The Role of Colchicine in the Prevention of Bleomycin-Induced Pulmonary Fibrosis in Rats

Original Article

Sarah A. Arakib[1], Mohamed Abdel Hay Autifi[2], Eman A. El-Sawaf[1], Alsayed A. Abdelhady[1] and Manal H. Al Badawi[1]

Department of Anatomy and Embryology, Faculty of Medicine, [1]Helwan University, [2]Al-Azhar University, Egypt

ABSTRACT

Introduction: Lung fibrosis is the most popular and serious sequel of interstitial inflammatory diseases and may lead to respiratory failure in critical cases with a mortality rate of 10% to 20%. Its etiology includes chemicals, infections, radiation, allergens, drugs such as bleomycin (BLM), and as a result of the coronavirus disease 2019 (COVID-19).

Aim of the Work: The purpose of the present work was to detect the preventive outcome of colchicine on lung fibrosis in rats.

Material and Methods: 24 male albino rats were subdivided into 4 groups, 6 rats per group; group I (control G), group II (colchicine G), group III (BLM G), and group IV (BLM+colchicine G). The lung fibrosis was done by injection of BLM intraperitoneally (dose; 0.5 mg\kg 2 times \ week for 3 weeks). BLM+colchicine group was taken BLM as in group III in addition colchicine was taken daily (dose; 1 mg/kg by oral gavage for 3 weeks). By the study period end (3 weeks), the rats were put under anesthesia and the thoracic wall was opened. The lungs were taken and prepared for H&E, Masson's trichrome, and immunohistochemical staining. Some specimens were prepared and examined by transmission electron microscope (TEM).

Results: The histological changes in the lungs of the BLM group were deformed pulmonary architecture, thickened interstitial walls, alveolar collapse, severe invasion by inflammatory cells, highly significant collagen fibrils deposition, severe positive reaction in the interstitial walls by anti-α-SMA, and degenerated pneumocytes I and II by TEM. The preventive group showed restoration of most of the normal lung architecture and a marked decrease in inflammatory and fibrotic changes.

Conclusion: Colchicine can lessen the histopathological and ultrastructural changes in the lungs resulting from BLM.

Received: 06 December 2024, **Accepted:** 20 December 2024

Key Words: α-SMA; bleomycin; clchicine; pulmonary fibrosis; rats.

Corresponding Author: Sarah A. Arakib, PhD, Department of Anatomy and Embryology, Faculty of Medicine, Helwan University, Egypt, **Tel.**: +20 10 0243 3933, **E-mail:** sarah.arakeeb@med.helwan.edu.eg

ISSN: 1110-0559, Vol. 48, No. 1

INTRODUCTION

Pulmonary fibrosis is the most recurrent lung interstitial disease, which can affect the interstitial septal walls with or without the alveoli[1]. Its etiology includes chemicals, allergens, and drugs such as bleomycin (BLM), and COVID-19[2].

BLM is an anticancer medication for Hodgkin's lymphoma treatment. Varied forms of lung toxicities have been described as a complication of BLM therapy. Pneumonitis is the most frequent one, and in serious cases may cause pulmonary fibrotic disease and respiratory failure, with a mortality rate of 10%-20%[3]. The mechanism of BLM toxicity may be due to oxidative distress and the release of cytokine[s][4].

Colchicine is an old drug extracted from the plant autumn crocus (colchicum autumnale). It is used as a main treatment for gouty arthritis due to its anti-fibrotic and anti-inflammatory actions. It has recently been used for many diseases other than arthritis such as familial Mediterranean fever, Behcet's disease, pericarditis, coronary artery disease, and other inflammatory and fibrotic conditions[5].

Colchicine is considered an important drug in treating pulmonary fibrosis[6]. It obstructs the functions of mononuclear cells in the blood and can block the tubulin, so it can inhibit the mitotic action[7]. It can also, inhibit the proliferation of the fibroblast and the formation of collagen fibrils[8]. Furthermore, colchicine can inhibit fibronectin and many growth factors[9].

COVID-19 is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first identified in December 2019 in Wuhan, China, and became a pandemic disease in March 2020. A large proportion of cases have mild symptoms or are asymptomatic. However, 20% of cases (mostly elderly and those with comorbidities) develop severe acute respiratory distress syndrome (ARDS), which may lead to pulmonary fibrosis and respiratory failure[10].

During the COVID-19 pandemic, a lot of clinical experiments have studied colchicine's action in limiting the disease's progression and its complications[11].

AIM OF THE WORK

The experiment was done to detect the histopathological

DOI: 10.21608/ejh.2024.340347.2182

effect of BLM in developing lung fibrosis and the potential protective action of colchicine.

MATERIALS AND METHODS

Animals

Twenty-four male adult Sprague–Dawley albino rats were used in this experiment, each weighing about 200 gm. They were settled in cages of medium size,4 rats \ cage, and left for nearly a week for adaptation and had easy access to food and water. They have stayed under regular periods of dark and light.

Ethical considerations

The protocol for this research was accepted by the Animal Research Ethics Committee, Helwan University, School of Medicine (number of the acceptance; 43-2022).

Chemicals

- Colchicine: It was obtained from Sigma-Aldrich Co. (Germany with catalog number; ZXB-07-102), and taken orally by the gavage (the dose:1 mg/kg daily)[12].
- Bleomycin (BLM) sulfate: It was obtained from Sigma-Aldrich Co. (Germany with catalog number; ZXB-03-110), and was injected intraperitoneally 2 times\ a week for 3 weeks (the dose: 0.5 mg\kg in 0.5 ml saline)[13].

Animal groups

The rats were subdivided into 4 subgroups (6 rats \ subgroup).

Control group (Group I): They were only injected with intraperitoneal saline.

Colchicine group (Group II): They were taken colchicine only in the previously prescribed way and dose.

BLM group (Group III): They were injected with BLM as described previously for developing pulmonary fibrosis, and no other medications were taken.

BLM + Colchicine group (Group IV): This group acted as a preventive group. They have received the BLM and the colchicine in the same previously prescribed doses. The colchicine was taken three days before and during BLM intake.

After 3 weeks (the study ending), the rats were put under anesthesia. The thoracic wall was opened and the lung specimens were carefully taken.

Histological studies

Tissue preparation for light microscopy (LM) studies

The lung specimens were put in formalin (10%) for one night for fixation. Then, they were dehydrated in alcohol of ascending grades, after that, xylol was used for their clearing and finally put in blocks of paraffin. Serial thin slices were done and stained by Hematoxylin and Eosin (H&E)[14]. Others were stained by Masson's trichrome for the identification of collagen fibrils[15].

Immunohistochemical preparation

Some sections were fixed on positive slides for staining with anti-alpha smooth muscle actin antibody (anti-α-SMA).

After the deparaffinization of sections, antigen retrieval was done. The sections were incubated in 3% hydrogen peroxide for 5 minutes to quench endogenous tissue peroxidase. The sections were incubated in the primary antibody (anti-actin antibody; actin, smooth muscle Ab-1 in dilution of 1:200) at room temperature. The secondary antibodies used were En-Vision + System HRP anti-rabbit. After that, counter-staining was done using Hematoxylin[16].

Tissue preparation for transmission electron microscopy (TEM) study

Small pieces of 1 mm^3 of the lung tissues were taken. They were first prefixed in 3% fresh glutaraldehyde for nearly 45 minutes, then secondary fixation was done by Osmium tetroxide. After that, the specimens were dehydrated in ethanol or acetone of ascending concentrations, then infiltrated, embedded, polymerized, and finally cut into semithin and ultrathin sections[14].

UItrathin sections (60-100 nm thickness) were cut by a new glass knife to make a ribbon of sections. After sectioning, grids were stained with uranyl acetate followed by lead citrate to enhance contrast[14].

Morphometric Studies

A. Assessment of the average lung weight.

B. Assessment of the average percentage of areas of collagen fibrils in Masson's trichrome sections (by the image analyzer).

