Journal of Experimental Pharmacology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Bleomycin-Induced Fibrosis and the Effectiveness of *Centella Asiatica* as a Treatment

Noni Novisari Soeroso[1], Muhammad Ichwan[2], Arlinda Sari Wahyuni[3], Causa Trisna Mariedina[4], Yabestin Alfrianus Pakpahan[1]

[1]Department of Pulmonology & Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara, Prof. dr. Chairuddin P. Lubis Universitas Sumatera Utara Hospital, Medan, Indonesia; [2]Department of Pharmacology & Therapeutic, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [3]Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Correspondence: Noni Novisari Soeroso, Email noni@usu.ac.id

Introduction: Plant treatment has been used for thousands of years and has been proven to treat acute and chronic diseases. The function of the traditional plant *Centella asiatica* is as an antimicrobial agent, anticancer, antioxidant, and therapeutic gene in healing wounds and inflammation. Lung fibrosis caused by bleomycin can develop into chronic lung disease, which ends in tissue death if not treated immediately. The purpose of this study is to predict and explain the impact of *Centella asiatica* extract on model rats exposed to bleomycin in their lungs as a treatment or anti-fibrinolysis.

Methods: This research is an analytical study with a randomized in-vivo experimental design divided into 3 groups of 5 male Wistar rats aged 10 weeks. Negative control group (K) with intratracheal induction of bleomycin alone. The positive group was given intratracheal bleomycin 4 mg/kg/BB on days 0 and 21 and added *Centella asiatica* induction at 400 mg (P1) on days 15 to 49. The other positive group was given intratracheal bleomycin 4 mg/kg/BB on days 0 and 21 and added *Centella asiatica* induction at 800 mg (P2) on days 15 to 49. Data were collected according to findings of lung histology analysis of rat samples.

Results: In the interalveolar septum group, there was a difference in Masson's Trichrome staining results in groups K and P1 with $p<0.05$ ($p=0.036$). However, there was no difference in histopathological staining results in groups K and P2 ($p>0.05$).

Conclusion: The induction of bleomycin 4 mg/kg/BB was proven to cause fibrosis in the lungs of rats, and *Centella asiatica* extract was used as a treatment. Therefore, further research regarding antifibrotic drugs is hoped to reduce fibrotic areas significantly.

Keywords: bleomycin, pulmonary fibrosis, *Centella asiatica*, treatment

Introduction

Fibrosis is the most common interstitial lung disease defined by regular interstitial pneumonia histological pattern.[1] Idiopathic pulmonary fibrosis is a lung disorder where scar tissue is found in the lungs, however, the cause is still unknown. Pulmonary fibrosis patients may have a poor prognosis compared to other malignancies. Untreated pulmonary fibrosis can progress to CPD (Chronic Lung Disease), resulting in tissue death.[2] Data on the incidence of pulmonary fibrosis varies, in the United States, it is 6.8–8.8 per 100,000 population per year, Europe 0.22–7.4 per 10,000 population, and the incidence of fibrosis increases with age.[3,4]

Bleomycin is a compound produced by *Streptomyces verticillus* bacteria, and the bacteria is often used in trial animal interventions. Bleomycin is often used in chemotherapy, but this compound has the side effect of being toxic to lung cells. The mechanism of bleomycin toxicity has been tested in vitro. Side effects of using bleomycin include nausea, fever, vomiting, and allergies. Treatment with bleomycin can also cause interstitial pneumonitis and pulmonary fibrosis.[5] Animal models are crucial to the study of disease, and lung pathobiology is studied using many different models. Over time, numerous models of pulmonary fibrosis have been created. Common techniques include silica or asbestos, radiation damage, bleomycin, transgenic mice, and gene transfer via fibrogenic cytokines. As of right now, bleomycin is the mechanism employed to cause experimental lung fibrosis in animals.[6]

Received: 6 May 2024
Accepted: 10 September 2024
Published: 24 September 2024

Regrettably, lung damage resulting from pulmonary fibrosis is irreversible and permanent. The lungs may function better if the condition is identified early and treated. The majority of pulmonary fibrosis therapies aim to enhance quality of life and reduce symptoms. Lung illness fibroproliferative is common and has a high death rate. Inflammation, mesenchymal cell proliferation, and the deposition of interstitial matrix constituents including collagen and fibronectin are all involved in the pathophysiology of fibrotic lung disease. Although commonly used, corticosteroids and other immunosuppressive drugs have only shown mediocre effectiveness. The finding that pulmonary fibrosis is histologically characterized by an increase in lung mesenchymal cells and an accumulation of lung matrix that they secrete forms the basis for evaluating antifibrotic medicines for the treatment of lung fibrosis. In both human fibrotic lung histology sections and animal models of lung fibrosis, a myofibroblast-like phenotype is predominant. Lung myofibroblast-like cells proliferate in the airspaces of both acute and chronic pulmonary fibrosis, migrating through breaches in the basement membrane in response to transforming growth factor (TGF)-b1 and other cytokines, depositing collagen, fibronectin, proteoglycans, and other matrix components. In the interstitium, fibroblast growth and matrix deposition also take place, but to a lesser degree. In response to lung damage, increased mitogens and chemoattractants are released, mediating myofibroblast proliferation and migration.[7]

