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Research Article
Up-regulation of Antioxidant Status in Chronic Renal Failure Rats Treated with Mesenchymal Stem Cells and Hematopoietic Stem Cells

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Abstract

Background and Objective: Renal failure patients may benefit from kidney transplantation, but the use of immunosuppressive drugs can cause many complications. Stem cell treatment may be a solution to avoid these complications. The current work was an attempt to participate in investigation of the oxidative status of Chronic Renal Failure (CRF) before and after treatment with pluripotent stem cells. **Materials and Methods:** Sixty four rats were divided into 8 groups. Group I (GI) rats injected only with doxorubicin to initiate CRF and groups II-VII (GII-GVII) rats injected with doxorubicin and treated with stem cells as the following: GII treated with Mesenchymal Stem Cells (MSCs), GIII treated with MSCs and an immunosuppressor, GIV treated with Hematopoietic Stem Cells (HSCs), GV treated with HSCs and an immunosuppressor and GVI treated with MSCs and HSCs, GVII treated with MSCs, HSCs, growth factors extract and an immunosuppressor. GVIII as a negative control (normal rats treated with PBS). **Results:** The obtained results showed an increase in the oxidants (MDA) and a decrease in the antioxidant parameters (GSH, SOD and CAT) in GI. These parameters restored their normal values after treatment with stem cells (GII-VII) when compared with the normal group (GVIII). Also, the electrophoresis protein patterns of the stem cells treated rats (GII-VII) were similar to that of the normal group (VIII). **Conclusion:** It can be concluded that stem cells treatment can regenerate the damaged kidney cells and can correct the disturbance in the antioxidant status.

Key words: Mesenchymal Stem Cells (MSCs), hematopoietic, immunosuppressor, antioxidants, growth factor, chronic renal failure

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Renal diseases are groups of the most common diseases. Despite the advances in kidney transplantation, the shortage of donor organs limits treatment for the End Stage Renal Damage (ESRD) patients[1]. Chronic Kidney Disease (CKD) is characterized by a decrease of the Glomerular Filtration Rate (GFR) to less than 60 mL/min/1.73 mt^2, persisting for 3 months or more due to kidney cell damage regardless the cause of damage[2] and the kidney gradually loss its function[3].

Kidney transplantation may be one of the benefit solutions for the patients with renal failure, but the use of immunosuppressive drugs can cause many complications such as; encephalopathy, movement disorders, opportunistic infections, neoplasms, myopathy and progression of atherosclerosis[4]. Complications of renal transplantation are related to cardiovascular, renal, neurologic and gastrointestinal systems. Neurologic complications include stroke and posterior reversible encephalopathy syndrome, Central Nervous System (CNS) infections, neuromuscular disease, seizure disorders and neoplastic disease[5,6].

One of the main risk factors in CKD is oxidative stress, which occur when there is overproduction of Reactive Oxygen Species (ROS) or a reduction in antioxidant defense capacity. Oxidative stress is involved in the progression of renal injury, pathogenesis of atherosclerosis and exacerbation of disease burden in CKD patients[7].

Mesenchymal Stem Cells (MSCs) that function through multiple mechanisms (cell-based therapies) and have the potential to target the inflammatory and immunologic pathways in many kidney diseases have been considered a clinically relevant solution[8]. Because of their immunologic characteristics and differentiation potential, MSCs have been widely used in experimental models[9]. The principal immunomodulatory role of MSCs is based on their capacities to shift the balance of immunologic responses toward the generation of anti-inflammatory molecules[10]. In addition, MSCs display low immunogenic potential as they express small amounts of major histocompatibility class I (MHC-I) and class II (MHC-II) molecules[11]. Also, MSCs contributed to the regeneration of endothelial and mesangial cells[12]. Chemokines are a group of cytokines secreted by damaged cells after injury act as attractants, to recruit immune and stem cells to start the process of repair[13]. Stem cell characterized by self-renewal such as; activated B and T lymphocytes which can also undergo self-renewal[14].

