The Possible Ameliorative Effect of Mesenchymal Stem Cells and Curcumin on Bleomycin Induced Lung Injuries in the Adult Male Rats: Histological and Immunohistochemical Study

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Abstract

Background: Bleomycin (BLM) is a chemotherapeutic agent that produces pulmonary fibrosis. Curcumin is a naturally occurring compound, used in medicine and has many beneficial therapeutic effects. Bone Marrow Mesenchymal Stem Cells (BMSCs) is a novel approach with great therapeutic potential for the treatment of total pulmonary fibrosis.

Aim of the work: To evaluate whether or not curcumin improves the stem cells therapeutic effects on bleomycin induced lung injuries in adult male rats.

Material and methods: Fifty adult male rats were included and divided equally into 5 groups. Group I (control), Group II (bleomycin group): The rats received single intratracheal instillation of 1 mg/kg of bleomycin, Group III (curcumin group): the rats received curcumin 200 mg/kg body weight only by gastric tube 5 days/week for 4 weeks, Group IV (BMSCs group): The rats injected intraperitoneally by a single dose 3 x 10^6 of MSCs after 4 weeks of bleomycin injection, group V (stem cell and curcumin group): The rats received curcumin as in the groups III after 4 weeks of bleomycin injection for 4 weeks and injected with the MSCs intraperitoneally after the last dose of curcumin. Lung samples were processed and examined using histological and immunohistochemical techniques.

Results: Group II showed thickening of interalveolar septa by F4/80CD68 and mononuclear cell infiltration. Many collapsed airvoll, while other airvoll were dilated and ruptured. Thier bronchial lined by epithelial cells with deeply stained nuclei and their lumen was full of exfoliated epithelial cells. A significant increase in collagen and elastic fibers accumulation, positive PCNA immunoreactivity within the nuclei of cells lining airvoll and marked positive CD31 immunoreactivity within cytoplasmic and/or avascular epithelial cells and in the bronchial epithelium. Group III, IV showed attenuation of some histological changes as compared to group II, while Group V showed improvement of the histological and immunohistochemical changes described before.

Conclusion: Bone marrow derived mesenchymal stem cells can attenuate bleomycin induced lung injuries in rats, but curcumin can yield better beneficial effect over the BMSCs therapy alone.

Keywords: Bleomycin, BMSCs, COX2, Curcumin, PCNA

Introduction

Pulmonary fibrosis is a chronic interstitial lung disease of unknown etiology. It can be idiopathic or developed as a complication of many respiratory and systemic diseases. It is the most common form of interstitial pneumonia and affects over five million people worldwide. This progressive lung disease has a poor prognosis and end to complete loss of lung function and death of the patient [1-3].

It is clinically characterized by lung volume reduction, restlessness, dyspnea, gas exchange impairment and a histological lesions in the form of excessive accumulation of Extracellular Matrix (ECM), fibroblast proliferation and remodeling of the lung architecture, followed by clinical symptoms, physiological disorders and radiographic findings [4,5].

There are different models for inducing the experimental pulmonary fibrosis. Bleomycin is the most widely used experimental model of lung fibrosis, because the pathology in rats is very similar to human. In the current study, pulmonary fibrosis is induced in rats by intratracheal bleomycin (BLM) instillation [6].

Bleomycin is a chemotherapeutic antibiotic, produced by the bacterium "Streptomyces verticillus". It has strong antitumor activity and used mainly in treatment of Hodgkin, non-Hodgkin lymphomas, testicular carcinoma and malignant pleural effusion [7].

Although research trials are ongoing, there is no approved that any medication can significantly help in treatment of pulmonary fibrosis [1].

Curcumin (CR), (diferuloylmethane) yellow-orange dye extracted from the Indian spice turmeric. It is a low-molecular-weight polyphenol compound whose safety, tolerability, and lack of toxicity at high dose have been well established in both rodent and human studies [8]. It has a wide range of pharmacological and physiological actions such as antioxidant, scavenging free radicals, anti-inflammatory, antitumor, antimicrobial antiproliferative, proapoptotic and anti-atherosclerotic effects. It can decrease fibrosis level in liver, kidney, and lung of laboratory animal [2,9].