Statistical analysis

The mean (the average) and standard deviation (SD) were assessed by a statistical software program (SPSS). ANOVA was done, and then comparing the groups was done by Post Hoc. test. The data significance was identified by the probability value (*P. value*). The difference was non-significant at $P.>0.05$, significant $P.\leq 0.05$, and highly significant $P.\leq 0.001$.

RESULTS

Gross inspection of the lungs of group I (control) and group II (Colchicine) appeared pinkish, and uniform in texture, with no areas of hyperemia or hemorrhage (Figures 1 A,B). The lungs of group III (BLM group) appeared grossly swollen, and hyperemic, with hemorrhagic areas (Figure 1C). The lungs of group IV (preventive group) were pinkish colored lung with minimal hyperemia and hemorrhagic areas (Figure 1D).

LM study of the H&E specimens of the lungs of the group I (control), revealed normal pulmonary parenchymatous

tissue consisting of; alveolar sacs, alveolar ducts, and alveoli. The septal walls appeared thin. The bronchioles were lined by a continuous normal epithelial layer with a surrounding continuous smooth muscle layer (Figure 2A). The colchicine group (group II) showed a similar control histological picture (Figure 2B). The group III (BLM group) displayed severe destruction of the pulmonary parenchymatous tissue with loss of the normal appearance and intra-alveolar exudation with the subsequent collapse. The septal walls were hypertrophied with hemosiderin, and exudation was seen within. It was invaded markedly by inflammatory cells. The bronchiolar epithelium was seen desquamated in some specimens. Others showed cellular debris and hemorrhage inside the bronchi and bronchioles. Also, there was severe inflammatory cellular invasion around the bronchioles and the blood vessels. The walls of the bronchial arterioles were seen hypertrophied (Figures 2 C, 3A,B). Group IV (BLM+Colchicine) showed an obvious reduction in the histological changes; the pulmonary architecture has been restored consisting of; alveolar sacs, alveolar ducts, and alveoli. The thickness of the septal walls was markedly decreased in most areas, with localized areas of thickened walls (Figure 2D).

Masson's trichrome sections of the lungs of group I (control group), and group II (Colchicine group) showed minimal green-colored collagen seen in the thin interstitial septal walls, surrounding the bronchioles and the bronchial arterioles. Normal pulmonary parenchymatous tissue of alveolar sacs, alveolar ducts, and alveoli was also seen (Figures 4 A,B). Sections in group III (BLM group) showed increased fibrous green color content in the severely thickened interstitial septal walls, around the arterioles and the bronchioles (Figure 4C). Group IV (BLM+ Colchicine group) showed minimal green collagen in the interstitial septal walls (Figure 4D).

Immunohistochemical study of the lungs of the control and colchicine groups stained with anti-α-SMA showed a positive reaction in the bronchiolar smooth muscles and the wall of the bronchial arterioles, and the reaction was negative in the cells lining the alveoli (Figures 5 A,B). Group III (BLM group) showed a severe positive reactivity in the alveolar lining, and in the interstitial septal walls (Figure 5C). Group IV (preventive group) showed minimal positive reaction in the alveolar lining and in the interstitial septal walls (Figure 5D).

Ultrastructural study of the lungs of the control group displayed distended alveoli with thin septal walls in between. The septal walls contained small capillaries, and they were lined by pneumocytes (type I and II). Pneumocytes I were thinned cells with electron lucent flat nuclei. The blood-air barrier was seen continuous and consisted of; endothelial cells, pneumocytes I, and basal lamina in between. Pneumocytes II were cubical cells with electron lucent rounded nuclei. Their cytoplasm showed numerous lamellar bodies and mitochondria. The Pneumocytes II had characteristic surface microvilli (Figures 6,7A, 8A). The colchicine group showed a similar control picture (Figures 7B, 8B).

The BLM group displayed notable damage to the pneumocytes. The pneumocytes I were irregular and shrunken, with dark small nuclei, and the blood-air barrier was irregular and not continuous. The pneumocytes II displayed irregular small electron dense nuclei. Their cytoplasm showed empty lamellar bodies. The interstitial septal walls were severely thickened with deposition of collagen fibrils. It showed invasion by fibroblast and inflammatory cells as lymphocytes and eosinophils (Figures 7C, 8C, 9, 10 A,B).

TEM examination of the lungs of the preventive group showed restoring the natural alveolar architecture. The alveolar pneumocytes were seen as rather healthy. The pneumocytes I showed rather normal electron lucent nuclei. The blood-air barrier has restored its continuous shape without any interruption or irregularities. The nuclei of pneumocytes II were electron lucent but showed slight irregularities. The cytoplasm revealed numerous filled lamellar bodies and mitochondria. The microvilli were seen nearly normal (Figures 7D, 8D, 11).

Morphometric results

Assessment of the average lung weight

The average lung weight was 1.2 ± 0.05 gm in the control group,1.4 ± 0.24 gm in the colchicine group, 2.3 ± 0.32 gm in the BLM group, and 1.4 ± 0.06 gm in the preventive group. There was a high significance increase in the average lung weight in the BLM group relative to the control (*P value* <0.001). Also, there was a non-significant increase in the average lung weight of the preventive group in relation to the control (*P value*=1.000), and a high significance decrease in relation to the BLM group (*P value* <0.001) (Table 1, Bar chart 1).

Assessment of the average percentage of areas of collagen fibrils in Masson's trichrome sections

The average percentage of areas of collagen fibrils in Masson's trichrome sections was 4.8 ± 0.48 in the control group,4.5 ± 0.46 in the colchicine group, 18.8 ± 1.03 in the BLM group, and 8.1 ± 0.60 in the preventive group. There was a high significance increase in the BLM group relative to the control group (*P value* <0.001). Also, there was a high significance increase in the preventive group relative to the control group (*P value* <0.001), and a high significance decrease relative to the BLM group (*P value* <0.001) (Table 2, Bar chart 2).

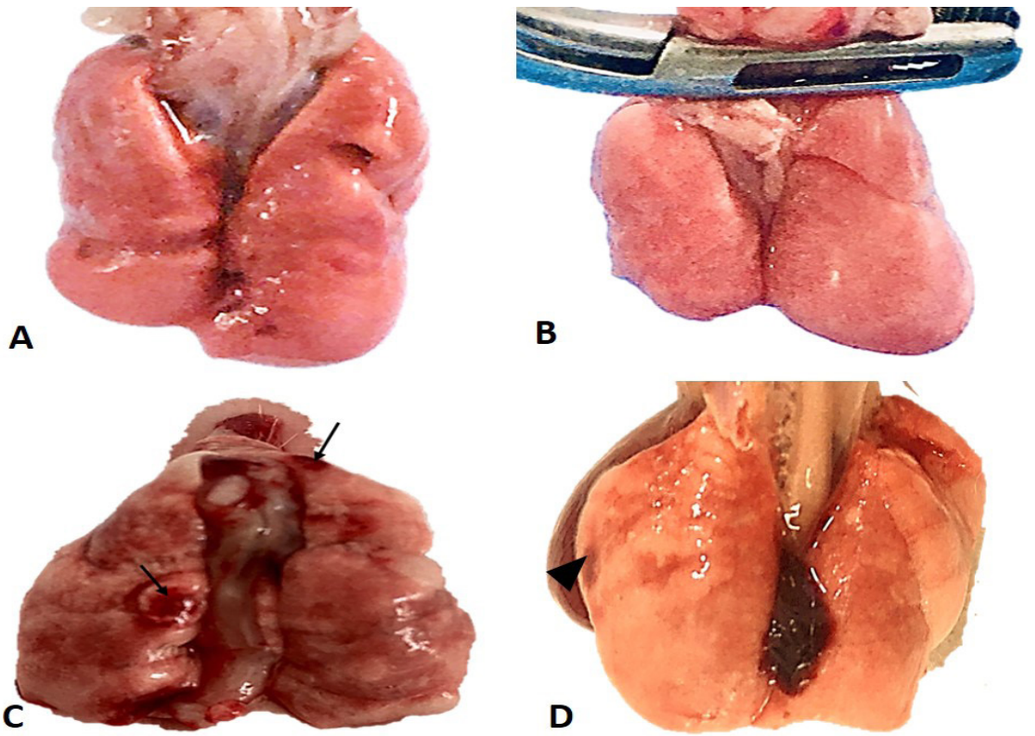


Fig.1{A-D}: Gross images of the lungs of the rats. A; The control group shows a pinkish-uniform lung. B; The colchicine group shows a similar control gross picture. C; The BLM group shows a swollen, hyperemic lung with areas of hemorrhage (arrows). D; The BLM+ Colchicine group shows a pinkish-colored lung with minimal hyperemia and hemorrhagic area (arrowhead).

