Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221-1691.377407

Impact Factor® 1.51

Modulatory effect of *D*-pinitol on bleomycin-induced pulmonary fibrosis in rats

Yu-Ling Duan, Zhi-Hua Wang, Yan-Xia Huo, Yang Zhang, Xiao-Ran Wu, Cui-Ke Gong, Lin-Lin Bai✉

Department of Respiration Medicine, Xingtai People's Hospital, Xingtai, Hebei Province, China

ABSTRACT

Objective: To assess the effect of *D*-pinitol on pulmonary fibrosis induced by bleomycin.

Methods: Sprague-Dawley rats received intratracheal bleomycin (6 IU/kg) to induce pulmonary fibrosis, followed by administration of either *D*-pinitol (5, 10, or 20 mg/kg) or vehicle or methylprednisolone (10 mg/kg) over 28 days after bleomycin administration. Lung function, biochemical parameters, serum biochemistry, mRNA expressions, and histological features were observed.

Results: *D*-pinitol at 10 and 20 mg/kg significantly ($P<0.05$) attenuated bleomycin-induced bronchoalveolar lavage fluid, decreased myeloperoxidase, nitric oxide, malondialdehyde levels, and increased glutathione and superoxide dismutase level. *D*-pinitol also improved lung function (enhanced pause, frequency of breathing, expired volume, and tidal volume). Besides, *D*-pinitol significantly ($P<0.05$) upregulated *Nrf2* and downregulated mRNA expressions of *TGF-β*, *collagen-1*, and *Smad-3*. Furthermore, considerably less inflammation (peribronchial, perivascular, and total), Ashcroft, and interstitial fibrosis scores were observed in the *D*-pinitol group.

Conclusions: *D*-pinitol exerts its effect against bleomycin-induced pulmonary fibrosis *via* antioxidative and anti-fibrotic pathways.

KEYWORDS: Antioxidant; Bleomycin; Collagen-1; *D*-pinitol; Pulmonary fibrosis; Smad-3; TGF-β

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a parenchymal respiratory illness characterized by fibrosis and inflammation in the lung. It is an aggressive, chronic disease associated with an unceasing decrease in lung function, high lethality, and respiratory failure[1]. It has been estimated that around 0.09-4.51 per 100 000 population suffered from IPF worldwide, with the region-wise incidence being 0.57-4.51 in Asia-Pacific, 2.40-2.98 in North America, and 2.40-2.98 in Europe[1]. The increased prevalence is associated with the increased economic burden with an estimated annual cost of $20 000 (United States Dollar in 2016) for a patient with IPF in North America, which is 2-3 times the national expenditure on healthcare[2].

Interstitial pneumonia is characterized by myofibroblasts and fibroblast clusters, extracellular matrix and collagen accumulation, and heterogenous fibrosis, which result in a distorted lung architecture and possible honeycombing of lungs[3]. The specific pathogenesis of IPF is not yet completely evaluated, current hypotheses point toward an abnormal repairing process in response to various environmental and host factors[4]. Various other factors, including genetics, are important in developing and maintaining IPF. They lead to modification in the epithelium, making it more susceptible to injuries due to environmental factors[5]. The damage to the epithelium disrupts the capillary barrier of alveoli, thereby causing the proteins (such as fibrin) to leak into its spaces, abnormal vascular remodeling, and activation of the coagulation cascade[1,5].

Significance

Bleomycin is a chemotherapeutic agent, and its long-term administration causes idiopathic pulmonary fibrosis (IPF). The current work evaluated the effect of *D*-pinitol, a cyclic polyol, against bleomycin-induced IPF in experimental rats. It could exert its effect *via* antioxidant and anti-fibrotic pathways, which will deliver valuable information to researchers as a possible alternative for IPF treatment.

✉To whom correspondence may be addressed. E-mail: bll160950@outlook.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2023 *Asian Pacific Journal of Tropical Biomedicine* Produced by Wolters Kluwer-Medknow.

How to cite this article: Duan YL, Wang ZH, Huo YX, Zhang Y, Wu XR, Gong CK, et al. Modulatory effect of *D*-pinitol on bleomycin-induced pulmonary fibrosis in rats. Asian Pac J Trop Biomed 2023; 13(5): 205-213.

Article history: Received 19 March 2023; Revision 10 April 2023; Accepted 17 May 2023; Available online 29 May 2023

Furthermore, a rapid increase in free radicals occurs, which further causes the upregulation of transforming growth factor-β (TGF-β)[6].

The treatment of IPF mainly comprises immunosuppressant like azathioprine and prednisolone with the addition of *N*-acetylcysteine[7]. However, this therapy was documented to be linked with a higher risk of hospitalization and death[7]. As a result of these findings, new therapeutics were developed using nintedanib and pirfenidone, which have been demonstrated to ameliorate elevated forced vital capacity and halt further development of IPF in both mild and severe cases[8]. However, several adverse effects are associated with using both nintedanib and pirfenidone, including gastrointestinal adverse effects, photosensitivity rash, and increased liver enzyme levels, some of which led to therapy discontinuation[9]. In several preclinical studies, alternative medicines such as traditional Chinese medicine, Korean herbal medicine, and ayurvedic medicine are quite effective against IPF[10]. A clinical trial on IPF patients using traditional Chinese medicine showed promising results in reduced acute exacerbations, relieved IPF symptoms, and delayed pulmonary deterioration[11]. Because these medications have few to no noticeable side effects, they are good candidates as therapeutic agents for IPF[11].

Cyclitol, a cyclic polyol, is a group of naturally occurring substances in many plants, and their byproducts are largely employed as traditional medicines to combat numerous diseases such as diabetes, cancer, *etc*[12]. One of the cyclic polyols, Inositol, has been reported to alleviate lipopolysaccharide-induced IPF and acute respiratory distress syndrome by regulating the signaling of hypoxia-inducible factor 1-alpha[13]. *Sutherlandia frutescens* is a tropical plant native to regions in southern Africa and has been reported for a wide range of health benefits. *D*-pinitol, a cyclic polyol is found abundantly in the leaves of *Sutherlandia frutescens*, a tropical plant in southern Africa and the Asian region. It is used as a traditional medicine due to its anti-inflammatory, antioxidant, and immunomodulatory activity[14]. *D*-pinitol has previously demonstrated anti-fibrotic activity against renal tubulointerstitial fibrosis by decreasing oxidative stress *via* activation of nuclear factor E_2-related factor 2 (Nrf2)[15]. Furthermore, *D*-pinitol has been shown to have a potential antitumor agent against human lung cancer cells[16]. Therefore, the current study was conducted to evaluate the potential of this cyclic polyol on IPF.

