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Role of Bone Marrow Transplantation in Reducing the Dangerous Effect of Multi-Walled Carbon Nanotubes or/and Gamma Rays on Male Rats

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ABSTRACT

Aim of the work: this study evaluated the efficacy of bone marrow (BM) transplantation to protect male rats from dangerous effect of multi-walled carbon nanotubes (MWCNTs) or/and γ-irradiation on the hematopoietic and lung tissues. **Materials and methods:** experimental animals were divided into 8 groups each consist of 6 male albino rats. Control group, BM-injected group, MWCNTs-injected group, 5Gy γ-irradiated group, 5Gy γ-irradiated+ MWCNTs-injected group, MWCNTs-injected+ BM-injected group, 5Gy γ-irradiated+ BM-injected group and 5Gy of γ-irradiated+ MWCNTs-injected + BM-injected group. All the treated animal groups were sacrificed after 28 days of the treatments. Blood components, MDA and GSH levels in the lung tissue were analyzed. The histopathological study in the lung tissue was also recorded. **Results:** exposure to MWCNTs or/and γ-radiation induced a significant decrease in certain blood components (WBCs, RBCs, Hb content, HCT value and PLT count). Furthermore, a significant elevation in MDA level and a significant decrease in GSH content were detected in the lung tissue. The histopathological changes after exposure of rats to MWCNTs recorded perivasculitis, atelectasis and interstitial pneumonia. Also, γ-radiation represented more collapsed and thickened walls in the alveoli, thickened bronchiolar walls with partial epithelial lining and foci of pulmonary hemorrhage in the lung tissue. The effect of BM transplantation after MWCNTs or/and γ-radiation ameliorated the values of blood components, MDA and GSH levels in the lung tissue. The improvement occurred by BM transplantation in rats treated with MWCNTs or/and exposed to γ-radiation were also recorded. The lung tissue showed numerous alveoli with thin interalveolar septa, alveolar sacs and terminal bronchioles with highly folded mucosa. **Conclusion:** treatment with BM transplantation improved the most deleterious parameters obtained in the blood and lung tissue of MWCNTs exposed or/and γ-irradiated rats.

Keywords: multi-walled carbon nanotubes, γ- Radiation, bone marrow, male rats.

INTRODUCTION

Carbon nanotubes (CNTs) are of great research interest due to their unique physicochemical properties, represent an important class of engineered nanomaterials. Cancer development due to fiber-like straight type of multi-walled carbon nanotubes has raised concerns for human safety because of its shape similar to asbestos [1]. The exposure to CNTs substantially induces harmful effects on the lungs, including inflammatory granulomas and lung fibrosis in animal models [2]. In a study, intracellular ROS production was five times in MWCNTs-treated cells than control levels [3]. Inhalation of MWCNTs during their manufacture or aspirating 25mg of MWCNTs in lung tissues of mice may cause oxidative stress and GSH deficiencies [4].

Radiation is one of the most important environmental factors and has hazardous effects on health, which include oxidative stress [5], immune dysfunction [6] and hematopoietic system dysfunction [7]. One of the recognized delayed effects of such exposures is lung injury, characterized by respiratory failure as a result of pneumonitis that may subsequently develop into lung fibrosis [8].

Bone marrow is the primary hematopoietic tissue in mammals producing all blood cells [9]. Autologous or syngeneic and allogeneic bone marrow transplantation has been increasingly efficacious in the treatment of malignant and lymph hematopoietic and solid malignancies, as well as in non-malignant disorders such as thalassemia and immunodeficiency, though it is also associated with high treatment-related morbidity and mortality [10]. Also, the preclinical and clinical studies have demonstrated that bone marrow stromal cells (MSCs) can be used for tissue repair [11]. The pluripotent stem cells play a pivotal role in tissue development and maintenance by replenishing the depletion of cells

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caused by ionizing radiation and damaging factors or that occurs physiologically during tissue [12].

In the current study, we aimed to evaluate the efficacy of BMT in reducing the dangerous effect of MWCNTs and γ-irradiation on the hematopoietic and lung tissues in rats.

MATERIALS AND METHODS

Experimental animals

Adult male albino rats of pure strain *(Rattus rattus)* ranging from 110-150 body weight were obtained from the animal house of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. The animals were maintained on a commercial standard pellet diet and tap water *ad libitum*. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Multi-walled carbon nanotubes (MWCNTs)

Multi-walled carbon nanotubes were provided by Egyptian Petroleum Research Institute (EPRI). They were synthesized by using a chemical vapour deposition (CVD) method [13]. Some of the physicochemical characteristics of the MWCNTs are as follows: purity ≥ 93%; average diameter 30 nm; length ≥1 μm, and metal impurities, carbon oxide, Magnesium oxide and amorphous carbon 7%. MWCNTs were determined with transmission electron microscopy (TEM) to be 1 to 2μm in length (Fig.1 A), with a diameter of 10 to 30nm (Fig. 1 B). The MWCNTs were suspended in aqueous solution (1% Tween 80 or DMEM containing 10% fetal bovine serum) and then sonicated for 16 cycles of 5 seconds ultrasonication with a 6 seconds pause in an ice bath using an ultrasonic disruptor (200 W, JY 92-IIN, Scientz, Ningbo, China). Animals were injected intravenously (i.v.) through the caudal vein with a single dose of 60 mg/kg body weight [14].

