the rats who were solely exposed to cigarette smoke.

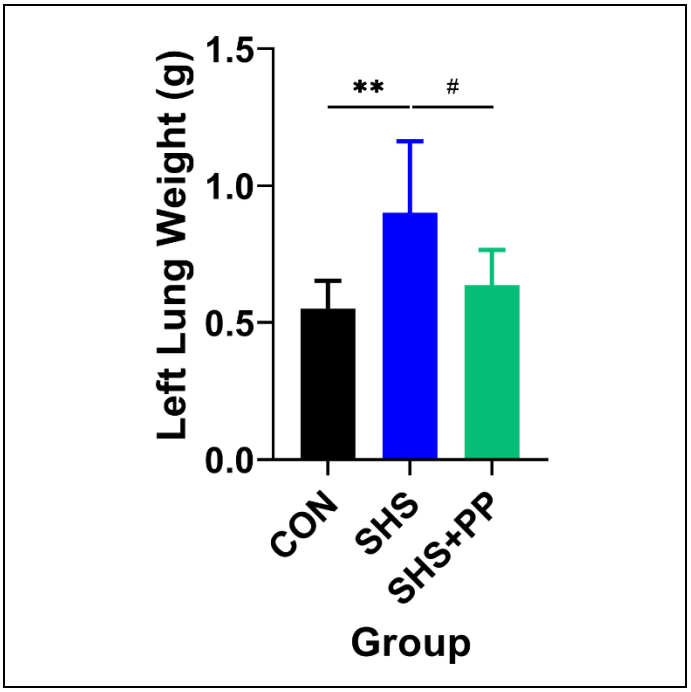

Figure 1. Effects of *Peperomia pellucida* extract on left lung weight. CON group = 6 rats, SHS group = 7 rats, SHS+PP group = 7 rats. Data are presented as mean ± SD. $^{**}p<0.01$ indicates significant differences between the CON group and the SHS group. $^{\#}p<0.05$ indicates significant differences between the SHS group and the SHS+PP group. CON: Control group; SHS: Secondhand smoke-exposed group; SHS+PP: Secondhand smoke-exposed group treated with *P. pellucida* extract; PP: *P. pellucida* extract.

3.4. Cigarette smoke-induced fibrogenesis is inhibited by *Peperomia pellucida* extract

The histological observations confirmed the damage to lung tissue caused by exposure to cigarette smoke and the effect of *P. pellucida* extract (Figure 4). The sections of lung tissue in the control group showed a normal structure of alveoli separated by thin interalveolar septa, bronchioles, and blood vessels. However, exposure to secondhand smoke led to inflammatory responses characterized by alveolar hemorrhage, thick-walled alveoli, and infiltration of leukocytes in the alveoli, bronchioles, and vasculature. Treatment with 400 mg/kg *P. pellucida* extract markedly attenuated the structure of lung tissue compared to the SHS group ($p < 0.05$).

3.5. Discussion

Continuous exposure to cigarette smoke results in damage to the alveoli and subsequent unregulated healing and repair of lung tissue due to the accumulation of ECM, including collagen [5,22–25]. Cigarette smoke exposure leads to an elevation in the permeability of the epithelium layer in the airways. This process damages the protective barrier and causes direct exposure of the underlying subepithelial tissue to reactive chemicals and free radicals [26]. The exposure initiates an inflammatory reaction that, if prolonged, might result in structural epithelial dysfunction, such as the remodeling observed in pulmonary fibrosis [27]. Inflammation is a crucial factor in the activation of fibroblasts, a biological mechanism vital for advancing lung fibrosis [28].

The gene expression that plays a role in the immune process and inflammatory response is regulated by the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [29]. Activation of the NF-κB pathway by cigarette smoke exposure induces transcription of pro-inflammatory cytokines, chemokines, and inflammatory mediators such as TNF-α, IL-1β, IL-6, IL-12p40, and cyclooxygenase-2 (COX-2) and also activates macrophages via the classical pathway (M1), so that M1 macrophages produce IL-1, IL-6, IL-12, TNF-α, and chemokines which play a role in the inflammatory process [30,31]. TNF-α is a potent pro-inflammatory cytokine that acts as a key molecule in the cellular and molecular interactions that regulate the fibrotic process [32]. Lung tissue injury caused by exposure to cigarette smoke can activate the NF-kB pathway and increase TNF-α production [30]. Our study suggests that cigarette smoke exposure increases the expression of TNF-α in the lung tissue. However, it is alleviated by the administration of *P. pellucida* extract.