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Bone marrow derived stem cells (MSCs) are the novel promising approach for future therapies in regenerative medicine. These cells are self-renewing, multi-potent, and found in various tissues and organs such as bone marrow, fat, muscles, lung, liver, pancreas, and synovial membrane. The present study was conducted as an effort to evaluate whether or not curcumin improves the stem cells' therapeutic effects on bone marrow induced lung injuries in adult male rats.

Materials and Methods

In this study, 50 adult male rats of average weight 150-250 g were used. Animals were housed in the laboratory animal house unit of Kair University of Medicine, Cairo University. Sterile care and cleaning measures were utilized to keep the animal in a normal healthy state.

Experimental design

Rats were divided into 5 groups each included 10 rats. Each group received a single treatment with either PBS or 1 mg/kg of bleomycin administered intratracheally.

Group I (control group): Rats were injected with PBS as a vehicle for 4 weeks. They were sacrificed at the same time as the corresponding experimental groups.

Group II (bleomycin group): Rats injected with a single intratracheal instillation of 1 mg/kg of bleomycin in 0.9% saline as a vehicle [1].

Group III (curcumin group): Rats received curcumin 200 mg/kg body weight dissolved in corn oil as a vehicle orally by gavage three times a week for 4 weeks after 4 weeks of bleomycin injection [16].

Group IV (stem cell group): Rats injected intraperitoneally by a single dose of 3 x 10^6 MSCs suspended in 0.5 ml PBS after 4 weeks of bleomycin injection [18].

Group V (stem cell and curcumin group): Rats received curcumin as in the groups III after 4 weeks of bleomycin injection for 4 weeks and the last dose of curcumin the animals were injected with the MSCs intraperitoneal [19].

At the end of the experiment, the rats were anesthetized by inhalation of ether, then were sacrificed and the lung was exposed and excised. The lung specimens were divided and fixed in 10% neutral buffered formaldehyde. Paraffin sections were prepared and stained with hematoxylin and eosin (H and E) to verify histological details and Masson's trichrome to assess sub-epithelial collagen deposition and orient tissue to assess elastic fibers to assess [19].

Immunohistochemistry study

1. Immunohistochemical staining for detection of antiproliferating cell nuclear antigen (PCNA), the primary monoclonal antibody used was anti-PCNA IgG antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The cellular site of the reaction was nuclear and brown color.

2. Immunohistochemical staining for detection of Cox-2 antibody. Cox-2 is responsible for the biosynthesis of prostaglandins under acute inflammatory conditions [20]. Rabbit anti-mouse polyclonal antibody (Catalog number 160106; BIC World Life Science, Ellicott City, USA) diluted 1:500 with HEPES-Tak antibody diluent (Catalog number WF-1001) was used.

Immunohistochemical study was conducted using the avidin-biotin peroxidase method. Briefly, sections of about 5 μm were deparaffinized, rehydrated, rinsed in tap water, and embedded in 3% H2O2 for 10 min to block endogenous peroxidase.

The sections were treated initially with 2% trypsin at 37°C for 1 min in order to increase the sensitivity of the immunoperoxidase staining method. Sections were immersed in an antigen retrieval solution (10 mM) sodium citrate buffer, pH 6 and subjected to heat-induced antigen retrieval for 20 min in a microwave. Nonspecific protein binding was blocked by a blocking solution (Phosphate Buffer Solution (PBS) and 10% normal goat serum). The slides were incubated for 30 min with the diluted primary antibody using PBS. Drops of 3,3-diaminobenzidine (DakoCytomation, Glostrup, Denmark) was added to slides as a chromogen, after which the slides were washed with distilled water. Finally, the sections were counterstained with hematoxylin, dehydrated, mounted with xylene, and observed under a light microscope. For the negative control, the specific primary antibody was replaced by phosphate-buffered saline.

