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Article

# Theophylline attenuates bleomycin-induced oxidative stress in rats: The role of IL-6, NF-κB, and antioxidant enzymes

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The purpose of this study was to evaluate the antifibrotic and antioxidant roles of theophylline (Theo), a bioactive compound, in bleomycin (BLM)-induced pulmonary fibrosis in Wistar albino rats. Assigned into 4 groups were 32 Wistar albino rats, comprising the control group (administered 0.9% isotonic saline), BLM group (treated with BLM at a dose of 2.5 mg/kg), BLM+Theo group (treated with Theo at a dose of 75 mg/kg + BLM at a dose of 2.5 mg/kg), and Theo group (treated with Theo at a dose of 75 mg/kg). In the BLM group, a significant decrease was observed in the catalase and glutathione peroxidase enzyme activities, and reduced glutathione (GSH) ($p < 0.05$, $p< 0.05$, $p< 0.001$, respectively), while the malondialdehyde (MDA) levels ($p< 0.001$) were significantly elevated when compared to the control group. However, the MDA levels in the BLM+Theo group were also significantly higher than in the control group ($p< 0.01$). Similarly, the GSH levels were significantly higher in the BLM+Theo group than in the BLM group ($p< 0.05$). The results indicated that Theo reduced the BLM-induced activation of nuclear factor-kappaB (NF-κB) and decreased interleukin-6 (IL-6) levels, together with significant amelioration of the immunohistochemical and histopathological architecture in the lung tissues. It was concluded that the administration of Theo had a positive effect on the GSH level, and activation of NF-κB and IL-6 expression, which were significant proinflammatory markers in the BLM-treated rats.

**Keywords:** Theophylline. Bleomycin. IL-6, NF-κB. Antioxidant enzymes.

## INTRODUCTION

Bleomycin (BLM), a member of the glycopeptide group of antibiotics, is a chemotherapeutic drug that has been used clinically for various human malignancies (Kalayarasan, Sriram, Sudhandiran, 2008). It is among the anti-cancer drugs that are commonly used in the treatment of many types of tumors, such as breast, prostate cancer, and ovarian tumors. However, its efficiency is limited due to the potential improvement of pulmonary fibrosis (PF) (Kabel, Omar, Elmaaboud, 2016). PF is a progressive disorder that is characterized by the disruption of the normal tissue architecture and lung function. It results in the excessive deposition of the extracellular matrix and synthesis in the distal airspace (Lucattelli *et al*., 2016). PF can be induced by a variety of etiological

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factors, such as inflammation, oxidative stress, epithelial-mesenchymal transition and immunodeficiency, which lead to damage of the alveolar epithelial cell, and fibroblast proliferation, which results in the abnormal deposition of the extracellular matrix and tissue remodeling (Zhang *et al*., 2016).

BLM cytotoxicity results from the induction of free radicals that cause DNA to breakdown and result in cell death (Nikbakht *et al*., 2015). BLM changes the balance between the oxidants and antioxidant defense system in the lungs. In this specific organ, the selective absence of BLM hydrolase activity results in a high degree of BLM-induced oxidative stress (Cuzzocrea *et al*., 2007). Oxidative stress plays a primary role in BLM-induced fibrosis and pulmonary inflammation, since an excess of reactive oxygen species (ROS) can non-specifically oxidize cellular macromolecules, such as DNA, proteins, and lipids, leading to tissue injury (Liu *et al*., 2012). Another role of the ROS produced by BLM is to activate nuclear factor-kappaB (NF-κB), a transcription factor that regulates many cytokine genes, such as, tumor necrosis factor-α (TNF-α), interleukin-1b (IL-1b), and IL-6, and macrophage inflammatory protein-2. TNF-α and IL-1b may, in turn, also activate NF-κB (Serrano-Mollar *et al*., 2003).

Methylxanthines are a group of phytochemical compounds that belong to a class of alkaloids. In particular, they are purine alkaloids because they contain nitrogen atoms in their heterocyclic structure (Bartella *et al*., 2019). Theophylline (Theo) is a methylxanthine drug that is used in therapy due to its powerful antiinflammatory action and has also been used to treat patients with airway diseases due to its constituted effect as a bronchodilator (Cordella *et al*., 2019). Theo (1,3-dimethylxanthine) is a natural compound present in some plants, such as cocoa beans and tea (Rao *et al*., 2018).

This study was designed to evaluate the potential antiinflammatory and antifibrotic roles of Theo on the histopathological and immunohistochemical alterations, interleukin-6 (IL-6) and NF-κB expression, and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and reduced glutathione (GSH) levels in BLM-induced PF in male Wistar rats.

