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Trigonelline mitigates bleomycin–induced idiopathic pulmonary fibrosis in mice

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ABSTRACT

Objective: To evaluate the effect of trigonelline on bleomycin-induced idiopathic pulmonary fibrosis (IPF) and to explore its underlying mechanisms using network pharmacology.

Methods: IPF was induced in C57BL/6 mice by a single intratracheal instillation of bleomycin (5 mg/kg). Trigonelline was administered at doses of 25, 50, and 100 mg/kg/day orally from the 2nd day post-bleomycin induction up to the 14th day. In IPF-induced mice, lung coefficient, immune cell infiltration in bronchoalveolar lavage fluid, and oxidative stress were measured. Histological alterations in lung tissues were also assessed. Moreover, network pharmacology approach was conducted to reveal molecular interactions of bleomycin and trigonelline with targets of IPF.

Results: Trigonelline treatment reduced bleomycin-induced oxidative stress and immune cell infiltration, and mitigated physiological changes in the lung tissues of mice. Moreover, trigonelline alleviated bleomycin-induced histological alterations in lung tissues. Network pharmacology analysis showed that bleomycin and trigonelline interacted with IPF targets, such as NFKB1, HDAC2, HIF1A, and TLR4.

Conclusions: The interaction of trigonelline with key IPF targets and its ameliorative effects on lung damage and oxidative stress highlight its potential in treating IPF. It may be considered an antifibrotic agent for further clinical development.

KEYWORDS: Trigonelline; Idiopathic pulmonary fibrosis; Antifibrotic; Bleomycin; Network pharmacology

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive

interstitial lung disease characterized by aberrant fibrotic remodeling of lung tissue leading to impaired lung function and ultimately respiratory failure[1,2]. The prevalence of IPF is approximately 200–400 cases per million for males and 130–200 cases per million for females[3]. The conventional treatment of IPF includes extensive use of corticosteroids and other symptomatic treatments, aiming to slow the disease progression, prevent infections, and enhance the patient's quality of life[4]. The addition of cytotoxic medications like cyclophosphamide and azathioprine to steroids is discouraged due to a lack of proven extension of life expectancy or improvement in lung function. Clinical studies have revealed the adverse effects of such addition of cytotoxic drugs to steroids in treating IPF[5]. With the availability of only two marketed antifibrotic drugs pirfenidone (PFD) and nintedanib, the quest for novel drugs to alleviate IPF

Significance

Idiopathic pulmonary fibrosis is a progressive and life-threatening condition, often worsened by treatments like bleomycin. Current therapies, including pirfenidone, aim to manage the disease but have limitations. In this study, trigonelline significantly reduced fibrosis and oxidative stress in mice with bleomycin-induced idiopathic pulmonary fibrosis. It may be used to develop as an antifibrotic agent for the treatment of idiopathic pulmonary fibrosis.

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continues.

Trigonelline (TGN), *N*-methyl nicotinic acid, is a naturally occurring alkaloid abundant in various plant sources like coffee beans, fenugreek seeds, and traditional Chinese medicines[6,7]. TGN possesses multiple biological activities including antioxidant[8–10] and anti-inflammatory[9,10], as well as inhibition of the epithelial to mesenchymal transition in renal tubular epithelial cells[11]. These properties make it an attractive candidate to be explored for its druggability as an antifibrotic agent in treating IPF.

In this study, we sought to unravel the therapeutic potential of TGN in the context of IPF using a comprehensive preclinical approach combined with systems biology investigation to elucidate its mode of action. Firstly, we employed a well-established murine model of IPF, induced by intratracheal bleomycin (BLM) administration, to investigate the effects of TGN treatment on the key pathological features of IPF which included measuring white blood cell count in bronchoalveolar fluid, oxidative stress markers in lung tissue, and histology, *etc.* Furthermore, to elucidate the molecular mechanisms of the effects of TGN, we employed network pharmacology to uncover potential target proteins and pathways that may contribute to its antifibrotic actions.

2. Materials and methods

2.1. In silico studies

2.1.1. Target prediction and gene set enrichment analysis

Target prediction and gene set enrichment analysis were conducted using the online servers Superpred, Digipred, and SwissTargetPrediction to identify probable protein targets of the selected compound, applying probability scores of ≥ 0.7 and ≥ 0.09 , respectively. The UniProt database (<https://www.uniprot.org/>) was then utilized to obtain the gene IDs of the predicted protein targets[12]. To analyze the protein-protein interaction, the set of proteins was submitted as input to the search tool for the Retrieval of Interacting Genes/Proteins database STRING v.11.0 (<https://string-db.org/>).