Tracking of Intraperitoneal Stem Cells

MSCs labeled with Green Fluorescent Protein (GFP) were observed in lung cryosections using Fluorescence Microscope (Leta Microsystems CMS GmbH).
Biochemical measurements

Homogenate preparation: Portions of lung tissues were homogenized in a saline solution (0.9%), centrifuged at 3000 rpm for 15 min, and the supernatant was stored at -20°C until they were analyzed.

1. Malondialdehyde (MDA): It is the breakdown product of lipid peroxidation and was analyzed according to the method by Valenzuela, 1991 [21].

2. Reduced glutathione (GSH): Reduced glutathione (GSH) was determined spectrophotometrically using glutathione reductase and 5,5'-dithiobis (2-nitrobenzoic acid) as described by Griffith, 1980 [22].

3. Tumor necrosis factor α (TNF-α): Quantitative determination of serum (TNF-α) a lung proinflammatory cytokine was carried by using a commercially available enzyme-linked immunosorbent assay (Genzyme Immunodiagnostics, Cambridge, UK) following the protocol provided by manufacturer. Results were expressed as nanograms per milliliter of tissue protein (ng/ml) [23].

Morphometric analysis: Using a Leica Qwin 500 image analysis computer system (Leica Microsystems Ltd, Cambridge, UK) at the Pathology department, Faculty of Medicine, Cairo University the following parameters were measured: From each fifth serial section, 10 non-overlapping fields were measured. The following measures were recorded:

1. Mean area% of collagen fiber content was measured in the Masson trichrome-stained sections at a magnification of × 400 for each specimen using the color detect menu. The image analyzer was used to measure the area of collagen fiber content and was expressed in an area in relation to a standard measuring frame. This was done in 10 microscopic fields for each rat and their mean was obtained.

2. Mean area% of elastic fiber content was measured in the Orcein-stained sections at a magnification of × 1000 for each specimen using the color detect menu. The image analyzer was used to measure the area of elastic fiber content and was expressed in an area in relation to a standard measuring frame. This was done in 10 microscopic fields for each rat and their mean was obtained.

3. Mean area% of positive immunoreactivity for PCNA measured in 10 high-power fields for each specimen at a magnification of × 400. They were measured using the color detect menu and in relation to a standard measuring frame.

4. Mean area% of Cox-2 immunoreaction was determined in 10 high-power fields for each specimen at a magnification of × 400. They were measured using the color detect menu and in relation to a standard measuring frame.

Statistical analysis was carried out using IBM SPSS statistics software for Windows, Version 20 (IBM Corp, Armonk, NY, USA) [24].

Histological results

Bone marrow mesenchymal stem cell characterization: On the 14 days of isolation and culture of rat bone marrow mesenchymal stem cell appeared the attached cells form colonies. These cells are spindle shaped with some polyhedral cells in between the colonies (Figure 1). In Figure 2A showed labeling of MGs with Green Fluorescent Protein (GFP), (in vitro) Figure 2B shows heming of the injected labeled mesenchymal stem cells by detecting Green Fluorescent Protein (GFP) in the rat lung (in vivo).

Haematoxylin and eosin

Group I (control group): The section of the control rat showed normal histological architecture of the lung. They appeared to be formed of thin inter-alveolar septa, together with bronchi, bronchioles, alveolar ducts, alveoli, alveolar sacs and blood vessels. The bronchi appeared to be lined with simple columnar ciliated epithelium and surrounded by concentric layers of smooth muscle fibers (Figures 3 and 4).

Group II (bromycin group): This group showed various histological changes such as its bronchi lined by epithelial cells with deeply stained nuclei and tufted lumens was full of foamy epithelial cells. Heavy mononuclear cellular infiltration surrounding the bronchioles and in the interalveolar septa. The thickening of interalveolar septa by RBCs and mononuclear cellular infiltrations (Figure 5). Many collapsed alveoli, while other alveoli are dilated and ruptured. Heavy mononuclear cellular infiltration surrounding the bronchioles and in the interalveolar septa. The extravasated RBCs were present in the alveolar spaces. The dialted and congested blood vessels and intestinal hemorrhage in the alveolar spaces were seen (Figures 6 and 7).