## MATERIAL AND METHODS

### Experimental Animals

In this study, a total of 32 male Wistar albino rats (aged 8 weeks, weighing 200 ± 50 g) were obtained from the Van Yuzuncu Yil University, Medical Faculty, Experimental Animal Research Center. The rats were individually housed in clean cages at normal temperature (22 ± 2 °C) and a light/dark photoperiod of 12:12, and supplied with drinking water and food ad libitum. The experimental protocol was conducted following the general principles of the animal ethics committee of Van-YYU (YUHAD-YEK, Date: 31/05/2018/, Decision number: 2018-05).

### Experimental design

The rats were randomly assigned into 4 equal groups (8 rats in each group), comprising the control group: administered the same volume of intratracheal (i.t.) instillation 0.9% sterile isotonic saline solution instead of BLM, calculated according to body weight. BLM group: administered a single injection of BLM at a dose of 2.5 mg/kg of body weight via i.t. injection on the first day. BLM+Theo group: administered a single i.t. instillation injection of BLM at a dose of 2.5 mg/kg on the first day, in addition to an intraperitoneal (i.p.) injection of Theo at a dose of 75 mg/kg each day. Theo group: administered an i.p. injection of Theo at a dose of 75 mg/kg body weight each day. The treatment course lasted for 14 days for all of the groups. At the end of the experimental period, all of the male Wistar albino rats were anesthetized and euthanized. The lungs were then removed for histopathological and immunohistochemical examinations and SOD, CAT, GSH-Px, MDA, and GSH analyses.

### Preparation of lung homogenates

Lung tissues were removed and washed with physiological saline (NaCl 0.9%), and the tissue samples were stored at –65 °C until analysis. Tissue samples (0.5 g) were homogenized in 5000 μL of ice-cold buffer:

(10 nmol/L Tris-HCl, pH 7.4) and (1 mmol/L EDTA, 0.32 mol/L) sucrose, using an IKA Ultra Turrax T25 homogenizer (Staufen, Germany) and a glass porcelain Bandelin Sonupuls 20 KHz ultrasonic homogenizer (Heinrichstraße, Berlin, Germany) for 8 min and then the homogenate was centrifuged at 9500 rpm for 30 min (Xia *et al*., 1994). All of the processes were performed at 4 °C, and then the clear supernatants were used for determination of the antioxidant enzyme (SOD, CAT, and GSH-Px), MDA, and GSH levels of the lung tissues.

### Antioxidant enzymes determination

The clear supernatant obtained from the lung tissue homogenate was used to assess the SOD, CAT, and GSH-Px levels. Tissue SOD (EC 1.15.1.1) enzyme activity was estimated at 505 nm using the method described by Sun *et al.* (1988). GSH-Px (E.C.1.11.1.9) tissue enzyme activity was assayed at 340 nm using the method of Paglia and Valentine (1967). CAT (EC 1.11.1.6) tissue enzyme activity was determined at 240 nm according to the method of Aebi (1984). The results were presented as IU/g.

### MDA and GSH estimation

The lung tissue GSH level was determined at 412 nm using the method of Rizzi *et al*. (1988). Data were presented as μmol/g. The lung tissue MDA level was assayed using the modified method of Jain *et al*. (1989), based on the reaction between MDA and thiobarbituric acid. The MDA was estimated at 532 nm. Data were presented as nmol/g.

### Histopathological analysis of the lung

Lung tissues for histopathological assessment were fixed in a 10% (v/v) formalin solution for 48 h before being processed for the histopathological analysis, and then washed under tap water for 10 h. In the routine tissue follow-up, the tissues were passed through an xylene and alcohol series, and embedded into paraffin blocks. Next, 4-μm-thick slices were cut from each block, and the sections were placed onto microscope slides. Sections to be used for histopathological examination were stained with hematoxylin and eosin (H&E) and examined under a Leica DM 1000 light microscope (Leica Microsystems, Wetzlar, Germany). They were assessed as none (−), mild (+), moderate (++), and severe (+++), based on the histopathological findings.

### Immunohistochemistry

All of the sections were mounted on adhesive poly-L-lysine-coated slides for the immunoperoxidase assay and passed through an alcohol and xylol series, deparaffinized, and then dehydrated. They were washed with phosphate buffer saline (PBS, pH 7.2) for 5 min and stored in 3% hydrogen peroxide for 10 min to inactivate any endogenous peroxidase. After washing with PBS for 5–10 min, they were incubated for 5 min with a protein block that was compatible with all primer and secondary antibodies to prevent nonspecific ground staining. At the end of the incubation, the primary antibody (IL-6), rabbit polyclonal IgG NF-κB p65, and PBS in the control group were distilled without washing off the block solution remaining on the tissue sections. In relation to the primer, the antibody was allowed to stand at room temperature for 1 h. It was washed twice with PBS for 5 min each time and then incubated with biotinylated secondary antibody for 10–30 min at room temperature. The sections were then washed again with PBS after being allowed to stand in streptavidin peroxidase for 10–30 min, and then washed in the same manner with PBS. After washing, 3,3'-Diaminobenzidine chromogen was added to the sections, and they were allowed to stand for 5–10 min to take in the chromogenic substance. The sections were kept in Mayer's hematoxylin for background staining for 1–2 min and then washed under tap water. After passing through an xylose and alcohol series, Entellan was dropped on the sections and the slides were closed with coverslips, and examined under a Leica DM 1000 light microscope (Leica Microsystems). The sections were evaluated as no (−), mild (+), moderate (++), and severe (+++), depending on their immunopositivity.

