EVALUATION OF MESENCHYMAL STEM CELL THERAPY IN DOXORUBICINE-INDUCED CARDIOTOXICITY IN ADULT FEMALE ALBINO RATS

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ABSTRACT

Background: Doxorubicin (DOX) is a commonly used anticancer drug despite its dose-dependent cardiotoxic effects. The possibility of using stem cells to repair DOX-induced cardiotoxicity may open new therapeutic options. **Aim of the work:** To evaluate the role of mesenchymal stem cells (MSC) in recovery of both the structure and function of DOX-induced cardiotoxicity. **Material and Methods:** The study used ninety adult female albino rats divided into three equal groups; one control treated with saline, DOX group treated with doxorubicin 2.5 mg/kg every other day for two weeks, and DOX-MSC group treated with the same DOX regimen then injected one week after last DOX dose with DAPI-labeled male umbilical cord blood MSCs (UCB-MSCs). All three groups were evaluated at the 3rd and 6th weeks of the study by physical status, cardiovascular assessment in vivo, serum biochemical tests [malondialdehyde (MDA), total anti-oxidant capacity (TAC) and cardiac troponin I (cTnI)]**. Results:** DOX caused deterioration in general rat health, significant prolongation of QT interval and serum TAC, also caused an increase in serum MDA and cTnI. These findings aggravated at the 6th week in DOX group. Significant improvement of previous parameters in DOX-MSC group compared to DOX was detected at the 6th week. Homing of injected MSCs in rat hearts was confirmed by RT- PCR for male-specific *Sry* gene. **Conclusion and Recommendations:** UCB-MSCs have reparative effects on the damaged heart by ameliorating oxidative stress and relatively improving the function of the failing heart. This approach is worthy of serious consideration for clinical use.

KEYWORDS*:* Doxorubicin, cardiomyopathy, cardiotoxicity, mesenchymal stem cells, umbilical cord blood mesenchymal stem cells, oxidative stress.

1. INTRODUCTION

Cancer is a leading cause of morbidity and mortality worldwide. It is estimated that there will be 13.1 million deaths due to cancer in 2030. However, over the last 20 years, the survival rate of cancer patients has significantly increased due to the great progress of modern cancer therapy. To achieve these results, however, a considerable price has been paid in terms of side effects associated with intensive anticancer treatment ***(Cardinale and Sandri, 2010).***

Anthracyclines, including doxorubicin (DOX) have become one of the most effective chemotherapy treatments for a wide range of malignant tumors such as hematological cancers, lymphomas and various solid tumors such as breast cancer, small cell lung carcinoma and ovarian cancer ***(Shi et al., 2011).*** Despite its broad-spectrum antineoplastic activity, adverse events, particularly cardiotoxicity, has limited the use of conventional doxorubicin in clinical practice ***(Oliveira et al., 2013).***

The molecular mechanisms involved in chronic anthracycline cardiotoxicity remain a major topic of discussion. Several mechanisms, such as apoptosis, alteration of iron and calcium homeostasis, have been described, but the exact mechanism is not yet been fully understood. Oxidative stress is believed to be the most critical mediator of such myocardial damage due to DOX therapy ***(Richard et al, 2011 & Angsutararux et al., 2015).***

The recognition that the adult heart in animals and humans contains a pool of resident primitive cells that are self-renewing and that regenerate myocytes and coronary vessels in vivo raises the question of whether the cardiotoxic effects of DOX are directed primarily to this stem cell compartment [cardiac progenitor cells (CPCs)] being more

sensitive to oxidative stress and die rapidly by apoptosis ***(De Angelis et al., 2010).***

Many efforts have been made to prevent cardiac toxicity in patients with cancer, but these current therapies cannot guarantee permanent cardiac protection. One of their main limitations is that they do not promote myocardium regeneration which drives researchers to find new ways to solve those problems ***(De Angelis et al., 2016).*** Long ago, mesenchymal stem cells (MSCs) attracted interest for their possible use for both cell and gene therapies because of their capacity for self-renewal and multipotentiality for differentiation. These cells can be induced, either in vitro or in vivo, to differentiate terminally into various tissue types including cardiomyocytes ***(Lin et al., 2010).***

Cell transplantation is emerging now as a potential therapy to treat heart failure ***(Loffredo et al., 2015).*** It is hypothesized that the activation (or inhibition) of (a) signaling pathway(s) by introduced MSCs may restore the regenerative potential of cardiac stem cells and, hopefully, may avoid anthracycline-induced cardiotoxic effects ***(Ezquer et al., 2015).***

Consequently, the strategy of stem cell therapy may allow aggressive chemotherapy followed by stem cell repopulation which can rescue the cardiomyopathic heart ***(Gho et al., 2013).***

2. AIM OF THE WORK

Evaluate the role of UCB-MSC in recovery of both the structure and function of DOX cardiotoxic effects in adult female albino rats.

3. MATERIAL AND METHODS

Ninety adult female albino rats weighing 150–200g were included in the study, and were obtained from the animal house of faculty of medicine, Zagazig University. Principles of laboratory animal care followed recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals **(*Institute of Laboratory Animal Resources, 2011*)**, and Zagazig University – Faculty of Medicine Institutional animal house. Before starting the experiment, all animals subjected to 14 days of passive preliminaries for house acclimatization to ascertain their physical wellbeing and to exclude any diseased animal. Animals were bred in the animal house in well-ventilated rooms under controlled temperature (21-23 ^{o}C) and humidity (55±2%) in a 12 h light-dark cycle within standard plastic cages of proper sizes and were fed ad libitum. After housing acclimatization, the rats were randomly divided into three equal groups:

Group I (control): Thirty control rats that were injected every other day with 1 mL of normal saline intra-peritoneally (IP) for two weeks and served as positive control group ***(Elmadbouh et al., 2015).*** **Group II (DOX group):** Thirty rats that received 6 injections of 2.5 mg/kg B.W (i.p.) of doxorubicin (DOX) every other day over a period of 2 weeks to reach a cumulative dose of 15 mg/kg. This schedule has been previously found consistent and reproducible for induction of DOX cardiomyopathy ***(De Angelis et al., 2010; Shafik et al., 2011; Elmadbouh et al., 2015).*** **Group III (DOX- MSC group):** Thirty rats that received DOX as the above regimen of group II and then left for one week without medication before being injected with mesenchymal stem cell (MSC) therapy in a single dose of 5× 10^{6} MSC per rat. This one-week gap before injecting MSCs is essential to ensure adequate elimination of DOX from rat bodies so as not to damage introduced stem cells with its cytotoxic action (***Speth et al., 1988; Gustafson et al., 2002)***, also this week is required to ensure development of cardiomyopathy stigmata before starting evaluation of rats ***(Arola et al., 2000 & De Angelis et al., 2010).*** At the 3rd week from the start of the experiment (one week after the last dose of DOX half of rats from all groups were evaluated by the following parameters: 1) The physical condition of animals regarding decreased activity, the presence of ascites, body weight, and mortality rate ***(Burkholder et al., 2012).*** 2) Rat electrocardiography (ECG) and arterial blood pressure in live urethane-anesthetized animals by PowerLab Data Acquisition and Analysis System (ADInstruments). 3) Blood samples were

collected from the carotid artery cannula inserted in anesthetized live rats from all groups before heart excision ***(Parasuraman et al., 2010).*** Samples were allowed to clot for 30 minutes at room temperature, centrifuged at 4000 rpm for 15minutes, and then the top yellow serum layer is pulled by a sterile pipette without disturbing the white buffy layer ***(Bertinchant et al., 2003).*** Serum samples collected in Eppendorf tubes were kept frozen at -80o C to be assayed later for the oxidative stress markers (MDA and TAC) by spectrophotometry and cardiac troponin I by ELISA. Then the remaining half of rats of group III (DOX-MSC) was injected with MSCs in a single dose.