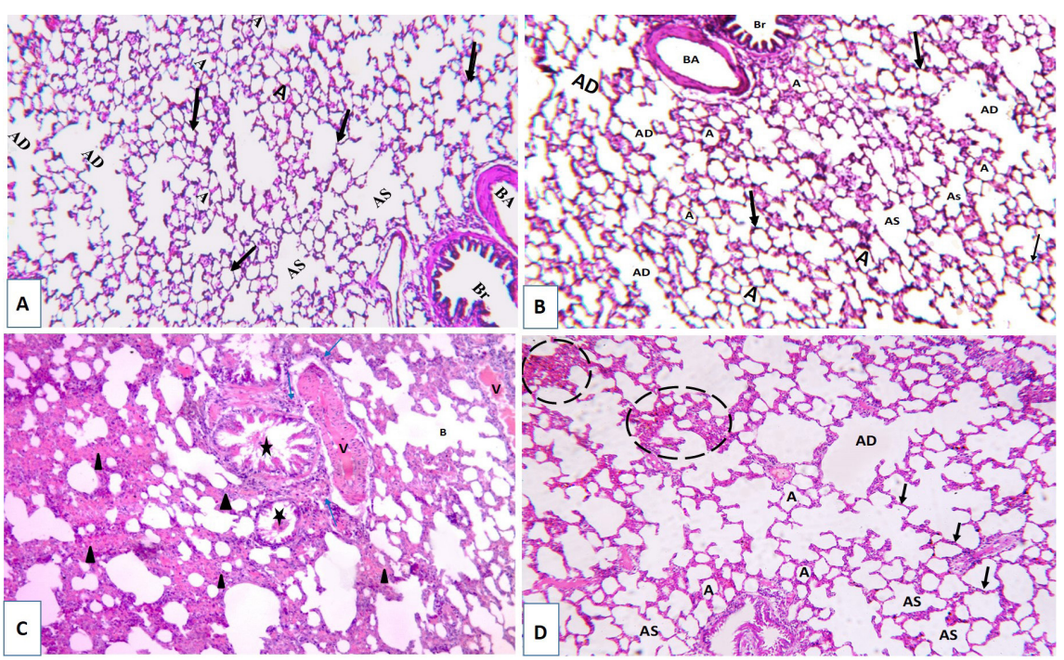


Fig.2{A-D}: LM images of sections in the lungs stained by H&E showing: A; Control group shows normal pulmonary parenchymatous tissue formed of alveolar sacs, alveolar ducts, and alveoli. The alveoli are surrounded by thin septal walls (black arrows). B; Colchicine group showing a similar control picture. C; BLM group shows the distortion of the pulmonary parenchymatous tissue with alveolar exudation and collapse (arrowhead), inflammatory cell invasion around the bronchioles and the blood vessels (blue arrows), cellular debris inside the bronchioles (star), vascular congestion (V), and emphysematous bullae (B). D; BLM + Colchicine group shows restored normal pulmonary parenchymatous tissue formed of alveolar sacs, alveolar ducts, and alveoli. The thickness of the interstitial septal walls is markedly decreased in most areas (arrows), and local areas of thickened septa (dotted circles). Alveolar sacs (AS), Alveolar ducts (AD), Alveoli (A) (H&E x100)

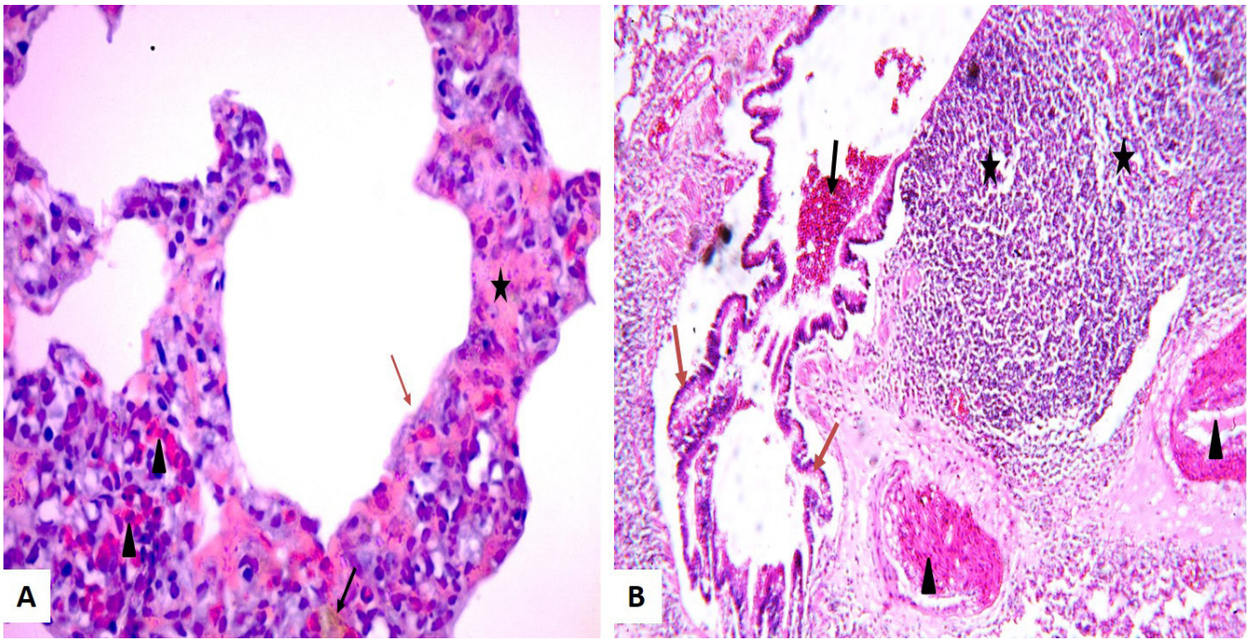


Fig. 3{A-B}: LM images of sections in the lungs of the BLM group stained with H&E showing: A; thickened interalveolar interstitial septum (red arrow), interstitial secretions (star), inflammatory cells (arrowhead), and hemosiderin (black arrow). B; bronchial epithelium shedding (red arrows), hemorrhage inside the bronchioles (black arrow), lymphocytes surrounding bronchioles (stars), and hypertrophied bronchial arterioles (arrowhead). (H&E x400)