### Statistical analysis

Results were presented as the mean ± standard deviation. Analysis of variance (ANOVA) was performed

for the statistical analyses, and the statistical comparisons among the groups were performed using the the post-hoc Tukey test for the normally distributed data, followed by the Bonferroni test for the non-normally distributed data, using IBM SPSS Statistics for Windows 23.0 (IBM Corp., Armonk, NY, USA). Histological evaluation was performed with relevant tests for the categorical data.

## RESULTS

### Histopathological findings

The control group was detected to have normal histological appearance of the lung tissues (Figure 1-A). Lung tissues were examined in the BLM group, and the peribronchial and bronchiolar mononuclear cell infiltrations, interstitial pneumonia, interstitial peribronchial and peribronchiolar fibrosis, and mild desquamation in the bronchial and bronchiolar epithelium, and severe hyperemia in the interstitial vessels were observed (Figure 1-B). In the BLM+Theo group, mild interstitial pneumonia, moderate hyperemia in the vessels, and desquamation in the bronchial-bronchiolar epithelium were observed (Figure 1-C). A statistically significant difference ($p< 0.05$) was found when compared to the BLM group. In the histopathological examination of lung tissues in the Theo group, it was observed that the lung parenchyma and in subpleural sites had a normal histological appearance (Figure 1-D). The histopathological findings are summarized in Table I.

