Histological Study on the Promising role of Mesenchymal Stem Cell Therapy in Paraquat Induced Lung Injury in Adult Male Albino Rats

Eman A. Abd Fattah, Assmaa O. Selim, Eman M. Mohamed, Basant T. Abdelbaki
Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

*Corresponding author:
Eman M. Mohamed
Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Postal code: 44519
Tel.: +201226283878
Email address: emanmosallam79@gmail.com

Abstract

Background: Paraquat (PQ) is a widely used herbicide. Several cases of chronic poisoning have been reported in recent years that associated with variety of adverse effects. The most serious one is pulmonary fibrosis with no effective medication up to now. Recently, exogenous stem cells have brought new hope and a great promise in repair of numerous diseases.

The objective: So, the present study aimed to detect the histological and immunohistochemical changes induced by PQ and to investigate the possible repairing effect of stem cells on PQ induced lung injury in adult male albino rats.

Materials and methods: 36 rats were divided into 3 groups: control group (I), PQ group (II) received a single dose 20 mg/kg orally and PQ & stem cells group (III) received PQ as group II and bone marrow derived mesenchymal stem cells at dose of 5x10⁵/200μl PBS intravenous at 24 and 48 hours after PQ ingestion. After 4 weeks, histological, immunohistochemical and morphometric studies were performed.

Results: Histologically, PQ treated rats displayed many collapsed alveoli with thickened inter-alveolar septa and marked cellular infiltration. Immunohistochemically, Alpha smooth muscle actin (α-SMA) were expressed profusely in the fibrotic lesions and a marked reduction in pro-surfactant protein–B (SPB) immune-expression was also obvious. While, stem cells treated rats exhibited a considerable degree of preservation of the alveolar architecture and also for α-SMA and SPB immune-expression which were proven morphometrically. In conclusion: Stem cells denote a remarkable therapeutic effect ameliorating Parquats’ inflammatory and fibrotic changes in lung.

Keywords: Paraquat, Mesenchymal stem cells, α-SMA, Surfactant-B, Lung, rat.

Introduction
Paraquat (PQ) is a widely used herbicide in agriculture. It is considered a safe substrate when used appropriately. However, several acute and chronic poisoning cases have been reported in recent years [1]. The inhalation and ingestion are the common routes of human exposure leading to its accumulation in the lung and to a less extent in the liver and kidney. So, lung damage and pulmonary fibrosis are the most frequent injuries [2].
Lung injury caused by PQ may be due to alveolar epithelial damage, causing hemorrhage and edema, or due to myofibroblasts infiltration into the alveolar septa and their differentiation into fibroblasts causing fibrosis [3]. Researchers suggested that fibrosis started in rats 2 hours after PQ poisoning [4]. Although, the mechanism of PQ toxicity has not been fully understood, the oxidative stress with generation of reactive oxygen species (ROS) is considered a main factor. Also, inflammatory, alveolar type II and endothelial cells secrete many chemical mediators and cytokines aiding in injury progression [5]. There is no specific antidote or effective treatment proposed for PQ toxicity. So, its toxicity or poisoning management has been predominantly limited either to decrease its absorption or to increase its elimination [6]. Recently, stem cell therapy has been applied in the treatment of many organ diseases. The regenerative capacity of lung by endogenous stem cell begins when extensive damage occurs. But, it is not always aids in full recovery. So, researchers pay attention on the exogenous stem cells for treatment of several lung diseases [7]. So, the present study aimed to detect the histological and immunohistochemical changes induced by PQ and to investigate the possible repairing effect of stem cells on PQ induced lung injury in adult male albino rats.

Materials and methods

Animals

Thirty six healthy adult male albino rats (aged 8-10 weeks and weighted 180-200gm) were obtained and housed in plastic cages with stainless steel wire-bar lid in the Animal house, Faculty of Medicine, Zagazig University under standard conditions (temperature 23±2ºC, humidity 50±5%, 12:12h light/dark cycle). The rats were left to acclimate for 1 week prior to any use in the experiment. All the ethical issues were considered based on the medical research ethics committee of Zagazig University (Egypt).

Reagent

Paraquat (PQ) was obtained in the form of powder (CAS Number: 75365-73-0) from Sigma Aldrich (St. Louis, MO, USA).

