Research article

Mesenchymal stem cells alleviate hydrochloric acid-induced lung injury through suppression of inflammation, oxidative stress and apoptosis in comparison to moxifloxacin and sildenafil

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A B S T R A C T

Introduction: Acute lung injury (ALI) is a severe life-threatening disease causing uncontrolled pulmonary inflammation and oxidative damage. There are still no effective therapies for this disease. The aim of this study was to evaluate the protective role of mesenchymal stem cells, moxifloxacin, sildenafil or a combination of moxifloxacin and sildenafil against hydrochloric Acid (HCl) - induced ALI.

Methods: HCl or saline was injected intra-tracheally and after 2 h, moxifloxacin, sildenafil, moxifloxacin + sildenafil or mesenchymal stem cells were injected. After 7 days, rats were sacrificed for evaluation of the blood chemistry and inflammation via determination of the level of oxidative stress markers, apoptosis and the histo-pathological alterations by H&E.

Results: In HCl-injected rats, there were a significant increase in total white blood cells (WBCs), lymphocytes, malondialdehyde (MDA) and *caspase-3* gene expression. Also, there were a significant decrease in superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and Hemeoxygenase-1 (HO-1) gene expression in lung tissue. On the other hand, treatment of lung injured rats with mesenchymal stem cell, moxifloxacin, sildenafil or a combination of moxifloxacin and sildenafil showed a significant decrease in WBCs and lymphocytes and ameliorated the histopathological changes. MDA level in lung tissue was only significantly lowered in rats treated with moxifloxacin alone or in combination with sildenafil or MSCs. GSH was just increased in rats treated with moxifloxacin, sildenafil or with MSCs. Antioxidant parameters and gene expression of *HO-1* and *caspase-3* were significantly modulated in rats treated with MSCs.

Conclusion: MSCs ameliorated the toxic effects of HCl through their ability to decrease inflammation, oxidative stress, and apoptosis in acute lung injury.

1. Introduction

Acute lung injury (ALI) is a serious form of lung disease. This disease has a variety of causes, including sepsis, trauma, drug toxicity, aspiration, multiple blood transfusion, ischemia, and acute pancreatitis (Standiford and Ward, 2016). ALI pathology is characterized by the destruction of the epithelium–capillary interface, the extravasation of protein-rich fluid, the release of pro-inflammatory cytokines, chemokines, and the infiltration of neutrophils. These changes lead to flooding of the alveolar spaces, which disrupts gas exchange and results in significant hypoxemia (Meng et al., 2019). Gastric aspiration is a high-risk condition for lung injury. Its effect range from subclinical pneumonitis to alveolar damage and progressive respiratory failure, according to the volume of aspirate,

with fibrosis development in some patients (Ayala et al., 2018). The aspirate contents may contain low pH stomach fluid, bacteria, blood or food particles. Among the gastric contents, hydrochloric acid (HCl) has the most important impact on lung injury. Gastric aspiration often occurs in patients in the Intensive Care Unit (ICU) (Alluri et al., 2017). Aspiration also occurs in patients with altered levels of consciousness due to trauma, cerebral vascular ischemia, or metabolic encephalopathies (Mizushina et al., 2019). To date there is no effective therapy for ALI, so in this study we used sildenafil or in combination with moxifloxacin or mesencymal stem cells as therapy for the disease.

Moxifloxacin is a synthetic antibacterial agent that belongs to the fluoroquinolone family. It is well established in the treatment of pneumonia or acute exacerbation in chronic obstructive pulmonary disease

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(COPD) (Minov et al., 2018). The antimicrobial activity of moxifloxacin against Gram-negative and Gram-positive bacteria is based on their ability to inhibit topoisomerases (Beisswenger et al., 2014). Moreover, moxifloxacin was reported to have immunoregulatory effects via suppression of neutrophilic migration and generation of pro-inflammatory cytokine from monocytes (Lee and Chae, 2011).

Phosphodiesterase 5 PDE5 catalyzes the hydrolysis of cyclic guanosine monophosphate (cGMP). c-GMP is a second messenger and has a major role in various cellular processes, like inflammation (Gokakin et al., 2013). PDE5 inhibitors seem to be especially applicable in curing pulmonary diseases because PDE5 activities are largely increased in oxidative stress and inflammatory processes (Kosutova et al., 2018). Sildenafil is a selective inhibitor for PDE5 leading to an increase in the concentration of c-GMP and cyclic adenosine monophosphate (cAMP). Sildenafil has been demonstrated to decrease oxidative stress and the inflammatory response through the inhibition of superoxide formation (Hemmati et al., 2015).

