

Histological Study on The Possible Therapeutic Effect of Mesenchymal Stem Cells Versus Their Derived Exosomes in Carbon Tetrachloride Induced Lung Injury in Adult Male Albino Rat

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ABSTRACT

Background: Lung fibrosis is a deadly, progressive illness. CCl₄ is a xenobiotic used to cause lung fibrosis. Mesenchymal stem cells (MSCs) garnered interest as a possible therapeutic option. Research has demonstrated exosomes' (EXOs) potential in tissue regeneration.

Aim: Evaluate the possible therapeutic effects of mesenchymal stem cells versus their derived exosomes on induced lung fibrosis in rat.

Material: 46 adult male rats were used. 10 rats were used for preparation of MSCs and isolation of exosomes. 36 Rats were separated into 4 groups, Group I (control) $n=15$, Group II ($n=7$): received intraperitoneal injection (IP) of 30% CCl₄ of 3 ml/kg body weight in olive oil twice weekly for 8 weeks. Group III ($n=7$): received CCl₄ like group II. Then each rat received 1×10^6 MSCs in 150 μ L PBS once through the tail vein at the beginning of week 9. Group IV ($n=7$): received CCl₄ like group II. Then received 150 μ g of EXOs diluted in 150 μ L PBS once through tail vein at the beginning of week 9. At week 10, rats were scarified. Lung specimens underwent histological, morphometric, and statistical analysis.

Results: CCl₄ led to mononuclear infiltration, congested blood capillaries, extravasation, thickening in interalveolar septa and increased deposition of collagen. Disrupted microvilli and empty lamellar bodies were present. MSCs & EXOs improved the previous histological changes. However, EXOs resulted in a significant improvement

Conclusion: Both MSCs and EXOs had significant effects in improving CCl₄ induced injury in the lung, but EXOs were better.

Key Words: Exosomes, Lung fibrosis, MSCs, rat.

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INTRODUCTION

Lung fibrosis is the final step of a lot of lung conditions^[1] Lung inflammation, autoimmune disorders, hypersensitivity pneumonitis, drug-induced interstitial lung disease, infection, and cancer are among the causes of lung fibrosis^[2].

Nonetheless, one of the most prevalent and severe types of pulmonary fibrosis, with a high death and morbidity rate, is idiopathic lung fibrosis (IPF). Regardless of the cause, lung fibrosis cannot be cured since lung scarring cannot be undone once it has started^[3,4].

The liquid form of carbon tetrachloride (CCl₄) is colorless, transparent, fireproof, and volatile. Through eating, inhalation, and cutaneous absorption, CCl₄ readily

enters the body^[5]. CCl₄ is a commonly used xenobiotic to induce toxicity in experimental animal studies. It is widely known as a hepatotoxic, nephrotoxic, and pulmonary toxic agent^[6].

An experimental model that closely resembles oxidative stress in various pathophysiological scenarios is animal carbon tetrachloride poisoning. It has been shown that the pathophysiology of common lung conditions such cystic fibrosis, asthma, and chronic obstructive pulmonary disease is comparable to that of CCl₄.^[7] Pulmonary fibrosis, the final stage of many lung inflammatory diseases, is a severe and debilitating illness with a terrible prognosis^[8].

Recently, because of their capacity for differentiation and immunomodulatory action, MSCs have garnered a lot of interest as a possible therapeutic option. Clinical trials

and new research are being conducted to apply MSCs as a regenerative treatment for a variety of illnesses, including organ fibrosis^[9].

Exosomes are small extracellular vesicles (30-150 nm) secreted by various types of cells and play a pivotal role in communication between cells. They transport bioactive molecules such as lipids, proteins and RNAs, which can modulate the function of recipient cells.^[10] Exosomes' potential for tissue regeneration and repair has been brought to light by recent research. Specifically, stem cell-derived exosomes or other healing cells have demonstrated potential in the treatment of a number of ailments^[11].

AIM OF THE STUDY

This study aimed to compare the histological effects of mesenchymal stem cells versus their derived exosomes on the structure of lung in CCl₄ induced lung fibrosis in rats. By evaluating lung morphology, inflammation, fibrosis, and regenerative markers, we aim to elucidate the potential benefits and underlying mechanisms of exosome therapy in lung fibrosis. This research could provide valuable insights into new therapeutic strategies for managing lung fibrosis.

Aim To evaluate the possible therapeutic effects of MSC versus their derived exosomes on induced lung fibrosis in rat model.

MATERIALS AND METHODS

Materials

1. Animals used in experiment

The current study was performed on 36 adult male albino rats, 4 to 5 months age. Their weight was about 180-200 grams. The rats were acquired from the Faculty of Medicine Ain Shams' animal house. Rats were kept at the Medical Research Centre at Faculty of Medicine, ASU. During a week in a typical laboratory setting with a 12-hour light-dark cycle and temperatures between 22 and 24°C for environmental adaption. They were kept in cages made of plastic covered with mesh wire. They were provided with a steady, nutrient-dense feed and unrestricted access to water.

ETHICAL CONSIDERATION

The experimental work was carried out in compliance with Ain Shams University's Scientific Research Ethical Committee's Animal Care requirements. (FMASU R79/2025) (28-3-2025) organized and run under the International Council on Harmonization (ICH) and Islamic Organization for Medical Science (IOMS) guidelines, as well as the US Office for Human Research Protections and US Code of Federal Regulations and is covered by Federal Wide Assurance No. FWA 00017585.

2. Study Interventions:

Ten male albino rats used for preparation of mesenchymal stem cells and exosomes.

3. Isolation and culture of ADSCs:

Ten male albino rats of one month age weighing 70-80gm used as a source of MSCs at Ain Shams University's Stem Cell Research Lab, Histology Department, Faculty of Medicine. According to earlier publications, MSCs were cultivated at 37 °C, with saturated humidity and 5% CO₂, in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS), 500 U/ml penicillin, and 500 µg/ml streptomycin^[12].

4. Isolation and characterization of exosomes:

Isolation of exosomes was done by ultracentrifugation method according to *Coughlan et al.*^[13]. Ultracentrifugation was done at National Research Center, Cairo, Egypt by ultracentrifuge machine (Sorval-MTx150, made in Japan). Resuspension of isolated exosomes in PBS (50–200 µl) was done for downstream use and were stored at -80°C. Characterization of exosomes^[14] was done using transmission electron microscope (TEM), JEOL- 1010; 80kv, at Al- Azhar University in the Regional Center of Mycology and Biotechnology (RCMB), (Figure I).