Bone marrow derived-mesenchymal stem cells (BM-MSCs)

These cells were labeled with PKH-26 (red fluorescence cell linker). They were provided by The Stem Cell Research Unit in Biochemistry Department, Kasr Al-Ainy Faculty of Medicine, Cairo University.

Detection of stem cells homing

Lung tissue sections were examined with a fluorescent microscope (Olympus BX50F4, No. 7M03285, Tokyo, Japan) to detect and trace the cells stained with PKH-26 in Biochemistry Department, Faculty of Medicine, Zagazig University.

Experimental Design

Rats were equally divided into three groups as follow:

Group I: (Control Group) which was further subdivided equally into 3 subgroups; the negative control group (IA) received no treatment, the vehicle control group (IB) received a single dose of 0.5 ml sterile saline (solvent of PQ) using intra-gastric tube and the vehicle control group (IC) received 0.5 ml phosphate buffer saline (PBS) (suspension of MSCs) intravenous through tail vein.

Group II: (PQ group) received a single dose 20 mg/kg body weight of PQ diluted in 0.5 ml sterile saline using intra-gastric tube [8].
Group III: (PQ + BM-MSCs) received PQ as in group II then the rats received $5 \times 10^5/200\mu l$ of PBS fluorescent labeled bone marrow derived MSCs intravenously through the tail vein at 24 & 48 hr after PQ ingestion [9& 10]. At the end of the experiment (4 weeks after PQ ingestion), rats of all groups were fasted overnight then anaesthetized and were sacrificed by intraperitoneal injection of thiopental (50 mg/kg). Their thoracic cages were opened, lungs were dissected out and samples from the lower left lobes of the lungs were taken.

**Histological Study**

**Light microscopic study**

Specimens from the lower left lobes of the lungs from each animal were fixed in 10% saline formalin and processed to prepare 5 μm thick paraffin sections for Haematoxylin and Eosin (H&E) stain and Mallory’s trichrome (MT) stain [11]. For Immunohistochemical staining, Paraffin sections (4 μm) were dewaxed, hydrated, and microwave-treated, and then blocked in a normal mouse serum. The sections were incubated with the specific primary antibody overnight (4°C) then with biotinylated secondary antibodies followed by streptavidin conjugated-horseradish peroxidase. Staining was completed by incubation with 3, 3’-diaminobenzidine (DAB).

Immunohistochemical staining was done with anti- alpha smooth muscle Actin (α-SMA) antibody (rabbit polyclonal antibody; No. ab5694; dilution 1/50; Abcam, Cambridge, UK) to localize myofibroblast [12] and anti-pro-surfactant protein–B (SPB) (rabbit polyclonal antibody; No. ab40876; dilution 1/50; Abcam, Cambridge, UK) to detect surfactant secreting cells [13].

**Electron microscopic study**

Specimens were fixed in glutaraldehyde and post fixed in osmium tetroxide. Then, they were dehydrated, embedded in resin and the ultrathin sections (Leica ultra-cut UCT) were stained with uranyl acetate and lead citrate [14]. Sections were examined and photographed by (JEOL JEM 1010 transmission electron microscope; Jeol Ltd., Tokyo, Japan) in the Regional Center of Mycology and Biotechnology (RCMB), Al- Azhar University, Egypt.

**Image analysis and morphometric studies**

Leica Quin 500 Image Analyzer (Leica Ltd., Cambridge, UK) in the Image Analyzing Unit of the Pathology Department, Faculty of Dentistry, Cairo University (Egypt) was used. Alveolar septal thicknesses were performed in H&E stained sections. Positive cells in anti SPB immune stained sections were counted. In addition, the area% of collagen fibers in MT stained sections and +ve α-SMA immunoreactivity in immunostained sections were also measured. These measurements were done in 10 nonoverlapping high power fields using binary mode.

**Statistical analysis:**

The obtained data were expressed as means± standard deviation (SD) and compared using one-way analysis of variance (ANOVA). P value <0.05 were considered statistically significant [15].

**Results**

**Fluorescent microscopic evaluation**

Fluorescence microscopy image revealed PKH26 labeled cells as bright red dots in lung sections (Fig. 1).
Histological results

Group I (the control group)

Examination of all control subgroups showed nearly similar histology. Therefore, the negative control figures only were presented. H&E-stained sections revealed the normal lung architecture; rounded or polygonal alveoli and alveolar sac (Fig. 2A). Patent alveoli with thin inter-alveolar septa were observed. Their lining epithelium composed of squamous cells (pneumocytes type I) and cuboidal cells (pneumocytes type II) (Fig. 2B). Mallory’s trichrome-stained sections revealed minimal collagen fibers in the interalveolar septa, around the bronchioles and blood vessels (Fig. 2C).