The MSCs can be isolated from nearly all kinds of human tissues. Among these are HMSCs from adipose tissue[15], peripheral blood[16], heart[17] and lung[18]. The MSCs display significant differences in their proliferative capacities according to their originating tissue. For example, UC-MSCs exhibit a higher proliferation potential than BM-MSCs[19]. The mean doubling time of the UC-MSCs is about 24 h and remained almost constant for up to 10 cell passages. However, the population doubling time of BM-MSCs reached approximately 40 h and considerably increased already after 6 cell passages[20]. Likewise, adipose tissue-derived MSCs also demonstrated an elevated growth rate compared to BM-MSCs[21]. Morphologically BM-MSCs are larger in size than adipose tissue-derived MSCs which also accompanied by an earlier *in vitro* senescence of BM-MSC[22].

Insulin-like growth factor-1 (IGF-1) is one of the key cytokines secreted by MSCs[23] and may play an important role in proliferation, migration and differentiation of MSCs during transplantation[24].

Therefore, the aim of the present study was to investigate the role of isolated stem cells and insulin like growth factor-1 extract in improvement of the antioxidant status in CRF rats injected intraperitoneally with HSCs and MSCs.

MATERIALS AND METHODS

Study area: The present study was started on 2013 and the experiments were carried out in Faculty of Science, Damietta University; Egypt and the results calculated and discussed in Faculty of Science University of Tabuk; Saudi Arabia.

Isolation of hematopoietic stem cells (HSCs) (CD^{+}_{34}) from cord blood: Positive selection of Hematopoietic Stem Cells (HSCs) fractions from human cord blood carried out using an immune magnetic positive selection method (Manual EasySep® Protocol Using Purple EasySep® Magnet)[25].

Mesenchymal stem cells (MSCs) identification (CD^{-}_{34}): Up to 2×10^6 cells were sorted by a Magnetic-Activated Cell Sorting (MACS) system, which consists of magnetic beads conjugated to an antibody against CD^{-}_{34} and the cells were harvested using 0.25% trypsin and pelleted. The cells were washed and labeled with anti-biotin magnetic beads and then passed through a magnetic column where CD^{-}_{34} cells were retained, while unlabeled cells passed and used Manual EasySep® Protocol Using Purple EasySep® Magnet[25].

Growth factor extraction: Human umbilical cord, uterine, placental and uterine curettage tissues were used as source of growth factors. These tissues were gently flushed with 50 mL of cold (4°C) Phosphate Buffered Saline (PBS), then cut into

1-2 cm pieces and 100 mg of the tissue were homogenized in 500 mL of 10 mM Tris-HCl buffer (pH 7.4) with a Homogenizer (Glass-col Terre haute, USA) (high speed for 2 min at 4°C). The homogenate was centrifuged at 13500xg for 45 min at 4°C using cooling centrifuge. The clear supernatant (without tissue debris) was then filtered through 0.45 µm filters and used in the detection methods[26]. The growth factor extract was sterilized and filtered using filter syringe 0.4 µm in sterilized fume hood. The growth factor from placental was the most effective and therefore was used in this study.

Experimental animal of the study

Animals: All experiments were performed on 64 adult male Sprague Dawley Albino rats purchased from Theodore Bilharzia Institute, Giza, Egypt with body weights of 70-120 g. Rats were housed in steel mesh cage (8 rats/cage) and maintained for two weeks acclimatization periods on commercial standard diet and tap water. The rats were divided into eight groups, 8 animals each. The most important observations were recorded such as the reduction in the activity and appetite.