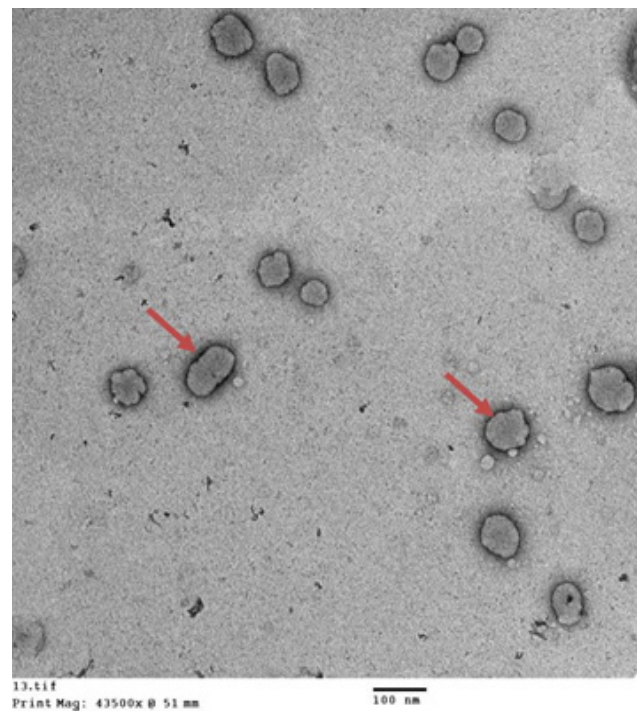


Fig. I: An electron photomicrograph showing MSCs derived exosomes of different diameters. They have rounded to oval shape. Notice phosphotungstic acid (↑) is concentrated on the membrane of exosomes.

(TEM x 100,000)

Methods

Animal grouping

Four groups of the remaining thirty-six rats were formed.

- Group I (Control Group) ($n=15$):

Three subgroups were formed out of them.

Subgroup Ia: Five rats were left without any interference then sacrificed when the experiment is over.

Subgroup Ib: Five rats were injected by 150 μ L PBS in tail vein once at the beginning of week nine then sacrificed at the end of week ten.

Subgroup Ic: Five rats were injected intraperitoneal by olive oil 3 ml/kg body weight twice per week for 8 weeks then scarified at the end of week ten.

- Group II (CCl4 group) ($n=7$):

This group included seven rats. Rats received intraperitoneal injection of 30% CCl4 (Sigma, St Louis, USA) at a dose of 3 ml/kg body weight in olive oil twice weekly for eight weeks to induce lung fibrosis^[8] Then at the end of week ten rats were scarified.

- Group III (MSCs group) ($n=7$):

Rats were given intraperitoneal injection (IP) of 30% CCl4 for eight weeks to induce lung fibrosis like group II. Then each rat received 1×10^6 MSCs in 150 μ L PBS once through the tail vein at the beginning of week nine then sacrificed at the end of week ten^[8].

- Group IV (EXOs group) ($n=7$):

Rats received IP of 30% CCl4 for eight weeks to induce lung fibrosis like group II. Then each rat received 150 μ g of EXOs diluted in 150 μ L PBS once through tail vein at the beginning of week nine then sacrificed at the end of week ten^[15].

Histological studies

All of the rats were given ether anesthesia and sacrificed at the end of the experiment. Lungs were exposed, and Samples were gathered. In order to prepare paraffin blocks, half of the samples were fixed using a 10% formalin solution. H&E staining and Sirius Red (for collagen fibers) were applied to 5 μ m thick paraffin slices. The remaining half of the samples were quickly fixed in 2.5 glutaraldehyde for 24 hours after being chopped into tiny pieces (1 mm³). The specimens were prepared for the TEM analysis. A light microscope was used to view semithin (1 μ m) sections that had been dyed with 1% toluidine blue in borax^[16]. At Ain Shams University's Faculty of Science, ultrathin sections (50 nm) were inspected and captured on camera using a JEM 1200 EXII TEM.

Morphometrical and statistical analysis:

1. The mean thickness of interalveolar septum in H & E sections.
2. The mean area % of collagen fibers in Sirius Red sections.

The LEICA Q win Image Analyzer from the Histology Department of the Faculty of Medicine at Ain Shams University performed these measurements. Five distinct, non-overlapping fields were used to measure the parameters in five sections from each of the five rats in each group. Mean $\pm$ SD was used to express the values.

The SPSS software (version 20) was used for statistical analysis, comparing the means of each group using ANOVA, or one-way analysis. *P values* less than 0.05 were regarded as significant differences.

RESULTS

General observation:

Group II (CCL4) rats showed decreased activity and general weakness. Rats in the group III (MSC) and Group IV (exosome) exhibited a generally good condition.

Mortality rate:

1 rat in Group II (CCL4) passed away during this study, resulting in a mortality rate of 20%, while no deaths were observed in the other groups. Dead Rats had been replaced.

I. Histological results

A. Light microscopic findings:

H&E staining

Analysis of the lung stained with H&E of group I (control) revealed patent alveolar spaces and sacs separated by thin interalveolar septa. Alveoli were lined mostly by pneumocyte type I that has a flat nucleus, and few type II pneumocyte with a spherical nucleus. (Figure 1A &B). Bronchioles were seen lined with simple epithelium and dome shaped club cells (Figure 1A). Examination of group II (CCl4 group) showed many narrow and collapsed alveoli and thick interalveolar septa populated with mononuclear cellular infiltration (Figure 1C, D, E &F). Dilated alveoli were noticed in other areas. In addition, disruption of endothelial lining of capillaries with extravasation of RBCs in the septa was seen (Figure 1D &F). Bronchioles showed disruption of epithelial lining with sloughed cells in their lumen (Figure 1C &E). Congested blood vessels were also found (Figure 1C &E). Examination of group III (MSCs group) showed some patent alveoli & alveolar sacs (Figure 1G &H). Many other alveoli appeared narrow & collapsed with thick interalveolar septum (Figure 1G &H). Bronchioles appeared nearly similar to control group (Figure 1G). Mononuclear cellular infiltration and

with extravasation of RBC in interalveolar septa were still found (Figure 1G & H). Examination of group IV (EXOs group) showed many patent alveoli and alveolar sacs lined by pneumocyte type I and pneumocyte type II and separated by thin interalveolar septa (Figure 1I & J).

Bronchioles showed more or less normal lining with club cells (Figure 1I). Some few narrow-collapsed alveoli with thick interalveolar septa were noticed (Figure 1J). Some congested blood vessels and mild mononuclear cellular infiltration were also still seen (Figure 1I & J).