Fig. (1): a section of stem cell treated lung showing PKH26 labeled cells appearing as bright dots within the alveolar wall (arrow). (Fluorescent Microscope).

Fig. (2): Photomicrograph of histological sections of the control rat (group I). (A&B): Normal lung architecture with rounded or polygonal alveoli (a) and alveolar sacs (S) are observed. The lining epithelium is composed of squamous type I pneumocytes with flat nuclei (arrow) and cuboidal type II pneumocytes with rounded nuclei (arrow head). Note, thin inter-alveolar septa (curved arrow) are nearly in all fields. (C): Blue staining for minimal amount of collagen fibers (arrow) is seen in the inter-alveolar septa, around bronchiole (B) and the blood vessel (BV). [H&E (A, B) Mallory’s Trichrome (C)].
Immunohistochemical-stained sections for alpha smooth muscle actin (α-SMA) revealed weak positive immunoreaction within the inter-alveolar septa and at smooth muscle knobs (Fig. 3A), and sections stained for pro-surfactant protein–B (SPB) recruited a normal distribution of positively immune-expressed cells. Pneumocytes type II secreting surfactant-B showed a brown cytoplasm (Figure 3B).

Fig. (3): Photomicrograph of immunohistological sections of the control rat (group I). (A): Cells within the inter-alveolar septa and at smooth muscle knob show a weak positive α-SMA immunoexpression (arrow). (B): Normally distributed cells in the alveolar wall show a positive SPB cytoplasmic immunoexpression (arrow).

Electron microscopic examination showed patent alveoli with thin inter-alveolar septa containing few interstitial cells. Pneumocytes type I with attenuated cytoplasm and flat eucromatic nuclei and pneumocytes type II with centrally placed nuclei were seen in the alveolar wall (Fig.4A). Type II cells appeared with rounded nuclei, well defined lamellar bodies, numerous mitochondria and also obvious microvilli (Fig.4B). Normal blood air barriers with attenuated cytoplasm of type I pneumocytes, fused basal lamina and the cytoplasm of the capillary endothelial cells were good clarified (Fig. 4C).
Fig. (4): Electron micrograph of lung sections from the control rats (group I). (A): An alveolus (A) lined by pneumocyte type I (P1) with flat euchromatic nucleus (n1) and pneumocyte type II (P2) with centrally placed nucleus (n2) is observed. Thin inter-alveolar septa (S) containing interstitial cells (Ic) and blood capillary (C) are seen. (B): Pneumocyte type II (P2) with central euchromatic nucleus (n), defined lamellar bodies (arrow) and many mitochondria (m) are seen. Microvilli (arrow head) appear on its surface. (C): Blood air barrier normally formed of attenuated cytoplasm of pneumocyte type I (thick arrow), fused basal lamina (arrow head) and cytoplasm of capillary endothelial cell (thin arrow).

Group II (PQ group)

Examination of H&E-stained sections from the PQ treated lung sections of adult male albino rats revealed marked changes; most of the alveoli were collapsed with marked thickening of the inter-alveolar septa. Extensive inflammatory cellular infiltrations around the bronchioles and infiltrating their epithelium with some luminal exfoliated cells were seen (Fig. 5A) and also congested blood vessels were observed (Fig. 5B). Mallory’s trichrome stained-sections revealed extensive deposition of collagen fibers in inter-alveolar septa (Fig. 5C), around bronchioles and blood vessels (Fig. 5D).

Fig. (5): Photomicrograph of histological sections of the PQ treated rat (group II). (A&B): Collapsed alveoli (ca) with thickened inter-alveolar septa (arrow) are well observed. Marked inflammatory cellular infiltration (IF) are seen around the bronchiole (B) and infiltrating its epithelium (arrow head) with luminal exfoliated cells (wavy arrow). Congested blood vessel (BV) is also observed. (C &D): Blue staining for extensive amount of collagen fibers (arrow) in the inter-alveolar septa, around the bronchiole (B) and the blood vessel (BV). [H&E (A, B) Mallory’s Trichrome (C, D)].
Immunohistochemical-stained sections for α-SMA revealed strong positive immunoreaction within the inter-alveolar septa (Fig. 6A), and sections stained for SPB showed few positive immune-expressed cells in the alveolar wall in most of the lung sections (Fig. 6B).

