The Effect of Ginkgo Biloba Extract on Lung Fibrosis in Rats

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Abstract

Background: Pulmonary fibrosis (PF) is a fatal fibrotic lung disease with limited treatment options. The objective of this study was to determine the anti-fibrotic effect of *Ginkgo biloba* aqueous extra

ct (GAE) and possible underlying mechanisms in pulmonary fibrosis in adult male albino rats. **Methods**: Adult male albino rats (170-200 g) were divided into 4 groups (5 animals each) as follows: group (1) normal rats served as control, (2) Bleomycin (BLM)-induced lung fibrosis animals as positive control (2.5 mg/kg/weekly, intratracheally) for 4 weeks, (3) Pulmonary fibrosis-modeled animals were treated with GAE (200 mg/kg/day, orally) for 4 weeks and (4) Pulmonary fibrosis-modeled animals were treated with GAE (400 mg/kg/day, orally) for 4 weeks. **Results:** results revealed that the administration of GAE markedly improved the lung fibrosis disorders; this was monitored from the significant reduction in the values of Liver enzymes Alanine aminotransferase (ALAT) and Aspartate aminotransferase (ASAT) (50.4±3.3, 82.6±8.2). kidney function urea and creatinine (46.8±1.5, 0.82±0.045) and phosphorus (5.8±0.2 B) as well as lung MDA coupled with remarkable raise in Calcium (9.1±0.7C) and lung TAC activity. Moreover, the histopathological findings showed a therapeutic potential of GAE which succeeded in prevention of pulmonary

fibrosis. **Conclusion:** GAE exhibited anti-fibrotic property that may be mechanized through the radical scavenging and antioxidant characteristics of its active constituent's especially high phenolic content; reflecting a promising potency of GAE as pulmonary protective supplement

Key words: Pulmonary fibrosis, Bleomycin, *Ginkgo biloba*, Rat.

تاثير مستخلص الجنكه بيلوبا على التليف الرئوي في الفئران

المستخلص:

يُعدّ التليف الرئوي (PF) مرضًا رئويًا تليفيًا قاتلًا، وخيارات علاجه محدودة .هدفت هذه الدراسة إلى تحديد التأثير المضاد للتليف لمستخلص الجنكة بيلوبا المائي (GAE) والآليات الكامنة المحتملة في التليف الرئوي لدى ذكور الجرذان البيضاء البالغة .الطريقة: قُسِّمت ذكور الجرذان البيضاء البالغة (١٧٠-٢٠٠ غرام) إلى أربع مجموعات (خمسة حيوانات لكل مجموعة) على النحو التالي: المجموعة (١) فئران سليمة كمجموعة ضابطة، (٢) حيوانات مُصابة بتليف رئوي مُستحث بالبليومايسين (BLM) كمجموعة ضابطة إيجابية (٢,٥ ملغم/كغم أسبوعيًا، داخل القصبة الهوائية) لمدة ٤ أسابيع، (٣) عولجت الحيوانات المُصممة لتليف الرئة بـ لمستخلص الجنكة (٢٠٠ ملغم/كغم يوميًا، عن طريق الفم) لمدة ٤ أسابيع، و(٤) عولجت الحيوانات المُصممة لتليف الرئة بـ لمستخلص الجنكة (٤٠٠ ملغم/كغم يوميًا، عن طريق الفم) لمدة ٤ أسابيع .النتائج: أظهرت النتائج أن إعطاء لمستخلص الجنكة قد أدى إلى تحسن ملحوظ في اضطرابات تليف الرئة؛ وقد تم رصد ذلك من خلال الانخفاض الكبير في قيم إنزيمات الكبد ألانين أمينوترانسفيراز (ALAT)وأسبارتات أمينوترانسفيراز3.3 ± 50.4) (ASAT) ، ٨٢,٦ .(8.2 ± وظائف الكلى اليوريا والكرياتينين (٤٦,٨ ± ١,٥، ٠,٨٢ ± ٠,٠٤٥) والفوسفور 5.8) ± 0.2 B)بالإضافة إلى MDA الرئة إلى جانب زيادة ملحوظة في الكالسيوم ± 9.1) (0.7 C)ونشاط TAC الرئة .وعلاوة على ذلك، أظهرت النتائج النسيجية المرضية