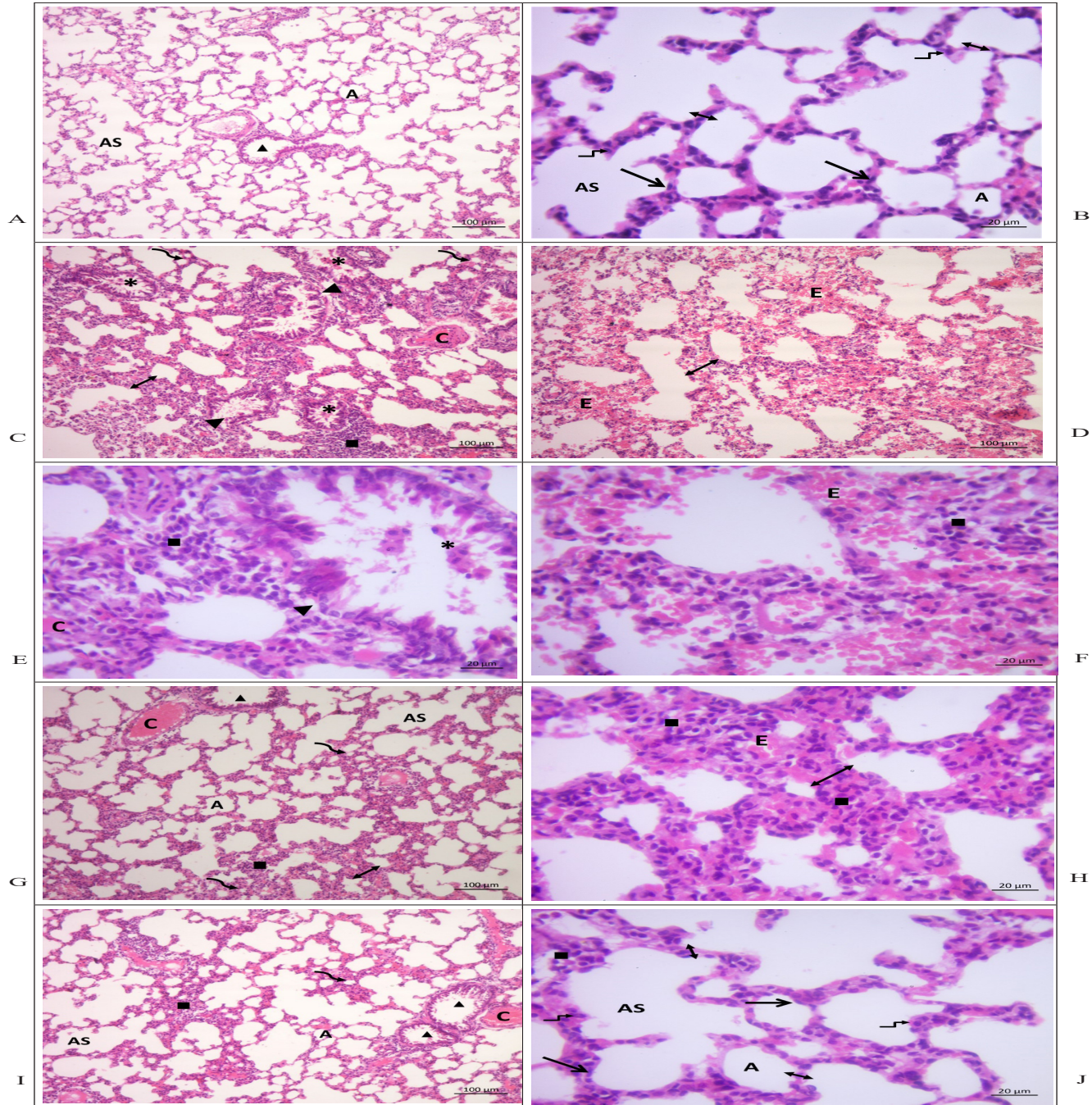


Fig. 1: photomicrographs of H & E stained lung tissue from different studied groups. [A & B]: group I(control): A, showing patent alveolar spaces(A) and alveolar sacs (AS) and bronchioles (▲) lined by simple epithelium and club cells x100. B, showing alveoli lined by pneumocyte type I (↑) and pneumocyte type II (armed arrow) and separated by thin interalveolar septa (↑). x400 [C, D, E and F]: group II (CCL4): showing many narrow and collapsed alveoli (curved arrow) and thick interalveolar septa (↑) with mononuclear infiltration (■) and extravasation of RBC (E). Disruption of epithelial lining of bronchioles (▲) with sloughed cells in the lumen (*) are seen. Congested blood vessels and capillaries (C) are also found. [G & H]: group III (MSCs): showing some patent alveoli (A) & alveolar sacs (AS). Many other alveoli appear narrow & collapsed (curved arrow) with thick interalveolar septum (↑). Bronchioles (▲) are lined by simple epithelium and club cells. Mononuclear cellular infiltration (■) and extravasation (E) are still seen [I&J]: group IV (exosome): showing many patent alveoli (A) and alveolar sacs (AS) lined by pneumocyte type I (↑) and pneumocyte type II (armed arrow) and separated by thin interalveolar septa (↑). Bronchioles (▲) are seen lined by simple epithelium and club cells. Some narrow-collapsed alveoli with thick interalveolar septa are seen (curved arrow). Some congested blood vessels (C) and mild mononuclear cellular infiltration (■) are still seen. (C,D,G & I X 100 and E,F,H&JX400).

Sirius Red staining

Sirius Red-stained sections of lung of group I (control) showed fine few collagen fibers between the alveoli (Figure 2A). Group II showed notable rise in collagen fibers deposition between the alveoli and in the wall of the bronchioles and blood vessels (Figure 2B & Table 1).

Also, group III showed significant increase in the collagen fibers deposition between the alveoli and in the wall of the bronchioles as compared to the control (Figure 2C & Table 1). While, Group IV showed significant decrease in collagen fibers deposition between the alveoli, in the wall of the bronchioles and blood vessels as compared to group II (Figure 2D & Table 1).

