ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Therapeutic potential of *Aronia melanocarpa* in the prevention of bleomycin-induced lung fibrosis in rats

Metin Bağcı[1], Taha Ulutan Kars[1]*, Hasan İbrahim Kozan[2], Seda Yılmaz[1], Sümeyye Uçar[3], Aslı Okan Oflamaz[4], Seher Yılmaz[5], Züleyha Doğanyiğit[4], and Abdulkadir Baştürk[1]

[1]Hematology Clinic, Konya City Hospital, Health Science University, Konya, Türkiye

[2]Department of Food Processing, Meram Vocational School, Necmettin Erbakan University, Konya, Türkiye

[3]Department of Anatomy, Faculty of Medicine, Erciyes University, Kayseri, Türkiye

[4]Department of Histology and Embryology, Faculty of Medicine, Bozok University, Yozgat, Türkiye

[5]Department of Anatomy, Faculty of Medicine, Bozok University, Yozgat, Türkiye

Abstract

Introduction: Pulmonary fibrosis is a progressive and life-threatening condition frequently associated with chemotherapeutic agents, such as bleomycin (BLE). *Aronia melanocarpa* extract (AME), a potent antioxidant derived from black chokeberry, has shown promising anti-inflammatory and anti-fibrotic effects in various pre-clinical models.

Objective: This study aims to evaluate the protective and therapeutic effects of AME in a rat model of BLE-induced pulmonary fibrosis.

Methods: A total of 60 rats were divided into six groups: control, fibrosis (BLE only), positive control (BLE + methylprednisolone), AME-only, AME + BLE (AME administered concurrently with BLE), and BLE + AME (AME administered after fibrosis induction). Lung tissues were analyzed histologically and biochemically for inflammation, fibrosis, and oxidative stress markers.

Results: AME administration significantly reduced alveolar wall thickening, hemorrhage, cellular infiltration, and collagen deposition. These effects were more pronounced in the AME + BLE group, indicating a potential prophylactic advantage. In addition, AME restored antioxidant enzyme levels and suppressed lipid peroxidation.

Conclusion: AME exhibits both preventive and therapeutic effects against BLE-induced lung injury. Its polyphenol-rich composition and antioxidative properties support its potential as a low-cost, low-toxicity candidate in pulmonary fibrosis management.

Keywords: *Aronia melanocarpa*; Bleomycin; Lung fibrosis; Oxidative stress; Antioxidant; Animal model

***Corresponding author:**
Taha Ulutan Kars
(tahaulutankars@gmail.com)

Citation: Bağcı M, Kars TU, Kozan HI, *et al*. Therapeutic potential of *Aronia melanocarpa* in the prevention of bleomycin-induced lung fibrosis in rats. *Eurasian J Med Oncol*. 2025;9(4):248-257. doi: 10.36922/EJMO025170148

Received: April 26, 2025

1st revised: July 19, 2025

2nd revised: August 3, 2025

Accepted: August 11, 2025

Published online: September 22, 2025

1. Introduction

Fibrosis is a global health issue leading to chronic organ failure through excessive extracellular matrix accumulation. It commonly affects organs, such as the lungs, liver, and kidneys.[1,2] Pulmonary fibrosis arises when tissue repair mechanisms are disrupted or

when damaging stimuli persist. Key contributors include proinflammatory cytokines, inflammatory cell migration, and myofibroblast activation.[3]

Bleomycin (BLE) is an antitumor antibiotic used in the treatment of germ cell tumors and Hodgkin lymphoma. However, it can cause life-threatening interstitial pulmonary fibrosis in up to 10% of patients.[4-6] Oxidative stress plays a central role in its pathophysiology, and antioxidants may offer therapeutic benefit.[5-7] The mechanism involves migration of inflammatory cells and release of proinflammatory mediators in the lungs.[8-11] BLE also induces fibroblast recruitment from bone marrow, and the fibroblast expresses type I collagen and chemokine receptors, such as C-X-C chemokine receptor type 4 and C-C chemokine receptor type 7.[12] Resistance to BLE-induced lung injury observed in athymic mice further highlights the role of inflammation.[8]

Although glucocorticoids are used to manage BLE-induced lung injury,[13] treatment responses vary. While some forms, such as hypersensitivity pneumonitis or organizing pneumonia, may respond well to corticosteroids, interstitial fibrosis often does not.[14] Therefore, antioxidant and anti-inflammatory agents are being explored as alternatives. Several herbal extracts have shown promise in this context.