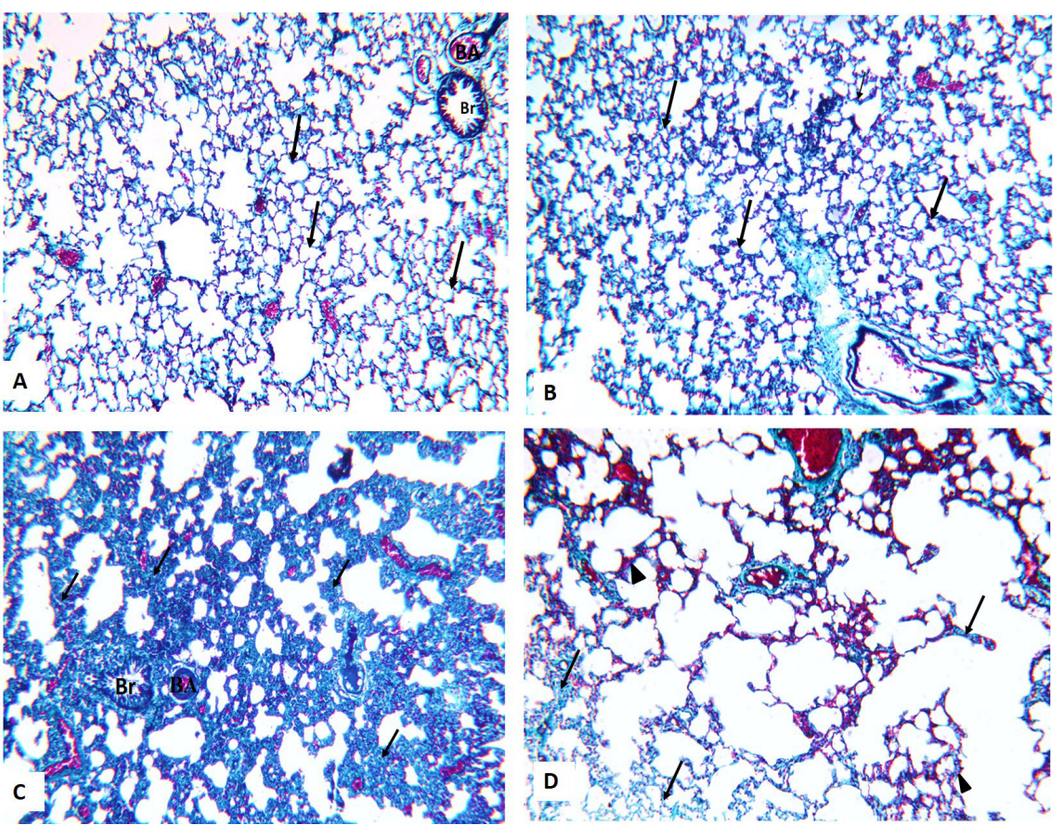


Fig. 4{A-D}: LM images of sections in the lung of rats stained by Masson's trichrome. A; The Control group shows little green-colored collagen in the thin interstitial septal walls (arrows), surrounding the bronchioles (Br), and the bronchial arteriole (BA). B; Colchicine group shows a similar control picture. C; BLM group shows severe collagen deposition in the thickened septal walls (arrows), surrounding the bronchioles (Br) and bronchial arteriole (BA). D; BLM + colchicine group shows a marked decrease in green-colored collagen (arrows), and a decrease in the thickness of interstitial walls (arrowheads). (Masson's trichrome x100)

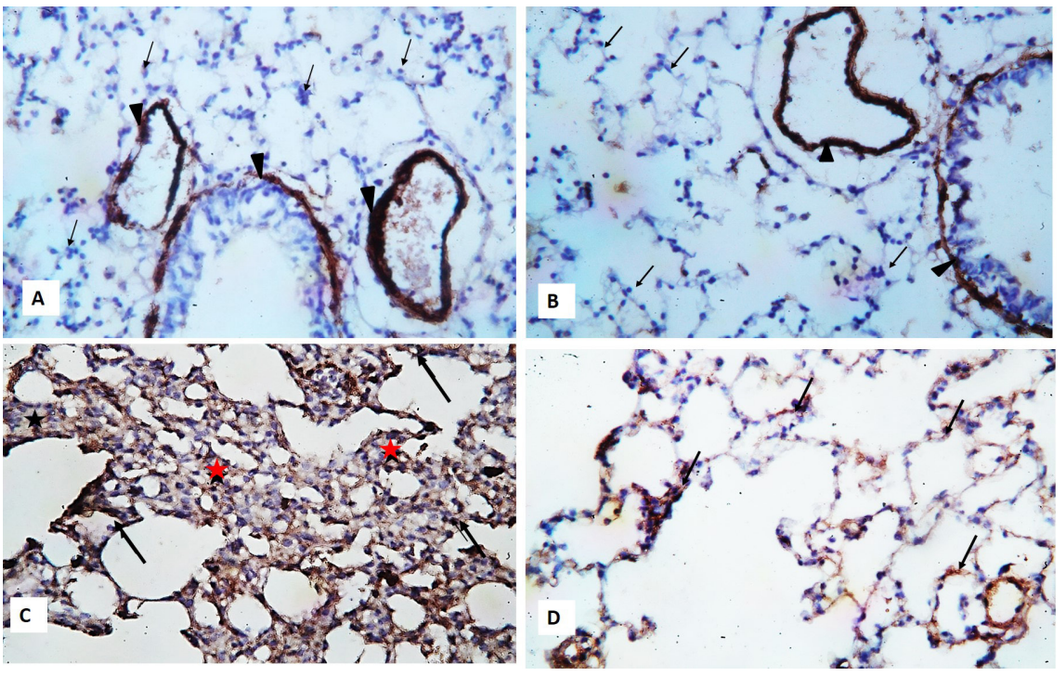


Fig. 5 {A-D}: LM images of sections in the lungs stained by anti-α-SMA showing: A; The control group shows a positive reaction in the muscles surrounding the bronchiole, and the bronchial arterioles (arrowhead). A negative reaction was seen in the alveolar walls (black arrows). B; Colchicine group showing a similar control reaction. C; BLM group showing severe positive cytoplasmic reactivity in the thickened septa (stars), and the alveolar lining cells (black arrow). D; BLM + colchicine group shows little positive cytoplasmic reactivity in the interstitial septal walls, and the alveolar lining cells (black arrow) Anti-α-SMA x400

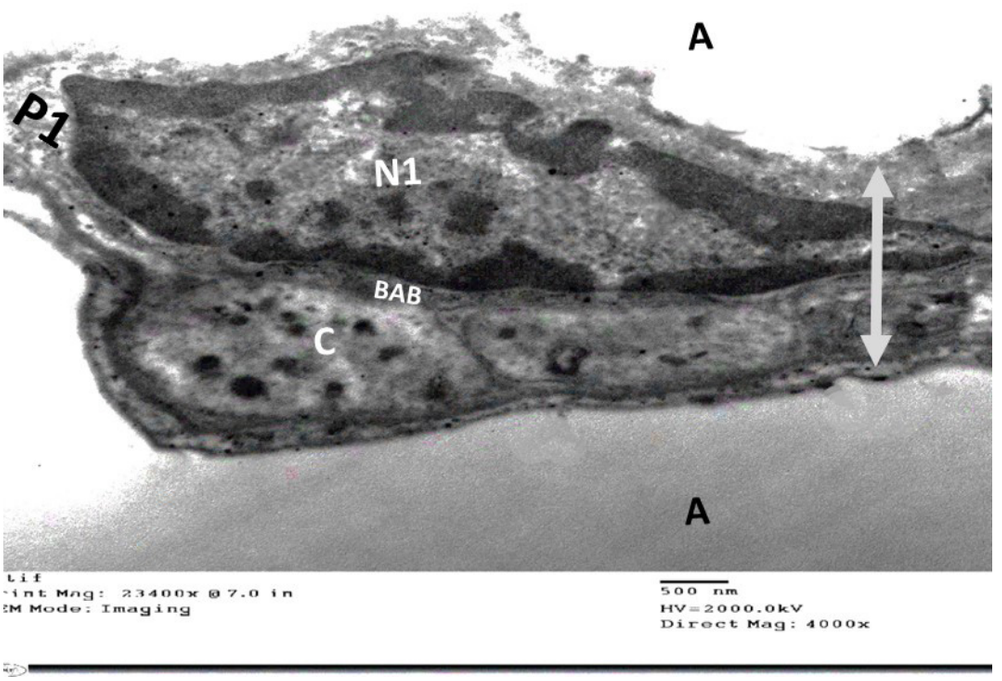


Fig. 6: A TEM image of a lung section of the control group revealing a thin interstitial septal wall ( ) lined by pneumocyte I (P1), and shows small capillaries (C) with intervening blood-air barrier (BAB). The pneumocyte I (P1) was seen as a flat cell with a flat pale nucleus (N1). Notice the preserved distended alveolar space (A). (TEM x4000)