Three weeks later (6 weeks from the start of the experiment): all remaining rats were evaluated again by the same previous parameters ***(Arola et al., 2000; De Angelis et al., 2010).*** Heart tissue samples from group III (DOX- MSC group) were also used for detection and localization of mesenchymal stem cells in myocardial tissue by detecting "sry" gene located on Y chromosome of male baby umbilical cord blood stem cells within heart tissues of female rats using PCR and gel electrophoresis ***(Wu et al., 2012).***

1- Preparation, injection, and tissue detection of mesenchymal stem cells (MSC):

Fresh umbilical cord blood (UCB) was collected on term delivery of male neonates with mothers' consent. Since the umbilical cord is discarded after birth, the cells are easily accessible without ethical concerns ***(Moretti et al., 2009).*** A blood collection bag system containing citrate phosphate dextrose (CPD) anticoagulant was used and the unit was stored at 22±4oc till processing. Separation of cells was preferably done after four-hour storage at 4 o C ***(Bieback et al., 2004 & Baksh et al., 2007).***

To isolate mononuclear cells (MNCs), the UCB unit was diluted 1:3 with phosphate-buffered saline (PBS)/2 mM EDTA. 1.5 volume of diluted blood was carefully loaded onto 1 volume of Ficoll-Hypaque solution. After density gradient centrifugation, MNCs were removed from the interphase and washed two to three times with PBS. Cell counts were performed manually using hemocytometer ***(Wang et al., 2004).*** UCB-derived MNCs were set in culture at a density of 1×10^6 /cm^2 into T culture flask of 25ml capacity (Falcon, Becton Dickinson, Heidelberg, Germany) with serum-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and glucose (4.5 g/l) with L-glutamine and 1% penicillin-streptomycin-Amphotericin B Mixture (10 IU/10 IU/25 ug, 100 ml) in 5% CO2 in a 37ºC incubator (Heraeus, Langenselbold, Germany) ***(Freshney, 2010).*** When cultures reach 80-90% confluence, cultures were washed twice with PBS and cells were trypsinized with 0.25% trypsin/ EDTA (Trypsin 1:250, EDTA 1 mM, Lonza Bioproducts, Belgium) for 10-15 min at 37 °C. After centrifugation (at 2400 rpm for 20 min), cells were suspended in DMEM at a concentration of 5×10^6/ml and incubated in another culture flask (Falcon). The resulting cultures were referred to as first-passage cultures ***(Alhadlaq and Mao, 2004).*** Cell viability testing was carried out using the trypan blue dye exclusion test as described before ***(Haasters et al., 2009).***

Characterization of MSC: MSCs in culture were characterized by their adhesiveness and fusiform shape detected by inverted microscope ***(Rochefort et al., 2005 & Moretti et al., 2009),*** and by determination of surface markers of MSCs i.e. the positive expression of CD105 surface marker and the negative expression of CD34 surface marker ***(Baksh et al., 2007).***

After three cell culture passages, stem cells were washed six times with PBS and treated with trypsin for 3 min to generate a single-cell suspension, washed three times with DMEM, resuspended in minimal volume of the serum-free DMEM, counted, and prepared for injection into tail vein of female albino rats in a single dose of 15×10^6 MSCs/ inoculum ***(Caplan and Dennis, 2006 & Ma et al., 2014).***

Experimental rat groups were examined through DNA extraction from heart tissue of

sacrificed female rats, after PCR amplification, for human sry gene. This method was done to detect the expression of sex determination region on the Y chromosome (sry gene) in recipient female rats' hearts. Results were to be compared to the results of the sry gene of male rats ***(Pulavendran et al., 2011).***

2- In vivo cardiovascular system evaluation:

Animals were anesthetized with urethane (Ethyl carbamate) at a concentration of 50 mg/ml, and given to rats in a dose of 1.2 g/kg IP. Anesthetized rats were placed on a warm pad in a supine position. The right common carotid artery was isolated after midline neck incision and cannulated with insertion of a Millar Mikro-tip pressure transducer (1.4F sensor, 2F catheter; Millar Instruments, Houston, TX) ***(Mungrue et a., 2002).*** Electrocardiograms (ECGs) were recorded with subcutaneous electrodes in anesthetized rats. Electrodes were inserted subcutaneously into left hind limb, left forelimb and right forelimb in subsequent order. The lead II ECG is recorded from the needle electrode inserted into the right forelimb ***(van Acker et al., 1996).***

3- Statistical analysis of results:

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0 ***(SPSS Inc., 2009).***

Quantitative data were expressed as mean ± SD (Standard deviation). **One way analysis of variance (ANOVA) *F*-test test** was used to calculate difference between quantitative variables in more than two groups in normally distributed data. **Kruskal Wallis test** was used to calculate difference between quantitative variables in more than two groups in not normally distributed data. This is followed by **Least Significance Difference test (LSD)** for multiple comparisons between groups. **Paired sample T test** was used to calculate difference between quantitative variables in the same group at 3 weeks and 6 weeks. Qualitative data were represented as frequencies and relative percentages. **Chi square test** was used to calculate difference between qualitative variables. Survival rates were calculated and differences between the groups were assessed with the **log-rank (Mantel–Haenszel) test.** The significance Level for all above mentioned statistical tests is fixed at 5% level of probability (P-value) where P value of >0.05 indicates non-significant results, P value of <0.05 indicates significant results and P value of <0.01 indicates highly significant results.

4. RESULTS

I) Characterization of Isolated Cord Blood-Derived mesenchymal stem cells (MSCs):

1- Microscopical appearance:

Overtime, small round-shaped cells in culture change into larger spindle shaped cells with high proliferation and differentiation capacity. After three passages in culture, the cell population showed characteristic adhesiveness, and displaying typical fibroblast-like morphology (fusiform shape) detected by inverted microscope (figure 1).

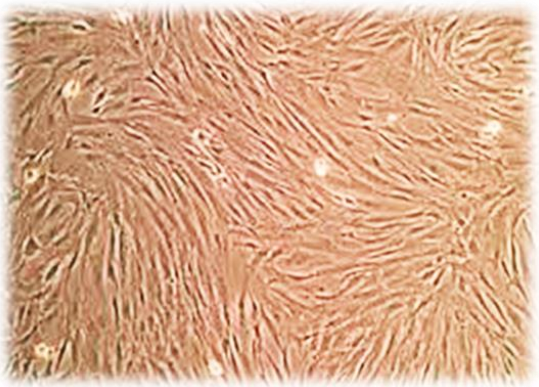


Fig. 1: A photomicrograph of mesenchymal stem cells culture with fusiform/ spindle shape and 80% confluence in the 2nd week by inverted microscope (x200).

2- Expression of surface markers of MSCs:

Majority of isolated cells (about 63%) showed only CD105 positive expression and negative expression of CD34 surface markers, while few cells (about 36%) expressed the adhesion/stem cell marker CD34 and were further separated by MACS column. This phenotype of culture-expanded UC-stem cells conformed to the criteria for MSCs (figure 2).

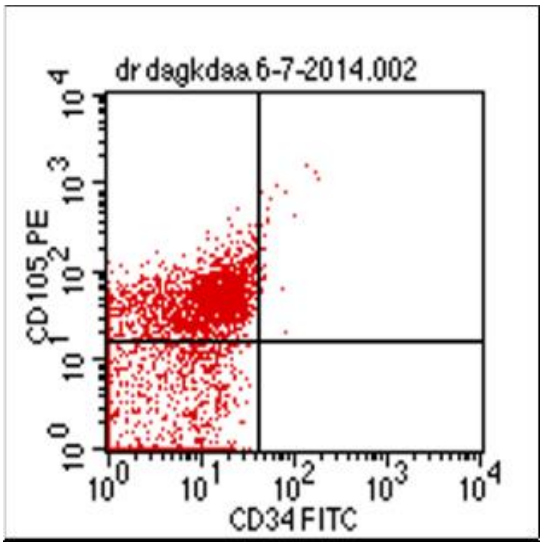


Fig.2: Graphical demonstration of umbilical cord MSC (UCB-MSCs) surface markers

II) Tracing incorporation and distribution of Injected MSCs:

Specific *Sry* gene band (with a size of 254 bp) was detected only in cardiac tissue of group III female rats injected with UCB-MSCs confirming homing of such MSCs into injured cardiac tissues.

III) Physical examination results:

1- General appearance, health and activity:

The general appearance and behavior of all groups of animals was recorded during the time course of the present study.

This observation revealed that control rats were inquisitive and active and were observed moving around the cage, eating, drinking and interacting with cage mates; particularly after being stimulated by having their cage picked up and moved from the rack.

One week after completion of doxorubicin treatment in both groups II and III (at the 3rd week evaluation); the animals' fur became scruffy and developed a light yellow tinge.

On the 6th week evaluation (four weeks from the last DOX dose), animals in the DOX group (II) appeared to be sicker, weaker, anorexic and lethargic with dull or sluggish movements compared with the control group. Anorexia, while not directly observable, was

Table (1): Shows effect of DOX on physical parameters in all studied groups. DOX- administered groups (II, III) showed significant (P< 0.001) decrease in body weight compared to control group (I), significant ascites (P<0.05) and non-significant death rate at 3rd week, DOX group (II) showed significant (P< 0.001) decrease in body weight, significant ascites (P<0.05) and significant death rate (P<0.05) compared to control and DOX-MSC and control groups at 6th week.

	Group l (control)		**Group II (DOX)**		**Group III (DOX-MSC)**	
	At 3rd week	***At 6th week***	***At 3rd week***	***At6th week***	***At 3rd week***	***At 6th week***
Body weight in grams	180 ± 17.9	163.3±22.5	138.3±14.7[a]	125 ± 7.3 [a]	133.3±15.1 [a]	165± 23.5 [b]
Ascites:						
Nil	100%	100%	--- [a]	--- [a]	--- [a]	--- [a b]
Moderate	---	---	100%	---	100%	100%
Sever	---	---	---	100%	---	---
Death rate:						
Survived	27 (90%)	27 (100%)	26 (86.7%)	19 (73.1%) [a]	25(83.3%)	24(96%)
Dead	3(10%)	0 (0%)	4 (13.3%)	7 (26.9%) [a]	5(16.7%)	1(4%)[b]

[a] **significant versus control (P<0.05)** [b] **significant versus DOX group (II) (P<0.05)**

indicated when there was a lack of feces in a cage that has not just been cleaned. There was also obvious red exudate around the eyes of group II (DOX) by the end of 6th week of the study.

