



## Sildenafil, a Selective Inhibitor of type 5 phosphodiesterase, attenuates Bleomycin-induced Lung Fibrosis in Rat

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**ABSTRACT:** The present study aimed to investigate effects of sildenafil on bleomycin (BLM)-induced lung fibrosis in rat. Animals were divided into five groups and treated according to the following treatments: group 1 received daily normal saline, intraperitoneally (ip); group 2 received a single dose of BLM (7.5 IU/kg), intratracheally, and then received normal saline like the control group; groups 3, 4 and 5 were BLM groups which received sildenafil (5, 10 and 25 mg/kg/day ip, respectively) from 1 week before to 3 weeks after BLM administration. Finally, the animals were killed and the changes of hydroxyproline (HP) and histology were evaluated in lung tissue. The results showed that HP level was significantly lower in the sildenafil treated rats as compared to BLM group. In addition, the HP changes were well related with the pathological findings. This study suggests that blocking of type 5 phosphodiesterase may prevent progression of BLM-induced lung fibrosis.

**Key words:** Pulmonary fibrosis, phosphodiesterase inhibitors, oxidative stress, bleomycin, sildenafil

### INTRODUCTION

Pulmonary fibrosis (PF) is one of the most common respiratory diseases; it is characterised pathologically by excessive accumulation of extracellular matrix (ECM) around the alveoli and remodelling of the lung architecture. Overall, imbalance between the accumulation and breakdown of ECM is a major reason for the incidence of PF. When the normal balance between ECM deposition and turnover is shifted toward deposition or away from breakdown, excessive ECM accumulates (Wynn and Ramalingam, 2012; Todd *et al.*, 2012). Furthermore, oxidative stress has a critical role in the pathogenesis of PF (Yildirim *et al.*, 2004).

Several animal experimental models have been used in PF studies (Moore and Hogaboam, 2008). Bleomycin (BLM) has been widely used in animal models to study the mechanisms involved in PF and evaluate potential therapeutic agents (Wynn, 2011). In this model, the changes in connective tissue components such as collagen, fibronectin and hyaluronan have been confirmed as markers of PF severity (Hernnas *et al.*, 1992; Westergren-Thorsson *et al.*, 1993; Broekelmann *et al.*, 1991; Khalil *et al.*, 1989). Sildenafil is a selective inhibitor of type 5 phosphodiesterase (PDE5), which is expressed in vascular smooth muscles, platelets and bronchial smooth muscles (Lin *et al.*, 2006; Wallis *et al.*, 1999). This drug is approved for use in treating idiopathic pulmonary arterial hypertension and erectile

dysfunction (Galie *et al.*, 2005). Sildenafil has been demonstrated to decrease oxidative stress and the inflammatory response through the inhibition of superoxide formation (Rodriguez-Iturbe *et al.*, 2005; Shukla *et al.*, 2005). In addition, it can improve lung diffusion capacity, aerobic performance, exercise ventilation efficacy and pulmonary haemodynamics (Guazzi *et al.*, 2004; Lewis *et al.*, 2007).

Based on the available evidence, the aim of this study was to investigate the effects of sildenafil on a pulmonary fibrotic model induced using BLM.

### MATERIALS AND METHODS

#### A. Reagents

All chemicals were obtained from Merck (Darmstadt, Germany) unless otherwise stated. BLM and sildenafil (Cipla Co. Ltd., India), ether and ketamine (Rotexmedica Co., Germany) and the Sirius Red Total Collagen Detection Kit (Chondrex Co., USA) were used in this study.

#### B. Animals

Wistar rats (190-250 g) were obtained from the animal house and research centre of the Jundishapur University of Medical Sciences, Ahvaz, Iran. All animals were housed under controlled ambient temperature (22±2°C) and humidity (40-70%) with a 12 h light/dark cycle and food and water available ad libitum.

Animals were acclimated to the housing conditions for 1 week before the experiments. All experiments were approved by the Animal Care and Use Committee of the Jundishapur University of Medical Sciences, which complies with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

#### C. Experimental design and sampling

Animals were randomly divided into five groups, seven in each, and treated for 28 days according to the following treatments: group 1 (control group) received daily normal saline solution, intraperitoneally (ip); group 2 (positive control) received a single dose of BLM (7.5 IU/kg), intratracheally, and then received normal saline solution like the control group; groups 3, 4 and 5 were BLM groups which received sildenafil (5, 10 and 25 mg/kg/day ip, respectively) from 1 week before to 3 weeks after BLM administration.

#### D. Hydroxyproline (HP) assay

HP contents, as an index of pulmonary collagen, were determined using the Sirius Red Total Collagen Detection Kit (Chondrex Co.) according to the manufacturer's instructions. Results were expressed as HP  $\mu\text{g/g}$  tissue.