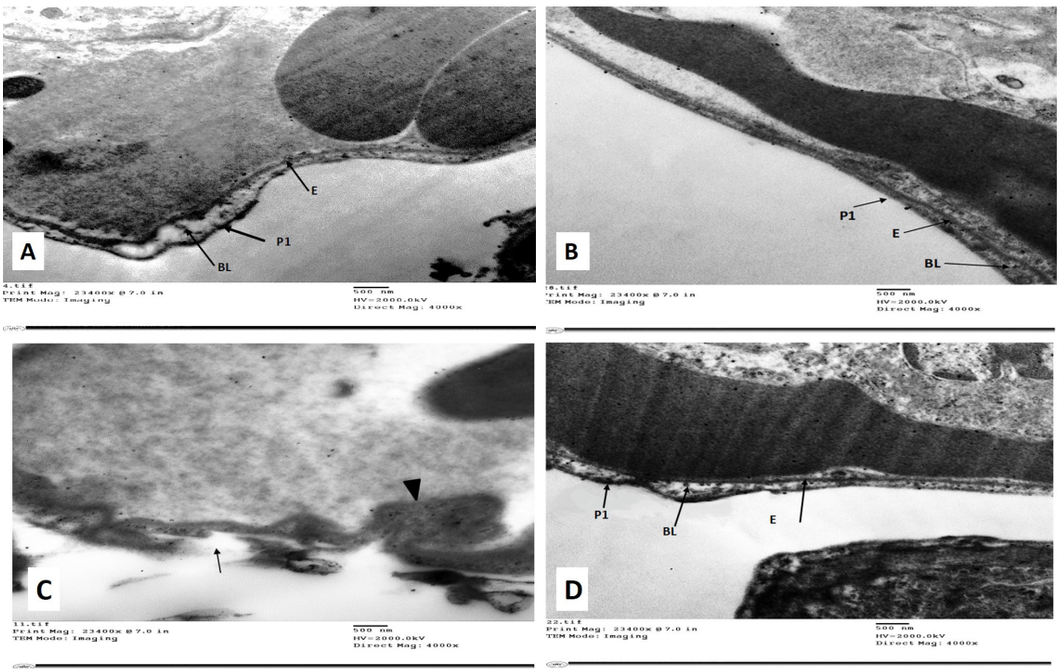


Fig. 7 {A-D}: TEM images of lung sections of the rats showing: A; The control group shows the blood-air barrier consisting of; endothelial cells (E), pneumocyte I (PI), and basal lamina in-between (BL). B; Colchicine group shows a similar control picture. C; BLM group shows irregular (arrowhead) and interrupted (arrow) blood air barrier. D; BLM + colchicine group shows restored normal blood-air barrier, which is formed of; endothelial cells (E), pneumocyte I (PI), and basal lamina in-between (BL). (TEM x4000)

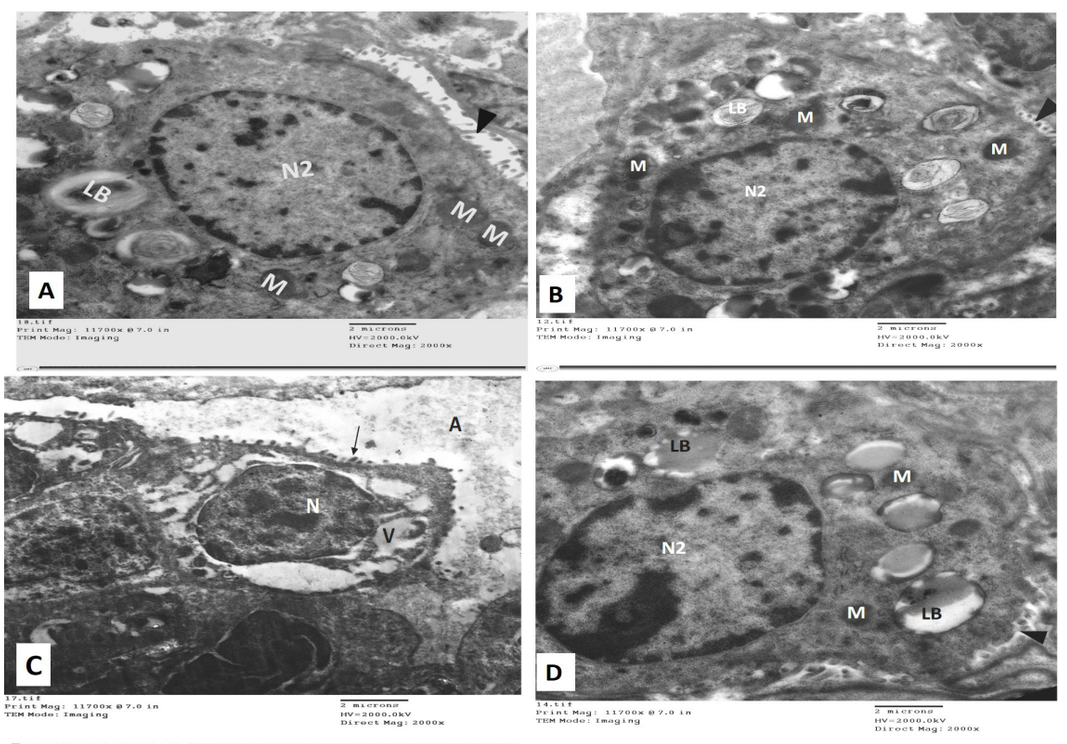


Fig. 8{A-D}: TEM images of lung sections of rats showing: A; The Control group shows pneumocyte II having a rounded euchromatic nucleus (N2) with numerous lamellar bodies (LB) and mitochondria (M) in the cytoplasm. Notice; the surface microvilli (arrowhead). B; The colchicine group has a similar control picture. C; BLM group; pneumocytes II appears wasted, having a small nucleus (N) with heterogeneous chromatin, multiple vacuoles (V) in the cytoplasm, and no organelles seen. The cell surface shows short microvilli (arrow). Note the Exudate in the alveolar space (A). D; The BLM + colchicine group shows pneumocyte II with a pale slightly irregular nucleus (N2). The cytoplasm contains nearly normal lamellar bodies (LB) and many mitochondria (M). Notice, the appearance of the microvilli (arrowhead). (TEM x2000)

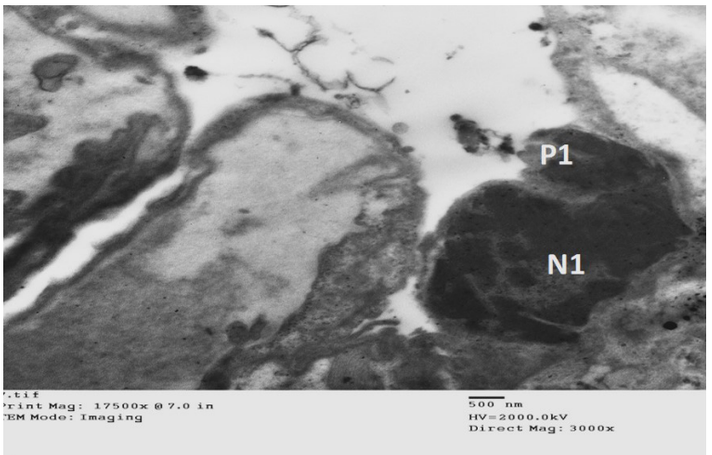


Fig. 9: A TEM image of a lung section of the BLM group showing pneumocyte I (P1), which appears compressed, abnormal shape, with a dark irregular nucleus (N1). (TEM x3000)

