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Original Research Article

Protective Effects of *Peperomia pellucida* Extract Against Secondhand Smoke-Induced Pulmonary Fibrosis via Antioxidant and Anti-inflammatory Pathways

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ABSTRACT

Secondhand smoke exposure (SHSE) represents a significant external risk factor, which contributes to pulmonary fibrosis onset by activating inflammatory, fibrogenic, and oxidative stress mechanisms. This study explores the efficacy of *Peperomia pellucida* (PP) in mitigating lung injury caused by SHSE in Wistar rats. Twenty animals were randomly grouped to one of three cohorts: control (CON), SHSE, and SHSE with PP extract (SHSE+PP). Rats were exposed to secondhand smoke for 4 consecutive weeks. The treatment group received PP extract (400 mg/kg) for 1 week before and during 4 weeks of SHSE. Lung tissues were evaluated via histopathology, immunohistochemistry, and glutathione (GSH) assays. SHSE significantly increased lung weight, histopathological scores, and profibrotic (TGF-β) and pro-inflammatory (TNF-α and IL-6) cytokines ($p < 0.05$). A marked reduction in GSH levels was also observed, indicating increased oxidative stress. In contrast, rats treated with PP extract showed significant improvements in all parameters, including reduced cytokine expression, improved lung architecture, and restored GSH levels ($p < 0.05$). These effects are likely mediated by bioactive compounds in PP, such as phenolics, phenylpropanoids, sesquiterpenes, and chlorophyll derivatives, which inhibit NF-κB and activate the Nrf2 pathway, thereby reducing inflammation, fibrosis, and oxidative damage. This is the first report demonstrating the protective effect of the extract in SHSE-induced pulmonary fibrogenesis. The results highlight the action of PP as a protective alternative medicine and support further investigation into its clinical application for preventing secondhand smoke-related chronic lung diseases.

Keywords: Pulmonary Fibrosis, *Peperomia pellucida*, Secondhand Smoke Exposure.

Introduction

Environmental tobacco smoke exposure is widely acknowledged as a major contributing factor of chronic and irreversible pulmonary fibrotic remodeling.[1,2] This pathological process, known as idiopathic pulmonary fibrosis (IPF), results in a marked declining respiratory function, reduced life expectancy, and poor quality of life, with nearly half of patients dying within three to five years after diagnosis.[3] Given its established links to various pulmonary conditions—including asthma, IPF, lung carcinoma, and chronic obstructive pulmonary disease (COPD)—secondhand smoke remains a pressing public health challenge. The 2021 Global Adult Tobacco Survey (GATS) reported that 74.2% of adults are exposed to secondhand smoke in restaurants, 59.3% at home, and 44.8% in workplaces.[4]Pulmonary fibrosis resulting from secondhand smoke exposure is initiated by epithelial injury, followed by dysregulated repair processes and aberrant tissue remodeling.[5,6]

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Transforming growth factor-beta (TGF-β) serves as the central mediator in the development of IPF by stimulating myofibroblast activation, promoting extracellular matrix (ECM) accumulation, and preventing its degradation, which contributes to fibrotic tissue formation.[7] Additionally, pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) and are key drivers in fibrogenesis by inducing fibroblast activation and matrix deposition.[8,9] In addition, oxidative stress mediates IPF's pathogenesis by enhancing TGF-β overexpression and inflammatory responses, which in turn impair endogenous antioxidant defenses.[10]

Despite being approved by the Food and Drug Administration (FDA) for IPF treatment, the clinical efficacy of pirfenidone and nintedanib remains constrained due to a range of significant adverse effects. Pirfenidone commonly causes gastrointestinal discomfort, photosensitivity reactions, liver toxicity, and fatigue. Nintedanib, on the other hand, is frequently associated with diarrhea, elevated liver enzymes, bleeding risk, and cardiovascular complications. Moreover, the high cost of these therapies significantly limits their accessibility, particularly for patients in resource-limited settings.[11,12] These limitations highlight pressing need for the development of innovative, more cost-effective, and safer therapeutic compounds for IPF management.

Peperomia pellucida, a small herbal plant from the *Peperomia* genus, which is known for its biological and chemical diversity within the *Piperaceae* family, is widely distributed across South, North, Central America, and Asia.[13] In Indonesia, *P. pellucida* thrives in humid environments. Extensive research has demonstrated the plant's capacity to exert both anti-inflammatory and antioxidant effects, which are largely ascribed to its diverse array of bioactive constituents.[14,15] Prior investigations into *Piperaceae* species, including *Piper nigrum*, *Piper betle*, and *Piper longum*, have shown that these extracts can inhibit IL-6, TNF-α and TGF-β, while also alleviating oxidative stress through

radical scavenging mechanisms.[16,17] However, no studies have explored the potential of *P. pellucida* in preventing lung fibrosis progression. The objective of the study was to assess the potential of this affordable Indonesian herbal plant (*P. pellucida*) as an innovative preventive agent to prevent lung fibrogenesis in Wistar rats induced by secondhand smoke exposure through modulation of inflammatory, fibrotic, and oxidative pathways.

Materials and Methods

Plant Collection and Identification

The aerial stems and leaves parts of *P. pellucida* were harvested on July 10, 2023 from, Taman Flora Bratang, Surabaya, Indonesia (7°17'38.4"S 112°45'42.3"E), and were subsequently authenticated by Dr. Ratna Yulianti, a taxonomist from the East Java Provincial Government, Health Office, Batu Materia Medica Herbal Laboratory Technical Implementation Unit, Indonesia. A voucher specimen was deposited at the same institution (No. 000.9.3/2684/102.20/2023).

Initial processing involved wet sorting to remove extraneous materials such as weeds and debris, followed by thorough rinsing under running water. The cleaned plant material was then cut into smaller segments and dried at 50 °C using an oven until the moisture content was reduced below 10%. Following the drying process, the material was milled into a fine powder.

Extraction of Plant Material

Extraction was performed using a maceration technique with 96% ethanol, employing 500 grams of powdered *P. pellucida* aerial parts and 5 liters of solvent (1:10 w/v ratio), over three consecutive 24-hour cycles at ambient temperature.[13] The resulting filtrate was concentrated and stored at 5 °C for preservation. The powdered simplicia, which was prepared from collected plant material, received a Certificate of Production and Quality Testing issued by the Batu Materia Medica Herbal Laboratory (No. 400.7.21.4/2382/102.20/2023).