2. Materials and methods

2.1. Animals

Male rats of strain Sprague-Dawley with weights ranging from 180 to 220 g were obtained from the Xingtai People's Hospital animal house. They were maintained at (24 ± 1) ℃ under a relative humidity of 45%-55% with a 12:12 h dark/light cycle throughout the experimental period. Rats were provided with standard pellet chow and filtered water.

2.2. Ethical statement

The Institutional Animal Ethics Committee of Xingtai People's Hospital approved the protocol of the experiment [approval no. LL2022 (BD15)] and all the experiments were performed as per the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.3. IPF model establishment

Induction of IPF was performed according to the method reported before[17]. Bleomycin (BLM) hydrochloride (Biochem Pharmaceutical Industries Ltd., India) at a dose of 6 IU/kg (in 0.9% NaCl) was administered to overnight fasted rats. Rats were randomly assigned to the following groups (n=16):

BLM control group received distilled water (10 mg/kg); MP10 group received methylprednisolone (MP, 10 mg/kg); P5 group received *D*-pinitol 5 mg/kg (purity 95%, Sigma Chemical Co., St Louis, MO, USA); P10 group received *D*-pinitol 10 mg/kg; P20 group, received *D*-pinitol 20 mg/kg.

The following groups did not receive BLM:

Normal group received distilled water (10 mg/kg); sham control group received intratracheal administration of saline followed by treatment of distilled water (10 mg/kg); *per se* group received *D*-pinitol (20 mg/kg).

All treatments lasted for 28 d. The doses of *D*-pinitol (5 or 10 or 20 mg/kg)[18] and methylprednisolone (10 mg/kg)[6] were selected based on previous reports. *D*-pinitol and methylprednisolone were prepared daily as a fresh solution in distilled water.

2.4. Lung function and peripheral blood oxygen measurements

The whole-body flow-through plethysmography (EMKA Technologies, France) was used to evaluate the respiratory dynamics, whereas the peripheral pulse Ox sensor (ChoiceMMed, V1.0CF3, MD300CF3, China) was used to determine *in-vivo* peripheral blood oxygen content[17].

2.5. Serum and bronchoalveolar lavage fluid (BALF) biochemical analysis

At the end of the experiment (on the 28th day), rats were anesthetized with ether for blood withdrawal by using the retro-orbital puncture method to collect the blood specimen, followed by separation of serum, which was further used for the analysis of lactate dehydrogenase and alkaline phosphatase using reagent assay kits procured from Accurex Biomedical Pvt. Ltd., Mumbai,

India. Simultaneously, BALF was also collected to assess the total cell counts and levels of superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), hydroxyproline, myeloperoxidase (MPO), and total protein as per the methods reported previously[19,20].

2.6. Lung biochemical analysis

Rats were sacrificed by the cervical dislocation method, and lungs specimens were collected to determine the SOD, GSH, MDA, NO, and total protein levels.

2.7. Reverse transcriptase (RT)–PCR

Another specimen of lungs was maintained at –70 ℃ to determine *collagen-1*, *Nrf2*, *Smad-3*, and *TGF*-β mRNA expressions using total RNA Extraction kit and One-step reverse transcription polymerase chain reaction kit (MP Biomedicals India Private Limited, India) as per previously described method[21,22]. The gene expression (Supplementary Table 1) was assessed semi-quantitatively by generating densitometry data for band intensities in different sets of experiments and analyzing the gel images using the software Image J program (Version 1.33, USA). The band intensities were compared with constitutively expressed *β-actin*.

2.8. Histological analysis

The remaining specimen of lung tissue was subjected to histopathological analysis using picro-sirius red and hematoxylin and eosin stains as described previously[17,23]. Sections were examined under a light microscope (Olympus DP71, DP-BSW Ver.03.03, Olympus Medical Systems India Private Limited, India) to obtain a general impression of the histopathology features of the specimen and infiltration of cells in epithelium and sub-epithelium. The intensity of histological aberrations in the nasal and spleen tissue was graded as Grade 0 (not present or very slight); Grade 1 (mild); Grade 2 (moderate); and Grade 3 (severe) as described in the literature[6].

2.9. Statistical analysis

Data analysis was conducted using GraphPad Prism 5.0 software (GraphPad, San Diego, CA) and reported as mean ± standard error mean (SEM) and a value of $P<0.05$ was considered statistically significant. During one way ANOVA, a *post hoc* analysis was conducted using Tukey's multiple ranges (for parametric tests) and the Kruskal-Wallis test (non-parametric tests).

3. Results

3.1. Effect on body weight and lung index

There was no significant difference in body weight or lung index between the *per se* group and the sham or the normal groups ($P>0.05$). BLM significantly ($P<0.05$) reduced body weight and increased relative lung weight compared with the sham and normal groups. *D*-pinitol at 10 and 20 mg/kg treatment significantly ($P<0.05$) ameliorated alteration in body weight and relative lung weight compared with the BLM control group. *D*-pinitol at 5 mg/kg did not demonstrate any significant change (Table 1).

3.2. Effect on percent oxygen saturation

There was no significant difference in the percentage of oxygen saturation between the *per se* group and the sham or the normal groups ($P>0.05$). BLM significantly ($P<0.05$) decreased the percentage of oxygen saturation compared to the sham and normal groups. Treatment with *D*-pinitol at 10 and 20 mg/kg significantly increased the percentage of oxygen saturation ($P<0.05$). *D*-pinitol at 5 mg/kg did not demonstrate any significant change (Table 1).

3.3. Effect on levels of serum alkaline phosphatase and lactate dehydrogenase

There was no significant difference in alkaline phosphatase or lactate dehydrogenase between the *per se* group and the sham or the normal groups ($P>0.05$). Serum alkaline phosphatase and lactate

Table 1. Effect of *D*-pinitol on body weight, relative lung weight, pulse oximetry, serum ALP, and LDH (n=6).