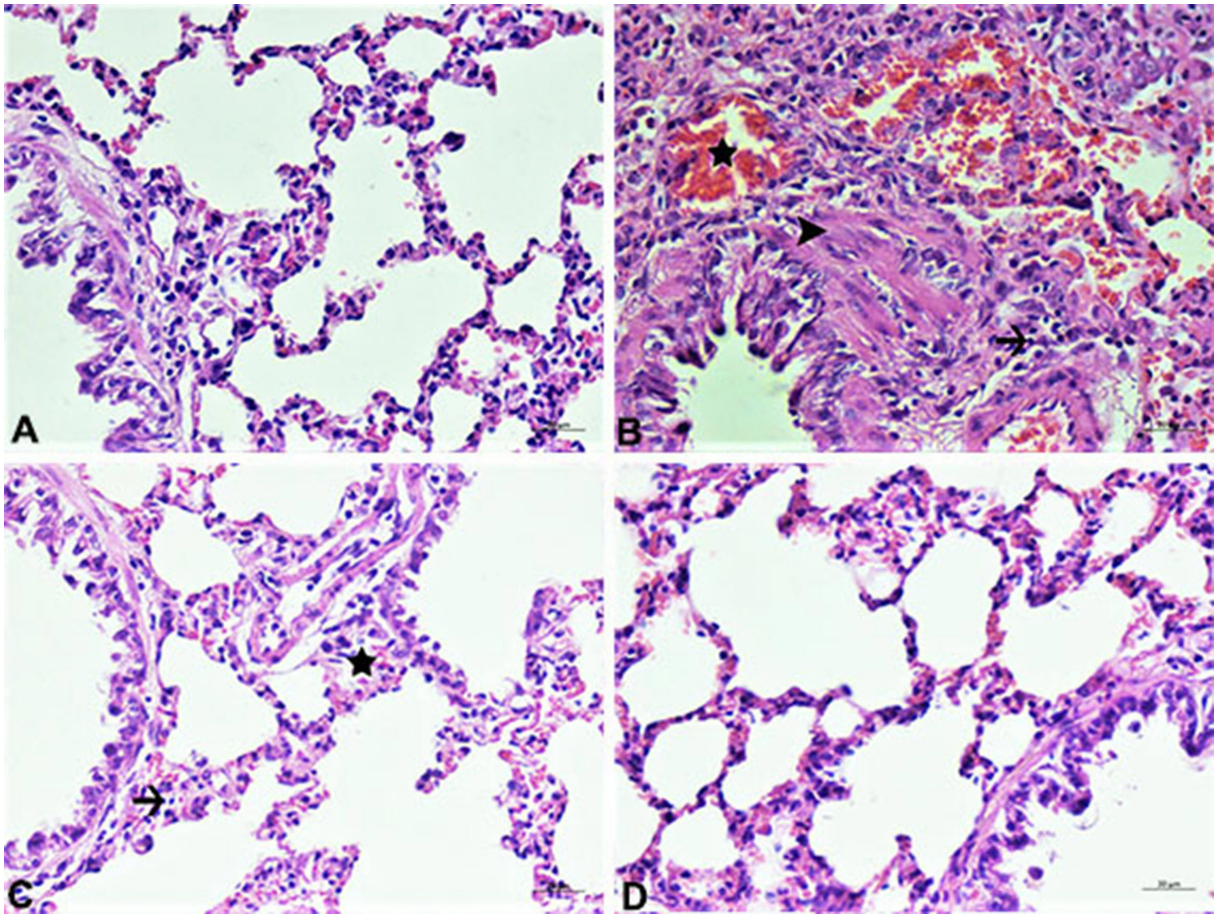


**FIGURE 1 -** The lung tissue, control group, normal histological appearance (A), section taken from the lungs of rats given BLM, BLM group, peribronchiolar and interstitial fibrosis (arrowheads), peribronchiolar cell infiltration (arrow), interstitial pneumonia, severe level hyperemia in the vessels (star) (B), section taken from the lungs of rats given both BLM and Theo, BLM+Theo group, mild level of interstitial pneumonia (star), moderate hyperemia in the vessels (star) (C), Theo group, normal histological appearance (D), H&E, Bar: 20 µm.

**TABLE I -** Scores of immunohistochemical and histopathological findings

| Parameters | Control | BLMs | BLMs+theo | Theo |
|---|---|---|---|---|
| **Peribronchial-bronchiolar and interstitial fibrosis** | - | +++ | + | - |
| **Interstitial pneumonia** | - | +++ | + | - |
| **Peribronchial-bronchiolar inflammatory cells** | - | +++ | + | - |
| **Hyperemia in the vessels** | - | +++ | ++ | - |
| **IL-6** | - | +++ | + | - |
| **NF-κB** | - | +++ | + | - |

(-) No change, (+) Mild change, (++) Moderate change, (+++) Severe change

### Immunohistochemical results

As a result of the immunohistochemical analyses of the lung tissues, in the control group, negative IL-6 and NF-κB expression was determined (Figure 2,3-A). In the BLM group, the lung tissues showed severe IL-6 expression in the bronchial-bronchiolar epithelium, perivascular, peribronchial-peribronchiolar, and interstitial tissues (Figure 2-B). Intracytoplasmic NF-κB expression was observed in the lung tissues, especially in areas with peribronchial-peribronchiolar cell infiltration in the mononuclear cells (Figure 3-B). In the lung tissues of the BLM+Theo group, mild expression of IL-6 was detected in the bronchial-bronchiolar epithelium, perivascular, peribronchial-peribronchiolar and interstitial tissues (Figure 2-C). A statistically significant difference was found when compared to the BLM group ($p< 0.05$). Intracytoplasmic NF-κB expression was observed at mild levels in regions of cell infiltration in the mononuclear cells. A statistically significant difference ($p< 0.05$) was detected when compared to the BLM group (Figure 3-C). The lung tissue in the Theo group exhibited negative IL-6 and NF-κB expressions (Figure 2,3-D). The immunohistochemical findings are summarized in Table I.

Table I presents the histopathological and immunohistochemical (IL-6 and NF-κB) scores of the effects of BLM and Theo on the lung tissues of rats with BLM-induced PF.