An earlier study found that the aqueous extract of *Centella asiatica* inhibited the activities of human respiratory epithelial cells that proliferated. The more *Centella asiatica* extract used, the greater inhibition will be shown because the impact of inhibition is dose-dependent. It was discovered by Coldren et al that the *Centella asiatica* extract inhibited the growth of fibroblast cells.[8] Human breast cancer cells underwent apoptosis when exposed to *Centella asiatica* extracts, according to a recent study by Babykutty et al.[9] Asiaticosside and madecassoside, the active ingredients in *Centella asiatica* extract, have been shown by Sampson et al to have an anti-proliferative impact on keratinocyte cells.[10]

Madagascar and the surrounding areas of the Indian Ocean are home to the traditional tropical Asian plant known as *Centella asiatica*, or pegagan. Urban belongs to the Umbelliferae family; it is often referred to as gotu kola or horseshoe grass, and it is widely distributed in tropical and subtropical regions of the world's southern and northern hemispheres. In Shennong's Classic of Materia Medica, it was initially included as a pharmaceutical and described as a medium-grade medication. The dried entirety herb is utilized as pharmaceutical, is biting and impactful in flavor, cold in nature, with channel tropism of liver, spleen, and kidney. It has the ability to disperse edema, accelerate diuresis, clear heat, activate blood, and stop death.[11] According to the most recent research, the drug has a variety of pharmacological properties, including nerve assurance and anti-inflammatory, anti-oxidant, anti-fibrosis, and anti-cancer actions. In the past, *Centella asiatica* was used to treat skin infections. It has the effect of slowing down the growth of scar tissue and speeding up the healing process. Another studies in Europe show that the *Centella asiatica* plant can support healing in the treatment of wounds and ulcers.[12] This plant contains four types of acid, namely asiatic acid, madecasic, asiaticoside, and madecoside, and the pharmacologically active compound pentacyclic triterpene. *Centella asiatica* extract can be an antimicrobial, anticancer, antioxidant, and therapeutic agent in wound healing and inflammation.[13,14] This study aims to predict and explain bleomycin-induced fibrosis and the effectiveness of *Centella asiatica* as a treatment.

Method

This research is an analytical study with an in vivo randomized experimental design in 3 groups of rats, each with 5 rat models. The study was carried out at the Animal Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. The study's inclusion criteria were Wistar rats (white) male at 10 weeks old, weighing 200–250 grams, healthy and active, and macroscopically no morphological abnormalities. The exclusion criteria in this study were rats that died during the research, neither sick nor infected. This research was approved by the Faculty of Medicine, Universitas Sumatera Utara, Research Ethics Committee, Number: 1239/KEPK/USU/2022.

In animal experiments, comply with the rules stated in Republic of Indonesia Law number 18, 2009 concerning Animal Husbandry and Animal Health; Chapter VI about Veterinary Public Health and Animal Welfare article 66 concerning animal welfare, and also following the IACUC guidelines in the Institutional Animal Care and Use Committee Guidebook.

The *Centella asiatica* extract was purchased from Haldin Pacific Semesta Ltd., a company that provides herbs and other plants extracts (product name: Gotu Kola Powder Extract, code: 10000069). This company has been certified ISO

14001 and GMP certificate by Indonesian Food and Drug Authority. Considering the best practice that has been applied in the company, we are convinced that the preparation of the extract, starting from the identification of the plants, growing, harvesting, drying and extraction processes have been done properly following the high standard requirement.

Research Procedure

The bleomycin dose used is 4 mg/kg/BB intratracheally. The dosage of *Centella asiatica* extract used was 400 and 800 mg. The negative control group was given intratracheal bleomycin only, the positive group was given intratracheal bleomycin 4 mg/kg/BB on days 0 and 21, added *Centella asiatica* induction at a dose of 400 mg on days 15 to 49 and the other positive group was given intratracheal bleomycin 4 mg/kg/BB day 0 and day 21, added *Centella asiatica* induction at a dose of 800 mg day 15 to 49.

On the 50th day, all groups of experimental animals will be terminated using ketamine. After cleaning with airflow, the samples were stained with Haemotoxyline-eosin (HE) staining in 4 groups of model rats. HE staining was carried out using Hematoxylin-Mayer reagent, Eosin solution, and Masson trichome. Histopathological examination using a light microscope with 100 times magnification. The features of fibrosis are described using a modification of the Aschroft scale. Trichome Masson staining is described utilizing a modified Ashcroft scale (Figure 1).

The features of fibrosis are described utilizing a modified Ashcroft scale. The degree of fibrosis is graded as 0 if the lungs are normal, and grade 1 if there is minimal fibrous thickening in the walls of the alveoli or bronchioles. Grade 2 if the septum of the alveoli shows clear fibrosis, the thickness of the septum is ≥3x than normal, and the lung structures of