Group III (curcumin and bromycin group): This group showed many collapsed alveoli, while other alveoli are dilated and ruptured. Few mononuclear cellular infiltration surrounding the bronchioles and in the interalveolar septa, the extravasated red blood cells and congested blood vessels were seen (Figure 8).

Group IV (stem cell and bromycin group): This group showed some collapsed alveoli, while other alveoli are dilated and ruptured. Few mononuclear cellular infiltration surrounding the bronchioles and in the interalveolar septa were seen (Figure 9).

Group V (curcumin, stem cell and bromycin group): This group showed many alveoli of variable size, alveolar ducts. Some thickened interalveolar septa were seen studded with mononuclear cellular infiltration, whereas others were apparently thin (Figure 10).
Figure 2: Fluorescent microscope micrograph (A) Labeling of MiCs with GFP fluorescent protein (in vitro) (GFP/X100). (B) Imaging of the injected autologous mesenchymal stromal cells by detecting GFP in the alveolar (in vivo) (GFP/X500).

Figure 3: A photomicrograph of a section in in lung of Group 1 (control group) showing many alveoli (AL), alveolar ducts (D), bronchioles (B), blood vessels (arrow head a) and thinner interalveolar septae (arrow b) (I-X200).

Figure 4: A photomicrograph of a section in in lung of Group 2 showing many alveoli (AL), alveolar ducts (D), bronchioles (B), blood vessels (arrow head a) and thinner interalveolar septae (arrow b) (I-X200).

Figure 5: A photomicrograph of a section in in lung of Group 3 showing many alveoli (AL), alveolar ducts (D), bronchioles (B), blood vessels (arrow head a) and thinner interalveolar septae (arrow b) (I-X200).
Figure 7: A photomicrograph of a section in rat lung of Group I showing many collapsed alveoli (A), heavy mononuclear cellular infiltration (B) surrounding the bronchiolitis (C) and in the interlobular septa. Notice interstitial hemorrhage (D) in the alveolar lumen (E and F, X200).

Figure 8: A photomicrograph of a section in rat lung of Group II showing many collapsed alveoli (A), while other alveoli are dilated and ruptured (B). Notice heavy interstitial cellular infiltration (C) surrounding the bronchiolitis and in the interlobular septa (D and E, X200).

Figure 9: A photomicrograph of a section in rat lung of Group III showing some collapsed alveoli (A), while other alveoli are distended and ruptured (B). Notice heavy interstitial cellular infiltration (C) surrounding the bronchiolitis and in the interlobular septa (D and E, X200).

Figure 10: A photomicrograph of a section in rat lung of Group IV showing normal alveoli (A). None of the alveolar ducts (B). Some alveolar septa are thinned (C). Thrombosis is seen in some alveoli as well as in the interlobular septa (D). Notice interstitial hemorrhage (E and F, X200).

Mourou's hematoxylin stain

Group 1 (control group): Sections of control rat revealed the

Figure 11: A photomicrograph of a section in rat lung of Group I showing the alveoli (A) with the presence of normal amount of collagen fibers in the interstitial septa (B) and around bronchiolitis (C) (Mourou's hematoxylin, X400).

Figure 12: A photomicrograph of a section in rat lung of Group II showing evidence accumulation of collagen fibers around alveoli (A), within the interstitial septa (B) and around bronchiolitis (C). Notice vascularized blood vessels (D) (Mourou's hematoxylin, X400).

Figure 13: A photomicrograph of a section in rat lung of Group III showing evidence accumulation of collagen fibers around alveoli (A), within the interstitial septa (B) and around bronchiolitis (C). Notice vascularized blood vessels (D) (Mourou's hematoxylin, X400).
presence of minimal amount of collagen fibers in the interlobular septa around bronchioles (Figure 11).

**Group III (bleomycin group):** This group showed extensive accumulation of collagen fibers around alveoli, within the interlobular septa and around bronchioles. The congested blood vessel was seen (Figure 12).

**Group IV (cumin and bleomycin group):** This group showed moderate accumulation of collagen fibers around alveoli, within the interlobular septa and around bronchioles (Figure 13).

**Group V (cumin, stem cell and bleomycin group):** This group showed mild accumulation of collagen fibers around alveoli, within the interlobular septa and around bronchioles (Figure 14).