On the other hand these findings were all absent from group III (DOX-MSC) which showed much better activity, appetite, and general heath.

2- ***Body weight:***

The result of the present study revealed that there was a highly significant difference of mean values of body weight among different studied groups at both 3rd and 6th week evaluation of rats (where p<0.001 and p=0.004 respectively) by analysis of variance (ANOVA) (table 1). There was a highly significant decrease in body weight of the DOX group (group II) compared to the control group at both three and six weeks of the study (p<0.001 and p=0.004 respectively). Regarding DOX-MSC group (group III), highly significant reduction of weight compared to control group was observed only in the 3rd week (p<0.001) before injecting MSCs. On comparing the groups II and III (DOX and DOX-MSC respectively), there was non-significant different in body weight at the 3rd week (p>0.05), while in the 6th week, there was a significant increase of body weight (p=0.003) in group III (DOX-MSCs) compared to DOX group (group II). Using paired t test to compare results of the same group in 3rd and 6th weeks, it was found that there was no significant change in body weight between the two periods (3rd and 6th weeks) regarding control (groups I), which maintained weight gain throughout the study, and DOX (group II) (p>0.05), while there was a significant increase in body weight of group III (DOX-MSC) (p= 0.001) in the 6th week compared to the 3rd week.

3- ***Presence of ascites:***

The present study revealed that ascites (abdominal effusion due to blood congestion as

a sign of heart failure) was observed only in both of the doxorubicin-treated groups (group II and III), while virtually no abdominal effusion was detected in controls throughout the study. Estimated volume of ascites in our study was considered as follows: Mild: 10-25ml (+)/ moderate: 26-40ml (++)/ severe: 40-55ml (+++). At the 3rd week of the study both doxorubicin-treated groups; groups II (DOX) and III (DOX-MSC), showed moderate ascites compared to normal control group. By the 6th week the DOX group (group II) animals developed grossly enlarged abdomens and relatively severe ascites while the ascites in group III (DOX-MSC) was partially resolved to be mild (figure 3).

Table (2): Shows effect of DOX on hemodynamics and ECG parameters in all studied groups. No significant change was detected in systolic, diastolic and mean arterial pressure between along with HR all studied groups. QT interval showed significant prolongation in DOX- administered groups (II, III) ($P<0.01$, $P<0.05$) compared to control group at 6th week.

	Group l (control)		**Group II (DOX)**		**Group III (DOX-MSC)**	
	At 3rd week	***At 6th week***	***At 3rd week***	***At 6th week***	***At 3rd week***	***At 6th week***
Systolic BP	110 ± 11.44	113.7 ±9.9	109.7 ± 9.79	110.1 ± 14.6	102.5 ± 10.89	114.9 ± 4.95
Diastolic BP	75.3 ± 17.25	84.3 ±13.7	81.67 ± 9.87	74.7 ± 17.73	62.17 ± 13.96	81.1 ± 8.25
Mean ABP	91.83 ± 13.4	100 ±12.3	95.5 ± 8,62	91.6 ± 15.41	81.5 ± 11.62	98 ± 5.85
HR	251.3 ± 65.5	284.8 ±21.7	276.8 ± 31.6	244.3 ± 27.6	235 ± 38.74	264.9 ± 36.84
QRS complex in /ms	18.33 ± 1.97	16.67 ± 2.5	13.5± 3.62	15 ± 4.88	15.33± 3.67	13.8 ± 2.62
QT interval /ms	35.5 ± 5.05	35 ± 3.35	33 ± 9.98	49.1 ± 8.03[a]	47.17 ± 7.65 [ab]	43.6 ± 5.34[a]
PR interval /ms	46.17 ± 2.48	48.83 ± 5.19	42 ± 4.69	46.9 ± 4.28	47.33 ± 5.35	42.4 ± 6.6

[a] significant versus control (P<0.05) **[b] significant versus DOX group (II) (P<0.05)**

4- Survival rates of different groups:

The present study revealed that during the post treatment period, the survival rate, using Mantel–Haenszel test, showed insignificant difference among studied groups at the 3rd week evaluation ($p>0.05$). On the contrary, the survival rate was significantly different ($p= 0.002$) at the 6th week being least in the DOX-group (group II) [73.1%] than in the control (group I) [100%] and MSC-treated group (group III).

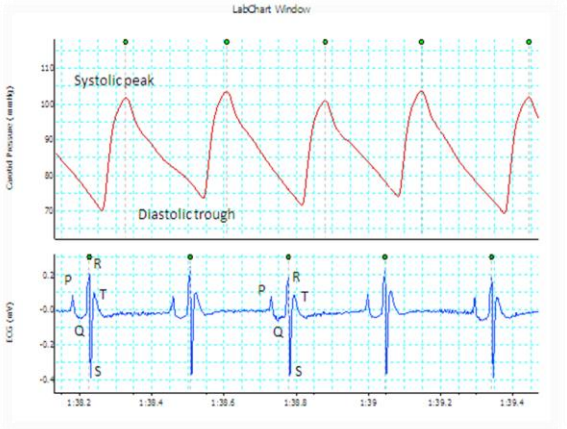
IV) In vivo Cardiovascular assessment:

1- Blood pressure and heart rate:

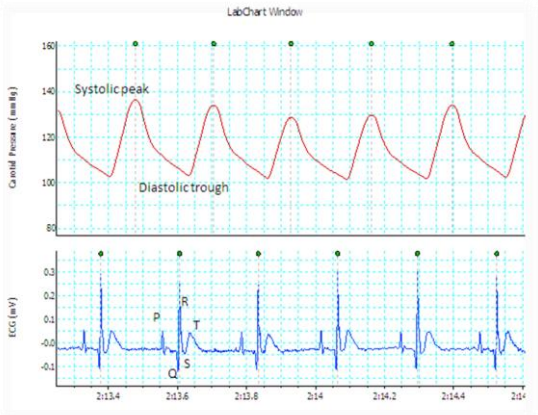
Statistical analysis using analysis of variance (ANOVA) revealed no significant difference ($p>0.05$) among studied groups at both 3rd and 6th week evaluation regarding values of arterial blood pressure [ABP] (systolic, diastolic and mean arterial blood pressures [MAP]) and the heart rate (HR). However with paired t test, comparing values of the same group at the 3rd and 6th weeks, there was a significant improvement of both systolic and mean arterial blood pressure ($p=0.006$ and $p=0.02$ respectively) in only group III (DOX-MSC) between the 3rd and 6th week (before and after injection of MSCs). There was no significant difference between the 3rd and 6th week in any of the studied groups regarding diastolic BP and heart rate.

2- Electrocardiography (ECG):

Regarding ECG intervals, as seen in rat ECG tracing [figure 4 (a) and (b)], with ANOVA test, there was no significant difference among the studied groups in neither the QRS complex nor the PR interval at both the 3rd and the 6th week of the study ($p>0.05$). Only the QT interval was found to be significantly different among the three groups at 3rd week of the study ($p=0.02$**)** and highly significant in difference among the studied groups at 6th week ($p=0.001$).



(a) Systolic BP: 115 mmHg, Diastolic BP: 70mmHg, HR: 245bpm, QT interval: 39ms, R amplitude: 0.79 mv.



(b) Systolic BP: 130 mmHg, Diastolic BP: 95 mmHg, HR: 266bpm, QT interval: 63ms, R amplitude: 0.58 mv.

Fig. 4: (a) Normal ECG tracing of a control rat (below) with normal arterial blood pressure tracing (above), (b) arterial blood pressure and ECG of a rat from DOX group at the 6th week with lower ECG amplitude and prolonged QT interval.

Least significant difference (LSD) test for QT interval at the 3rd week revealed a significant prolongation of QT interval in DOX-MSC group (group III) compared to control (group I)

(p=0.02) and to the DOX group (group II) (p=0.007), but no significant difference between control (group I) and DOX (group II) groups. At the 6th week, there was still a significant prolongation of QT interval in group III compared to group I (p=0.01) and a highly significant prolongation in the group II compared to group I (p<0.001) with the most prolonged QT interval found in DOX group (group II) (49.1 ± 8.03 ms). There was no significant difference between groups II and III (p>0.05). Using paired t test, apart from the significant prolongation of QT interval of group II (DOX) in the 6th week compared to the 3rd week, there was no significant difference in ECG components between the 3rd and the 6th week in any of the studied groups (p>0.05).