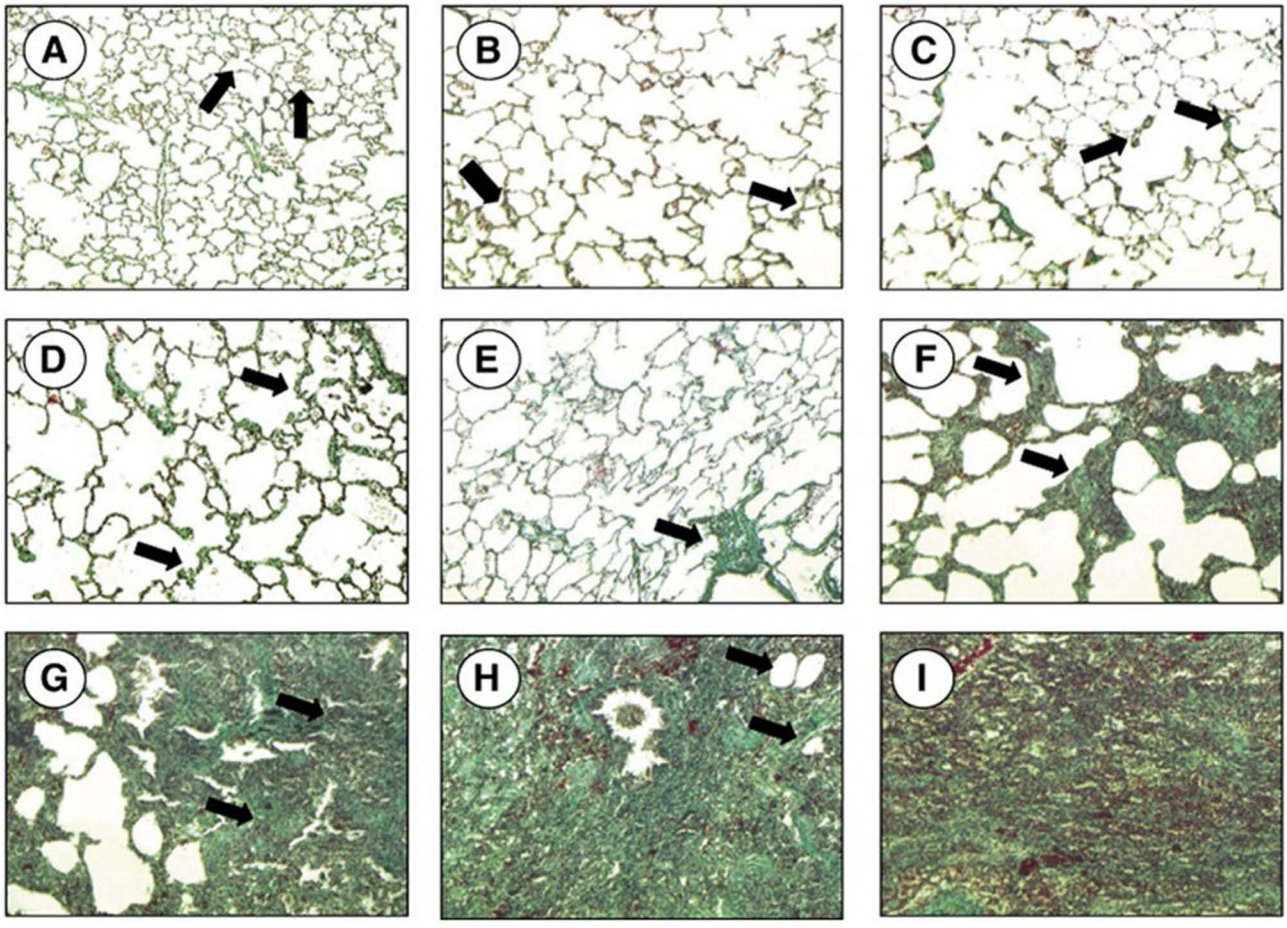

Figure 1 Fibrosis Description with the Modified Ashcroft Scale. Fibrosis was induced in rats after intratracheal administration of bleomycin. (**A**) Grade 0, normal lung. (**B**) Grade 1, isolated alveolar septa with gentle fibrotic changes. (**C**) Grade 2, fibrotic changes of alveolar septa with knot-like formation. (**D**) Grade 3, contiguous fibrotic walls of alveolar septa. (**E**) Grade 4, single fibrotic masses. (**F**) Grade 5, confluent fibrotic masses. (**G**) Grade 6, large contiguous fibrotic masses. (**H**) Grade 7, airbubbles. (**I**) Grade 8, fibrous obliteration.[15]

some of the alveoli are enlarged, but there is no visible fibrosis. Grade 3 if the alveoli septum shows clear fibrosis, the thickness of the septum is ≥3x than normal, and the lung structure of some of the alveoli is enlarged, but there is no visible fibrosis. Grade 4 if the alveoli septum is varied, the lung structure is a single fibrotic mass. Grade 5 if the alveolar septum is varied, the pulmonary structure of a confluent fibrotic mass, ≥10% and ≤50% of the visual field, widespread structural damage. Grade 6 if the alveolar septum is varied, mostly deformed, lung structure: large fibrotic mass, ≥50% visual field, pervasive structural damage. Grade 7 if the alveoli septum has no shape lung structure: alveoli are fused with a fibrotic mass, still visible air bubbles. Grade 8 if complete fibrosis in all lung fields.[16] The following is the flow of this research (Figure 2).

Result

In the negative control group (K) in the interalveolar septum, it was seen that the histopathology in the rats was fewer thick (FT) (0–25%) in 0 rats, partly thick in 3 rats (66.70%) and mostly thick (MT) as many as 2 rats (42.90%). Whereas in Trichrome Masson (TM), it is seen that the alveoli septum varies the structure of the single fibrotic masses with a degree of 4 as many as 1 specimens (20%), whereas in the alveoli septum varies fibrotic lung structures above 10% but below 50% of the field of view, damage to the structure widespread with 5 degrees as many as 2 specimens (40%), in the alveoli septum varies. Most changed shape, large fibrotic lung structure above 50% of the viewing field, a vast structure of 2 specimens (40%) (See Table 1 Figure 3a and b).