Fig. (6): Photomicrograph of immunohistological sections of the PQ treated rats (group II). (A): Cells within the inter-alveolar septa (arrow) show a strong positive α-SMA immunoexpression. (B): A few positive SPB cytoplasmic immunoexpression (arrow) in some alveolar walls are observed.

Electron microscopic examination revealed major changes when compared with the control group. Thick inter-alveolar septa with many cells, blood capillaries and also collagen fibers were observed (Fig. 7A). Pneumocytes type II appeared with irregular heterochromatic nuclei, regular and irregular empty lamellar bodies forming vacuoles in the cytoplasm. Abundant collagen fibers were also observed (Fig. 7B). Deformed blood air barriers in the form of extensive separations containing collagen fibers between the basal lamina of type I & endothelial cells were obvious. Wide irregular cytoplasm of capillary endothelial cells were also noticed (Fig. 7C).

Fig. (7): Electron micrograph of lung sections from the PQ treated rats (Group II). (A): Thick inter-alveolar septa (S) with many interstitial cells (Ic), collagen fibers (thick arrow) and blood
capillaries (C) are observed. Pneumocyte type II (P2) with irregular nucleus (n), empty lamellar bodies (thin arrow) and ill identified microvilli (arrow head) are seen. (B): Pneumocyte type II (P2) with irregular nucleus (n) and some regular empty lamellar bodies (thin arrow) but others irregular (arrow head) forming vacuoles in the cytoplasm are observed. Abundant amount of collagen fibers (thick arrow) is noticed.(C): deformed blood air barrier with cytoplasm of pneumocyte type I (thick arrow), well separated (S) two basal lamina (arrow head) containing collagen fibers (Co) and relatively wide irregular cytoplasm of capillary endothelium (thin arrow) is seen.

**Group III (PQ + BM-MSCs)**

Examination of H&E-stained sections of this group showed an improvement or nearly normal lung structure, but less than the control. They revealed normal alveoli and thin inter-alveolar septa (Fig. 8A). The lining alveolar epithelium composed of defined squamous cells (pneumocytes type I) and cuboidal cells (pneumocytes type II) but some septal thickening and collapsed alveoli were still present (Fig. 8B). While, Mallory’s trichrome-stained sections revealed minimal blue staining for collagen fibers in the inter-alveolar septa, around the bronchioles and blood vessels (Fig. 8C).

![Photomicrograph of histological sections of the PQ with MSCs treated rat (group III).](image)

(A & B): Apparently normal alveoli (a), alveolar sacs (S) and relatively thin inter-alveolar septa (curved arrow) are seen. Squamous type I pneumocyte with flat nuclei (thin arrow) and other cuboidal type II pneumocyte with rounded nuclei (arrow head) are seen lining the alveolar walls. Note, few collapsed alveoli (star) with relatively thick inter-alveolar septa (thick arrow) (C): Blue staining for minimal amount of collagen fibers (arrow) in the inter-alveolar septa, around the bronchioles (B) and blood vessels (BV). [H&E (A, B) Mallory’s Trichrome (C)].

Some or few fields of lung sections revealed relatively weak and few positive immunoreactions for α-SMA within the inter-alveolar septa (Fig. 9A). Relative normal positive immune-expressions for SPB secreting cells in some fields were observed (Fig. 9B).
Fig. (9): Photomicrograph of immunohistological sections of the PQ with MSCs treated rat (group III). (A): Some cells within the inter-alveolar septa (arrow) show a weak positive α-SMA immunoexpression. (B): Some alveolar walls show few cells with positive SPB cytoplasmic immunoexpression (arrow).

The electron microscopic examination revealed nearly normal structures. Apparently normal alveoli with relatively thin inter-alveolar septa containing minimal amounts of collagen fibers were observed (Fig. 10A). Pneumocytes type II with irregular nuclei, clear lamellar bodies and numerous mitochondria were seen (Fig. 10 A & B). Nearly normal blood air barriers were observed, but irregular attenuated cytoplasm of type I pneumocytes, little separation in some sites between two basal lamina of both cells (type I & endothelial) containing a few amount of collagen fibers were detected (Fig. 10C).

