

Histological and Immunohistochemical Study of the Possible Therapeutic Effect of Mesenchymal Stem Cells, their Exosomes And Vitamin E on Experimentally Induced Cardio-Toxicity in Adult Male Albino Rats

O.K.Helal¹, A.E.Mansy¹, O. M.Aly¹, N.A.Salama¹

¹Histology and Cell Biology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
E-Mail:Noher.ahmed@gmail.com

Abstract

Cardiotoxicity is an important lethal complication of several cancer therapeutic agents. Clearly, DNA damaging chemotherapy treatments may cause damage to both cancer and healthy cells to generate toxic side-effects. We aimed to demonstrate and to compare the possible therapeutic effect of bone marrow mesenchymal stem cells, their Exosomes and Vitamin E on induced cardio toxicity in adult male albino rats. This work was performed on 60 adult male albino rats subdivided into 5 groups Group I (-ve control) Group III (CP plus BM-MSCs) Group IV (CP plus Exosomes group) Group V (CP plus Vitamin E) Ten rats were used to isolate the BM-MSCs and their Exosomes. Tissue sections were prepared for light microscope examination by staining with Hematoxyline & Eosin and Masson's Trichrome, and for immunohistochemical staining for proliferating cell nuclear antigen (PCNA). The use of bone marrow mesenchymal stem cells (BM-MSCs) after administration of Cyclophosphamide (CP) showed marked reduction in the cardiac toxic effect which appeared in improvement of CP induced pathological changes on the cardiac muscle fibers detected by light microscopic examination while the use of their exosomes and vit. E after administration of CP, showed moderate and mild improvement of CP induced pathological changes on the cardiac muscle fibers, respectively. Bone marrow mesenchymal stem cells, their exosomes and vit.E have beneficial effect in CP induced cardiotoxicity while statistical analysis revealed that bone marrow mesenchymal stem cells was superior to exosomes and vit. E as a potent therapeutic agent against Cyclophosphamide cardiac toxicity. We speculate that the beneficial effect cannot be explained only by antioxidant effect but also by the immunogenic and pleiotropic effects of MSCs

1. Introduction

Cardiac toxicity is one of the life-threatening complications of cancer therapy. Several reports suggest that cardiomyocytes are produced throughout one's lifetime, although at low frequency compared with rapidly renewing tissues such as bone marrow (1).

Cyclophosphamide (CP) is a nitrogen mustard alkylating agent with potent antineoplastic, immunosuppressive and immunomodulatory properties and possibly the most widely used antineoplastic agent. Its use has long been established in cancer therapy and in pretransplant stem cell conditioning regimens. Cyclophosphamide's effect on both cell-mediated and humoral immunity has made it particularly appealing in the off-label treatment of several refractory autoimmune conditions. (2).

Chronic cardio toxicity associated with CP is characterized by progressive heart failure developing from weeks to years after therapy (3).

The metabolism of anti-cancer drugs can lead to more active anticancer metabolites but those metabolites can likewise contribute to the observed cardio toxicity. Regarding cyclophosphamide metabolites, both hydroxy cyclophosphamide and acrolein are shown to be more cardio toxic than the parent drug (4).

Stem cells are capable of differentiating into cells of the same type, which in turn give

rise to other kinds of cells (5). Stem cells are classified on the basis of their origin and potential to differentiate. Based on origin, these cells are of two types: embryonic stem cells (ESCs) and adult stem cells

Bone marrow mesenchymal stem cells (BM-MSCs) have been used for cardiovascular regenerative therapy for decades. These cells are established as one of the potential therapeutic agents, following several tests in animal models and clinical trials. MSCs are identified to help in cardiac regeneration by either revitalizing the cardiac stem cells or vascularizing the arteries and veins of the heart (6).

Exosomes are 40–100-nm micro vesicles with a bi-lipid membrane and cargo of abundant proteins and RNAs. They are now recognized as natural vehicles involved in intercellular communication by protein and RNA delivery. Exosomes released by Bone Marrow Mesenchymal Cells (BMSCs) provide a novel source and great potential donor cell for regenerative medicine. (7).

Vitamin E (Vit E), a lipophilic vitamin, has a strong antioxidative effect and is reported to be effective for the primary and secondary prevention of cardiovascular (CV) diseases. It may prevent the increase of reactive oxygen species (ROS) produced by oxidative damage of lipids in cellular components and tissues. ROS can attack double bonds in polyunsaturated fatty acids in cellular

components and thus induce lipid peroxidation, which may result in more oxidative damage. (8).

The aim of this study was to evaluate and compare the possible beneficial effect of bone

2. Patient and Method

This work was performed on 60 adult male albino rats of weight range 180-200 grams for each were utilized in this work. The animals have been housed in clean well-ventilated cages. Every 5 rats were housed in a separate cage under strict care and hygiene to keep them in normal and healthy conditions. Free access to food and water was allowed.

The rats were divided into 5 groups as follow:

Group I (-ve control) (10 rats): subdivided into:

Ia (1 rat): Rat received no medication.

Ib (3 rats): Each rat in this subgroup was injected intraperitoneally once with 0.5 ml normal saline (vehicle for CP).

Ic (3 rats): Each rat in this subgroup was injected intraperitoneally once with 0.5 ml PBS (vehicle for stem cells).

Id (3 rats): Each rat in this subgroup was injected intraperitoneally once with 0.5 ml DMEM (vehicle for exosomes).