Animals and Ethical Approval

This experiment was ethically reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Wijaya Kusuma Surabaya (Ethical Clearance No. 79/SLE/FK/UWKS/2023) in Indonesia. Twenty adult male Wistar rats (7–13 weeks old; 170–230 g) were obtained from the Faculty of Medicine, Universitas Wijaya Kusuma Surabaya. Prior to the initiation of experimental procedures, the animals were habituated for 7 days under standard laboratory conditions, i.e., a 12-hour light an dark cycle, a controlled room temperature of 21 ± 5 °C, and relative humidity of 50 ± 5%. The animals had free access to tap water and standard rodent feed. The study employed a post-test-only control group approach.

Animal Grouping and Intervention

Twenty male Wistar rats were randomly assigned to three distinct experimental cohorts: a control group (CON; n = 6), a group exposed to secondhand smoke (SHSE; n = 7), and a group exposed to secondhand smoke and treated with *P. pellucida* extract (SHSE+PP; n = 7). The animals were housed in standardized cages measuring 33 × 27.5 × 13 cm, with three rats accommodated per cage. Environmental parameters were tightly controlled. Exposure to secondhand smoke was conducted in a custom-designed chamber using commercial, non-filtered cigarettes, each containing 1.99 mg of nicotine and 38.93 mg of tar.[18]

The control group was provided with a standard diet over a five-week duration. Rats in the SHSE group were exposed to secondhand smoke at a dose of one cigarette/rat/day for a continuous period of four weeks. In the SHSE+PP group, *P. pellucida* extract was given orally (400 mg/kg body weight (BW)) for one week preceding secondhand smoke exposure. This was followed by concurrent administration of both cigarette smoke (1 cigarette/rat/day) and the plant extract daily over the subsequent four weeks. Body weight of all animals were monitored on a weekly basis throughout the study to assess overall health and observe any therapeutic effects associated with the treatment. On the final day of treatment, the rats were fasted for 12 hours before being euthanized using intramuscular injection of ketamine-xylazine. Lung tissues were collected following euthanasia. The left lung was dissected, weighed, and immersed in 10% formalin for histopathological assessments, including hematoxylin and eosin staining and immunohistochemistry.[20,21] Concurrently, the right lung was perfused with phosphate-buffered saline via the right ventricle and subsequently stored at -40 °C for the analysis of glutathione (GSH) content.[22]

Hematoxylin and Eosin Staining

Subsequently, the left lung was transversely sectioned and embedded into tissue cassettes for further processing. The tissue was processed using a Thermo Scientific STP 120 for 16 hours, during which dehydration was performed using graded ethanol concentrations, followed by clearing with xylene. The processed tissue was subsequently infiltrated with molten paraffin and embedded to form paraffin blocks. The paraffin-embedded lung tissues were sectionat at 4 µm and affixed onto glass slides using a water bath method. Hematoxylin and eosin staining was performed following standard histological procedures.[23] Microscopic evaluation was performed at 100× magnification using an Olympus BX43 microscope, with digital imaging facilitated by the Olympus DP21 image processing system. Lung injury and fibrosis severity were evaluated using the Klopfleisch histopathological scoring system.[24]

Immunohistochemistry Staining

Immunohistochemical analysis was conducted on 4 µm-thick sections obtained from paraffin-embedded lung tissues. Sections underwent standard deparaffinization and rehydration procedures, then treated with 3% H_2O_2.[25] To minimize nonspecific binding, the sections were first treated with a protein blocking solution and incubated overnight with primary antibodies that target IL-6 (1 mg/mL; 1:400 dilution; cat. no. ab6672; Abcam, MA, USA), TGF-β (0.5 mg/mL; 1:500 dilution; cat. no. ab215715; Abcam), and TNF-α (1 mg/mL; 1:100 dilution; cat. no. ab6671; Abcam) at 4 °C. After primary incubation, a secondary antibody was applied for an hour at 37 °C, and immunoreactivity was detected using 0.05% diaminobenzidine (DAB). The sections were then counterstained and washed with phosphate-buffered saline. Coverslips were applied to the slides prior to microscopic evaluation. Immunoreactivity was examined under an Olympus BX43 microscope at 1000× magnification, and the expression level of IL-6, TGF-β, and TNF-α were semi-quantitatively assessed using the Immunoreactive Score (IRS) method.[26]

Antioxidant Assay

The right lung samples were thoroughly homogenized in ice-cold phosphate-buffered saline added with a protease inhibitor. The homogenized mixture was subsequently centrifuged at 10,000 × g for 15 minutes at 4 °C, after which the supernatant was taken. GSH concentrations were quantified using a Rat GSH ELISA kit, following the manufacturer's standardized protocols (Bioassay Technology Laboratory, Rat Glutathione, EA0113Ra). Optical density values were assessed with a microplate spectrophotometer set to a wavelength of 450 nm.

Statistical Analysis

GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) was used to analyze data. Distribution of data was evaluated using the Shapiro–Wilk test. For data meeting normality criteria, intergroup comparisons were performed using one- or two-way analysis of variance (ANOVA), with post hoc evaluation conducted using Fisher's LSD test when appropriate. In cases of non-parametric distribution, the Kruskal–Wallis test was carried out, followed by Dunn's method for subsequent pairwise analyses. A p-value of < 0.05 demonstrated statistical significance. Quantitative data were presented as mean ± standard error of the mean (SEM).

Results and Discussion

Protective Effect of P. pellucida Extract on Body Weight

Figure 1 shows BW differences in rats across the CON, SHSE, and SHSE+PP groups during the treatment period. On day 0 (D0) of the intervention, the baseline BW did not differ much across the

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experimental groups (p = 0.981), indicating that the initial BW conditions of the rats were relatively homogenous across all groups. At both days 7 (D7) and 14 (D14), BW remained statistically comparable between the SHSE (p = 0.999) and SHSE+PP groups (p = 0.868). By day 21 (D21), a marked increased in mean BW was noted in SHSE+PP group relative to the SHSE group (p = 0.040). This pattern became increasingly evident on days 28 (D28) and 35 (D35), with the SHSE group exhibiting a significantly reduced body weight compared to the CON group on D28 (p = 0.007) and D35 (p = 0.004). Meanwhile, on D28 and D35, the SHSE+PP group, which received *P. pellucida* extract, had significantly higher BW (p = 0.002 and p = 0.000, respectively) relative to SHSE group. These results indicate that oral delivery of 400 mg/kg BW of *P. pellucida* extract confers a protective effect against secondhand smoke-induced weight reduction. All animals in the CON group showed normal BW increase.