Cell-based therapy with mesenchymal stem cells (MSCs) is a potentially attractive option for treating patients with acute lung injury due to its efficacy for reduction of the magnitude of lung injury and enhance recovery from lung injury (Matthay, 2015). MSCs are multipotent stromal cells that can be isolated from bone marrow (BM), adipose tissue, umbilical cord, placenta, periodontal ligament, and dental pulp and may differentiate into adipocytes, chondrocytes, and osteoblasts (Antoniou et al., 2018). MSCs are attractive for clinical therapy due to their ability to differentiate into a variety of cell types, provide trophic support, and modulate innate immune response (Mauri et al., 2017). The aim of the current study was to evaluate the protective role of MSCs, sildenafil, and a combination of moxifloxacin and sildenafil in acute lung injury. Moxifloxacin was used as a standard drug for lung injury. In this study, the gastric acid aspiration was induced in the rats by using hydrochloric acid (El-Hamid et al., 2017).

2. Materials and methods

2.1. Reagents

HCl was purchased from Sigma® (Cat No, H1758). Moxifloxacin® was obtained from a local pharmacy as tablets (Moxiflox, 400 mg/tablet, Eva pharma©, Egypt). Moxifloxacin tablets were ground and each 100 mg of the tablet was dissolved in 100 ml of distilled H_2O. Sildenafil® was purchased from Pfizer Inc. (Sildenafil citrate, 100 mg/tablet, Pfizer, Egypt) and was dissolved in 100 ml of distilled H_2O.

2.2. Isolation, culture, and expansion of bone marrow mesenchymal stem cells

Isolation and culturing of mesenchymal stem cells (MSCs) was done by following the previously reported protocols (Abdel Aziz et al., 2007; Friedenstein et al., 1970). All rats were handled according to the guidelines of the Animal Ethics Committee at Zoology Department in Faculty of Science, Mansoura University, Egypt. The rats were anesthetized by halothane, then the skin was sterilized with 70% ethyl alcohol before cutting it. The femurs and tibia of male rats were carefully cut off from adherent soft tissues. Then they were put into a sterilized falcon tube containing 70% ethyl alcohol for 1–2 min. The bones were immersed in a falcon tube with Phosphate buffered saline (Cat no. BE17-516F, Lonza, USA) for washing. The bones were taken to laminar air flow for bone marrow extraction. The two ends of the bones were cut using sterile scissors. Bone marrow was flushed by Dulbecco's modified Eagles medium (DMEM) (Cat no. BE12-719F, Lonza, Belgium) supplemented with 10% fetal bovine serum (Cat no.10270, Gibco, USA) and 1% PEN-STREP (10.000 U penicillin -10.000 μg streptomycin/ml) (Cat no. DE17-602E, Lonza, USA). The marrow plugs were cultured in 20 ml complete media and incubated at 37 °C in a 5% humidified CO_2 incubator (Shel lab, USA). After 24h, the old media were discarded for

removing unattached cells. MSCs were differentiated from other bone marrow cells by their ability to attach to tissue culture polystyrene (flask, $75cm^2$, Greiner Bio-One). Cells were subcultured by using 0.25% Trypsin/ethylenediamine-tetraacetic acid (Cat no. BE17-161E, Lonza, Belgium).

2.3. Animals

Sixty male Sprague-Dawley rats (12-weeks old) weighted 180–200g. Rats were housed at regular (12 h light/dark cycle) at thermally-controlled facility. Rats received *ad libitum* water and food for one week prior to the experimental work. All rats were handled according to the guidelines of the Animal Ethics Committee at Zoology Department in Faculty of Science, Mansoura University, Egypt.