V) Serum Biochemical Profile:

1- Myocardial damage biomarker: Cardiac troponin I (cTn-I):

Analysis of of mean values of serum cardiac troponin I (cTnI) revealed statistically significant difference among studied groups in both the 3rd and the 6th weeks of the study where p<**0.001** and p=**0.01** respectively (table3).

Table (3): Shows effect of DOX on Cardiac troponin I, MDA and TAC in all studied groups. DOX-administered groups (II, III) showed significant (P< 0.05) increase in cTn I and significant (P< 0. 01) increase in MAD along with significant (P< 0.001) decrease in TAC compared to control at 3rd week. DOX-MSC treated group (III) showed significant (P<0.01) decrease in cTn I and significant (P< 0.001) decrease in MAD along with significant (P<0.01) increase in TAC compared to DOX group (II) at 6th week.

	Group l (control)		Group II (DOX)		Group III (DOX-MSC)	
	At 3rd week	*At 6th week*	*At 3rd week*	*At 6th week*	*At 3rd week*	*At 6th week*
Cardiac troponin I	53.28 ± 3.6	50.3 ± 4.4	72.62 ± 3.4 [a]	88.2 ± 17.2 [a]	78.48 ± 6.52 [a]	66.84 ± 18.3 [b]
MDA	15.84 ± 2.9	14.82 ± 2.8	25.31 ± 3.3 [a]	35.74 ± 3.4 [a]	24.96 ± 4.5 [a]	15.66 ± 1.2 [b]
TAC	1.02 ± 0.10	1.07 ± 0.13	0.04 ± 0.03 [a]	0.08 ± 0.02 [a]	0.05 ± 0.02 [a]	0.65 ± 0.35 [ab]

[a] **significant versus control (P<0.05)** [b] **significant versus DOX group (II) (P<0.05)**

At the 3rd week, significant rise in cTnI in both DOX and DOX-MSC groups compared to the control group (p=0.01 and p=0.03 respectively) with non-significant difference between groups II and III (p>0.05) [table 7(a)]. At the 6th week the level of cTnI in group III (DOX-MSC) was comparable to that of the control group (I) with non-significant difference (p>0.05), while the level of cTnI continued to rise in group II (DOX) to be significantly higher than both the control (p=0.02) and the DOX-MSC group (III) (p<0.001). Paired t test showed the statistically significant decline in level of cTn I in the DOX-MSC group (III) in the 6th week of the study (3weeks after MSCs injection (p= **0.03**).

2- Oxidative stress parameters:

a- Malondialdehyde (MDA):

Statistical analysis of mean values of serum malondialdehyde (MDA) among the studied groups using ANOVA showed highly significant difference at both the 3rd and the 6th week of the study with p=0.003 and p<0.001 respectively (figure 5). Least significant difference (LSD) test for comparison of the

mean values of MDA in-between groups after three weeks revealed that there was a highly significant rise of MDA in DOX-treated groups (groups II and III) (p=0.002). The rise of MDA serum level was also highly significant at the 6th week in the DOX group (group II) compared to the other two groups [control (I) and DOX-MSC (III) groups] (p<0.001 for both). The DOX-MSC group and the control group showed serum values with statistically insignificant difference (p>0.05). Paired t test showed statistically significant rise of MDA in the DOX group (group II) at the 6th week than the 3rd (p=0.002). On the contrary, the MDA significantly decreased in the DOX-MSC group (group III) after treatment with MSCs (at the 6th week) (p= 0.001).

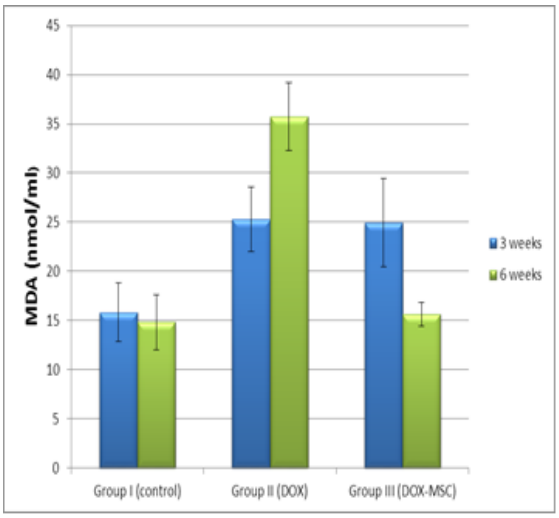


Fig. 5: Bar chart showing graphical comparison among different studied groups as regards malondialdehyde (MDA) at both the 3rd and the 6th week of the study.

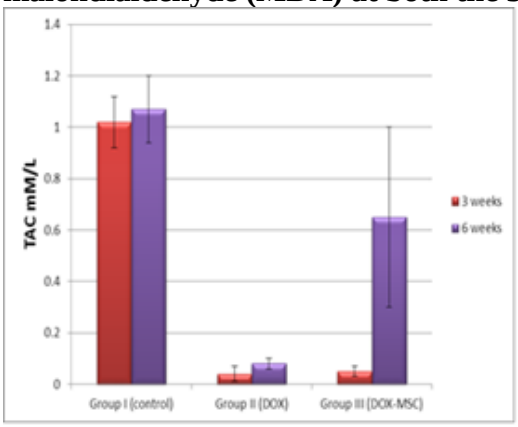


Fig. 6: Bar chart showing graphical comparison among different studied groups as regards total antioxidant capacity (TAC) at both the 3rd and the 6th week of the study.

b- Total antioxidant capacity (TAC):

The statistical analysis of the total antioxidant capacity (TAC) using **Kruskal–Wallis test** revealed highly significant difference of mean values among the three studied groups at both the 3rd (p=0.009) and the 6th (p=0.001) week of the study (figure 6).

The least significant difference (LSD) test at the 3rd week showed a highly significant decline of TAC in both group II (DOX) and group III (DOX-MSC) [0.04 ± 0.03mM/l and 0.05 ± 0.02 mM/l respectively] compared to control group [1.02 ± 0.10 mM/l] with p<0.001. There was a non-significant difference between groups II and III. The value of TAC after six weeks was still lower in both groups II and III than the control (p<0.001 and p=0.01 respectively). However, TAC was significantly higher in MSC-treated group (group III) [0.65 ± 0.35mM/l] than in non-treated group (group II) [0.08 ± 0.02 mM/l] with p<0.001.

5. DISCUSSION

Doxorubicin (DOX) is classified as an anthracycline antibiotic and is used in chemotherapy to treat many types of cancer. Its clinical use is limited by the risk of severe cardiotoxicity which can lead to progressive irreversible congestive heart failure particularly by multiple cumulative doses exceeding 550 mg/m2 ***(Chatterjee et al., 2010).*** However, Doxorubicin is still a well-established and a highly effective anti-neoplastic agent used to treat several adult and pediatric cancers such as solid tumors, leukemia, lymphomas and breast cancer ***(Octavia et al., 2012).***

Myocardial protection and repair are old dreams of physicians and remain as important goals to enhance the beneficial antitumor activity of doxorubicin as well as to remove the risk of its induced cumulative cardiotoxicity ***(Ahmed and EL-Maraghy, 2013)***. Unfortunately, no effective treatment currently exists for DOX-induced cardiomyopathy ***(Razmaraii et al., 2016).***

The hypothesis that the anthracycline anti-tumour agents inadvertently deplete the resident cardiac stem cells pool, with subsequent loss of the of cardiac capability of regeneration and demonstration of symptoms of cardiotoxicity, has been supported by several experimental studies ***(De Angelis et al., 2010 & Li et al., 2010)***.

As a result, using stem cells to treat DOX-induced cardiotoxicity and its subsequent manifestation s of heart failure has been studied preclinically for long time before being tried in the clinical setting, where a flurry of small, mostly uncontrolled trials indicate that stem cell therapy may be feasible in patients ***(Ezquer et al., 2015)***.

The present study has been designed and conducted to determine the possible curative effects of umbilical cord blood mesenchymal stem cell (UCB-MSC) against the DOX-induced cardiotoxicity in female adult albino rats. Studied rats were randomly divided into three groups; group I (saline control), group II (DOX), and group III (DOX-MSC) that's treated with MSC one week after DOX treatment. Intraperitoneal administration of DOX at a total cumulative dose of 15 mg/kg in 6 equal doses (2.5 mg/kg per 48 hours) over a period of two weeks has induced physical, hemodynamic, ECG, biochemical and histopathological evidences that validate the development of DOX cardiomyopathy in the rat model and this dose has been found to be consistent with previous models of DOX-induced cardiomyopathy ***(Siveski-Iliskovic et al., 1994; Arola et al., 2000; De Angelis et al., 2010 & Elmadbouh et al., 2015).***

Female rats were used as they lack the male-specific sex determining region Y (*Sry* gene) that was used for detection of homing of male-origin MSCs ***(Conconi et al., 2006)*** injected via tail vein at the third week of the study and this systemic delivery of MSCs has been reported by several groups ***(Di at al., 2012; Mohammadi Gorji et al., 2012; Oliveira et al., 2013; Pinarli et al., 2013 & Ammar et al., 2015).***

Intravenous (IV) injection of stem cells has the advantage of being minimally invasive, and it allows for wide distribution of cells throughout the body. It could also have integral beneficial

effects in DOX- treated patients with the inflammatory suppressive activity of MSCs on other systems like the brain, reducing tumor necrosis factor-α (TNF-α) production by microglial cells, and the liver; managing tissue-derived oxidative stress ***(Tulubas et al., 2013 & Ezquer et al., 2015)***.