Aronia melanocarpa extract (AME) is rich in bioactive polyphenols, including proanthocyanidins, anthocyanins, phenolic acids, and flavonols.[15,16] Major anthocyanins include cyanidin-3-glucoside and its derivatives,[17] whereas flavonols, such as quercetin and isorhamnetin derivatives, are also abundant.[18] Proanthocyanidins comprise (−)-epicatechin and (+)-catechin,[19] and phenolic acids, such as chlorogenic acid, are dominant.[20] AME has demonstrated antioxidant and anti-inflammatory properties and has been shown to prevent liver fibrosis in pre-clinical models.[21]

This study aims to evaluate the potential of AME in preventing and treating BLE-induced pulmonary fibrosis in rats.

2. Methods

The experimental design involved the utilization of two primary chemical agents: BLE (Blemicin, Koçak Farma, Türkiye), recognized for its fibrosis-inducing properties, and AME, chosen for its established antioxidant, anti-inflammatory, antibacterial, and antiproliferative effects. AME was the focus of the investigation, assessed for both its preventive and therapeutic roles against BLE-induced pulmonary fibrosis.

2.1. Preparation of *A. melanocarpa* extract

Fresh *Aronia* fruits were thoroughly washed and manually crushed using a fine-mesh strainer to separate seeds and skins. The resulting pomace was collected, and 50 g of pomace was blended with 500 mL of distilled water. The mixture was homogenized at 900 rpm for 2 min using a laboratory homogenizer (WiseTis HG–15D, Daihan Scientific, Korea), stirred for 1 h at room temperature, and filtered through filter paper (Whatman No. 1, Cytiva, USA). The filtrate was concentrated under vacuum using a rotary evaporator (Laborota 4001, Heidolph, Germany) and stored at 4°C in amber glass bottles to preserve the polyphenolic content and antioxidant stability.

To confirm the consistency of antioxidant composition across batches, the extract was evaluated for its total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging capacity using standardized methods. These analyses ensured the reproducibility of biochemical activity *in vivo*.

2.2. Animal model and ethical approval

Healthy male Sprague Dawley rats (n = 60, 180–200 g) were obtained from the Erciyes University Experimental Research Center. All experimental procedures complied with ethical standards and were approved by the Erciyes University Local Animal Ethics Committee (Protocol no: 23/119). Every effort was made to minimize animal suffering and to use the minimum number of animals required to ensure statistical validity. Animals were maintained under standard conditions with controlled temperature (24 ± 2°C), humidity (60 ± 5%), and a 12-h light/dark cycle. Rats had free access to standard pellet feed and tap water throughout the study.

2.3. Experimental design

The rats were randomly divided into six groups (n = 10 per group) as follows:

(i) Control: No treatment
(ii) Fibrosis group (BLE): Received a single intratracheal dose of BLE (5 mg/kg)
(iii) Positive control: Received BLE + methylprednisolone (4 mg/kg/day orally)
(iv) AME only: Received AME only (100 mg/kg/day orally)
(v) AME + BLE: AME was administered simultaneously with BLE from day 0 (preventive effect)
(vi) BLE + AME: AME was initiated nine days after BLE administration (therapeutic effect).

A. melanocarpa extract and methylprednisolone treatments were continued for 28 days via oral gavage. BLE was administered intratracheally under mild anesthesia (ketamine/xylazine) using a microsyringe. At the end of the study period, all animals were euthanized under deep

anesthesia, and lung tissues were collected for biochemical and histological analyses.

