Pharmacogn. Res., 2025; 17(2):532-542.
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Original Article

Syringic Acid Ameliorates Bleomycin Induced Pulmonary Inflammation and Fibrosis in Rats via Maintenance of Endogenous Antioxidants and Downregulation of Pro-Inflammatory Markers

Bhupatiraju VS Lakshmi[1,*], Krupa Varam Bethala[2]

[1]Department of Pharmacology, School of Allied and Healthcare Sciences, Malla Reddy University, Hyderabad, Telangana, INDIA.
[2]Department of Pharmacology, School of Pharmacy, KPJ Healthcare University College, Nilai, MALAYSIA.

ABSTRACT

Background: Chronic administration of Bleomycin (BLM), a chemotherapeutic drug, has been linked to Idiopathic Pulmonary Fibrosis (IPF). It has been observed that syringic acid, a phenolic compound, has antiapoptotic, anti-inflammatory and antioxidant properties. **Objectives:** To assess syringic acid's therapeutic potential against lung fibrosis caused by BLM and determine potential mechanism of action. **Materials and Methods:** Sprague-Dawley rats were inducted into IPF after receiving 7.5 IU/kg of BLM intratracheally. Syringic acid (50 mg/kg, p.o.) was administered to rats for 14 days, after which different parameters in lung and Bronchoalveolar Lavage Fluid (BALF) were measured. **Results:** Altered BALF differential cell counts, elevated lung index, hydroxyproline, NO, MDA levels and reduction in GSH, GPx, SOD and CAT in group receiving BLM were evidence of pulmonary toxicity. Administering 50 mg/kg of syringic acid significantly reduced (p<0.001) the changes brought about by BLM. The expression of TNF-α was greatly reduced by syringic acid when it was stimulated by BLM. The BLM-treated group's histological analysis revealed significant lung damage with alveolar septal thickening, interstitial infiltration, collapsing alveolar gaps and an elevated Szapiel score. Syringic acid lessened these effects. Syringic acid group (p<0.01) markedly reduced Szapiel score, collagen deposition, lung edema and fibrotic alterations. It also inhibited infiltration of myofibroblasts and inflammatory cells, primarily macrophages and lymphocytes. This, in turn, improves control of oxidant and pro-inflammatory markers (TNF-α) to decrease collagen deposition during pulmonary fibrosis. **Conclusion:** Finally, it is concluded that Syringic acid can protect the lung against BLM-induced pulmonary oxidative stress, inflammation and fibrosis.

Keywords: Anti-fibrotic, Anti-inflammatory, Antioxidant, Bleomycin, Pulmonary fibrosis, Syringic acid.

Correspondence:
Dr. Bhupatiraju V.S. Lakshmi
Professor and HOD, Pharmacology, School of Allied and Healthcare Sciences, Malla Reddy University, Hyderabad-500100, Telangana, INDIA.
Email: drbvslakshmi@allareddyuniversity.ac.in

Received: 21-10-2024;
Revised: 04-12-2024;
Accepted: 17-02-2025.

INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) is a distinct type of slowly developing, chronic lung disease with no known cause that is linked to oxidative stress, inflammation and the buildup of fibroblasts and myofibroblasts. In the early stages of the disease, this can result in abnormal extracellular collagen deposition.[1] Regretfully, the prognosis for IPF is bleak and pirfenidone remains the only viable medication, even after much research. The 5-year death rate is 80% in the absence of lung transplantation. Novel treatment agents with enhanced efficacy are therefore required.[2]

Many plant-derived bioactive chemicals, including vinblastine, camptothecin and paclitaxel, have been identified and are being utilized extensively to treat different kinds of cancer in the previous few decades. Creating more effective treatment agents with increased action against lung cancer is a requirement. Nowadays, there is a lot of interest in functional foods and nutraceuticals for the prevention of several chronic illnesses, like cancer and cardiovascular disease.[3] The two most significant classes of bioactive substances and secondary metabolites found in plants are flavonoids and phenolic acids.[4]

Syringic Acid (SA), also known as 4-hydroxy-3, 5-dimethoxybenzoic acid, is a significant phenolic chemical that occurs naturally and is present in a wide variety of plants and foods. Swiss chard, dates, walnuts, olives, spices and pumpkin are the primary sources of SA.[5,6] It has been reported that SA is present abundant in cereals such as barley, maize, millet, oat,

DOI: 10.5530/pres.20251990

rice, rye, sorghum and wheat[7] and in plants like *Raphanus sativus* L.,[8] *Hemidesmus indicus*[9] *Tagetese recta* Linn. flower.[10] SA has been shown to have a number of biological properties, including antioxidant, antiproliferative,[11] anti-endotoxic,[12] and anti-cancer activity.[13] The administration of SA could suppress hepatic fibrosis in chronic liver injury.[14] SA decreased proliferation in leukemia cells and induced apoptosis by raising the level caspase 3, 8 and 9 activities.[15]

Because intratracheal BLM treatment in rats results in alveolar cell injury, an inflammatory response, fibroblast proliferation and collagen content deposition, Bleomycin (BLM)-induced pulmonary fibrosis is a commonly used animal model of human IPF.[16] Reactive Oxygen Species (ROS) and proteolytic enzymes are known to be released by activated inflammatory cells that accumulate in the lungs, causing parenchymal damage and escalating the severity of injury.[17] Research evaluating various antioxidant compounds as a preventative measure in BLM-induced lung fibrosis in rats, including N-acetylcysteine, erdosteine,[18] caffeic acid phenethyl ester,[17] melatonin,[19] *Ginkgo biloba*,[20] cordyceps,[21] and resveratrol,[22] discovered that these compounds typically prevented or lessened lung fibrosis based on Ashcroft's criteria and lung hydroxyproline content.

Consequently, using antioxidant techniques to prevent or treat BLM-induced lung fibrosis may make sense. We investigated SA's ability to prevent IPF using this approach. We assessed the histological and biochemical evidence of lung fibrosis brought on by exposure to BLM and we investigated inflammatory markers and oxidative stress in a model of injured lungs in rats.

MATERIALS AND METHODS

Chemicals and kits

Bleomycin was obtained from Chemex Company (Argentina), bovine serum albumin, Ellman's reagent (DTNB), thiobarbituric acid and Bradford reagent were purchased from Sigma–Aldrich. Ammonium molybdate, butylated hydroxytoluene, trichloroacetic acid, buffered formalin, HCl and perchloric acid were purchased from Merck Company. Commercial Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD) kits were purchased from RANSEL, Randox Com, UK. TNF-α commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit was provided by Xpressbio life scientific products.