2.4. Experimental design

Animals were randomly divided into six groups and each group included 10 rats (Charan and Kantharia, 2013). gp1: negative control and received saline into trachea. gp2:(positive control) lung injury was induced by injected 0.1 Normality (N) HCl in trachea (2 ml/kg) (El-Hamid et al., 2017). gp3: lung injury was induced as described in gp2 and after 2 h, rats received moxifloxacin (10 mg/kg/I.P. twice daily/week) (El-Hamid et al., 2017). gp4: lung injury was induced as described in gp2 and after 2 h, rats received sildenafil (10 mg/kg/day/I.P./week) (Hemmati et al., 2015). gp5: lung injury was induced as described in gp2 and after 2 h rats received sildenafil (10 mg/kg/day/I.P./week) and moxifloxacin (10 mg/kg/I.P. twice daily/week). gp6: lung injury was induced as described in gp2 and after 2 h, rats received BM-MSCs (1×10^6) cells suspended in 0.2 ml media intravenous via penile vein, single dose (Mauri et al., 2017). At the end of the treatment period, rats were sacrificed one week after anesthesia and blood were withdrawn in the EDTA-containing tube for blood cell count (CBC) and lung tissues were taken for biochemical study and gene expression.

2.5. Biochemical study

The lung tissues were excised from each animal, and washed with 0.9 % NaCl solution, 0.2 gram of tissue was homogenized for determination of superoxide dismutase (SOD) (Cat no. SD 2521) (Nishikimi et al., 1972), catalase (CAT) (Cat no. CA 2517) (Aebi, 1984), glutathione reduced (GSH) (Cat no. GR 2511) (Beutler, 1963) and Malondialdehyde (MDA) (Cat no. MD 2529) (Ohkawa et al., 1979). The analysis was performed according to the manufacturer's instructions.

2.6. Gene expression by real-time PCR

Real-time PCR was used to detect the gene expression of the *caspase-3* and *heme oxygenase-1* (HO-1). Total RNA was extracted from 0.025 g of tissues using the RNeasy® Mini Kit (Cat no. 74106) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed into cDNA using RT2SensiFAST™ cDNA Synthesis Kit (Cat. No. BIO-65053) following the manufacturer's instructions. Real-time PCR was performed using SensiFAST™ SYBR® No-ROX Kit (Cat. No. BIO-52066). The reaction mixture contained 10 μl SensiFAST SYBR® No-ROX mix (2x), 1 μl reverse primer, 1 μl forward primer (Table 1), 3 μl cDNA product and 5 μl of nuclease-free water. The amplification of the gene was performed by using the following: initial denaturation at 95 °C for 3 min, (denaturation at 94 °C for 20 s, annealing at 58 °C for 30 s and extension at 60 °C for 30 s 40 cycles). Then the samples were subjected to PIKOREAL96 Real-time thermal cycler (Thermo Fisher, USA). Target gene expression was normalized to the housekeeping gene GAPDH (Table 1). The relative expression of each target gene was calculated according to $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 1
Primer sequence that used in real-time PCR.

The gene	Sequences (5'-3')	Accession No.	Product Size (bp)
GAPDH	F: TGACTTCAACAGCAACTCCCA R: AGGGCCTCTCTCTTGCTCTC	NM _017008.4	211
Caspase-3	F: GGCCGACTTCCTGTATGCTT R: CGTACAGTTTCAGCATGGCG	NM _012922.2	110
Hemoxygenas-1(HO-1)	F: TCACCTTCCCGAGCATCGAC R: TCACCCTGTGCTTGACCTCG	NM _012580.2	99

2.7. Histopathological analysis

Sections of lung tissues (5 μm) were prepared for hematoxylin and eosin (H&E). The stained sections were evaluated by a blind pathologist for evaluation of any histopathological abnormalities using a light microscope (Leica Microsystems, Germany).

2.8. Statistical analysis

Statistical analysis was carried out by Statistical Package for the Social Sciences SPSS program (version 19; SPSS, Chicago, Illinois, USA. All values were presented as mean ± standard error of the mean (SEM). Differences were considered to be significant at $p < 0.05$. One-way analysis of variance (ANOVA) and post-hoc test were used to determine differences between groups (Snedecor and Cochran, 1980).

3. Results

3.1. MSC-treated, as well as the drug-treated, decreased the number of white blood cells (WBCs) and lymphocytes

Rats injected with HCl (gp1) alone showed a significant increase in both WBCs and lymphocytes in comparison to the rats treated with any of the drugs or MSCs ($p < 0.05$) (Table 2) (Fig. 1).