2.4. Antioxidant and phenolic analysis of *A. melanocarpa* extract

The antioxidant activity of AME was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[22] Absorbance was measured at 517 nm using a ultravialot–visible light spectrophotometer (8453 Diode Array Spectrophotometer, Agilent Technologies, USA), and results were expressed as percentage inhibition of DPPH radicals. The TPC was assessed using the Folin-Ciocalteu reagent method with results expressed in mg gallic acid equivalents (GAE)/100 mL at 750 nm.[23] TFC was evaluated using an aluminum chloride colorimetric method and expressed as mg catechin equivalents (CE)/100 mL at 510 nm. All samples were analyzed in triplicate to ensure reproducibility.[24]

2.5. Histological analysis

Following euthanasia, lung tissues were immediately fixed in 10% neutral-buffered formalin for 24 h. Following fixation, samples underwent dehydration through a graded ethanol series (70%, 80%, 95%, and 100%), clearing in xylene, and paraffin embedding. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E) for general histopathological assessment, and with Masson's trichrome for collagen detection.

Histological parameters, including alveolar wall thickening, inflammatory infiltration, and intra-alveolar hemorrhage, were scored semi-quantitatively, ranging from 0 (none) to 3 (severe).[25] Fibrosis extent and collagen deposition were further quantified using ImageJ software, based on color thresholding of trichrome-stained slides.[26] All slides were independently evaluated by two blinded histologists.

2.6. Biochemical analysis

Pulmonary tissue homogenates were prepared in phosphate-buffered saline (PBS) (PBS; pH 7.4) and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatants were collected for measurement of malondialdehyde (MDA), total oxidant status (TOS), and total antioxidant status (TAS). Commercial enzyme-linked immunosorbent assay kits (Sunredbio, China) were used, and absorbance was recorded at 450 nm using a microplate reader. MDA and TOS results were expressed as nmol/mL, whereas TAS was reported as U/mL. All values were normalized to protein content determined via the Bradford assay.

2.7. Statistical analysis

Data analysis was conducted using GraphPad Prism (Version 8, Dotmatics, United Kingdom). The Shapiro–Wilk test was used to assess normality. Normally distributed data were analyzed using one-way analysis of variance followed by Tukey's *post hoc* test. Non-normally distributed data were evaluated using the Kruskal–Wallis test followed by Dunn's multiple comparisons. Results were expressed as mean ± standard deviation, and $p<0.05$ was considered statistically significant.

3. Results

3.1. Antioxidant and phenolic properties of *A. melanocarpa* extract

The antioxidant potential of AME was confirmed through *in vitro* biochemical assays. The DPPH radical scavenging activity was found to be 75.96 ± 4.34%, indicating a potent capacity in free radical neutralization. TPC was measured at 106.56 ± 1.68 mg GAE/100 mL, whereas TFC was 14.63 ± 1.12 mg CE/100 mL. These values support the strong antioxidant profile of AME and provide a biochemical rationale for its potential anti-fibrotic effects.

3.2. Histological findings

Lung tissues stained with H&E showed marked histopathological alterations in response to BLE administration. The control and AME-only groups displayed normal alveolar architecture with no signs of bleeding, inflammatory cell infiltration, or alveolar wall thickening. In contrast, the BLE group showed extensive intra-alveolar hemorrhage, dense cellular infiltration, and significant alveolar septal thickening. The positive control group (BLE + methylprednisolone) also exhibited pronounced tissue damage, although with partial amelioration compared to BLE alone (Figure 1).

Among the treatment arms, the AME + BLE group demonstrated the most favorable histological profile, with notably reduced inflammatory infiltration and preserved alveolar spaces. The BLE + AME group, in which AME was introduced after fibrosis onset, showed moderate improvement but remained inferior to the co-administration strategy.

Histological injury scores (Table 1) supported these observations. The BLE group had significantly higher scores in all three parameters: bleeding (1.98 ± 0.23), cellular infiltration (1.5 ± 0.2), and alveolar wall thickening (1.88 ± 0.17). Compared to this group, the AME + BLE group had significantly lower values ($p<0.05$): bleeding (1.66 ± 0.22), infiltration (1.63 ± 0.35), and thickening (1.25 ± 0.28). Notably, only the AME + BLE group