Groups	Body weight (g)	Relative lung weight	O_2 saturation	Serum ALP (U/L)	Serum LDH (U/L)
Normal	238.80 ± 2.61	0.0083 ± 0.0002	96.00 ± 0.37	53.75 ± 1.81	952.20 ± 34.77
Sham	237.20 ± 2.33	0.0076 ± 0.0001	96.17 ± 0.17	52.95 ± 1.35	1015.00 ± 34.88
BLM control	159.20 ± 2.64$^{\#,\&}$	0.0399 ± 0.0009$^{\#,\&}$	87.33 ± 0.21$^{\#,\&}$	169.10 ± 2.04$^{\#,\&}$	2045.00 ± 36.26$^{\#,\&}$
BLM + MP10	230.00 ± 3.30*	0.0106 ± 0.0004*	93.67 ± 0.33*	63.62 ± 1.37*	1052.00 ± 28.71*
BLM + P5	170.00 ± 2.68$^{\#,\&}$	0.0366 ± 0.0013$^{\#,\&}$	89.33 ± 0.21$^{\#,\&}$	152.00 ± 2.49$^{\#,\&}$	1926.00 ± 72.20$^{\#,\&}$
BLM + P10	181.20 ± 1.89*	0.0222 ± 0.0006*	91.50 ± 0.22*	109.10 ± 1.48*	1652.00 ± 49.52*
BLM + P20	220.70 ± 1.94*	0.0134 ± 0.0003*	93.67 ± 0.33*	79.74 ± 1.86*	1343.00 ± 17.75*
Per se	238.70 ± 2.86	0.0073 ± 0.0001	94.50 ± 0.22	54.93 ± 1.04	1056.00 ± 35.35

One way ANOVA (*post-hoc* test: Tukey's multiple ranges) was used for data analysis. $^{\#}P<0.05$ compared with normal; $^{\&}P<0.05$ compared with sham; $^{*}P<0.05$ compared with the BLM control group. BLM: bleomycin; MP: methylprednisolone; P: *D*-pinitol; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.

dehydrogenase were significantly (P<0.05) increased in the BLM control group. *D*-pinitol at 10 and 20 mg/kg significantly reduced serum alkaline phosphatase and lactate dehydrogenase (P<0.05). *D*-pinitol at 5 mg/kg did not demonstrate any significant change (Table 1).

3.4. Effect on lung function

There was no significant difference in lung function between the *per se* group and the sham or the normal groups (P>0.05). Significantly higher peak expiratory flow, frequency of breathing, enhanced pause, and significantly lower peak inspiratory flow, expired volume, and tidal volume were seen in the BLM control group compared with the sham and normal groups (P<0.05). *D*-pinitol at 10 and 20 mg/kg significantly decreased peak expiratory flow, breathing frequency, enhanced pause, and significantly increased peak inspiratory flow, expired volume, and tidal volume (P<0.05). *D*-pinitol at 5 mg/kg did not demonstrate any significant change in lung function (Table 2).

3.5. Effect on BALF differential cell count

There was no significant difference in cell count between the *per se* group and the sham or the normal groups (P>0.05). The BLM control group showed a significant (P<0.05) increase in cell counts (total lymphocytes, neutrophils, monocytes, and eosinophils) in the BALF compared with the sham and normal groups. *D*-pinitol at 10 and 20 mg/kg reduced cell counts in BALF significantly (P<0.05). *D*-pinitol at 5 mg/kg did not demonstrate any significant change in differential cell count (Table 3).

3.6. Effect on hydroxyproline and MPO levels in the BALF and lung

There was no significant difference in hydroxyproline or MPO between the *per se* group and the sham or the normal groups (P>0.05). Significantly higher hydroxyproline and MPO levels were observed in the BALF and lung of the BLM control group (P<0.05). *D*-pinitol at 10 and 20 mg/kg significantly decreased levels of hydroxyproline and MPO in the BALF and lung (P<0.05). *D*-pinitol at 5 mg/kg did not demonstrate any significant change in levels of hydroxyproline and MPO in the BALF and lung (Figure 1A and 1B).

3.7. Effect on oxido–nitrosative stress in the BALF and lung

There was no significant difference in antioxidant indices between the *per se* group and the sham or the normal groups (P>0.05). The BLM control group had significantly (P<0.05) higher NO and MDA levels and significantly (P<0.05) lower SOD and GSH levels in the BALF and lung compared with the sham and normal groups. *D*-pinitol at 10 and 20 mg/kg significantly decreased MDA and NO, and significantly increased GSH and SOD (P<0.05). *D*-pinitol at 5 mg/kg did not demonstrate any significant change in these levels in BALF and lung (Figure 1C-1F).

Table 2. Effect of *D*-pinitol on lung function test (n=6).

Groups	AUC [PEF (m/s)]	AUC [PIF (m/s)]	AUC [EV (m)]	AUC [TV (m)]	AUC [f (bpm)]	AUC (P_{enh})
Normal	287.30 ± 5.47	556.40 ± 7.38	30.24 ± 1.24	38.68 ± 0.70	4712.00 ± 156.10	25.63 ± 1.17
Sham	290.80 ± 12.02	562.60 ± 5.52	29.95 ± 0.80	38.27 ± 0.98	4421.00 ± 115.90	25.38 ± 1.24
BLM control	626.50 ± 15.55$^{\#,\&}$	184.00 ± 6.19$^{\#,\&}$	14.36 ± 0.85$^{\#,\&}$	23.44 ± 0.84$^{\#,\&}$	8023.00 ± 89.61$^{\#,\&}$	42.06 ± 1.38$^{\#,\&}$
BLM + MP10	382.70 ± 29.03*	514.40 ± 13.77*	24.09 ± 0.80*	36.30 ± 0.98*	5114.00 ± 188.60*	31.30 ± 0.98*
BLM + P5	578.00 ± 15.82$^{\#,\&}$	197.70 ± 7.04$^{\#,\&}$	14.83 ± 1.31$^{\#,\&}$	25.69 ± 1.47$^{\#,\&}$	7775.00 ± 167.50$^{\#,\&}$	41.65 ± 0.90$^{\#,\&}$
BLM + P10	509.00 ± 26.08*	353.20 ± 8.64*	22.34 ± 0.52*	27.45 ± 0.64*	6355.00 ± 119.90	36.32 ± 1.35*
BLM + P20	401.00 ± 20.95*	474.70 ± 11.45*	23.80 ± 1.07*	31.99 ± 0.57*	5506.00 ± 77.88*	27.86 ± 1.18*
Per se	312.90 ± 20.53	556.30 ± 7.68	32.30 ± 0.64	35.07 ± 1.20	5510.00 ± 59.99	24.92 ± 1.73