Experimental groups

- **Group I: Rats with Chronic Renal Failure (CRF) model:** each rat was injected intra-peritoneally with 15 mg doxorubicin (adriamycin)/kg b.wt., one dose as positive control[27]
- **Group II: Rats with CRF treated with MSCs:** One week after inducing CRF in the rats, each rat was injected intraperitoneally with one dose of MSCs (100 µL of cell suspension contain 1×10^{6})[28]
- **Group III: Rats with CRF treated with MSCs and an immunosuppressor:** CRF, was induced in rats of this group as in group I and after one week, each rat was inoculated intraperitoneally with one dose MSCs (100 µL cell suspension contain $1x10^{6}$ cell) and were given daily 5 mg cyclosporine a/kg b.wt./day as immunosuppressor for 30 days[29].
- **Group IV: Rats with CRF treated with HSCs:** CRF rats were intraperitoneally injected with single dose of HSCs (100 µL of cells suspension contain 2×10^{4} cells)
- **Group V: Rats with CRF treated with HSC sand an immunosuppressor:** CRF rats were intraperitoneally injected with one dose of HSCs (100 µL of cells suspension contain 2×10^{4} cells) and were given daily 5 mg cyclosporine a/kg b.wt./day as immuno-suppressor drug for 30 days[29]
- **Group VI: Rats with CRF treated with MSCs and HSCs:** One week after inducing CRF in the rats of group I, each rat was inoculated intraperitoneally with one dose of HSCs (100 µL of cells suspension contains 2×10^{4} cells) and MSCs one dose (100 µL of cells suspension contains 1×10^{6} cells)
- **Group VII: Rats with CRF treated with MSCs, HSCs, growth factors extract and an immunosuppressor:** One week after induction of CRF in rats of this group, each rat was inoculated intraperitoneally with one dose of MSCs, one dose of HSCs (100 µL of cells suspension contain 2×10^{4} cells) and sterilized growth factor crude extract (200 µL) daily intraperitoneally injected and 5 mg cyclosporine/kg b.wt./day as immunosuppressor drug for 30 days
- **Group VIII: Negative control:** Rats were injected intraperitoneally with 100 µL/kg b.wt./daily physiological saline solution for 30 days as negative control

Collection of samples

Collection of blood samples: At the end of the experiment (after 25 days) rats of each group were sacrificed and one part of blood samples was collected and then centrifuged for obtaining sera and another part was collected using EDTA as anticoagulant for obtaining plasmas.

Collection of kidney samples: Kidney samples were quickly excised, rinsed with isotonic saline solution and dried with filter paper. The kidney samples were weighed and stored at -20°C in plastic vials containing 0.5 mL of ice cold sterile isotonic saline solution.

Preparation of kidney homogenates: A piece of kidney tissue (0.14 g) was homogenized in 1 mL of PBS pH 7.4 using a Teflon pestle connected to homogenized motor (25 strokes per minute at 1000 rev/min) and the homogenate was diluted to became 10% (w/v). The homogenate was centrifuged at 13000 rpm for 30 min at 4°C. The supernatant was used for biochemical analyses.

Biochemical analyses: Reduced glutathione (GSH) in the blood was determined by the method of Beutler *et al.*[30]. The GSH levels in kidney homogenate were estimated by the method proposed by Moron *et al.*[31]. Malondialdehyde (MDA) in kidney homogenate was measured by the thiobarbituric acid assay according to the method of Ohkawa *et al.*[32] and MDA in red blood cells were determined by the method of Stocks and Dormandy[33]. Catalase activity in serum was assayed

according to the method of Aebi[34]. Superoxide dismutase (SOD) activity in kidney homogenate was determined by the method of Rest and Spitznagel[35]. Red blood cell of SOD activity was determined by the method of Winterbourn *et al.*[36]. The total proteins were determined by the method of Lowry *et al.*[37]. The total lipids in serum and tissue extract were determined by the method of Knight *et al.*[38]. Serum albumin was determined by the method of Doumas *et al.*[39]. Hemoglobin (Hb) concentration was determined according to the method of Drabkin and Austin[40]. Protein fractionation was done by using one-dimensional polyacrylamide gel electrophoresis according to the method of Laemmli[41].

Statistical analysis: Statistical analysis was conducted with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The results were expressed as Mean±SD. Correlation tests were made by Graph pad prism 5. The p-values $\leq$0.05 was said to be *Significant and $\leq$0.001 highly **Significant.