إمكانات علاجية لـ لمستخلص الجنكة والتي نجحت في الوقاية من تليف الرئة. في الختام، أظهر GAE خاصية مضادة للتليف يمكن آليًا من خلال خصائص إزالة الجذور الحرة ومضادات الأكسدة لمكونه النشط ذي المحتوى الفينولي العالي بشكل خاص؛ مما يعكس فعالية واعدة لـ لمستخلص الجنكة كمكمل غذائي وقائي للرئة.

الكلمات المفتاحية: التليف الرئوي، بليومايسين، الجنكة بيلوبا، الجرذان.

Introduction

Pulmonary fibrosis (PF) is a chronic lung disorder that may be produced by infections, toxic agents, immune-mediate, or might be idiopathic; it frequently progressive with marked interstitial lung damage, ultimately fibrosis, and loss of the lung elasticity ended by respiratory failure **(Thuwaini *et al.,* 2021)**. Bleomycin (BLM) is a common fibro genic agent, provoking an initial adult respiratory distress syndrome-like injury with subsequent strong fibro proliferative response, severe abnormalities of the alveolar surfactant system accompanied by epithelial metaplasia **(Chaudhary *et al.,* 2006)**. Furthermore, bleomycin is capable to cause cell damage independent from its influence on DNA, by inducing lipid peroxidation **(Eroschenko, 2005)**. The pathogenesis of PF is complex and the urokinase-type plasminogen activator (uPA)/plasminogen system participates in the repair process, where the balance between the activating enzyme uPA, and its inhibitor PAI-1, is a critical determinant of the amount of scar development that follows **(Gunther *et al.,* 2003)**. During acute and chronic inflammatory lung diseases, the normal fibrinolytic activity in the alveolar space is inhibited by increased levels of plasminogen activator inhibitor 1 (PAI-1). Transgenic mice having increased fibrinolytic activity due to genetic deficiency of PAI-1 develop less fibrosis after bleomycin-induced lung inflammation **(Shetty *et al.,* 1996)**.

Several studies demonstrated that natural medicinal plants with potent antioxidant activity and potential protective effects can alleviate the damage of

oxidative stress associated diseases through inhibition of ROS generation and improvement of antioxidant defense mechanisms **(Forni *et al.,* 2019)**.

Ginkgo biloba (GB) is one of the world's most extensively used herbal medicines, having been used for hundreds of years **(Ude *et al.,* 2013)**.

Ginkgo biloba extract (GBE), a therapeutic drug, has anti-inflammatory and antioxidant effects that protect cells from harmful substances **(Anhui *et al.,* 2025)**. Ginkgo biloba contain plenty of bioactive components, making it a chemically diversified plant **(Tabassum *et al.,* 2022)**. GB extract includes sugars, amino acids, organic acids, polysaccharides, sterols and inositols. The active constituents have various chemical structures, including flavonoids, terpenoids, and small amounts of organic acids **(Maclennan *et al.,* 2002 & Serrano-García *et al.,* 2013)**. It is the most widely herbal treatment, it helps in scavenging free radical; lowering oxidative stress; and helps in the treatment of several cardiovascular diseases (CVD), cancer, inhibit the apoptosis of myocardial cells and protect the myocardium, decreasing oxidative stress and repressing inflammatory reaction **(Kuller *et al.,* 2010; Chen *et al.,* 2019 & Barbalho *et al.,* 2022)**. Most of the studies have attributed the cardio protection of GB to enhanced antioxidant activity **(Wang *et al.,* 2016)**. Therefore, the current study was carried out to investigate the protective potential of GAE ameliorate the pathophysiological induced pulmonary fibrosis, and to assess anti-oxidative damage in the rats intoxicated with Bleomycin.