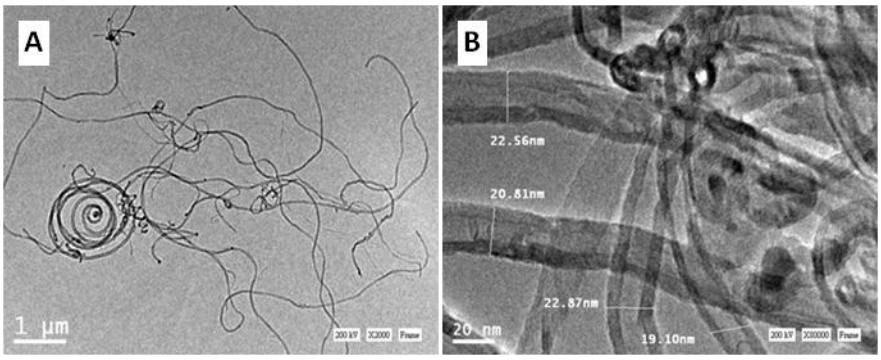

Fig. 1. A&B. Transmission electron microscopy of multi wall carbon nanotubes.

Radiation treatment

Whole body γ-irradiation was performed with a Canadian (Cesium-137) Gamma Cell-40 at NCRRT, Cairo, Egypt; at a dose rate of 1.2 R/second. Rats were exposed to 5Gy of whole body γ- radiations delivered as an acute single dose.

Bone Marrow (BM) transplantation

Donors and recipients were chosen from the same inbred strain, brother to brother (isologues or synergic or allogeneic transplantation). Femur bones were dissected out and cleaned. The ends of the bones were chipped by a bone nibbling forceps. Then the marrow was blown out of the femur into isotonic solution under sterilized conditions inside a laminar flow cabinet. The marrow was collected into a sterile container surrounded by ice cubes, and mixed by drawing and expelling it several times from the syringe without needle in order to avoid mechanical damage to the cells. Total viable cells of about 75 $\times 10^6 \pm 5\%$ were injected intravenously through the caudal vein [15].

Groups of animals under investigation

Forty eight male albino rats were divided into 8 groups of 6 animals in each as follows:

G1: control rats.

G2: rats were injected with BMCs (75×10^6) through the caudal vein.

G3: rats were injected with MWCNTs (60 mg/kg bw) through the caudal vein.

G4: rats were exposed to 5Gy of γ-radiation.

G5: rats were exposed to 5Gy γ-radiation and injected caudally with MWCNTs.

G6: rats were injected caudally with MWCNTs then injected with BMCs.

G7: rats were exposed to 5Gy of γ-radiation then injected caudally with BMCs one hour later [16].

G8: rats were exposed to 5Gy γ-radiation and injected caudally with MWCNTs then injected with BMCs one hour post treatment.

After 28 days of final treatments all groups of animals were sacrificed.

Blood and lung tissue from animals of each group were collected and used for the proposed studies.

Hematological analyses

After collecting blood samples in a CBC container containing the anticoagulant EDTA, CBC samples were placed in the hematology mixer machine for ten minutes, and then samples were counted using the cell counter device (ABX Micros 60, Horiba ABX, France) to determine the blood parameters: WBCs, RBCs counts, Hb concentration, HCT value and PLT count.

Biochemical analyses

The lung glutathione (GSH) content was determined according to the method of **Beutler *et al.*** [17]. Malondialdehyde (MDA) level, the end product of lipid peroxidation was determined in the lung tissue according to the method of **Yoshioka *et al*.** [18].

Histopathological studies

The lung was excised, fixed in 10% formalin for 48 hours, then dehydrated, processed and embedded in paraffin blocks, for histopathological observations, sections of (5-6 µm) thickness from lung tissues were stained with haematoxylin and eosin (H&E) according to the method of **Drury and Wallington** [19]. For connective-tissue fibrillae detection Mason's stain was used according to **Carson** [20].

Statistical analyses

Multiple group comparison was done using one-way analysis of variance using SPSS/PC software program (version 14.0; SPSS Inc., Chicago, IL, USA). All values were represented as mean ± S.E of six animals. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS

1- Hematological parameters

Results represented in Table 1 showed the effect of BMCs transplantation on blood components of rats treated with MWCNTs or/and 5Gy γ-radiation. It was noticed that the number of **WBCs** count decreased significantly in rats exposed to MWCNTs or/and 5Gy γ-radiation compared to the control group with percent of changes -46.29%, -44.40% and -48.14% respectively and **PLT** count with percent of changes -47.18%, -48.50% and -28.63% respectively. On the other hand, no significant changes were recorded in **WBCs** and **PLT** counts in BMCs transplantation group compared to the control group. Meanwhile, BMCs transplanted in rats exposed to MWCNTs or/and 5Gy γ-radiation represented partial amelioration in the values of **WBCs** and **PLT** with percent of changes (-9.25%, 32.40% and -35.37% respectively) and **PLT** count with percent of changes (-2.44%, -26.70% and -1.23% respectively) compared to the control group.

Also, in table 1 data recorded significant decreases in the values of **RBCs** count of rats exposed to MWCNTs or/and 5Gy γ-radiation with percent of changes -34.88%, -21.25% and -22.50% respectively, **Hb** content with percent of changes -35.30%, -27.20% and -33.33% respectively and **HCT** value with percent of changes -26.65%, -27.60% and -26.87% respectively compared to the control group. On the other hand, no significant changes were recorded in **RBCs** count, **Hb** content and **HCT** value in BMCs transplantation group compared to the control group. Meanwhile, BMCs transplanted in rats exposed to MWCNTs or/and 5Gy γ-radiation represented partial amelioration in the values of **RBCs**, **Hb** and **HCT**. The values of RBCs count had percent of changes -23.87%, -10.00% and -13.75% respectively, **Hb** content had percent of changes -13.60%, -12.20% and -23.80% respectively and **HCT** value had percent of changes +3.99%, -2.10 % and +20.33% respectively compared to the control group.

2- Biochemical parameters

Results obtained in table 2 showed that the lung MDA level increased significantly with percent of changes +124.62%, +102.19% and +138.23% respectively and decreased significantly in GSH content with percent of changes -48.83%, -49.09% and -65.27% respectively in rats exposed to MWCNTs or/and 5Gy of γ-radiation compared to the control group. On the other hand, BMCs transplantation group had nearly normal value of the lung MDA levels and GSH content when compared to the control group. When rats were transplanted with BMCs after exposure to MWCNTs or/and 5Gy γ-radiation, there was amelioration in the values of MDA level with percent of changes -11.19%, +15.96% and +4.60% respectively and amelioration in the values of GSH content with percent of changes -20.62%, -18.54%and -25.33% respectively when compared to the control group.