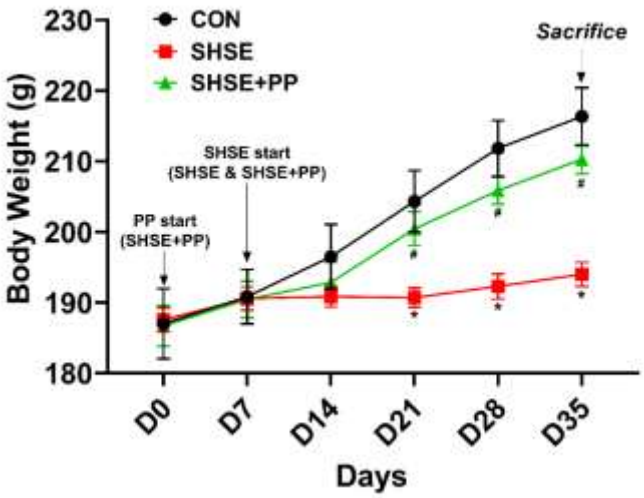

Figure 1: Effects of *Peperomia pellucida* (PP) extract and secondhand smoke exposure (SHSE) on body weight. Values are expressed as mean ± SEM (n = 6–7 per group). *p < 0.05 vs. control (CON); $^{\#}p$ < 0.05 vs. SHSE group, determined by one-way ANOVA followed by Fisher's Least Significant Difference (LSD) post hoc test. CON: control group; SHSE: secondhand smoke-exposed group; SHSE+PP: SHSE group treated with PP extract.

The observed reduction in BW suggests the negative impact of passive smoke exposure on the physical condition of the rats. This effect is likely associated with reduced food consumption and metabolic dysregulation triggered by systemic inflammation and oxidative insult resulting from toxic constituents in cigarette smoke.[29] Previous studies have also supported these findings, showing that cigarette smoke exposure increases the generation of free radicals and pro-inflammatory cytokines, which can affect appetite and body metabolism.[30,31]

Body weight reduction as a result of lung disease has been associated with poor prognosis in interstitial lung diseases like IPF. According to Kalininskiy *et al*. (2022), a reduction in BW during the first year following an IPF diagnosis is closely linked to lower survival rates and diminished quality of life in affected individuals.[32] Body weight loss in IPF patients is considered an indicator of therapy failure and faster disease progression.[33] These findings emphasize the importance of maintaining a healthy BW in individuals with lung diseases caused by smoke exposure to improve their prognosis. Thus, preventing BW loss due to passive smoke exposure could serve as an intervention in preventing the progression of severe lung diseases.

Effect of P. pellucida on Secondhand Smoke-Induced Lung Mass Changes

To examine the protective potential of *P. pellucida* extract toward secondhand smoke-induced changes in lung mass—reflective of inflammation and fibrotic remodeling—absolute left lung weights were measured in the experimental rats. The SHSE group demonstrated a marked elevation in left lung weight relative to the control (CON) group (Figure 2), with a p-value of < 0.001. This condition represents structural changes in lung tissue due to inflammation, oxidative stress, and fibrosis caused by cigarette smoke exposure.[34] Previous studies have shown that cigarette smoke exposure contributes to increased lung tissue mass, which is associated with pulmonary edema and pathological changes in lung structure due to chronic inflammation and oxidative stress.[35] Additionally, research by Fang *et al*. (2019) also found that increased lung weight in cigarette smoke-exposed mouse models indicated interstitial fibrosis.[36] Our study demonstrates that the SHSE group exhibited a significant increase in lung weight, which may indicate inflammatory infiltration, edema, and extracellular matrix accumulation. These possibilities are further confirmed by histopathological analysis.

In the SHSE+PP group, a significant reduction in left lung organ weight was observed relative to the SHSE group (p < 0.001), reflecting the protective capacity of *P. pellucida* extract in mitigating structural lung injury induced by secondhand smoke exposure. The observed effect is likely attributed to the bioactive constituents of *P. pellucida*, including flavonoids and phenolic compounds, which possess well-documented antioxidant and anti-inflammatory properties. Lan *et al*. (2024) demonstrated that plant extracts rich in flavonoids attenuate oxidative stress and inflammatory responses in lung tissue, contributing to a reduction in fibrosis and tissue swelling.[37] Similarly, the present findings corroborate earlier study, which showed that administration of herbal extracts in cigarette smoke-exposed rats led to reduced lung weight and improved structural changes in lung tissue.[38] Taken together, these results support the properties of *P. pellucida* in mitigating structural lung injury associated with secondhand smoke exposure.

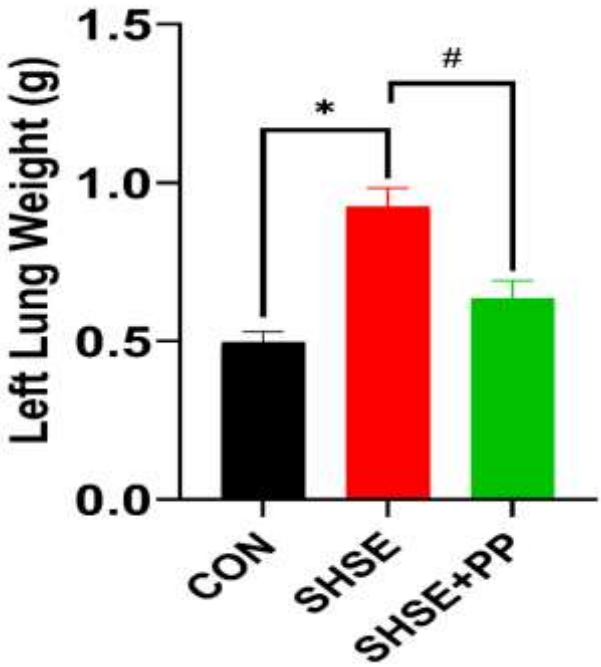

Figure 2: Effects of *Peperomia pellucida* (PP) extract and secondhand smoke exposure (SHSE) on left lung weight. Values are expressed as mean ± SEM (n = 6–7 per group). *p < 0.05 vs. control (CON); $^{\#}p$ < 0.05 vs. SHSE group, determined by one-way ANOVA followed by Fisher's Least Significant Difference (LSD) post hoc test. CON: control group; SHSE: secondhand smoke-exposed group; SHSE+PP: SHSE group treated with PP extract.