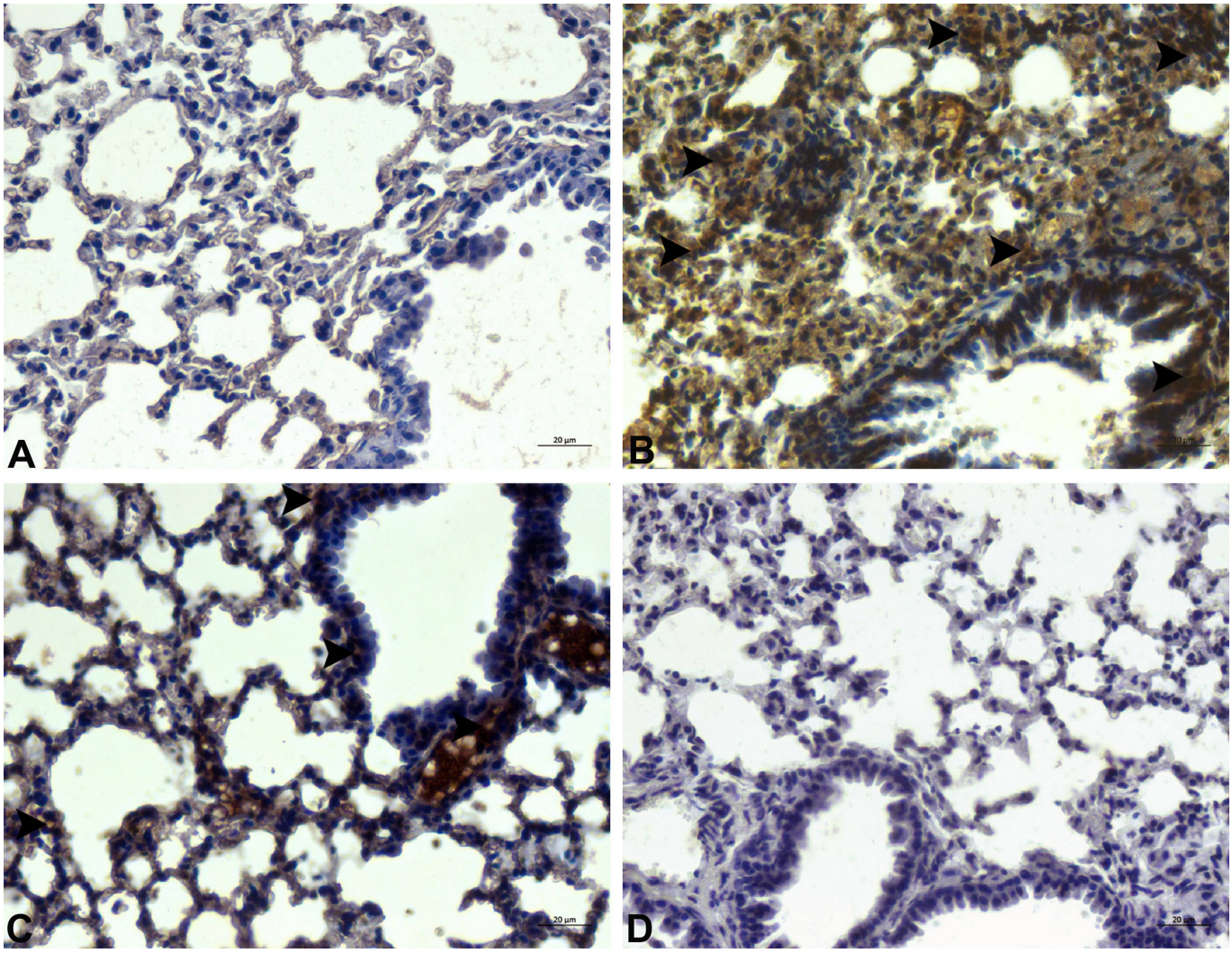


**FIGURE 2 -** Lung tissue, control group, negative expression of NF-κB and IL-6 (A), BLM group (arrowheads), severe IL-6 expression determined in the bronchial-bronchiolar epithelium, perivascular, peribronchiolar, and interstitial tissues (B), BLM+Theo group, bronchial-bronchiolar epithelium, perivascular, peribronchiolar, and interstitial tissues IL-6 expression detected at mild levels (C), Theo group, no obvious expression of NF-κB or IL-6 (D), IHC-P, Bar: 20 µm.