2.1.2. Network analysis

Based on the enrichment analysis, protein targets involving pathways of “IPF” are selected. The datasheet was built for the interaction between TGN, selected protein targets, and pathways, and the network between them was constructed using Cytoscape 3.6.1 software. The network was analyzed as a directed graph using the ‘Network Analyzer’ tool. The topological parameter ‘edge count’ was applied to quantify the degree of connectivity among nodes. The node size in the network was set from “low values to small sizes” and node color from “low values to blue color and high values to green color” based on edge

count topological parameter for both settings[13,14].

2.2. Chemicals and reagents

TGN was procured from Yarrow Chem Products Pvt. Ltd., Mumbai, India; PFD (Batch No. B22310E001) was procured from Umedica Laboratories R and D Centre, Mumbai, India; BLM (HDC22076) was gifted by Cipla Ltd. Verna, Goa. All the other chemicals used were of analytical grade. Other chemicals used for the biochemical estimations were purchased from AK Scientific, Hyderabad.

2.3. Animals

Healthy male C57BL/6 mice (6–8 weeks old, and weight 17–20 g) were obtained from the Department of Pharmacology, R.C. Patel Institute of Pharmaceutical Education Research Shirpur, Maharashtra, India. They were housed in clean propylene laboratory cages. The standard housing conditions included $(22 \pm 2)^\circ\text{C}$ temperature, 50%–60% relative humidity, and a 12 h/12 h of light/dark cycle. The mice were fed with standard pelletized feed supplied by Nutrivet Life Science, Pune, India, and water was provided *ad libitum*. The acclimatization duration was one week before the actual experimentation.

2.4. Study design

Briefly, animals were randomly assigned into six groups ($n=8$). Mice were anesthetized with urethane (1.25 g/kg i.p.)[15] by intraperitoneal injection. Then, 5 mg/kg of BLM in sterile 0.9% NaCl was intratracheally injected into the mice of group II–VI to induce pulmonary fibrosis[16]. The mice of the control group (Group I) were given the same volume of sterile saline instead of BLM solution. From day 2, following the intratracheal administration of BLM, the mice of respective treatment groups were treated orally with PFD (100 mg/kg/day) and TGN ($25, 50, \text{ and } 100\text{ mg/kg/day}$). On the 15th day, the lung tissues of all group animals were collected after being sacrificed for further analysis.

2.4.1. White blood cell counts in bronchoalveolar fluid

On the 15th day post BLM challenge, the mice were weighed and sacrificed using an overdose of urethane (1.2 g/kg i.p.)[15]. After tracheal cannulation, the lungs were lavaged with 1 mL of phosphate-buffered saline (PBS) three times, and the bronchoalveolar fluid was pooled[17]. The total cell counts were determined in the bronchoalveolar fluid using Horiba (Yumizen H500 cell analyzer).

2.4.2. Determination of body weight and lung coefficient

Subsequently, the lungs were harvested and rinsed with ice-cold PBS and blotted on filter paper before being weighed. The lung

tissues were then stored at -80°C until further analysis. Evaluation of lung injury severity was accomplished through the computation of the lung coefficient, determined by the ratio of lung weight (in mg) to body weight (in g).

2.4.3. Tissue preparation

The lung tissues were divided into 3 parts: one section of lung tissues that were isolated and immersed in phosphate buffer (pH 7.4) for biochemical estimation, one section in 10% KCl solution for lipid peroxidation estimation, and one section in formalin solution (10%) for histology. For biochemical estimation, the tissues were homogenized in separate tubes, and stored at -80°C for the measurement of enzymatic activity. It is known that antioxidant enzymatic activity is stable in hemolysates stored in such cold conditions[18].

2.4.4. Biochemical analysis

The oxidative stress-related markers like glutathione (GSH)[19], malondialdehyde (MDA), a thiobarbituric acid reactive substance as a tissue marker for lipid peroxidation[20], catalase activity[21], and superoxide dismutase (SOD)[22] were determined using the protocols published earlier[23].