3.2. Antioxidant and oxidative stress parameters were improved in treated groups

In a moxifloxacin-treated rats (gp3), the activity of catalase (CAT), level of reduced glutathione (GSH) and activity of superoxide dismutase (SOD) were significantly increased ($p < 0.05$) in comparison to HCl-injected group (gp2). Sildenafil treatment of lung injury led to a significant increase in the activities of SOD ($p = 0.001$), CAT ($p = 0.001$) and the content of reduced glutathione GSH (p = 0.001) (Table 3) while no significant change in the level of MDA ($p = 0.5$).

The SOD and CAT activities were attenuated in the rats treated with both Sildenafil and Moxifloxacin (gp5) (Table 3) ($p = 0.002$, $p = 0.003$). Moreover, the combination treatment decreased the level of MDA ($p = 0.001$) without a significant change in the level of GSH. The rats that were treated with MSCs (gp6) showed a significant amelioration for the negative effect caused by HCl injection via the increase in the activities of both CAT and SOD with a significant increase in the level of GSH and a decrease in the level of MDA in lung tissue ($p = 0.001$) (Fig. 2).

3.3. MSCs induced the expression of HO-1 and Caspase-3 genes

The relative expression of *hemeoxygease-1 (HO-1)* was significantly increased in Moxifloxacin (gp3, $p = 0.002$), sildenafil (gp4, $p = 0.001$), in combination treatment (gp5, $p = 0.002$) as well as in the MSCs treated rats (gp6, $p = 0.001$). The relative expression of *caspase-3* was significantly decreased in Moxifloxacin (gp3, $p = 0.001$), sildenafil (gp4, $p = 0.002$), in combination treatment (gp5, $p = 0.001$) as well as in the MSCs treated rats (gp6, $p = 0.001$) (Table 4) (Fig. 3).

Table 2
White blood cells and lymphocytes in control and treated groups. MOX = moxifloxacin, SILD = Sildenafil, MSCs = Mesenchymal stem cells. Bold p values means that $p < 0.05$.

| Groups	gp1[a]	gp2[b]	gp3[c]	gp4[d]	gp5[e]	gp6[f]	P value
Treatment	Saline	HCl	MOX	SILD	MOX + SILD	MSCs	
WBCs	8.27 ± 0.07	25.48 ± 0.6	14.85 ± 0.21	9.35 ± 0.66	9.7 ± 0.57	13.17 ± 0.27	a *vs* b, $p = $ **0.004** b *vs* c, $p = $ **0.000** b *vs* d, $p = $ **0.001** b *vs* e, $p = $ **0.001** b *vs* f, $p = $ **0.002**
Lymphocyte	9.36 ± 0.09	15.94 ± 0.55	12 ± 0.43	5.95 ± 0.31	7.9 ± 0.55	9.66 ± 0.28	a *vs* b, $p = $ **0.001** b *vs* c, $p = $ **0.001** b *vs* d, $p = $ **0.002** b *vs* e, $p = $ **0.001** b *vs* f, $p = $ **0.001**

3.4. Histopathological findings in the lung tissues

In our present study, the histological examination of the lung tissue in control group showed normal lung tissues without hemorrhage or inflammation (Fig. 4A) the positive control group (HCl-injected rats) showed presence of vasculitis (black arrow) and hemorrhage was noticed within the alveoli or within the perivascular areas (arrowhead) (Fig. 4B) in comparison to the healthy rats (Fig. 4A). On the other hand, moxifloxacin led to an increase in the alveolar spaces and a moderate decrease of interstitial thickening (black arrow) (Fig. 4C).

Our results in sildenafil-treated group showed a marked decrease in inter-alveolar thickening with minute hemorrhage (black arrow) (Fig. 4D). The mixed treated group showed a marked decrease in necrotic, vascular and infiltrative lesions and a marked increase in alveolar spaces (Fig. 4E). In MSCs-treated group showed a marked increase in the ventilation spaces and a decrease in the interstitial fibrosis, inflammatory cells infiltration (Fig. 4F).