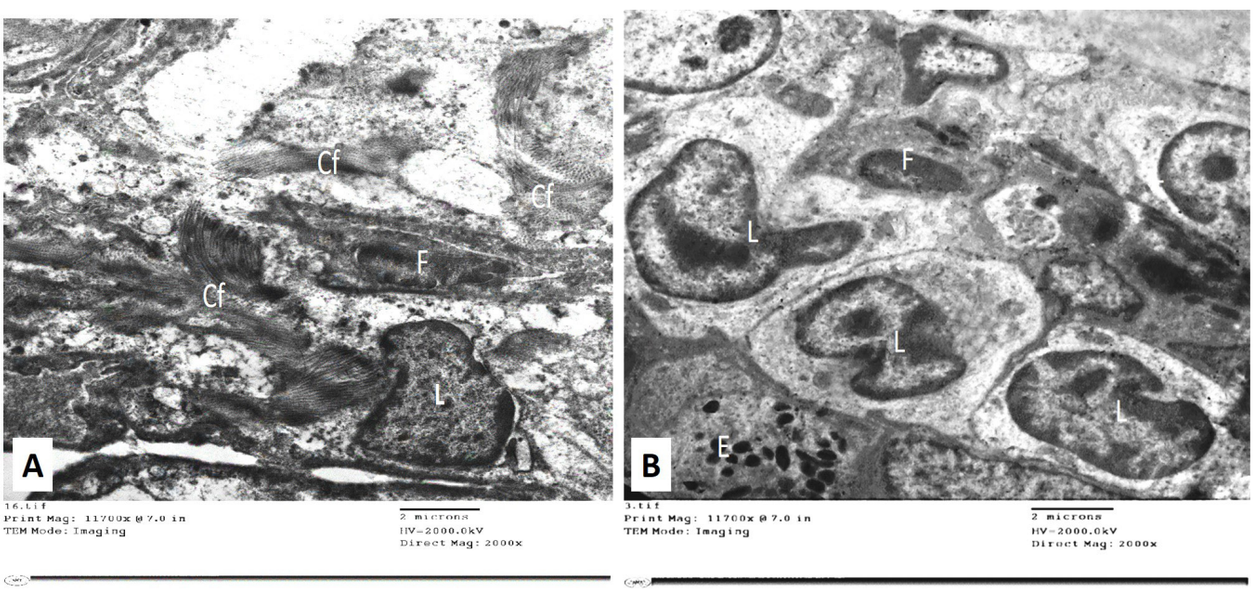


Fig. 10 {A, B}: TEM images of lung sections of the BLM group showing thickened septal walls. A; showing a fibroblast (F) surrounded by collagen fibrils (Cf), with lymphocytic infiltration (L). B; showing marked lymphocytic (L), eosinophil (E), and fibroblast (F)infiltration. (TEM x2000)

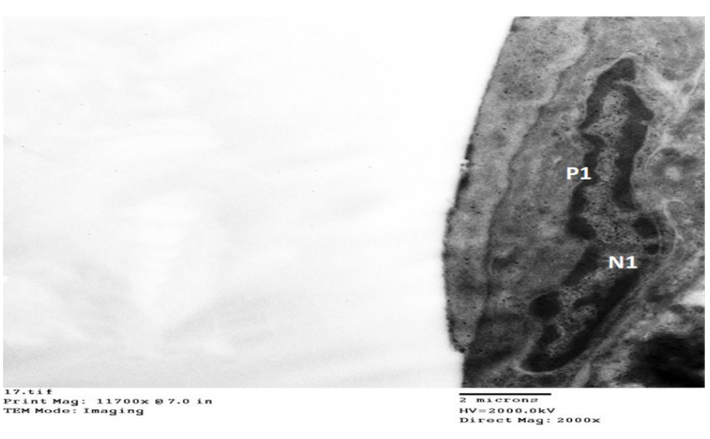


Fig. 11: A TEM image of a lung section of the preventive group showing restored normal pneumocyte (P1); flat cell with pale flat nucleus (N1). (TEM x2000)

Table 1: The average lung weight in grams in the different groups

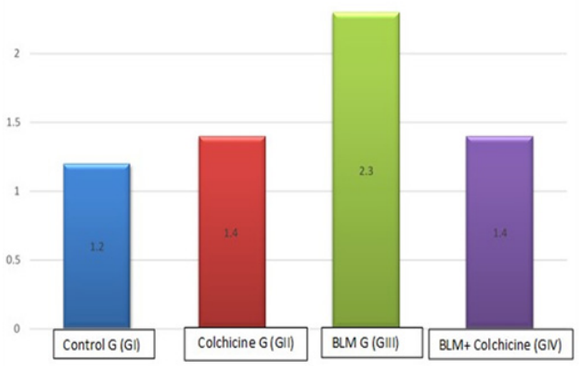
Group	Mean ± SD.	p^a	p^b
G I (control G.)	1.2 ± 0.05		
G II (Colchicine G.)	1.4 ± 0.24	1.000	<0.001**b
G III (BLM G.)	2.3 ± 0.32	<0.001**a	
GIV (BLM+ Colchicine G.)	1.4 ± 0.06	1.000	<0.001**b

F: ANOVA
* Significance difference $p \leq 0.05$
** High significance $p < 0.001$
a: relative to the control group
b: relative to the BLM group

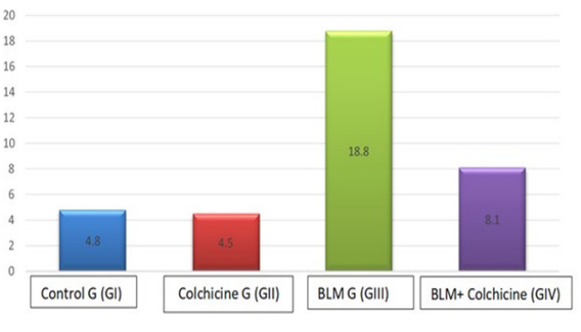
Table 2: The average percentage of areas of collagen fibrils in Masson's trichrome sections in the different groups

Group	Mean ± SD.	p^a	p^b
G I (control G.)	4.8 ± 0.48		
G II (Colchicine G.)	4.5 ± 0.46	1.000	<0.001**b
G III (BLM G.)	18.8 ± 1.03	<0.001**a	
GIV (BLM+ colchicine G.)	8.1 ± 0.60	<0.001**a	<0.001**b

F: ANOVA
* Significance difference $p \leq 0.05$
** High significance $p < 0.001$
a: relative to the control group
b: relative to the BLM group



Bar chart 1: The average lung weight in grams in the different groups.



Bar chart 2: The average percentage of areas of collagen fibrils in Masson's trichrome sections in the different groups.

DISCUSSION

Pulmonary fibrosis is one of the commonest lung diseases, as its prevalence is 13/100000 in females, and 20/100000 in males[17]. The idiopathic type is the most common form, which appears as progressive, chronic interstitial pneumonic disease, ends with the proliferation of the fibroblasts and collection of collagen in the lung parenchymatous tissue, caused by injury of the alveolar epithelium, and inflammation of the interstitium[18]. Our work aimed to study the changes in the lung caused by BLM and the potential protective action of colchicine. The BLM is preferred as a model for the development of laboratory lung fibrosis due to its immediate and continuous poisonous action on the lung[19].

In the ongoing study, the lungs of the control and the colchicine groups appeared grossly pinkish, and uniform in texture. In contrast, the lungs of the BLM group seemed to be swollen and hyperemic, with emphysematous bullae and hemorrhagic areas. The lungs in the preventive group appeared less swollen and decreased hyperemia. Kitzerow *et al*., (2022) found the same outcomes[20].

In the current work, studying of H&E sections of the BLM group displayed damaged lung parenchymatous tissue, collapsed alveoli, severely thickened interstitial septal walls, and marked inflammatory reaction in the form of fluid exudation, and severe invasion by inflammatory cells.

These outcomes were consistent with Zakaria *et al*., (2021) who found that the BLM can cause stimulation of the macrophages to produce many cytokines such as IL-1. These cytokines can activate and proliferate the fibroblasts and finally result in the accumulation of collagen fibrils[19]. Moodley *et al*., (2009) also stated that the invasion of the lung parenchymatous tissues by inflammatory cells is considered as a direct effect of BLM[21].

In the present work, the bronchioles were seen as abnormal as; desquamation of the bronchiolar epithelium, interruption of the muscular layer, cellular debris and hemorrhage in their lumens, and inflammatory cellular invasion seen surrounding them.