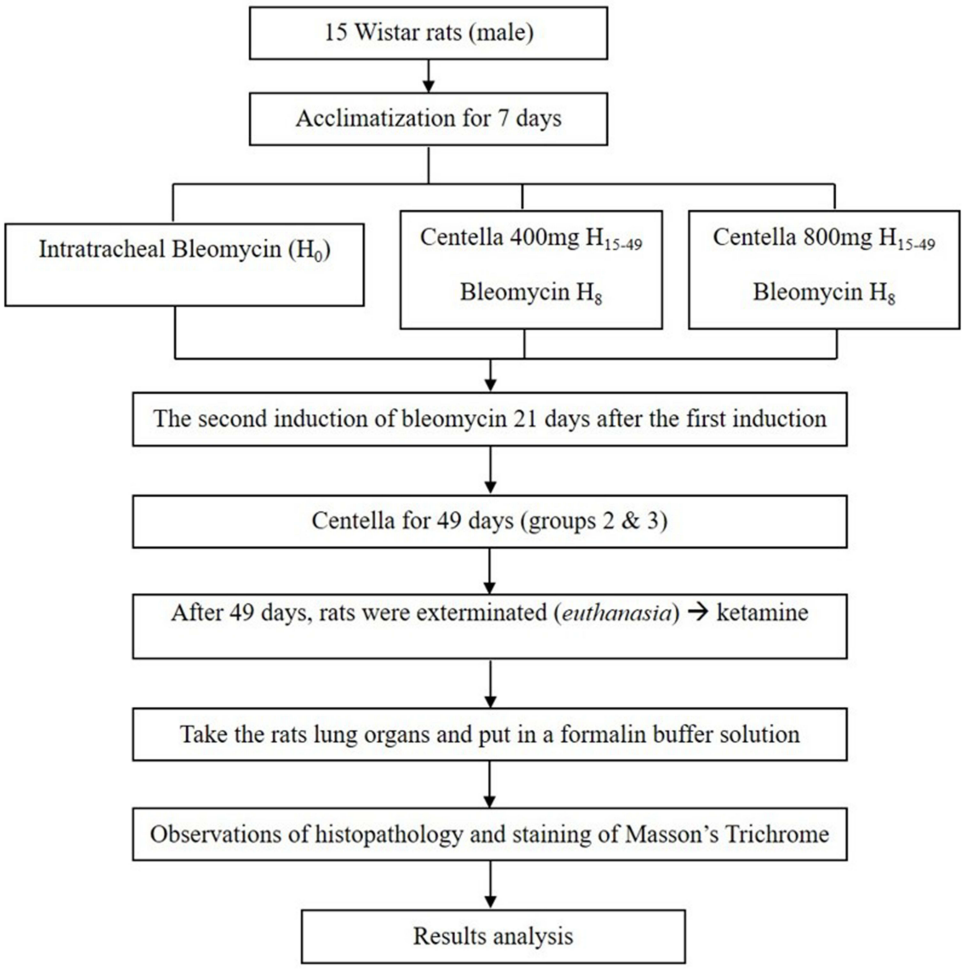

Figure 2 Research Flow.

Table 1 Results of Histopathology and Trichrome Masson Staining of Alveoli Samples

Sample Code	Alveoli						Interalveolar Septum					
	K		P1		P2		K		P1		P2	
	HP	TM	HP	TM	HP	TM	HP	TM	HP	TM	HP	TM
1	Mostly Thick		Partly Thick		Partly Thick		Partly Thick	5	Partly Thick	4	Mostly Thick	6
2	Partly Thick		Partly Thick		Partly Thick		Mostly Thick	6	Partly Thick	4	Mostly Thick	3
3	Mostly Thick		Partly Thick		Partly Thick		Mostly Thick	4	Partly Thick	4	Fewer thick	3
4	Partly Thick		Partly Thick		Partly Thick		Partly Thick	5	Mostly Thick	4	Fewer thick	3
5	Partly Thick		Partly Thick		Partly Thick		Partly Thick	6	Partly Thick	4	Partly Thick	4

Notes: FT: Fewer thick (0–25%), PT: Partly thick (26–74%), MT: Mostly thick (75–100%), P1: Induction of bleomycin with *Centella asiatica* 400 mg (Control Group), P2: Induction of bleomycin with *Centella asiatica* 800 mg (Control Group), K: Negative Control Group.
Abbreviations: HP: Histopathology, TM: Trichrome Masson.

In the interalveolar septum of P1, it was seen that the histopathology of rats was partly thick (PT) in 4 rats (80%) and mostly thick (MT) in 1 rat (20%), Whereas in Trichrome Masson (TM) it was seen that the alveoli septum varies the structure of the single fibrotic masses with 4 degrees found in 5 rats (100%) (See Figure 4a and b).

In the interalveolar septum of P2, it was seen that the histopathology was partly thick (PT) in 1 rat (20%), mostly thick (MT) in 2 rats (40%), and partly thick in 2 rats, whereas in Trichrome Masson (TM) septum alveoli fibrosis is visible, the thickness of the septum is more than 3x than normal, the structure of the lungs on some of the alveoli are enlarged, there is no visible fibrosis with grade 3 in 3 rats, while in Trichrome Masson (TM) the septum alveoli is seen varying in the structure of a single lung grade 4 fibrotic masses in 1 rat, in the alveolar septum varied. Most of them were