BLM-induced lung fibrosis in experimental animals[23] We purchased thirty-two male Sprague Dawley rats, weighing between 150 and 250 g, from Sanzyme Ltd., in GaghanPahad, Hyderabad. The rats were kept in conventional settings, with 12 hr cycles of light and dark, 24±1°C temperature and 65±10% humidity, inside cages made of polypropylene. Water from the tap and standard pelletized feed were given. The Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2020-21/MPCOL/9) authorized the protocols for every pharmaceutical trial. The rats were divided into four groups equally and at random and they received the following care:

Group 1: The animals received normal saline (0.5 mL/d, p.o.) for 14 days,

Group 2: Syringic acid group; 50 mg/kg p.o. was given for 14 days.

Group 3: BLM was applied intratracheally (7.5 units/kg, single dose) on day 1. Normal saline (0.5 mL/d, p.o.) was given for 14 consecutive days,

Group 4: BLM was applied intratracheally (7.5 units/kg, single dose) on day 1. Syringic acid (50 mg/kg/d, p.o.) was given for 14 consecutive days.

The rats were weighed and then anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg) i.p., followed by a single intratracheal injection of BLM hydrochloride (7.5 IU/kg body weight in 0.25 mL phosphate buffered saline, Nippon Kayaku, Japan). The control group received the same amount of intratracheal saline by the same route. All rats were sacrificed after 14 days of BLM injection. After sacrificing the animals by an overdose of anesthesia, the lung tissue specimens were rapidly and meticulously harvested for biochemical and histopathological analysis. The lung tissue samples were cleaned in cold saline and weighed to determine the lung/body mass index. By dividing the lung weight (g) by the body weight (g) and multiplying the result by 100, the lung index was calculated. The right section of the lung was placed in liquid nitrogen and stored at −70°C until the assay for Thiobarbituric Acid-Reactive Substances (TBARS) a lipid peroxidation product, Superoxide Dismutase (SOD), reduced Glutathione (GSH), Catalase (CAT) and Glutathione Peroxidase (GPx) contents. The left part of the lung was placed in formaldehyde solution for routine histopathological examination by light microscopy.

Determination of hydroxyproline in the lung tissue

A colorimetric assay was used to determine the left lung's total collagen content.[24] The left lung was dried at 80°C until it achieved a steady weight. The dry lung was hydrolyzed overnight in a glass vial with 12-N HCl at 120°C while being vacuum-operated. The sample volume was adjusted to 30 mL using distilled water and the pH was adjusted to 7 using NaOH. After combining the sample solution (1.0 mL) with 1.0 mL of chloramine T solution (0.05 mol/L), the mixture was incubated for 20 min at room temperature. After adding a 20% dimethyl benzaldehyde solution to the mixture, it was incubated for 20 min at 60°C. Every sample was analyzed by measuring its absorbance at 557 nm. The results are expressed in micrograms of hydroxyproline per gram of wet lung weight using the standard hydroxyproline curve.[25]

Biochemical assays

Two hundred milligrams of frozen lung tissue specimen was dissected into pieces on dry ice, homogenized in 1.15% KCl buffer

(1:9, w/v) using a manual glass homogenizer for approximately 5 min and flushed with centrifugation for approximately 10s to remove large debris. The supernatant was used for analysis.

Using a commercial kit, the activity of SOD was measured (RANSOD kit, Randox Com, UK). For SOD, absorption was measured at 412 nm and reported as (IU/mg protein). Goth's colorimetric approach was used to measure the Catalase (CAT) activity. After homogenised lung tissue was incubated with H_2O_2, the reaction was stopped after 10 min by mixing in ammonium molybdate. While the ammonium molybdate reacts with the sample's residual H_2O_2 to form a yellow complex, the CAT enzyme breaks down the H_2O_2 in the samples to produce H_2O and O_2. Using a spectrophotometer, absorption was measured at 410 nm. The expression for CAT activity was (μmol H_2O_2/min/mg protein).[26] A commercial kit (RANSEL kit, Randox Com, UK) based on the Paglia and Valentine method[27] was used to measure the GPX activity. At 340 nm, absorption was measured and (IU/mg of protein) was the expression for enzyme activity. Using Ellman's approach, the GSH level in lung tissue was determined. Ellman's reagent was applied to 40 μL of lung tissue homogenate after it had been combined with 2 mL of buffer phosphate. The GSH content of the yellow complex was measured using a spectrophotometer set to 410 nm and was reported as (nmol/mg protein).[28] Thiobarbituric Acid Reactive Substances (TBARS) production was used to measure the amount of lipid peroxidation.[29] To summarize, 0.8 mL of a solution containing 0.25N HCl, 0.375% (w/v) thiobarbituric acid and 15% (w/v) Trichloroacetic Acid (TCA) was mixed with 0.2 mL of homogenized tissue. Centrifugation was used for 5 min at 5000 RPM to precipitate and remove the protein. After being transferred to test tubes containing 0.02% butylated hydroxyl toluene, the supernatants were bain-marie heated for 15 min at 100°C. After that, samples were chilled and centrifuged for 5 min at 2000 RPM to get rid of the precipitant. When measuring absorption at 532 nm, MDA concentrations were reported as (nmol/mg protein).

Determination of Nitric Oxide (NO) The Griess method was used to determine the nitrite levels, which serve as an indicator of NO generation. First, acetonitrile (1:2, v/v) was used to deproteinize tissue samples. Subsequently, 100 μL of supernatant and 100 μL of Griess reagent were applied to a microplate well. After that, samples were incubated in microplate wells for 30 min at 37°C and the absorbance of the samples was eventually found at 546 nm. Based on a typical linear curve established by 0-150 mol/mL sodium nitrite, the quantity of nitric oxide metabolites in the samples was ascertained. For tissue samples, the data were given in moles per gram (g) and per litre (L).[30,31]

Bronchoalveolar lavage Fluid differential cell count

The process of obtaining bronchoalveolar lavage fluid involved injecting 3 mL of saline three times, for a total of 9 mL and then gently aspirating the fluid out of the lung after inserting an intratracheal catheter into a trachea. The recovery ratio of lavage fluid with this catheter was roughly 80% and did not show any discernible variation across the groups. Using a hemocytometer, the total number of cells in the bronchoalveolar lavage fluid was determined. Smear slides were produced and stained with Giemsa solution to allow for differential counts of leukocytes in the Bronchoalveolar lavage fluid. Three hundred cells were counted differently for each sample.