Many molecules, including integrins, selectins, and chemokine receptors, known to be involved in the tethering, rolling, adhesion, and transmigration of leukocytes from the bloodstream into tissues are also expressed on MSCs ***(Van Linthout et al., 2011).***

The umbilical cord blood MSCs (UCB-MSCs), in particular, are characterized with cell surface profile of adhesion molecules correlating with their faster lung clearance and homing into damaged tissues than other types of MSCs ***(Nystedt et al., 2013)***, in addition, they have reported significant cardiomyogenic potential (30–50%) ***(Gopinath et al., 2010).*** In the present study, homing of injected UCB-MSCs was confirmed by detecting the male specific *Sry* gene within female rat myocardium DNA by PCR and electrophoresis (***Pulavendran et al., 2011).***

Compared to other mesenchymal stem cells, UCB stem cells have unique properties that UCB-MSCs have a longer telomere length, which correlates to their higher proliferative potential, they have not been exposed to immunological challenge, they are less likely to carry somatic mutations than other adult cells, furthermore, animal studies indicated that they do not have the risk to form teratomas like embryonic cells ***(van de Ven et al., 2007).***

Manifestations of depressed physical activity and declined general health were apparent one week after completion of DOX treatment (at the 3rd week) in both DOX-treated groups (II and III) and became much more evident three weeks later (at the 6th week) only in group II (DOX) with marked anorexia, low activity and dull or sluggish movements compared with the control group. These findings pass in parallel with those obtained by several other studies including ***Cove-Smith et al. (2014) and Elmadbouh et al. (2015).***

Weight loss throughout the study was another important physical index of DOX-induced cardiomyopathy and heart failure. Despite the developed ascites, significantly lower ultimate body weight of the DOX group (II) in relation to the other two groups (control and DOX-MSC groups) was observed.

Similar results of decrease in the final body weight of the doxorubicin-treated rats were previously obtained by ***Alimoradi et al. (2012), Ahmad and Elmaraghy (2013), Ammar et al. (2015)*** and ***Razmaraii et al, (2016).***

Previous studies have reported that DOX impaired general health and prevented the progression of body weight in rats due to a decrease in appetite and food consumption ***(Ghibu et al., 2012; Spivak et al., 2013 & Arafa et al., 2014),*** which was also observed in this study, in addition to the DOX-induced inhibition of protein production ***(Kwok and Richardson, 2004).***

However, on evaluation of group III (DOX-MSC group) in the 6th week of the study, the MSC- treated rats showed improved general physical condition with much better activity, appetite, and general heath which is in line with the results of ***Mohammadi Gorji et al. (2012), Pinarli et al. (2013)*** and ***Elmadbouh et al. (2015)***.

Moreover, body weight has been significantly improved in group III (DOX-MSC) in the 6th week (after MSCs injection) compared to the 3rd week of the study and gained more final body weight compared to DOX group. The improved final body weight of MSC- treated toxic rats has been found before by ***De Angelis et al. (2010), Di et al. (2012), Oliveira et al., 2013,*** and ***Ammar et al. (2015).***

Development of ascites in both of the doxorubicin-treated groups (group II and III) by the 3rd week of the study and its progression only in the DOX group by the 6th week was an important sign of venous congestion and has been reported by several previous studies to be a characteristic evidence of DOX-induced heart failure ***(Mohan et al., 2006; Yilmaz et al., 2006 & Merlet et al., 2013).***

Although ascites is associated with the development of heart failure, the possibility of DOX-mediated peritonitis cannot be ruled out, that's why some researchers have dismissed the accumulation of ascitic fluid as evidence of DOX-mediated heart failure ***(Hayward and Hydock, 2007).***

However, developed ascites due to drug-induced peritonitis was previously suggested to be avoidable when DOX is administered in multiple doses at low dose concentrations ***(Teraoka et al., 2000)*** as adopted in the present study.

On the other hand, the ascites in group III (DOX-MSC) was partially resolved by the 6th week of the study to be mild reflecting the improved DOX-induced heart failure due to therapeutic effects of MSCs.

Decreased volume of ascites in DOX-treated rats with injection of MSCs was noticed in previous studies as well (***De Angelis et al., 2010; Di et al., 2012 & Pinarli et al., 2013).***

The survival rate, in the present study, was significantly declined in the DOX-group (group II) at the 6th week [73.1%] compared to the control (group I) [100%] and MSC-treated group (group III) [96%]. These survival rate results were reproducible with those of ***Merlet et al. (2013), Warpe et al. (2014)*** and ***Elmadbouh et al. (2015).***

Higher mortality has been found in literature with single large dose of DOX administration ***(Hayward and Hydock, 2007),*** while much lower mortality has been associated with more chronic courses ***(Cove-Smith et al., 2014).***

Burkholder and colleagues (2012) reported that deaths which occurred 3 weeks after completion of the treatment and onwards were included in the calculation of cardiac failure-associated mortality as deaths occurred earlier were thought to be due to poor general conditions associated with bone marrow depression.

That's why the high mortality in the DOX group by the 6th week was a result of deteriorated heart failure that was incompatible with life due to well noted anorexia ***(Richard et al., 2011)*** with advanced nephropathy and protein catabolism ***(Abdel-Raheem et al., 2013).***

Improved survival in group III with MSC intervention shown in the present study was comparable to the results obtained by ***Di et al. (2012), Oliveira et al. (2013), Pinarli et al. (2013)*** and ***Ammar et al. (2015).***

These results indicate that MSCs improved weight gain and the general health status and have a positive influence on a variety of factors required for improved survival length and quality. These therapeutic benefits of MSCs are largely dependent on their capacity to act as a trophic factor pool combating different degenerating and debilitating conditions ***(Wei et al., 2013).***

Several in vivo cardiovascular parameters were evaluated in the current study to evaluate recovery of DOX-induced impaired ventricular function with the injected UCB-MSC.

This study revealed no significant difference among studied groups at both 3rd and 6th week evaluation regarding indices of arterial blood pressure (systolic, diastolic and mean arterial blood pressures) and the heart rate (HR).

Similarly, no significant change in arterial blood pressure values among DOX and other studied groups was obtained by ***Hayward and Hydock (2007), Richard et al. (2011)*** and ***Colak et al. (2012)*** and absence of significant change in heart rate was showed also by other previous studies ***(Richard et al., 2011 & Warpe et al., 2015).***

Other studies demonstrate significant decline in blood pressure indices in the DOX-treated groups despite similar DOX dosing regimens ***(Ammar et al., 2015; Elmadbouh et al., 2015 & Razmaraii et al, 2016),*** but this difference could be due to that these studies used male Wister or Sprague Dawley rats instead of female albino rats used in this study with both gender and species differences ***(Martignoni et al., 2006 & Vijay et al., 2015).***

Warpe et al. (2015) also obtained significant decline in blood pressure; in addition to using male Wister rats, he induced cardiomyopathy by a different dosing regimen.

Similarly, others got significant HR changes following DOX treatment and this difference from the current study could be attributed to the use of male rats as well as higher cumulative DOX dose of 18mg/kg ***(Shafik et al., 2011 & Ahmad and Elmaraghy, 2013)***, or the use of single large dose of 20mg/kg ***(Colak et al.; 2012),*** or just gender-related variation in drug response despite similar dosing regimens ***(Vijay et al., 2015 & Razmaraii et al, 2016).***

Evaluation of ECG revealed that only the QT interval was found to be significantly longer in the DOX-treated animals at the 3rd and the 6th weeks of the with significant prolongation of QT interval in group II (DOX) in the 6th week compared to the 3rd week.

Dose-dependent significant changes in the ECG verified the marred cardiac contractility and conduction after doxorubicin intoxication and imply negative chronotropic effect in general ***(Li et al., 2009).*** Additionally general metabolic and electrolyte abnormalities associated with developed heart failure contribute particularly to the QT interval prolongation ***(Hunter et al., 2008).***

Previous reports of DOX cardiotoxicity in laboratory animals have indicated prolongation of only QT interval, consistent with the present study ***(Rahimi_Balaei et al., 2010).***

On the other hand, ***Colak et al. (2012),*** using single dose of 20mg/kg DOX, didn't warrant that the DOX treatment might cause a heart conduction disorder.

Conversely, there was a significant improvement of both systolic and mean arterial blood pressure values in group III (DOX-MSC) between the 3rd and 6th week (before and after injection of MSCs).