Table 1. The effect of BMCs transplantation on WBCs (10e3/ UL), PLT (10e3/UL) count, RBCs ($10e^6$/ UL) count, Hb (g/dL) content and HCT % of rats treated with MWCNTs or/and 5Gy γ-radiation.

Groups / Parameters		Control	MWCNTs	5Gy	MWCNTs +5Gy	BMCs	MWCNTs +BMCs	5Gy + BMCs	MWCNTs+ 5Gy+ BMCs
WBCs	Mean ±SE	10.80 ± 0.60	5.80 ±0.43 [a, d]	6.00 ±0.60 [a, d]	5.60 ±0.21 [a, d]	11.00 ±0.40 [b, c]	9.80 ±0.09 [a, b, c, d]	7.30 ±0.70 [a, b, c, d]	6.98 ±0.35 [a, b, c, d]
	% change vs control		-46.29	-44.40	-48.14	+1.80	-9.25	-32.40	-35.37
PLT	Mean ±SE	379.70 ±35.30	200.54 ±11.70 [a, c, d]	195.50 ±10.00 [a, b, d]	271.00 ±21.21 [a, b, c, d]	380.20 ±36.00 [b, c]	370.43 ±12.62 [a, b, c, d]	278.30 ±18.00 [a, b, c, d]	375.00 ±11.90 [a, b, c, d]
	% change vs control		-47.18	-48.50	-28.63	+0.10	-2.44	-26.70	-1.23
RBCs	Mean ±SE	8.00 ±0.40	5.21 ±0.35 [a, c, d]	6.30 ±0.30 [a, b, d]	6.20 ±0.28 [a, b, d]	8.20 ±0.30 [b, c]	6.09 ±0.26 [a, b, d]	7.20 ±0.20 [a, b, c, d]	6.90 ±0.15 [a, b, c, d]
	% change vs control		-34.88	-21.25	-22.50	+2.50	-23.87	-10.00	-13.75
Hb	Mean ±SE	14.70 ±0.60	9.50 ±2.80 [a, c, d]	10.70 ±0.50 [a, b, d]	9.80 ±0.53 [a, c, d]	15.00 ±0.50 [b,c]	12.70 ±0.45 [a, b, c, d]	12.90 ±0.40 [a,b,c, d]	11.20 ±0.38 [a, b, d]
	% change vs control		-35.30	-27.20	-33.33	+2.00	-13.60	-12.20	-23.80
HCT %	Mean ±SE	41.30 ±1.40	30.30 ±2.54 [a, d]	29.90 ±1.40 [a, d]	30.20 ±1.05 [a, d]	41.80 ±0.90 [b, c]	39.65 ±0.62 [a, b, c, d]	40.40 ±1.30 [a, b, c, d]	32.90 ±0.95 [a, b, c, d]
	% change vs control		-26.65	-27.60	-26.87	+1.20	+3.99	-2.10	+20.33

Each value represents mean ± SE of 6 rats. [a]: Significantly different from control group ($p \leq 0.05\%$)
[b]: Significantly different from MWCNTs group ($p \leq 0.05\%$)
[c]: Significantly different from 5Gy group ($p \leq 0.05\%$)
[d]: Significantly different from BMCs group ($p \leq 0.05\%$)

Table 2. The effect of BMCs transplantation in rats treated with MWCNTs or/and 5Gy γ-radiation on MDA levels (n mol/g) and GSH (mg/gm) content in the lung tissue.

Group / Parameters		Control	MWCNTs	5Gy	MWCNTs +5Gy	BMCs	MWCNTs+ BMCs	5Gy + BMCs	MWCNTs +5Gy+ BMCs
MDA	Mean ±	15.065 ±0.59	33.84 ±0.76 [a, c, d]	30.46 ±0.56 [a ,b, d]	35.89 ±0.72 [a, b, c, d]	13.47 ±0.41 [b, c]	13.39 ±0.62 [b, c]	17.47 ±0.66 [a, b, c, d]	15.76 ±0.29 [b, c, d]
	% change vs control		+124.62	+102.19	+138.23	-10.59	-11.19	+15.96	+4.60
GSH	Mean ±	3.83 ±0.21	1.96 ±0.22 [a, d]	1.95 ±0.14 [a, d]	1.33 ± 0.17 [a, b, c, d]	3.99 ±0.18 [b, c]	3.04 ±0.16 [a, b, c, d]	3.12 ±0.12 [a, b, c, d]	2.86 ±0.14 [a, b, c, d]
	% change vs control		-48.83	-49.09	-65.27	+4.18	-20.62	-18.54	-25.33

Legends as in table1

3- Lung histopathological observations

A- Hematoxylin and eosin (H&E) staining

The lung tissue showed numerous alveoli with thin interalveolar septa, terminal bronchiole lined by simple columnar ciliated epithelium alternating with non-ciliated cells and surrounded by thin muscle layer (**Fig.2 A&B**). Treatment of control rats with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) after 28 days showed perivasculitis, atelectasis and interstitial pneumonia (Fig.**3 A & B**). On the other hand, exposure of rats to 5Gy γ-radiation represented more collapse and thickened wall in alveoli, thickened bronchiolar wall with partial epithelial lining and foci of pulmonary hemorrhage (**Fig. 4 A&B**). Meanwhile, the exposure of rats to 5Gy γ-radiation and treatment with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) recorded atelectasis and congested blood vessel (**Fig. 4 C**).