Measurement of tumor necrosis factor-α

Tumor necrosis factor-α concentration was measured using an enzyme-linked immunosorbent assay kit (Xpress Bio Life scientific products). The determinations were done according to the test kit instructions.

Histopathological studies

Inflammatory and fibrotic evidence in histopathology studies

The tissue from the left lung was put in 10% buffered formalin and blocked in paraffin for histopathological analysis. Hematoxylin and Eosin (H&E) and Masson's Trichrome (MT) were used to stain 4 μM sections to assess collagen deposition, alveolar thickness and inflammatory cell infiltration. The Szapiel score[32] graded alveolitis and inflammation from 0 to 3, while the Ashcroft score[33] measured fibrotic lesions from 0 to 8. Grading of inflammatory and fibrotic lesions was done on three slides per rat, with ten fields each slide.

Statistical analysis

The Data were analyzed using GraphPad Prism 8 (GraphPad Software Inc.). The result was expressed as Mean±SEM and analyzed using one-way ANOVA followed by Tukey–Kramer multiple comparison tests. Previously, the normal distribution

Table 1: Effect of Syringic acid on Bleomycin induced body changes in animals.

Treatment Groups	Before treatment (W0) (g)	After treatment (W1) (g)	% (W1-W0)/W1.
Control	183.31± 8.8	196.61±8.2	6.76
Syringic acid	165.38±14.1	179.35±18.8	7.78
BLM	201.54±19.6	168.36±16.4	-19.70
Syringic acid+ BLM	245.33±32.1	253.38±34.8	3.17

Data are presented as Mean±SEM (n = 8). BLM-Bleomycin.

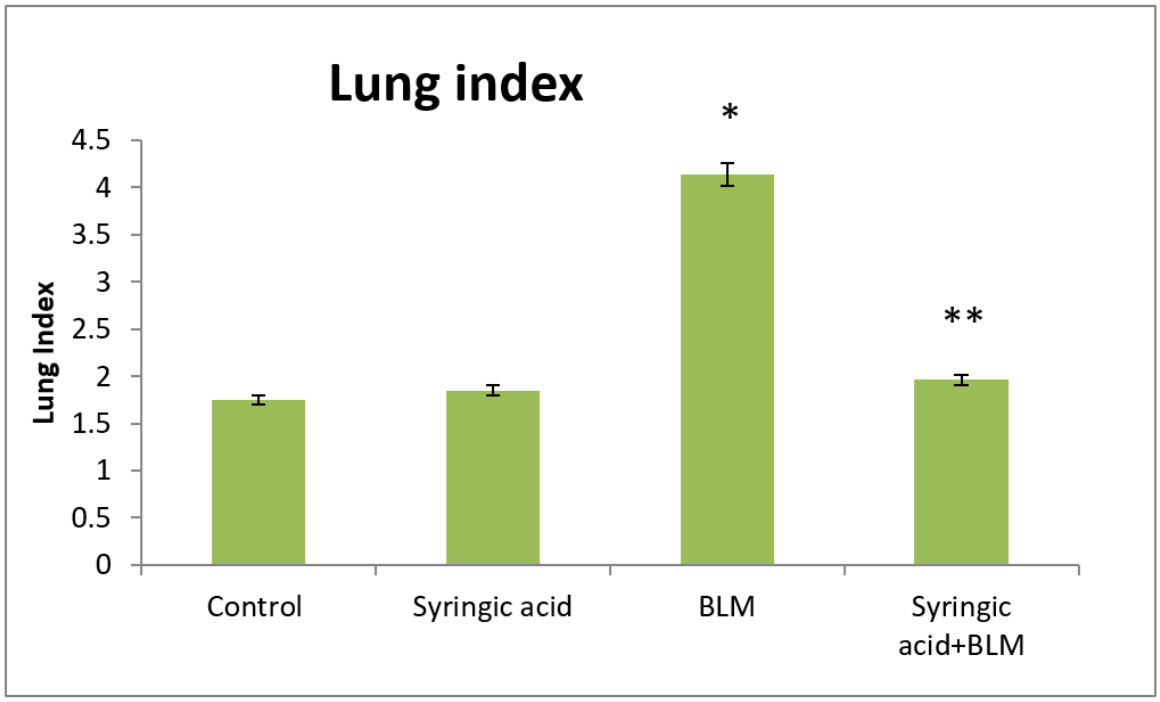

Figure 1: Effect of Syringic acid on the rat lung index of BLM-induced pulmonary fibrosis. Values are presented as mean±SEM (n=8). * p<0.001 Indicate significant difference compared with the control group; ** p<0.01 vs BLM-treated rats. BLM: Bleomycin.

of data was evaluated. Differences were considered statistically significant at (p<0.05).

RESULTS

Effect of syringic acid on body weight and lung index

BLM application caused significant body weight loss after 14 days compared with the control group (p<0.001). BLM significantly increased lung index at 14th day compared with control group (p<0.001). Syringic acid administration increased body weight compared with BLM group (p<0.01) (Table 1). When compared to the BLM group, syringic acid (50 mg/kg) dramatically reduced the lung index (Figure 1).

Hydroxyproline assay

Using hydroxyproline measurements, we calculated the amount of collagen deposited in the lung tissues. Bleomycin markedly raised the amount of hydroxyproline in the rat lungs as compared to the control group (p<0.001). The group treated with syringic acid plus bleomycin had a substantial decrease in hydroxyproline content as compared to the group treated with BLM (p<0.01) (Figure 2).

Antioxidant defense system markers

When compared to the control group, BLM reduced the activity of the enzymes SOD, CAT and GPX at the same level (p<0.001). Syringic acid pretreatment at a dose of 50 mg/kg boosted the activity of CAT, GPX and SOD. These effects demonstrated a strong recovery and were comparable to the BLM group (p<0.01) (Table 2). When compared to the control group, BLM decreased the levels of GSH in lung tissue (p<0.001). GSH levels were elevated by 50 mg/kg (p<0.001) of syringic acid. Syringic acid 50 mg/kg (p<0.01) reduced lung tissue MDA and NO levels, while BLM raised MDA and NO levels (p<0.001) (Figure 3).