2.4.5. Measurement of hydroxyproline (HYP) content

The quantification of collagen content was ascertained by measuring HYP levels in lung tissue homogenate, using HCl Acid in Ehrlich's Solution according to the modified HYP protocol[24]. Briefly, a lung tissue homogenate was prepared in distilled water (10 mg/mL). To the homogenate, 6 N HCl (1:10) was added and hydrolyzed at 110°C for 24 h. The resulting solution was brought to room temperature, and the pH was adjusted to 7.0-7.5 with 10 mol/L NaOH. The sample was then diluted to 1 mg/mL with distilled water. Meanwhile, HYP (0-200 $\mu\text{g/mL}$ in distilled water) was used as a standard to estimate the HYP content in the tissue. Next, 100 μL of each standard and sample was transferred into a 96-well plate, and 50 μL of chloramine T solution was added to each well. The plate was incubated for 25 min at room temperature. After incubation, 50 μL of Ehrlich's reagent was added to each well, and the plate was incubated for an additional 90 min at 60°C . The plate was then cooled to room temperature, and 150 μL of 2-propanol was added to each well to stop the reaction. The absorbance was read at 560 nm, and the HYP content was estimated using a standard calibration curve of the HYP standard.

2.4.6. Histopathological staining

The lower lobe of the right lung was fixed in 4% paraformaldehyde overnight, followed by dehydration in a series of graded ethanol solutions. Subsequently, the sample was embedded in paraffin and sectioned to a thickness of 5 μm . Staining procedures, including

hematoxylin and eosin (H & E) staining, as well as Masson's trichrome staining, were carried out on these paraffin-embedded sections[25]. Histopathological alterations and areas of positive staining in the lung tissues were examined. The microscopy was done using a Motic BA410E microscope (Leica DM 1000 LED, Leica Microsystems) and images were captured using the Motic Image Plus 2.0 software (magnification: $10\times$).

2.4.7. Ashcroft's scoring

A paraffin lung section, stained with H & E method, was meticulously examined by a blinded pathologist. Each consecutive field was individually evaluated for the extent of interstitial fibrosis and assigned a score ranging from 0 to 8 according to a predefined severity scale[26]. Upon comprehensive assessment of the entire section, the average score of all fields was determined as the fibrosis score for that section and reported to two decimal places.

2.5. Statistical analysis

The data was analyzed using GraphPad Prism software version 8.0 and presented as mean $\pm$ standard error mean (SEM). Group comparisons were conducted through one-way ANOVA, followed by *post hoc* comparisons using the Bonferroni test. A significance level of $P < 0.05$ was used to determine statistical significance.

2.6. Ethical statement

The experiment protocol was approved by the Institutional Animal Ethics Committee, approval number (IAEC/2022-23/03).

3. Results

3.1. In silico studies

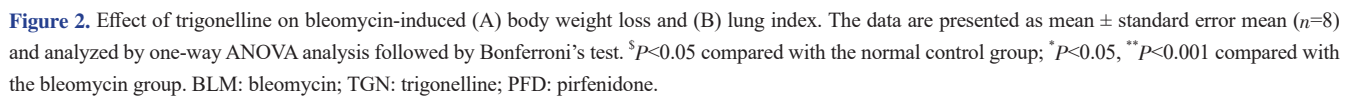
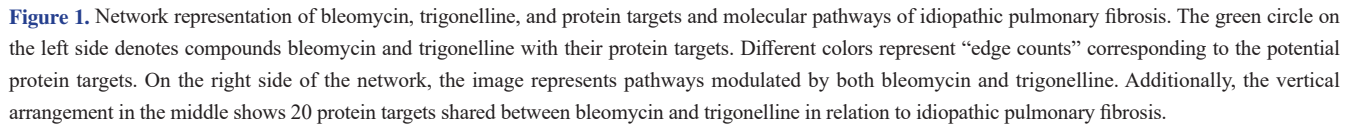
Retrieval of bioactive targets of TGN, BLM, and IPF was obtained using literature and various target prediction databases and results are summarized in Supplementary Tables 1, 2, and 3, respectively.

3.1.1. Targets related to BLM

Based on the literature and target prediction databases, BLM was found to target 69 protein molecules involved in the IPF (Supplementary Table 4).

3.1.2. Targets related to TGN

Based on the literature and target prediction databases, TGN was found to target 72 protein molecules that are involved in the IPF (Supplementary Table 4).



3.1.3. Targets (genes) enrichment and network analysis

BLM was found to hit 69 protein molecules and the pathway enrichment analysis showed these molecules were involved in 100 molecular pathways, of which 26 pathways were associated with IPF (Supplementary Table 5). The signaling pathways such as HIF-1, Notch, Toll-like receptor, ErbB, VEGF, RAS, and MAPK were found to score the least FDR value and higher gene count. Figure 1 represents the BLM-target-pathway network.