RESULTS

In Table 1, the levels of GSH in erythrocytes and kidney tissue are highly significantly decreased in rats of CRF group (GI) compared to GSH levels in RBCs and kidney tissue homogenate of the control group (GVIII). After treatment with either MSCs or HSCs in presence or absence of growth factor and cyclosporine a, GSH levels in RBCs and kidney tissue homogenate restored nearly their normal values with non-significant variations between the treated groups (GII-VII). However, MDA levels either in erythrocytes or kidney tissues homogenate are highly significantly increased in CRF rats (GI) compared to the control group (GVIII). This elevation returned nearly to the normal values of control group (GVIII) in all treated groups with MSCs and HSCs with non-significant variations between the treated groups (GII-VII) except groups III and IV.

Table 2 illustrates the highly significant decrease in the activities of catalase and SOD in kidney tissues homogenate and activity of SOD in RBCs of CRF group (GI) compared to their values in the control group (GVIII). These enzymes nearly restored their normal activities after treatment the rats with MSCs and HSCs in presence of growth factor and cyclosporine a as an immunosuppressor (groups II-VII) with non-significant variations in SOD between the treated groups (GII-VII).

It is observed that the total protein in serum and kidney tissue homogenate and levels of total lipids and albumin in serum of CRF rat group (GI)are highly significantly decreased compared to the corresponding values in the control (GVIII). All these parameters nearly returned to its normal values in groups (GII-VII) after treatment with MSCs and HSCs with and without growth factors and cyclosporine a (Table 3).

Table 1: Mean values of GSH and MDA in erythrocytes and kidney tissue homogenates of rats in groups I- VIII

Groups	GSH (mmoles/l cells)	GSH (mg g^{-1} tissue)	MDA (nmole g^{-1} tissue)	MDA (mmoles mL^{-1} packed cells)
Group I	2.49±1.33**	0.62±0.66**	11.85±2.38**	21.66±3.37**
Group II	6.60±3.67^{ns,a}	2.22±0.43^{ns,b}	3.83±1.24^{ns,b}	11.87±4.90^{ns,b}
Group III	5.11±2.84^{ns,a}	1.98±.59 ^{ns,a}	4.08±1.58^{*,a}	15.73±6.16^{ns,a}
Group IV	7.49±7.27^{ns,b}	1.53±0.22^{ns,b}	4.13±3.39^{*,b}	12.43±7.18^{ns,a}
Group V	4.33±2.44^{ns,a}	1.83±0.04^{ns,b}	3.43±1.73^{ns,b}	13.45±2.04^{ns,a}
Group VI	4.67±2.61^{ns,a}	1.77±0.69^{ns,b}	3.41±1.03^{ns,b}	16.46±3.94^{ns,a}
Group VII	6.76±4.26^{ns,a}	2.09±0.69^{ns,b}	3.67±0.05^{ns,a}	13.70±3.09^{ns,a}
Group VIII	9.88±4.74	2.26±0.49	3.70±1.29	14.39±2.75

The values represented as Mean±SD, (n = 8), ns non-significant (p>0.05), *Significant (p<0.05) and **Highly significant (p<0.001) when compared groups I-VII with group VIII, [a]: Significant (p<0.05), [b]: Highly significant (p<0.001), [c]: Non-significant (p>0.05) when compared groups II-VII with group I, Group I: Positive control, Group VIII: Negative control, GSH: Glutathione, MDA: Malondialdehyde

Table 2: CAT in kidney tissue homogenate and SOD activities in erythrocytes and kidney tissue homogenate from rats of groups I- VIII