Total and differential cell count in Bronchoalveolar lavage fluid

The impact of syringic acid on total cell counts and the differential in bronchoalveolar lavage fluid between the experimental and control groups of rats is displayed in Table 3. When compared to control rats, bleomycin therapy resulted in a substantial increase in the overall cell count in the bronchoalveolar lavage fluid (p<0.001). The total cell counts in rats given syringic acid treatment stayed at levels comparable to those of control rats. Rats treated to bleomycin had significantly higher neutrophil and eosinophil counts in their lungs, according to the differential cell count. The increase in blood cells in the bronchoalveolar lavage caused by bleomycin was considerably decreased after a 14-day syringic acid treatment. Although the bleomycin-induced group had a lower percentage of lymphocytes and alveolar macrophages, syringic acid treatment dramatically reversed these effects (p<0.01).

Pro-inflammatory marker-Tumor necrosis factor-α concentration

Figure 4a, b shows the plasma levels of tumor necrosis factor-α. On day 14, the plasma levels of tumor necrosis factor-α protein in the rats in the bleomycin-administered group were still higher than those in the control group. At the conclusion of the trial, it

was discovered that syringic acid treatment reduced the increase in tumor necrosis factor-α level caused by bleomycin.

Histological changes

On day 14, histopathological abnormalities in the lungs were found using Masson's trichome staining (Figure 5) and hematoxylin and eosin staining (Figure 6). Typical open alveoli, interalveolar gaps with typical terminal bronchi, normal bronchiolar epithelial appearance, thin interalveolar septa, absence of inflammatory cells and fibrosis were all seen in normal lung tissues. Rats in the bleomycin-administered group displayed deformed tissue architecture, including moderate to severe bleeding, congestion, emphysema-related sloughing of the bronchial epithelium from the basement membrane, areas of increased alveolar thickening, increased fibrosis and leukocyte accumulation in the alveolar walls. In contrast to the bleomycin-treated group, the lungs of the rats treated with syringic acid exhibited fewer leukocytes and less thickening of the alveoli.

When localizing collagen as a distinct area in a histological preparation, Masson staining is seen to be a dependable technique. In comparison to the control group, the bleomycin-treated group showed larger fibrotic regions and an elevated grade of collagen deposition. In contrast to the bleomycin group, collagen formation was noticeably reduced in syringic acid. The Szapiel examination's pathology score was used to determine the lung sections' semi-quantitative assessment. On day 14, the bleomycin-induced group's Szapiel core was discovered to be considerably higher than that of the control group. On the fourteenth day, the Szapiel scores of the group

Table 2: Oxidative stress status in animals subjected to various treatments.

Biochemical estimations	Control	Syringic acid	BLM	Syringic acid+BLM
Malondialdehyde level (nmoles/mg protein)	1.50±0.55	1.45±0.48	2.98±1.02*	1.34±0.32**
Reduced glutathione (nmol/mg protein)	25.61±1.17	25.61±1.17	7.51±097*	21.58±2.64**
Glutathione peroxidase (IU/mg protein)	81.23±0.98	83.23±2.25	20.27±4.21*	79.52±3.69**
Superoxide dismutase (IU/mg protein)	74.58±1.85	72.15±3.32	32.88±2.52*	70.64±3.02**
Catalase (μM H_2O_2/mg tissue/min)	84.69±2.15	87.26±3.58	29.52±2.95*	87.37±1.98**

Values are presented as mean±SEM (n=8). *p<0.001 Indicate significant difference compared with the control group; ** p<0.01 vs BLM-treated rats. BLM: Bleomycin.

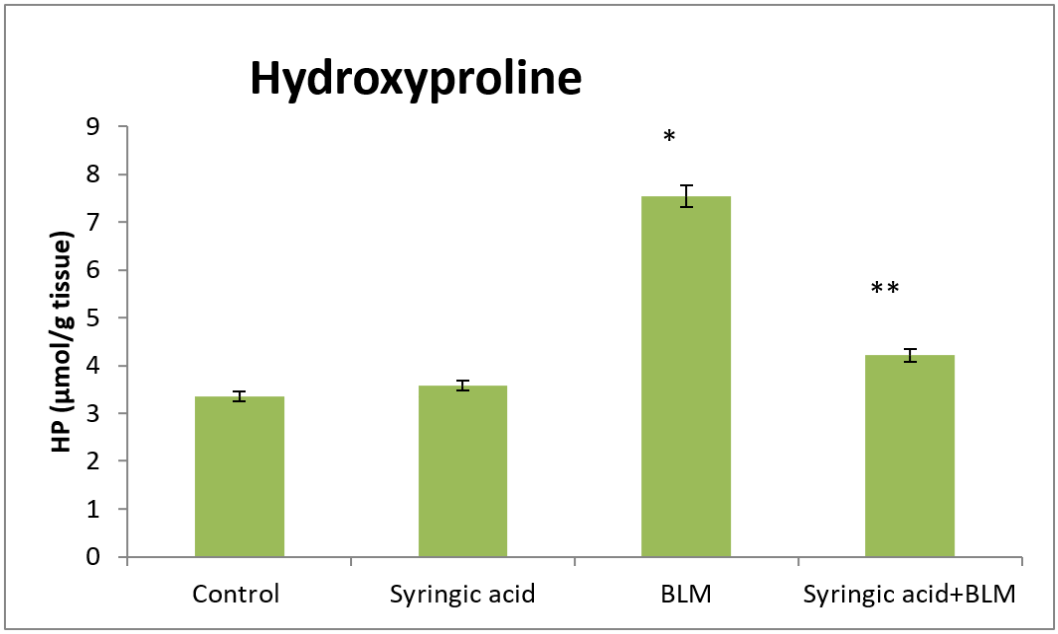

Figure 2: Effect of Syringic acid on hydroxyproline content in the rat lung index of BLM-induced pulmonary fibrosis. Values are presented as mean±SEM (n=8). * p<0.001 Indicate significant difference compared with the control group; ** p<0.01 vs BLM-treated rats. BLM: Bleomycin.