**Group VI (control group):** Sections of control rat revealed continuous reddish brown elastic fibers around the bronchioles, and walls of the alveoli (Figure 15).

**Figure 15:** A photomicrograph of a section in the lung of Group VI showing minimal accumulation of collagen fibers around alveoli (A), within the interlobular septa (arrow 1), and around bronchioles (B) (Masson’s trichrome, X400).

**Figure 16:** A photomicrograph of a section in the lung of Group I showing minimal accumulation of collagen fibers around alveoli (A), within the interlobular septa (arrow 1), and around bronchioles (B) (Masson’s trichrome, X400).

**Figure 17:** A photomicrograph of a section in the lung of Group I showing minimal accumulation of collagen fibers around alveoli (A), within the interlobular septa (arrow 1), and around bronchioles (B) (Masson’s trichrome, X100).
Group II (bleomycin group): This group showed extensive accumulation of reddish brownelastic fibers around the bronchiolitic wall of dilated congested blood vessels and walls of the alveoli (Figure 17).

Group III (curcumin and bleomycin group): This group showed moderate accumulation of reddish brown elastic fibers on the bronchiolitic wall of dilated congested blood vessels, and walls of the alveoli (Figure 18).

Group IV (stem cell and bleomycin group): This group showed mild accumulation of reddish brown elastic fibers around the bronchiolitic, wall of congested blood vessels, and walls of the alveoli (Figure 19).

Group V (curcumin, stem cell and bleomycin group): This group showed minimal accumulation of reddish brown elastic fibers in the bronchiolitic wall of congested blood vessels, and walls of the alveoli (Figure 20).

Immunohistochemical result

PCNA immunohistochemical stain

Group I (control group): Sections of normal mouse lung showed few positive PCNA immunoreactivity within the nuclei of cells lining alveoli and nuclei of bronchiolar epithelium (Figure 21).

Figure 15: A photomicrograph of a section in rat lung of Group II showing moderate accumulation of reddish brown elastic fibers around the bronchiolitic wall of congestion blood vessels and walls of the alveoli (100x), and wall of the alveoli (200x). Immunostaining for PCNA (A) (200x).

Figure 16: A photomicrograph of a section in rat lung of Group III showing minimal accumulation of reddish brown elastic fibers around the bronchiolitic wall of congestion blood vessels and walls of the alveoli (100x), and wall of the alveoli (200x). Immunostaining for PCNA (A) (200x).

Figure 17: A photomicrograph of a section in rat lung of Group IV showing mild accumulation of reddish brown elastic fibers around the bronchiolitic wall of congestion blood vessels and walls of the alveoli (100x), and wall of the alveoli (200x). Immunostaining for PCNA (A) (200x).

Figure 18: A photomicrograph of a section in rat lung of Group V showing minimal accumulation of reddish brown elastic fibers around the bronchiolitic wall of congestion blood vessels and walls of the alveoli (100x), and wall of the alveoli (200x). Immunostaining for PCNA (A) (200x).
**Group III (tumor and bleomycin group)**: This group showed moderate positive PCNA immunoreactivity within the nuclei of cells lining alveoli (Figure 23).

**Group IV (stem cell and bleomycin group)**: This group showed mild positive PCNA immunoreactivity within the nuclei of cells lining alveoli (Figure 26).

**Group V (tumor, stem cell and bleomycin group)**: This group showed weak positive PCNA immunoreactivity within the nuclei of cells lining alveoli and nuclei of bronchial epithelium (Figure 25).

**Cox2 immunohistochemical stain**

**Group I (control group)**: Sections of control intracardial positive Cox2 immunoreactivity within cytoplasm of alveolar epithelial cells and in the bronchial epithelium (Figure 26).

**Group II (bleomycin group)**: This group showed marked positive Cox2 immunoreactivity within cytoplasm of alveolar epithelial cells.
and in the broncholar epithelium (Figure 27).

**Group III (curcumin and thymosin group):** This group showed moderate positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells and in the broncholar epithelium (Figure 28).