Group II (CP-treated) (+ve control) (10 rats): The rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg dissolved in normal saline on the first day of the experiment and were sacrificed on day 10.

Group III (CP plus BM-MSCs) (10 rats): The rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by a single intraperitoneal dose of 2×10^6 cells/kg BM-MSCs dissolved in PBS injected on day 10 of the experiment, and were sacrificed on day 40.

Group IV (CP plus Exosomes group) (10 rats): The rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by a single intraperitoneal dose MSC-Exosomes 0.5 mg dissolved in DMEM injected on day 10 of the experiment, and were sacrificed on day 40.

Group V (CP plus Vitamin E) (10 rats): The rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by vitamin E which was given three times in a week in a dose (100 mg/kg body weight) by gastric gavage, and were sacrificed on day 40.

Ten rats were used to isolate the BM-MSCs and their Exosomes.

Cyclophosphamide monohydrate (Endoxan) 1 gm vial was purchased in a white powder form from Baxter Oncology GmbH,

marrow mesenchymal stem cells, their derived exosomes and Vitamin E in treatment of experimentally induced acute cardio-toxicity in adult male albino rats.

Germany. It was dissolved in 50 ml normal saline (0.9% NaCl) and injected intraperitoneally (I.P) once in a dose of 90 mg/kg on the first day of the experiment and were sacrificed on day 10. (9).

The BM-derived MSCs were prepared in the Stem cell unit, central lab, Kasr Al-Ainy Faculty of Medicine, and were used with a dose of 2×10^6 cells/kg BM-MSCs dissolved in 0.5 ml phosphate buffer saline (PBS) for each rat and were injected intraperitoneally on day 10 of the experiment and were sacrificed on day 40. (10).

MSC-Exos were prepared in the Stem cell unit, central lab, Kasr Al-Ainy Faculty of Medicine, and were used at dose of 0.5 mg at concentration (100 µg protein /mL dissolved in Dulbecco's Modified Eagle Medium "DMEM") injected intraperitoneally on day 10 of the experiment and were sacrificed on day 40. (10,7).

Vit E 400 mg was purchased in a capsule form from Pharco Pharmaceuticals, Alexandria, Egypt. It was given three times in a week in a dose (100 mg/kg body weight) by gastric gavage. (8).

Protocol of isolation and culture of bone marrow mesenchymal stem cells (MSCs) (11)

Equipment for cell isolation & culture:

Transmitted light and incident inverted microscopy (Axiovert 100-ZEISS).

Laminar flow cabinet (NUAIRE, Biological Safety Cabinet, Class II Type A/B3) equipped with UV light for decontamination.

Standard air CO2 incubator (Thermo).

40-mm nylon mesh filter (BD, Falcon, USA).

6- well and 24-well plastic cell culture plates (Costar, USA).

Heart specimens:

Specimens were fixed in 10% buffered formal saline and processed for paraffin sections of 5-7 µm thickness, mounted on glass slides for H & E, and Masson's Trichrome staining. Other sections were mounted on +ve charged slides for immunohistochemistry for proliferating cell nuclear antigen (PCNA).

II-Masson's Trichrome for detection of collagen fibers:

Sections were stained with Masson's Trichrome according to (12)

III- Immunohistochemical Staining of Proliferating Cell Nuclear Antigen (PCNA) (12):

PCNA is an auxillary protein of DNA-polymerase enzymes, necessary for DNA synthesis and is used as a standard marker in proliferating cells (Kerr et al., 2006). PCNA antibody is a mouse monoclonal antibody PC 10 (Novocastra, Milton, Keynes, USA). PCNA positive cells show brown nuclear deposits.

Immunostaining provides a way of identifying substances in tissues using antigen-antibody (Ag-Ab) reactions which can be made microscopically visible through the incorporation of a suitable label. Immunohistochemical staining for PCNA was done using the Avidin-Biotin immunoperoxidase polyclonal kit provided by Fremont, CA, USA).

Primary antibody: PCNA (Clone PC10):

It's a mouse monoclonal antibody (Lab. Vision Corporation Laboratories, CA, USA, catalogue number: MS106P). It was supplied as 1 ml of antibody (20 ug/ml). Storage at 4oC short term and -20oC long term, avoid freeze-thaw cycles, preservative:0.05% Sodium Azide. No special pretreatment was required for immunohistochemical staining of formalin fixed tissues.

Detection System for Antibody:

Histostain SP kit (LAB-SA system, Zymed Laboratories Inc, San Francisco, CA 94080, USA, catalogue number 95-9643). It is a broad-spectrum detection system that reacts with mouse, rabbit, guinea pig and rat primary

3- Results:

1-Haematoxylin and Eosin stained sections:

Group I (Control group):

Showed normal architecture of branching and anastomosing cardiac muscle fibers. Cardiomyocytes have central oval vesicular nuclei with acidophilic cytoplasm. Cardiac muscle fibers are separated by interfibrillar spaces containing fibroblasts. (Fig:1).

Group II (CP-treated):

Showed disorganized, disturbed and widely separated, atrophic muscle fibers with cytoplasmic vacuolation and vacuolated nuclei, dilatation of T tubules (SER) and pyknosis of many cardiac myocytes. Extravasation of red blood cells between cardiomyocytes with cellular inflammatory infiltrations are observed. (Fig:2).