#### E. Histopathology

Lung sections were fixed with 10% buffered paraformaldehyde and then dehydrated and embedded in paraffin. Specimens were stained with haematoxylin and eosin and observed via light microscopy. A semi-quantitative scoring of the staining was utilised for the assessment of fibrosis with a scoring system from 0 to

3+ (0: normal; +: presence of fibrosis involving less than 25% of the lung parenchyma; ++: lesions containing 25-75% of the lung, formation of fibrous bands or small fibrous mass; +++: lesions including more than 75% of the lung, severe fibrosis).

#### F. Statistical analysis

The results were presented as mean  $\pm$  standard error of the mean (SEM) and compared using one-way analysis of variance (ANOVA). Also, in order to evaluate correlations, Pearson test was used. A p-value  $< 0.05$  was considered statistically significant. The data were analysed using GraphPad Prism Version 5.01 software (GraphPad Software Inc., San Diego, CA, USA).

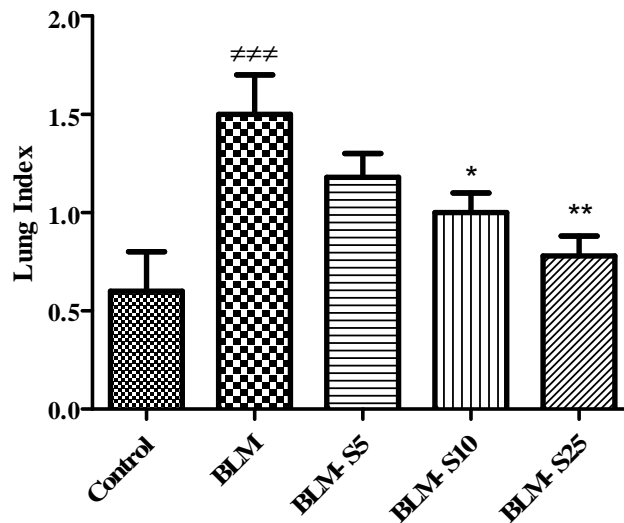
## RESULTS

#### A. Animal's lung weight index

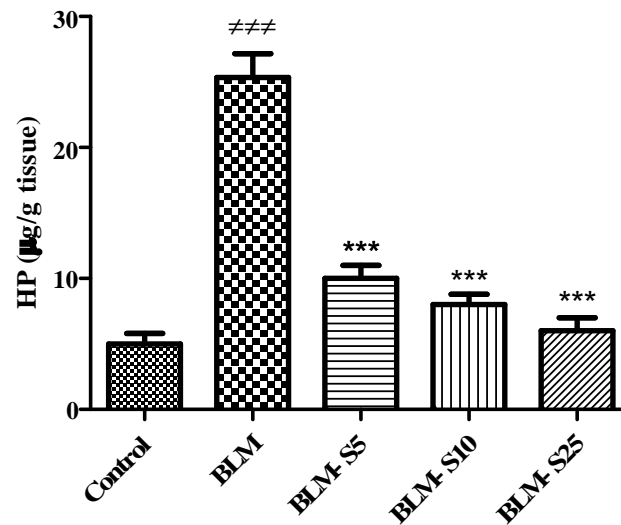
As shown in Fig. 1, a remarkable increase in the total net lung weight index (lung weight/body weight  $\times 100$ ) was observed in the BLM-treated animals when compared to the control group ( $P < 0.001$ ). Following sildenafil administration, a dose-dependent decrease in total net lung weight index was observed in comparison to the BLM-treated rats ( $r^2 = 0.738$ ,  $P < 0.001$ ).

#### B. HP assay

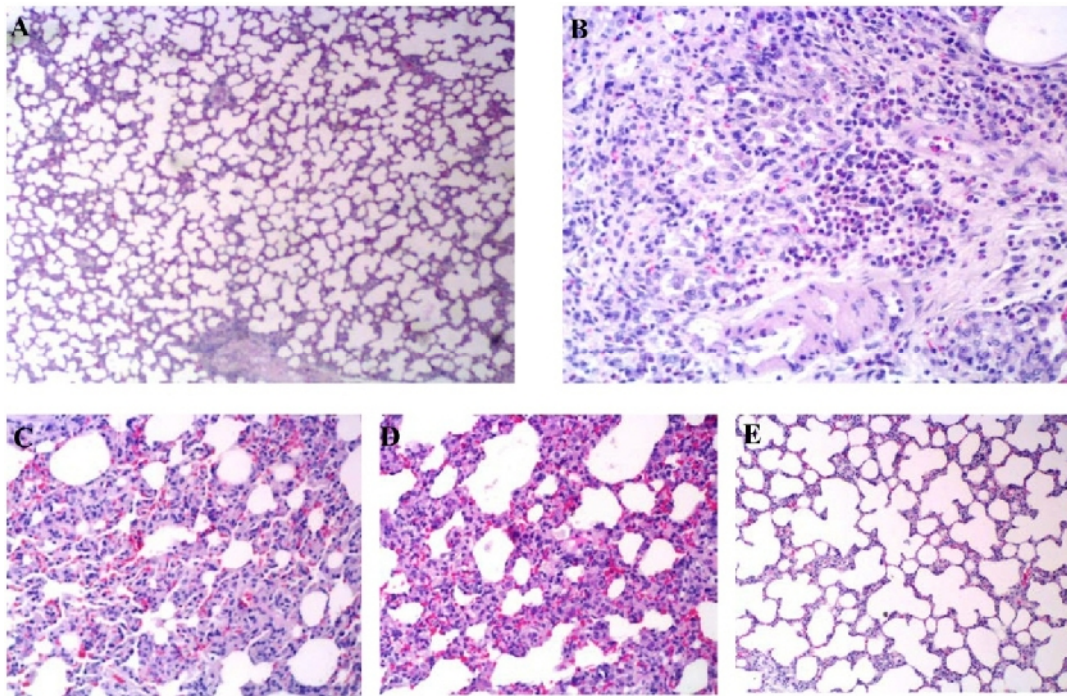
As shown in Fig. 2, administration of BLM significantly increased HP levels in comparison to the control group ( $P < 0.001$ ). Sildenafil treatment was able to reduce the increased contents of this marker at the employed doses of 5, 10 and 25 mg/kg. The best response was obtained at a dose of 25 mg/kg.