TGF-β1 plays a crucial role as a mediator in various aspects of lung inflammation, tissue healing, and fibrosis [33]. This cytokine that promotes fibrosis can attract fibroblasts and monocytes/macrophages and drive these cells to produce inflammatory and fibrotic cytokines such as TNF-α, IL-6, and TGF-β1 [34]. Furthermore, TGF-β1 stimulates ECM synthesis via activating fibroblasts [35]. As shown in Figure 3, the expression of TGF-β1 is elevated due to cigarette smoke exposure. Nevertheless, *P. pellucida* extract is capable of decreasing TGF-β1 expression.

A recent study has confirmed the correlation between matrix metalloproteinases (MMPs) and several factors that affect the healing of damaged tissue and the characteristics of epithelial and mesenchymal cells [36]. Dysregulated regulation and expression of matrix metalloproteinases (MMPs) may initiate uncontrolled restructuring in the pulmonary microenvironment of idiopathic pulmonary fibrosis (IPF) [37]. MMPs have traditionally been recognized as the primary agents for breaking down ECM/core microsome proteins. However, MMPs exhibit many functions, extending beyond ECM degradation. They also activate diverse bioactive substances, including growth factors, cytokines, and chemokines [37,38].

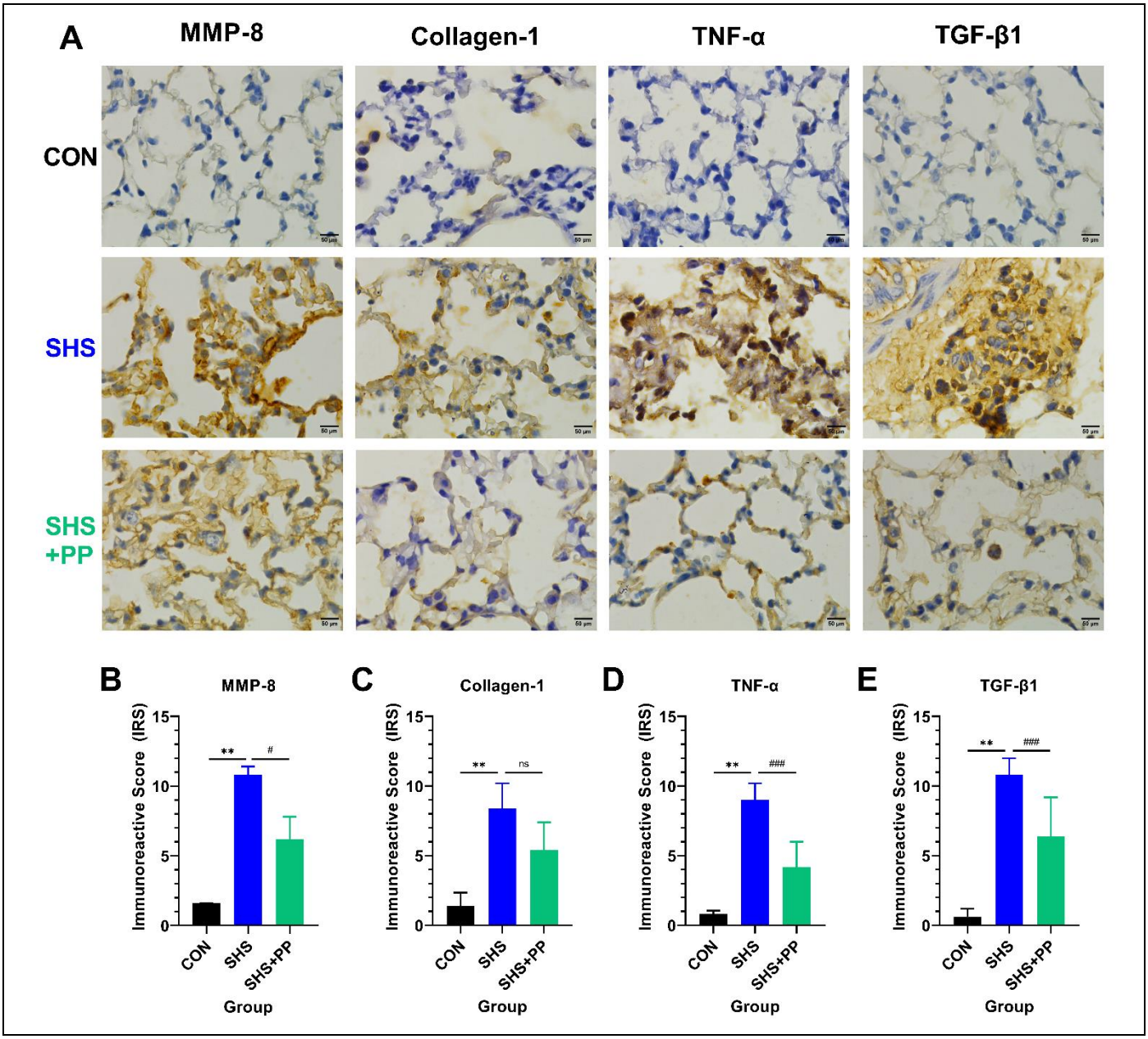

Figure 2. Effects of *Peperomia pellucida* (PP) extract on lung matrix metalloproteinase, extracellular matrix, inflammatory, and fibrotic status in a secondhand smoke exposure (SHSE)-induced pulmonary fibrogenesis model. Representative photomicrographs of MMP-8, collagen-1, TNF-α, and TGF-β1 immunohistochemical-stained lung sections (**A**). The CON group shows only a few positively stained MMP-8, collagen-1, TNF-α, and TGF-β1 in the lung interalveolar septa. The