Attenuation of Structural Lung Damage by P. pellucida in SHSE-Exposed Rats

To provide additional validation of the protective role of *P. pellucida* extract in counteracting structural lung alterations induced by secondhand smoke exposure, we performed histopathological analysis using H and E staining. The normal control group exhibited uniform lung tissue structure, with intact bronchiolar walls and alveolar septa (Figure 3A), while the SHSE group showed multiple pathological features, including alveolar hemorrhage, emphysema, alveolar wall thickening, and alveolitis (Figure 3B). These tissue alterations are consistent with the clinical features commonly seen in lung diseases caused by secondhand smoke exposure, in which long-term exposure can trigger chronic inflammation, oxidative stress, and scar tissue formation in the lungs.[35,36] The observed thickening of alveolar walls and inflammation within bronchi and vasculature further support the notion that secondhand smoke inflicts widespread structural injury, ultimately contributing to the development of IPF.[37–39]

In contrast, histopathological examination of the SHSE+PP group revealed milder structural changes, characterized by reduced alveolar wall thickening and decreased inflammatory cell infiltration (Figure 3C). This was further supported by a significantly lower Klopfleisch's score relatove to SHSE group ($p = 0.002$) (Figure 3D). These results indicate that *P. pellucida* may facilitate repair of lung architecture, likely through its anti-inflammatory and antioxidant properties. By attenuating these pathological processes, *P. pellucida* may inhibit the progression of pulmonary fibrogenesis triggered by secondhand smoke exposure.

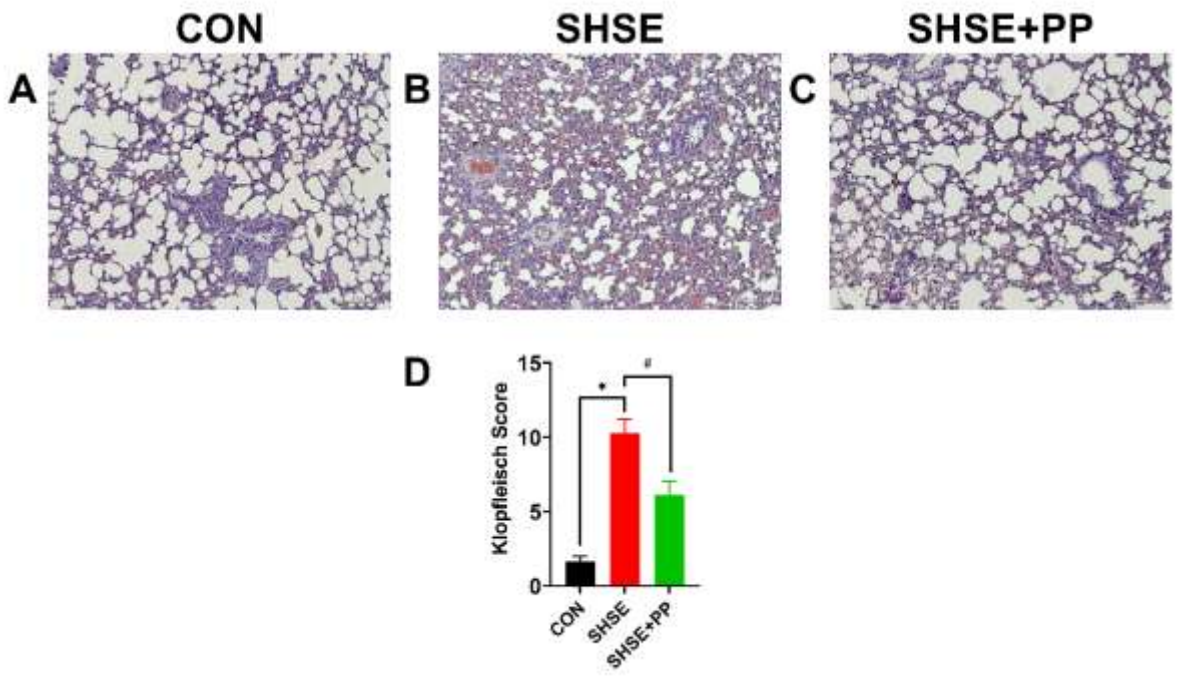

Figure 3: Histopathological evaluation of *Peperomia pellucida* (PP) extract on lung architecture in a rat model of pulmonary fibrosis induced by secondhand smoke exposure (SHSE). Representative hematoxylin–eosin–stained lung sections are shown for the control (CON), SHSE, and SHSE+PP groups (**A–C**, magnification ×100). The CON group exhibited normal alveolar and bronchiolar architecture. In contrast, the SHSE group showed severe structural disruption characterized by alveolar septal thickening, alveolitis, vasculitis, hemorrhage, and bronchiolitis. Administration of PP extract markedly preserved lung architecture, as evidenced by reduced alveolar wall thickening and minimal leukocyte infiltration in the alveolar and bronchiolar regions. Quantitative assessment using the Klopfleisch score (**D**) supports these findings, demonstrating significant histological improvement in the SHSE+PP group. Values are expressed as mean ± SEM (n = 6–7 per group). *$p < 0.05$ vs. control (CON); $^{\#}p < 0.05$ vs. SHSE group, determined by one-way ANOVA followed by Fisher's Least Significant Difference (LSD) post hoc test. CON: control group; SHSE: secondhand smoke-exposed group; SHSE+PP: SHSE group treated with PP extract.

P. pellucida Ameliorates SHSE-Induced Inflammatory and Fibrotic Cytokine Expression in Lung Tissue

To evaluate the inflammatory and fibrotic response in lung tissue, the expression of IL-6, TNF-α, and TGF-*β* were assessed using immunohistochemical staining (Figure 4A-I). Representative staining for IL-6 is shown in Figure 4A-C, for TNF-α in 4D-F, and for TGF-*β* in 4G-I. The SHSE group experienced a significant upregulation of IL-6 ($p = 0.001$), TNF-α ($p < 0.001$), and TGF-*β* ($p < 0.001$) relative to the CON group (Figure 4J-L). This data shows that secondhand smoke promotes the expression of these cytokines, as previously reported.[30,36,38–40] IL-6 is a crucial mediator in the early stage of pulmonary fibrogenesis, primarily by triggering and enhancing the expression of downstream cytokines like TNF-α and TGF-*β*.[8,41] TNF-α further exacerbates disease progression by upregulating proinflammatory mediators, facilitating myofibroblast differentiation, and promoting the infiltration of macrophages and neutrophils.[36,39] In addition to its proinflammatory role, TNF-α interacts synergistically with TGF-*β* to accelerate ECM deposition and fibrotic remodeling.[42,43] Moreover, TGF-*β*, a key profibrotic cytokine, promotes scar tissue formation and collagen synthesis in interstitial lung diseases. It also triggers epithelial–mesenchymal transition (EMT), facilitating fibroblasts deposition and boosting the expansion of connective tissue within the pulmonary environment.[40,42]