Table 3: **The total and differential blood cell counts of rats from various study groups.**

Experimental Groups	Total cells (x10^6 mL^{-1})	Macrophages (%)	Neutrophils (%)	Eosinophils (%)	Lymphocytes (%)
Control	0.69±0.15	87.25±1.48	5.68±0.98	0.84±0.12	12.59±1.15
Syringic acid	0.71±0.14	89.54±5.65	6.67±1.10	0.99±0.26	12.98±2.58
BLM	1.98±0.21*	58.54±4.51*	45.23±3.58*	4.98±1.78*	5.34±0.75*
Syringic acid + BLM	0.95±0.08**	79.25±2.15**	12.58±3.69**	2.69±0.57**	13.64±1.37**

Values are presented as mean±SEM (n=8). * p<0.001 Indicate significant difference compared with the control group; ** p<0.01 vs BLM-treated rats. BLM: Bleomycin.

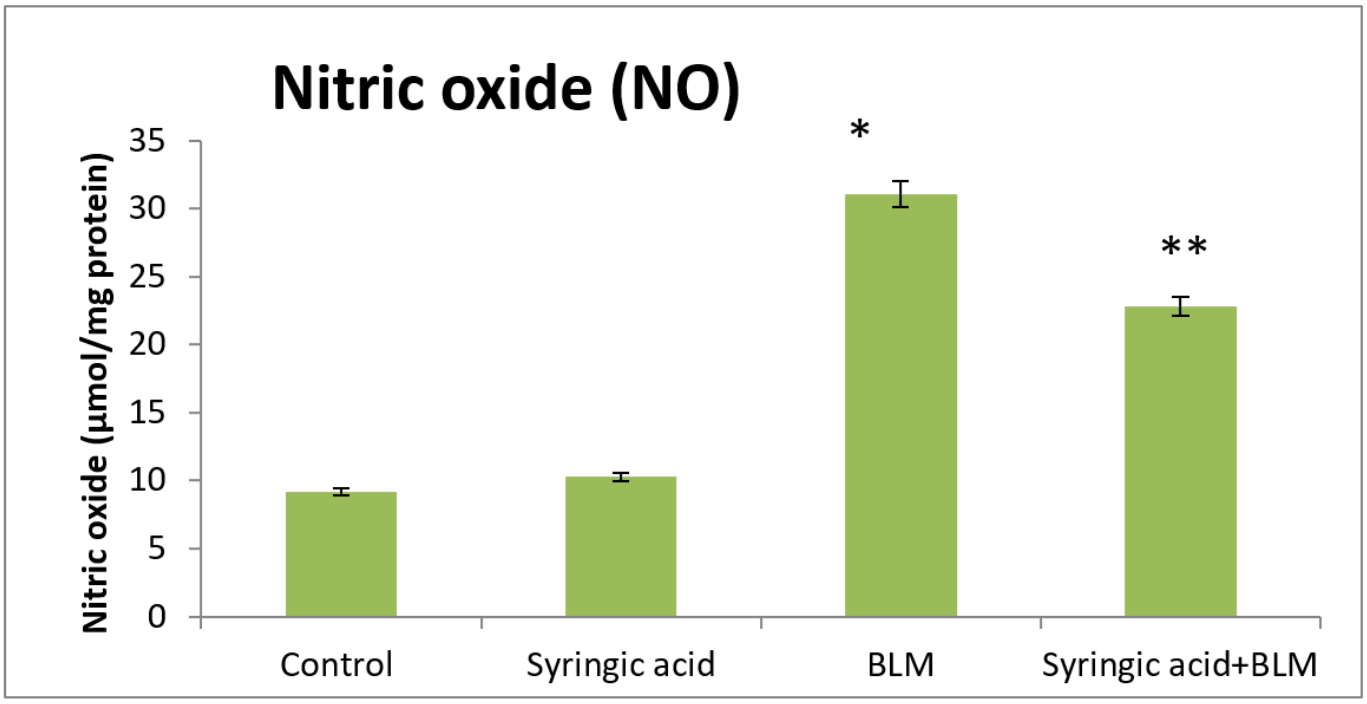

Figure 3: Effect of Syringic acid on the tissue oxidative stress markers (NO metabolite level) in animals. Values are presented as mean±SEM (n=8). * p<0.001 Indicate significant difference compared with the control group; ** p<0.01 vs BLM-treated rats. BLM: Bleomycin.

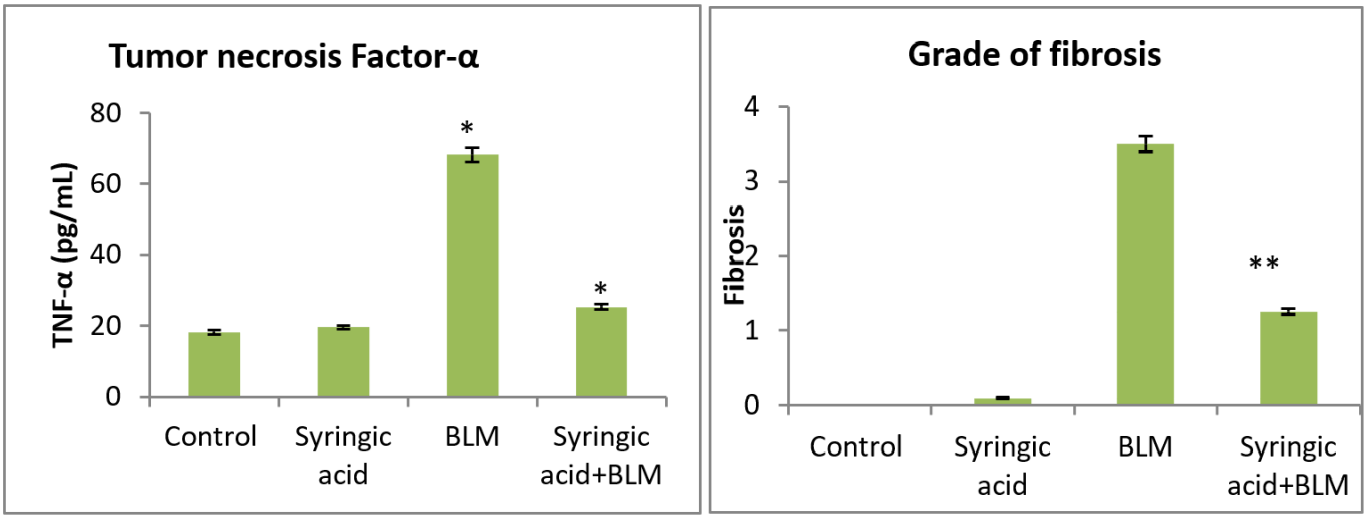

Figure 4: A and B: The tumor necrosis factor-α and grade of fibrosis in rats subjected to various treatments. Values are presented as mean±SEM (n=8). * p<0.001 Indicate significant difference compared with the control group; **p<0.01 vs BLM-treated rats. BLM: Bleomycin.

treated with syringic acid shown a notable decline in comparison to the group treated with bleomycin.