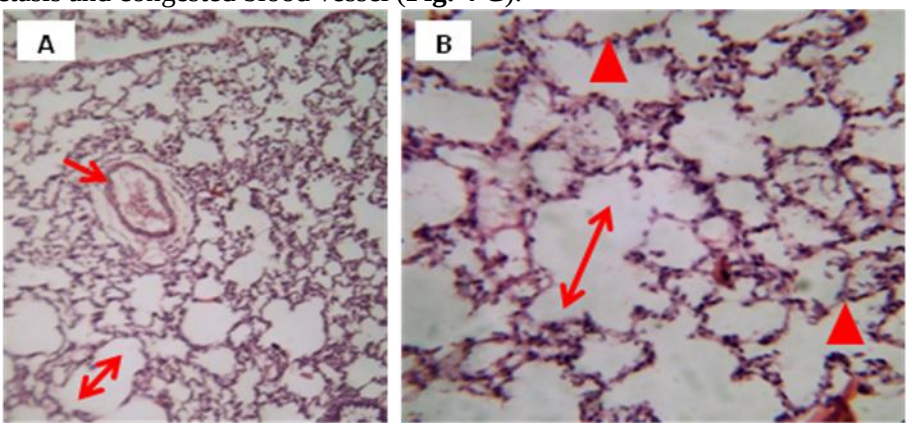

Fig.2. Photomicrographs of lung sections in control adult male albino rats showing numerous alveoli (↕) with thin interalveolar septa (▲), terminal bronchiole (↓) lined by simple columnar ciliated epithelium alternating with non-ciliated cells and surrounded by thin muscle layer. (H&E AX100 - BX 400)

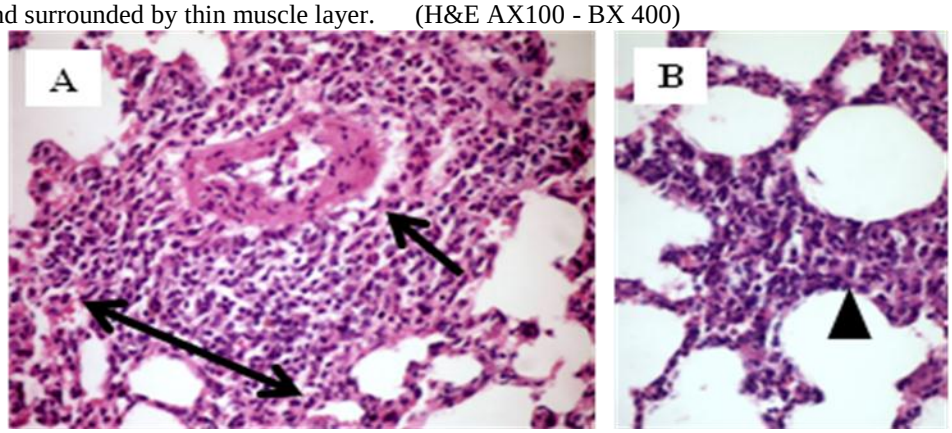

Fig. 3. Photomicrographs of lung sections in rats treated with 20 – 40 nm MWCNTs (60 mg/kg bw) 28 days post-treatment showing perivasculitis (↑), interstitial pneumonia (↔) in A, atelectasis in B (▲). (H&E X400)

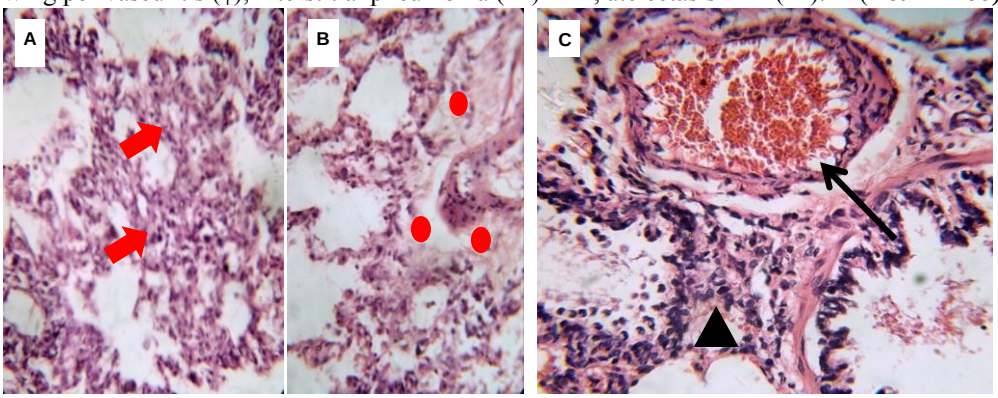

Fig.4. Photomicrographs of lung sections in rats exposed to 5Gy γ-radiation represented more collapse and thickened wall in alveoli (⬈) in A, thickened bronchiolar wall with partial epithelial lining and foci of pulmonary hemorrhage (●) in B. Notice: atelectasis (▲) and congested blood vessel (↑) in rats exposed to 5Gy γ-radiation and treated with MWCNTs in C. (H&E X 400).

The treatment of control rats with BMCs transplantation showed numerous alveoli with thin interalveolar septa and the terminal bronchiole exhibited highly folded mucosa (**Fig. 5 A**). The effect of BMCs transplantation injected in rats treated with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) showed numerous alveoli with thin interalveolar septa, terminal bronchiole exhibited highly folded mucosa (**Fig.5 B**). In addition, BMCs transplantation in rats exposed to 5Gy γ-radiation, lung tissue showed also numerous alveoli with thin interalveolar septa, alveolar sacs and bronchiole with highly folded mucosa (**Fig.5C**). When rats were exposed to 5Gy γ-radiation and treated with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) then treated with BMCs transplantation for 28 days, showed numerous alveoli with thin interalveolar septa, alveolar sacs and some atelectasis was also observed (**Fig. 6**).