Groups	CAT Activity (KU g^{-1} protein)	SOD in Tissue (Inhibition (%))	SOD activity (Units g^{-1} Hb)
Group I	537.63±304.86**	34.52±8.62**	177.87±59.10**
Group II	1084.50±86.79^{ns,b}	54.56±.39^{ns,a}	312.70±96.67^{ns,b}
Group III	1049.70±428.97^{*,b}	52.86±8.94^{ns,b}	395.00±47.84^{ns,b}
Group IV	1378.69±384.98^{ns,b}	59.27±9.97^{ns,b}	371.45±23.32^{ns,a}
Group V	1115.24±269.37^{ns,b}	49.29±17.54^{ns,b}	340.31±12.04^{ns,b}
Group VI	1175.47±231.65^{*,b}	47.44±4.98^{ns,b}	338.31±48.56^{ns,b}
Group VII	1033.66±164.04^{ns,b}	52.12±12.03^{ns,b}	365.06±16.18^{ns,b}
Group VIII	1214.23±254.12	43.08±5.35	450.06±103.20

The values represented as Mean±SD, (n = 8), ns non-significant (p>0.05), *Significant (p<0.05), **Highly significant (p<0.001) when compared groups I-VII with group VIII, [a]: Significant (p<0.05), [b]: Highly significant (p<0.001), [c]: Non-significant (p>0.05) when compared groups II-VII with group I. Group I: Positive control, Group VIII: Negative control

Table 3: Total protein in serum and kidney tissue homogenate and total lipids and albumin levels in serum from rats of groups I-VIII

Groups	Total protein in serum (mg%)	Total lipid (mg%)	Total protein in tissue (mg%)	Albumin (mg%)
Group I	31.64±11.57**	292.25±57.70**	140.63±14.79*	6.47±0.23**
Group II	62.70±1.67^{ns,b}	309.06±79.75^{*,a}	153.38±1.69^{ns,a}	7.14±0.79^{ns,a}
Group III	60.18±2.93^{ns,b}	306.24±30.71^{*,a}	147.88±10.74^{ns,a}	7.24±0.33^{ns,b}
Group IV	53.49±1.36^{ns,b}	333.45±1.43^{*,a}	150.50±7.31^{ns,a}	8.06±0.67^{ns,b}
Group V	57.49±3.57 ^{ns,b}	321.60±37.14^{*,a}	154.66±3.39^{ns,a}	7.24±0.29^{ns,b}
Group VI	45.22±1.11 ^{*,b}	316.21.75±1.86^{*,a}	145.03±0.06^{ns,a}	7.03±0.65^{ns,b}
Group VII	50.21±2.54^{ns,b}	310.96±28.71^{*,a}	146.09±4.06^{ns,a}	8.21±0.32^{ns,b}
Group VIII	62.02±0.74	370.89±32.72	157.63±5.63	7.50±0.53

The values represented as Mean±SD, (n = 8), ns non-significant (p>0.05), *Significant (p<0.05) and **Highly significant (p<0.001) when compared groups II-VII with group VIII, [a]: Significant (p<0.05), [b]: Highly significant (p<0.001), [c]: Non-significant (p>0.05) when compared groups II-VII with group I, Group I: Positive control Group, VIII: Negative control

Table 4: Kidney weight, kidney wt./b.wt. ratio and blood Hb levels in groups I-VIII

Groups	Kidney wt. (g)	Kidney wt./b.wt. ratio	Hb (g%)
Group I	0.30±0.02**	210.60±17.80**	8.18±1.43**
Group II	0.40±0.09^{ns,b}	157.69±25.44^{ns,b}	12.55±1.07^{ns,b}
Group III	0.35±0.08^{ns,a}	150.63±31.61^{ns,b}	10.93±1.65^{ns,a}
Group IV	0.38±0.03^{ns,b}	143.52±17.20^{ns,b}	11.29±2.88^{ns,a}
Group V	0.35±0.32^{ns,b}	154.74±16.91^{ns,b}	13.00±0.85^{ns,b}
Group VI	0.37±0.02^{ns,b}	141.65±12.03^{ns,b}	9.53±2.04^{ns,a}
Group VII	0.33±0.08^{ns,a}	148.09±10.54^{ns,b}	10.32±0.55^{ns,a}
Group VIII	0.43±0.05	138.83±58.28	12.82±3.29