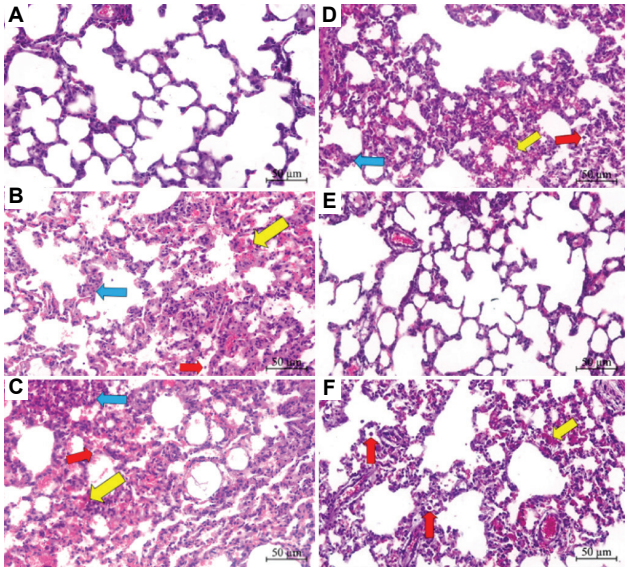

Figure 1. Hematoxylin and eosin staining images of the lung tissues of the experimental groups. (A) Control, (B) Positive control, (C) BLE, (D) AME, (E) BLE + AME, (F) AME + BLE. The yellow arrows indicate bleeding areas, the blue arrows indicate alveolar wall thickening, and the red arrows indicate cellular infiltration areas. Scale bar: 50 µm; magnification: ×20.
Abbreviations: AME: *Aronia melanocarpa* extract; BLE: Bleomycin.

Table 1. Histological injury scores observed in lung tissues

Groups	Bleeding	Cellular infiltration	Alveolar wall thickening
Control	0.30±0.08	0.15±0.10	0.35±0.13
Positive control	1.78±0.27[a]	1.11±0.24[a]	1.93±0.25[a]
BLE	1.98±0.23[a]	1.50±0.20[ab]	1.88±0.17[a]
AME	0.18±0.07[bc]	0.20±0.08[bc]	0.21±0.11[bc]
BLE+AME	2.16±0.13[abd]	2.10±0.17[abcd]	2.05±0.083[ad]
AME+BLE	1.66±0.22[ade]	1.63±0.35[abde]	1.25±0.28[abcde]

Notes: [a]$p<0.05$ compared to the control; [b]$p<0.05$ compared to the positive control; [c]$p<0.05$ compared to BLE; [d]$p<0.05$ compared to AME; [e]$p<0.05$ compared to BLE+AME; [f]$p<0.05$ compared to AME+BLE.
Abbreviations: AME: *Aronia melanocarpa* extract; BLE: Bleomycin.

showed a statistically significant reduction in alveolar wall thickening when compared directly to the BLE group, underscoring the superiority of early intervention.

3.3. Masson's trichrome staining and collagen deposition

Masson's trichrome staining revealed widespread collagen accumulation in the interstitial and perivascular areas of the BLE group, consistent with advanced fibrosis (Figure 2). All treatment groups exhibited higher collagen density than the control group ($p<0.05$), confirming the fibrogenic potential of BLE (Figure 3).

However, both AME-treated groups—whether administered simultaneously (AME + BLE) or after fibrosis onset (BLE + AME)—demonstrated significantly less collagen deposition than the BLE group ($p<0.05$). The extent of collagen reduction was comparable between the two AME protocols, suggesting that while both strategies confer benefit, early administration may additionally protect alveolar structures without significantly altering total collagen burden (Figure 3).

3.4. Biochemical markers of oxidative stress

As shown in Figure 4, BLE administration resulted in significant oxidative stress, evidenced by elevated MDA and TOS levels and reduced TAS in lung tissues. Compared to the control group, the BLE group reported a marked increase in MDA and TOS, and a corresponding decline in TAS ($p<0.05$), confirming oxidative injury.

A. melanocarpa extract treatment reversed these biochemical alterations. Both the AME + BLE and BLE + AME groups showed significantly lower MDA and TOS levels, along with increased TAS, approaching values comparable to the control group. These changes were statistically significant compared to the BLE group ($p<0.05$). While both regimens were effective, the AME + BLE group demonstrated slightly more favorable antioxidant parameters, although the difference was not statistically significant between the two AME-treated groups.