SHSE-treated group (SHS group) exhibits an abundance of positively stained brown cells in the interalveolar septa. The SHS+PP-treated group shows a few positively stained brown cells that are close to the control group (×1000, scale bar = 50 µm). An immunoreactive score analysis of the MMP-8 (**B**), collagen-1 (**C**), TNF-α (**D**), and TGF-β1 (**E**) immune reactivity. CON group = 6 rats, SHS group = 7 rats, SHS+PP group = 7 rats. Data are presented as median with interquartile range. $^{**}p<0.01$ indicates significant differences between the CON group and the SHS group. $^{\#}p<0.05$ and $^{\#\#\#}p<0.001$ indicates significant differences between the SHS group and the SHS+PP group. ns indicates not significant. CON: Control group; SHS: Secondhand smoke-exposed group; SHS+PP: Secondhand smoke-exposed group treated with *P. pellucida* extract; PP: *P. pellucida* extract.

While the production of MMPs is highly regulated, cytokines such as TGF-β and TNF-α can effectively regulate the synthesis of MMPs through manipulation of numerous cell signaling cascades [39,40]. Therefore, MMPs also regulate different immunomodulatory molecules implicated in the progression of pulmonary fibrosis; MMPs can both promote and suppress pulmonary fibrosis. MMP-8 is expressed by epithelial cells, macrophages, lymphocytes, fibroblasts, fibrocytes, dendritic cells, and mesenchymal stem cells (MSCs). MMP-8 has a function in pulmonary fibrosis by acting as a mediator that promotes fibrosis. In a study conducted by Craig et al. (2013), it was observed that the progression of lung fibrosis produced by bleomycin was reduced in mice that lacked MMP-8. In our study, we found that MMP-8 and collagen-1 increase following cigarette smoke exposure, indicating an imbalance of ECM. As