4. Discussion

Acute lung injury is characterized by severe inflammatory response leading to deterioration of gas exchange and till now there is no effective therapy for ALI. The main model to induce acute lung injury in animals is the administration of hydrochloric acid by intra-tracheal route according to Sahin et al. (2011). In our study, the lung injury was induced by applying the HCl model followed by administration of mesenchymal stem cells and compared their activities to the standard drugs for ALI treatment. There was an increase in WBCs count in positive group (HCl-injected) compared to other treated groups. The results are in agreement with the study of Imam et al. and (Imam et al., 2015) this increase may be due to the acute inflammation leading to leukocytes
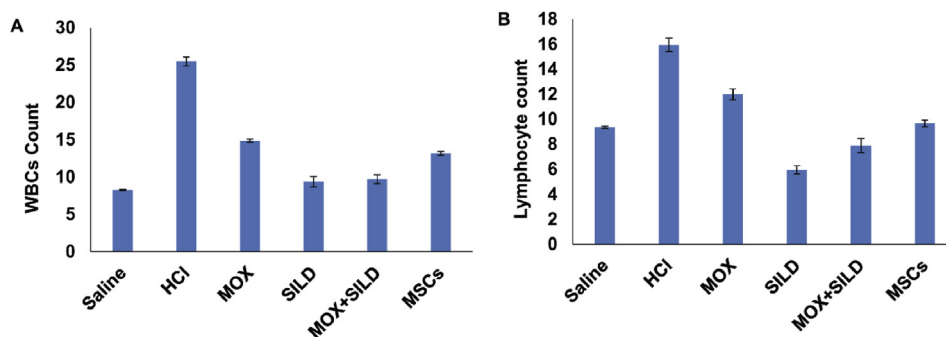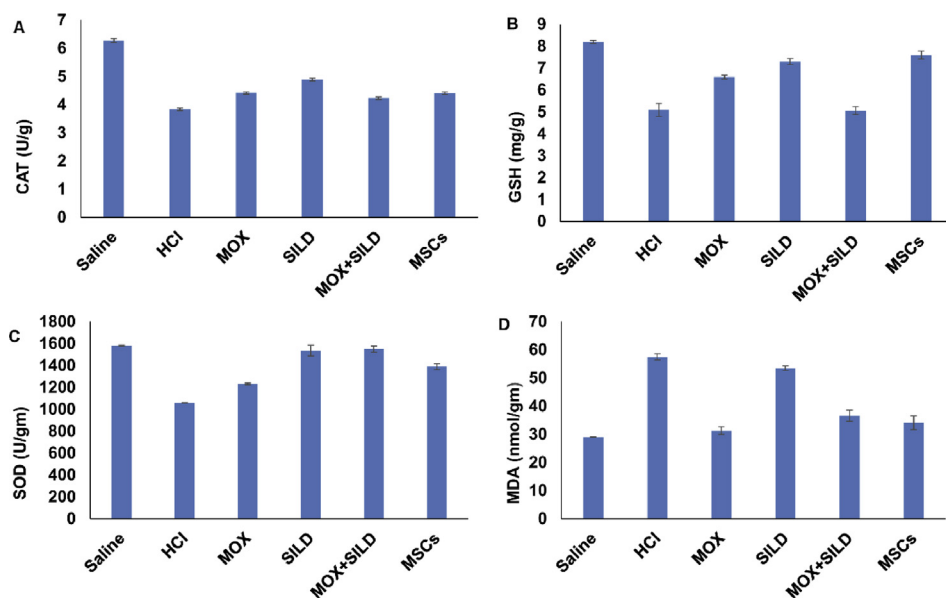
Fig. 1. (A) There was a significant decrease in WBCs count in different treated groups ($p < 0.05$) in all treated groups in comparison to HCl-injected group. (B) There was a significant decrease in lymphocyte count in different treated groups ($p < 0.05$) in comparison to HCl-injected group. MOX = moxifloxacin, SILD = Sildenafil, MSCs = Mesenchymal stem cells.

Table 3

CAT, GSH, SOD and MDA levels in lung tissues in control and different treated groups. MOX = moxifloxacin, SILD = Sildenafil, MSCs = Mesenchymal stem cells. Bold p values means that $p < 0.05$.