These results were consistent with Zakaria *et al*., (2021) who said that marked invasion of inflammatory cells around the bronchioles was observed in H&E sections due to the chemoattractant action of the cytokines[19].

In this study, the walls of the arterioles accompanying bronchioles were seen hypertrophied and their lumens were narrowed in the BLM group, which might be explained by Schroll *et al*., (2010) as a vascular resistance increase against pulmonary fibrosis caused by BLM[22].

In the present work, the Masson's trichrome sections of the BLM group revealed a large amount of collagen in the hypertrophied interstitium, surrounding the bronchioles and around the bronchial arterioles.

These results came with Zakaria *et al*., (2021) who said that severe collection of collagen fibrils in the interstitial septal walls and the bronchioles was defined by increasing the green color in sections of Masson's trichrome[19].

In the current study, ultrastructural examination of the BLM group revealed that the pneumocytes displayed notable corruption. Pneumocytes I had an abnormal shape, with dark small nuclei, and the blood-air barrier was seen as irregular and interrupted. The pneumocytes II showed severe damage. They had small nuclei with dark clumped chromatin and showed unfilled lamellar bodies in their cytoplasm.

These results were consistent with Zakaria *et al*., (2021) who revealed that pneumocytes I wasn't easily counted during EM study, due to the immediate toxicity of BLM on it. Also, the pneumocytes II atrophy may be due to the effect of oxygen-free radicals and lipid peroxidation[19].

In the concurrent research, the EM study of the BLM group showed notable infiltration of the interstitial septal wall with inflammatory cells mainly lymphocytes, also, many fibroblasts and collagen fibers deposition were noticed.

This was explained by Ishida *et al*., (2023) who stated that BLM administration leads to infiltration by inflammatory cells in the lung parenchymatous tissue which leads to activation of the fibroblasts[23].

Also, Kim *et al*., (2010) added that increasing lymphocytes in the lung parenchymatous tissue following the administration of BLM and suggested that the lymphocytes might be responsible for the pathogenesis of lung inflammation with the subsequent fibrosis[24].

Lung fibrosis management is still controversial. The ideal drug hasn't yet been found. The old drug colchicine has been proposed many times for the treatment of lung fibrosis, due to its effect against inflammation and fibrosis. During the COVID-19 spike, colchicine was a drug of interest for many clinical studies to limit the complications of the disease[11].

In our research, the H& E sections of the preventive group (taken the BLM and the Colchicine 3 days before BLM administration and continued during the period of BLM intake) revealed minimal damage in most sections with the restoration of the normal alveolar architecture, the interstitial walls were markedly decreased in thickness except minimal local areas of increased thickness and the inflammatory cells were relatively decreased.

The EM of the lungs of the preventive group showed rather normal pneumocytes. Most pneumocytes I and II appeared rather normal without having a major structural abnormality. The blood-air barrier appeared rather healthy with no interruption. The lamellate bodies in the pneumocytes II were seen as rather packed. The collagen fibers in the walls were obviously diminished.

Esther *et al*., (2020) explained the colchicine action in decreasing the edema and inflammation of the lung tissue by its anti-inflammatory, anti-mitotic, and anti-fibrotic pathways[25].

However, our results were against Douglas *et al*., (1998) who reported that prednisone and colchicine couldn't stop the progression of pulmonary fibrosis, and they are ineffective antifibrotic agents[26].

In the present work, immune staining with Anti-α-SMA showed that the control group and the colchicine group showed negative reactions in the septal walls and the alveolar cells, only the reaction was positive in the smooth muscles of the bronchioles and the bronchial arterioles. While the BLM group showed a strong positive cytoplasmic reactivity within the interstitial septal walls and the alveolar cells. The preventive group showed a decreased reaction relative to the BLM group. Mohamed *et al*., 2020 found the same reaction[27].

In our study, statistical analysis by assessment of the average lung weight displayed that the BLM group revealed a high-significance increase in the average lung weight relative to the control group. The preventive group revealed a non-significant discrepancy relative to the control group, and a high significance decrease relative to the BLM group. Kitzerow *et al*., 2022 had published the same results[20].

In this study, statistical analysis by assessment of the average percentage of areas of collagen fibrils in Masson's trichrome sections displayed that the BLM group showed a high significance increase relative to the control group. The preventive group displayed high significant increase relative to the control group, and high significant decrease relative to the BLM group.

Dina *et al*., 2021 stated that Masson's trichrome sections could be used in morphometric analysis by measuring the areas of green color stain to calculate the percentage of collagen fiber deposition[28].

CONCLUSION

It was found that colchicine has a great role in improving the histological changes of pulmonary fibrosis and is recommended to be used as a supportive drug in patients with serious lung inflammation with a risk of developing fibrosis.

ACKNOWLEDGMENT

The authors appreciate the technicians and workers who participated in this study.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Kreuter M, Ladner UM, Costabel U, *et al*. The Diagnosis and Treatment of Pulmonary Fibrosis. Dtsch Arztebl Int. 2021;5(118):152–162. https://doi.org/10.3238/arztebl.m2021.0018

2. Wilson MS and Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. Mucosal Immunol. 2009; 2(2):103–121. https://doi.org/10.1038/mi.2008.85

3. Victor GE, Iouri B, Ravindranath T, *et al*. Bleomycin-induced pulmonary toxicity and treatment with infliximab: A case report. Clin Case Rep. 2018; 6(10): 2011–2014. https://doi.org/10.1002/ccr3.1790

4. Sleijfer S. Bleomycin-induced pneumonitis. Chest.2001;120(2):617-624. https://doi.org/10.1378/chest.120.2.617

5. Leung YY, Hui LL, and Kraus VB. Colchicine --- update on mechanisms of action and therapeutic uses. Semin Arthritis Rheum. 2015;45(3):341–350. https://doi.org/10.1016/j.semarthrit.2015.06.013

6. Entzian P, Schlaak M, Seitzer U, *et al*. Anti-inflammatory and Antifibrotic Properties of Colchicine: Implications for Idiopathic Pulmonary Fibrosis. Lung.1997;175:41–51. https://doi.org/10.1007/pl00007555

7. Lu Y, Chen J, Xiao M, Li W, *et al*. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharm Res. 2012;29(11):2943-71. https://doi.org/10.1007/s11095-012-0828-z

8. Sun Y, Liang Y, Hu J, *et al*. Reduction of intraarticular adhesion by topical application of colchicine following knee surgery in rabbits. Sci Rep. (2014);4:6405. https://doi.org/10.1038/srep06405

9. Jin JL, Sun HL, Dong KK, *et al*. Colchicine attenuates inflammatory cell infiltration and extracellular matrix accumulation in diabetic nephropathy. American Journal of Physiology-Renal Physiology. 2009;297(1):200-209. https://doi.org/10.1152/ajprenal.90649.2008

10. Hama Amin BJ, Kakamad FH, Ahmed GS, et. al. Post COVID-19 pulmonary fibrosis; a meta-analysis study.Ann Med Surg.(2022);77:103590. https://doi.org/10.1016/j.amsu.2022.103590

11. Schlesinger N, Firestein BL, and Brunetti L. Colchicine in COVID-19: an Old Drug, New Use. Curr Pharmacol Rep. 2020;18:1–9. https://doi.org/10.1007/s40495-020-00225-6

12. Upuis J, Sirois MG, Rhe´aume E, *et al*. Colchicine reduces lung injury in experimental acute respiratory distress syndrome. PLoS ONE.2020; 15(12):1-15. https://doi.org/10.1371/journal.pone.0242318

13. Kamel E, Abdel Hady A, Abd-Elrahman A, *et al*. Evaluation of Role of Captopril and Erdosteine in Protection of the Lung against Bleomycin-Induced Injury in Rats. The Egyptian Journal of Hospital Medicine.2019;75(1): 1937-1945. https://dx.doi.org/10.21608/ejhm.2019.29117

14. Bancroft JD and Gamble M. Theory and Practice of Histological Techniques. 7th Edition, Churchill Livingstone of Elsevier, Philadelphia, 2013; P.:172-186.