Group III (CP plus BM-MSCs):

Showed nearly normal myocardial histological architecture, with vesicular centrally located nuclei and muscle fibers regularly arranged with mild spacing showing midfocal mononuclear cellular inflammatory infiltrations (Fig:3).

Group IV (CP plus Exosomes group):

Showed cardiac muscle fibers slightly disorganized and slightly distended with

antibody. This Histostain SP kit includes serum blocking solution, a biotinylated secondary antibody, a horseradish peroxidase streptavidin and a substrate chromogen mixture to demonstrate antigen in cells and tissues.

Control Slides:

Positive control section for PCNA: using anti-PCNA Ab which demonstrated brown nuclear reaction in section of human tonsil. Positive control tissues were indicative of correctly prepared tissues and proper staining techniques. A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation. If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative control sections for PCNA: Should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. Sections were processed in the previously mentioned sequence, but the primary antibody was not added and instead PBS, was added in this step. Omission of the primary antibody gave no staining reaction. Any staining observed on the specimen was probably due to non-specific protein binding or non-specific binding to other reagents.

cytoplasmic vacuolation and pyknosis in some cardiomyocytes. Minimal congestion of blood capillaries and hemorrhage between myocytes with mild mononuclear cellular infiltrations without dilatation are noticed (Fig:4).

Group V (CP plus Vitamin E):

Showed cytoplasmic vacuolation, pyknosis in some cardiomyocytes which show mild degeneration with mononuclear cellular infiltration, large area of hemorrhage and widely separated muscle fibers are observed (Fig:5).

2- Masson's Trichrome:

Group I (Control group):

Showed minimal collagen fibers accumulation between cardiac muscle fibers. (Fig:6).

Group II (CP-treated):

Showed marked collagen fibers accumulation around and between cardiac muscle fibers (Fig:7).

Group III (CP plus BM-MSCs):

Showed minimal collagen fibers accumulation between cardiac muscle fibers (Fig:8).

Group IV (CP plus Exosomes group):

Showed mild collagen fibers accumulation between cardiac muscle fibers (Fig:9).

Group V (CP plus Vitamin E):

Showed marked perivascular collagen fibers accumulation. (Fig:10).

3- Proliferating cell nuclear antigen (PCNA):

Group I (Control group):

Showed positive nuclear immunoreaction in vesicular nuclei. (Fig:11).

Group II (CP-treated):

showed negative nuclear immunoreaction in vesicular nuclei. (Fig:12).

Group III (CP plus BM-MSCs):

Showed strong positive nuclear immunoreaction in vesicular nuclei (Fig:13).

Group IV (CP plus Exosomes group):

Showed moderate positive nuclear immunoreaction in vesicular nuclei (Fig:14).

Group V (CP plus Vitamin E):

Showed hardly positive nuclear immunoreaction in vesicular nuclei. (Fig:15)

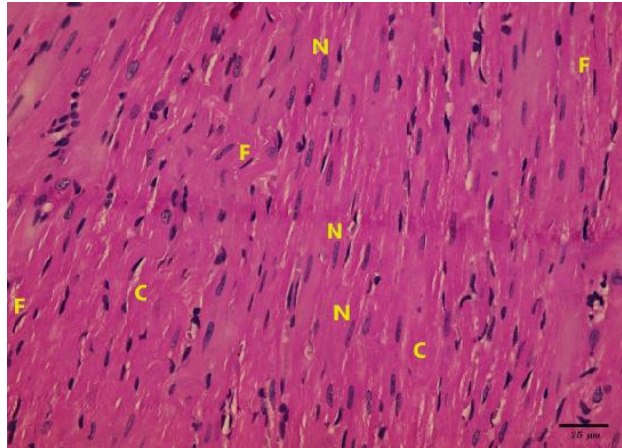


Fig. (1): Photomicrograph of a longitudinal section in cardiac muscle of a rat in group I (Control group) showing normal architecture of branching and anastomosing cardiac muscle fibers. Cardiomyocytes with central oval vesicular nuclei (N) and acidophilic cytoplasm (C). Cardiac muscle fibers are separated by interfibrillar spaces containing fibroblasts (F). (H&E X 400)

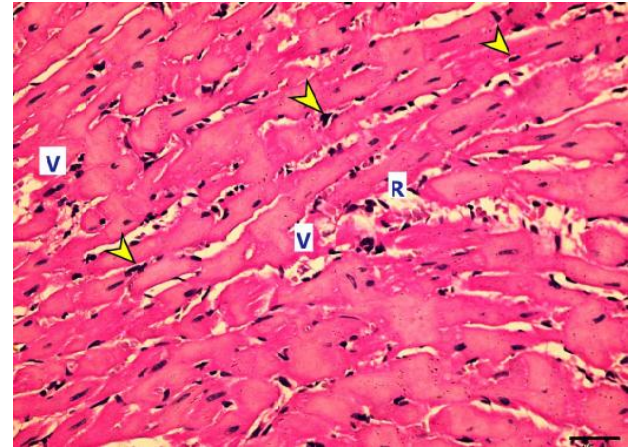


Fig. (2): A photomicrograph of a longitudinal section in cardiac muscle of a rat in group II (CP-treated) showing disorganized, atrophic, disturbed and widely separated muscle fibers (arrows) with cytoplasmic vacuolation and vacuolated nuclei (V), dilatation of T tubule (astric) and pyknosis (arrow head) of many cardiac myocytes. Extravasation of red blood cells with cellular inflammatory infiltrations (R) between cardiomyocytes are observed. (H&E X 400)