4. Discussion

In the present study, we demonstrated that AME exerts both preventive and therapeutic effects in a rat model of BLE-induced pulmonary fibrosis. Histopathological examination revealed that alveolar wall thickening, intra-alveolar hemorrhage, and inflammatory cell infiltration were significantly reduced in groups receiving AME. These findings were particularly prominent when AME was administered concurrently with BLE, indicating that early intervention may have a more substantial protective effect.

The pathophysiology of BLE-induced lung injury is closely associated with oxidative stress, inflammation, and progressive collagen deposition. Consistent with this, our BLE-only group reported markedly elevated levels of MDA, a lipid peroxidation product, and reduced levels of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione. These results align with previous studies that confirmed BLE-induced oxidative imbalance as a key driver of fibrogenesis.[15,17]

A. melanocarpa extract is well-known for its high content of polyphenols, including anthocyanins and procyanidins, which contribute to its potent antioxidant

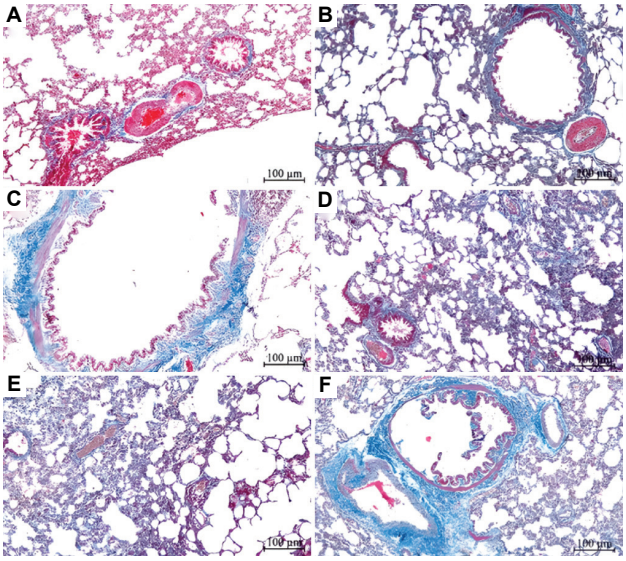

Figure 2. Masson's trichrome staining images of lung tissues in experimental groups. (A) Control, (B) Positive control, (C) BLE, (D) AME, (E) BLE + AME, (F) AME + BLE. Scale bar: 100 µm; magnification: ×10.
Abbreviations: AME: *Aronia melanocarpa* extract; BLE: Bleomycin.

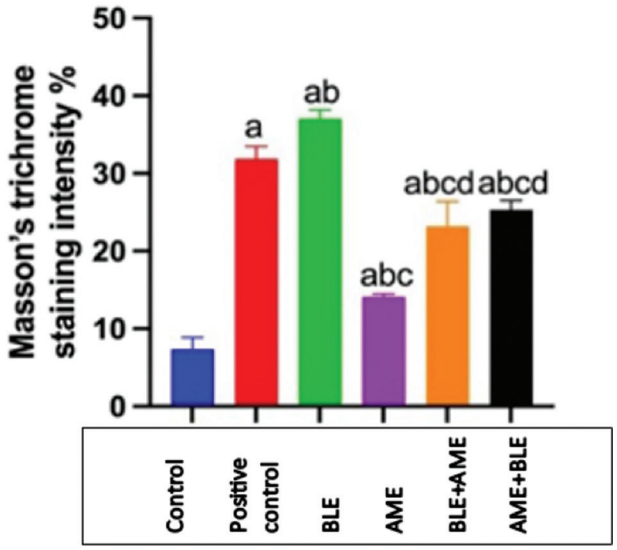

Figure 3. Masson's trichrome staining intensity of lung tissues in experimental groups. Notes: [a]$p<0.05$ compared to the control; [b]$p<0.05$ compared to the positive control; [c]$p<0.05$ compared to BLE; [d]$p<0.05$ compared to AME. Data shown in bar graphs are expressed as mean ± standard deviation.
Abbreviations: AME: *Aronia melanocarpa* extract; BLE: Bleomycin.

capacity.[15-20] Previous studies have shown that these compounds suppress proinflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, and inhibit fibrotic pathways, including transforming growth factor-β (TGF-β1)/small mothers against decapentaplegic (Smad) and mechanistic target of rapamycin signaling.[27-31] In our study, DPPH radical scavenging activity, TPC, and TFC