One way ANOVA (*post–hoc* test: Tukey's multiple ranges) was used for data analysis. $^{\#}P$<0.05 compared with normal; $^{\&}P$<0.05 compared with sham; $^{*}P$<0.05 compared with the BLM control group. PEF: peak expiratory flow; PIF: peak inspiratory flow; EV: expired volume; TV: tidal volume; f: frequency of breathing; P_{enh}: enhanced pause.

Table 3. Effect of *D*-pinitol on BALF differential cell count (n=6).

Groups	Total cell count (×10^6/mL)	L (×10^6/mL)	N (×10^6/mL)	M (×10^6/mL)	E (×10^6/mL)
Normal	12.67 ± 0.33	5.17 ± 0.48	2.83 ± 0.31	1.67 ± 0.33	3.00 ± 0.37
Sham	14.67 ± 0.61	5.17 ± 0.48	3.00 ± 0.37	3.00 ± 0.37	3.50 ± 0.43
BLM control	79.83 ± 1.30$^{\#,\&}$	19.83 ± 0.87$^{\#,\&}$	20.50 ± 0.76$^{\#,\&}$	14.00 ± 0.37$^{\#,\&}$	25.50 ± 0.43$^{\#,\&}$
BLM + MP10	18.50 ± 0.67*	6.67 ± 0.80*	6.33 ± 0.49*	2.67 ± 0.33*	2.83 ± 0.31*
BLM + P5	74.17 ± 0.87$^{\#,\&}$	18.50 ± 0.56$^{\#,\&}$	18.83 ± 0.48$^{\#,\&}$	13.83 ± 0.48$^{\#,\&}$	23.00 ± 0.73$^{\#,\&}$
BLM + P10	45.33 ± 1.26*	12.83 ± 0.60*	12.50 ± 0.43*	7.17 ± 0.48*	12.83 ± 0.48*
BLM + P20	23.00 ± 1.03*	9.17 ± 0.40*	6.50 ± 0.43*	3.50 ± 0.43*	3.83 ± 0.48*
Per se	16.00 ± 1.75	5.83 ± 0.60	3.83 ± 0.48	2.83 ± 0.48	3.50 ± 0.43

One way ANOVA (*post–hoc* test: Tukey's multiple ranges) was used for data analysis. $^{\#}P$<0.05 compared with normal; $^{\&}P$<0.05 compared with sham; $^{*}P$<0.05 compared with the BLM control group. BALF: bronchoalveolar lavage fluid; L: lymphocytes; N: neutrophils; M: monocytes; E: eosinophils.

3.8. Effect on Nrf2, collagen-1, Smad-3, and TGF-β mRNA expression in the lung

There was no significant difference between the *per se* group and the sham or the normal groups (P>0.05). Significantly (P<0.05) downregulated *Nrf2* mRNA and upregulated *collagen-1*, *Smad-3*, and *TGF-β* mRNA expressions were seen in the lungs of the BLM control group compared with sham and normal groups. *D*-pinitol at 10 and 20 mg/kg significantly upregulated the *Nrf2* mRNA and downregulated *collagen-1*, *Smad-3*, and *TGF-β* mRNA expression (P<0.05). *D*-pinitol at 5 mg/kg did not show any significant change (Figure 2).