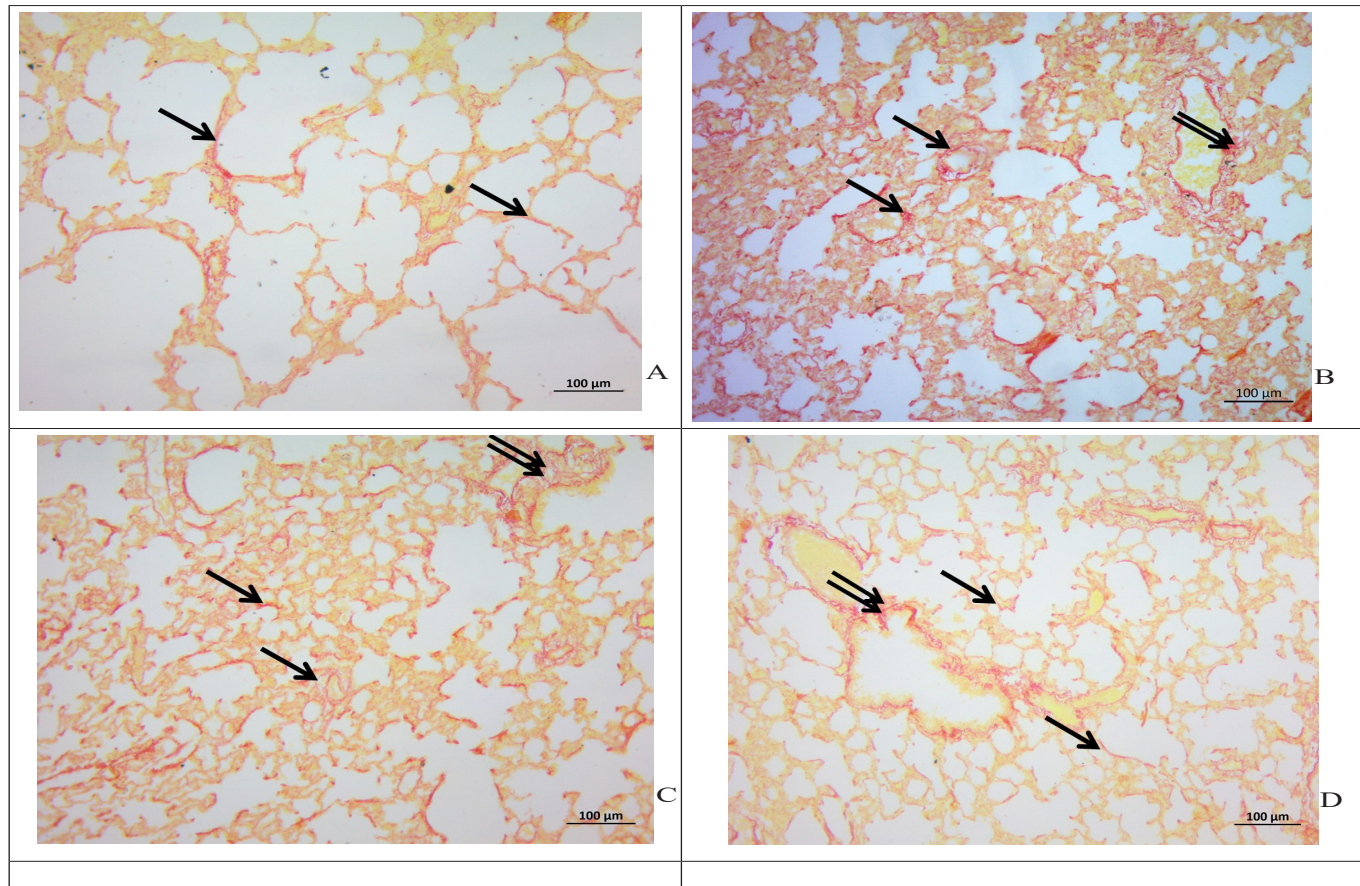


Fig. 2: photomicrographs of lung tissue stained with Sirius Red from different studied groups.

[A]: group I(control): showing fine few collagen fibers between the alveoli (↑). [B]: group II (CCL4 treated): showing apparent increase in the collagen fibers between the alveoli (↑), and in wall of the bronchioles (↑↑). [C]: group III (MSCs treated): showing apparent increase in the collagen fibers between the alveoli (↑), and in the wall of the bronchioles (↑↑). [D]: group IV (exosome treated): showing few collagen fibers between the alveoli (↑), but still apparent increased collagen in wall of the bronchioles (↑↑). X100.

Table 1: Showing mean thickness of interalveolar septum and mean area % of collagen in different studied groups:

	Group1 (control)	Group2 (CCL4)	Group 3 (MSCs)	Group 4 (exosomes)
Mean thickness of interalveolar septum	2.92± 0.54	10.78±1.95 a * c*d *	7.17±1.07 a * b* d *	3.51±0.85 a ns b * c *
Mean area % of collagen	1.97± 0.62	17.78±1.91 a * c*d*	7.50±1.57 a* b* d*	3.87±1.13 a* b* c*

Data are presented as mean ± standard deviation (SD).

Significance at * $P < 0.05$. (a: vs. Group1, b: vs. Group2, c: vs Group3 and d: vs Group4).

Toluidine blue-stained semithin sections

Control group showed alveolar spaces with thin interalveolar septum, lined mostly by simple squamous type I pneumocyte having flat nuclei, and few dome shape type II pneumocytes having rounded nuclei, and vacuolated cytoplasm (Figure 3A). While, group II showed alveolar spaces with very thick interalveolar septum and apparent increase in type II pneumocyte, some of them appeared with dark small nuclei. Foamy macrophage could be noticed in some sections (Figure 3B & C). Congested blood vessels,

extravasated RBC and mononuclear cellular infiltration with many lymphocytes with dark rounded nuclei were found (Figure 3C). Most of alveolar spaces were closely obliterated (Figure 3B). Group III showed alveoli with relatively thin interalveolar septum, lined by Flat type I pneumocyte and apparently increased type II pneumocyte with vesicular rounded nuclei (Figure 3D). In group IV, alveoli with relatively thin interalveolar septum, lined by an apparently normal type I pneumocyte and few dome shaped type II pneumocyte were seen (Figure 3E).