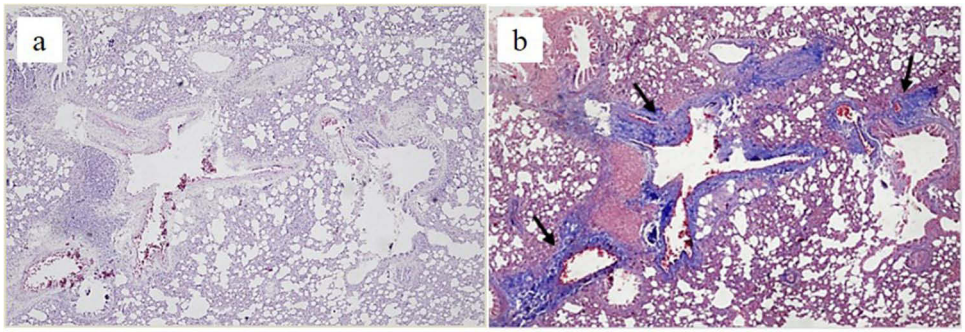

Figure 3 (a) Negative control with HE staining, **(b)** Negative control with TM staining. The degree of fibrosis score 5. Increased fibrosis with real damage to the structure of the lungs and the formation of fibrous bands or small fibrous masses.

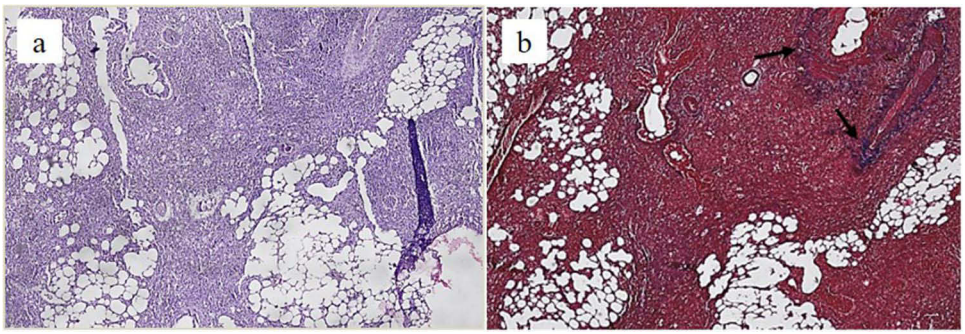

Figure 4 (a) P1 with HE staining, **(b)** P1 with TM staining. The degree of fibrosis score 3. Thickening moderate walls without real damage to the form of lungs.

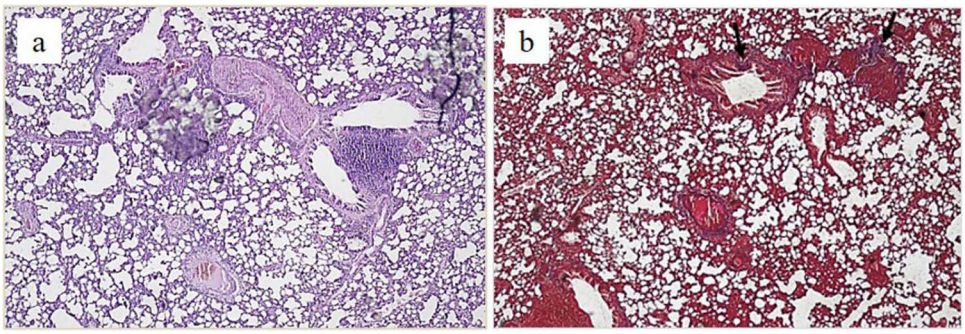

Figure 5 (a) P2 with HE staining, **(b)** P2 with TM staining. The degree of fibrosis score 2. There is no fibrosis.

deformed, the lung structure was a large fibrous mass over 50% of the field of view, and pervasive structural damage was found in 1 rat (See Figure 5a and b).

Data analysis of the correlation between the treatment group and the control group was carried out on histopathological staining, obtaining the following results:

Table 2 shows that in the alveoli group, there was no difference in histopathological staining outcome in groups K and P1 ($p > 0.05$) in group K. A total of 3 samples resulted in histopathology in the PT category, and 2 samples resulted in histopathology in the MT category. Meanwhile, in group P1, 5 samples resulted in histopathology in the form of PT, and none had histopathology in the MT category.

Furthermore, in the interalveolar septum group, the Chi-Square test results showed no difference in histopathological staining outcomes in groups K and P1 ($p > 0.05$). In group K, 2 samples resulted in histopathology in the MT category, and 3 samples PT. Meanwhile, in group P1, 1 sample resulted in histopathology in the form of MT and 4 samples in the PT category.

Apart from histopathology, the interalveolar septum was also stained with TM. Findings from the Mann–Whitney test indicate that there is a difference between group K and P1's TM staining results (p-value <0.05), where group P1 has a higher average of 5.2 ± 0.83, and K has an average of 4 ± 00.

Table 3 test shows that in the alveoli group, there is no difference in histopathological staining outcomes in groups K and P2 ($p > 0.05$) in group K, 3 samples resulted in histopathology in the PE category and 2 in the ME category.

Table 2 Correlation Between the P1 Treatment Group and the Control Group on Histopathology and TM Staining

			Group				Total	*P*
			K		P1			
			N	%	N	%		
Alveoli	Histopathology	Partly Thick (PT)	3	37.50%	5	62.50%	8	0.444
		Mostly Thick (MT)	2	100.00%	0	0.00%	2	
Interalveolar Septum	Histopathology	Mostly Thick (MT)	2	66.70%	1	33.30%	3	1,000
		Partly Thick (PT)	3	42.90%	4	57.10%	7	
	TM	Mean±SD	5.2 ± 0.83		4.0±0.00			0.036*
		4	1	16.70%	5	83.30%	6	
		5	2	100.00%	0	0.00%	2	
		6	2	100.00%	0	0.00%	2	

Table 3 Correlation Between the P2 Treatment Group and the Control Group on Histopathology and TM Staining