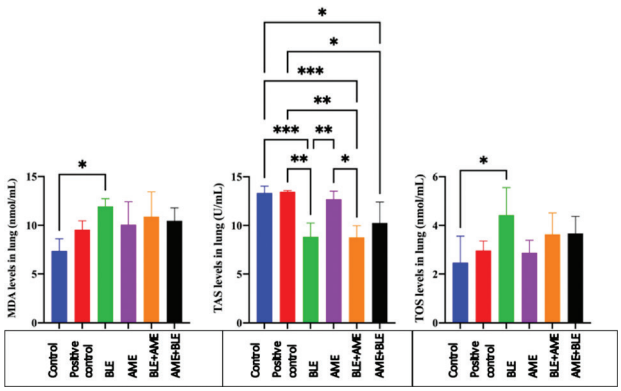

Figure 4. Results of MDA, TAS, and TOS in lung tissues in experimental groups. Notes: *$p<0.05$; **$p<0.005$; ***$p<0.001$. The Shapiro-Wilk test was applied to evaluate the normality and lognormality of the data. For normally distributed data, one-way analysis of variance was used for multiple comparisons between groups. The Kruskal-Wallis test was used for multiple comparisons between groups for non-normally distributed data. $p<0.05$ indicates a statistically significant difference.
Abbreviations: AME: *Aronia melanocarpa* extract; BLE: Bleomycin; MDA: Malondialdehyde; TAS: Total antioxidant status; TOS: Total oxidant status.

assays confirmed the strong antioxidant properties of AME. These biochemical attributes may underlie its protective effects against BLE-induced tissue damage. In groups where AME was administered concurrently with BLE, both histological injury scores and oxidative stress markers showed more favorable results than in the group receiving AME after BLE exposure. This suggests that AME's benefits are more pronounced when used preventively, potentially by mitigating the early oxidative burst and cytokine activation that precede fibrotic remodeling.

Recent studies have identified cyanidin-3-galactoside, a major anthocyanin in *A. melanocarpa*, as a key bioactive compound contributing to its antioxidant and anti-inflammatory properties. Notably, an experimental study in 2021 demonstrated that cyanidin-3-galactoside significantly attenuated particulate matter 10-induced pulmonary injury, suggesting its potential relevance across different models of lung injury, including BLE-induced fibrosis.[31] Although the present study did not include a targeted component analysis to isolate cyanidin-3-galactoside or other specific anthocyanins, the observed anti-fibrotic effects of AME may, at least in part, be attributed to these compounds. Future investigations incorporating detailed phytochemical profiling and mechanistic assays are warranted to clarify the individual contributions of cyanidin-3-galactoside and other polyphenols to the protective effects observed in this model.

Glucocorticoids have long been the primary treatment for BLE-induced lung injury,[32] but their effectiveness is

limited in cases with established fibrosis.[13,14] Numerous agents have been explored in pre-clinical studies—including sirolimus, gefitinib, melatonin, and metformin—with varying levels of success.[33-61] However, many of these compounds are synthetic and costly, and some carry adverse effect profiles. In contrast, AME offers a natural, economical, and potentially safer alternative.

The histopathological features observed in our model—alveolar wall thickening, hemorrhage, and leukocyte infiltration—are consistent with the early exudative and later fibrotic phases of BLE-induced lung injury. These phases mimic the progression observed in human idiopathic pulmonary fibrosis (IPF), particularly the UIP subtype.[62] Although our study did not include immunohistochemical or molecular pathway analyses, the observed histological improvements in AME-treated groups are suggestive of anti-fibrotic modulation, likely involving antioxidant and anti-inflammatory mechanisms.

The order of the AME administration also warrants discussion. The superiority of the BLE + AME group compared to the BLE-only group supports AME's therapeutic potential, whereas the even better outcomes in the AME + BLE group underscore its preventive efficacy. These findings may have translational relevance, suggesting that early dietary or pharmacological intervention with polyphenol-rich compounds, such as AME, could help attenuate or delay fibrotic progression.

One strength of our study is the inclusion of multiple evaluation parameters, including histological grading, antioxidant enzyme profiling, and detailed group comparisons. In addition, our study design, which incorporated both preventive and delayed treatment strategies, provides a comprehensive evaluation of AME's potential therapeutic window.