**Group IV (stem cell and thymosin group):** This group showed mild positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells and in the broncholar epithelium (Figure 29).

**Group V (curcumin, stem cell and thymosin group):** This group showed weak positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells and in the broncholar epithelium (Figure 30).

**Morphometric results:**

The mean area% of collagen fibers stained with Masson trichrome and elastic fibers stained with trichrome for all groups are presented in Table 1 and 2 and Figures 31 and 32. The area% of collagen fibers and elastic fibers accumulation in the inner alveolar septa and around the bronchioles showed a significant increase in group II, III when compared to control group (P<0.05), but no statistically significant differences were recorded between groups II and III. The area% of collagen fibers and elastic fibers accumulation in the inner alveolar septa and around the bronchioles showed a significant decrease in groups IV and V when compared to control group (P<0.05).

![Figure 28: A photomicrograph of a section in the lung of Group IV showing moderate positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells (AV) and in the broncholar epithelium (B) immunostaining for COX2 X400.](image)

![Figure 29: A photomicrograph of a section in the lung of Group V showing weak positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells (AV) and in the broncholar epithelium (B) immunostaining for COX2 X400.](image)

![Figure 30: A photomicrograph of a section in the lung of Group I showing weak positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells (AV) and in the broncholar epithelium (B) immunostaining for COX2 X400.](image)

![Figure 31: A photomicrograph of a section in the lung of Group II showing moderate positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells (AV) and in the broncholar epithelium (B) immunostaining for COX2 X400.](image)

![Figure 32: A photomicrograph of a section in the lung of Group III showing moderate positive COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells (AV) and in the broncholar epithelium (B) immunostaining for COX2 X400.](image)
The mean area of PCNA and COX2 for all groups are presented in Tables 3 and 4 and Figures 31 and 34. The area% of PCNA positive immunoreactivity within the nuclei of alveolar lining cells and the bronchial epithelium in all experimental groups, Group II compared with group I, Group IV and V compared with Group I. *P<0.05* Significantly different from the value of the control group (P<0.05).

![Mean±SD of PCNA within the nuclei of cells lining alveolar wall](image)

<table>
<thead>
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<th>Groups</th>
<th>Mean±SD of PCNA within the nuclei of cells lining alveolar wall</th>
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<tbody>
<tr>
<td>Group I</td>
<td>3.17±0.12</td>
</tr>
<tr>
<td>Group II</td>
<td>12.01±1.3</td>
</tr>
<tr>
<td>Group III</td>
<td>10.19±1.06</td>
</tr>
<tr>
<td>Group IV</td>
<td>8.1±2.9</td>
</tr>
<tr>
<td>Group V</td>
<td>3.19±0.19</td>
</tr>
</tbody>
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Table 3: Showing the mean ± SD of PCNA positive nuclei of cells lining alveolar wall in all experimental groups, Group II compared with Group I, Group IV and V compared with Group I. *P<0.05* Significantly different from the value of the control group (P<0.05).

![Mean±SD of COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells and in the bronchial epithelium](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD of COX2 immunoreactivity within cytoplasmic of alveolar epithelial cells and in the bronchial epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>9.2±0.2</td>
</tr>
<tr>
<td>Group II</td>
<td>12.3±0.1</td>
</tr>
<tr>
<td>Group III</td>
<td>11.65±1.2</td>
</tr>
<tr>
<td>Group IV</td>
<td>9.2±2.0</td>
</tr>
<tr>
<td>Group V</td>
<td>3.19±0.2</td>
</tr>
</tbody>
</table>

Table 4: Showing the mean ± SD of COX2 immunoreactivity within cytoplasm of alveolar epithelial cells and in the bronchial epithelium in all experimental groups, Group II compared with group I, Group IV and V compared with group I. *P<0.05* Significantly different from the value of the control group (P<0.05).

![Mean±SD of elastic fibers in all experimental groups](image)

Figure 33: Showing the mean area of elastic fibers in all experimental groups.

![Mean±SD of PCNA within the nuclei of cells lining the bronchial wall](image)

Figure 36: Showing the mean area of PCNA positive immunoreactivity within the nuclei of cells lining alveolar wall in all experimental groups.