			Group				Total	*P*
			K		P2			
			N	%	N	%		
Alveoli	Histopathology	Partly Enlarged (PE)	3	37.50%	5	62.50%	8	0.444
		Mostly Enlarged (ME)	2	100.00%	0	0.00%	2	
Interalveolar Septum	Histopathology	Fewer Thick (FT)	0	0.00%	2	100.00%	2	0.223
		Mostly Thick (MT)	2	50.00%	2	50.00%	4	
		Partly Thick (PT)	3	75.00%	1	25.00%	4	
	TM	Mean±SD	5.2 ± 0.83		3.8±1.30			0.149
		3	0	0.00%	3	100.00%	3	
		4	1	50.00%	1	50.00%	2	
		5	2	100.00%	0	0.00%	2	
		6	2	66.70%	1	33.30%	3	

Notes: PE: Partly Enlarged (extent of fibrosis <50%), ME: Mostly Enlarged (extent of fibrosis >50%), FT: Fewer thick (0–25%), MT: Mostly thick (75–100%), PT: Partly thick (26–74%).

Meanwhile, in group P2, 5 samples resulted in histopathology in the form of PE, and none resulted in histopathology in the form of ME.

In the Interalveolar Septum group, the Chi-Square test findings revealed no difference in the results of histopathological staining in groups K and P2 ($p>0.05$). Histopathology has a total of FT, MT and PT with 0, 2, and 3 samples respectively. Meanwhile, in group P2, the histopathological picture was obtained in the form of categories 2 FT, 2 MT, and 1 PT.

Apart from histopathology, the Interalveolar Septum group also had staining with TM. Based on the Man Whitney test results, there was no significant difference between the TM staining results in groups K and P2 ($p>0.05$). The mean of 5.2 ± 0.83 was greater in the P1 group, while the mean of 3.8 ± 1.30 was found in group K.

Discussion

During the first week after bleomycin was given, the response was mainly characterized by an increase in the positive group's rats' body weight. In the repeated bleomycin model, each period provided similar histologic features observed in the single treatment model, namely inflammation, alveolar and interstitial fibrosis, alveolar space loss, and bronchoalveolar hyperplasia. The chronic fibrosis process becomes increasingly interstitial, the alveoli thickening often lined by cuboidal bronchoalveolar epithelial cells. The alveolar spaces are often filled with fine granular homogeneous amphophilic material, often accompanied by infiltration of macrophages and multinucleated giant cells and neutrophils. In conclusion, the histopathological changes observed with single and repeated bleomycin treatment were very similar to other studies. This study used 400 mg and 800 mg of pegagan extract to prevent fibrosis, using bleomycin 4 mg/kg/BB.

Using histological and Trichrome Masson staining, the study's positive control group and negative control group at 400 mg and 800 mg doses, respectively, showed varying degrees of fibrosis and thickness. The study's statistical tests revealed a significant difference between the P1-treated group and the control group. However, there was a difference in thickness between the negative and control groups.

Bleomycin can induce lipid peroxidation, which can lead to cellular damage even in the absence of its effects on DNA. This substance is vital to the lungs and has the potential to harm alveolar cells and inflame the lungs. Following bleomycin induction, interstitial edema is observed in the lungs, which is characterized by the presence of immunological

and inflammatory cells. This can lead to the development of pulmonary fibrosis, characterized by increased production and influence of collagen and other matrices. This process involves a number of polypeptide mediators that have the ability to induce fibroblast proliferation or excessive collagen deposition, although more research is still required on these.[5]

Acute neutrophilic infiltration precedes the shift to chronic inflammation dominated by lymphocytes in response to intratracheal bleomycin. Neutrophils are unlikely to directly affect fibrogenesis since they decline to control levels prior to the onset of severe fibrosis. Nevertheless, they might have a part in causing the inflammation that results in pulmonary fibrosis.[17]

The primary ways that *Centella asiatica* helps respiratory conditions include pulmonary fibrosis, chronic obstructive pulmonary disease, lung damage, and certain anti-lung cancer effects. Pulmonary fibrosis can be brought on by a number of lung traumas. It is defined by an excessive build-up of extracellular matrix and the activation of fibroblasts/myofibroblasts. This leads mainly to increasing organ dysfunction, with different degrees of inflammation and fibrosis.[18,19] The TGF-β1 and NLRP3 pathways can effectively improve pulmonary fibrosis by reducing collagen buildup, and reducing the amounts of inflammatory factors.[20,21] Asiatic acid has an anti-fibrotic action by inhibiting Smad3, which raises the expression of Smad7 mRNA and protein. The inhibition of the TGF-β/Smad downstream signaling pathway stops the deposition of matrix and the activation of myofibroblasts. Reduced production of TGF-β-mediated profibrotic factors, including collagen I, α-SMA, CTGF, and plasminogen activator inhibitor-1 (PAI-1), is observed upon inhibition of the TGF-β/Smad signaling pathway. Asiatic acid and asiaticoside, two of *Centella asiatica's* active ingredients, have an anti-inflammatory primary mechanism of action when treating respiratory disorders. This aligns with the previously established pathogenic mechanisms of the ailment. Furthermore, it is important to closely monitor the possible therapeutic effects of asiatic acid and *Centella asiatica* in the treatment of lung cancer. Further research should be done on apoptotic mechanism induction and tumor cell differentiation inhibition.