Materials and methods

Materials

Chemicals

Bleomycin was obtained from Sigma Aldrich (St. Louis, MO, und Diagnostic mbH, Germany, serum ALAT and ASAT activity were ascertained. The levels of 4 creatinine, serum urea calcium, phosphorus, MDA and TAC were measured with kits

purchased from Biodiagnostic, Dokki, Giza, Egypt) The supplier of phenylhydrazine was Sigma in St. Louis, USA.

Materials

The source of *Ginkgo biloba* was Imtenan Health Shop in Obour City, Cairo, Egypt's Industrial Area.

Methods

Extraction

The following procedure was used to create the aqueous *Ginkgo biloba* extract: For two days, a mixture of 0.5 g bee glue and 10 mL deionized water was stored in a refrigerator at 4 ± 1 °C. After that, the sample was centrifuged for 20 minutes at 10,000 rpm. After filtering the supernatant using a Whatman #41 paper filter, the water extract was placed in an oven drier set at 50 °C for 24 hours, resulting in a semisolid extract. The water extract was preserved for additional examination in a freezer **(Paviani *et al.,* 2013)**.

HPLC analysis of phenolic constituents

Using an Agilent 1260 series, the HPLC (high-performance liquid chromatography) test was performed for GAE screening. A Kromasil C18 column with a 4.6 mm id and a 250 mm id was used for the separation (5 m). Water and acetonitrile were the main components of the mobile phase, which had a flow rate of 1 ml/min and a trifluoroacetic acid concentration of 0.05% (A and B). The linear gradient was used to program the mobile phase at the following intervals: 0 min (82% A), 0 min to 5 min (80% A), 5-8 min to 6 min, 5-8 min to 12 min, 85% A, and 15-16 min (82% A). At a wavelength of 280 nm, the multiwavelength detector was observed. Then, all the sample solutions were exposed to an injection of adequate volume (10 ml). A constant 35°C was kept in the column temperature.

Animals and experimental design

The study was approved by the ethical committee of Al-Azhar University in Assiut, Egypt. Male albino rats weighing between 170 and 200g were procured from the Animal Colony of the National Research Centre in Giza, Egypt. The animals were housed in appropriate plastic cages and allowed unrestricted access to food and water for a week prior to the experiment to allow for acclimatization. They were provided with human care in accordance with the standard institution's criteria for the care and use of experimental animals. Following the animals' acclimation to the circumstances of the experimental room, they were split into four groups at random, each with five animals. As a normal control, the first group of healthy animals received a conventional food and an intraperitoneal injection of 1 ml of isotonic saline without any therapies, the second group Bleomycin-induced lung fibrosis animals as positive control (2.5 mg/kg/weekly, intratracheally) for 4 weeks, third group Pulmonary fibrosis-modeled animals were treated with GAE (200 mg/kg/day, orally) for 4 weeks and fourth group pulmonary fibrosis-modeled animals were treated with GAE (400 mg/kg/day, orally) for 4 weeks according to **Moeller et al., (2008)**.

Blood and tissue sampling

Following an overnight fast and weight measurement at the conclusion of the treatment period, blood samples were taken from the retro-orbital plexus using sterile, heparinized glass capillaries. Each blood specimen was split into two parts: the first was collected for hematological measurements in a heparinized tube, and the second part underwent a 10-minute cool centrifugation at 3000 rpm to separate and divide the sera into aliquots, which were then stored at -80°C until biochemical measurements could be performed right away. Following blood collection, the animals were quickly sacrificed by abrupt decapitation, and all of the rats' lungs were removed. The lungs from each group

were then dried, rolled in aluminum foil, and stored at -80°C to evaluate oxidative stress markers. The remaining portions of each group's lungs were then submerged in a 10% v/v formaldehyde-saline buffer for histopathological analysis.