Fig. (10): Electron micrograph of lung sections from the PQ + MSCs treated rats (Group III). (A): Apparently normal alveolus (A) with relatively thin inter-alveolar septum (S) containing minimal amount of collagen fibers (thin arrow). Pneumocyte type II (P2) appears with irregular nucleus (n) and lamellar bodies (thick arrow) in its cytoplasm. (B): Pneumocyte type II (P2) with
irregular nucleus (n), lamellar bodies (thick arrow) and numerous mitochondria (m) are seen. Note, minimal amount of collagen fibers (Co) (C): A nearly normal blood air barrier is observed; irregular attenuated cytoplasm of type I pneumocyte (thick arrow), fused basal lamina (arrow head) but little separation (S) in some sites between two basal lamina of both cells (type II & endothelial) containing few amount of collagen fibers (bifid arrow). Notice red blood cell in the capillary lumen (R).

**Morphometric and statistical results**

Our statistically analyzed results for alveolar septa l thicknesses, number of anti-SPB immune-stained cells, area percentage of positive collagen fibers and anti α-SMA immune reactivity in different groups were summarized in (Table 1).

**Table 1. Alveolar septal thicknesses, number of anti-SPB immune-stained cells, area percentage of positive collagen fibers and anti α-muscle Actin immune reactivity in different groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tbody>
<tr>
<td>Alveolar septa thickness (µm)</td>
<td>7.13± 2.8</td>
<td>11.7± 4.3 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2± 2.7 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti SPB cell count</td>
<td>17.93 ± 1.07</td>
<td>6.93 ± 0.83 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0 ± 0.67 &lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>area % of collagen fibers</td>
<td>2.6± 2.8</td>
<td>14± 4.3 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5± 2.04 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>area % of α smooth muscle actin</td>
<td>1.87± 1.7</td>
<td>16±5.07 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25±1.5 &lt;sup&gt;b&lt;/sup&gt;</td>
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*: Significant difference (P < 0.05).

**Discussion**

Paraquat (PQ) is a well-known herbicide widely used in the world. Unfortunately, its excessive use causes damage to the lung and other organs [16]. The lungs are preferentially targeted owing to the rapid uptake and accumulation of PQ in lung cells, especially in the alveolar epithelium. PQ is similar in its chemical structure to amines that have a transport system in types I and II alveolar cells; so, it can enter into them followed by release of free radicals, which lead to cell death [17].

In the current work, hematoxylin and eosin stained sections from adult rats' lungs of PQ treated group revealed that the alveolar lining cells had darkly stained nuclei and many alveoli were collapsed with thick interalveolar septa. Similar changes were seen by Zhao et al. [18] who explained them by degeneration of type II pneumocytes which affect surfactant secretion leading to airway collapse and chronic obstructive pulmonary disease.

The thickened inter alveolar septa seen in our study was confirmed morphometrically when compared to the other groups. Riahi et al. [19] mentioned that the PQ lung injury induce
adhesion and infiltration of neutrophils and macrophage that generate cytokines and chemokines, which lead to endothelial damage, expansion of vascular permeability and edema.

Glavin et al. [20] founded fibroblasts and collagen fibers in the inter-alveolar septa. These findings were in the same line with our findings of increased collagen, both by light microscope using Mallory’s trichrome stain and electron microscope and confirmed morphometrically by a significant increase in their area %. Also, increased collagen fibers were approved by a significant increase in the mRNA expression of transforming growth factor beta (TGF-b), connective tissue growth factor, matrix metalloproteinase (Mmp) and collagen type I and III after PQ poisoning [21] and Endothelin-1(ET-1); which stimulates fibroblast growth [22]. Type II cells normally secrete prostaglandin E2, to suppress fibroblast growth, whose absence due to cell degeneration enhances fibroblast growth and collagen synthesis [2].

In the current work, vascular congestion, exfoliated bronchiolar cells and heavy cellular infiltration were detected. They were similar to that observed by Grommes and Soehnlein [23] who reported that the influx of inflammatory cells into the interstitium and broncho-alveolar space is recruited from the circulation when local defenses are overwhelmed by injurious or toxic substances. These inflammatory cells can damage epithelium and sub-epithelial structures by secreting several chemotactic substances with the result of excessive fibro-proliferation, which occur due to ineffective repair and epithelial regeneration [24&25]. Unfortunately, fibrosis affects the mechanics of the blood vessels and increases arterial stiffness that may lead to pulmonary hypertension [26].