The values represented as Mean±SD, (n = 8), ns non-significant (p>0.05), *Significant (p<0.05) and **Highly significant (p<0.001) when compared groups II-VII with group VIII, [a]: Significant (p<0.05), [b]: Highly significant (p<0.001), [c]: Non-significant (p>0.05) when compared groups II-VII with group I. Group I: Positive control Group, VIII: Negative control

Table 5: Correlations between tissue MDA, erythrocyte MDA, SOD activity in RBC'S and albumin with GSH in RBCs, GSH in tissue, MDA in tissue, MDA in RBCs, SOD activity in RBC'S and albumin for studied group of rats with Chronic Renal Failure (CRF)

Parameters	MDA (nmole/g tissue)	MDA (mmoles/ml packed cells)	SOD activity in RBC'S (Units/gHb)	Albumin (mg%)
GSH (mmoles/L cells)	r = -0.2799	r = -0.3700	r = 0.3342	.....
	p = 0.0125	p = 0.0013	p = 0.0035	
GSH (mg g^{-1} tissue)	r = -0.3342	r = -0.3385	.....	r = 0.3577
	p = 0.0035	p = 0.0031		p = 0.0019
MDA (nmole/g tissue)	.....	r = 0.2887	r = -0.2136	.....
		p = 0.0103	p = 0.0451	
MDA (mmoles/mL packed cells)	r = 0.2887	.....	r = -0.3041	r = -0.3266
	p = 0.0103		p = 0.0073	p = 0.0042
SOD activity in RBC'S (Units/g Hb)	r = -0.2136	r = -0.3041	.....	r = 0.3690
	p = 0.0451	p = 0.0073		p = 0.0013
Albumin (mg%)	.....	r = -0.3266	r = 0.3690	.....
		p = 0.0042	p = 0.0013	

Number of rats = 64

From Table 4, it is observed that kidney weight/b.wt. ratio is highly significantly increased in CRF rats (group I), while Hb level is highly significantly decreased compared to its level in the corresponding control (group VIII). These changes are nearly corrected in groups (II-VII) after treatment with MSCs and HSCs with and without growth factors and cyclosporine a.

Figure1 shows SDS-PAGE for kidney tissue homogenate of CRF rats. Significant differences between the homogenates of those of treated rats and chronic renal failure rats are shown. The arrows show 5 bands with molecular weights 80, 58, 54, 46 and 39 KDa in lane 1 (CRF group), these bands disappeared in other treated rats groups as in lanes 2-7 which is nearly as in lane 8 (the control group (GVIII)).

Figure 2 shows that the mean survival times of rats of all treated groups increased compared with that of rats of the untreated group (GI).

All possible correlations between the different investigated parameters are described in Table 5. The results showed significant and highly significant negative correlations between GSH either in tissue or RBCs and SOD either in tissue or RBCs ve. MDA either in tissue or RBCs. Also, there are negative correlations between Hb, albumin, total proteins and total lipids versus MDA.