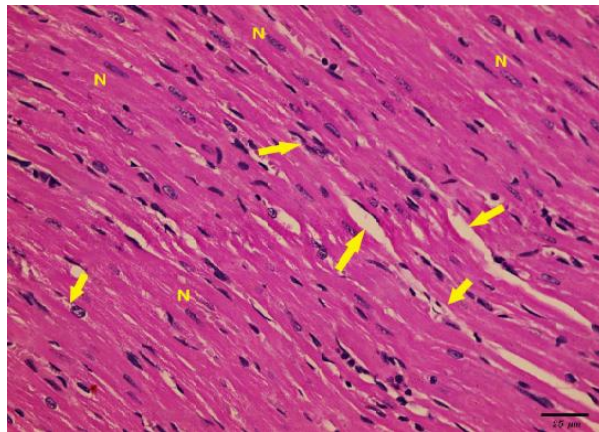


Fig. (3): Photomicrograph of a longitudinal section in cardiac muscle of a rat in group III (CP plus BM-MSCs) showing nearly normal myocardial histological architecture, with vesicular centrally located nuclei (N) and muscle fibers regularly arranged with mild spacing showing midfocal mononuclear cellular inflammatory infiltrations (yellow arrow). (H & E X 400)

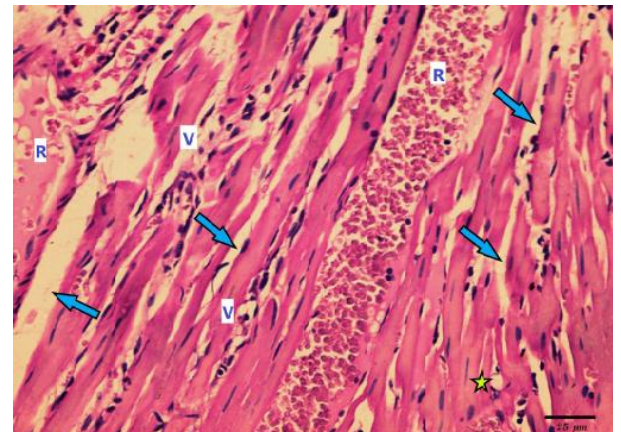


Fig. (4): Photomicrograph of a longitudinal section in cardiac muscle of a rat in group IV (CP plus Exosomes group) showing: Cardiac muscle fibers slightly disorganized and slightly distended with cytoplasmic vacuolation (V) and pyknosis (arrow head) in some cardiomyocytes. Minimal congestion of blood capillaries and hemorrhage between myocytes with mild mononuclear cellular infiltrations without dilatation are noticed (R). (H & E X 400)

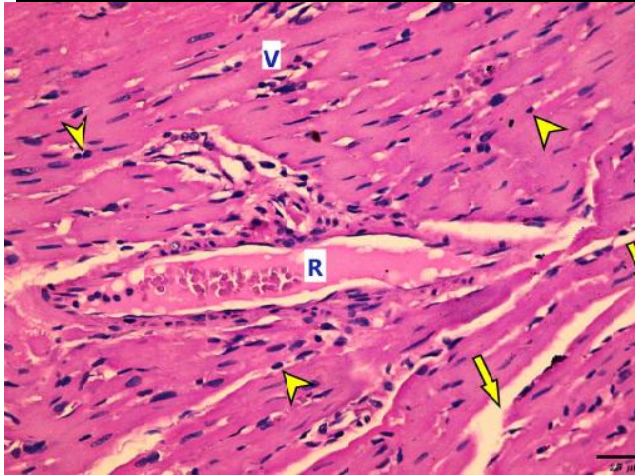


Fig. (5): A photomicrograph of a longitudinal section in cardiac muscle of a rat in group V (CP plus Vitamin E) showing cytoplasmic vacuolation (V) and pyknosis (arrow head) in some cardiomyocytes which show mild degeneration with mononuclear cellular infiltration and large area of hemorrhage (R). Widely separated muscle fibers (yellow arrow) are observed. (H&E X 400)

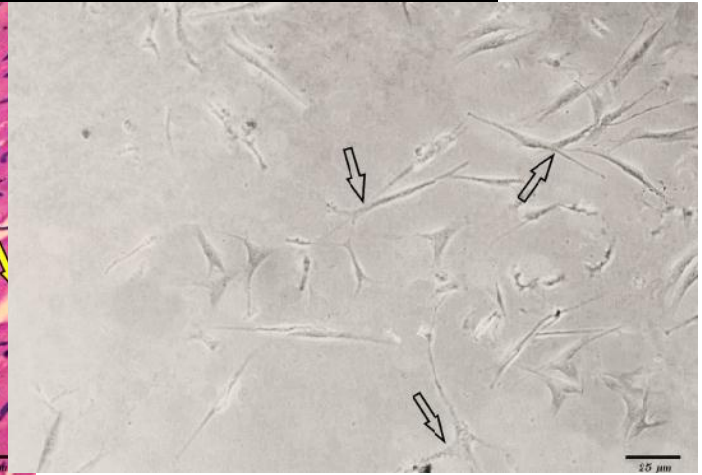


Fig. (6): A photomicrograph of a longitudinal section in cardiac muscle of a rat in group I (control group) showing minimal collagen fibers accumulation between cardiac muscle fibers (arrows). (Masson's Trichrome X 400)