15. Masson P. Trichrome staining and their preliminary technique. J Tech Meth.1929;12:75-90. https://doi.org/10.1007/BF01003791

16. Abu-Deif EE, Abdelrahim EA and Abdelrahim KM. Histological modifications aging in male albino rat. J. Cytol. Histol.(2016);7(2):408. http://dx.doi.org/10.1097/01.EHX.0000473759.06552.e4

17. Keeley EC, Mehrad B and Strieter RM. Fibrocytes: bringing new insights into mechanisms of inflammation and fibrosis. Int J Biochem Cell Biol.2011;42:535–542. https://doi.org/10.1016/j.biocel.2009.10.014

18. Raghu G, Collard HR, Egan JJ, *et al*. Idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med.2011;183(6):788-824. https://doi.org/10.1164/rccm.2009-040gl

19. Zakaria DM, Zahran NM, Arafa SA, *et al*. Histological and Physiological Studies of the Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Bleomycin Induced Lung Fibrosis in Adult Albino Rats. Tissue Eng Regen Med. 2021;18(1):127-141. https://doi.org/10.1007/s13770-020-00294-0

20. Kitzerow O, Zucker IH, Lisco SJ, *et al*. Timeline of Multi-Organ Plasma Extravasation After Bleomycin-Induced Acute Lung Injury. Frontiers in Physiology.2022;13:777072. https://doi.org/10.3389/fphys.2022.777072

21. Moodley Y, Atienza D, Manuelpillai U, *et al*. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. Am J Pathol.2009;175(1):303-313. https://doi.org/10.2353/ajpath.2009.080629

22. Schroll S, Arzt M, Sebah D, *et al*. Improvement of bleomycin-induced pulmonary hypertension and pulmonary fibrosis by the endothelin receptor antagonist Bosentan. Respir Physiol Neurobiol.2010;170(1):32-36. https://doi.org/10.1016/j.resp.2009.11.005

23. Ishida Y, Kuninaka Y, Mukaida N, *et al*. Immune Mechanisms of Pulmonary Fibrosis with Bleomycin. Int J Mol Sci.2023;24(4):3149. https://doi.org/10.3390/ijms24043149

24. Kim SN, Lee J, Yang HS, *et al*. Dose-response Effects of Bleomycin on Inflammation and Pulmonary Fibrosis in Mice. Toxicol Res.2010;26(3):217-222. https://doi.org/10.5487/TR.2010.26.3.217

25. Esther F, Ariel K and Yaron I.Introducing variability in targeting the microtubules: Review of current mechanisms and future directions in colchicine therapy. Pharmacology Research & Perspectives. (2020);8(4):e00616. https://doi.org/10.1002/prp2.616

26. Douglas WW, Ryu JH, Swensen SJ, *et al*. Colchicine versus prednisone in the treatment of idiopathic pulmonary fibrosis. A randomized prospective study. Members of the Lung Study Group. Am J Respir Crit Care Med.1998;158(1):220-225. https://doi.org/10.1164/ajrccm.158.1.9709089

27. Mohamed SE, Asmaa ME, Doaa EE, *et al*. Histological and Immunohistochemical Study to Evaluate the Effects of Metformin versus Green Tea Extracts on Bleomycin Induced Lung Injury in Adult Male Albino Rats. Egyptian Histology Journal.2020; 44(1);34-47. https://dx.doi.org/10.21608/ejh.2020.25595.1260

28. Dina MZ, Noha MZ, Samia AA, *et al*. Histological and Physiological Studies of the Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Bleomycin Induced Lung Fibrosis in Adult Albino Rats. Tissue Eng Regen Med.2021;18(1):127–141. https://doi.org/10.1007/s13770-020-00294-0

الملخص العربى

دور الكولشيسين فى منع التليف الرئوى الناتج عن البليوميسين فى الجرذان

سارة عبدالمنعم عراقيب[١]، محمد عبد الحى عطيفى[٢]، ايمان احمد الصواف[١]، السيد عبد الرحمن عبد الهادى[١]، منال حمدى البدوى[١]

قسم التشريح والاجنة، كلية الطب، [١]جامعة حلوان، [٢]جامعة الازهر

الخلفية: يعتبرالتليف الرئوى من اكثرالامراض شيوعًا وقد يؤدي إلى فشل فى الجهاز التنفسي في الحالات الحرجة بمعدل وفيات يتراوح بين ١٠٪ إلى ٢٠٪ ، هناك اسباب كثيرة للتليف الرئوى منها المواد الكيميائية، والالتهابات، والإشعاع، والمواد المسببة للحساسية، والأدوية مثل البليوميسين ، ونتيجة لمرض فيروس كورونا الأخير.
الهدف من البحث: تم تصميم هذاالبحث لمعرفة التأثير الوقائي للكولشيسين على تليف الرئة في الجرذان
التجربة: جرى استخدام ٢٤من الجرذان الذكورالبيضاء حيث تم توزيعهم إلى ٤ فئات(٦ جرذان فى كل فئة): الفئة الأولى (الفئة الضابطة): تم حقن الجرذان بمحلول ملحى داخل غشاء البطن فقط و لم تتلق اى ادوية اخرى، الفئة الثانية (فئة الكولشيسين فقط): اخذت الجرذان فى هذه الفئة الكولشيسين فقط بجرعة ١مجم / كجم عن طريق الفم ،الفئة الثالثة (فئة البليوميسين):تم اعطاءالجرذان دواءالبليوميسين فقط عن طريق الحقن بجرعة قدرها ٠,٥ مجم / كجم بداخل محلول ملحى داخل غشاء البطن مرتين اسبوعيا لمدة٣ اسابيع بهدف اصابتها بداء التليف الرئوى ،الفئة الرابعة (فئة البليوميسين والكولشيسين): تم اعطاء الجرذان البليوميسين بنفس الجرعة السابقة وتم اعطاؤها ايضا الكولشيسين كعلاج وقائى بجرعة ١مجم / كجم بالفم قبل البليوميسين بثلاثة ايام واثناءتناوله.
في ختام البحث، خضعت الجرذان للتخدير وتم فتح جدارالصدرللحصول على الرئتين. جرى تثبيت ومعالجة جزء من العينات باستخدام صبغة الهيماتوكسلين والايوسين بالاضافة الى صبغة الماسون تريكوم و صبغة هستوكيميائية المناعية لإظهار الخلايا العضلية الملساء، بعد ذلك تم فحص العينات بواسطة المجهر الضوئى، كما خضعت باقى العينات لمعالجات اضافية بهدف دراستها بواسطة المجهرالالكترونى النافذ.
النتائج: اظهر الفحص الهستولوجى للانسجة ان استخدام الكولشيسين ادى الى تقليل التليف الرئوى الناتج عن البليوميسين تمثل ذلك فى استعادة البنية الطبيعية لانسجة الرئه جزئيا،الى جانب انخفاض ملحوظ فى سمك الحاجزالموجود بين الحويصلات الهوائية، كما لوحظ ايضا انخفاض نسبى فى تسلل الخلايا الالتهابية وارتشاح السوائل عبر الانسجة، بالاضافة الى ذلك استعادة الشعيبات الهوائية طابعهاالطبيعى، وقداظهر فحص العينات فى المجموعة الوقائية تحسنا ملحوظا فى نمط الخلايا الرئوية مع ظهورطبيعى للاجسام الصفاحية الموجودة بالخلايا الرئوية من النوع الثانى ، كما استعاد الحجز الهوائى الدموى مظهره الطبيعى مع انخفاض ملحوظ فى ترسب الكولاجين داخل النسيج البينى
الاستنتاج: وجد أن الكولشيسين له تأثير كبير على تحسين تغيرات الانسجه المصاحبة للتليف الرئوي ويوصى باستخدامه كدواء مساعد في المرضى الذين يعانون من التهاب خطير في الرئة مع خطر الإصابة بالتليف الرئوى.