Clinical investigations have demonstrated the therapeutic effects of *Centella asiatica* and its triterpenoid content on neurological and skin illnesses. Improved mitochondrial function, anti-oxidative stress, anti-inflammatory, and anti-apoptotic properties are all possible with *Centella asiatica*. However, given the paucity of patients and the weak quality of the data, more clinical research is desperately needed. Together with additional components including centellose, centelloside, and madecassoside, this plant contains many pentacyclic triterpenoids, such as madecassic acid, brahmoside, and asiaticoside.[22,23] Its pharmacological activity is mostly attributed to triterpenes, primarily madecassic acid, asiatic acid, madecassoside, and asiatic oxide. According to a clinical investigation, *Centella asiatica* significantly enhanced the cognitive function of stroke patients. Patients were split into three groups and given daily doses of 1000 mg, 750 mg, and 3 mg of folic acid, respectively, along with *Centella asiatica* extract. For six weeks, the patient received treatment for the acute infarction phase of a stroke. When the MoCA-Ina test was used to assess the patients' cognitive performance, the therapy group received the highest score out of the three. Nevertheless, there were no discernible differences in AST and ALT levels when compared to the control group.[24] Additionally, *Centella asiatica* has been shown to cause gene expression changes that are compatible with its potential application in the treatment of connective tissue diseases, including microangiopathy and wound healing.[8]

Furthermore, preclinical research on *Centella asiatica* and its triterpenoids revealed other beneficial properties, such as (1) safeguarding the retinal vasculature, (2) lowering toxic and medication side effects, (3) lowering drug resistance, and (4) encouraging the regeneration of periodontal tissue. Osteolytic bone disease, leukemia, migraine, glaucoma, periodontitis, and oral submucosal fibrosis can all be relieved by *Centella asiatica*. Triterpenoid group "madecosside" is another potent ingredient in pegangan plants.[25] Through the synthesis of collagen, madecosside plays a crucial part in the repair of cell injury. Thirty percent of the protein in mammals' bodies is made up of collagen. Collagen fibers therefore aid in the repair of injured or wound tissue. In primary fibroblasts produced from human keloids, madecassoside showed decreased keloid development and enhanced wound healing.[26]

Asiatic acid acts as an antiseptic and has potential as an antifungal. Free radical damage can also be prevented by the body using this chemical. In most cases, this chemical is utilized to treat wounds. This compound can also help the neuroglia regeneration process, increase wound healing, stimulate granulation, induce changes in gene expression,

improve memory, increase anti-inflammatory activity, inhibit acetylcholinesterase activity, and increase anti-apoptotic activity.[27]

The limitation of this study is that there were rat samples that died during the evaluation and monitoring process of bleomycin and *Centella asiatica* administration, so maintaining and monitoring the rats became a challenge in this research process, in addition to other literature that is still limited regarding research on bleomycin and *Centella asiatica* on the respiratory system is also a limitation and at the same time a challenge in this study. Further studies are necessary to understand the compounds of *Centella asiatica* that are responsible for the anti-proliferative and anti-fibrotic effects on human respiratory epithelial cells.

Conclusion

Based on this research, *Centella asiatica* (Pegagan) extract as a treatment for fibrosis in the lungs of rats given bleomycin. According to this study's findings, it also proves that bleomycin 4 mg/kg/BB can cause fibrosis in the lungs of rats. The positive group was given intratracheal bleomycin 4 mg/kg/BB on days 0 and 21, *Centella asiatica* induction at 400 mg on days 15 to 49, and other positive groups. Intratracheal treatment of bleomycin 4 mg/kg/BB on days 0 and 21, added 800 mg dose of *Centella asiatica* induction on days 15 to 49, based on staining, showed significant results as a treatment for fibrosis. Asiatic acid has been shown in previous studies to have anti-fibrotic action by inhibiting Smad3, which increases the expression of Smad7 mRNA and protein. Inhibition of the TGF-β/Smad downstream signaling pathway stops matrix deposition and myofibroblast activation. And in another study it was mentioned that *Centella asiatica* water extract showed an inhibitory effect on the proliferation activity of human respiratory epithelial cells. The inhibitory effect was dose-dependent, the higher the dose of *Centella asiatica* extract the more inhibition was observed. While Bleomycin can induce lipid peroxidation, which can cause cell damage even without its effect on DNA. This substance is very important for the lungs and has the potential to harm alveolar cells and cause lung inflammation. After bleomycin induction, interstitial edema was observed in the lungs, which was characterized by the presence of immunological and inflammatory cells. This can lead to the development of pulmonary fibrosis. Based on the theories and research that have been conducted and the results obtained from this study, further research is needed on different samples to understand the *Centella asiatica* compounds responsible for the anti-fibrotic effects on human lungs, which will be able to reduce the area of fibrosis significantly.

Acknowledgment

The authors would like to thank the staff of the Pharmacology and Anatomical Pathology Laboratory at the Faculty of Medicine Universitas Sumatera Utara with the deepest feeling, for the guidance and knowledge they have provided, which has been very valuable for the success of this research.

Funding

This research was financed by the Universitas Sumatera Utara Research Institute in compliance with the terms of the TALENTA Universitas Sumatera Utara research contract Number: 6789/UN5.1.R/PPM/2021, which was issued on June 16, 2021.

Disclosure

The authors report no conflicts of interest in this work.