The enrichment analysis of 72 protein molecules targeted by TGN was found to modulate 50 molecular pathways. Among 50 pathways, 12 were associated with IPF (Supplementary Table 6). The signaling pathways such as HIF-1, Wnt, AMPK, TGF- β , mTOR, Relaxin, Toll-like receptor, and IL-17 were found to score the least FDR. As shown in Figure 1, the TGN protein targets and pathways network are depicted.

The common targets between BLM and TGN were found to be 20 (as shown in Figure 1) and the enrichment analysis demonstrated that these 20 protein molecules were involved in 7 common molecular pathways of IPF *viz.*, HIF-1, Notch, IL-17, Toll-like receptor, TNF, B cell receptor signaling pathways and pathways in cancer (Figure 1 and Supplementary Table 7).

3.2. In vivo studies

3.2.1. Effect of TGN on body weight and lung index

A significant decrease in body weight and increased lung coefficient were observed in the BLM-treated group compared to the control group ($P<0.05$). TGN administration significantly prevented the weight loss caused by BLM in mice with IPF in a dose-dependent manner as compared to the BLM group ($P<0.001$), indicating its

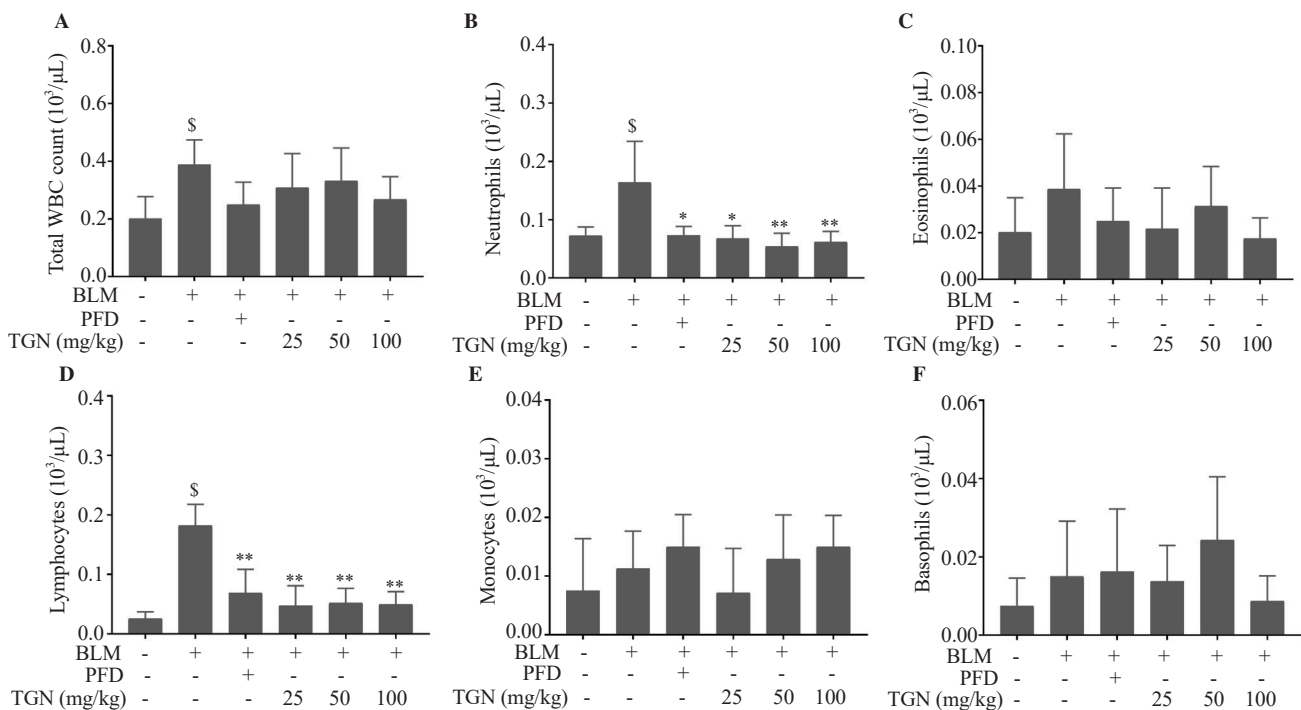


Figure 3. Effect of trigonelline on bleomycin-induced white blood cells in the bronchoalveolar fluid. The data are presented as mean $\pm$ standard error mean ($n=8$) and analyzed by one-way ANOVA analysis followed by Bonferroni's test. $^{\$}P<0.05$ compared with the normal control group; $^*P<0.05$, $^{**}P<0.001$ compared with the bleomycin group.