In the present study, the SHSE+PP group demonstrated a marked decrease of IL-6 ($p = 0.016$), TNF-α ($p = 0.003$), and TGF-*β* ($p < 0.001$) expression compared to the SHSE group (Figure 4A-L). While previous investigation have reported the anti-inflammatory and antifibrotic activities of other *Piperaceae* species, particularly through the downregulation of TNF-α, TGF-*β*, and IL-6, the current study is the first one that examine the protective activities of *P. pellucida* in preventing secondhand smoke-induced pulmonary fibrogenesis.[16,17]

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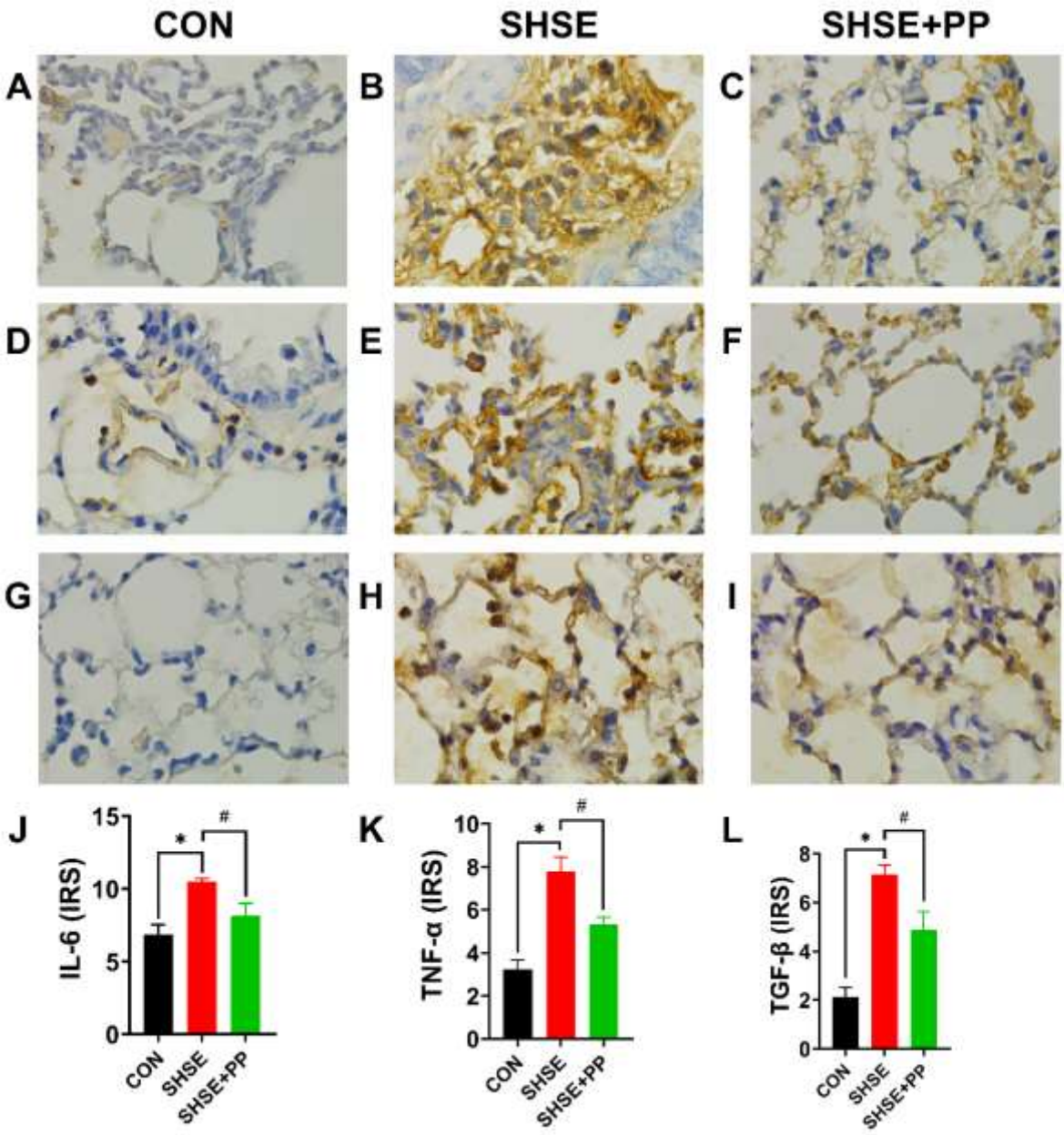

Figure 4: Immunohistochemical assessment of *Peperomia pellucida* (PP) extract on inflammatory and fibrotic markers in lung tissue of rats exposed to secondhand smoke (SHSE). Panels A–C, D–F, and G–I depict representative staining for IL-6, TNF-α, and TGF-β, respectively. In the control (CON) group, minimal immunopositivity is observed in interalveolar regions. Conversely, SHSE exposure markedly increases the number of positively stained cells. Treatment with PP extract (SHSE+PP group) substantially reduces staining intensity, closely resembling the CON group (magnification: ×1000). Quantitative analysis using immunoreactivity scores for IL-6 (J), TNF-α (K), and TGF-β (L) corroborates these histological findings. Results are expressed as mean ± SEM (n = 6–7 per group). *p < 0.05 vs. CON; $^{\#}p$ < 0.05 vs. SHSE, determined by one-way ANOVA with Fisher's Least Significant Difference (LSD) post hoc test. CON: control group; SHSE: secondhand smoke-exposed group; SHSE+PP: SHSE group treated with PP extract.