Biochemical determinations

The Schimadzu spectrophotometer (UV–vis 1201, Japan) was used for all biochemical assays Aspartate Amino Transferase (AST) and Alanine Amino Transfers (ALT) were estimated in accord with Reitman and Franke (1957), following the methods of **Searcy *et al.,* (1967) and Bohmer, (1971)** kidney function parameters including serum urea and creatinine, respectively were estimated. Calcium and phosphorus were measured according with **Gindler & King, (2015)** and **Goodwin, (1970), respectively.**

Oxidative stress markers of lung tissue

The method of **Ohishi & Yagi (1979)** were used to estimate the amounts of lung malondialdehyde (MDA). Total Antioxidant Capacity (TAC) was determined as mentioned by **Miller *et al.*, (1993).**

Histopathological examination

All animal lung samples were preserved in 10% neutral formalin, rinsed under running water, dried with ethanol, cleared with xylene, and embedded in paraffin. Hematoxylin and eosin (H&E) were then used to stain sections (5 µm thick) for the histological analysis **(Ashry et al., 2023)**.

Statistical analysis

Using the statistical analysis system's general linear model approach, all data were subjected to a one-way analysis of variance (ANOVA) **(SAS, 1982).** The Waller-Duncan k-ratio was used to assess the importance of the variations

between the various treatment groups **(Steel and Torrie, 1980)**. Every significance claim was predicated on the likelihood that $p \leq 0.05$.

Results

HPLC analysis of phenolic constituents

Table 1 displays the results of the HPLC method used to quantify GAE. Most of the 16 phenolic compounds were discovered using HPLC analysis in GAE. Among the chemicals found were high amounts of Querctin, Rutin, Gallic acid and Chlorogenic acid in GAE, with respective values of (21.19, 29.69, 41.79 and 124.67 µg/ml) Methyl gallate, Naringenin, Rosmarinic acid and Cinnamic acid had the lowest values of GAE, with respective values of (0.12, 4.81, 2.40, 1.18 µg/ml) (Table 1 and Fig. 1).

Table 1. The main phenolic constituents of the GAE (*Ginkgo biloba* aqueous extract) discovered by the HPLC screening

Constituents	Area	Concentration (µg/ml = µg/ 6.8mg)	Concentration (µg/g)
Gallic acid	576.78	41.79	417.94
Chlorogenic acid	889.69	124.67	1246.69
Catechin	0.00	0.00	0.00
Methyl gallate	2.07	0.12	1.17
Coffeic acid	168.70	10.10	100.99
Syringic acid	0.00	0.00	0.00
Rutin	204.23	29.69	296.93
Ellagic acid	0.00	0.00	0.00
Coumaric acid	474.44	17.33	173.25
Vanillin	3.80	0.13	1.28
Ferulic acid	110.92	6.42	64.15
Naringenin	49.72	4.81	48.07
Rosmarinic acid	26.98	2.40	23.97
Daidzein	121.38	6.94	69.40
Querctin	163.60	21.19	211.87
Cinnamic acid	54.42	1.18	11.78

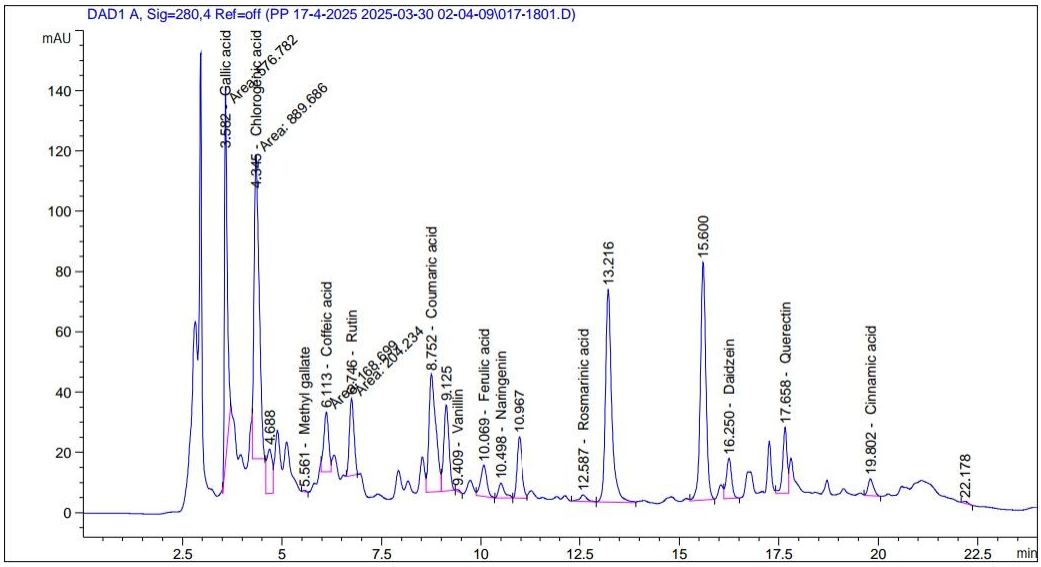


Fig.١. The results of HPLC screening of phenolic ingredients of GAE (*Ginkgo biloba* aqueous extract)