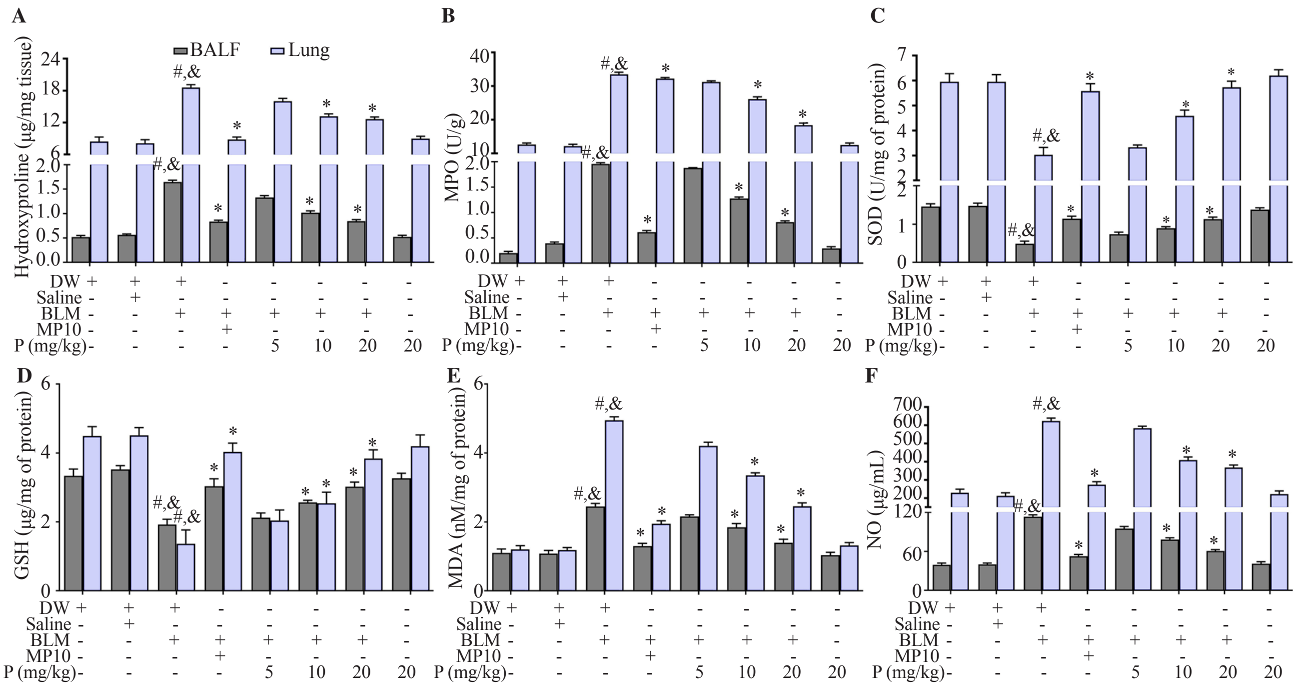

Figure 1. Effect of *D*-pinitol on hydroxyproline (A), MPO (B), SOD (C), GSH (D), MDA (E), and NO (F) in BALF and lung (n=6). One way ANOVA (*post-hoc* test: Tukey's multiple ranges) was used for data analysis. $^{\#}P$<0.05 compared with normal (received DW at 10 mg/kg); $^{\&}P$<0.05 compared with sham (received saline followed by DW); $^{*}P$<0.05 compared with the BLM control group (received BLM at 6 IU/kg and DW). DW: distilled water; BLM: bleomycin; MP: methylprednisolone; P: *D*-pinitol; GSH: glutathione; SOD: superoxide dismutase; NO: nitric oxide; MDA: malondialdehyde; MPO: myeloperoxidase.

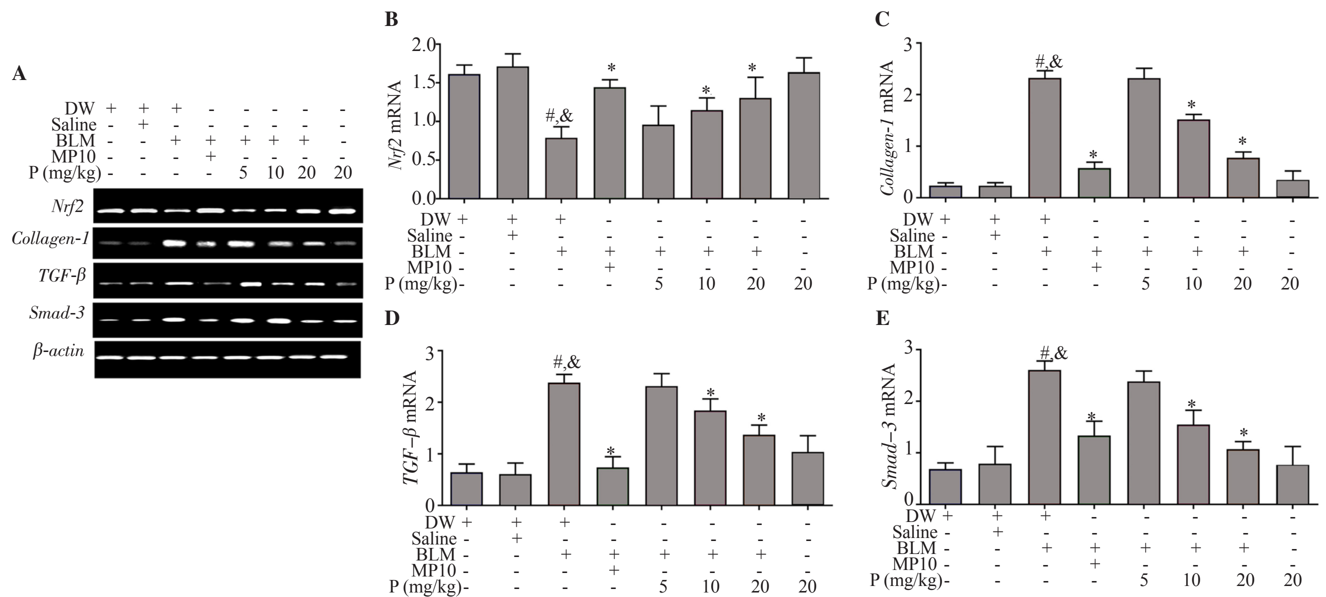

Figure 2. Effect of *D*-pinitol on *Nrf2*, *collagen-1*, *TGF-β*, and *Smad-3* mRNA expression in the lung (n=6). A: gel image; B: *Nrf2*; C: *collagen-1*; D: *TGF-β*; E: *Smad-3*. One way ANOVA (*post-hoc* test: Tukey's multiple ranges) was used for data analysis. $^{\#}P$<0.05 compared with normal; $^{\&}P$<0.05 compared with sham; $^{*}P$<0.05 compared with the BLM control group.

Figure 3. Effect of *D*-pinitol on airway histology of rats in the lung using hematoxylin and eosin staining (A-F) depicting peribronchial inflammation (black arrow) and perivascular inflammation (red arrow) and picro-sirius red staining (G-L) depicting interstitial fibrosis (black arrow) (n=3) at 40×.

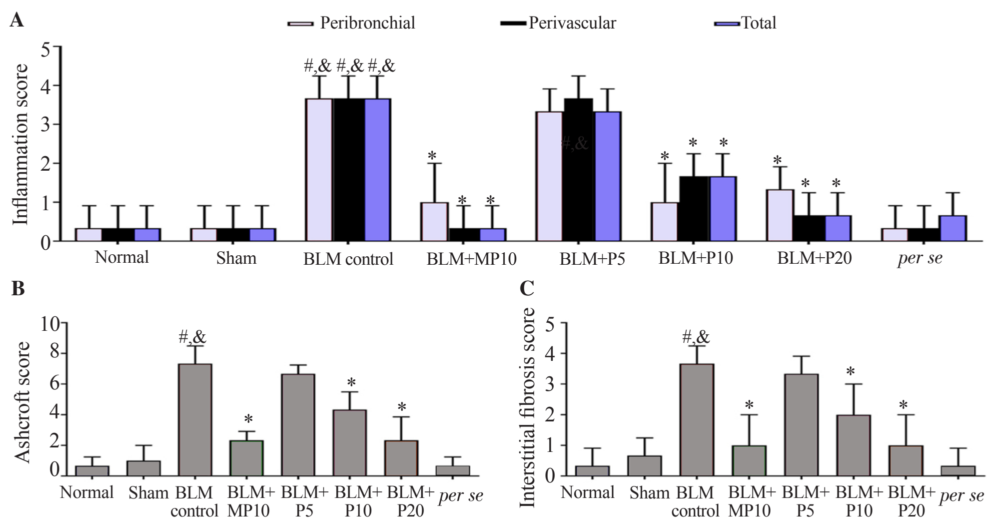

Figure 4. Effect of *D*-pinitol on inflammation score (A), Ashcroft score (B), and interstitial fibrosis score (C) (n=3). Non-parametric one way ANOVA (*post-hoc* test: Kruskal-Wallis test) was used for data analysis. $^{\#}P$<0.05 compared with normal; $^{\&}P$<0.05 compared with sham; $^{*}P$<0.05 compared with the BLM control group.