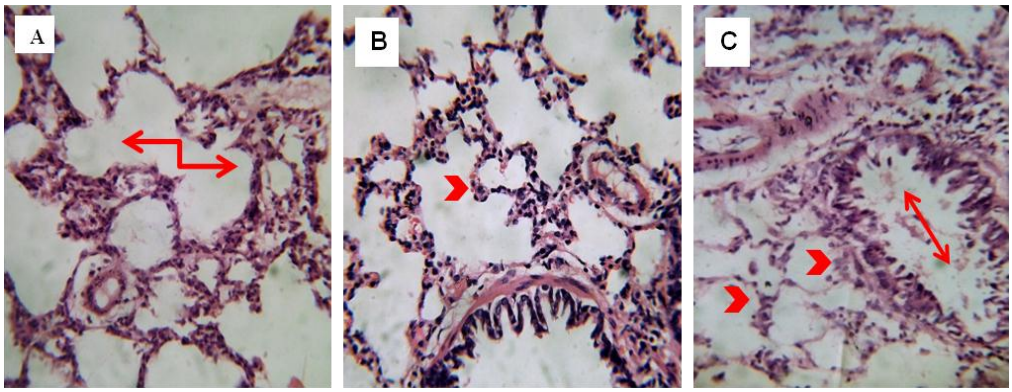

Fig. 5. Photomicrographs of lung sections in rats A. showing numerous alveoli (broken arrow) with thin interalveolar septa when treated with BMCs. B. recording numerous alveoli with thin interalveolar septa and terminal bronchiole with highly folded mucosa when treated with MWCNTs and BMCs. C. showing numerous alveoli (➤) with thin interalveolar septa and alveolar sacs and a bronchiole with highly folded mucosa in rats exposed to γ-radiation and treated with BMCs (↕) (C). (H&E X 400)

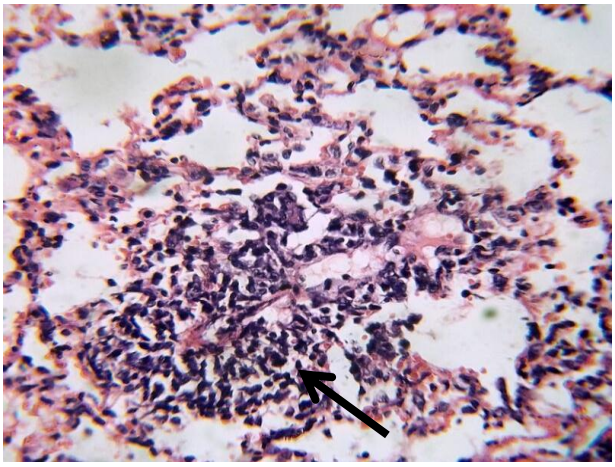

Fig. 6. Photomicrograph of lung section in rats exposed to 5Gy γ-radiation and treated with MWCNTs then BMCs transplantation showing numerous alveoli with thin interalveolar septa and alveolar sacs. Notice some atelectasis was observed (↑). (H&E X400)

B- Masson's trichrome staining

For demonstrating collagen fibers deposition in lung tissue Masson's trichrome stain was used. Control adult male albino rat lung showed faintly stained collagen fibers in pulmonary intersititium around bronchioles and alveolar sacs (**Fig. 7 A**). The treatment of rats with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) after 28 days recorded moderately stained collagen fibers deposition in and around perivascular, peribronchiole blood vessels, alveolar sacs and in-between alveoli (**Fig. 7 B**). In addition, exposure of experimental rats to 5Gy γ-radiation represented intensely stained collagen fibers deposition

surrounding congested pulmonary blood vessel, bronchiole, alveolar sacs and in-between alveoli (**Fig. 7 C**). On the other hand, exposure of rats to 5Gy γ-radiation and treated with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) showed moderately stained perivascular and peribronchiole collagen fibers deposition. Also, deposition of collagen fibers around the blood vessels, alveolar sacs and in-between alveoli was observed (**Fig. 7 D&E**).