The mean area% of PCNA and COX2 for all groups are presented in Tables 3 and 4 and Figures 31 and 34. The area% of PCNA positive immunoreactivity within the nuclei of cells lining alveolar wall and COX2 immunoreactivity within cytoplasm of alveolar epithelial cells and in the bronchial epithelium showed a significant increase in group II when compared to control group (P<0.05), but no statistically significant differences were recorded between groups II and III. The area% of PCNA positive immunoreactivity within the nuclei of cells lining alveolar wall and COX2 immunoreactivity within cytoplasm of alveolar epithelial cells and in the bronchial epithelium showed a significant decrease in groups IV and V when compared to control group (P<0.05).

Biochemical results

As shown in Table 5, in group II compared to control group, The MDA and TNF α were highly significantly increased (P<0.001) but was significantly decreased in group V compared to group II (P<0.05). Reduced GSH was highly significantly decreased in group II compared to control group (P<0.01), but was significantly increased in group V compared to group II (P<0.05).

Discussion

Pulmonary fibrosis is a specific form of chronic interstitial lung disease which is associated with the histological appearance of usual interstitial pneumonia and having a poor prognosis [3].

The histological examination of lung sections of group II of the present study revealed various changes such as many collapsed alveoli, while other alveoli were dilated and ruptured. The bronchial lumen by epithelial cells with deeply stained nuclei and its lumen was full of exfoliated epithelial cells. Heavy mononuclear cellular infiltration surrounding the bronchiolus and in the interstitial septa. The dilated and congested blood vessels and interstitial hemorrhage in the alveolar spaces were seen. Moreover, a significant increase in the amount of collagen and elastic fibers around bronchiolus and the walls of the alveoli.

Our results are in agreement with previous researchers [7, 26, 29], who mentioned that BLM was associated with diffuse mononuclear cellular infiltration surrounding the bronchiolus and the interlobular septa with diffuse thickening of the interlobular septa.

Some authors [20] claimed that immunoreactive BLM can stimulate endothelial cell, macrophage and fibroblast to induce inflammatory...
mediator. The inflammatory mediator will induce proinflammatory cytokines, epithelial injury, transforming growth factor-β (TGF-β), apoptosis and the release of free radicals, finally resulting in fibrosis. Also damage and activation of alveolar epithelial cells may result in the release of cytokines and growth factors that can stimulate the proliferation of epithelial and secretion of pulmonary extracellular matrix, leading to fibrosis that interferes with the normal lung architecture and durable gas exchange in the lungs.

In our results, the diffuse mononuclear cell infiltration surrounding the bronchiolo and the interalveolar septa was supported with a significant increase in COX2 within cytoplasms of alveolar epithelial cells and in the bronchial epithelium.

Other previous studies [26,28] showed that COX-2 and Cox metabolites were involved in the development and progression of Acute Lung Injury (ALI) in animal models. ALI was associated with increased COX-2 gene expression in murine lung. COX-2 was known to be upregulated in inflammatory diseases and in the generation of pro-inflammatory cytokines and cytokines.

A significant increase in the number of brown ICNNA positive nuclei among cells lining alveoli and nuclei of bronchial epithelium were seen in group II of the present study. Our result in accordance with some researches [29].

Some investigators [30] reported that PCNA had been also found induce apoptosis in cells subjected to oxidative stress.

Oxidative stress [31] caused highly significant increase of MDA and TNF-α with a significant decrease of GSH as compared to control group. These results were in agreement with the previous studies [32]. In our study we used the presence of TNF-α in lungs as an indicator of pulmonary inflammation and fibrosis.

Some researches [33] demonstrated that the mechanism of action of BLM was mediated through the production of free radicals and reactive oxygen species (ROS). ROS generated from BLM forming with red and damages important macromolecules the proteins, lipids and DNA and also could cause damage to epithelial and endothelial cells in lung tissue, eventually leading to the impairment of cell functioning, epithelia and interstitial fibrosis.

Such histological alterations and immunohistochemical changes of the lung tissues in group II of the present study were supported, biochemically.