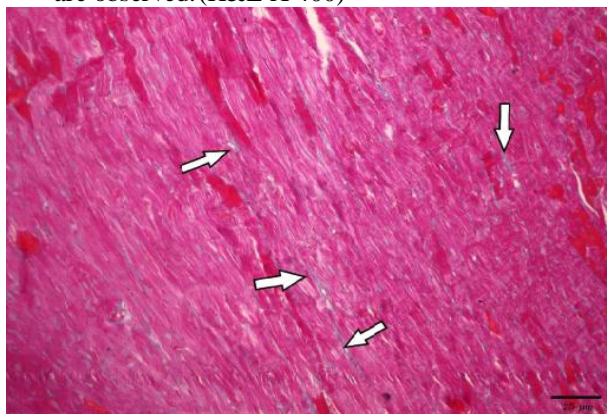


Fig. (7): A photomicrograph of a longitudinal section in cardiac muscle of a rat in Group II (CP-treated) showing marked collagen fibers accumulation around and between cardiac muscle fibers (arrows). (Masson's Trichrome X 400)

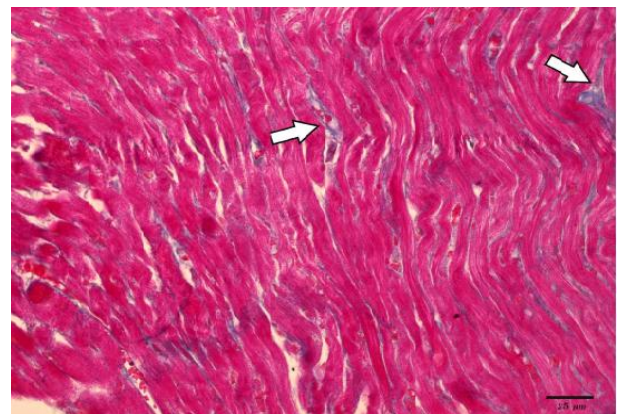


Fig. (8): A photomicrograph of a longitudinal section in cardiac muscle of a rat in group III (CP plus BM-MSCs) showing minimal collagen fibers accumulation between cardiac muscle fibers (arrows). (Masson's Trichrome X 400)

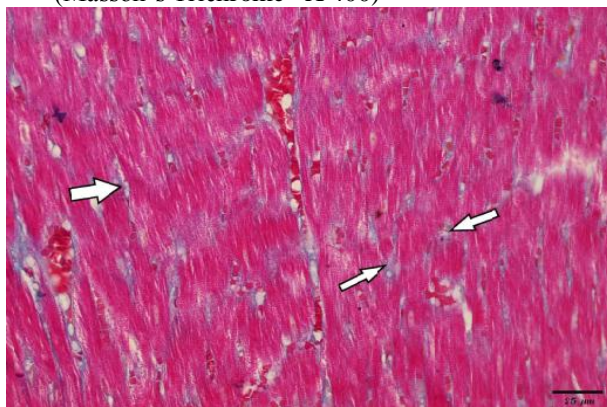


Fig. (9): A photomicrograph of a longitudinal section in cardiac muscle of a rat in group IV (CP plus Exosomes group) showing mild collagen fibers accumulation between cardiac muscle fibers (arrows). (Masson's Trichrome X 400)

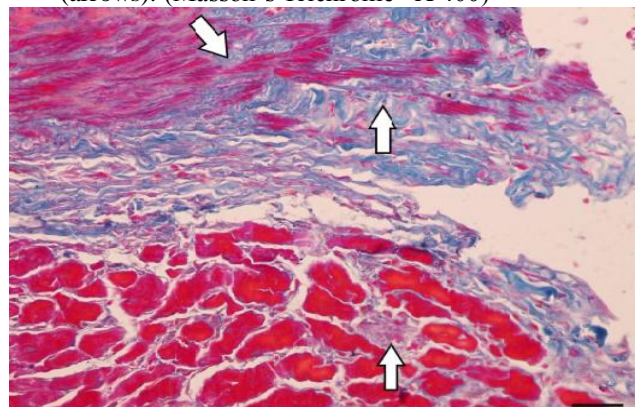


Fig. (10): A photomicrograph of a longitudinal section in cardiac muscle of a rat in Group V (CP plus Vitamin E) showing marked perivascular collagen fibers accumulation around dilated blood vessel (R). (Masson's Trichrome X 400)

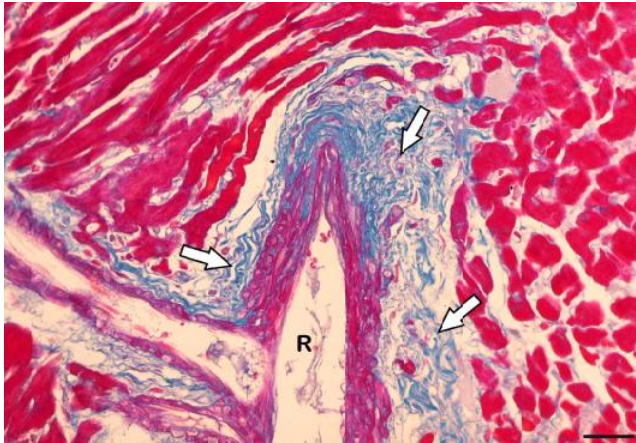


Fig. (11): Photomicrographs of PCNA immune-stained cardiac muscle sections of a rat in group I (control group) showing positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