DISCUSSION

One popular experimental model used to examine pathophysiology and investigate novel medications for the treatment of IPF is the BLM-induced PF model.[34] Many studies suggest that oxidative stress and inflammation in the respiratory system may be linked to the development of lung fibrosis.[35]

The findings supported the involvement of oxidative stress (shown by decreased GSH) and inflammation (neutrophil infiltration) in the BLM-induced lung damage. The reported alterations were considerably reduced by syringic acid.

Weight loss is a well-known side effect of BLM administration in the treatment of cancer and pulmonary fibrosis models.[35] In line with earlier research, BLM given intratracheally reduced animal weight and raised body lung index in our investigation.[36] According to previous research, BLM's effects on hunger and protein catabolism may be the cause of these effects.[37] Previous research indicates that during the early stages of pulmonary fibrosis, the inflammatory responses stimulate and facilitate damage to the cells that line capillaries and alveolar epithelium. Later on, fibroblasts promote the formation of Extracellular Matrix (ECM) and collagen secretion.[38] Fibrosis results from excessive collagen remodelling and deposition, which compromises the lung tissue's structural integrity.

Since hydroxyproline makes up the majority of collagen fibres, the tissue lung's hydroxyproline content reveals the amount of collagen and the degree of pulmonary fibrosis.[39] In the current investigation, intratracheal injection of BLM considerably elevated lung hydroxyproline levels, in line with findings by Huang *et al.*[40] and Ramezani *et al.*[41] Syringic acid treatment

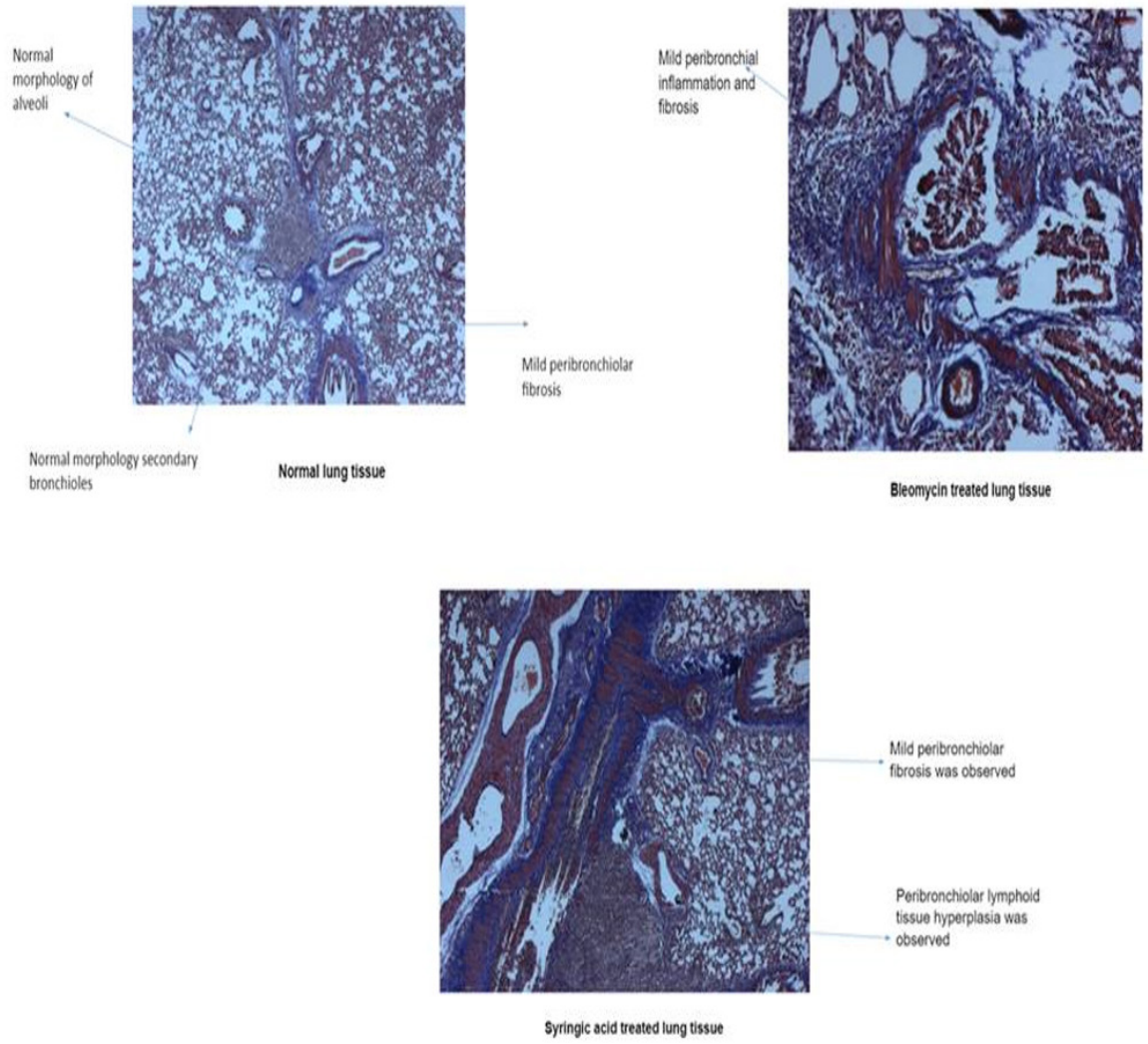

Figure 5: a) Normal lung tissue showing normal open pattern of alveoli and interalveolar space with minimal collagen deposition. b) Bleomycin treated group showing extensive collagen deposition as compared to control group lungs. c) Syringic acid tretaed lungs showing mild collagen deposition as compared to bleomycin treated lungs. (Masson's trichome staining, 10X).