The current study revealed that BLM (G.2) administration resulted in a significant increase in the levels of serum ASAT, ALAT, urea, creatinine, and phosphorus matched with a marked drop in calcium level were (245,151.4, 86.5, 1.78, 7.9 and 7.5 mg/dl) respectively, compared with those of the control (G.1) values were (76.2, 45.5, 46.4, 0.69, 5.4 and. 9.4 mg/dl) respectively. Meanwhile, these highly disturbed levels of biochemical markers were improved significantly post treatment with GAE 200 mg/kg (G.3) were (90.5, 59.9, 53.4, 0.91, 6.1 and 8.8 mg/dl). Finally, GAE 400 mg/kg (G.4) were (82.6, 50.4, 46.8, 0.82, 5.8 and 9.1 mg/dl). this improvement was noticed to be the highest regarding the GAE treated rats (Table 1).

Table 2. Serum biochemical markers of normal, BLM, and BLM~GAE treated rats

	Control	BLM	BLM~GAE low	BLM~GAE high
ALAT (U/L)	45.5±5.4^{cd}	151.4±7.8 ^{a}	59.9±8.1^{b}	50.4±3.3^{c}
ASAT (U/L)	76.2±5.7 ^{d}	245±16.9 ^{a}	90.5±11.5 ^{b}	82.6±8.2 ^{c}
Urea (mg/dl)	46.4±3.7 ^{c}	86.5±4.14 ^{a}	53.4±2.2 ^{b}	46.8±1.5 ^{c}
Creatinine (mg/dl)	0.69±0.04 ^{d}	1.78±0.2 ^{a}	0.91±0.1 ^{b}	0.82±0.045 ^{c}
Calcium (mg/dl)	9.4±1.22 ^{a}	7.5±0.9 ^{b}	8.8±0.6^{a}	9.1±0.7^{a}
Phosphorus (mg/dl)	5.4±0.2 ^{c}	7.9±0.4^{a}	6.1±0.3 ^{b}	5.8±0.2 ^{c}

Data are presented as mean ± standard error; within each raw, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; BLM (Bleomycin), GAE (*Ginkgo biloba* aqueous extract*)*.

Data in Figure (2) show noticeable alterations in values of the lung oxidative (MDA) and total antioxidant capacity (TAC) markers in the BLM group (G.1) were (16, 6 µmol/g as compared with control group (G.1) as the lung MDA level were (5, 16 µmol/g) elevated significantly, while the lung TAC level showed a significant decrease. Favorably, post-treatment of BLM intoxicated animals GAE resulted in a considerable elevation in the antioxidant indicators (TAC) coupled with a significant decrease in the lung oxidative ones (MDA); this amelioration was very clear and promising post treatment with GAE.