Groups	gp1[a]	gp2[b]	gp3[c]	gp4[d]	gp5[e]	gp6[f]	P value
Treatment	Saline	HCl	MOX	SILD	MOX + SILD	MSCs	
CAT (U/g)	6.28 ± 0.07	3.84 ± 0.05	4.42 ± 0.04	4.9 ± 0.05	4.24 ± 0.041	4.41 ± 0.042	a *vs* b, p = **0.001** b *vs* c, p = **0.002** b *vs* d, p = **0.001** b *vs* e, p = **0.003** b *vs* f, p = **0.001**
GSH (mg/g)	8.2 ± 0.07	5.09 ± 0.29	6.6 ± 0.09	7.3 ± 0.14	5.06 ± 0.18	7.6 ± 0.19	a *vs* b, p = **0.001** b *vs* c, p = **0.004** b *vs* d, p = **0.001** b *vs* e, p = 1.000 b *vs* f, p = **0.001**
SOD (U/gm)	1581.7 ± 3.1	1058.4 ± 2.2	1232 ± 8.5	1536.6 ± 49.48	1549.9 ± 29.5	1388.3 ± 27.4	a *vs* b, p = **0.001** b *vs* c, p = **0.002** b *vs* d, p = **0.001** b *vs* e, p = **0.002** b *vs* f, p = **0.001**
MDA (nmol/gm)	28.9 ± 0.11	57.5 ± 1.13	31.3 ± 1.35	53.44 ± 0.72	36.57 ± 1.95	34.05 ± 2.41	a *vs* b, p = **0.002** b *vs* c, p = **0.001** b *vs* d, p = 0.5 b *vs* e, p = **0.001** b *vs* f, p = **0.001**

Fig. 2. (A) There was a significant increase in CAT activity in different treated group in comparison to HCl-injected group ($p < 0.05$). (B) There was a significant increase in GSH in moxifloxacin, sildenafil and MSCs treated groups ($p < 0.05$) in comparison to HCl-treated group but there was no significant change in combination (moxifloxacin + sildenafil) treated group ($p = 1.000$). (C) SOD activity was significantly increased in different treated group in comparison to HCl-injected group ($p < 0.05$). (D) The level of MDA was significantly decreased in moxifloxacin, combination (moxifloxacin + sildenafil) and MSCs treated groups but there was no significant change in sildenafil treated group ($P = 0.5$). MOX = moxifloxacin, SILD = Sildenafil, MSCs = Mesenchymal stem cells.

migration and damaged tissues. In moxifloxacin treated group, WBCs count decreased and this is due to the modulation of the immune system in addition to its antimicrobial properties. The same findings were reported by Müller et al. (Müller-Redetzky et al., 2014). On the other hand,

Table 4
Fold of change in *Hemeoxygenase-1* and *Caspase-3 expression* in the different treated groups. MOX = moxifloxacin, SILD = sildenafil, MSCs = mesenchymal stem cells. Bold p values means that p<0.05.