The QT interval prolongation didn't respond to MSC treatment in the 6th week of the study and this can be simply explained by the fact that prolongation of QT interval is mainly attributed to electrolyte disturbance accompanying heart failure (mainly hyperkalemia) rather than direct myocardial damage ***(Hunter et al., 2008).***

There was statistically significant rise of the biomarker of cardiac injury; cTnI, in DOX-treated groups (II and III) at the 3rd week and in DOX group (II) only in the 6th weeks of the study. Several other studies support that cTnI was raised in the presence of the diffuse cardiac injury induced by DOX even after cessation of treatment ***(Pinarli et al., 2013; Reagan et al., 2013 & Cove-Smith et al., 2014).***

Cardiac troponin I is a specific cardiac marker as it has not been reported to be expressed outside of cardiac tissue ***(Alvarez et al., 2012).*** cTnI can be also considered a sensitive and reliable marker of myocardial damage with relevant clinical and prognostic implications ***(Cardinale et al., 2010).***

Serum cTnI results following one- and four-week intervals after DOX treatment indicated progression of cardiac injury beyond the dosing period consistent with the recognized pathogenesis of doxorubicin cardiomyopathy which involves active proteolytic cleavage of myocardial contractile proteins ***(Ky et al., 2014).*** The elevation of cTnI can be also related to coronary endothelial dysfunction which is known to occur in heart failure patients, but cardiomyocyte injury may also be due to acute left ventricular stretch which may cause proteolysis and release of the cTnI ***(Feng et al., 2007).*** In addition, it could be a direct consequence of the cardiomyocyte degenerative process ***(Jaffe and Wu, 2012).***

As well, it is well documented in clinical studies that the spectrum of cardiotoxicity with anthracycline agents includes coronary diseases with subsequent myocardial ischemic effects ***(Swerdlow et al., 2007).***

The specific mechanisms of doxorubicin cardiotoxicity are complex and remain unclear. However, these mechanisms are related mainly to the excessive production of reactive oxygen species (ROS) in the mitochondria that cause cellular oxidative stress ***(Abdel raheem et al., 2013).*** In turn, doxorubicin-induced increased oxidative stress is considered the main universal mechanism to the development of myocyte apoptosis and the overall deterioration of ***Angsutararux et al., 2015).***

Evaluation of oxidative stress status in the present study was performed via estimation of serum malondialdehyde (MDA) and the total

antioxidant capacity (TAC). Measuring oxidative stress systemically in serum is more representative of the generalized DOX-induced oxidative stress and provides a perspective on the systemic redox state of patients subjected to chemotherapy and its involvement in the aggravation of patient toxicity thus being more clinically applicable ***(Panis et al., 2012).***

Evaluation of serum MDA showed a highly significant rise in DOX-treated groups (II and III) at the 3rd week and statistically significant rise only in the DOX group (II) at the 6th week of the study. This rise of serum MDA is parallel with the results of ***Warpe et al. (2015).*** Others reported high local cardiac tissue MDA in doxorubicin-treated rats ***(Al-Harthi et al., 2014 & Elmadbouh et al., 2015)***.

Malondialdehyde is one of the end products of DOX-induced lipid peroxidation Increase in the levels of MDA is suggestive of increased oxidative stress and reduced antioxidant defenses which result from the generation of free radicals ***(Elberry et al., 2010).***

There are two mechanisms by which DOX can generate reactive free radicals. One involves one electron reduction of the drug to a semiquinone free radical intermediate ***(Minotti et al., 2004)***, and the second mechanism is dependent upon interaction of DOX with metal ions, especially iron ***(Neilan et al., 2007)***.

Several studies that have highlighted lipid peroxidation as the primary mechanism underlying DOX-induced cardiac toxicity and pointed out the importance of measuring MDA and other Thiobarbituric Acid Reactive Substances (TBARS) in evaluating doxorubicin- mediated cardiotoxicity in both experimental animals ***(El-Sayed et al., 2011; Richard et al., 2011& Colak et al., 2012)*** and human patients ***(El-Dakroory et al., 2013).***

Similarly, this study revealed highly significant decrease of TAC in both DOX-treated groups (DOX and DOX-MSC) compared to control at the 3rd week of the study. In the 6th week, TAC significantly continued to decrease in the DOX group. Similar decline in serum total antioxidant capacity with DOX treatment was found by ***El-Sayed et al. (2011)*** and ***Al-Harthi et al. (2014).***

The rise of oxidative stress products level, as a major cardiotoxic mechanism, should be associated with a decline in total body antioxidant capacity due to consumption of its components ***(El-Sayed et al., 2011***). Inversely, there was marked decline in level of MDA of DOX-MSC group in the 6th week to be comparable to that of the control owing to the effects of injected MSCs. Regarding level of TAC in the DOX-MSC group, despite its statistically significant rise in after MSC injection, it was still significantly less than the control level but significantly higher than that of the DOX group.

These results of both MDA and TAC reflect highly significant improvement of the redox status of cardiomyocytes and amelioration of the DOX- induced oxidative stress, the major mechanism of cardiotoxicity, after MSC injection systemically into rats ***(Di et al., 2012 & Liu et al., 2012***).

Accordingly, the use of this kind of cell-based therapy is highly versatile because almost all therapies were successful (partial recovery or maintenance of cardiac function) given diverse factors, including (i) time and route of administration of MSCs, (ii) number of doses of MSCs, (iii) source of MSCs, and (iv) grade of cardiac injury induced by doxorubicin. Unfortunately, the duration of the beneficial effect induced by MSC administration has not been tested (***Ezquer et al., 2015).***

6. CONCLUSION AND RECOMMENDATIONS

The results of the present study reflected the cardiotoxic effects of DOX that's mainly mediated by oxidative stress and free radical formation as seen in rise of MDA and decrease in TAC. These toxic effects manifest as deteriorated general health, impaired myocardial function, rise in serum cTnI, and marked affection of myocardial tissue by histopathological evaluation.

Systemic injection of UCB-MSCs into tail vein of adult female albino rats significantly ameliorated these toxic manifestations, led to

marked functional and structural cardiac improvement and stopped the progression towards heart failure with amelioration of DOX-induced oxidative stress which raise the possibility that doxorubicin-induced cardiac failure is a stem cell disease and show the reparative effects of MSCs on the damaged heart.

It's recommended to pay more attention to the stem cell therapeutic modality with the importance of performing further studies to evaluate the mechanism and extent of its repairing effect and elucidate the possibility of its clinical application.

7. REFERENCES

1. **Abdel‐Raheem, I. T., Taye, A. and Abouzied, M. M. (2013).** Cardioprotective Effects of Nicorandil, a Mitochondrial Potassium Channel Opener against Doxorubicin‐Induced Cardiotoxicity in Rats. Basic & clinical pharmacology & toxicology, 113(3), 158-166.
2. **Ahmed, L.A. and EL-Maraghy, S.A. (2013).** Nicorandil ameliorates mitochondrial dysfunction in doxorubicin-induced heart failure in rats, possible mechanism of cardioprotection. Biochemical pharmacology, 86(9), 1301-1310.
3. **Alhadlaq, A. and Mao, J. (2004).** Mesenchymal stem cells, isolation and therapeutics. Stem Cells Development, 13,436–448.
4. **Al-Harthi, S.E., Alarabi, O.M., Ramadan, W.S., Alaama, M.N. et al. (2014).** Amelioration of doxorubicin induced cardiotoxicity by resveratrol. Molecular Medicine Reports, 10, 1455-1460.
5. **Alimoradi, H., Barzegar-Fallah, A., Hassanzadeh, G., Mohammadi-Rick, S. et al. (2012).** The cardioprotective effects of an antiemetic drug, tropisetron, on cardiomyopathy related to doxorubicin. Cardiovascular toxicology, 12(4), 318-325.
6. **Alvarez, I., Uribe, A. and Duarte, S. (2012).** Biomarkers of heart failure in small animals. Journal of Veterinary Medicine, 24, 59-70.
7. **Ammar, H.I., Sequiera, G.L., Nashed, M.B., Ammar, R.I. et al. (2015).** Comparison of adipose tissue-and bone marrow-derived mesenchymal stem cells for alleviating doxorubicin-induced cardiac dysfunction in diabetic rats. Stem cell research & therapy, 6(1), 1.
8. **Angsutararux, P., Luanpitpong, S. and Issaragrisil, S. (2015).** Chemotherapy-induced cardiotoxicity, overview of the roles of oxidative stress. Oxidative medicine and cellular longevity, Article ID 795602, 13 pages
9. **Arafa, M.H., Mohammad, N.S., Atteia, H.H. and Abd-Elaziz, H.R. (2014).** Protective effect of resveratrol against doxorubicin-induced cardiac toxicity and fibrosis in male experimental rats. Journal of physiology and biochemistry, 70(3), 701-711.
10. **Arola, O.J., Saraste, A., Pulkki, K., Kallajoki, M. et al. (2000).** Acute doxorubicin cardiotoxicity involves cardiomyocyte apoptosis, Cancer Research 60,1789–1792.
11. **Baksh, D., Yao, R., and Tuan, R. S. (2007).** Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem cells, 25(6), 1384-1392.
12. **Bertinchant, J.P., Polgeb, A., Juana, J.M., Oliva-Laurairea, M.C. et al. (2003).** Evaluation of cardiac troponin I and T levels as markers of myocardial damage in doxorubicin-induced cardiomyopathy rats, and their relationship with echocardiographic and histological findings. Clinica Chimica Acta, 329 (1–2), 39–51.
13. **Bieback, K., Kern, S., Klüter, H. and Eichler, H. (2004).** Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem cells, 22(4), 625-634.
14. **Burkholder, T., Foltz, C., Karlsson, E., Linton, C.G. et al. (2012).** Health Evaluation of Experimental Laboratory Mice. Current Protocols in Mouse Biology, 2, 145–165.
15. **Burkholder, T., Foltz, C., Karlsson, E., Linton, C.G. et al. (2012).** Health Evaluation of Experimental Laboratory Mice. Current Protocols in Mouse Biology, 2, 145–165.
16. **Caplan, A.I. and Dennis, J.E. (2006).** Mesenchymal stem cells as trophic mediators. Journal of cellular biochemistry, 98(5), 1076-1084.
17. **Cardinale, D. and Sandri, M.T. (2010).** Role of biomarkers in chemotherapy-induced cardiotoxicity. Progress in cardiovascular diseases, 53(2), 121-129.
18. **Cardinale, D., Colombo, A., Torrisi, R., Sandri, M.T. et al. (2010).** Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. Journal of Clinical Oncology, 28(25), 3910-3916.
19. **Chatterjee, K., Zhang, J., Honbo, N. and Karliner, J. S. (2010).** Doxorubicin cardiomyopathy. Cardiology, 115(2), 155-162.
20. **Colak, M.C., Parlakpinar, H., Tasdemir, S., Samdanci, E. et al. (2012).** Therapeutic effects of ivabradine on hemodynamic parameters and cardiotoxicity induced by doxorubicin treatment in rat. Human & experimental toxicology, 31(9), 945-954.