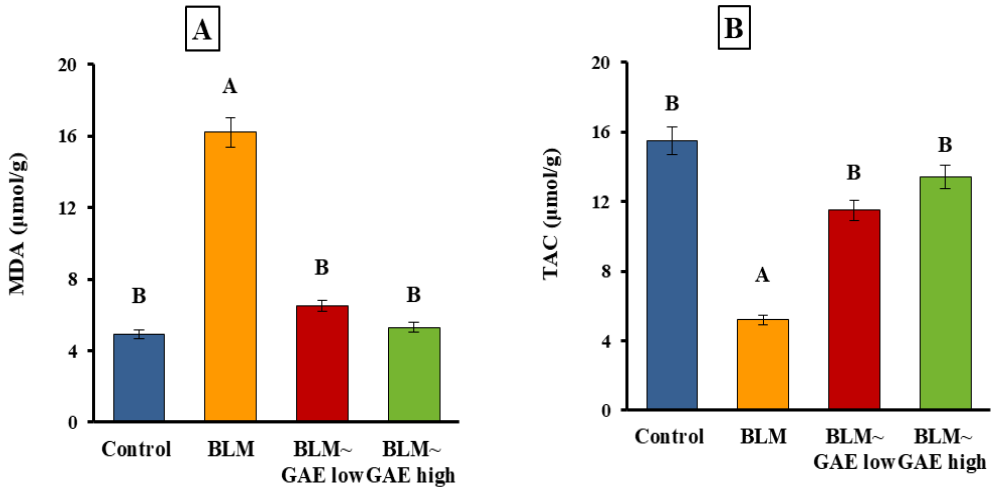
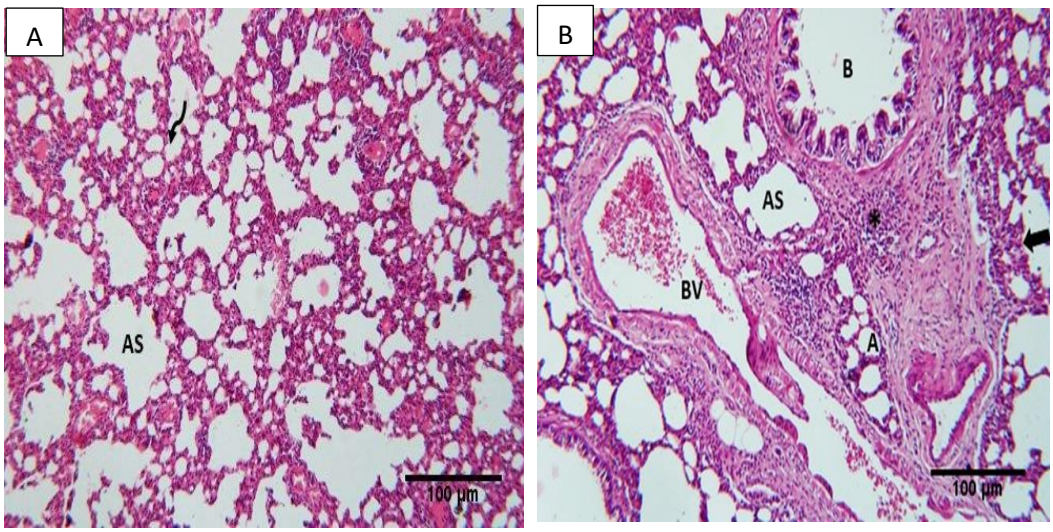


Figure 2: Effect of BLM and GAE on lung MDA (A) and lung TAC. Data are presented as Mean ± SD using one-way ANOVA and a post hoc test (Duncan) at $p \leq 0.05$. Columns sharing the same symbol are not significant. BLM (Bleomycin), GAE (*Ginkgo biloba* aqueous extract*)*

Histopathological findings

Sections stained with H&E from control lung tissues demonstrated the normal architecture of the lung. The sections comprised blood vessels, lung alveoli and alveolar sacs separated by thin interalveolar septa. Alveoli spaces were covered

mostly by flat cells with flattened nuclei (pneumocytes type-I), and cuboidal shape cells with rounded nuclei mainly at the angles (pneumocytes type-II) (Fig. 1A). The pulmonary fibrosis showed that the bronchioles showed folded mucosa covered with simple columnar ciliated epithelium, spirally arranged SMs, and adventitia of areolar connective tissue (CT). There were thick interalveolar septa with narrowing of alveolar space. The septa contained numerous inflammatory cells. Congested blood vessels and blood capillaries with intravascular fibrosis were noticed in all sections (Figure 1B). The lung tissues of group (3) revealed remarkable healing with reduced lymphocytic infiltration and normal alveoli with interalveolar septa (Fig. 1C). The group (4) tissues showed mild thickened interalveolar septa that was significantly less than group (3), with mild lymphocytic infiltration (Fig. 1D).