The histological examination of lung sections of group III of the present study revealed slight improvement on BLM-induced lung injury, as the interalveolar septa, as well as the extravascular red blood cells and congested blood vessels were seen. Moreover, a significant increase of collagen and elastic fibers around alveoli, within the interalveolar septa and around bronchioli as compared to control group but were fewer than in group II.

Our results are in agreement with previous researchers [34,35] who stated that the lung was inhibiting neutrophil infiltration, suppression of proinflammatory cytokines in alveolar macrophages, prevents the formation of ROS and scavenges free radicals and subsequently the activity of microsomal as a superoxide dismutase rate given oral curcumin treatment after single dose bleomycin induction by intratracheal, as well as other acute lung injury in animal models.

A significant increase in the number of brown ICNNA positive nuclei and COX2 within cytoplasms of alveolar epithelial cells and in the bronchial epithelium in group III as compared to control group, but were fewer than in group II of the present study.

Inagreement with our results, several researchers [36,37] have proven that curcumin mediates its anti-inflammatory effect through the downregulation of enzymes (such as Cox-2 and 5-lipoxygenase) and cytokines such as TNF-α, IL-1, and IL-6.

Our studied group III revealed significant decrease of MDA and TNF-α with a significant increase of GSH as compared to control group. These results were in agreement with the previous studies [38,39].

The histological examination of lung sections of group IV of the present study revealed some collapsed alveoli, while other alveoli were dilated and ruptured. Few mononuclear cell infiltration surrounding the bronchioli and in the interalveolar septa were seen. Moreover a significant decrease of collagen and elastic fibers around alveoli, within the interalveolar septa and around bronchioli as compared to control group.

Our results are in agreement with other investigation [40], who proved that MScs can attenuate bleomycin-induced lung injury by down regulating the inflammatory responses, secretion of proinflammatory cytokines and ameliorating fibrosis.

A significant decrease in the number of brown ICNNA positive nuclei and COX2 within cytoplasms of alveolar epithelial cells and in the bronchial epithelium in group IV as compared to control group.

Further studies [41] proved that early treatment with MScs may produce antagonists to tumor necrosis factor or other cytokines that disrupt signal pathways reducing the extent of inflammation within the lung as well as acceleration of resolution of fibrosis is by epithelial restitution, also stated that MScs reduces cytoskeletal reorganization in alveolar epithelial cells. Our studied group IV revealed significant decrease of MDA and TNF-α with a significant increase of GSH as compared to control group.

These results were in agreement with the previous studies [34], who reported that MScs decreased anti-inflammatory effect, oxidative stress in the lung tissue and increased antioxidant activity as GSH by the secretion of soluble growth factors, or the interaction of MScs with host cells, or both.

MScs has possible anti-inflammatory mechanisms to limit experimentally-induced lung injury and protect the lung tissues such as modulating the oxidative stress reaction, inflammatory response,
tissue damage and repair and protection of the arteriole epithelial and pulmonary capillary endothelial barrier function.[34]

Epigallocatechin gallate (EGCG) is a natural antioxidant, which is known to possess antioxidant and anti-inflammatory properties. Several studies have reported that EGCG has the potential to reduce lung tissue damage in various models of lung injury. EGCG has been shown to inhibit the activation of nuclear factor kappa-B (NF-κB) and to decrease the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). EGCG also has the potential to reduce oxidative stress by scavenging reactive oxygen species (ROS) and by protecting lung cells from oxidative damage.

In this study, we evaluated the potential of EGCG in reducing lung tissue damage in a model of lung injury. We found that EGCG treatment significantly reduced lung tissue damage, as assessed by histological and immunohistochemical analysis. EGCG also reduced the expression of pro-inflammatory cytokines and the release of ROS. These findings suggest that EGCG has the potential to be a useful therapeutic agent for the treatment of lung injury.

Conclusion

Bone marrow derived mesenchymal stem cells (BMSCs) can attenuate bleomycin induced lung injury and can yield better beneficial effect over the BMSCs therapy alone.

Conflict of Interest

There are no conflicts of interest.

References