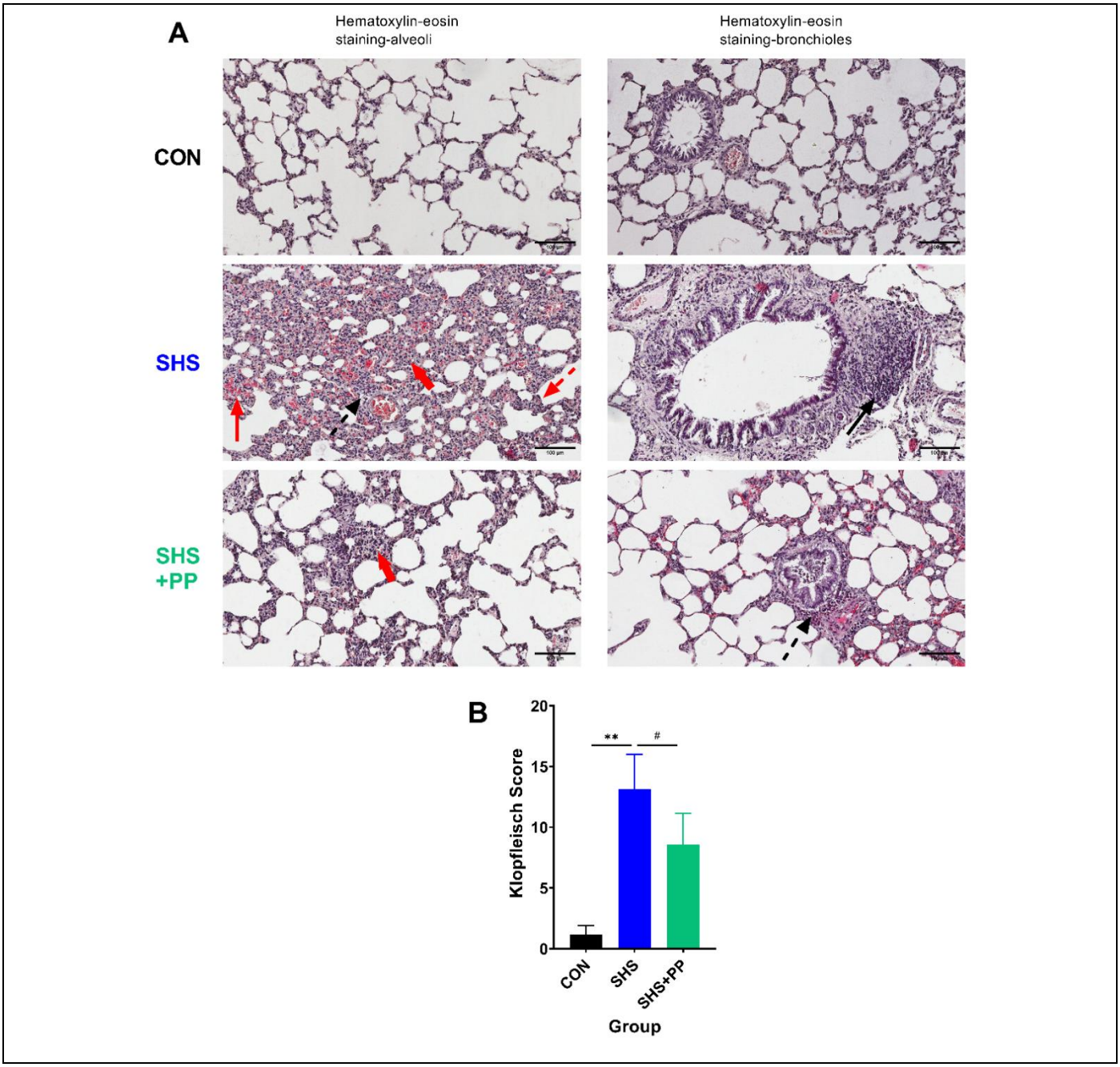

Figure 3. Representative photomicrographs of rat lung tissues stained with hematoxylin and eosin (A). The CON group shows normal architecture of thin-walled alveoli and bronchioles without leukocyte infiltration and hemorrhage. The secondhand smoke-exposed group (SHS group) reveals a disrupted architectural arrangement of the lung with markedly thickened alveolar septa (red dashed arrow), alveolitis (thick red arrow), vasculitis (black dashed arrow), hemorrhage (thin red arrow), and bronchiolitis (black arrow). The secondhand smoke exposure and *Peperomia pellucida* extract-treated group (SHS+PP group) show many thin-walled alveoli with minimal infiltration of leukocytes in the alveoli (red thick arrow) and bronchioles (black dashed arrow) (×400, scale bar = 100 µm). The histological scoring among various studied groups (B). CON group = 6 rats, SHS group = 7 rats, SHS+PP group = 7 rats. Data are presented as median with interquartile range. **p<0.01 indicates significant differences between the CON group and the SHS group. #p<0.05 indicates significant differences between the SHS group and the SHS+PP group. CON: Control group; SHS: Secondhand smoke-exposed group; SHS+PP: Secondhand smoke-exposed group treated with *P. pellucida* extract; PP: *P. pellucida* extract.

previously reported, MMPs are not solely responsible for breaking down ECM, such as collagen, since we found out that both MMP-8 and collagen-1 are significantly elevated.

Interestingly, other MMPs may be responsible for the development of pulmonary fibrogenesis following exposure to cigarette smoke. In a human study, levels of MMP-9 in the sputum remain elevated after cessation of cigarette smoke exposure, which may contribute to the lung damage typical of chronic obstructive pulmonary disorder (COPD)[42]. Furthermore, a recent study suggests that COPD and IPF may coexist in one individual who is susceptible to cigarette smoke, indicating a pathophysiological

overlap[43]. This hypothesis of pathophysiological overlap may be confirmed by a study done by Liu et al. (2017), in a mouse model of bleomycin-induced pulmonary fibrosis, the expression of MMP-9 in the lung was increased [44]. Additionally, the study also showed that tissue inhibitor of metalloproteinase-1 (TIMP-1) played a crucial role in the pathophysiology of pulmonary fibrosis [44].