3.9. Effect on lung histological aberrations

Figure 3A, B, G&H depict normal lung architecture in the sham and normal groups without any evidence of inflammatory infiltration and interstitial fibrosis. BLM caused inflammatory changes reflected by distorted morphology of lung including thickened intra-alveolar septa, alveolar injury, inflammatory infiltration, and fibrotic changes (Figure 3C&I). *D*-pinitol at 10 and 20 mg/kg effectively reduced inflammation infiltration, lung injury and peribronchial and interstitial fibrosis (Figure 3E-F & K-L). The BLM control group showed significantly (P<0.05) higher inflammation (peribronchial, perivascular, and total) (Figure 4A), Ashcroft, and interstitial fibrosis scores (Figure 4B & C) than the sham and normal groups. *D*-pinitol at 10 and 20 mg/kg significantly (P<0.05) reduced inflammation, Ashcroft score, and interstitial fibrosis. *D*-pinitol at 5 mg/kg did not significantly change inflammation, Ashcroft score, and interstitial fibrosis (Figure 4).

4. Discussion

The finding of our study suggests a potential effect of *D*-pinitol against pulmonary fibrosis. It is also noted that the effect of *D*-pinitol at 20 mg/kg on lung function, biochemical parameters, serum biochemistry, mRNA expressions, and histological features was almost comparable to the effect of methylprednisolone, which is utilized clinically for treating pulmonary fibrosis.

IPF is mainly characterized by dysregulated healing of wounds in response to micro-injuries leading to fibrogenesis[1]. Extracellular matrix accumulation in the lung is characteristic of the disease[4]. This accumulation leads to the remodeling of the lung texture and the thickening of alveolar walls, ultimately decreasing gas exchange[4].

Intratracheal administration of BLM causes breaks in single or double DNA strands, leading to apoptosis and free radical formation[24]. These cause the death of epithelial cells, fibroblast accumulation, and increased inflammatory cell infiltration, which ultimately causes fibrosis development[24] and decreased gaseous exchange; thus, it is crucial to evaluate the oxygenated level of hemoglobin[25]. Percentage of oxygen saturation is a vital marker for checking blood oxygen content, which has been reported to decrease with the administration of BLM[17]. Furthermore, as a result of inflammation, lung edema occurs, leading to an increase in relative lung weight[26]. The body weight has been also reported to decrease with the administration of BLM[27]. Consistent with these studies, our study shows that BLM administration resulted in decreased body weight, percent oxygen saturation, and increased relative lung weight and cell infiltration in BALF in rats. *D*-pinitol treatment markedly reduced relative lung weight and cell infiltration in BALF and increased body weight and percent oxygen saturation. This result shows that *D*-pinitol could ameliorate BLM-induced lung inflammation and injury.

The early phase of IPF is associated with increased free radicals[24]. These free radicals upregulate TGF-β1 expression, an essential cytokine in cellular function[6,28]. This causes an increase in fibroblasts, thereby contributing to the pathogenesis of pulmonary fibrosis[29]. A homolog, Smad-3, is responsible for inducing pulmonary fibrosis by forming a transcriptional activation complex[6]. This complex binds to TGF-β and other elements to repress peroxisome proliferator-activated receptor gamma transcription, which regulates inflammation[6,30]. The inflammatory activity increases due to the decreased transcription of peroxisome proliferator-activated receptor gamma[6]. This complex promotes the production of collagen-1, which deposits in the interstitium leading to the decreased tensile strength of the interstitium[6,10]. In the current investigation, the elevated *collagen-1*, *Smad-3*, and *TGF-β* mRNA expressions were associated with BLM administration. The decrease in these mRNA expressions caused by *D*-pinitol further indicates its protective effect against pulmonary fibrosis. Furthermore, *D*-pinitol also upregulated the mRNA expression of *Nrf2*, which has been evidenced in controlling inflammation and fibrosis *via* the suppression of various proinflammatory molecules and regulating oxidative stress *via* regulating detoxifying enzymes in the body[31].

During the induction and maintenance of IPF, oxidative stress plays an important role[24]. GSH and SOD are important antioxidants for scavenging the body's free radicals[32,33]. A study in IPF patients noted a highly decreased level of GSH[32]. A decreased level of SOD has also been noted in fibrotic areas of interstitial pneumonia lungs[33–35]. Furthermore, it was noted that IPF patients had increased levels of MPO, MDA, and NO levels, which are markers of oxidative stress[33]. Consistent with this, the current study also showed increased MPO, MDA, and NO levels and decreased SOD and GSH levels in the lung and BALF of rats treated with BLM. However, these levels were considerably normalized by the administration of *D*-pinitol, which further points to its effect on pulmonary fibrosis *via* its antioxidant effect.

Cyclic polyol demonstrated a wide array of activities[12] and previously reported amelioration of IPF by reducing sphingosine kinase 1 signaling, enhancing TGF-β/Smad, and inhibiting inflammation[36–38]. A study on the effect of quercetin in an *ex-vivo* setting in IPF patients concluded that patients could benefit from quercetin as it decreased inflammatory markers and increased Nrf2 activity[32]. A pilot study on stable IPF patients found that quercetin in combination with dasatinib may improve physical function in these patients[39]. In the current study, *D*-pinitol demonstrated potent antioxidant and anti-fibrotic activities. It is also reported that *D*-pinitol has potential against nephrotoxicity, renal tubulointerstitial fibrosis, and diabetic cardiomyopathy[15,40]. *D*-pinitol has been reported to be a potential treatment in type-2 diabetes mellitus patients as it prevented resistance to insulin and acted as an anti-hyperglycemic agent[41].