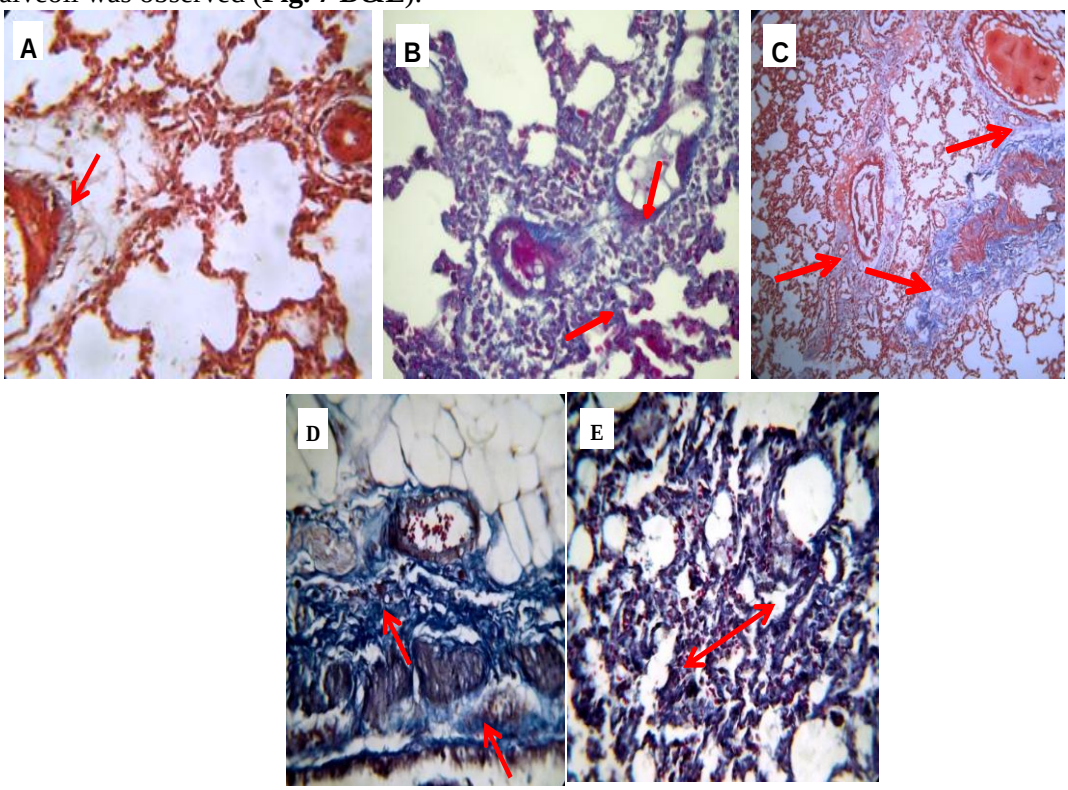

Fig. 7. Photomicrographs of lung sections in rats showing A. Faintly stained collagen fibers in pulmonary intersititium around bronchioles (↓) and alveolar sacs in control section. **B.** Rats treated with MWCNTs showing moderate stained collagen fibers deposition (↑) in perivascular, peribronchiole in (↑) and around blood vessels, alveolar sacs and in-between alveoli. **C.** Rats exposed to 5Gy γ-radiation showing intensely stained collagen fibers (↓) surrounding congested pulmonary blood vessel, bronchiole, alveolar sacs and in-between alveoli (↓). **D&E.** Rats exposed to 5Gy γ-radiation and treated with MWCNTs showing moderately stained collagen fibers deposition (↑) in perivascular, peribronchiole and around blood vessels, alveolar sacs and in-between alveoli (↔). (Masson's trichrome stain X400)

When control rats were injected with BMCs, faintly stained collagen fibers around bronchioles blood vessels and alveolar sacs were detected (**Fig. 8A**). The treatment of rats with BMCs transplantation and 20 – 40 nm MWCNTs (60 mg/kg bw) showed moderately stained collagen fibers, around bronchioles, blood vessels and alveolar sacs (**Fig. 8B**).

Meanwhile, experimental rats exposed to 5Gy γ-radiation and treated with BMCs transplantation showed faintly stained collagen fibers around bronchioles, blood vessels, alveolar sacs and in-between alveoli (**Fig. 8C**). When rats were exposed to 5Gy γ-radiation and treated with 20 – 40 nm MWCNTs (60 mg/kg bw) then BMCs transplantation, moderately stained perivascular and peribronchiole collagen fibers deposition around blood vessels, alveolar sacs and in-between alveoli were noticed (**Fig 8D**).

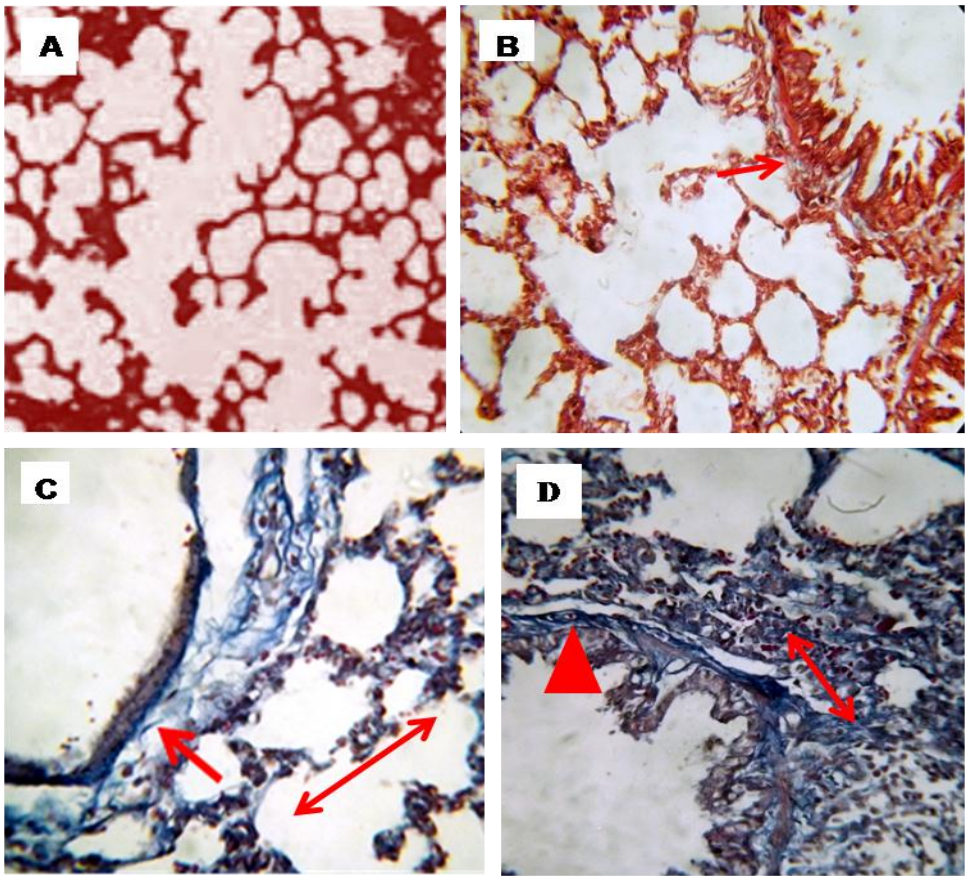

Fig. 8. Photomicrographs of lung sections in rats treated with BMCs transplantation showing A. Faintly stained collagen fibers (↑) around bronchioles blood vessels and alveolar sacs. **B.** Rats treated with MWCNTs then BMCs transplantation revealing moderately stained collagen fibers around bronchioles (↔), blood vessels and alveolar sacs (↑). **C.** Rats exposed to 5Gy γ-radiation and treated with BMCs transplantation showed faintly stained collagen fibers (→) around bronchioles, blood vessels, alveolar sacs and in-between alveoli. **D.** Rats exposed to 5Gy γ-radiation and treated with MWCNTs then BMCs transplantation showing moderately stained perivascular and peribronchiole collagen fibers deposition around blood vessels, alveolar sacs (▲) and in-between alveoli (↔). (Masson's trichrome stain X400)

DISCUSSION

Carbon nanotubes are of great research interest due to their unique physicochemical properties and represent an important class of engineered nanomaterials. MWCNTs consist of several stacked SWCNTs and exhibit diameters up to 100 nm and lengths up to several micrometers. Changes in structural and physicochemical properties of CNTs can lead to changes in biological activities including ROS generation; one of the most frequently reported CNTs-associated toxicities. Oxidative stress seems to be the primary cause behind the side effects of MWCNTs in humans because it induces inflammation by the activation of the transcription factors of oxidative stress-responsive [21].