P. pellucida's anti-inflammatory properties are likely due to its constituent bioactive compounds, including phenolics, phenylpropanoids, sesquiterpenes, and chlorophyll derivatives, which are knows to inhibit the principal transcription factor regulating pro-inflammatory cytokines expression, NF-κB.[13,34,44,45] Notably, Ho *et al*. (2024) reported that 2,4,5-trimethoxystyrene ether and dillapiole, compounds isolated from *P. pellucid*a extract, have affinity to NF-κB p65 subunit through the serine 276-phosphorylation site, thereby preventing its activation.[46] This inhibition resulted in a substantial downregulation of TNF-α and IL-6 expression. Moreover, *P. pellucida* extract has demonstrated anti-inflammatory effects *in vivo*, as evidenced by its ability to attenuate inflammation in a rat model of periodontitis.[47]

With regards to fibrotic signaling, the SHSE+PP group exhibited a notable suppression of TGF-β expression relative to the SHSE group (Figure 4G-I, 4L). This downregulation suggests that *P. pellucida* extract may attenuate fibrosis by interfering with TGF-*β*-mediated pathways. TGF-*β*, as a central modulator of fibrosis, evokes its biological activities primarily via the SMAD signaling pathway and EMT induction, contributing to fibroblast proliferation and ECM deposition.[42,43] Flavonoids abundant in *P. pellucida* have been shown to attenuate fibrotic responses by blocking the TGF-β type I receptor,

thereby inhibiting the following nuclear migration and SMAD2/3 phosphorylation.[48] For instance, apigenin, quercetin, and kaempferol have been documented to inhibit TGF-*β*-induced collagen synthesis and EMT in various fibrosis models, including pulmonary, hepatic, and renal fibrosis.[42,49] Li *et al*. (2022) demonstrated that flavonoid-rich juice significantly reduced TGF-*β* expression and SMAD2/3 activity, resulting in downregulation of collagen I and alpha-smooth muscle actin in an animal model of lung fibrosis.[29]

P. pellucida Enhances Antioxidant Defense Against SHSE-Induced Oxidative Stress

Secondhand smoke comprises a complex array of harmful substances that stimulate the production of free radicals, while simultaneously impairing endogenous antioxidant defenses.[18,38] In our study, rats exposed to SHSE exhibited a substantial reduction in lung GSH levels compared to the CON group ($p < 0.001$; Figure 5). GSH serves as the primary antioxidant responsible for detoxifying reactive oxygen and nitrogen species, and its depletion serves as a sensitive biomarker of oxidative damage in pulmonary tissues.[50,51] The observed decline in GSH among secondhand smoke-exposed animals likely contributes to enhanced inflammatory and fibrotic responses within pulmonary tissue. Oxidative stress is known to activate redox-sensitive transcriptional regulators, enhancing the expression of proinflammatory mediators.[5,18,38]

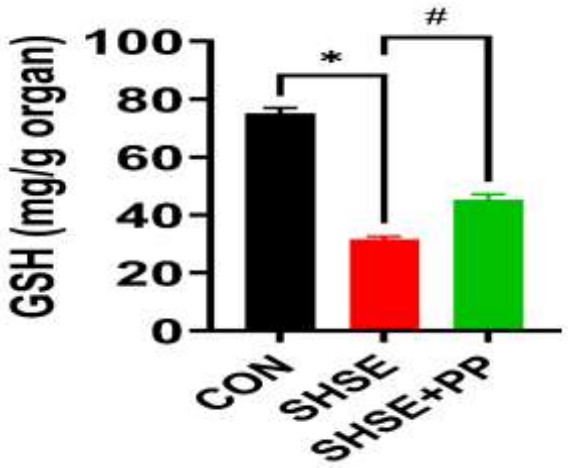

Figure 5: Effects of *Peperomia pellucida* (PP) extract and secondhand smoke exposure (SHSE) on antioxidant activity measured by lung tissue glutathione (GSH) level. Values are expressed as mean ± SEM (n = 6–7 per group). *$p < 0.05$ vs. control (CON); $^{\#}p < 0.05$ vs. SHSE group, determined by one-way ANOVA followed by Fisher's Least Significant Difference (LSD) post hoc test. CON: control group; SHSE: secondhand smoke-exposed group; SHSE+PP: SHSE group treated with PP extract.

These cytokines further exacerbate lung injury by promoting immune cell recruitment, epithelial damage, and the amplification of local inflammation. In addition, sustained oxidative stress is known to enhance the expression of TGF-*β*, the primary profibrotic cytokine that mediates ECM accumulation and EMT.[29,34,42]

This study found that GSH levels were significantly upregulated in the SHSE+PP group relative to rats exposed only to secondhand smoke ($p < 0.001$). The restoration of GSH levels suggests that *P. pellucida* confers antioxidant protection, likely through its diverse array of bioactive compounds, including phenolics, sesquiterpenes, chlorophyll derivatives, and phenylpropanoids.[14,44,46] Among these compounds, flavonoids are particularly notable for their capacity to scavenge free radicals and stimulate endogenous antioxidant responses, particularly through nuclear factor erythroid 2–related factor 2 (Nrf2) signaling. Nrf2 activation increases the transcription of genes associated with cytoprotection and antioxidant defense, including superoxide dismutase and heme oxygenase-1, both of which are responsible for counteracting oxidative stress within pulmonary tissues.[10,52] Through activation of Nrf2 pathway, *P. pellucida* may enhance the endogenous antioxidant defense system, thereby attenuating oxidative stress. This reduction in oxidative insult has been closely linked to the downregulation IL-6 and TNF-α, as well as the profibrotic mediator TGF-*β*.[10,40] Furthermore, previous studies have shown that supplementation with GSH precursors led to reduced oxidative burden and concomitant suppression of IL-6, TNF-α, and TGF-*β* levels in experimental models and clinical cases of IPF.[53,54]

Conclusion

This study demonstrates the protective role of *P. pellucida* extract on pulmonary fibrogenesis in rats exposed to secondhand smoke by modulating inflammatory, fibrotic, and oxidative pathways. The extract significantly inhibits the progression of fibrosis, as evidenced by improved histopathological scores, downregulation of TNF-α, IL-6, and TGF-*β* expression, and restoration of GSH levels in lung tissue. These findings are particularly important given the global burden of secondhand smoke exposure and its association with chronic lung diseases. These results demonstrate the protective activities of *P. pellucida* as a novel candidate for preventing the initiation and progression of pulmonary fibrosis. Future research should aim to isolate specific bioactive compounds, elucidate their molecular targets, and validate these effects in clinical or long-term models to facilitate the translational application of these findings in respiratory medicine.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Bellou V, Belbasis L, Evangelou E. Tobacco Smoking and Risk for Pulmonary Fibrosis. Chest. 2021;160(3):983–993.
2. Yu QY, Tang XX. Irreversibility of Pulmonary Fibrosis. Aging Dis. 2022;13(1):73.
3. Longitudinal lung function and gas transfer in individuals with idiopathic pulmonary fibrosis: a genome-wide association study. Lancet Respir Med. 2023;11(1):65–73.
4. WHO. Global Adult Tobacco Survey (GATS) Fact Sheet Indonesia 2021. 2021.
5. Estornut C, Milara J, Bayarri MA, Belhadj N, Cortijo J. Targeting Oxidative Stress as a Therapeutic Approach for Idiopathic Pulmonary Fibrosis. Front Pharmacol. 2021;12.
6. Sgalla G, Iovene B, Calvello M, Ori M, Varone F, Richeldi L. Idiopathic pulmonary fibrosis: pathogenesis and management. Respir Res. 2018;19(1):32.
7. Soetedjo FA, Kristijanto JA, Agusaputra H, Kusumaningtyas MJ. *Peperomia pellucida* Extract Ameliorates Secondhand Smoke Exposure-Induced Lung Fibrogenesis via Regulation of Matrix