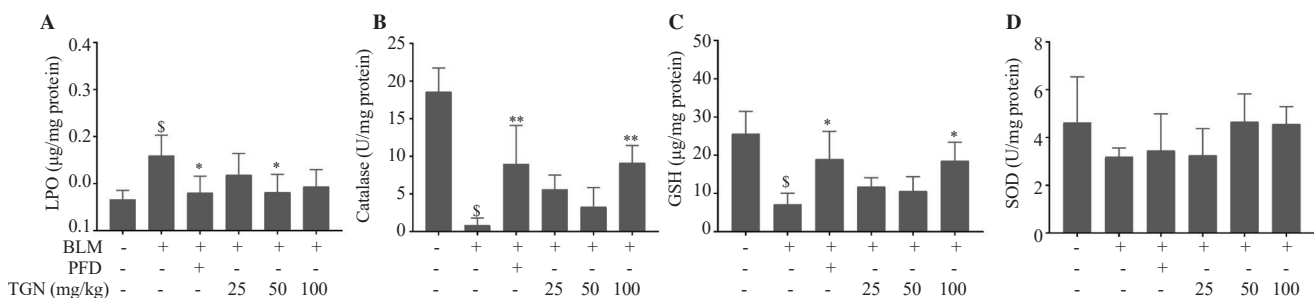


Figure 4. Effect of trigonelline on bleomycin-induced oxidative stress markers. The data are presented as mean $\pm$ standard error mean ($n=8$) and analyzed by one-way ANOVA analysis followed by Bonferroni's test. $^{\$}P<0.05$ compared with the normal control group; $^*P<0.05$, $^{**}P<0.001$ compared with the bleomycin group. LPO: lipid peroxidation; GSH: glutathione; SOD: superoxide dismutase.

protective effects on the overall health of mice. Treatment with TGN 50 and 100 mg/kg/p.o. showed a significant reduction in lung coefficient, reflecting a decrease in the stiffness of lung tissue and its fibrosis burden ($P<0.05$) (Figure 2), which was comparable to the effect of PFD.

3.2.2. Effect of TGN on immune cell profiles

In the bronchoalveolar fluid, BLM administration showed a significant increase in total WBC ($P<0.05$), neutrophil ($P<0.05$), and lymphocyte ($P<0.05$) counts. TGN treatment caused a significant decrease in lymphocytes ($P<0.001$) and neutrophils ($P<0.001$), but a decrease in WBC count was seen with no statistical significance, suggesting a shift towards a less inflammatory microenvironment. However, other immune cells including eosinophils, monocytes,

and basophils remained largely unaffected by both BLM and TGN (Figure 3).

3.2.3. Effect of TGN on oxidative stress markers

BLM treatment significantly increased lipid peroxidation ($P<0.05$) as indicated by increased MDA levels. TGN (50 mg/kg) administration significantly ($P<0.05$) reduced MDA levels, whereas, TGN (25 and 100 mg/kg) also showed an observable decrease in MDA levels (Figure 4A). BLM treatment also significantly decreased the catalase activity ($P<0.05$) and GSH level ($P<0.05$) compared to the normal control (Figure 4B and 4C), while treatment with BLM and TGN (100 mg/kg) showed increased catalase and GSH level ($P<0.05$), indicating its potential to mitigate oxidative stress in IPF. The SOD levels were also decreased by BLM and