**Fig. 1.** Effects of sildenafil (S) on lung index (lung weight/body weight  $\times 100$ ) in BLM-induced lung damage in adult male rat. Statistical analysis used one-way ANOVA with Tukey's test. Data are expressed as means  $\pm$  SEM,  $n = 7$  for each group. ###Significantly different from control group ( $P < 0.001$ ). \*Significantly different from BLM group ( $P < 0.05$ ); \*\*Significantly different from BLM group ( $P < 0.01$ ).



**Fig. 2.** Effects of sildenafil (S) on HP contents in BLM-induced lung damage in adult male rat. Statistical analysis used one-way ANOVA with Tukey's test. Data are expressed as means  $\pm$  SEM,  $n = 7$  for each group. ###Significantly different from control group ( $P < 0.001$ ); \*\*\*Significantly different from BLM group ( $P < 0.001$ ).



**Fig. 3.** Effect of sildenafil on histological changes in BLM-induced lung damage in adult male rat (Stained with hematoxylin-eosin,  $\times 400$ ). (A) Control group; (B) BLM group; (C) Sildenafil group (5mg/kg), (D) Sildenafil group (10mg/kg) and (E) Sildenafil group (25mg/kg).

### C. Histology

Following administration of BLM, some histopathological changes in the lung were observed compared to the control group, including alveolar collapse, thickened interalveolar septa and the

infiltration of inflammatory cells (Fig. 3). Sildenafil prevented the histopathological changes resulting from BLM in a dose-dependent manner. It should be noted that the pathological findings were well related with the HP changes.

## DISCUSSION

The present study revealed the protective effects of sildenafil in PF induced by BLM. In addition, our data showed that the decrease of fibrotic changes after administration of different doses of sildenafil is associated with HP changes. These findings are in agreement with other studies showing a similar association in the reduction of fibrosis via sildenafil in corporal fibrosis (Ferrini *et al.*, 2007; Sirad *et al.*, 2011), systemic sclerosis (Golglazier *et al.*, 2005), and pulmonary sarcoidosis (Milman *et al.*, 2008). The existing evidence could be associated with the role of PDE5 in oxidative stress and the inflammatory process. Previous studies have demonstrated the role of oxidative mechanisms in the pathogenesis of BLM-induced pulmonary fibrosis. Reactive oxygen species (ROS) are produced by BLM-initiated inflammation after the oxidation of the BLM-Fe (II) complex and is activated by inflammatory cells. These processes lead to lung injury and fibroblast differentiation and proliferation in the alveolar septa. These activated fibroblasts produce ECM protein and collagen deposition which distorts the lung's architecture and results in respiratory deficiency (Sleijfer, 2001). In contrast, recent studies have indicated that PDE inhibitors can decrease the formation of ROS by increasing cAMP and cGMP in various cells (Mohammadi *et al.*, 2011). For instance, Milani *et al.* (2005) reported that the administration of PDE inhibitors to diabetic rats increased antioxidant capacity. In addition, the prevention of oxidative damage through pentoxifylline as a PDE5 inhibitor in oxidative stress-induced embryotoxicity has been reported. cGMP as a mediator in the inflammation process can trigger cGMP/cGMP-dependent protein kinase I (cGKI) pathway, which is effective in improving cardiac fibrosis (Patrucco *et al.*, 2014). This pathway may also be involved in the therapeutic effect of sildenafil in PF. In addition to role of the cAMP and/or cGMP, these potential effects could be mediated by nitric oxide (NO) donors (Mohammadi *et al.*, 2011).

## CONCLUSION

Taken collectively, our findings showed that sildenafil inhibited BLM-induced PF. This effect is possibly related to the anti-inflammatory, anti-oxidative capability involved in inhibition of PDE5 enzyme. However, its exact mechanisms need to be verified in future studies.

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