Metalloproteinase, Inflammatory, and Fibrotic Cytokines: A Pre-Clinical Study. Pharm Sci Asia. 2024;51(3):223–232.
8. Li Y, Zhao J, Yin Y, Li K, Zhang C, Zheng Y. The Role of IL-6 in Fibrotic Diseases: Molecular and Cellular Mechanisms. Int J Biol Sci. 2022;18(14):5405–5414.
9. Hou J, Ma T, Cao H, Chen Y, Wang C, Chen X, Xiang Z, Han X. TNF-α-induced NF-κB activation promotes myofibroblast differentiation of LR-MSCs and exacerbates bleomycin-induced pulmonary fibrosis. J Cell Physiol. 2018;233(3):2409–2419.
10. Wang Y, Wei J, Deng H, Zheng L, Yang H, Lv X. The Role of Nrf2 in Pulmonary Fibrosis: Molecular Mechanisms and Treatment Approaches. Antioxidants. 2022;11(9):1685.
11. Gulati S, Luckhardt TR. Updated Evaluation of the Safety, Efficacy and Tolerability of Pirfenidone in the Treatment of Idiopathic Pulmonary Fibrosis. Drug Healthc Patient Saf. 2020;12:85–94.
12. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, Brozek JL, Collard HR, Cunningham W, Homma S, Johkoh T, Martinez FJ, Myers J, Protzko SL, Richeldi L, Rind D, Selman M, Theodore A, Wells AU, Hoogsteden H, Schünemann HJ; American Thoracic Society; European Respiratory society; Japanese Respiratory Society; Latin American Thoracic Association. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med. 2015;192(2):e3–e19.
13. Kartika IGAA, Riani C, Insanu M, Adnyana IK. *Peperomia pellucida* extracts stimulates bone healing in alveolar socket following tooth extraction. J Tradit Complement Med. 2022;12(3):302–307.
14. Gomes PWP, Barretto H, Reis JDE, Muribeca A, Veloso A, Albuquerque C, Teixeira A, Braamcamp W, Pamplona S, Silva C, Silva M. Chemical Composition of Leaves, Stem, and Roots of *Peperomia pellucida* (L.) Kunth. Molecules. 2022;27(6):1847.
15. Fakayode AE, Emma- Okon BO, Morakinyo AE, Fajobi AO, Akinyele KN, Oyedapo OO. Investigations of Ameliorative Potentials Extract of *P. pellucida* on Salt - Fructose Induced Dyslipidemia in Wistar Rats. Trop J Nat Prod Res. 2023;7(9):4112–4116.
16. Okoh S, Iweriebor B, Okoh O, Okoh A. Bioactive constituents, radical scavenging, and antibacterial properties of the leaves and stem essential oils from *Peperomia pellucida* (L.) kunth. Pharmacogn Mag. 2017;13(51):392.
17. Finato AC, Fraga-Silva TF, Prati AUC, de Souza Júnior AA, Mazzeu BF, Felippe LG, Pinto RA, Golim M de A, Arruda MSP, Furlan M, Venturini J. Crude leaf extracts of *Piperaceae* species downmodulate inflammatory responses by human monocytes. PLoS One. 2018;13(6):e0198682.
18. Soetedjo FA, Kristijanto JA, Khamidah N. Attenuation of Cigarette-Smoke-Induced Oxidative Stress By Oat Diet. Indones J Health Sci. 2024;8(1):91–96.
19. Kurniati I, Tjahjono K, Prasetyo A, Miranti P. I. *Muntingia calabura* Modulates Alveolar Matrix Metalloproteinase-9 (MMP-9) and Decreases The Alveolar Diameter in Sprague-Dawley Rats Exposed to Cigarette Smoke. Trop J Nat Prod Res. 2025;9(4):1853.
20. Ofori M, Danquah CA, Asante J, Ativui S, Doe P, Abdul-Nasir Taribabu A, Nugbemado IN, Mensah AN. *Betulin* and *Crinum asiaticum* L. bulbs extract attenuate pulmonary fibrosis by down regulating pro-fibrotic and pro-inflammatory cytokines in bleomycin-induced fibrosis mice model. Heliyon. 2023;9(6):e16914.
21. Khwaldeh A, Shraideh Z, Badran D, Shoiab A, Alsarhan AA, Al-Fawaeir S, Alzbeede A, Aljamal A. Hepatoprotective Effects of Honey Against Tobacco Smoking Toxicity in Wistar Rats. Trop J Nat Prod Res. 2024;8(5):7220–7224.
22. ThermoFisher Scientific. ELISA Sample Preparation Protocols Rat Lung Perfusion. *https://www.thermofisher.com/id/en/home/references/protocols/cell-and-tissue-analysis/elisa-protocol/elisa-sample-preparation-protocols/rat-lung-perfusion.html*.
23. Zhou X, Moore B. Lung Section Staining and Microscopy. Bio Protoc. 2017;7(10).
24. Klopfleisch R. Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology - a systematic review. BMC Vet Res. 2013;9(1):123.
25. Lee Y-H, Seo D-S, Lee MJ, Cha H-G. Immunohistochemical characterization of oxidative stress in the lungs of rats exposed to the humidifier disinfectant polyhexamethylene guanidine hydrochloride. J Toxicol Pathol. 2019;32(4):311–317.
26. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue – a review. Diagn Pathol. 2014;9(1):221.
27. Mishra P, Pandey C, Singh U, Gupta A, Sahu C, Keshri A. Descriptive statistics and normality tests for statistical data. Ann Card Anaesth. 2019;22(1):67.
28. Lee S, Lee DK. What is the proper way to apply the multiple comparison test? Korean J Anesthesiol. 2018;71(5):353–360.
29. Li Y, Wang L, Zhang Q, Tian L, Gan C, Liu H, Yin W, Ye T. Blueberry Juice Attenuates Pulmonary Fibrosis via Blocking the TGF-β1/Smad Signaling Pathway. Front Pharmacol. 2022;13:825915.
30. Guo J, Meng X, Zheng Y-M, Zhao S-K, Qiang C, Zhou L-B. Cigarette Smoke Mediates Nasal Epithelial Barrier Dysfunction via TNF-α. Am J Rhinol Allergy. 2023;37(6):646–655.
31. Li Y, Jiang Q, Wang L. Appetite Regulation of TLR4-Induced Inflammatory Signaling. Front Endocrinol (Lausanne). 2021;12777997.
32. Kalininskiy A, Rackow AR, Nagel D, Croft D, McGrane-Minton H, Kottmann RM. Association between weight loss and mortality in idiopathic pulmonary fibrosis. Respir Res. 2022;23(1):377.
33. Lee JK, Chung C, Kim J, Cho HS, Kim HC. Clinical impact of weight loss on mortality in patients with idiopathic pulmonary fibrosis: a retrospective cohort study. Sci Rep. 2023;13(1):5774.
34. Thakur D, Taliaferro O, Atkinson M, Stoffel R, Guleria RS, Gupta S. Inhibition of nuclear factor κB in the lungs protect bleomycin-induced lung fibrosis in mice. Mol Biol Rep. 2022;49(5):3481–3490.
35. Kim J, Cho Y, Oh G, Park H, Yang MJ, Park C, Kim Y, Choi K, Go R, Kim M. Repeated intratracheal instillation of whole-cigarette smoke condensate to assess lung damage in a rat model. Environ Toxicol. 2024;39(4):2304–2315.
36. Fang L, Cheng Q, Zhao F, Cheng H, Luo Y, Bao X, Li Y, Liang X, Huang Y, Xu J, Han J, Tang Y, Tang S, Liu W, Luo Z, Feng D. Cigarette smoke exposure combined with lipopolysaccharides induced pulmonary fibrosis in mice. Respir Physiol Neurobiol. 2019;266:9–17.
37. Lan Y-W, Chen C-E, Huang T-T, Huang T-H, Chen C-M, Chong K-Y. *Antrodia cinnamomea* extract alleviates bleomycin-induced pulmonary fibrosis in mice by inhibiting the mTOR pathway. Biomed J. 2024;47(6):100720.
38. Dianat M, Radan M, Badavi M, Mard SA, Bayati V, Ahmadizadeh M. Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress. Respir Res. 2018;19(1):58.
39. Serré J, Tanjeko AT, Mathyssen C, Vanherwegen A-S, Heigl T, Janssen R, Verbeken E, Maes K, Vanaudenaerde B, Janssens W, Gayan-Ramirez G. Enhanced lung inflammatory response in whole-body compared to nose-only cigarette smoke-exposed mice. Respir Res. 2021;22(1):86.
40. Cha S-R, Jang J, Park S-M, Ryu SM, Cho S-J, Yang S-R. Cigarette Smoke-Induced Respiratory Response: Insights into Cellular Processes and Biomarkers. Antioxidants. 2023;12(6):1210.
41. Juhl P, Bondesen S, Hawkins CL, Karsdal MA, Bay-Jensen A-C, Davies MJ, Siebuhr AS. Dermal fibroblasts have different extracellular matrix profiles induced by TGF-β, PDGF and IL-6 in a model for skin fibrosis. Sci Rep. 2020;10(1):17300.
42. Lee JH, Massagué J. TGF-β in developmental and fibrogenic EMTs. Semin Cancer Biol. 2022;86(Pt 2):136–145.