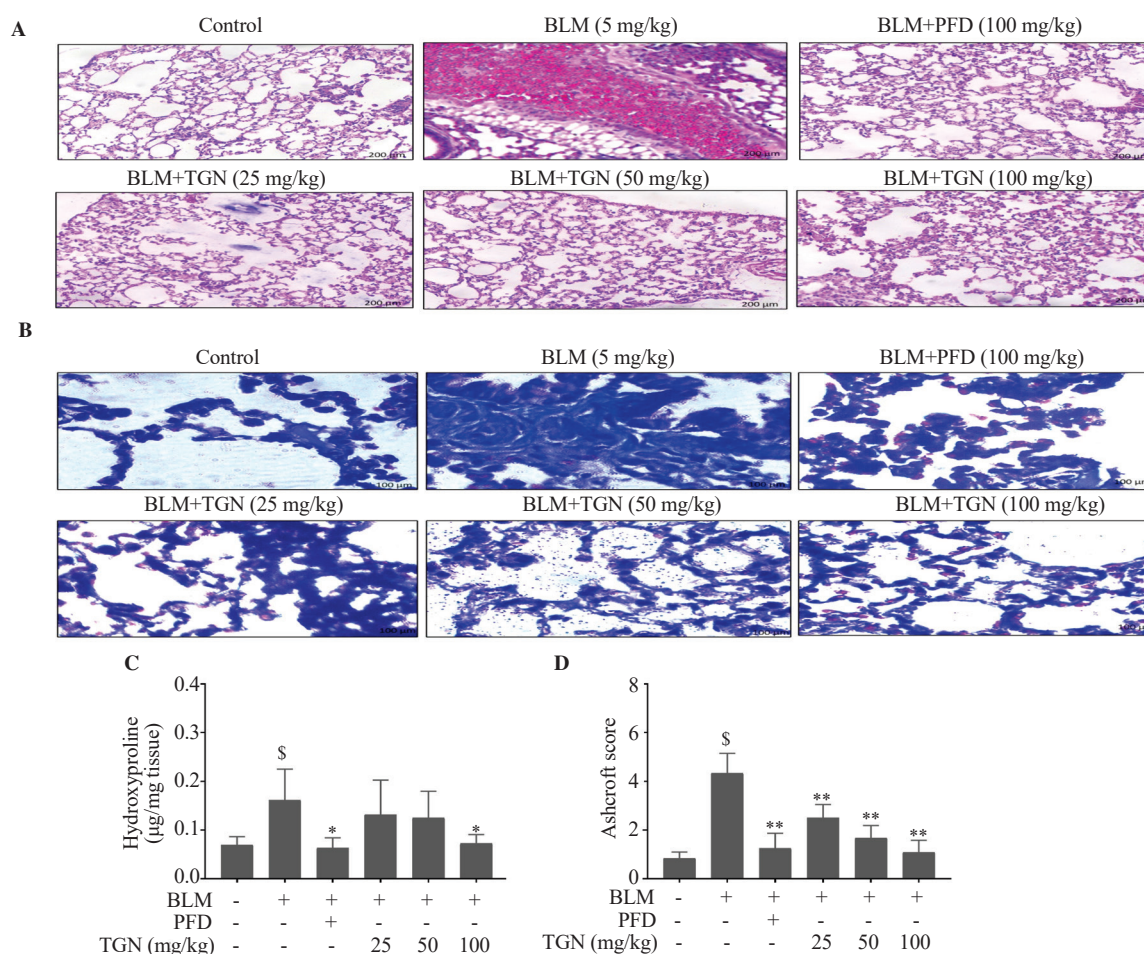


Figure 5. Histological analysis of lung tissue in different treatment groups stained with (A) hematoxylin and eosin and (B) Masson's trichrome at 10× magnification. The scale bar is 200 µm in (A) and 100 µm in (B). The control group shows normal lung architecture with minimal interstitial thickening and collagen deposition. The bleomycin (5 mg/kg) group displays severe fibrosis with dense fibrotic tissue, marked interstitial thickening, and substantial collagen accumulation. The bleomycin group treated with pirfenidone (100 mg/kg) exhibits reduced fibrosis with better-preserved alveolar structures and decreased collagen deposition. The bleomycin group treated with trigonelline (25 mg/kg) shows moderate fibrosis reduction and partial restoration of alveolar structures. The bleomycin group treated with trigonelline (50 mg/kg) demonstrates further fibrosis attenuation with better-aligned alveolar spaces and reduced collagen. The bleomycin group treated with trigonelline (100 mg/kg) reveals minimal fibrosis with near-normal alveolar architecture and minimal collagen deposition, closely resembling the control group. (C) Effect of trigonelline on hydroxyproline content and (D) grading of lung fibrosis by Ashcroft scoring. The data are presented as mean ± standard error mean ($n=8$) and analyzed by one-way ANOVA analysis followed by Bonferroni's test. [§] $P<0.05$ compared with the normal control group; ^{*} $P<0.05$, ^{**} $P<0.001$ compared with the bleomycin group.

increased by TGN, but the effect was found to be non-significant (Figure 4D).

3.2.4. Effect of TGN on HYP level

BLM treatment significantly increased ($P<0.05$) the collagen deposition in lung tissues as evidenced by increased HYP level. Treatment with TGN (100 mg/kg) significantly reduced HYP levels ($P<0.05$), showing its efficacy in mitigating fibrosis. TGN at 25 and 50 mg/kg did not show a significant reduction in HYP level (Figure 5C).