The main chemical profile of *P. pellucida* includes alkaloids, flavonoids, sterols, tannins, saponins, triterpenoids, phenols, azulenes, carotenoids, depsides, and quinones [45]. These compounds are related to the analgesic, anti-inflammatory, antipyretic, bactericidal, and fungicidal potential of this species [15]. Previous study suggests that oral administration of *P. pellucida* extract also helps the healing process by accelerating the inflammatory process which is characterized by a decrease in the number of polymorphonuclear (PMN) leukocytes [18].

Flavonoids are a type of polyphenol chemicals that are naturally present in plants. These compounds possess anti-inflammatory properties and exert their effects by blocking enzymes and transcription factors that control the production of inflammatory mediators [46]. Flavonoids hinder the activity of the transcription factor NF-κB, a key player in the development of lung fibrosis. Therefore, utilizing flavonoids could be a promising approach to mitigate the advancement of pulmonary fibrosis. Administration of an NF-κB inhibitor effectively suppressed the activation of fibroblasts and resulted in decreased levels of TNF-α, IL-6, IL-1β, and IL-18 [47]. A separate investigation documented that blocking the NF-κB pathway had a safeguarding impact against bleomycin-induced lung fibrosis in mice [48]. Quercetin, as an example of flavonoid, administration hinders the TGF-β signaling pathway, leading to a decrease in the advancement of renal fibrosis [49]. It also reduces the elevated levels of MMP-7 in mice with bleomycin-induced pulmonary fibrosis [50].

4. CONCLUSION

Overall, the SHSE-induced fibrogenesis process is inhibited by administration of *P. pellucida* extract. The ECM imbalance induced by cigarette smoke exposure is ameliorated by oral administration of *P. pellucida* extract, which is indicated by a decrease in MMP-8 and collagen-1 expression. Moreover, the inflammatory and fibrotic processes are alleviated by this medicinal extract, which is indicated by a decrease in TNF-a and TGF-β1 expression. Through observation of histopathological slides, *P. pellucida* extract is capable of treating cigarette smoke-induced lung fibrogenesis. These promising results might be related to the chemical and biological diversity of *P. pellucida*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ethics approval

This research was reviewed and approved by the Ethical Committee, under the approval number 80/SLE/FK/UWKS/2023.

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