Exposure to ionizing radiation whether accidental or during radiotherapy, leads to a serious systemic damage to various cellular and sub cellular structures and initiates a cascade of events that are based not only on direct DNA damage, but also other effects including oxidative damage that leads to alteration of tissue physiological functions. Radiation induces free radicals and increases in membrane lipid peroxidation and this impairs the antioxidative defence mechanism. Ionizing radiation causes the body to produce large amounts of ROS. Imbalance between production of ROS and antioxidant defense can result in oxidative stress [22]. Also, **Rezaeyan *et al.*** [23] showed that oxidative stress played an important role in the pathogenesis and progression of γ-irradiation-induced cellular damage.

In the present study, exposure of rats to 5Gy of γ-radiation recorded a significant decrease in hematological parameter (WBCs, PLT, RBCs counts, Hb content and HCT %) in comparison with control group, which may be due to alteration in bone marrow as well as hemopoietic system.

These results were in line with the findings of **Elshater *et al.*** [24] who reported that radiation exposure induced decline in hematological parameters (RBCs, Hb, HCT, PLT and WBCs). Also, **Marusyk *et al.*** [25] revealed that the blood system is the most sensitive target-organ of radiation. The decrease in Hb content and Hct% may be due to induction of changes by radiation in erythrocyte membrane emphasize the formation of free radicals. The effect of free radicals on erythrocyte membrane may contribute to the eventual leak of hemoglobin out of the cells. The reduction of leukocytes is the most classic indicator of radiation damage. Whole-body γ-irradiation was found to induce direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and reduced production of the blood cellular elements [26]. The previous results are in agreement with **Zhang *et al.*** [27] who showed that mice exposed to 7.5Gy TBI induced damage to the hematopoietic system.

In the present study, treatment of rats with a single dose of MWCNTs 20 – 40 nm MWCNTs (60 mg/kg bw) after 28 days post-treatment recorded a significant decrease in hematological parameters (RBCs, Hb, HCT, WBCs and PLT). A similar observation was obtained by **Mocan** [28] who revealed that the decrease in human RBCs is due to extended hemolysis for RBCs as well as absence of hemagglutination following exposure to MWCNTs, which induce the release of oxidative stress products like H_2o_2. Also, total and differential leukocyte, RBCs and PLT counts decreased in rats at 28 days post exposure to 22 mg/m^3 MWCNTs [29]. Similarly, **Pothmann *et al.*** [30] reported that significant decreases in hematological parameters (relative eosinophil and absolute neutrophil count, RBCs count, Hb, HCT and platelets count) in rats exposed to 5.0 mg/m^3 MWCNTs. Moreover, **Cartwright *et al.*** [4] recorded that aspirating 25µg of MWCNTs developed neutrophilia in male mice.

Moreover, the present study showed that ionizing radiation at a dose level of 5Gy γ-radiation seriously stimulated oxidative stress. This was evident from the significant elevation in the levels of MDA as well as reduction of GSH content in the lung tissue. The same results were obtained by **Azab *et al.*** [26] who showed that radiation exposure induced radiolysis of water in the aqueous media of the cells which leads to production of hydroxyl radicals (˙OH). Hydroxyl radical interact with the polyunsaturated fatty acids in the lipid portion of biological membranes initiating the lipid peroxidation and finally damaged the cell membranes. The oxidative stress which happened after γ-irradiation exposure may be due to the overproduction of MDA and the reduction on auto antioxidant GSH. Since, glutathione has protection role against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation [31]. Similarly, ionizing radiation induced a decrease in the membrane fluidity, disordering of membrane lipids, strengthening of the hydrogen bonding of the phosphate groups of lipid head-groups and caused lipid peroxidation. Moreover, **Abd El-Rahman *et al*.** [32] exposed male albino rats to 6Gy of whole body IR and recorded high LPO marker (MDA) associated with reduction in the content of reduced GSH in the lung tissue.

Results of the present study indicated that treatment of male albino rats with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) after 28 days recorded a significant increase in the levels of MDA and decrease in the level of GSH in the lung tissue. These results are in accordance with the findings of **Shvedova *et al.*** [33] who showed that (0.25–1 mg/kg) MWCNTs given by aspiration of mice caused a decreased in the level of GSH and an increased the level of MDA in the lung tissue. **Ryman-Rasmussen *et al.*** [34] also, reported that (100 mg/m^3) MWCNTs generated ROS and depleted antioxidants levels in exposed rat lung epithelial cells. Similarly, **Cartwright *et al.*** [4] found that MWCNTs-induced oxidative stress as reflected in GSH content of lung tissue when mice was exposed to 25mg of MWCNTs.

Bone marrow transplantation is the treatment of choice for many leukemias and solid tumors [35]. The present results discerned that injection of BMT after exposure to MWCNTs or/and γ-radiation led to a significant elevation of hematological parameters compared to the treated group. These results are in accordance with **El-Ganzuri *et al.*** [36] who confirmed the possible protective effect of BMCs transplantation on the hematopoietic tissue in irradiated rats. BMT after γ-radiation exposure showed that an elevation of bone marrow lymphocyte count is due to proliferation of stromal cells. In addition,, MSCs, one of the many types of adult stem cells, also have a high self-renewal capability for tissue engineering research including the treatment of hematopoietic diseases and lung diseases. Also, ability of carbon nanotube-based nanofibrillar surfaces, having enhanced cell adhesion and growth factor adsorption similar to the

extracellular matrix, to boost stem cell differentiation [37].