43. Ye Z, Hu Y. TGF-β1: Gentlemanly orchestrator in idiopathic pulmonary fibrosis (Review). Int J Mol Med. 2021;48(1):132.
44. Alves NSF, Setzer WN, da Silva JKR. The chemistry and biological activities of *Peperomia pellucida* (*Piperaceae*): A critical review. J Ethnopharmacol. 2019;232:90–102.
45. Lisdiana L, Widiatningrum T, Kurniawati F. Molecular Docking Bioactive Compound of Rambutan Peel (*Nephelium lappaceum* L) and NF-κB in the Context of Cigarette Smoke-Induced Inflammation. Trop J Nat Prod Res. 2022;6(10):1654–1659.
46. Ho KL, Yong PH, Wang CW, Lim SH, Kuppusamy UR, Arumugam B, Ngo CT, Ng ZX. In vitro anti-inflammatory activity and molecular docking of *Peperomia pellucida* (L.) Kunth extract via the NF-κB and PPAR-γ signalling in human retinal pigment epithelial cells. Bioorg Chem. 2024;153:107969.
47. Nasution DLI, Tjahajawati S, Indriyanti R, Amaliya. Anti-inflammatory effectiveness of *Peperomia pellucida* (L.) Kunth in rats induced with periodontitis. Biochem Biophys Rep. 2024;40:101856.
48. Wang M-C. Natural plant resource flavonoids as potential therapeutic drugs for pulmonary fibrosis. Heliyon. 2023;9(8):e19308.
49. Cuijpers I, Sthijns MMJPE, van den Bogart VAR, Katsburg J, Leenders CFM, Troost FJ. Quercetin, Kaempferol and Capsaicin Counteract the TGF-β1-Induced Upregulation of αSMA and Collagen in Myoblasts. Int J Mol Sci. 2025;26(11):5151.
50. Dalle-Donne I, Garavaglia ML, Colombo G, Astori E, Lionetti MC, La Porta CAM, Santucci A, Rossi R, Giustarini D, Milzani A. Cigarette smoke and glutathione: Focus on in vitro cell models. Toxicol in Vitro. 2020;65:104818.
51. van de Wetering C, Elko E, Berg M, Schiffers CHJ, Stylianidis V, van den Berge M, Nawijn MC, Wouters EFM, Janssen-Heininger YMW, Reynaert NL. Glutathione S-transferases and their implications in the lung diseases asthma and chronic obstructive pulmonary disease: Early life susceptibility? Redox Biol. 2021;43:101995.
52. Hao W, Li M, Cai Q, Wu S, Li X, He Q, Hu Y. Roles of NRF2 in Fibrotic Diseases: From Mechanisms to Therapeutic Approaches. Front Physiol. 2022;13:889792.
53. Zhang X-L, Cao Y, Zheng B. Efficacy of N-acetylcysteine plus pirfenidone in the treatment of idiopathic pulmonary fibrosis: a systematic review and meta-analysis. BMC Pulm Med. 2023;23(1):479.
54. Kulshrestha R, Pandey A, Jaggi A, Bansal S. Beneficial effects of N-acetylcysteine on protease-antiprotease balance in attenuating bleomycin-induced pulmonary fibrosis in rats. Iran J Basic Med Sci. 2020;23(3):396–405.