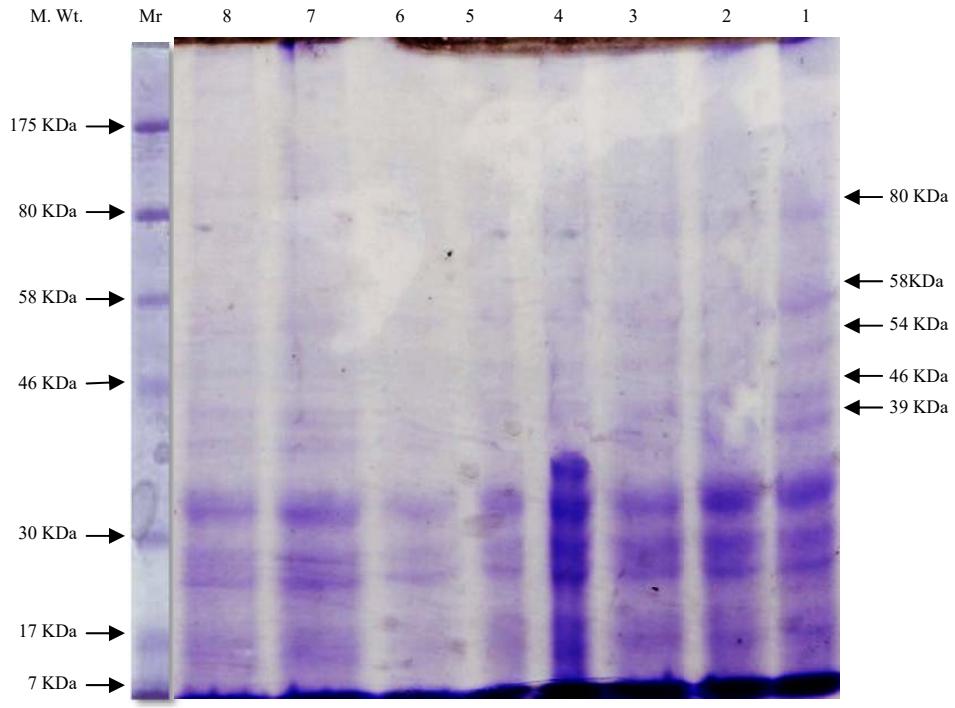

Fig. 1: SDS-PAGE (12%) for proteins of kidney tissue homogenates of groups I –VIII

Lane 1: Kidney tissue homogenate from Chronic Renal Failure (CRF) rats, Lane 2: Kidney tissue homogenate from CRF rats treated with MSC, Lane 3: Kidney tissue homogenate from CRF rats treated with MSCs and cyclosporine a, Lane 4: Kidney tissue homogenate from CRF rats treated with HSCs, Lane 5: Kidney tissue homogenate from CRF rats treated with HSCs and cyclosporine a, Lane 6: Treated with MSCs and HSCs, Lane 7: Kidney tissue homogenate from CRF rats treated with MSCs, HSCs, growth factor extract and cyclosporine a, Lane 8: Kidney tissue homogenate of control group rats, Mr: Protein marker

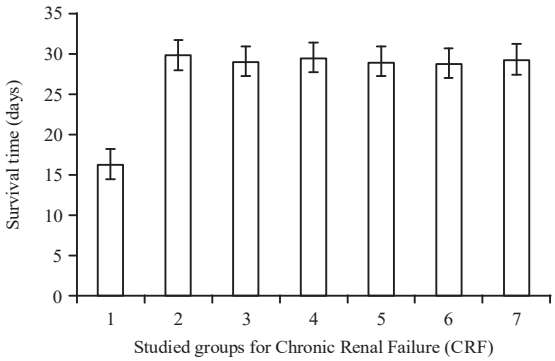

Fig. 2: Mean survival time of rats with chronic renal failure

DISCUSSION

Doxorubicin (adriamycin) treatment led to severe damage in the kidney tissues, such as; the decrease in kidney weight[42], CRF progression with glomerular and interstitial changes, destruction in the morphology of kidney cell membrane and a significant aggravated glomerular injury[43]. All these complications in CRF may be attributed to the increase in urinary protein excretion in hypertensive rats accompanied by damage in structure of the kidney which was followed by nephritis and necrosis in kidney tissue[44].

In the current study, doxorubicin treated rats showed an increase in MDA levels as a result of the increase in oxygen species (O_2^-) production due to the inflammatory cells infiltration in rats with CRF. Several studies showed that nephrotoxicity induced by doxorubicin is mainly caused by oxidative stress leading to inducing damage to renal tubular epithelial cells[45,46]. Nephrotoxic action of doxorubicin is considered to be due to generation of semiquinone radicals, which in turn react with molecular oxygen at an early stage after administration and causes development of progression of renal injury. Also, formation of a variety of vasoactive mediators can be promoted by oxidative stress. These mediators can affect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient and thus reducing the glomerular filtration rate[47].