In the present study, ultrastructurally, pneumocyte type II cells appeared with irregular heterochromatic nuclei, vacuolated cytoplasm and empty or irregular lamellar bodies. These results were in accordance with Dinis-oliveira et al. [27] who explained that PQ induces DNA fragmentation of alveolar cells that affects the membrane transport proteins, immune responses and major signal pathways. Also, PQ up regulates apoptosis-associated genes in the alveolar cells such as trithorax (TRX) and heme oxygenase-1 (HO-1) in prolonged intoxication. In addition, free radicals produced by PQ oxidative process results in damage of the mitochondria with affection of energy production needed for the metabolic pathways [28]. These free radicals also cause fragmentation and vacuolation of the cells by release of lysosomal enzymes into the cytoplasm [29].

In the current work, thickened air barrier was obvious in PQ group with excessive collagen fibers interspersed. This thickening was attributed to thickening of the alveolar and the vascular basal lamina by oxidative modification of their protein and carbohydrate content [30]. It was also explained by excessive production of transforming growth factor b1 (TGF-b1) which stimulates fibroblast to produce type I and VI collagen fibers [31].

Mesenchymal stem cells (MSCs) charactarized by expression of low levels of human leucocytic antigen (HLA) class I and class II allowing the escape of recognition. Furthermore, they suppress T cell proliferation and so, do not elicit an immune response in the recipients. These advantages of MSCs motivated many researchers to study their therapeutic effects on many organs [32]. Homing of MSCs to certain organ in response to signals is a multi-step process that includes cell attachment and rolling in the vessel lumen, adhesion and extravasation across the vascular endothelium then migration through the tissue stroma [33].

In the present study, bone marrow mesenchymal stem cells (BM-MSCs) treated group revealed obvious improvement in lung structure. Apparently normal alveoli with thin inter-alveolar septa, few amounts of collagen fibers were present in most lung fields and these results confirmed statistically. The results were consistent with previous studies on the beneficial therapeutic effects of stem cells [34& 35]. Our light microscope findings were supported by the
ultrastructural results such as, nearly normal type II cells with their lamellar bodies, few interstitial cells and also a characteristic blood air barrier.

Several previous biochemical studies elucidated different stem cells mechanisms; the major one was antioxidant activity. The treatment with BM-MSCs tended to normalize most of the up-regulated oxidative stress markers as malonaldehyde (MDA) and myeloperoxidase (MPO) and the down-regulated antioxidant as superoxide dismutase (SOD), catalase (CAT) and Glutathione reductase (GR) by obstructive lung diseases [36]. Another mechanism was anti-inflammatory by reducing the pro-inflammatory cytokines as tumor necrosis factor-α (TNF-α), interlukin- (IL) 1&6 and secreting anti-inflammatory agents as IL-10 and IL-13 [34&37]. Also, BM-MSCs can release prostaglandins (PGE2) acting on the EP2 and EP4 receptors on the macrophages, increasing the IL-10 production that can beneficially modulate the host immune response and reduce the inflammation [38& 39].

The ability of BM-MSCs in attenuating lung fibrosis occurs by inhibition of the pro-fibrotic cytokines, TNF-α and IL-1 through a paracrine mechanism [40]. Moreover, they induced changes in the fibroblast characterized by decreasing collagen and increasing hyaluronic acid production in fibroblasts co-cultured with MSCs [41]. Also, acceleration of fibrosis resolution occurs through epithelial restitution and subsequently secretion of surfactant protein C (SP-C) and Secretoglobin (cytokine-like secretory protein predominantly expressed in airway epithelial cells) that have anti-inflammatory and anti-fibrotic activities [42& 43]. Additionally, MSCs can facilitate lung repair and regeneration by their cytoprotective secretions [44].