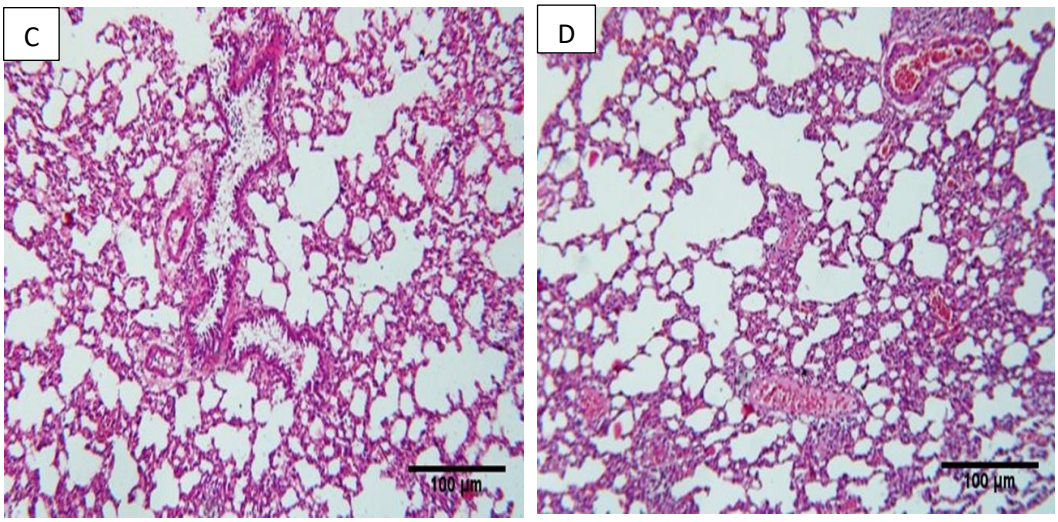


Figure (3A-D). (A) Photomicrograph of normal lung tissue of the control group showing normal histological appearance of pulmonary tissue with normal alveoli and alveolar space (AS) and thin interalveolar septa (curved arrow). (B) The lung tissue of the BLM-induced lung fibrosis group showing congested blood vessels (BV), inflammatory cells infiltration (*), wide alveolar space (AS) and thickness in interalveolar septa (arrow). The adventitia of bronchiole (B) is infiltrated with inflammatory cells. (C) The lung tissue of the BLM+GAE low group showing approximately normal tissue with remarkable improvement in the pulmonary tissue with normal alveoli, alveolar space, and interalveolar septa. (D) The lung tissue of the BLM+ GAE high group showing significant repair compared to with mild thickness of interalveolar septa and reduced inflammatory cells infiltration

Discussion

Pulmonary fibrosis is a deadly and progressive lung disorder. Despite numerous studies, a few drugs are available to treat this disorder **(Puglisi *et al.*, 2016)**. The bleomycin-induced animal model of PF is widely used to investigate new anti-fibrotic compounds **(Serrano-Mollar *et al.*, 2003)**. Several agents with potent

antioxidant and anti-inflammatory activities exhibit protective effects against bleomycin-induced lung injury **(Liang *et al.,* 2011 & Mansouri *et al.,* 2019)**. Therefore, this study provided evidence that the anti-fibrotic treatment feature of GAE may offer a novel alternative therapy for the treatment of pulmonary fibrosis through enhances improvement of liver and kidney function and restoration of the impaired oxidative stress.

BLM cytotoxicity encloses oxidative stress as one of the mechanisms of the induced lung tissue injury **(Allawzi *et al.,* 2019)**, where BLM can bind Fe (II) forming a complex, which is subsequently oxidized to Fe (III) in presence of O_2, resulting in the reduction of oxygen to free radicals and production of reactive oxygen species (ROS), such as, O_2, hydroxyl radicals, and Fe (III). Then, this bleomycin complex binds to the DNA helix through a nucleophilic bond, resulting in DNA strand breaks. In addition, membrane lipid peroxidation and subsequent membrane damage occur. This suggests that GAE suppresses lipid peroxidation and membrane disruption, stabilizing myocyte membranes and decreasing leakiness in pulmonary fibrosis. Additionally, histological examination of the lung tissue in normal control animals revealed an intact, united cell membrane free of edema, inflammation, and inflammatory cell infiltration; in contrast, coagulative myonecrosis, inflammation, and inflammatory cell infiltration were revealed in the rats' lung fibrosis BLM. Conversely, rats administered GAE exhibited edema, reduced permeability of inflammatory cells, and condensed myonecrosis. GAE appears non-toxic to lung fibrosis when paired with hemodynamic and biochemical recovery as well as histological salvage, most likely because the endogenous antioxidant defense against BLM has been restored.