21. **Conconi, M.T., Burra, P., Di Liddo, R., Calore, C. et al. (2006).** CD105 (+) cells from Wharton's jelly show in vitro and in vivo myogenic differentiative potential. International journal of molecular medicine, 18(6), 1089-1096.
22. **Cove-Smith, L., Woodhouse, N., Hargreaves, A., Kirk, J. et al. (2014).** An integrated characterization of serological, pathological, and functional events in doxorubicin-induced cardiotoxicity. Toxicological Sciences, 140(1), 3-15.
23. **De Angelis, A., Piegari, E., Cappetta, D., Marino, L. et al. (2010).** Anthracycline Cardiomyopathy Is Mediated by Depletion of the Cardiac Stem Cell Pooland Is Rescued by Restoration of Progenitor Cell Function. Circulation, 121,276-292.
24. **De Angelis, A., Urbanek, K., Cappetta, D., Piegari, E. et al. (2016).** Doxorubicin cardiotoxicity and target cells, a broader perspective. Cardio-Oncology 2, 2.
25. **Di, G.H., Jiang, S., Li, F.Q., Sun, J.Z. et al. (2012).** Human umbilical cord mesenchymal stromal cells mitigate chemotherapy-associated tissue injury in a pre-clinical mouse model. Cytotherapy, 14(4), 412-422.
26. **Di, G.H., Jiang, S., Li, F.Q., Sun, J.Z. et al. (2012).** Human umbilical cord mesenchymal stromal cells mitigate chemotherapy-associated tissue injury in a pre-clinical mouse model. Cytotherapy, 14(4), 412-422.
27. **Elberry, A.A., Abdel-Naim, A.B., Abdel-Sattar, E.A., Nagy, A.A. et al. (2010).** Cranberry (Vaccinium macrocarpon) protects against doxorubicin-induced cardiotoxicity in rats. Food and chemical toxicology, 48(5), 1178-1184.
28. **Elmadbouh, I., Mansour, M., Nabeh, M., Faried, W. et al. (2015).** Atorvastatin improves cardiac function and remodeling in chronic non-ischemic heart failure, A clinical and pre-clinical study. The Egyptian Heart Journal, 67, 289–298.
29. **El-Sayed, E.M., El-azeem, A.S.A., Afify, A.A., Shabana, M.H. et al. (2011).** Cardioprotective effects of Curcuma longa L. extracts against doxorubicin-induced cardiotoxicity in rats. Journal of Medicinal Plants Research, 5(17), 4049-4058.
30. **Ezquer, F., Gutiérrez, J., Ezquer, M., Caglevic, C. et al. (2015).** Mesenchymal stem cell therapy for doxorubicin cardiomyopathy: hopes and fears. Stem cell research & therapy, 6(1), 116.
31. **Feng, J., Schaus, B.J., Fallavollita, J.A., Lee, T.C. (2007).** Preload induces troponin I degradation independently of myocardial ischemia. Circulation, 103(16), 2035-2037.
32. **Freshney, R.I. (2010).** Culture of Animal Cells, A Manual of Basic Technique. John Wiley & sons, Hoboken, New Jersey, USA.
33. **Ghibu, S., Delemasure, S., Richard, C., Guilland, J.C. et al. (2012).** General oxidative stress during doxorubicin-induced cardiotoxicity in rats: absence of cardioprotection and low antioxidant efficiency of alpha-lipoic acid. Biochimie, 94(4), 932-939.
34. **Gho, J.M., Kummeling, J.M., Koudstaal, S., Doevendans, P.A. et al. (2013).** Cell Therapy, a Novel Remedy for Dilated Cardiomyopathy? A Systematic. Journal of Cardiac Failure, 19, 494-502.
35. **Gopinath, S., Vanamala, S.K., Gondi, C.S. and Rao, J.S. (2010).** Human umbilical cord blood derived stem cells repair doxorubicin-induced pathological cardiac hypertrophy in mice. Biochemical and biophysical research communications, 395(3), 367-372.
36. **Gustafson, D.L., Rastatter, J.C., Colombo, T. and Long, M.E. (2002).** Doxorubicin pharmacokinetics, macromolecule binding, metabolism, and excretion in the context of a physiologic model. Journal of pharmaceutical sciences, 91(6), 1488-1501.
37. **Haasters, F., Prall, W., Anz, D., Bourquin, C. et al. (2009).** Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing. Journal of Anatomy, 214, 759–767.
38. **Hayward, R. and Hydock, D. S. (2007).** Doxorubicin cardiotoxicity in the rat: an in vivo characterization. Journal of the American Association for Laboratory Animal Science, 46(4), 20-32.
39. **Hunter, J.D., Sharma, P. and Rathi, S. (2008).** Long QT syndrome. Continuing Education in Anaesthesia, Critical Care & Pain, 8(2), 67-70.
40. **Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (2011).** Guide for the Care and Use of Laboratory Animals. 8th ed. National Academy Press, Washington DC, PP. 21-55. http,//grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf.
41. **Jaffe, A.S. and Wu, A.H. (2012).** Troponin Release-reversible or irreversible injury? Should we care?. Clinical Chemistry, 58, 148–150.
42. **Kwok, J.C. and Richardson, D.R. (2004).** Examination of the mechanism (s) involved in doxorubicin-mediated iron accumulation in ferritin: studies using metabolic inhibitors, protein synthesis inhibitors, and lysosomotropic agents.Molecular Pharmacology, 65(1), 181-195.