Also, BMT is critically important for tumorigenesis post irradiation cellular events [38]. In the present study the effect of BMCs transplantation on rats treated with MWCNTs or/and 5Gy γ-radiation showed a partial amelioration in the values of MDA levels and GSH content. Accordingly, BMT causes elevation in RBCs, the important source of GSH which can ameliorate the GSH depletion in blood and organs and hence oxidative stress**.** These results are in agreement with **Soliman *et al.*** [39] who found that in irradiated rats receiving BMCs transplantation significantly depressed LPO in serum and conversely elevated GSH as compared to the irradiated group. These findings coincide with present results where the GSH was elevated significantly in IR rats after BMCs transplantation compared to the irradiated group.

The lung is one of the most sensitive tissues to ionizing radiation. In the present study, exposure of rats to 5Gy γ-radiation, the lung tissue exhibited a more collapse and thickened walls of the alveoli, thickened bronchiolar walls with partial epithelial lining and foci of pulmonary hemorrhage. It was also reported that lung histopathological changes following gamma radiation exposure may be due to OS and subsequent overproduction of ROS which was postulated as one of the most important mechanisms of radiation toxicity [40] The increased thickness of the inter alveolar walls observed in the experimental rats of this study could be explained by the presence of excess inflammatory cells, congested capillaries, increased interstitial connective tissue and the associated alveolar collapse. Similarly, **Abd El-Hady and Al Jalaud** [41] revealed that exposing rats to 3Gy γ- radiation induced many histopathological changes represented by narrow alveolar sacs with highly thickened and congested alveolar septae, highly thickened arterial and venous walls which appeared congested with hemolysed blood cells. Bronchial walls appeared highly thickened, some of pulmonary bronchioles showed debris of degenerated cells with granulomatous areas in the lung interstitium.

In the present study, exposure of rats to 5Gy of γ-radiation represented extensive amount of collagen fibres deposition surrounding congested pulmonary blood vessel, bronchiole and alveolar sacs and in between alveoli. Similarly, **Zhang *et al.*** [42] explained that the appearance of degenerated alveolar septae and debris of degenerated cells in the lung bronchioles may be due to highly affected DNA in the nuclei of their cells after exposure to γ-irradiation**.** In addition, increased collagen fibres after γ-irradiation exposure are in coincidence with those of **Zhang *et al.*** [42]**.** Radiation induced pulmonary fibrosis with high morbidity and mortality rates due to progressive breakdown of pulmonary architecture, which results in respiratory failure. Gamma radiation induces direct activation of an epithelial mesenchymal transition in type II alveolar epithelial cells**,** pulmonary interstitial area density, inflammatory response and the development of fibrosis [43].

Moreover, the present study indicated that treatment of rats with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) after 28 days showed perivasculitis, atelectasis and interstitial pneumonia. Inhalation of MWCNTs during their manufacture or incorporation into various commercial products may cause lung inflammation, fibrosis, and oxidative stress in exposed workers [4]. Also, treatment of rats with a single dose of 20 – 40 nm MWCNTs (60 mg/kg bw) showed moderate collagen fiber deposition around blood vessels, alveolar sacs and in-between alveoli post 28 days of exposure. This result is similar to that described in time of an intratracheal instillation of MWCNTs to rats at 7 days resulted in strong pulmonary toxicity effects, including focal peribronchiolar lymphoid aggregates, lymphoplasmocytic infiltration, fibrosis and diffuse alveolar damage [44]. Also, **Treumann *et al.*** [45] observed granulomatous inflammation in the lung of rats at 0.5 and 2.5 mg/ m^3 MWCNTs and inhalation of MWCNTs led to an increase of connective tissue (collagen, reticulin) which could lead to lung fibrosis. These studies suggested that induction of fibroblast proliferation and collagen production by CNTs might be a key determining factor of CNT-induced lung fibrosis [46]. Also, **Kasai *et al.*** [1] found that granulomatous changes in the lung were induced and focal fibrosis of the alveolar walls was observed in rats when they were exposed to whole-body inhalation of MWCNTs at 5mg/m^3. In addition, **Kinaret *et al.*** [47] noticed that mice exposed to MWCNTs by inhalation of 6.2–8.2 mg/m^3 for 4 days promoted strong accumulation of eosinophils in the lungs and recruited also a few neutrophils and lymphocytes.

Data of the present study indicated that the improvement occurred by BMCs transplantation in rats treated with MWCNTs or/and exposed to 5Gy γ-radiation were recorded. The lung tissues

showed numerous alveoli with thin interalveolar septa, alveolar sacs and a bronchiole with highly folded mucosa, recurrence of the normal distribution of collagen fibres around the bronchioles, blood vessels, alveolar sacs and in between alveoli. Similarly, **Murat *et al.*** [48] reported that in TBI, BM transplantation avoided alterations and interstitial pneumonia of effective lung (a different cell phenotype, may be attributable to lung production of marrow cytokines). In agreement with the findings of **Jiang *et al.*** [49] who showed that MSCs had a significant potential for clinical use in radiation-induced lung injury in rats. These results are in accordance with the findings of **Abd El-Hady and AlJalaud** [41] who revealed that BM MSCs had lung tissue radiotherapeutic effects against WBI (3Gy) in male albino rats. Moreover, **McMurray *et al*** [50] showed that MSCs cultured on a nanostructured substrate maintained their multipotency, the ability of a cell to differentiate into a limited number of cell types. While the evidence on the supporting role of cancer stem cells in CNTs tumorigenesis, due to the chronic exposure of CNTs [46].

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