3.2.5. Histopathological results

Normal lung tissue with no fibrosis was observed in the control group, whereas, in the BLM group, extensive collagen deposition and significant disruption of lung architecture were observed. By comparison, TGN groups showed a dose-dependent reduction in collagen deposition (Figure 5A and 5B). The extent of fibrosis was evaluated by Ashcroft's scale-based grades as shown in Figure 5D. The control group displayed a low Ashcroft scale grade, indicating minimal or no fibrosis. The BLM group showed a significantly higher Ashcroft score compared to the control group ($P<0.05$), reflecting the extensive fibrosis induced by BLM treatment. However, all TGN-treated groups demonstrated significantly lower Ashcroft scores compared to the BLM group ($P<0.001$).

4. Discussion

IPF is a progressive and ultimately fatal lung ailment that carries substantial mortality rates. BLM-induced lung fibrosis mouse model, widely used for IPF research, mimics the features of IPF typified by lung architectural degradation and collagen accumulation in pulmonary tissues[27]. The increased lung coefficient, elevated HYP levels, and pronounced alterations in pulmonary histopathology and ultrastructure observed in the model groups collectively affirm the successful establishment of the IPF model. The present study delves into the potential therapeutic utility of TGN in the context of IPF. By employing a multidimensional approach, encompassing preclinical experimentation, network pharmacology, and comprehensive assessment of IPF-related parameters, we provided preclinical efficacy data on the TGN's potential as an antifibrotic agent.

In the present study, it was found that BLM targeted 69 protein molecules and in the enrichment analysis, these protein targets were involved in 100 pathways, out of which 26 pathways were related to IPF. These pathways include HIF-1, PI3K-Akt, JAK-STAT, FoxO, Apelin, Notch, B cell receptor, ErbB, Ras, GTPase, MAPK, VEGF, p53, and TNF signaling pathways.

In addition, supported by literature, we can state that BLM induces

IPF by activating key signaling pathways including HIF-1, PI3K-Akt, JAK-STAT, FoxO, Apelin, Notch, B cell receptor, ErbB, Ras, MAPK, VEGF, p53, and TNF, which collectively drive fibroblast activation, myofibroblast differentiation, chronic inflammation, and extracellular matrix (ECM) remodeling.

While TGN targeted 72 protein molecules to modulate 50 molecular pathways, among these pathways, the HIF-1, Wnt, AMPK, TGF-beta, mTOR, Relaxin, Toll-like receptor, and IL-17 signaling pathways were found to be associated with IPF. As earlier discussed, these targets have a potential role in the pathogenesis of IPF. We found that a total of 20 protein molecules and 7 molecular pathways are commonly targeted by BLM and TGN. Out of these, NFKB1, HDAC2, H1F1A, and TLR4 targets have higher edge counts indicating more prominent involvement in IPF.

The NFKB1 regulates inflammatory responses and promotes the production of pro-fibrotic cytokines, exacerbating fibrosis[28]. HDAC2 affects gene expression by modifying chromatin structure, contributing to fibroblast activation and ECM remodeling[29]. As earlier discussed, HIF1A is critical under hypoxic conditions, enhancing fibroblast proliferation and survival, and promoting collagen production through ECM accumulation. TLR4 mediates the innate immune response to lung injury by recognizing damage-associated molecular patterns, leading to chronic inflammation, and driving fibrotic processes[30]. Collectively, these targets modulated by BLM were reversed by TGN, providing an antifibrotic mechanism observed in the *in vivo* studies.

Our study revealed that TGN administration exerted a significant impact on multiple facets of IPF progression. The lung coefficient is a key indicator of lung tissue rigidity and fibrotic load[31]. A reduced lung coefficient in TGN-treated mice indicates its efficacy in reducing collagen deposition in the lungs during IPF. It is noteworthy that this effect of TGN was comparable to the effect of PFD, a marketed antifibrotic drug prescribed for treating IPF. The inflammatory cell infiltration in lung parenchyma, notably lymphocytes, has consistently emerged as a widespread histological observation in IPF[32,33]. The substantial presence of such lymphocytes is likely influenced by a range of cytokines and chemokines known to be involved in IPF[34–36]. Neutrophils contribute to immune system regulation through the production of cytokines and reactive oxygen species, which can induce inflammation. Prolonged inflammation, however, can result in tissue and organ damage. Furthermore, neutrophils are implicated in fibrotic lung conditions[37,38]. Neutrophil elastase, for instance, participates in the remodeling of the extracellular matrix[39] and influences the proliferation of lung fibroblasts as well as the differentiation of myofibroblasts[40]. In the context of IPF, neutrophils play a role in regulating the equilibrium between matrix metalloproteinases and tissue inhibitors of metalloproteinases[41].