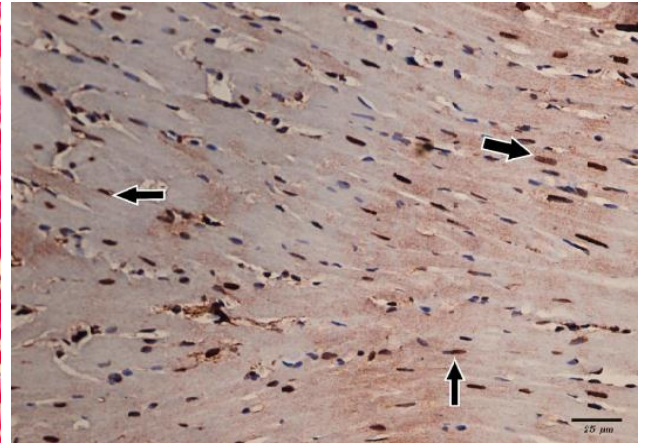


Fig. (12): Photomicrographs of PCNA immune-stained cardiac muscle sections of a rat in Group II (CP-treated) showing negative (weak brown) nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

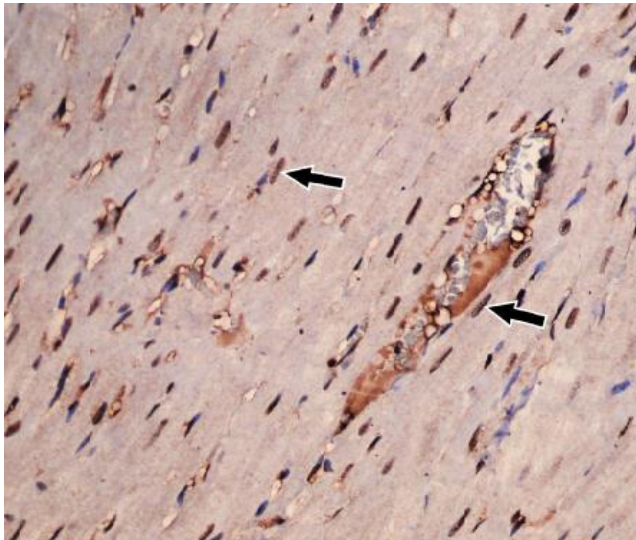


Fig. (13): Photomicrographs of PCNA immune-stained cardiac muscle sections of a rat in group III (CP plus BM-MSCs) showing strong positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

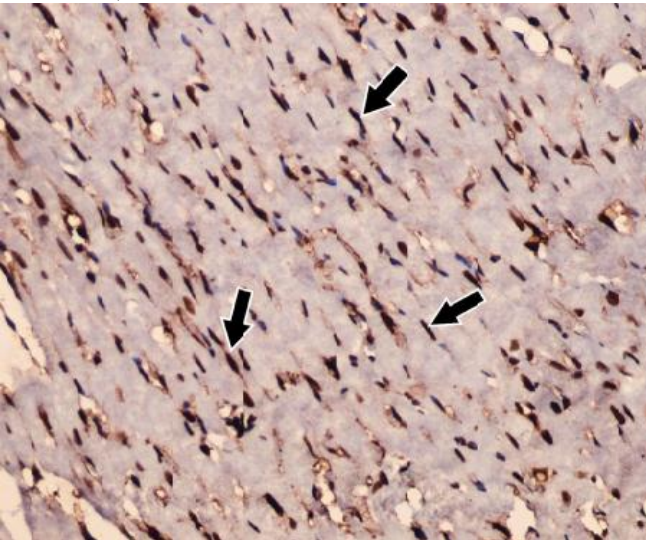


Fig. (14): Photomicrographs of PCNA immune-stained cardiac muscle sections of a rat in group IV (CP plus Exosomes group) showing moderate positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

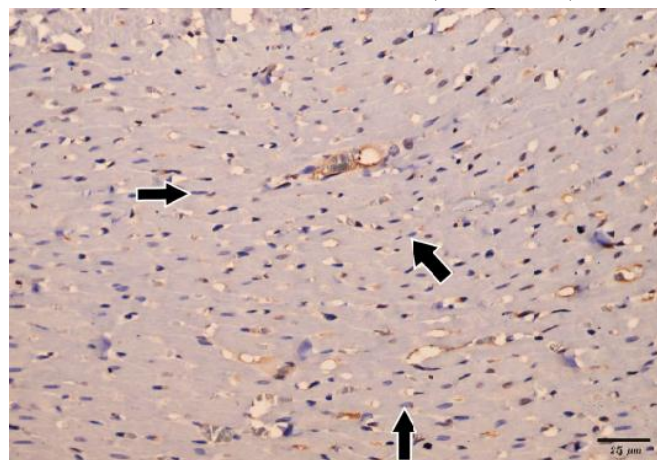


Fig. (15): Photomicrographs of PCNA immune-stained cardiac muscle sections of a rat in Group V (CP plus Vitamin E) showing hardly positive (few brown) nuclear immunoreactions in vesicular nuclei (arrow) can be detected. (PCNA x400)

The obtained results were expressed as mean value, SE (standard error) and the statistical difference between various groups was analyzed by the one-way ANOVA and the significance was set at $p < 0.05$.