However, certain limitations should be noted. First, the study was restricted to a single model of BLE-induced pulmonary fibrosis, which may not fully represent the spectrum of fibrotic lung disorders. Second, no gene or protein expression analyses were performed to confirm the modulation of key signaling pathways, such as TGF-β/Smad or nuclear factor-light-chain-enhancer of activated B cells, nor were collagen gene expression studies included. Finally, potential synergistic effects of AME with existing anti-fibrotic agents remain unexplored. Further studies addressing these aspects will be essential to fully elucidate AME's mechanisms of action and therapeutic potential.

Our results indicate that AME significantly attenuates BLE-induced pulmonary injury and oxidative stress in rats. The protective effect is more prominent when AME is administered simultaneously with BLE, suggesting its potential as a preventive agent. Given its strong antioxidant capacity, affordability, and low toxicity profile, AME may serve as a valuable candidate for further pre-clinical and clinical research in the context of pulmonary fibrosis.

5. Conclusion

In this experimental study, the protective and therapeutic effects of AME against BLE-induced pulmonary fibrosis were evaluated using a well-established rat model. Both histopathological and biochemical assessments demonstrated that AME significantly attenuated the fibrotic response in lung tissue, particularly when administered concurrently with BLE. Reductions in alveolar wall thickening, inflammatory infiltration, and collagen deposition were notable, alongside improved oxidative stress parameters, including decreased MDA and TOS levels, as well as enhanced TAS.

These findings support the hypothesis that AME exerts its protective effects through a combination of antioxidant and anti-inflammatory mechanisms, consistent with its high polyphenolic and flavonoid content. The study design also revealed a clear difference between preventive and therapeutic strategies, with simultaneous AME administration yielding more pronounced histological protection than delayed treatment. This highlights the importance of timing in anti-fibrotic interventions, particularly in diseases such as IPF, where early treatment may alter disease trajectory.

Given that cyanidin-3-galactoside, a principal anthocyanin in *A. melanocarpa*, has been shown to mitigate airborne particle-induced lung injury, future studies should aim to delineate its specific role in the anti-fibrotic activity observed in our model.

Compared to existing anti-fibrotic agents, such as corticosteroids and pirfenidone, AME offers a natural, well-tolerated, and potentially cost-effective alternative. While corticosteroids remain part of the standard care in certain pulmonary inflammatory conditions, their long-term side effects limit their use. AME, in contrast, may represent a safer complementary or adjunctive option for fibrosis prevention, especially in drug-induced lung injury.

In conclusion, AME is a promising candidate in the prevention and amelioration of drug-induced pulmonary fibrosis. Its strong antioxidant and anti-inflammatory profile, coupled with minimal toxicity, justifies further exploration in both pre-clinical and clinical settings. Early intervention with AME may provide a novel approach to mitigating fibrotic lung injury, potentially improving patient outcomes in various pulmonary disorders.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no conflicts of interest, financial or otherwise, that could be perceived to influence the outcomes or interpretation of this study. No commercial or institutional affiliations exist that might constitute a potential conflict. All authors confirm that they have no relationships with any entities that might have an interest in the submitted work.

Author contributions

Conceptualization: Metin Bağcı, Taha Ulutan Kars
Formal analysis: Metin Bağcı, Seda Yılmaz
Investigation: Hasan İbrahim Kozan, Seher Yılmaz, Aslı Okan Oflamaz, Sümeyye Uçar, Züleyha Doğanyiğit
Methodology: Metin Bağcı, Taha Ulutan Kars, Seher Yılmaz, Abdulkadir Baştürk
Writing–original draft: Metin Bağcı, Taha Ulutan Kars
Writing–review & editing: Metin Bağcı, Taha Ulutan Kars

Ethics approval and consent to participate

All procedures were approved by the Erciyes University Local Animal Ethics Committee (Protocol No: 23/119), in accordance with national guidelines (Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes, Official Gazette No. 26220, Türkiye). All efforts were made to minimize animal suffering and to use the minimum number of animals required for statistical validity.

Consent for publication

Not applicable.

Availability of data

Data are available upon request from the corresponding author.

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