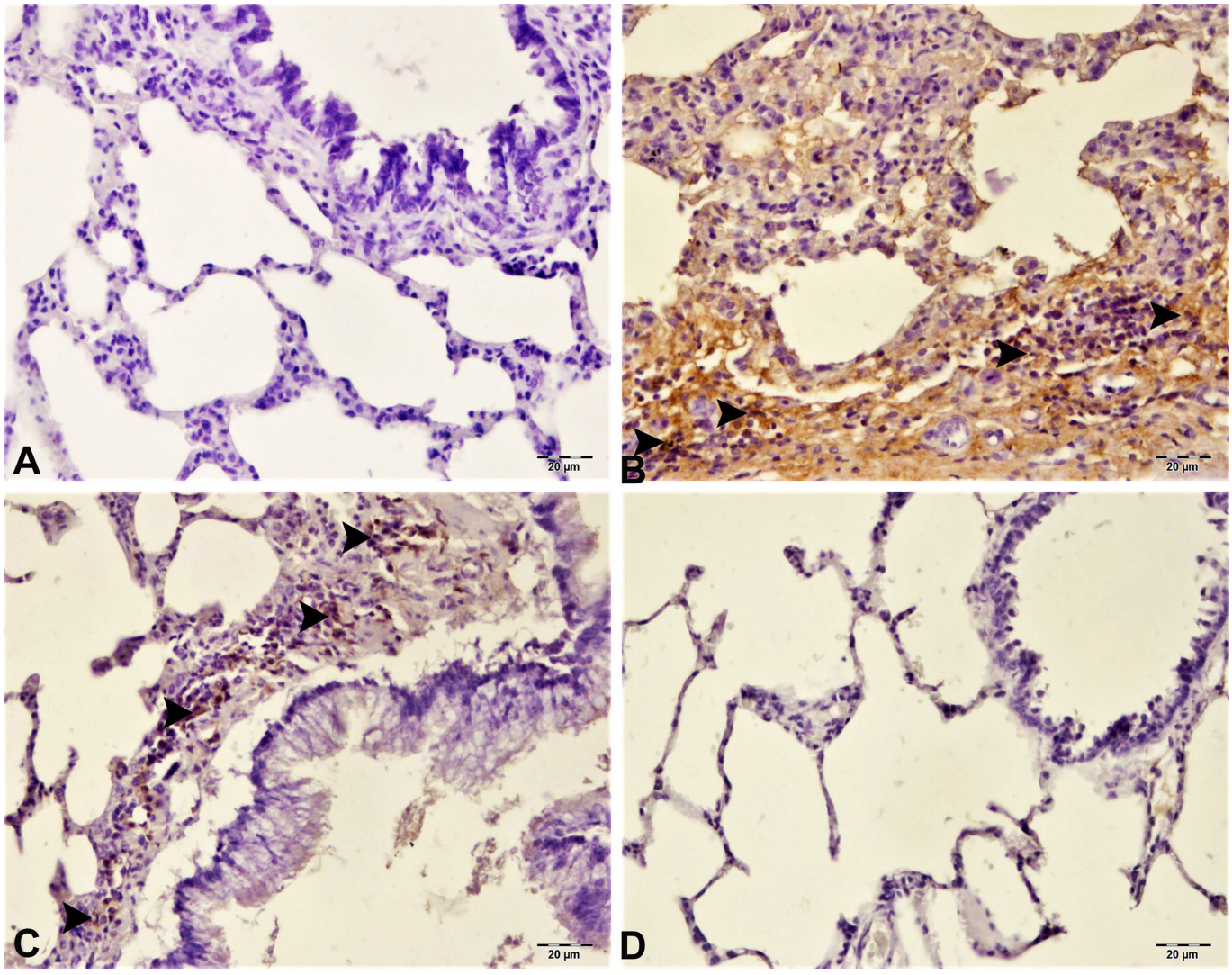


**FIGURE 3 -** Lung tissue, control group, negative expression of NF-κB (A), BLM group (arrowheads), intracytoplasmic NF-κB expression observed in areas with peribronchial-peribronchiolar cell infiltration in mononuclear cells (B), BLM+Theo group, intracytoplasmic NF-κB expression observed at mild levels in regions of cell infiltration in mononuclear cells (C), Theo group, negative expression of NF-κB (D), IHC-P, Bar: 20 µm.

## Biochemical results

BLM-induced oxidative stress was assessed using the antioxidant enzyme activity of SOD, GSH-Px, and CAT, and the MDA and GSH levels the in lung tissue samples, as shown in Table II.

In the BLM group, a significant reduction was observed in the GSH-Px and CAT enzyme activities, and GSH levels ($p< 0.05$, $p< 0.05$, $p< 0.001$, respectively), while the MDA levels ($p<0.001$) were significantly increased when compared to the control group. However, the MDA levels in the BLM+Theo group were also significantly higher than those in the control group ($p< 0.01$). Similarly, the GSH levels were significantly higher in the BLM+Theo group than in the BLM group ($p< 0.05$). The GSH-Px, CAT, and GSH levels in the Theo group were significantly lower than those in the BLM group ($p< 0.05$, $p< 0.01$, $p< 0.01$). Moreover, the Theo group demonstrated a significant difference in the MDA levels ($p< 0.001$).

**TABLE II -** The mean antioxidant enzymes (SOD, CAT and GSH-Px activities), MDA and GSH levels in control, BLMs, BLMs+theo and Theo groups in lung tissue samples

| Parameters | Control ($\overline{X}$ ± SD) | BLMs ($\overline{X}$ ± SD) | BLMs+theo ($\overline{X}$ ± SD) | Theo ($\overline{X}$ ± SD) |
|---|---|---|---|---|
| **GSH-Px (IU/g)** | 4.37 ± 0.48[c] | 3.52 ± 0.49[c,c1] | 3.77 ± 0.59 | 4.24 ± 0.50[c] |
| **CAT (IU/g)** | 25.99 ± 6.86[c] | 15.35 ± 3.72[c,b] | 20.17 ± 8.26 | 27.27 ± 7.93[b] |
| **SOD (IU/g)** | 253.18 ± 7.63 | 260.79 ± 10.27 | 256.85 ± 1.25 | 254.58 ± 6.80 |
| **GSH (µmol/g)** | 0.42 ± 0.069[a] | 0.30 ± 0.014[a,c,b] | 0.38 ± 0.036[c] | 0.39 ± 0.053[b] |
| **MDA (nmol/g)** | 8.66 ± 0.34[a,b] | 9.93 ± 0.54[a,a1] | 9.80 ± 0.64[b,b1] | 8.64 ± 0.61[a1,b1] |

a,a1: $p<0.001$, b,b1: $p<0.01$, c,c1: $p<0.05$ (Different letters, significant, differences between groups).