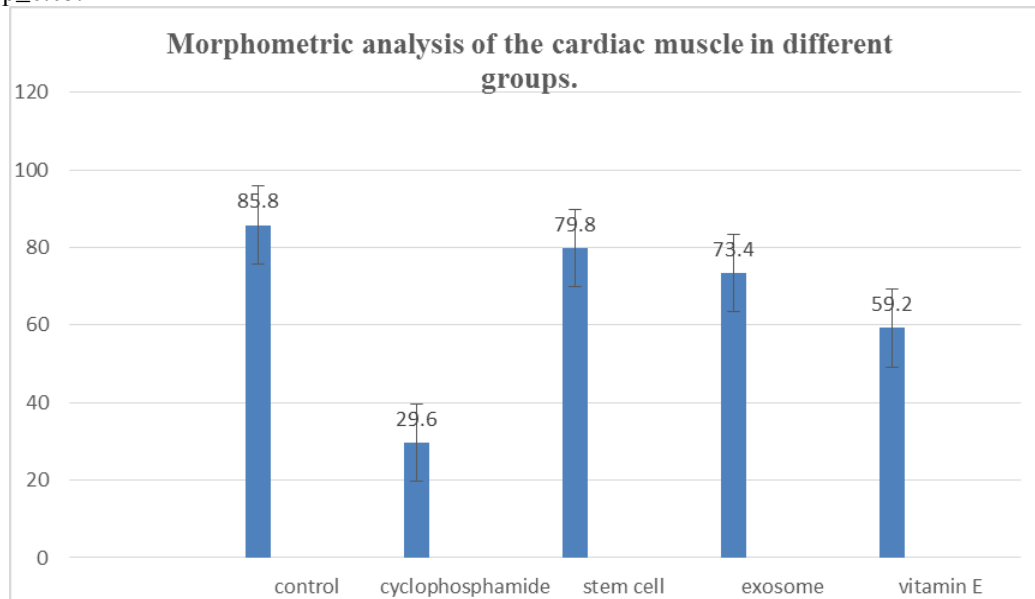


Fig. (16): Morphometric analysis of the cardiac muscle in different groups.

3- Discussion:

As the final infarct size in patients with acute myocardial infarction (AMI) predicts long-term clinical outcome, it is envisaged that the identification of means of minimizing this injury will reduce patient mortality and morbidity (13).

The CP group (group II) the rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg on the first day of the experiment and were sacrificed on day 10. In the present study this group showed disorganized, disturbed, atrophic and widely separated cardiac muscle fibers with vacuolated sarcoplasm, pyknotic nuclei, extravasation of RBCs between cardiomyocytes, irregular indented nucleus, apparent swollen and distorted mitochondria, lysis of myofibrils, marked collagen fibers accumulation between cardiomyocytes and negative nuclear PCNA immunoreaction in vesicular nuclei.

In agreement with these findings Birchall et al., (14), Sumandeep et al., (15) and Martin et al., (16) reported that histologically, there were diffuse interstitial edema, extravasation of erythrocytes with foci of band necrosis, nuclear extravasation or pyknosis, intracellular edema, acute pericarditis and hemorrhagic myocarditis with fibrin platelet microthrombi in capillaries and fibrin strands in the interstitium on ultrastructural examination.

Cardiac tissue regenerative medicine involves cardiomyocyte regeneration, neovascularization, and paracrine cytokines, which have anti-inflammatory, anti-apoptotic,

and anti-remodeling effects. During the last decade, stem cells have become promising candidates for regenerative medicine not only because of their capacity of differentiation toward cardiomyocyte and vascular cell lineages but also their capacity for releasing such paracrine factors (17).

Currently, MSCs have been known as promising tool for therapeutic purpose in clinic based on their several advantages including self-renewal, extensive in vitro expansion, immunomodulation property, engraftment capacity, multi-lineages differentiation potential including few ethical concerns as compared to embryonic stem cells. Moreover, increasing evidences have been shown that MSCs can be isolated from various cell types including adipose tissue, dental pulp, peripheral blood, placenta and umbilical cord. These unique biological properties of MSCs highlight great potential in several applications such as regenerative medicine, tissue engineering and cell-based therapy (18).

The stem cell group (group III) the rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by a single intraperitoneal dose of 2×10^6 cells/kg BM-MSCs injected on day 10 of the experiment, and were sacrificed on day 40. In the present study this group showed, nearly normal myocardial histological architecture, with vesicular centrally located nuclei, muscle fibers regularly arranged with mild spacing with midfocal mononuclear cellular inflammatory infiltrations, euchromatic nucleus with mild irregular outline, normal

mitochondria which were distributed between myofibrils, minimal collagen fibers accumulation between cardiac muscle fibers and strong positive nuclear PCNA immunoreaction in vesicular nuclei.

In agreement with these findings, Berry et al., (19), Helal et al., (20) and Ji et al., (21) reported that transplantation of autologous undifferentiated mesenchymal stem cells could be an effective method for myocardial regeneration after infarction, decreases fibrosis, apoptosis and left ventricular dilatation while increasing myocardial thickness. The decreased amount of fibrosis after MSCs injection was explained by Wen et al., (22) who mentioned that MSCs exert paracrine anti-fibrotic effects to attenuate ventricular remodeling through regulation of cardiac fibroblasts (CFB) proliferation.