This study also has several limitations. Firstly, the anti-

inflammatory effects of *D*-pinitol might play a vital role in anti-fibrotic effects against BLM-induced pulmonary fibrosis. BLM-induced pulmonary fibrosis is associated with the sequence of biphasic events, the preliminary acute inflammatory response occurred within an onset of 8-9 days lasting up to 14-16 days[17]. A previous study also evaluated the anti-inflammatory potential of glycosides against BLM-induced pulmonary fibrosis, where inflammatory markers (TGF-alpha and interleukins) were assessed on day 14[17]. An additional study is warranted to evaluate the anti-inflammatory potential of *D*-pinitol during BLM-induced pulmonary fibrosis. Another limitation of this study is only using a BLM model. Although the BLM-induced pulmonary fibrosis model has been widely used to evaluate potential therapies, a single animal model does not recapitulate the spectrum of human pulmonary fibrosis. For clinical implication, more different animal models including silica-induced pulmonary fibrosis model should be considered.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Funding

The authors received no extramural funding for this study.

Authors' contributions

YLD: concept, design, literature search, data analysis, manuscript preparation, and manuscript review. ZHW: concept, data acquisition, manuscript editing and manuscript review. YXH: literature search, data analysis, manuscript editing and manuscript review. YZ: concept, design, data analysis, manuscript editing and manuscript review. XRW: concept, literature search, data acquisition, manuscript preparation, and manuscript review. CKG: concept, data acquisition, manuscript preparation, and manuscript review. LLB: concept, design, literature search, data acquisition, data analysis, manuscript preparation, manuscript editing and manuscript review.

References

[1] Barratt SL, Creamer A, Hayton C, Chaudhuri N. Idiopathic pulmonary fibrosis (IPF): An overview. *J Clin Med* 2018; **7**(8): 201.

[2] Diamantopoulos A, Wright E, Vlahopoulou K, Cornic L, Schoof N, Maher TM. The burden of illness of idiopathic pulmonary fibrosis: A comprehensive evidence review. *Pharmacoeconomics* 2018; **36**(7): 779-807.

[3] Rabeyrin M, Thivolet F, Ferretti GR, Chalabreysse L, Jankowski A, Cottin V, et al. Usual interstitial pneumonia end-stage features from explants with radiologic and pathological correlations. *Ann Diagn Pathol* 2015; **19**(4): 269-276.

[4] Sgalla G, Iovene B, Calvello M, Ori M, Varone F, Richeldi L. Idiopathic pulmonary fibrosis: Pathogenesis and management. *Respir Res* 2018; **19**(1): 32.

[5] Yang IV, Schwartz DA. Epigenetics of idiopathic pulmonary fibrosis. *Transl Res* 2015; **165**(1): 48-60.

[6] Zhou Z, Kandhare AD, Kandhare AA, Bodhankar SL. Hesperidin ameliorates bleomycin-induced experimental pulmonary fibrosis *via* inhibition of TGF-beta1/Smad3/AMPK and IkappaBalpha/NF-kappaB pathways. *EXCLI J* 2019; **18**: 723-745.

[7] Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med* 2005; **353**(21): 2229-2242.

[8] Nathan SD, Albera C, Bradford WZ, Costabel U, Glaspole I, Glassberg MK, et al. Effect of pirfenidone on mortality: Pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. *Lancet Respir Med* 2017; **5**(1): 33-41.

[9] Kolb M, Richeldi L, Behr J, Maher TM, Tang W, Stowasser S, et al. Nintedanib in patients with idiopathic pulmonary fibrosis and preserved lung volume. *Thorax* 2017; **72**(4): 340-346.

[10]Kandhare AD, Mukherjee AA, Ghosh P, Bodhankar SL. Efficacy of antioxidant in idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *EXCLI J* 2016; **15**: 636-651.

[11]Guo S, Song Y, Feng J, Liu S, Li Y, Liu M, et al. Effects of Qizhukangxian granules on idiopathic pulmonary fibrosis: A randomized, double blind, placebo-controlled and multicenter clinical pilot trial. *J Tradit Chin Med* 2020; **40**(4): 674-682.

[12]Sánchez-Hidalgo M, León-González AJ, Gálvez-Peralta M, González-Mauraza NH, Martin-Cordero C. *D*-pinitol: A cyclitol with versatile biological and pharmacological activities. *Phytochem Rev* 2021; **20**: 211-224.

[13]Liang Y, Xu Y, Lu B, Huang Y, Xu S, Xie J, et al. Inositol alleviates pulmonary fibrosis by promoting autophagy *via* inhibiting the HIF-1alpha-SLUG axis in acute respiratory distress syndrome. *Oxid Med Cell Longev* 2022; **2022**: 1030238.

[14]Srivastava K, Tiwari M, Dubey A, Dubey A. *D*-pinitol-a natural phytomolecule and its pharmacological effect. *Int J Pharm Life Sci* 2020 **11**(5): 6609-6623.

[15]Koh ES, Kim S, Kim M, Hong YA, Shin SJ, Park CW, et al. *D*-Pinitol alleviates cyclosporine A-induced renal tubulointerstitial fibrosis *via* activating Sirt1 and Nrf2 antioxidant pathways. *Int J Mol Med* 2018; **41**(4): 1826-1834.

[16]Zhan T, Lou H. Synthesis of azole nucleoside analogues of *D*-pinitol as potential antitumor agents. *Carbohydr Res* 2007; **342**(6): 865-869.

[17]Kandhare AD, Bodhankar SL, Mohan V, Thakurdesai PA. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. *Chem Biol Interact* 2015; **237**: 151-165.

[18]Fan Y, Wang J, Feng Z, Cao K, Xu H, Liu J. Pinitol attenuates LPS-induced pneumonia in experimental animals: Possible role *via* inhibition

of the TLR-4 and NF-kappaB/IkappaBalpha signaling cascade pathway. *J Biochem Mol Toxicol* 2021; **35**(1): e22622.