## DISCUSSION

Histopathological examination of the lung tissue samples showed a significant reduction of fibrotic areas due to the protective properties of the Theo at a dose of 75 mg/kg. Sections of the lung tissues from the BLM+Theo group showed mild interstitial pneumonia, moderate hyperemia in the vessels, and desquamation in the bronchial-bronchiolar epithelium (Figure 1-C). These results demonstrated that Theo was able to mitigate the histopathological architecture caused by BLM administration. Theo was able to reduce BLM-induced PF in rats, and this useful effect was related to the inhibition of BLM-induced lung inflammation and the decrease of interstitial pneumonia, hyperemia, and infiltration of inflammatory cells in the lungs. In the current study, the fibrosis scoring analysis established that the PF detected in the BLM+Theo group was lower than that in the BLM group (Table I).

BLM cytotoxicity is mediated by 2 major structural components, comprising a bithiazole ring, which partly intercalates in the DNA helix, pyrimidine, and imidazole (Iyer *et al*., 2009). The lungs are constantly exposed to relatively higher oxygen pressure than other organs. Exogenous oxidants may elevate and activate inflammatory cells, resulting in the production of free radicals in the lungs (He *et al*., 2015).

The expression of IL-6 and NF-κB may play a key role in lung inflammation and fibrosis. In the present study, the results showed an attenuation of inflammation with the administration of Theo in addition to BLM.

Inflammation and structural cells, including neutrophils, macrophages, and epithelial cells, which are activated in the airways of patients with chronic obstructive pulmonary diseases (COPD), cause oxidative stress, which activate some transcription factors. For example, NF-κB activates several inflammatory genes and leads to an increase in the inflammatory response (Gallelli *et al*., 2017). Theo was originally used as a bronchodilator; however, lately it has been found to have antiinflammatory effects, dependent on the concentration. Selective molecular effect mechanisms, especially NF-κB inhibition, have been proposed for Theo (Chang *et al*., 2017). It has been reported that low-doses of Theo alleviated airway inflammation by reducing eosinophils, IL-8, and TNF-α in the sputum of patients with COPD (Shih *et al*., 2017).

Dong *et al*. (2012) examined lung tissue samples obtained from BLM-treated rats and observed that IL-6 levels were significantly increased when compared to the control group. The overexpression of cytokines, which have been associated with PF in animal models, especially IL-6, TGF-β1, and platelet-derived growth factor, has been known to induce the development of fibrosis. Another study by He *et al*. (2015) evaluated BLM-treated rats and found that the bronchoalveolar lavage fluid (BALF) IL6 and TNFα levels were higher than those in the control group. These data showed

that the administration of Tanshinone IIA reduced proinflammatory cytokine release and inflammatory cell infiltration in BLM-treated rats.

Proinflammatory cytokines, including IL-6 and TNF-α, which increase during the initial phase of PF, are essential for the development of initial pulmonary inflammation to PF (Zhang *et al*., 2016). TNF-α is an inflammatory cytokine that is secreted by activated alveolar macrophages and has been highly involved in the pathogenesis of PF, and seen as a potential use for therapeutic interventions. IL-6 released from alveolar macrophages is also involved in the pathogenesis of human and experimental PF (Serrano-Mollar *et al*., 2003). NF-κB is a transcription factor that is composed of p65 and p50 subunits of the Rel protein family. It activates the transcription of many cytokines, such as TNF-α, IL-1, and IL-6, which are thought to be important for the production of acute inflammatory responses. It has been found that oxidants, in addition to their direct damaging effects, may also up-regulate cytokine production by the activation of NF-κB, and therefore promote PF (Kalayarasan, Sriram, Sudhandiran, 2008).

Min *et al*., 2015 determined that there was a significant increase in lung tissue MDA and IL-6 levels ($p< 0.05p< 0.05$, respectively) in the BLM treatment group when compared to the control group. By contrast, decrease in the SOD ($p< 0.05$) and GSH ($p< 0.05$) in the BLM treatment group was found in their study. The angiotensinconverting enzyme 2 gene (ACE2uMSC) group also showed a significant reduction in the MDA levels and increase in GSH levels when compared with the uMSC group ($p < 0.05$). The expression of inflammation factor (IL6) was significantly reduced in the ACE2uMSC group when compared to that in the BLM group ($p < 0.05$). Another study by Altintas *et al*. (2016) reported that lung tissue SOD, GSH-Px, CAT, and GSH levels decreased significantly ($p < 0.001$ for all) in the BLM group than in the control group. In contrast, the MDA and IL-6 levels were significantly higher ($p < 0.001$ for all) in the BLM-induced lung fibrosis group when compared to the control group.