Groups	gp1[a]normal	gp2[b]	gp3[c]	Gp4[d]	Gp5[e]	Gp6[f]	P value
Treatment	Saline	HCl	MOX	SILD	MOX + SILD	MSCs	
HO-1	1.02 ± 0.03	0.31 ± 0.02	1.34 ± 0.04	1.99 ± 0.21	1.7 ± 0.24	2.4 ± 0.1	a *vs* b, $p = $ **0.004**
							b *vs* c, $p = $ **0.002**
							b *vs* d, $p = $ **0.001**
							b *vs* e, $p = $ **0.002**
							b *vs* f, $p = $ **0.001**
Caspase-3	1.04 ± 0.06	2 ± 0.16	0.41 ± 0.07	0.33 ± 0.01	0.72 ± 0.08	0.16 ± 0.07	a *vs* b, $p = $ **0.003**
							b *vs* c, $p = $ **0.001**
							b *vs* d, $p = $ **0.002**
							b *vs* e, $p = $ **0.001**
							b *vs* f, $p = $ **0.001**
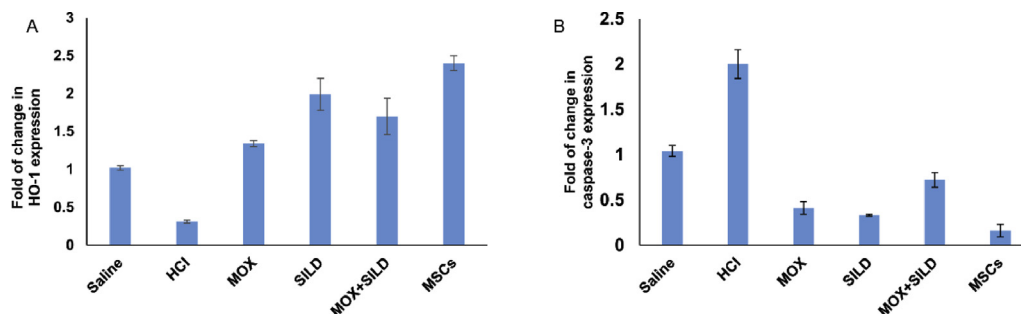
Fig. 3. (A) The relative expression of (*HO-1*) was significantly increased in different treated group ($p < 0.05$). (B) The relative expression of caspase-3 was significantly decreased in different treated group in comparison to HCl-injected group ($p < 0.05$).
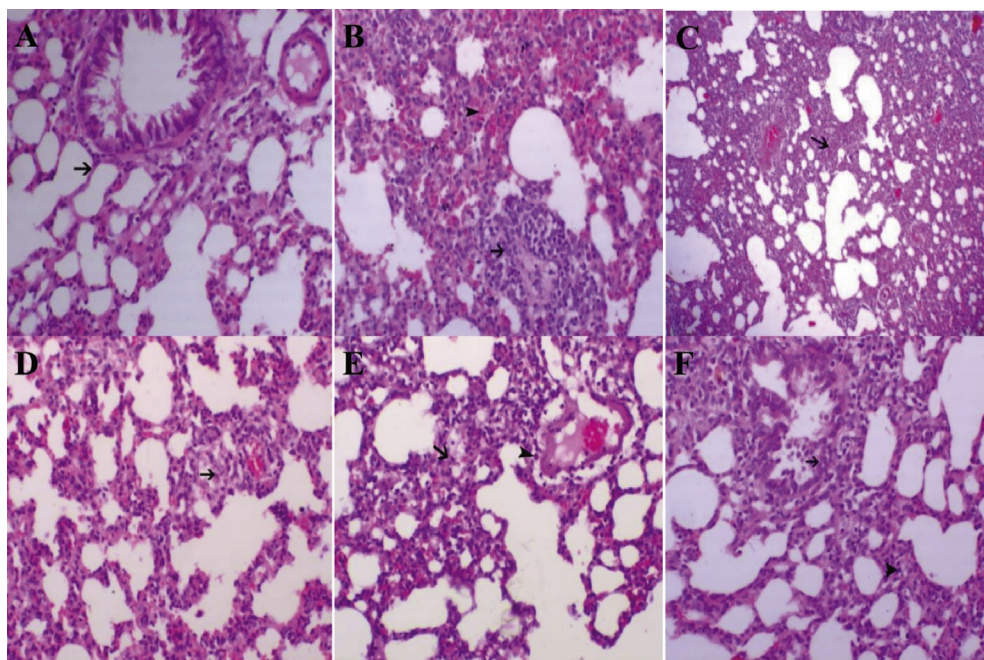
Fig. 4. Morphologic changes of rat lung. (A) Control showing normal lung tissue (HE, 200x). (B) HCl-injected rats showing vasculitis (black arrow) and alveolar hemorrhage (arrowhead) (HE, 200x). (C) rats treated with moxifloxacin standard drug after induction of lung injury with HCl showed an increase in the alveolar spaces and a moderate decrease of interstitial thickening (black arrow) (HE, 40x). (D) rats treated with sildenafil standard drug after induction of lung injury with HCl showed a marked decrease in inter-alveolar thickening with minute hemorrhage (black arrow) (HE, 200x). (E) rats treated with a combination of moxifloxacin and sildenafil after induction of lung injury with HCl showed a decrease in inter-alveolar thickening (black arrow) and mild perivascular inflammatory cell infiltration (Arrowhead) (HE, 200x). (F) rats treated with MSCs after induction of lung injury with HCl showed a marked decrease inter-alveolar thickening (arrowhead) and marked bronchial lining epithelium hyperplasia (black Arrow) (HE, 200x).

the most significant decrease in WBCs was found to be in sildenafil-treated group. The result is due to the inhibitory effect of sildenafil through the elevation of cyclic-guanosine monophosphate (cGMP) level. Moreover, Wang et al. showed that sildenafil was able to activate cGMP in acrolein-induced airway inflammation rat model (Wang et al., 2009).