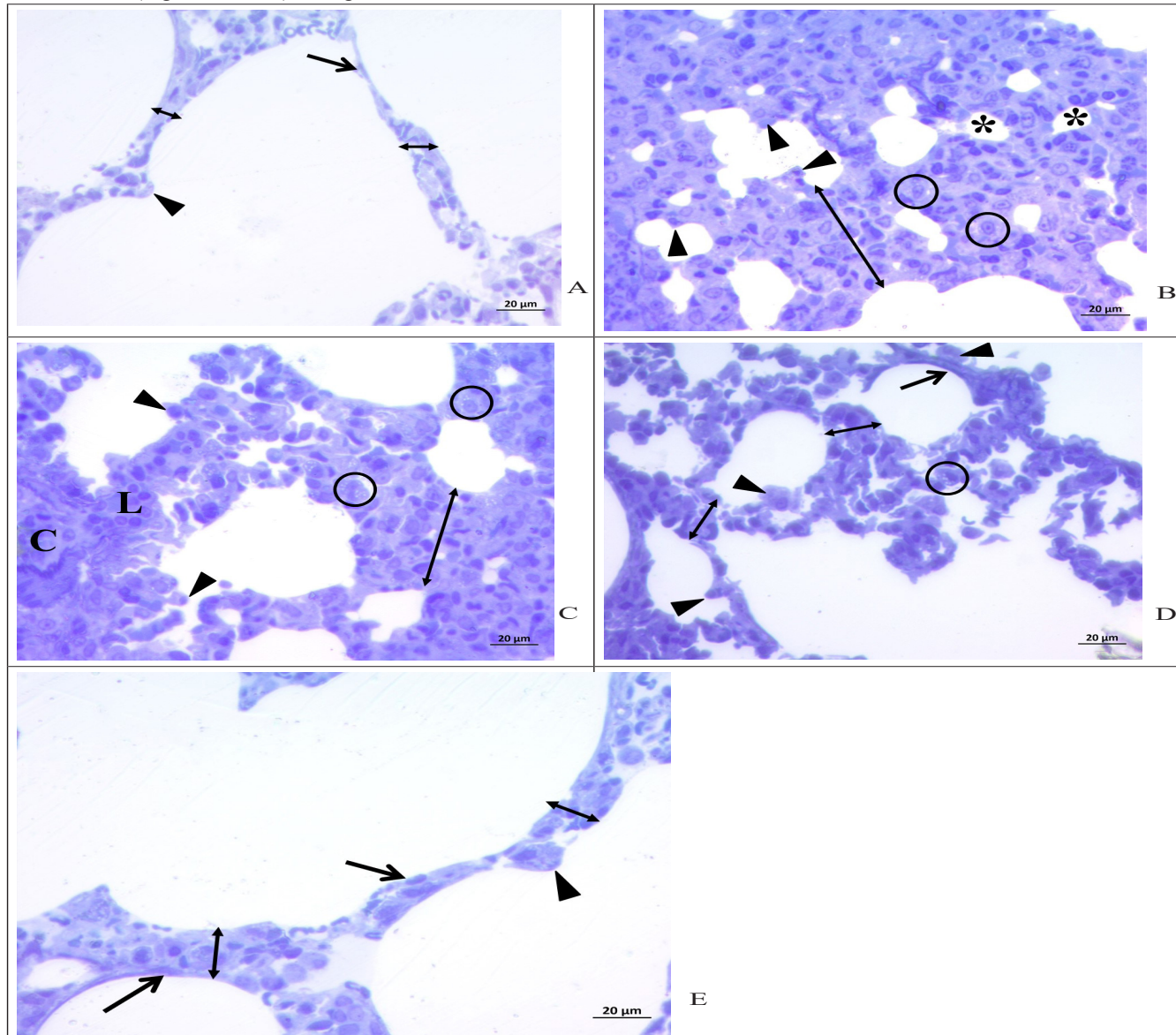


Fig. 3: photomicrographs of semithin sections of lung tissue stained with Toluidine blue stain from different studied groups: [A]: group I(control): showing alveolar spaces with thin interalveolar septum (↑), lined by simple squamous type I pneumocyte with flat nuclei (↑) and dome-shaped type II pneumocytes with vacuolated cytoplasm and rounded nuclei (▲).

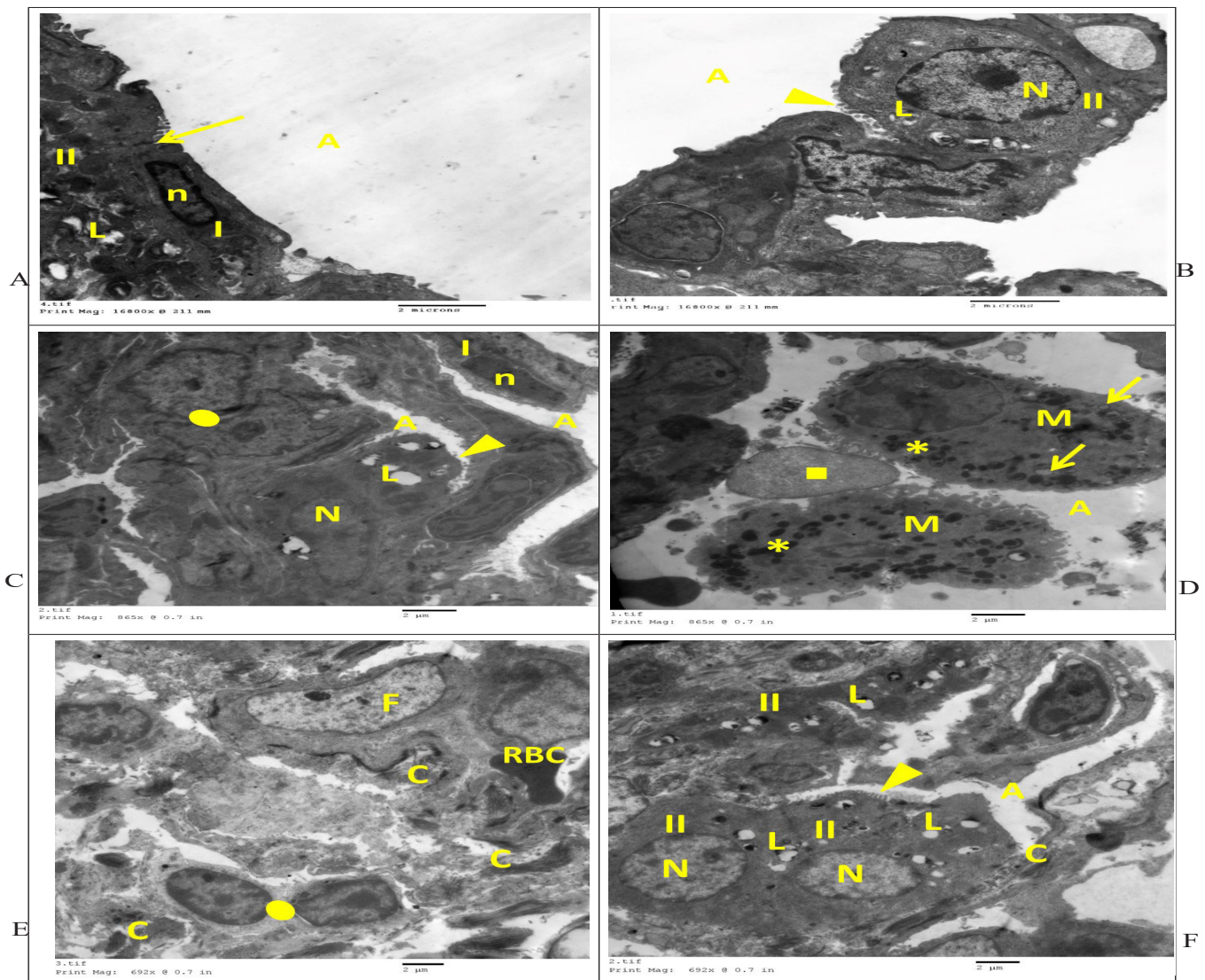
[B & C]: group II (CCL4 treated): showing narrow alveolar spaces with very thick interalveolar septum (↑). Foamy macrophages are seen in the septum (black circle). Apparent increase in type II pneumocytes, some are seen with dark small nuclei (▲). Congested blood vessels (C), extravasated RBC and mononuclear infiltration with many lymphocytes with dark rounded nuclei (L) are also found. Many alveolar spaces are closely obliterated (*). [D]: group III (MSCs treated): showing alveoli with relatively thin interalveolar septum (↑), lined by type I pneumocyte (↑) and apparently increased number of type II pneumocyte with vesicular rounded nuclei (▲). Some foamy macrophages in the septum (black circle) are still seen. [E]: group IV (exosome treated): showing alveoli with relatively thin interalveolar septum (↑), lined by type I pneumocyte (↑) and few type II pneumocyte (▲).

X400.