The alteration of immune cell profiles within the bronchoalveolar fluid following TGN treatment indicates its potential to modulate the immune microenvironment in the lung. The reduction in neutrophil counts coupled with a decrease in lymphocyte cell count suggests a shift towards a less inflammatory milieu. This immune modulation, in conjunction with its observed impact on oxidative stress markers, highlights that TGN can mitigate IPF-associated inflammation and injury.

The alignment of our histopathological findings, such as fibrosis grading as per Ashcroft's scale and Masson's trichrome staining, with the molecular insights gleaned from docking studies enhances our understanding of the effects of TGN. This convergence substantiates its potential to target specific molecular pathways underlying fibrogenesis and supports the observed histological improvements. The effects of TGN on other parameters were found to be similar and comparable to that of PFD in ameliorating fibrotic progression. Moreover, the multifaceted nature of TGN's actions, including its immunomodulatory^[42], anti-inflammatory, and antioxidant properties^[10], positions it as a versatile candidate for treating IPF.

The limitations and future perspectives of the study include the study utilized a BLM-induced lung fibrosis mouse model to investigate IPF, which, while widely accepted, may not fully capture the complexity of human IPF. The pathophysiological features of IPF in humans can differ from those induced by BLM in mice, potentially affecting the generalizability of the results to human patients. The study's findings suggest that TGN offers therapeutic benefits in managing BLM-induced lung fibrosis. Given that BLM is a common chemotherapeutic agent with known side effects including fibrosis, exploring TGN's role in mitigating these effects could provide a valuable strategy for improving patient outcomes in BLM therapy in cancer patients. Future research should investigate whether TGN can be effectively integrated into BLM treatment regimens to reduce fibrosis and associated lung damage, potentially enhancing the overall safety and efficacy of BLM-included therapies. Considering TGN's observed antifibrotic effects and its impact on multiple signaling pathways, it is plausible to explore its potential for use in cancer therapy, particularly in combination with BLM. Fibrosis is a common side effect of cancer treatments, and addressing this issue could improve patient's quality of life and treatment tolerability. Evaluating TGN in cancer therapy could open new avenues for its application, especially in combination with BLM or other chemotherapeutic agents. Future research can be focused on evaluating TGN's potential through a multi-faceted approach. *In vitro* studies are needed to explore TGN's effects on cellular mechanisms and its interaction with BLM. *In vivo* research should extend to animal models to assess TGN's efficacy in managing BLM-induced fibrosis and improving cancer treatment outcomes.

Molecular studies should investigate how TGN modulates key signaling pathways relevant to fibrosis and cancer using advanced techniques like transcriptomics and proteomics. Finally, clinical trials are essential to confirm TGN's safety, efficacy, and therapeutic benefits in combination with BLM for both fibrosis management and cancer therapy.

In conclusion, this study highlights TGN's potential as a significant antifibrotic agent in the management of BLM-induced IPF. TGN administration effectively prevented weight loss and reduced lung stiffness in a dose-dependent manner. It also decreased lymphocyte and neutrophil counts in bronchoalveolar fluid, reduced lipid peroxidation, and increased antioxidant levels (GSH, SOD, and catalase) in BLM-induced IPF mice. Additionally, TGN treatment markedly reduced HYP levels and ameliorated lung histological alternations. Future research can be focused on exploring molecular mechanisms and conducting clinical trials to establish TGN's therapeutic potential.

Conflict of interest statement

The authors declare no conflict of interest.

Funding

This study received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

SG performed literature searches, conducted experiments, data acquisition, statistical analysis, and manuscript preparation. CG and GM assisted in study design and performed data and statistical analysis, and manuscript review. VSP and HMP contributed to network pharmacology, prepared figures, and manuscript revision and drafting. SK, BD, BB, and CNK assisted in preparing the first manuscript draft and executed a literature search. CRP designed the concept and study, literature search, supervised experimental studies, data, and statistical analysis, manuscript preparation, editing, and review. All authors have read, contributed to, and approved the final version of manuscript.

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