References

1. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. *Am J Respir Crit Care Med*. 2000;161:646–664. doi:10.1164/ajrccm.161.2.ats3-00
2. Jennifer SY. *Genetic Modulators of Pulmonary Function in Cystic Fibrosis & Genetic Susceptibility Factors of Emphysema*. Leuven University Press; 2005.
3. Gripp KW, Ennis S, Napoli J. Exome analysis in clinical practice: expanding the phenotype of Bartsocas-Papas syndrome. *American Journal of Medical Genetics*. 2013;161:1058–1063. doi:10.1002/ajmg.a.35913

4. Guilherme RF, Xisto DG, Kunkel SL, et al. Pulmonary antifibrotic mechanisms aspirin-triggered lipoxin A4 synthetic analog. *American Journal of Respiratory Cell and Molecular Biology*. 2013;49(6):1029–1037. doi:10.1165/rcmb.2012-0462OC
5. Hay J, Shahzeidi S, Laurent G. Mechanisms of bleomycin-induced lung damage. *Archives of Toxicology*. 1991;65(2):81–94. doi:10.1007/BF02034932
6. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? *International Journal of Biochemistry and Cell Biology*. 2008;40(3):362–382. doi:10.1016/j.biocel.2007.08.011
7. Lasky JA, Ortiz LA. Antifibrotic therapy for the treatment of pulmonary fibrosis. *The American Journal of the Medical Sciences*. 2001;322(4):213–221
8. Coldren CD, Hashim P, Ali JM, Oh SK, Sinskey AJ, Rha CK. Gene expression changes in the human fibroblast induced by Centella asiatica triterpenoids. *Planta Medica*. 2003;69(8):725–732.
9. Babykutty S, Padikkala J, Sathiadevan PP, et al. Apoptosis induction of Centella asciatica on human breast cancer cells. *Afr J Tradit Complement Altern Med*. 2008;6(1):9–16.
10. Sampson JH, Raman A, Karlsen G, Navsaria H, Leigh IM. In vitro keratinocyte antiproliferant effect of Centella asiatica extract and triterpenoid saponins. *Phytomedicine*. 2001;8(3):230–235.
11. Fan Y, Yin X. Potential therapeutic targets and biological mechanisms of Centella asiatica on hepatic fibrosis: A study of network pharmacology. *Ann Transl Med*. 2021;9(11):932. doi: 10.21037/atm-21-2253
12. Maquart FX, Bellon G, Gillery P, Wegrowski Y, Borel JP. Stimulation of collagen synthesis in fibroblast cultures by a triterpene extracted from Centella asiatica. *Connect Tissue Res*. 1990;24(2):107–120. doi:10.3109/03008209009152427
13. Jayashree G, Kurup Muraleedhara G, Sudarslal S, Jacob VB. Anti-oxidant activity of Centella asiatica on lymphoma-bearing mice. *Fitoterapia*. 2003;74(5):431–434. doi:10.1016/S0367-326X(03)00121-7
14. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to Centella asiatica (L.) urban. *Journal of Ethnopharmacology*. 1995;48(1):53–57. doi:10.1016/0378-8741(95)01284-K
15. Hübner R-H, Gitter W, El Mokhtari NE, et al. Standardized quantification of pulmonary fibrosis in histological samples. *BioTechniques*. 2008;44(4):507–517. doi:10.2144/000112729
16. Ashcroft T, Simpson JM, Timbrell V. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. *J Clin Pathol*. 1988;41(4):467–470. doi:10.1136/jcp.41.4.467
17. Dorr RT. Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. *Semin Oncol*. 1992;19:3–8.
18. Selman M, Pardo A. Idiopathic pulmonary fibrosis: an epithelial/fibroblastic cross-talk disorder. *Respiratory Research*. 2001;3:1–8. doi:10.1186/rr175
19. Thannickal VJ, Toews GB, White ES, Lynch JP, Martinez FJ. Mechanisms of pulmonary fibrosis. *Annual Review of Medicine*. 2004;55:395–417. doi:10.1146/annurev.med.55.091902.103810
20. Nie Y, Sun L, Wu Y, et al. AKT2 regulates pulmonary inflammation and fibrosis via modulating macrophage activation. *The Journal of Immunology*. 2017;198(11):4470–4480. doi:10.4049/jimmunol.1601503
21. Tian R, Zhu Y, Yao J, et al. NLRP3 participates in the regulation of EMT in bleomycin-induced pulmonary fibrosis. *Experimental Cell Research*. 2017;357(2):328–334. doi:10.1016/j.yexcr.2017.05.028
22. Singh B, Rastogi RP. A reinvestigation of the triterpenes of Centella asiatica. *Phytochemistry*. 1969;8(5):917–921. doi:10.1016/S0031-9422(00)85884-7
23. Pizzorno JE, Murray MT. *Textbook of Natural Medicine*. 1999; 827–830.
24. Sun B, Wu L, Wu Y, et al. Therapeutic potential of Centella asiatica and its triterpenes: a review. *Frontiers in Pharmacology*. 2020;11(September):1–24. doi:10.3389/fphar.2020.568032
25. Irham WH, Tamrin, Marpaung L, Marpongahtun. Bioactive compounds in Pegagan leaf (Centella asiatica L. Urban) for wound healing. *Journal of Physics: Conference Series*. 2019;1232(1):6–11.
26. Song J, Xu H, Lu Q, et al. Madecassoside suppresses migration of fibroblasts from keloids: involvement of p38 kinase and PI3K signaling pathways. *Burns*. 2012;38(5):677–684. doi:10.1016/j.burns.2011.12.017
27. Yasurin P, Sriariyanun M, Phusantisampan T. Review: the bioavailability activity of Centella asiatica. *KMUTNB International Journal of Applied Science and Technology*. 2015;9(1):1–9. doi:10.14416/j.ijast.2015.11.001