The slightly disorganized and wide separation of some fiber's bundles explained by Tuby et al., (23) who stated that the organization of newly-formed contractile myofilaments in the cytoplasm in various degrees of maturation in cardiac cells. In some cells the myofilaments were dispersed in the cytoplasm and in others they were organized in clusters anchored to well-developed Z-lines, in certain cells the myofilaments were organized parallel to the longitudinal direction of the cells, resembling the morphological characteristics of mature intact cardiomyocytes. Some of the cells were also seen in a process of formation of typical intercalated disc between them.

In contrast, Grinnemo et al., (24) reported that implanted human MSCs into a cardiac ischemic xenomodel, accompanied by no improvement in myocardial function in treated animals compared to controls. This failure may be due to that human MSC required implantation into immune-incompetent animals as well as immunosuppression to survive, indicating that these cells are otherwise rejected.

Regardless of the benefits of MSCs, clinical application of MSC-based therapy is restricted. This restriction is attributed to the poor viability of the transplanted cells in the myocardium. Also, reactive oxygen species (ROS) is known to be a key mediator in cardiac dysfunction. ROS is known to hinder cell adhesion and stimulate cell detachment and death. Furthermore, the grafted cell may encounter ischemic conditions lacking nutrients and oxygen and consequently affecting cell viability (25).

The efficacy of cell therapies largely depends on the appropriate control of the fate and function of the engrafted cells. Therefore,

strategies for enhancing stem cell survival, proliferation and differentiation have become one of the hot topics of central interest. The application of adjuvant drug is a promising way to support the stem cell therapy (26).

Group IV (CP plus Exosomes group) the rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by a single intraperitoneal dose MSC-Exosomes at concentration 0.5 mg/mL injected on day 10 of the experiment, and were sacrificed on day 40. In the present study this group showed, cardiac muscle fibers slightly disorganized and slightly distended with cytoplasmic vacuolation and pyknosis in some cardiomyocytes. Minimal congestion of blood capillaries and hemorrhage between myocytes with mild mononuclear cellular infiltrations without dilatation was noticed, mild collagen fibers accumulation between cardiac muscle fibers, mitochondria with lost cristae leaving empty space. Other mitochondria were aggregated together in normal shape. Mild lysis in sarcomere, focal area of discontinuous intercalated disc and rarified sarcoplasm and moderate positive nuclear PCNA immunoreaction in vesicular nuclei.

Many of the studies performed to date used vesicles purified from cells cultured *in vitro*, which may have a different lipid composition, protein content, or other characteristics compared with those released *in vivo*. Thus, although they demonstrate a potentially useful therapeutic effect, they do not address the fundamental question of the *in vivo* relevance of native endogenous vesicles. This is difficult to determine without effective and specific tools to prevent micro vesicle or exosome release *in vivo*. There are some exciting leads in this area, with the proteins being implicated in exosome release (27).

However, the exact contingent of proteins involved is suspected to be cell type-specific and requires much more investigation. Similarly, the existence of a mechanism controlling the cell-specific delivery of exosomes is not well-established. A recent report suggests that selectivity of uptake is regulated by specific interactions between tetraspanins and integrins and can be controlled by altering the expression of different tetraspanins proteins. (28)

This raises the possibility that the capacity for targeting of exosomes can be harnessed for delivery to specific cells or organs but, again, much work remains to be done in this area. This and other basic aspects of exosome biology will require much deeper

understanding before their clinical application can be seriously considered.

In contrast, Lai et al. were the first to show that exosomes from MSCs are cardioprotective acutely (29). They used high-performance liquid chromatography (HPLC) to purify exosomes released by MSCs in culture and injected them into the tail veins of mice undergoing 30 min myocardial ischemia via ligation of the coronary artery (29). In injected mice, infarct size was significantly reduced 24 h later (29), and cardiac function was improved at 28 days (30).

Collectively, Costanza et al., (31) indicate that exosomes from several different sources can modulate post-ischemic angiogenesis, tissue protection and repair in animal models of heart and kidney ischemia, or ischemia/reperfusion and this is due to their potent paracrine effects.

Importantly, a dose-response study was performed and at least 4 µg/kg exosomes were required to observe significant benefit. Furthermore, the MSC exosomes also protected the isolated, perfused heart, meaning that protection was independent of circulating immune cells. In the hearts of treated animals, higher levels of ATP and NADH as well as lower levels of oxidative stress were observed but it is difficult to know whether this is a

4- References

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primary effect or is secondary to the smaller infarcts. (30).

Group V (CP plus Vitamin E) the rats in this group had received a single intraperitoneal injection of CP in a dose of 90 mg/kg, followed by vitamin E which was given three times in a week in a dose (100 mg/kg body weight) by gastric gavage. In the present study this group showed, cytoplasmic vacuolation, pyknosis in some cardiomyocytes with mild degeneration, mononuclear cellular infiltration, large area of hemorrhage, extravasation of red blood cells, widely separated muscle fibers and marked perivascular collagen fibers accumulation. The nucleus appeared with irregular outline, giant mitochondria with complete lysis of their cristae, focal lytic area of myofibrils, distortion of Z line with fragmented intercalated discs and hardly positive nuclear PCNA immunoreaction in vesicular nuclei.

In agreement with these findings, (32) reported that the group of mice, which treated with vit E and Cisplatin showed interrupted cardiac fibers with cellular infiltrations, pyknotic nuclei, decreased intercalated discs and increased amount of connective tissue between cardiac muscle fibers.

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