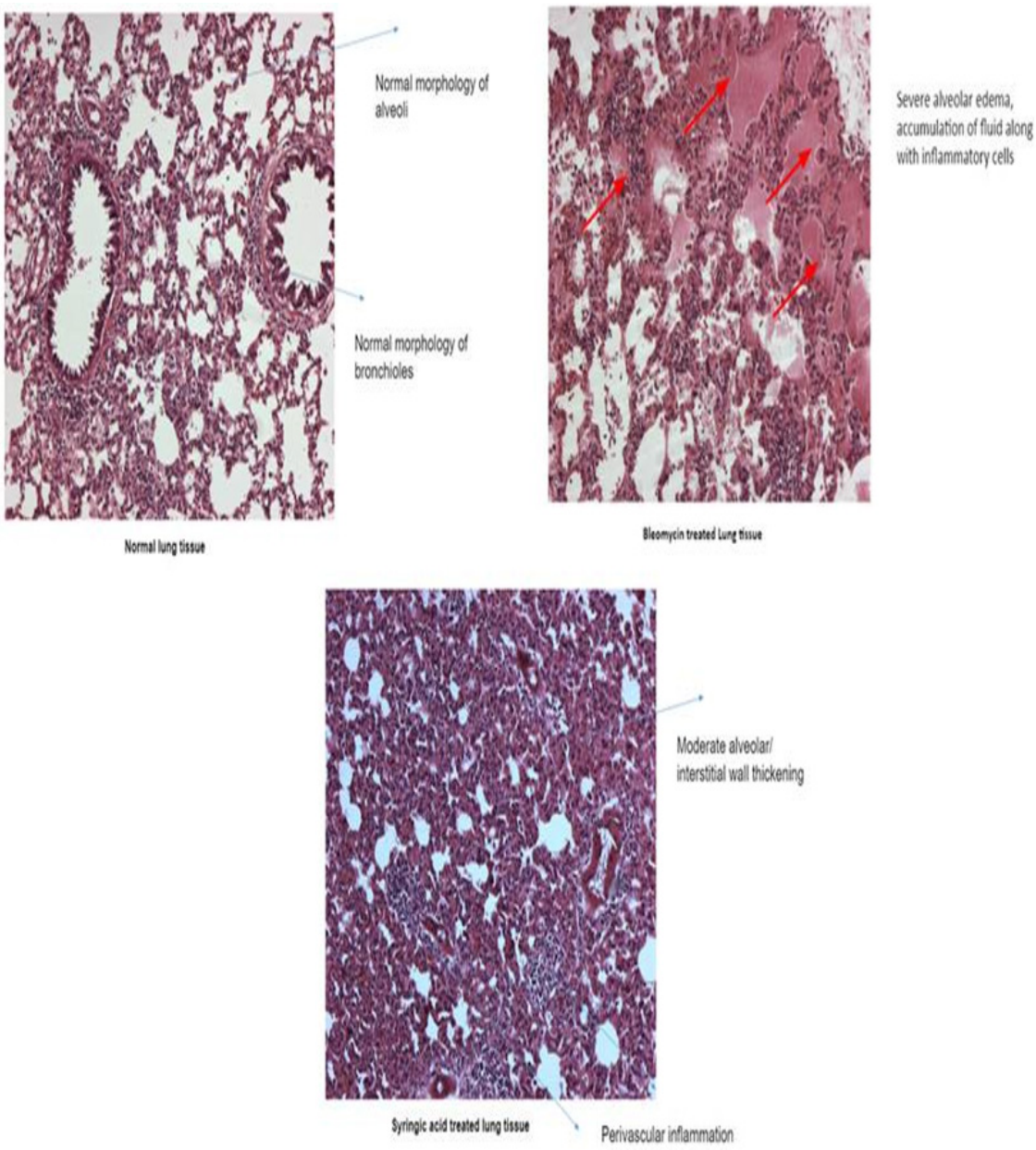

Figure 6: a) Control lung tissue showing normal open alveoli and interalveolar Space. b) Bleomycin-treated lungs showing macrophage infiltration, hemorrhages, congestion and thickening of alveolar lining. c) Syringic acid treated lungs showing decrease in the thickening of alveolar lining and decrease in the macrophage infiltration as compared to bleomycin tissue (Hematoxylin and Eosin staining, 10x).

considerably decreased the increase in hydroxyproline levels in the lung tissue.

Accordingly, a histological analysis of the lung tissues in the group that received BLM treatment revealed that the drug caused duct hyperplasia, collagen buildup and inflammatory cell penetration. In pulmonary fibrosis animal models, BLM enhances the generation of ROS.[42]

By employing Masson's trichome staining of lung sections for collagen deposition, this discovery was further supported. In the current study, bleomycin triggered the deposition, aggregation and deposition of collagen in the peribronchial and perialveolar tissues, obliterating the alveolar gaps as small fibrils. However, the group that received syringic acid treatment had significantly less collagen deposition, which may have been caused by syringic acid's inhibitory action in line with earlier studies assessing the fibrosis damage.[43]

The ability of BLM to produce ROS is one of the generally acknowledged reasons for the lung damage and tissue remodeling it induces. It is known that BLM binds DNA and Fe2+ to create a complex.[44,45] Redox cycling occurs in the DNA/Fe2+/BLM complex, producing ROS such hydroxyl and superoxide

radicals. We assessed the levels of oxidative stress markers in the current investigation. Apart from the inflammation and collagen deposition caused by BLM, we discovered that it also considerably increased the levels of lipid peroxide and significantly decreased the levels of antioxidant enzymes such as GPx, CAT and SOD when compared to the control group. These findings corroborated earlier research assessing the function of oxidative stress in BLM-induced lung fibrosis.[46]

Furthermore, we saw that syringic acid improved oxidative stress indicators such as CAT, MDA and SOD. Rat lung tissues in the syringic acid + BLM group had MDA content that was relatively similar to the control group. In these rats, concurrent administration of syringic acid effectively mitigated the inflammatory consequences of BLM therapy, or syringic acid's antioxidant properties produced an at least partially preventive impact. Reduced oxidative stress has been linked to anti-inflammatory benefits of most antioxidant medicines investigated for treatment of BLM-induced lung fibrosis models.[47,48]

Yinfang *et al*. reported that syringic acid had antioxidant qualities in this regard by lowering lipid peroxidation, which was indicated by a drop in the MDA level. Furthermore, by reducing oxidative stress, syringic acid is said to be a direct scavenger of free oxygen radicals in non-alcoholic fatty liver disease brought on by a high-fat diet. It has been established that NO plays a critical role in the aetiology of lung disorders, particularly pulmonary fibrosis. Peroxynitrite, a highly reactive nitrogen species, is created when NO interacts with superoxide free radicals, causing nitrosative stress and significant lung tissue damage. When compared to the BLM group, the group that received syringic acid saw a considerable drop in the level of NO.