B. TEM results:

Group I (control group) showed patent alveolar spaces lined by pneumocyte I with flat euchromatic nuclei and dome shaped pneumocyte II with euchromatic rounded nuclei, lamellar bodies and apical microvilli on their surfaces. Tight junctions between alveolar cells were noticed (Figure 4A & B). Tight junction between alveolar cells was noticed (Figure 4A). Group II (CCL4 group) showed collapsed narrow alveolar spaces lined by pneumocyte I with heterochromatic flat nuclei and pneumocyte II with round nuclei, empty lamellar bodies and disrupted apical microvilli (Figure 4C). Many macrophages with many lysosomes and phagosomes in their cytoplasm were noticed in the alveolar spaces and the septa (Figure 4D). Some proliferating type II pneumocyte were also found (Figure 4C& E). Many Fibroblasts with euchromatic nuclei were seen in interalveolar septa with collagen fibers deposition in many areas. Extravasated

RBCs were noticed (Figure 4E). Group III (MSCs group) showed collapsed narrow alveolar spaces with many type II pneumocytes (II) with euchromatic nuclei, apical microvilli, secondary lysosomes and some regenerating empty lamellar bodies in their cytoplasm (Figures 4F & G). Fibroblasts and many collagen fibers depositions were noticed (Figures 4F & G). Many macrophages with euchromatic indented nuclei and numerous lysosomes were also present. Other macrophage appeared having smooth cytoplasm with scanty lysosomes (Figure 4H). Group IV (EXOs group) showed patent alveolar spaces lined with type I pneumocyte with thin cytoplasm and euchromatic nucleus and dome shaped mature type II pneumocyte with electron dense large lamellar bodies, elongated electron dense mitochondria and apical microvilli. Macrophages with irregular outline and lysosomes were seen. Collagen fibers deposition was also found in some septa (Figures 4I & J).



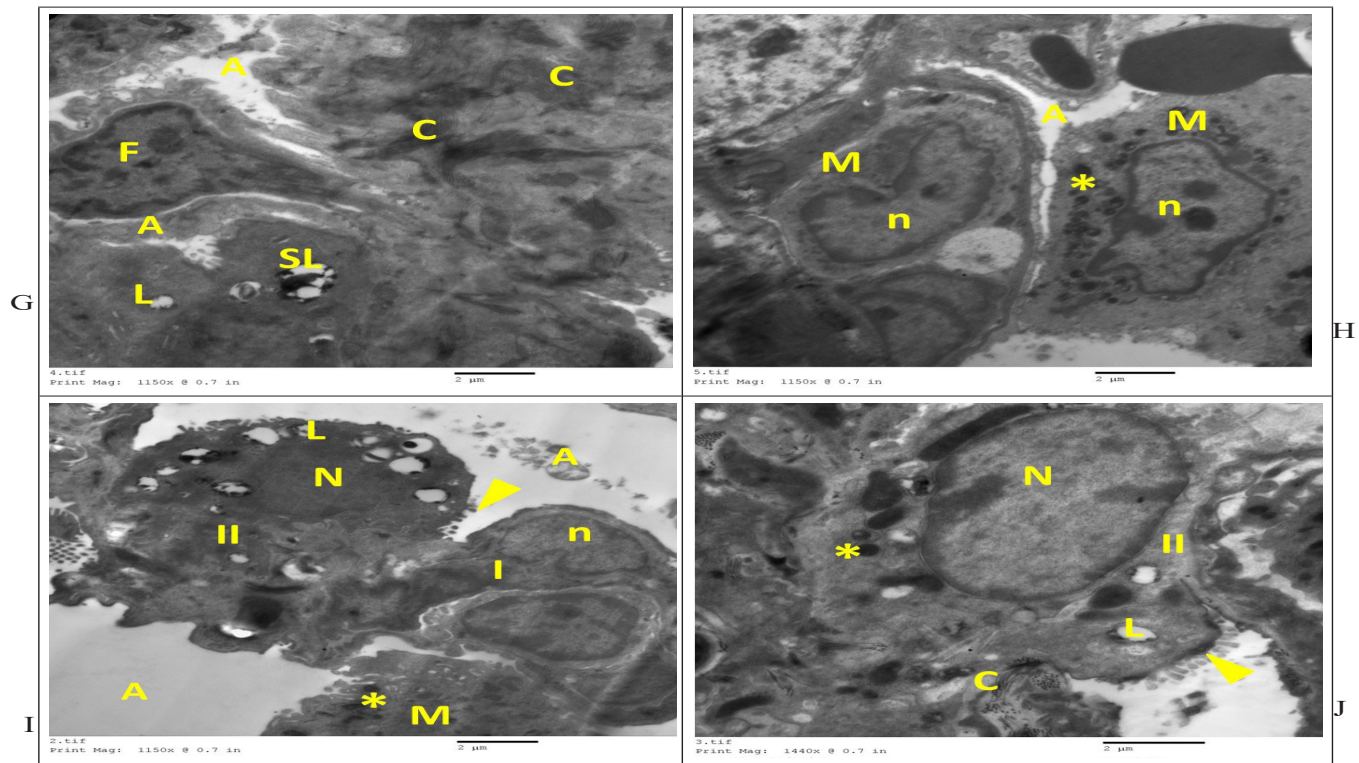


Fig. 4: Electron micrographs of lung tissue from different studied groups. [A & B]: group I(control): showing patent alveolar spaces (A) lined by pneumocyte I (I) with flat euchromatic nucleus (n) and dome shaped pneumocyte II (II) with euchromatic rounded nucleus (N), lamellar bodies (L) and apical microvilli (yellow Δ). Tight junction (↑) between alveolar cells is noticed. [C, D and E]: group II (CCL4 treated): showing narrow alveolar spaces (A) lined by pneumocyte I (I) with heterochromatic flat nucleus (n) and pneumocyte II (II) with rounded nucleus (N), empty lamellar bodies (L) and disrupted apical microvilli (yellow Δ). Many macrophages (M) with many lysosomes (*) and phagosomes (↑) in their cytoplasm are noticed in the alveolar spaces and the septa. Some apoptotic bodies (■) and proliferating type II pneumocytes (yellow dot) are also found. Many Fibroblasts with euchromatic nuclei (F) are seen in interalveolar septa with collagen fibers deposition (C) in many areas. Notice the extravasated RBC [F, G and H]: group III (MSCs treated): showing narrow alveolar spaces (A) with many type II pneumocytes (II) with euchromatic nuclei (N), apical microvilli (yellow Δ), secondary lysosomes (SL) and some empty lamellar bodies (L) in their cytoplasm. Fibroblasts (F) and many collagen fibers depositions (C) are noticed. Many macrophages (M) with euchromatic indented nuclei (n) and numerous lysosomes (*) were also present. Some macrophage appear with smooth cytoplasm and scanty lysosomes. [I and J]: group IV (exosome treated): showing patent alveolar spaces (A) lined with type I pneumocyte (I) with thin cytoplasm and euchromatic nucleus (n) and dome shaped type II pneumocyte (II) with lamellar bodies (L) and apical microvilli (yellow Δ). Part of macrophage (M) with irregular outline and lysosomes (*) is seen. Collagen fibers deposition (C) is found in some septa. TEM (C,D,E and F X 1500) and (A, B, G, H, I and J X 2000)

II. Morphometric results

The interalveolar septum's mean thickness considerably increased in CCL4 group when associated to group I and both

MSCs and EXOs groups. There was significant decrease in group IV (EXOs) when compared to MSCs group. However, non-significant difference is present between EXOs group and the control (Table 1 & Chart 1).