43. **Ky, B., Putt, M., Sawaya, H., French, B. et al. (2014).** Early increases in multiple biomarkers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab. Journal of the American College of Cardiology, 63(8), 809-816.
44. **Li, Y.M., Guo, Y.P. and Liu, Y. (2010).** Cancer chemotherapy induces cardiotoxicity by targeting cardiac stem cells. Journal of cellular and molecular medicine, 14(11), 2630-2632.
45. **Lin, Y.C., Leu, S., Sun, C.K., Yen, C.H. et al. (2010).** Early combined treatment with sildenafil and adipose-derived mesenchymal stem cells preserves heart function in rat dilated cardiomyopathy. Journal of Translational Medicine, 8, 88.
46. **Liu, H., McTaggart, S.J., Johnson, D.W. and Gobe, G.C. (2012).** Original article anti-oxidant pathways are stimulated by mesenchymal stromal cells in renal repair after ischemic injury. Cytotherapy, 14(2), 162-172.
47. **Loffredo, F.S., Steinhauser, M.L., Gannon, J. and Lee, R.T. (2015).** Bone Marrow-Derived Cell Therapy Stimulates Endogenous Cardiomyocyte Progenitors and Promotes Cardiac Repair. Cell Stem Cell, 17(1), 125-134.
48. **Ma, S., Xie, N., Li, W., Yuan, B. et al. (2014).** Immunobiology of mesenchymal stem cells. Cell Death & Differentiation, 21(2), 216-225.
49. **Martignoni, M., Groothuis, G.M. and de Kanter, R. (2006).** Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opinion on Drug Metabolism & Toxicology, 2(6), 875-894.
50. **Merlet, N., Piriou, N., Rozec, B., Grabherr, A. et al. (2013).** Increased beta2-adrenoceptors in doxorubicin-induced cardiomyopathy in rat. PloS one, 8(5), e64711.
51. **Minotti, G., Recalcati, S., Menna, P., Salvatorelli, E. et al. (2004).** Doxorubicin cardiotoxicity and the control of iron metabolism, quinone-dependent and independent mechanisms. Methods in Enzymology, 378, 340–361.
52. **Mohammadi Gorji, S., Karimpor Malekshah, A.A., Hashemi-Soteh, M.B., Rafiei, A. et al. (2012).** Effect of mesenchymal stem cells on doxorubicin-induced fibrosis. Cell J, 14(2), 142-151.
53. **Mohan, I.K., Kumar, K.V., Naidu, M.U.R., Khan, M. et al. (2006).** Protective effect of CardiPro against doxorubicin-induced cardiotoxicity in mice. Phytomedicine, 13(4), 222-229.
54. **Moretti, P., Hatlapatka, T., Marten, D., Lavrentieva, A. et al. (2009).** Mesenchymal stromal cells derived from human umbilical cord tissues, primitive cells with potential for clinical and tissue engineering applications. In Bioreactor Systems for Tissue Engineering II (pp. 29-54). Springer Berlin Heidelberg.
55. **Mungrue, I.N., Gros, R., You, X., Pirani, A. et al. (2002).** Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. The Journal of clinical investigation, 109(6), 735-743.
56. **Neilan, T.G., Blake, S.L., Ichinose, F., Raher, M.J. et al. (2007).** Disruption of nitric oxide synthase 3 protects against the cardiac injury, dysfunction, and mortality induced by doxorubicin. Circulation, 116(5), 506-514.
57. **Nystedt, J., Anderson, H., Tikkanen, J., Pietilä, M. et al. (2013).** Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. Stem Cells, 31(2), 317-326.
58. **Octavia, Y., Tocchetti, C.G., Gabrielson, K.L., Janssens, S., Crijns, H.J. and Moens, A.L. (2012).** Doxorubicin-induced cardiomyopathy, From molecular mechanisms to therapeutic strategies. Journal of Molecular and Cellular Cardiology, 52, 1213–1225.
59. **Oliveira, M.S., Melo, M.B., Carvalho, J.L., Melo, I.M. et al. (2013).** Doxorubicin Cardiotoxicity and Cardiac Function Improvement After Stem Cell Therapy Diagnosed by Strain Echocardiography.Journal of Cancer Science & Therapy, 5(2), 052–057.
60. **Panis, C., Herrera, A.C.S.A., Victorino, V.J., Campos, F.C. et al. (2012).** Oxidative stress and hematological profiles of advanced breast cancer patients subjected to paclitaxel or doxorubicin chemotherapy. Breast cancer research and treatment, 133(1), 89-97.
61. **Parasuraman, S., Raveendran, R., & Kesavan, R. (2010).** Blood sample collection in small laboratory animals. Journal of Pharmacology & Pharmacotherapeutics, 1(2), 87–93.
62. **Pinarli, F. A., Turan, N. N., Pinarli, F. G., Okur, A. et al. (2013).** Resveratrol and Adipose-derived Mesenchymal Stem Cells Are Effective in the Prevention and Treatment of Doxorubicin Cardiotoxicity in Rats. Pediatric Hematology and Oncology, 30 (3), 226-238.
63. **Pulavendran, S., Rose, C., and Mandal, A.B. (2011).** Hepatocyte growth factor incorporated chitosan nanoparticles augment the differentiation of stem cell into hepatocytes for the recovery of

liver cirrhosis in mice. Journal of nanobiotechnology, 9(1), 1.

64. **Razmaraii, N., Babaei, H., Nayebi, A.M., Asadnasab, G. et al. (2016).** Cardioprotective Effect of Phenytoin on Doxorubicin-induced Cardiac Toxicity in a Rat Model. Journal of cardiovascular pharmacology, 67(3), 237-245.
65. **Reagan, W.J., York, M., Berridge, B., Schultze, E. et al. (2013).** Comparison of cardiac troponin I and T, including the evaluation of an ultrasensitive assay, as indicators of doxorubicin-induced cardiotoxicity. Toxicologic Pathology, 41, 1146-1158.
66. **Richard, C., Ghibu, S., Delemasure-Chalumeau, S., Guilland, J.C. et al. (2011).** Oxidative stress and myocardial gene alterations associated with doxorubicin-induced cardiotoxicity in rats persist for 2 months after treatment cessation. Journal of Pharmacology and Experimental Therapeutics, 339(3), 807-814.
67. **Rochefort, G., Vaudin, P., Bonnet, N.,Pages, J., Domenech, J. and Charbord, P. (2005).** Influence of hypoxia on the domiciliation of mesenchymal stem cells after infusion into rats, possibilities of targeting pulmonary artery remodeling via cells therapies?. Respiratory Research, 6, 125.
68. **Shafik, A.N., Khodeir, M.M. and Fadel, M.S. (2011).** Animal study of Anthracycline-induced Cardiotoxicity and Nephrotoxicity and Evaluation of Protective Agents. Journal of Cancer Sciences and Therapy, 3, 096-103.
69. **Shi, J., Abdelwahid, E. and Wei, L. (2011).** Apoptosis in Anthracycline Cardiomyopathy. Current Pediatric Reviews, 7(4), 329–336.
70. **Speth, P.A.J., Van Hoesel, Q.G.C.M. and Haanen, C. (1988).** Clinical pharmacokinetics of doxorubicin. Clinical pharmacokinetics, 15(1), 15-31.
71. **Spivak, M., Bubnov, R., Yemets, I., Lazarenko, L. et al. (2013).** Doxorubicin dose for congestive heart failure modeling and the use of general ultrasound equipment for evaluation in rats. Longitudinal in vivo study. Medical ultrasonography, 15(1), 23–28.
72. **SPSS Inc. (2009).** PASW Statistics for Windows, Version 18.0. Chicago, SPSS Inc.
73. **Swerdlow, A.J., Higgins, C.D., Smith, P., Cunningham, D. et al. (2007).** Myocardial infarction mortality risk after treatment for Hodgkin disease, a collaborative British cohort study.Journal of the National Cancer Institute, 99(3), 206-214.
74. **Teraoka, K., Hirano, M., Yamaguchi, K. and Yamashina, A. (2000).** Progressive cardiac dysfunction in adriamycin - induced cardiomyopathy rats. European journal of heart failure, 2(4), 373-378.
75. Tulubas, F., Gurel, A., Oran, M., Topcu, B. et al. (2015). The protective effects of ω-3 fatty acids on doxorubicin-induced hepatotoxicity and nephrotoxicity in rats. Toxicology and industrial health,31(7), 638-644.
76. **van Acker, S.A., Kramer, K., Voest, E.E., Grimbergen, J.A. et al. (1996).** Doxorubicin-induced cardiotoxicity monitored by ECG in freely moving mice A new model to test potential protectors. Cancer chemotherapy and pharmacology, 38(1), 95-101.
77. **van de Ven, C., Collins, D., Bradley, M.B., Morris, E. et al. (2007).** The potential of umbilical cord blood multipotent stem cells for non hematopoietic tissue and cell regeneration. Experimental hematology, 35(12), 1753-1765.
78. **Van Linthout, S., Stamm, C., Schultheiss, H. P. and Tschöpe, C. (2011).** Mesenchymal stem cells and inflammatory cardiomyopathy: cardiac homing and beyond. Cardiology research and practice, 2011, Article ID 757154, 8 pages.
79. **Vijay, V., Han, T., Moland, C.L., Kwekel, J.C. et al. (2015).** Sexual dimorphism in the expression of mitochondria-related genes in rat heart at different ages. PloS one, 10(1), e0117047.
80. **Wang, H., Hung, S., Peng, S., Huang, C. et al. (2004).** Mesenchymal Stem Cells in the Wharton's Jelly of the Human Umbilical Cord. Stem Cells, 22, 1330–1337.
81. **Warpe, V.S., Mali, V.R., Arulmozhi, S., Bodhankar, S.L. et al. (2015).** Cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in wistar rats. Journal of Acute Medicine, 5(1), 1-8.
82. **Wei, X., Yang, X., Han, Z.P., Qu, F.F. et al. (2013).** Mesenchymal stem cells, a new trend for cell therapy. Acta Pharmacologica Sinica, 34(6), 747-754.
83. **Wu, J., Kodzius, R., Xiao, K., Qin, J. et al. (2012).** Fast detection of genetic information by an optimized PCR in an interchangeable chip. Biomedical microdevices, 14(1), 179-186.
84. **Yilmaz, S., Atessahin, A., Sahna, E., Karahan, I. et al. (2006).** Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology, 218(2), 164-171.