BM-MSCs could mobilize into circulation, accumulate at the site of inflammation and differentiate to form endothelial and epithelial-like phenotype cells as type alveolar I & II [45]. The differentiation of MSCs into alveolar cells was seen in the in vitro study and got new cells having lamellar bodies and secreting surfactant that reduces surface tension and prevent the collapse [46]. While other studies postulated that BM-MSCs secrete different soluble factors that play a vital role in repair, most likely through their anti-apoptotic, angiogenic and immunomodulatory properties in addition to anti-inflammatory and antioxidant activities [47]. MSCs restore cytoskeletal organization in alveolar epithelial cells. Fortunately, when BM-MSCs were co-cultured with type II cell, a significant increase in cytoprotective gene expression as HO-1 and metallothionein (MT) was detected on the third and fourth day after PQ exposures [48]. BM-MSCs were suggested to have a potential role in helping type II cell to acquire relative resistant to PQ in addition to inflammatory modulating antioxidant maintaining [28].

Regarding α-SMA immune reaction; in the present work, the PQ group revealed numerous positive immunoreexpressed cells in the inter-alveolar septa which may be myofibroblasts that was confirmed by a significant increase in the mean area % when compared to other groups. Myofibroblasts act as contractile cells, promoting the alveolar development during organogenesis and are located in the walls of venules and alveoli [49] but α-SMA-positive interstitial cells are not found within the healthy alveolar septa [50]. In response to tissue injury, quiescent fibroblasts are transformed to active myofibroblasts [51]. In addition, extra-pulmonary circulating fibrocytes have been reported to migrate to injured sites [52]. Myofibroblast differentiation is strictly regulated by numerous transcription factors and cytokines acting via numerous pathways having commonly pro-fibrotic influence [53]. These cytokines were primarily secreted by the local macrophages [54]. Mechanical regulation is another one as stiffening of extra-cellular matrix, may itself be an inducer of its differentiation without pro-fibrotic agents [55].

All the above were in line with Kulkarni et al. [56] who found that PQ treatment significantly led to up regulation of pro-fibrotic genes such as α-SMA, which is considered as a key marker.
for myofibroblasts. Also, the increased TGF expression in PQ toxicity represents a strong stimul
us of α-SMA expression indicating the differentiation of lung fibroblasts into myofibroblasts [57]. On the other hand, the alveolar epithelial cells lose their epithelial marker E-cadherin when chronically exposed to TGF-b and inflammation and transformed into fibroblast-like cells with increased expression of α-SMA, type I collagen, vimentin and desmin [58]. In the current work, we found a significant decrease in the area % of α-SMA in BM-MSCs group. It was mentioned that inhibition of TGF-b1 and epithelial mesenchymal transition (EMT) by cell therapy leads not only to suppress fibrosis but also to down regulate α-SMA which was elevated during the fibrosis process by TGF-b1 [35&59].

In this work, we found a significant decrease in the number of positive pro-surfactant protein–B (SPB) immunoexpressed cells in PQ treated group as compared to other groups. Surfactant protein-B is an essential lipid-associated protein found in lung surfactant. It is highly hydrophobic, rearranges lipid molecules in the fluid lining alveoli, avoiding contact with water so, the alveoli can more easily inflate [60 & 61]. The cause of surfactant-deficient state with PQ is its inhibition by leakage of plasma proteins through the damaged alveoli-capillary membrane [62]. However, the oxidative/nitrative modification of surfactant may result in a loss of surface activity, either from degradation or aggregation [63]. PQ intoxication leads to oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+, disrupting pulmonary surfactant synthesis [64&65].

Moreover, the levels of TNF-α and IL-1β were significantly increased in broncho-alveolar lavage fluid (BALF) of rats after PQ exposure [66]. IL-1β plays a key role in the development of acute lung injury and can inhibit fluid transport across the distal lung epithelium causing surfactant abnormalities [67]. Also, PQ reduces the concentration of the alveolar surface lipids (e.g. lecithin) which are responsible for decreasing alveolar surface tension. These surface lipids lead to decrease the ability of the alveoli to contract and expand accurately [68]. In our work, the significant increase in the area percent of SP-B immunoexpression in BM-MSCs group as compared to the PQ group may be due to restoring structure and function of pneumocytes type II. In accordance to Beers et al. [69] who found that TGF-b1 (which was increased in PQ group) inhibits surfactant component expression, we suggest that stem cell can restore surfactant function by their inhibiting effect on TGF-b1.

From the results of our study, we concluded that PQ can induce definite inflammatory and degenerative changes in lung tissues. On the other hand, BM-MSCs present some ameliorating effects on these PQ induced changes.

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