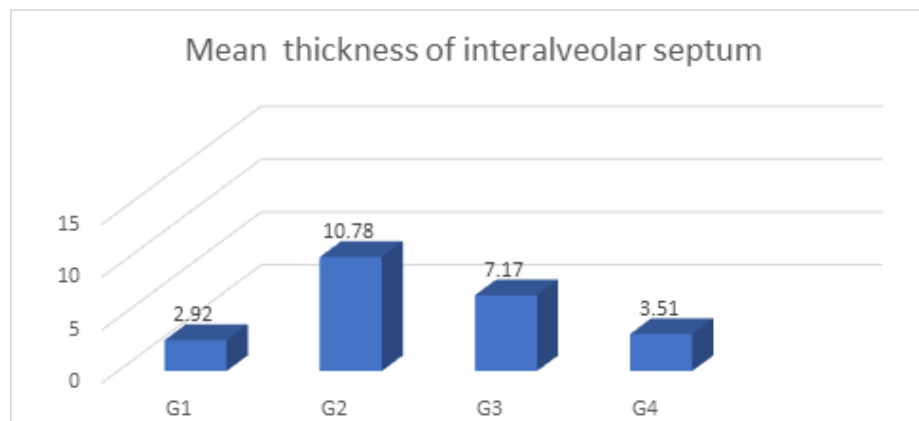


Chart 1: Mean thickness of interalveolar septum in different studied groups

Average area percentage of collagen fibers was significantly increased in CCL4 group compared to group I, MSCs and EXOs groups. Meanwhile, MSCs group revealed significant increase toward both control and EXOs groups,

but significant decrease towards CCL4 group. Moreover, EXOs group revealed significant increase towards the control, but significant decrease towards both CCL4 & MSCs groups (Table 1 & Chart 2).

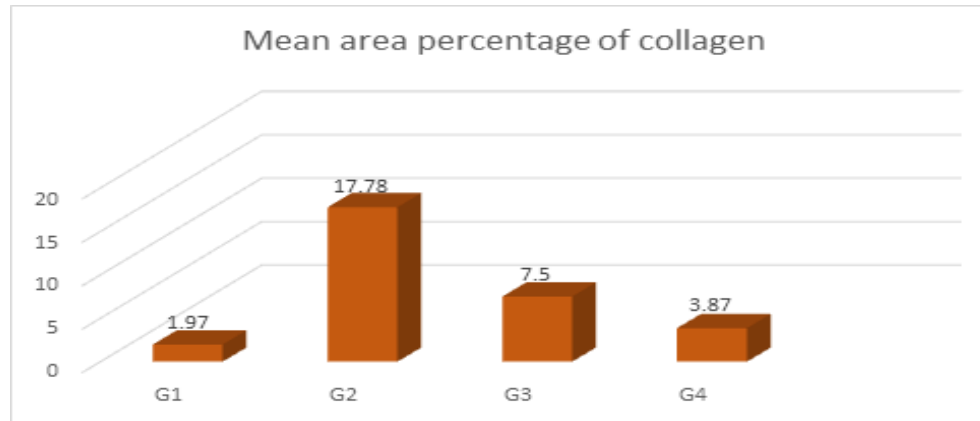


Chart 2: Mean area % of collagen in different studied groups.

DISCUSSION

A serious condition that affects the lung parenchyma is pulmonary fibrosis. As the fibrosis increases, the disease's pattern worsens and leads to severe restrictive lung disease^[17]. CCL4 is commonly used to induce toxicity in experimental animal studies^[6].

Histological and ultrastructural evaluation of lung tissue in this study revealed an obvious deterioration in CCL4-treated group, this was manifested by collapsed and narrow alveoli, thickened interalveolar septa with populated with mononuclear cell infiltration, extravasation of RBC. These results might be explained by degeneration of endothelial lining of capillaries and epithelial lining of alveoli with depletion of lamellar bodies and sloughing of club cells of bronchioles. These findings were in accordance with some researchers who reported that club cells secrete proteins that have immunomodulatory effect and inhibit macrophage activation and inflammation^[18].

TEM revealed apparent increase in macrophages with numerous lysosomes in their cytoplasm in the current study. It was found that necrotic materials activate macrophage (M1) with release of tumor necrosis factor, reactive oxygen species (ROS) and cytokines (IL1, IL6, and IL12) leading to inflammation^[17] which was confirmed by significant increase in thickness of alveolar wall in the current study.

Apparent increase in pneumocyte II (stem cells) was noticed in group II of the current study with appearance of proliferating cells, which was reported by previous studies that it might be due to renew and regenerate the damaged pneumocyte I. Furthermore, it was stated that release of ROS has a role as second messenger that stimulates cell proliferation^[19].

Also, TEM examination of this research showed macrophage with smooth cytoplasm and scanty lysosomes (most probably M2). Some authors cleared that in late phase, M2 activation involved repair and healing leads to fibrosis confirmed by significant increase in Sirius red stain. Also, they cleared that M2 macrophage activation resulted in release of interleukin 10 (IL-10) and transforming growth factor-beta (TGF-beta), that mediate its anti-inflammatory and healing roles^[17]. These findings agreed with previous studies indicated that CCL4 induced oxidative stress and inflammation, leading to pulmonary fibrosis and epithelial damage^[20]. Excess extracellular matrix (ECM) buildup in the interstitial tissue and basement membrane and increased activated mesenchymal cells (myofibroblasts) were a hallmark of fibrosis^[21]. Fibrosis was approved by the substantial rise in collagen area % due to increased deposition of collagen in alveolar walls and bronchioles. This was consistent with reports showing that chronic inflammation can activate fibroblasts and myofibroblasts, promoting ECM accumulation^[22,23]. Ultrastructural examination of lung sections in this study revealed type I pneumocytes with nuclear heterochromatin and cytoplasmic thinning, while type II pneumocytes exhibited disrupted lamellar bodies and apical microvilli. These changes might impair surfactant production and gas exchange, affecting respiratory function. It was stated that surfactant may play a part in controlling the liquid balances in the airways and the clearance rates of the bronchi, but it did not just preserve the stability of the alveoli and airways. Furthermore, oxidative stress directly harmed surfactant homeostasis and resulted in impaired surfactant synthesis from lipid peroxidation mechanisms^[24].