[19]Kandhare AD, Bodhankar SL, Singh V, Mohan V, Thakurdesai PA. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. *Biomed Aging Pathol* 2013; **3**(1): 23-30.

[20]Aswar U, Mahajan U, Kandhare A, Aswar M. Ferulic acid ameliorates doxorubicin-induced cardiac toxicity in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2019; **392**(6): 659-668.

[21]Kandhare AD, Shivakumar V, Rajmane A, Ghosh P, Bodhankar SL. Evaluation of the neuroprotective effect of chrysin *via* modulation of endogenous biomarkers in a rat model of spinal cord injury. *J Nat Med* 2014; **68**(3): 586-603.

[22]Mukherjee A, Zhang J, Kandhare AD, Bodhankar SL. Curcumin ameliorates vitamin A deficiency-induced urolithiasis in neonatal rats *via* inhibition of KIM-1/NGAL, Nrf2, and iNOs pathways. *Lat Am J Pharm* 2018; **37**(12): 2502-2511.

[23]Devkar ST, Kandhare AD, Zanwar AA, Jagtap SD, Katyare SS, Bodhankar SL, et al. Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: Decisive role of TNF-α, IL-1β, COX-II and iNOS. *Pharm Biol* 2016; **54**(11): 2394-2403.

[24]Tashiro J, Rubio GA, Limper AH, Williams K, Elliot SJ, Ninou I, et al. Exploring animal models that resemble idiopathic pulmonary fibrosis. *Front Med (Lausanne)* 2017; **4**: 118.

[25]Collins JA, Rudenski A, Gibson J, Howard L, O'Driscoll R. Relating oxygen partial pressure, saturation and content: The haemoglobin-oxygen dissociation curve. *Breathe* 2015; **11**(3): 194-201.

[26]Aubin Vega M, Chupin C, Pascariu M, Prive A, Dagenais A, Berthiaume Y, et al. Dexamethasone fails to improve bleomycin-induced acute lung injury in mice. *Physiol Rep* 2019; **7**(21): e14253.

[27]Chen L, Zhao W. Apigenin protects against bleomycin-induced lung fibrosis in rats. *Exp Ther Med* 2016; **11**(1): 230-234.

[28]Li W, Kandhare AD, Mukherjee AA, Bodhankar SL. Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats: Role of TGF-ß/Smads and Ang-1/Tie2 signaling pathways. *EXCLI J* 2018; **17**: 399-419.

[29]Yang W, Li X, Qi S, Li X, Zhou K, Qing S, et al. lncRNA H19 is involved in TGF-beta1-induced epithelial to mesenchymal transition in bovine epithelial cells through PI3K/AKT Signaling Pathway. *Peer J* 2017; **5**: e3950.

[30]Cui J, Wang G, Kandhare AD, Mukherjee AA, Bodhankar SL. Neuroprotective effect of naringin, a flavone glycoside in quinolinic acid-induced neurotoxicity: Possible role of PPAR-gamma, Bax/Bcl-2, and caspase-3. *Food Chem Toxicol* 2018; **121**: 95-108.

[31]Pompili S, Sferra R, Gaudio E, Viscido A, Frieri G, Vetuschi A, et al. Can Nrf2 modulate the development of intestinal fibrosis and cancer in inflammatory bowel disease? *Int J Mol Sci* 2019; **20**(16) :4061.

[32]Veith C, Drent M, Bast A, van Schooten FJ, Boots AW. The disturbed redox-balance in pulmonary fibrosis is modulated by the plant flavonoid quercetin. *Toxicol Appl Pharmacol* 2017; **336**: 40-48.

[33]Fois AG, Paliogiannis P, Sotgia S, Mangoni AA, Zinellu E, Pirina P, et al. Evaluation of oxidative stress biomarkers in idiopathic pulmonary fibrosis and therapeutic applications: A systematic review. *Respir Res* 2018; **19**(1): 51.

[34]Pan W, Meng LP, Su J, Yang ZB, Du WF, Xu ZW, et al. *Crotalaria ferruginea* extract attenuates lipopolysaccharide-induced acute lung injury in mice by inhibiting MAPK/NF-κB signaling pathways. *Asian Pac J Trop Biomed* 2021; **11**: 481-490.

[35]El-Sayed RA, El-Demerdash FM, El-Magd MA. Ginseng ameliorates pulmonary toxicity induced by silicon dioxide nanoparticles in rats. *Asian Pac J Trop Biomed* 2021; **11**: 254-262.

[36]Zhang X, Cai Y, Zhang W, Chen X. Quercetin ameliorates pulmonary fibrosis by inhibiting SphK1/S1P signaling. *Biochem Cell Biol* 2018; **96**(6): 742-751.

[37]Li XZ, Wang XL, Wang YJ, Liang QK, Li Y, Chen YW, et al. Total flavonoids of *Oxytropis falcata* Bunge have a positive effect on idiopathic pulmonary fibrosis by inhibiting the TGF-beta1/Smad signaling pathway. *J Ethnopharmacol* 2022; **285**: 114858.

[38]Kar S, Biswas S, Banerjee ER. Evaluating the ameliorative potential of plant flavonoids and their nanocomposites in bleomycin induced idiopathic pulmonary fibrosis. *Biomed Res Ther* 2016; **3**(7). doi: 10.7603/s40730-016-0032-6.

[39]Justice JN, Nambiar AM, Tchkonia T, LeBrasseur NK, Pascual R, Hashmi SK, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine* 2019; **40**: 554-563.

[40]Li XL, Xu M, Yu F, Fu CL, Yu X, Cheng M, et al. Effects of *D*-pinitol on myocardial apoptosis and fibrosis in streptozocin-induced aging-accelerated mice. *J Food Biochem* 2021; **45**(4): e13669.

[41]Kim JI, Kim JC, Kang MJ, Lee MS, Kim JJ, Cha IJ. Effects of pinitol isolated from soybeans on glycaemic control and cardiovascular risk factors in Korean patients with type Ⅱ diabetes mellitus: A randomized controlled study. *Eur J Clin Nutr* 2005; **59**(3): 456-458.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.