Bleomycin administered intratracheally causes interstitial inflammation in addition to the oxidative stress already discussed. This inflammation is accompanied by a notable increase in leukocyte recruitment. Leukocytes, including neutrophils, lymphocytes and macrophages, are important for tissue remodelling and inflammation.[49] In the bronchoalveolar lavage fluid, the bleomycin-treated group exhibited a significant decrease in macrophages and a significant rise in total cells, neutrophils and lymphocytes. This is consistent with earlier research by Gong *et al*. (2005)[50] and Sriram *et al*. (2009).[51] Rats treated with syringic acid had similar counts of neutrophils, lymphocytes, macrophages and total cells as the rats in the control group. Inhibited leukocytes recruitment, which directly impacted inflammation and tissue repair, might partly account for the preventive effect of Syringic acid on bleomycin-induced pulmonary fibrosis, which may be due to its ability to interfere with free radical-mediated reactions.

Furthermore, among the complex webs of cellular and molecular interactions that control the fibrotic process, tumour necrosis factor-α, a strong pro-inflammatory cytokine, functions as a key player.[52] According to El-Medany *et al*. (2005),[53] there was a notable increase in tumor necrosis factor-α expression in the group that received bleomycin in this investigation. Bleomycin is known to induce inflammation-mediated tissue injury, which may be brought on by the generation of free radicals, which could activate nuclear factor kappa-B and enhance the synthesis of tumour necrosis factor-α.[54,55] TNF-α expression was significantly decreased by syringic acid. NO release was inhibited by syringic acid. The lung interstitium experiences an excessive amount of collagen deposition because of an increase in fibroblast count. Inhibiting fibroblast proliferation and excessive collagen synthesis is one method of attenuating fibrosis. Sections of the lungs stained with Masson's trichome showed that the syringic acid-treated group had less collagen deposition.

According to this study, the pulmonary response to the bleomycin challenge involves both a decrease in lung antioxidant capacity and a quick onset of oxidative stress. By inhibiting inflammation and having the ability to scavenge reactive oxygen species, syringic acid's inhibitory impact decreased oxidative stress. Further research is necessary to elucidate the protective mechanism of syringic acid on this model and explore its impact on alternative animal models of lung fibrosis.

CONCLUSION

In lung tissue, bleomycin inoculation led to decreased antioxidant capacity, increased levels of inflammatory cytokines, fibrotic alterations and collagen accumulation; in contrast, syringic acid demonstrated pneumoprotective properties by boosting antioxidant defense, lowering inflammatory cytokine levels and preventing collagen accumulation. Accordingly, the current findings imply that syringic acid successfully guards against the lung damage brought on by bleomycin challenge.

ACKNOWLEDGEMENT

The authors express their gratitude to all technical support personnel involved in animal care and basic experiments. Moreover, the facilities provided by Malla Reddy University are sincerely appreciated.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Fig: Figure; **i.p.:** Intraperitoneal; **kg:** Kilogram; **mg:** Milligram; **mL:** Millilitre; **p.o.:** Per oral; **w/w:** Weight/weight; **w/v:** weight/volume; **NO:** Nitric oxide; **GSH:** Reduced glutathione; **MDA:** Malondialdehyde; **CAT:** Catalase; **SOD:** Superoxide dismutase; **GPx:** Glutathione peroxidase; **TNF-α:** Tumor necrosis factor alpha; **BLM:** Bleomycin; **BALF:** Bronchoalveolar lavage fluid; **GPx:** Glutathione peroxidase; **eNOS:** Endothelial nitric oxide

synthase; **EMT:** Epithelial-mesenchymal transition; **ECM:** Extracellular matrix; **H&E:** Hematoxylin and Eosin; **HMGB1:** High mobility group box 1 protein; **IPF:** Idiopathic pulmonary fibrosis; **IHC:** Immunohistochemical; **iNOS:** Inducible Nitric Oxide Synthase; **MDA:** Malondialdehyde.

SUMMARY

Pulmonary Fibrosis (PF) is a chronic, progressive, age-related interstitial lung disease with a high morbidity and mortality rate. Its pathological features include excessive collagen deposition, enlarged interstitial spaces between alveoli, thickened alveolar walls and inflammatory cell infiltration, resulting in widespread scarring, decreased lung compliance and even lung failure. There is currently no treatment and animal models of PF are still crucial tools for investigating its etiopathogenesis. Although the etiology of PF is complex and variable, the accepted hypothesis is that it is caused by abnormal wound healing of alveolar epithelial cells in response to repeated injury stimulation. Bleomycin (BLM)-induced PF is the most widely accepted model for developing new anti-fibrosis strategies and evaluating drug efficacy due to its low cost, ease of induction and good reproducibility. Rats were induced with intratracheal administration of Bleomycin and the effect of syringic acid was evaluated. There was alteration in Bronchoalveolar fluid differential cell counts, lung index, hydroxyproline content, antioxidant levels and inflammatory biomarkers. Szapiel score and Ashcroft scores were used to assess the extent of lung damage. Syringic acid significantly improved the histopathological injuries illustrated by hematoxylin and eosin-stained lung tissue sections. It significantly restored body changes, reduced the lung index, hydroxyproline content, inflammatory biomarkers. This study has demonstrated that Syringic acid inhibits inflammatory response as well as prooxidant damage in pulmonary tissue of PF rats via attenuation of inflammatory response and oxidative stress. These findings suggest that Syringic acid has a therapeutic potential for the management of pulmonary fibrosis.

ETHICS APPROVAL

The Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2020-21/MPCOL/9) authorized the protocol.

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Cite this article: Lakshmi BVS, Bethala KV. Syringic Acid Ameliorates Bleomycin Induced Pulmonary Inflammation and Fibrosis in Rats via Maintenance of Endogenous Antioxidants and Downregulation of Pro-Inflammatory Markers. Pharmacog Res. 2025;17(2):532-42.