Ginkgo biloba extracts (GBE), exhibit substantial free radical scavenging activity, GBE has been observed to regulate the expression of antioxidant enzymes, enhance mitochondrial function, and reduce lipid peroxidation, there

was a significant increase in the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in various brain regions **(Wankhade *et al.,* 2025)** .

The fact that oxidative stress plays a crucial role in the pathological development of pulmonary fibrosis has been referred to **Daniil *et al.,* (2008)**. Generation of ROS and reactive nitrogen species has been reported to be implicated, at least, in part in the pathogenesis of fibrosis **(Bargagl *et al.,* 2009)**. Markers of oxidative stress have been identified in the lungs of pulmonary fibrosis patients and aberrant antioxidant activity exacerbated pulmonary fibrosis in animal models **(Chitra *et al.,* 2013)**. In association, overproduction of NO has been demonstrated to play a key role in induction of pulmonary fibrosis in animal models and humans **(Kalayarasan *et al.,* 2008)**.

The increased lung MDA level in the lung of BLM-treated rats, recorded in the present study, agrees with previously documented results, demonstrating that BLM causes its pulmonary injury via oxidative stress **(Ju *et al.,* 2022)**. At the same time, the antioxidant defense system was negatively affected by BLM intoxication, where the activity of TAC content in the lung, were found to be significantly decreased by BLM intoxication **(Shahabi *et al.,* 2022).**

In this case, we suggest that bleomycin causes intracellular formation of ROS that attack the cell membrane and organelles, which causes an increase in the MDA level, and that GAE prevents this excessive level of ROS which decreases therefore the rate of MDA detected **(Pini *et al.,* 2010)**.

Ginkgo biloba extract (GBE) treatment improved cell viability, inhibited ferroptosis, and ultimately alleviated chronic obstructive pulmonary disease (COPD). The present findings suggest that GBE alleviates the progression of COPD through the inhibitory effect of the ferroptosis process and that GBE may be an effective treatment option for COPD **(Anhui *et al.,* 2025)**

GBE contains many constituents, with flavonoids and terpenoids as the main active components, which can exert antioxidant, antiplatelet, and anti-inflammatory effects **(Liu *et al.,* 2021; Ji *et al.,* 202*3*)**. GBE has also been reported to alleviate the fibrosis of liver, lung, and kidney **(Iraz *et al.,* 2006; Al-Attar, 2012; Lu *et al.,* 2015; Wang *et al.,* 2016).**

Our biochemical results are confirmed by the histopathological examination which proved that BLM causes pulmonary fibrosis marked alveolar wall thickness, fibrosis, inflammatory cells, and lymphoid aggregates, also the lung interstitum is disturbed with areas of hemorrhage. These findings are supported by another previous study **(Tzakaria *et al.,* 2021 & Ju *et al.,* 2022)**. The pathological importance of oxidative stress in BLM has been highlighted by previous studies **(Kellner *et al.*, 2017)**. Reactive oxygen species (ROS) react with macromolecules, leading to higher rates of lipid peroxidation **(Kwiecien *et al.*, 2014)**.

Conclusion

In conclusion, our data demonstrate that *Ginkgo biloba* aqueous extract (GAE) as antioxidant can help the host body face the manifestations of BLM-induced lung fibrosis; it achieved success in alleviating oxidative stress, amelioration and retrieving the lung structure and function back close to its healthy state. Therefore, *Ginkgo biloba* aqueous extract co-treatment can protect against BLM-induced lung fibrosis.

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