Many ways have been investigated for protection against doxorubicin. Previous results suggested that, hydroethanolic extract of *P. major* protected renal tissue against doxorubicin-induced renal inflammation[48]. In the present study, the

administration of stem cells to the rats of CRF decreased the levels of MDA, which might be due to kidney tissue regeneration. GSH, a major non-protein thiol in living organisms, has a multiple role as an antioxidant agent and functions as a scavenger of ROS, including hydroxyl radicals, singlet oxygen, nitric oxide and peroxynitrite[47]. The results in the current study indicated that doxorubicin administration decreased levels of GSH in the kidney. Also, activities of CAT and SOD were decreased in the renal tissue. This increase in renal MDA content and decrease in GSH levels as well as, decrease in SOD and CAT activities that found in rats with CRF are similar to the results obtained by Kode *et al.*[11], who concluded that renal failure increases oxidative stress in renal tissues and stem cells have an ameliorating effect on the oxidative stress via their antioxidant property. Also, Gwinner *et al.*[49] found a reduction in CAT and SOD activities in mice model. In the current work, CAT and SOD activities and GSH content were significantly increased near the normal in the groups treated with MSCs and HSCs in presence or absence of cyclosporine a as an immunosuppressor drug. In addition, treatment of the rats with both MSCs and HSCs and natural uterine tissue extract like growth factor-1 improved the levels of GSH in renal tissues compared to the group injected with doxorubicin without stem cell treatment. Also, treatment of rats with stem cells from umbilical cord improved the levels of the two antioxidant enzymes (SOD and CAT) in doxorubicin administered rats, a finding that confirm the results reported by other studies for CRF rats model[50]. The MSCs invoke only minimal immune reactivity and furthermore, may possess anti-inflammatory and immunomodulatory effects[51].

The observed improvement in body weight by stem cells injected into CRF rats may be due to the increase in the appetite to provide a source of protein and carbohydrates for tissue regeneration and gaining functionality again[52]. The increase in kidney weight by doxorubicin probably resulted from edema that is caused by the drug-induced acute tubular necrosis[53].

Treatment of CRF rats with MSCs and HSCs in the presence of insulin and insulin like growth factor-1 showed restoring kidney functionality and total lipid and albumin levels. Also, lowered Hb level was restored significantly to its normal values after treatment with the stem cells. Other previous studies demonstrated the role of stem cells in regeneration of damaged renal tissues[54]. This indicated that stem cells have a potential protective effect against doxorubicin-induced nephro-toxicity.

SDS-PAGE proteins for MSCs and HSCs, insulin and insulin like growth factor-1 in the treated groups were similar to those of the control rats, while, kidney tissue homogenate from CRF rats, has shown the presence of new proteins with molecular weights 80, 58, 54, 46 and 39 KDa and this may be due to the oxidative stress acute phase proteins (Fig. 1). It is clear from the results that, mixing of HSCs and MSCs in treatment or addition of growth factor and immunosuppressor have no significant enhancement effect on the action of MSCs in the different treated groups and this means that MSCs have the ability to express growth factors such as VEGF, HGF and IGF-1, which facilitate recovery of kidney injury[53].

The highly significant ($p<0.0001$) negative correlations between MDA and the antioxidant parameters showed the high role of the oxidants in disturbance of the antioxidant status.

CONCLUSION

Renal failure rat model showed high elevation in oxidative stress as represented by MDA and decrease in antioxidant such as GSH level and Cat and SOD activity. After treatment with MSCs and HSCs, MDA return to its normal level of GSH and the activities of Cat and SOD also return to their normal activity. From these results, it can be concluded that, stem cells can regenerate the damaged kidney cells and can correct the disturbance in antioxidant status.

SIGNIFICANCE STATEMENT

Renal diseases are the most common diseases and the solution of these diseases attracts the attention of the researchers. The results of the current study demonstrated the importance of stem cell therapy in treatment of renal diseases.

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