In the mixed treated group WBCs count decreased due to the dual action of moxifloxacin and sildenafil. In MSCs-treated group, the level of WBCs decreased. It has been reported that bone marrow mesenchymal

stem cells have an anti-inflammatory activity by reducing the influx of inflammatory cells in the injured tissue as reported in an endotoxin-induced ALI model in mice (Hao et al., 2015). Lymphocytes play a necessary role in the regulation of inflammation in different lung diseases. Our results showed that HCl-injected group increased in the count of lymphocytes due to pulmonary injury and dysfunction. While the different treatment groups showed a decrease. Our results are in agreement with Imam et al. (2015) but in the same time are in contrary to El-Hamid et al. (2017). The decrease may be due to inhibition of

lymphocytes infiltration in inflamed lung tissues in different treatment groups.

Antioxidant proteins (enzymatic (SOD and CAT) or non-enzymatic (GSH)) decrease the level of reactive oxygen species (ROS) (Pejic et al., 2006). Different reports confirmed the correlation between oxidative stress and the development of lung injury (Choi and Alam, 1996). In this study, HCl induced lung injury via the decrease in the level of SOD, CAT, and GSH. Moreover, HCl increased lipid peroxidation product (MDA). Sun et al., 2017 showed that lung injury was induced by decreasing the antioxidant parameters and increasing the MDA level in the sepsis model (Sun et al., 2017). On the other hand, the tested standard drugs in this study, as well as MSCs, ameliorated the injury induced by HCl via the attenuation of the level of antioxidant parameters and MDA. These findings may be due to the ability of the standard drugs and MSCs to modulate the level of free radicals as described by other researchers (El-Hamid et al., 2017; Gokakin et al., 2013; Shen et al., 2018). Furthermore, MSCs were reported to improve the level of cysteine and GSH (Iyer et al., 2010). Sildenafil, as a standard drug for ALI treatment, decreases the oxidative stress and inhibits the inflammation (Gokakin et al., 2013). Moxifloxacin has immunoregulatory effects, including suppression of neutrophilic migration and generation of a proinflammatory cytokine from monocytes (El-Hamid et al., 2017).

Hemeoxygenase-1 (HO-1) is a key player in the cellular antioxidant system (Choi and Alam, 1996). HO-1 is the rate-limiting enzyme in the heme degradation pathway and at the same time, it was found that its expression can be induced in response to oxidative stress. Here in this study, HCl induced the lung injury by decreasing the gene expression of HO-1 while its effect was ameliorated by MSCs as well as the standard drugs either alone or in combination. Zhang et al., 2017 showed that BM-MSCs upregulate the level of HO-1 leading to a decrease in ROS production (Z. H. Zhang et al., 2017). Zhang et al., 2019 explained the indirect mechanism of MSCs that trigger overexpression of Nrf2 gene and over expression of the antioxidant HO-1 (L. Zhang et al., 2019). Additionally, MSCs and standard drugs were able to decrease the lung injury via the downregulation of caspase-3 expression leading to the protection of alveolar epithelial cells from apoptosis (Kadry and Abdel-Megeed, 2017; Liu et al., 2018). Our result showed that the most significant decrease of caspase-3 in MSCs-treated group and this due to secretion of growth factors by MSCs, including Hepatocyte growth factor (HGF). HGF is a multifunctional factor that promotes angiogenesis and reduces apoptosis by improvement of B-cell lymphoma 2 (Bcl-2) expression and reduction of caspase-3 expression (Jiang et al., 2015).

In our present study, the histological examination of the lung tissue showed and confirmed the effectiveness of the MSCs and the standard drugs in improving the lung pathology. HCl induced lung pathological alteration via the recruitment of polymorphonuclear neutrophils (PMNs) (El-Hamid et al., 2017) while moxifloxacin improved the pathological alteration via its immunomodulatory activity and preventing the infiltration and migration of neutrophils (El-Hamid et al., 2017). It has been reported that sildenafil modulates the immune cell response via the regulation cGMP pathway and protein kinase G (Shih et al., 2013). The improvement in lung pathology after MSCs treatment could be due to the ability of MSCs to migrate into lung tissue and to promote tissue repair (Zheng et al., 2016). In conclusion, the present study suggests that BM-MSCs could limit HCl-induced acute lung injury by modulation inflammation, oxidative stress and apoptosis compared with other drugs.

Declarations

Author contribution statement

R. El-Demerdash, S. El-Metwaly, F. El-Senduny and A.F. Abdel-Aziz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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