In the current study MSC-treated rats showed partial improvement of lung tissue with some patent alveoli and a notable decline in the mean interalveolar septal thickness

in comparison to the CCl₄ group. Type II pneumocytes appeared more numerous and showed signs of regeneration (immature cells), such as euchromatic nuclei and preserved regenerating lamellar bodies. These findings suggest that MSCs exert a protective effect through paracrine signaling and immunomodulation, as previously reported^[25]. Several studies reported that MSCs improved lung fibrosis. Recently in 2024, it has been reported that MSCs improved lung fibrosis induced by amiodarone in rat lungs^[26]. Another study showed that two injections of MSCs appeared to be optimal for improving radiation induced lung fibrosis. They found that the inflammatory response was reduced, with reduction in the expression of Caspase-3 protein and an increase in the Bcl-2/Bax ratio^[27]. Moreover, MSCs attenuated rats' lung fibrosis through anti-inflammatory and anti-apoptotic effects. By limiting the expression of pro-inflammatory factors like IL-6 and interferon β and promoting the expression of several anti-inflammatory factors like IL-1, IL-6, and IL-10, MSCs have an anti-inflammatory effect. MSC-secreted IL-1 has the ability to degrade inflammasomes and reduce IL-1 β expression. To reduce the inflammatory condition in pulmonary tissue, MSCs can also increase the secretion of HGF, IL-10, and keratinocyte growth factor (KGF) and decrease NF- κ B nuclear transfer. Furthermore, MSCs secrete superoxide dismutase (SOD), an effective ROS scavenger that may protect the lungs from reactive oxygen damage and reduce TGF- β and collagen production, hence reducing the buildup of extracellular matrix and relieving lung fibrosis^[28].

Despite that, some residual fibrotic changes persisted in MSCs treated group, including collagen deposition and inflammatory infiltration. It was reported that MSCs treated rats show lower collagen deposition and decrease TGFB in injured lung tissues denoting that MSCs regulate TGFB level^[29].

Exosome-treated rats of the present study revealed the most favorable outcomes, with better preservation of alveolar architecture, minimal mononuclear infiltration, and reduced collagen deposition which was in accordance with some researches^[30]. Exosomes restore club cells which secrete proteins that suppress macrophage activation thus had anti-inflammatory effect^[18].

Ultrastructural analysis showed nearly normal morphology of type I and mature form of type II pneumocytes, with intact tight junctions and lamellar bodies, microvilli suggesting effective restoration of epithelial integrity and surfactant production. These results agreed with the evidence that indicated that exosomes derived from MSCs carry bioactive molecules including miRNAs (antifibrotic) by modulating activity of M2 macrophages, growth factors for repair, and anti-inflammatory cytokines (iL10 & TGFB) and that enhance their therapeutic effects^[31]. The exosomes' capacity to reduce fibrosis and promote tissue

repair without the risks associated with cell transplantation makes them a promising alternative for regenerative therapies^[32,33]. Furthermore, Statistical comparison (the significant differences in thickness of alveolar septa due to mononuclear infiltrates and area percentage of Sirius red stain for collagen) indicated that exosomes improved the lung architecture compared to MSCs. It was stated that beside the role of exosomes as a signaling molecule, they are not only more stable than MSCs, but they also have the same effects. Exosomes are less immunogenic and better tolerated than MSC treatment. With these benefits, there are more opportunities to treat diseases^[34]. Moreover, numerous functional proteins, mRNAs, miRNAs, and signaling lipids are found in exosomes. They are safer and more stable than their parent cells as we can reduce the risk of occlusion in microvasculature that might happen when administering viable cells as MSCs^[35].

CONCLUSION

This study showed that CCl₄ induced significant histological and ultrastructural damage in rat lungs, characterized by inflammation, fibrosis, and epithelial injury. Both MSC and exosome treatments showed therapeutic efficacy, with exosomes revealing better healing results.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

AUTHORS CONTRIBUTION

Faten A. Mahmoud: suggested research idea - conducting the experiment - writing - collecting references - investigating and statistically analyzing the results - photography - final review, Eman H. Eltantawy: conducting the experiment - writing - collecting references - investigating and statistically analyzing the results – photography, Samar Mohamed Fekry Mohamed Ezzat: shared in conduction of the experiment - writing - collecting references - investigating and statistically analyzing the results - photography - final review

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دراسة هستولوجية على التأثير العلاجي المحتمل للخلايا الجذعية الوسيطة مقابل الإكسوسومات المشتقة منها في إصابة الرئة المستحثة برابع كلوريد الكربون في ذكور الجرذان البيضاء البالغة

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المقدمة: تليف الرئة مرضٌ مميّزٌ ومتفقم. يُستخدم رابع كلوريد الكربون كمواد غريبة للتسبب في تليف الرئة. حظيت الخلايا الجذعية الوسيطة باهتمام كبير كخيار علاجي محتمل. وقد أثبتت الأبحاث قدرة الإكسوسومات على تجديد الأنسجة.

الهدف من الدراسة: تقييم التأثير الهستولوجي للخلايا الجذعية الوسيطة مقابل الإكسوسومات على التليف الرئوي المستحث برابع كلوريد الكربون في الجرذ

المواد: تم استخدام ست وأربعون جرذا ذكرا بالغا. تم استخدام عشرة جرذان لتحضير الخلايا الجذعية وفصل الإكسوسومات. وتم تقسيم ست وثلاثين جرذا الى أربع مجموعات، المجموعة الأولى (الضابطة) وعددهم خمسة عشر، المجموعة الثانية وعددهم سبع وتم حقنها داخل الغشاء البريتوني بـ ٣٠٪ رابع كلوريد الكربون بجرعة ٣ مل/كجم من وزن الجسم مذاب في زيت زيتون مرتين أسبوعيا لمدة ثمانية أسابيع. المجموعة الثالثة وعددها سبع وتم حقنها برابع كلوريد الكربون كما في المجموعة الثانية ثم تم حقنها بالخلايا الجذعية بجرعة 1×10^6 خلية في ١٥٠ ميكروليتر من المحلول الملحي المعدل بالفوسفات مرة واحدة في الوريد الذيلي في بداية الأسبوع التاسع. المجموعة الرابعة وعددها سبع وكذلك تم حقنها برابع كلوريد الكربون كما في المجموعة الثانية ثم حقنها بالإكسوسومات المحضرة في المحلول الملحي المعدل بالفوسفات بجرعة ١٥٠ ميكروجرام مرة واحدة في الوريد الذيلي في بداية الأسبوع التاسع وتم التضحية بجميع الجرذان في الأسبوع العاشر وتم تجميع العينات وتم عمل الدراسات والفحص النسيجي والاحصاء.

النتائج: التغيرات الناتجة عن رابع كلوريد الكربون ظهرت في شكل، إحتقان الشعيرات الدموية، تجمعات من الخلايا الخاصة بالالتهابات ونزيف وزيادة في سمك الحواجز بين الحويصلات الهوائية مع زيادة في كمية الكولاجين. كما ظهرت تغيرات في تركيب الخلايا المبطنة للحويصلات الهوائية بالميكروسكوب الإلكتروني مثل تضرر في الأهداب الدقيقة والأجسام الطبقيّة الفارغة. وقد أدى استخدام الخلايا الجذعية والإكسوسومات الى تحسن التغيرات الهيستولوجية السابقة ولكن التحسن كان أفضل مع الإكسوسومات.

الخلاصة: يمكن الاستنتاج أن استخدام الخلايا الجذعية والإكسوسومات أدى الى تحسن التلف المستحث برابع كلوريد الكربون في الرئة وكان تأثير الإكسوسومات أفضل.