The study of Serrano-Mollar *et al*. (2003) examined the protective effects of N-acetylcysteine (300 mg/kg) on the lung tissues of rats. They observed that lung tissue GSH levels were significantly decreased in the BLM group ($p< 0.05$), in addition to increased TNF-α levels and the immunohistochemical detection of NF-κB after exposure to BLM. The increased TNF-α levels may have been associated with its blocking effect on the activation of NF-κB. Following N-acetylcysteine (300 mg/kg) treatment, reduced levels of inflammatory cytokines and increased GSH levels were observed. In a different study, Liu *et al*., (2012) examined BALFs taken from rats, and determined that the IL-6 and MDA levels in BLM group were increased; however, the proinflammatory cytokine (IL-6) ($p< 0.01$) and MDA levels were significantly decreased with oxymatrine administration when compared with the BLM group. Another study by Ruan *et al*. (2020) examined BLM-induced inflammatory response and the protective effect of anlotinib on alveolar lavage fluid and in the lung tissues of mice. They determined that the concentration of inflammatory factor (IL-6) and MDA levels were significantly ($p< 0.05$) increased and the SOD enzyme activity was decreased after the injection of BLM. However, the IL-6 and MDA levels decreased and SOD enzyme activity increased with the administration of anlotinib when compared with the BLM group ($p< 0.05$).

In this study, the significant elevation in the IL-6 and MDA levels was consistent with those reported in the literature (Liu *et al*., 2012; Serrano-Mollar *et al*., 2003; He *et al*., 2015; Dong *et al*., 2012; Min *et al*., 2015; Altintas *et al*., 2016; Ruan *et al*., 2020). In the present findings, the significant decrease in the GSH-Px, CAT, and GSH levels in the lung tissues of rats with BLM-induced lung fibrosis were in agreement with the values obtained from earlier studies (Serrano-Mollar *et al*., 2003; Min *et al*., 2015; Altintas *et al*., 2016). The finding of significantly decreased SOD levels in the BLM group was not supported by the other research (Min *et al*., 2015; Altintas *et al*., 2016). However, decreased NF-κB levels were in agreement with the data of Serrano-Mollar *et al* (2003).

It is clear that BLM may cause injury to the epithelium through the formation of ROS. After which, T cells are recruited and release the factors that are responsible for the differentiation and recruitment of monocytes/macrophages (Ayaub *et al*., 2016). A large

number of free radicals in lung tissue cause injury due to inflammation and lipid peroxidation during early PF, which raises the proliferation of fibroblasts and alveolar-interstitial fibrosis (Zhang *et al*., 2016).

To the best of our knowledge, this was the first experimental study to examine the lung tissues of male Wistar albino rats with BLM-induced toxicity and demonstrate the protective antiinflammatory and antifibrotic roles of Theo on the SOD, GSH-Px, CAT, GSH, and MDA levels, histopathological and immunohistochemical alterations, and IL-6 and NF-κB expression.

In the current study, the role of proinflammatory cytokines, such as the expression of IL-6 in BLM-induced PF in rats, was assessed. Treatment with Theo significantly inhibited NF-κB expression when compared to treatment with BLM alone. BLM led to a significant reduction in the CAT and GSH-Px enzyme activities in the lung tissues when compared to the control group. Treatment with Theo had a significant effect on the antioxidant enzyme levels when compared to treatment with BLM alone. In the current study, the decrease in the CAT, GSH-Px, and GSH levels in the lung tissues of the BLM-treated rats might have been as a result of increased free radical formation and lipid peroxidation. Treatment with Theo significantly increased the GSH levels ($p < 0.05$) in the BLM-treated rats.

These findings suggested that treatment with Theo resulted in a significant increase in antioxidant enzymes (GSH-Px, CAT) and GSH levels as well as a significant decrease in MDA levels, and IL-6 and NF-κB expression, together with a decrease in inflammatory cells, and the fibrosis score connected with amelioration of the immunohistochemical and histopathological architecture compared when with BLM treatment alone. Moreover, these results indicated that Theo regulated the epithelium, perivascular, peribronchial-peribronchiolar, interstitial tissues, mononuclear cells, and the expression of fibrotic cytokines from the cells and played an important role in preventing lung fibrosis.

## CONCLUSION

The findings of the current study revealed that treatment with Theo was effective in decreasing lung injury induced by BLM in rats. Theo significantly attenuated PF, reduced MDA levels, and increased CAT and GSH-Px enzyme activities, and GSH levels. It was seen that the administration of Theo significantly improved BLMinduced histopathological and immunohistochemical alterations. In the current study, decreased inflammatory cytokine and IL-6 expression was related to the decrease in the degree of inflammation in the lung fibrosis. The lung-protective effects of Theo are linked with the inhibition of NF-κB and amelioration of interstitial pneumonia, hyperemia, and inflammatory cell infiltration in the lungs. Moreover, it has the ability to attenuate cytokines, contributing to its antifibrotic effect. The results of this study revealed that Theo may protect rats against BLM-induced PF by decreasing the expression of IL-6 and NF-κB as a result of its antiinflammatory effects. The present study demonstrated that abnormal free radical generation in BLMtreated rats was inhibited via treatment with Theo.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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